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Relationship between Chemokine *MCP-1* Gene Polymorphism and Severity of Aerobic Bacterial Infection in Burn Wound Patients

A Thesis
Submitted to the Council of the College of Medicine/ University of Kerbala in Partial Fulfillments of the Requirements for the Degree of Master in Medical Microbiology

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Supervisor Certification

We certify that this thesis entitled (Relationship between Chemokine *MCP-1* Gene polymorphism and Severity of Bacterial Infection in burn wound patients) was prepared under our supervision at the College of Medicine, University of Kerbala, as a partial fulfillment of the requirements for the Degree of Master in Medical Microbiology.

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We, the examiners committee, certify that we've read the M.Sc. thesis entitled:

"Relationship between Chemokine *MCP -1* Gene Polymorphism and Severity of Aerobic Bacterial Infection in Burn Wound Patients"

We have examined the student **(Hasan Fadhil Khudhair Mutar)** in its contents. In our opinion it is meets the Standards of thesis for the degree of Masters in Medical Microbiology and Immunology.

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Dedication

I dedicate this work

To……

The first teacher, our prophet Mohammed (may Allah bless him and his family).

The soul of my father and mother.

My family.

My brothers, sisters

my friends.

To those who lighted the way for me, my teachers

Hasan

2024

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Summary

Burn wound infections remain one of the most widespread bacterial infections in the world. These infections are predominantly caused by *Pseudomonas aeruginosa*. Following thermal injury, *MCP-1*, a myeloid-associated factor, is associated with worse clinical outcomes. The *MCP-1* gene's promoter region contains a functional genetic polymorphism at rs1024611 (-2518 A > G), which influences *MCP-1* expression and associated to a number of inflammatory diseases.

A total of 83 swab samples were collected from both sex (44 males and 39 females), whose ages ranged from (1-60) years, attending to Imam Al-Hussein Medical City, Karbala, Iraq, during the period extending from August 2023 to December 2023. In addition, 70 blood samples were collected in an EDTA tube, divided into 35 samples from burn wound patients and 35 samples from apparently healthy individuals at an age range of (1-52) years old. Blood samples were used to detect the *MCP-1* gene polymorphism. All bacterial isolates were subjected to cultural, microscopical, and biochemical examinations. The identification of these bacterial species and the antibiotic susceptibility test of *Pseudomonas aeruginosa* were confirmed by using the VITEK2 compact system. And *MCP-1*gene polymorphism was measured by amplification refractory mutational system (ARMS) by polymerase chain reaction technique.

The results showed that the percentage of burned males and females were 53.0% and 47.0% respectively. While in control (42.9%) as males and (57.1%) as females. Regarding sex, there were non significant differences (P=0.420) between the study's groups. Rates of infection were higher in patients under the age of 11 compared to other age groups, and there were non significant differences (P=0.575) between the control and patient groups.

According to the type of burn, scald burns comprised the largest percentage of burn injuries (53%), followed by flame burns (41%), and electric burns (6.0%) had the lowest percentage of injuries.

In regards to the degrees of burn severity, the second degree constituted the most common of the cases (66.3%), while the third degree burned (25.3%), and the second +third degree constituted only (8.4%). The current study showed that *Pseudomonas aeruginosa* was the commonest burn wound isolate (31.43%), then *Klebsiella pneumoniae* (20.00%), followed by *Escherichia coli* (14.29%). Also, this study showed high resistance rates of *Pseudomonas aeruginosa* to all antibiotics (100%), except for the susceptibility (100%) to Colistin only.

Regarding the *MCP-1* gene polymorphism (rs1024611), the present study showed that patients' AA genotype was higher than that of controls' (40.0% vs. 8.6%, respectively). The percentage of the AG genotype was found to be higher in the control group compared to the patient group (17.1% vs. 14.3%, respectively), However, GG genotype was higher percentage in controls than in patients (74.3% vs. 45.7%, respectively). There was a significant difference in genotypes (P=0.008) between cases and control groups. The "A" allele was increased in patients compared with control (47.2% vs. 17.2%, respectively), whereas the "G" allele was elevated in control compared with patients (82.8% vs. 52.8%, respectively). There were significant differences (P = 0.001) between them.

The result of this study has shown that the AA genotype was higher in patients compared to controls. Therefore, the *MCP-1* gene (rs1024611) polymorphism may have a protective role in burn wound bacterial infection. In addition, *Pseudomonas aeruginosa* is the most prevalent bacteria in burned patients and susceptible to Colistin only.

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List of Abbreviations

Code	Words
ARMS	**Amplification-refractory mutation system**
AST	**Antibiotic susceptibility test**
CCL2	**Chemokine C Ligand 2**
DNA	**Deoxyribonucleic Acid**
dNTPs	**Deoxynucleotide triphosphates**
DW	**Distill water**
E. coli	***Escherichia coli***
EDTA	**Ethylene diamine tetra acetic acid**
GBR	**Global Burn Registry**
GN	**Gram-negative**

HAI	**Hospital-acquired infections**
ID	**Identification**
K. pneumoniae	***Klebsiella pneumoniae***
LasA	**ElastaseA**
LasB	**ElastaseB**
MCP-1	***Monocyte chemoattractant protein-1***
MIC	***Minimum inhibitory concentration***
P. aeruginosa	***Pseudomonas aeruginosa***
PCR	**Polymerase chain reaction**
S. aureus	***Staphylococcus aureus***
SD	**Standard deviation**
S.epidermidis	***Staphylococcus epidermidis***
S. haemolyticus	***Staphylococcus. haemolyticus***
SNP	**Single Nucleotide Polymorphisms**
TBE	**Tris-Borate EDTA**
TBSA	**Total body surface area**
T3SS	**Type 3 secretion systems**
US	**United States**
UV	**Ultraviolet**
WHO	**World Health Organization**

Chapter One
Introduction and Literatures Review

1.1. Introduction

Burn injuries continue to be a major risk to the public's health because they are responsible for a significant percentage of deaths and disabilities that might have been avoided each year (Stokes and Johnson, 2017). Burn injuries, in comparison to other types of wounds, often have a larger wound size, a more irregular shape, more severe massive bleeding, and exudates from the wound sites, which makes them more susceptible to infection (Hu *et al*., 2023).

Burn infections, mostly caused by Gram-negative and Gram-positive bacteria (Aljanaby and Aljanaby, 2018). bacterial infections are the leading cause of death among burn patients. These infections are linked to burn unit contamination, which is a major problem in a burn ward (Kadhim *et al*., 2023). In comparison to the other bacteria that may cause infections in burn wounds, *Pseudomonas aeruginosa* is the most common and widespread of all bacteria that can cause infections in burn wounds. This is due to its preference for growing in moist environments and its ability to cause invasive infections when it grows within burn wound exudates (Kour *et al*., 2023). Multidrug-resistant *Pseudomonas aeruginosa* has emerged as a significant problem worldwide, posing a severe hazard to burn-infected patients (AL-Sabagh, Ghaima, AL-Dabbagh, 2023). Its possession of a large variety of virulence factors contributes significantly to the pathogenicity of the host, so the rapid detection of these bacteria plays a crucial role in controlling the diseases that cause them, especially burn injuries (Fakhry and Aljanabi, 2024).

Monocyte chemoattractant protein-1, belongs to the family of chemokines known as CC chemokines. This chemokine plays an essential role in the inflammation process by attracting or increasing the expression of other inflammatory factors and cells. It leads to the advancement of many disorders by this main mechanism of migration and infiltration of

inflammatory cells like monocytes/macrophages at the site of inflammation (Singh, Anshita and Ravichandiran, 2021). Functional genetic polymorphism at rs1024611 (-2518 A > G) in the promoter region of the *MCP-1* gene influences *MCP-1* expression and is linked to many inflammatory diseases (Lin *et al.*, 2021). Polymorphism rs1024611 located in the regulatory region of the *MCP-1* gene has previously been shown to be associated with increased *MCP-1* production (Shadrina *et al*., 2017).

The aim of the study

The aim of the study was to investigate the relationship between severity of bacterial infection and *MCP-1* gene polymorphism

1- Determination of clinical characteristics in the patients group

2. Isolation and identification of pathogenic bacteria in burn wounds by culture media, biochemical tests and the VITEK2 system and Detection of the antibiotic susceptibility of *P. aeruginosa* by using the VITEK2 system.

3-Detection of *MCP-1* gene polymorphism by the ARMS method using the PCR technique.

1.2. Literature Review

1.2.1. Definition of burn

Burns are defined as damage to the skin or other body tissues caused by severe heat or trauma. The World Health Organization (WHO) defines burns as skin injuries due to thermal, chemical, electric, or radiation-mediated entities. (Tasleem *et al*., 2023) Burn injury is an umbrella term for a trauma, most commonly affecting the skin or lung (Laggner *et al*., 2022).

Burns are one of the most common injuries in everyday life for people of every age (Chen *et al*., 2021). And they represent an important social and medical problem in the world (Gatea, Nedjat and Yekaninejad, 2019). They can seriously endanger humans' health and lives and they may cause disability and even psychological trauma in individuals. Such an event can also lead to an economic burden on the victim's families and society (Hameed, Ibraheam, and, Fakhir, 2019).

Burn injuries cause long-term, profound alterations that require attention to optimize quality of life (Jeschke *et al*., 2020).

1.2.2. Epidemiology

Burns form the fourth most common cause of injury after road traffic injuries, falls, and interpersonal conflicts (Obaid and Baiee, 2022). Worldwide, as a result of burn injuries, a significant number of hospitalized patients are affected and remain a major cause of morbidity and mortality, with significant economic consequences for healthcare (Obed *et al*., 2023).

The World Health Organization Global Burn Registry (GBR) a minimum dataset aligned with a centralized registry—was officially launched in 2018 to facilitate hospital-level collection of key prevention, care, and outcome data from burn-injured patients around the world in a standardized manner (Hebron *et al*., 2022). The World Health Organization (WHO) reports that burn injuries affect over 11 million people annually, with

180,000 people losing their lives as a result of burns (Markiewicz-Gospodarek *et al*., 2022).

Burns require medical care every 30 minutes in the United States, and every year, burn injuries kill between 4,000 and 6,000 people. Around 120 burn centers are located in the United States. The American Burn Association only certifies half of these burn centers. This implies that most individuals lack direct access to burn centers and require stabilization at a local hospital following their initial injury (Miller, 2023). According to reports from the American Burn Association 2019, the most common type of injury in the United States is flame burns (41%), followed by scalds (31%), chemical burns (3.5%), and electrical burns (3.6%), which are significantly less frequent. Burns in children under five are often scald injuries, and flame-related burns rise with age (Jeschke *et al*., 2020). Burn injuries are common in developing countries like Iraq, especially in rural and low-income areas (Hasan and Al-Humairi, 2022). About 7 million Indians suffer burn injuries annually (Roy *et al*., 2022). Nearly 3,000 individuals die every year in Bangladesh from burns (Bailey *et al*., 2019).

1.2.3. Etiology

There are many factors that place some individuals at greater risk for burn injury and poor outcomes (Spiwak, Sareen, and Logsetty, 2022). Due to etiology, burns can be divided into thermal, chemical, electrical, and others, such as radiation (Lachowski *et al*., 2023).

1.2.3.1. Thermal burn

Thermal burns are wounds to the skin produced by exposure to high temperatures, such as flames, hot surfaces, or hot liquids (Sonbul *et al*., 2021). Hot surfaces, often characterized by their limited surface area but depth, exert influence on the underlying skin tissues. Flame burns may cause lesions that affect several functional areas, causing direct and indirect thermal damage to the upper respiratory tract and the whole body

(Dimitrova, 2023). Flame burns cause some of the most psychologically and physically damaging type of trauma. Patients with flame burns are more likely to die and get multiorgan failure than those with scald burns (Lixia *et al*., 2021). Scalds affects all age groups, with the children being the most common (Mobayen *et al*., 2021). In Iraq, flames and scalds were the most common causes of burns (Lami and Al Naser, 2019).

1.2.3.2. Chemical burn

The term "chemical burn" refers to the irritation and devastation of human tissue resulting from chemical exposure, usually through direct contact with the chemical or steam (Agbenorku *et al*., 2015).The etiology and outcomes of chemical burns differ from country to country, depending on factors such as the composition of the local population, the distribution of industries, and the geographic and social environments. (Eftekhari *et al*., 2023).

The National Fire Protection Society has classified 300 of the more than one million known chemical compounds as extremely hazardous. Chemicals may be categorized into acid, alkali, organic, and inorganic substances. Acid causes coagulative necrosis while alkalis produce extensive denaturation of tissue protein. These produce deeper burns than acid burns (Akelma and Karahan, 2019). Acids and alkalis are the most common types of causative agents involved in chemical burns. Representative agents for alkalis are sodium hydroxide and potassium hydroxide, whereas representative agents for acids are sulfuric acid, hydrochloric acid, and hydrofluoric acid (Koh, Lee, and Kim, 2017).

1.2.3.3. Electrical burn

Different degrees of burns can result from the conversion of electricity into heat (Di *et al*., 2022). Electrical burns are extremely dangerous injuries that can result in deep burns, severe morbidity, and long-term effects (Gandhi, Parashar, and Sharma, 2022). Contact electrical burns are more

severe than other forms of contact burn injury (Marín *et al*., 2020). Electrical burns are considered the most serious public health issue due to their higher death rates (Mobayen and Sadeghi, 2022). The effects of electricity on the body are determined by seven factors.type of current (alternating current or direct current), amount of current, path of current, duration of current, contact area, and body resistance and voltage (Li *et al*., 2017).

Generally, there are two categories for electrical burns: high-voltage (≥1000 V) and low-voltage (<1000 V) (Ding *et al* 2020). Low voltage injuries are often limited to the immediate surrounding area; however, high-voltage injuries have been linked to deep underlying tissue damage that resembles crush injuries (Boyd *et al*., 2019).

1.2.3.4. Radiation burn

The skin has a certain tolerance to radiation. Excessing this tolerance will result in severe radiation burns of varying degrees. Sunburn from ultraviolet (UV) light is the most common type of radiation burn (Waghmare, 2013). Overexposure to UV radiation from the sun is hazardous to the skin (Camponogara and Oliveira, 2022). Sunburn is the reddening of the skin after overexposure to UV radiation from the sun, because the skin cannot be protected by melanin when exposed to an excessive amount of sunlight (Sánchez-Pérez *et al*., 2019).

Compared to ultraviolet A radiation (UV A), ultraviolet B (UV B) is more energetic , and it is predominantly responsible for sunburn in humans (Becker *et al*., 2020). Ultraviolet (UV) radiation impairs the outermost layer of the body, namely, the skin. UVB rays are known as "the burning rays" and account for 4%–5% of all UV radiation. It is a thousand times more potent at causing sunburn than UVA and is responsible for photo-induced skin damage (Tanaka, Uchi, and Furue, 2019). Most sunburns are first-degree or superficial second-degree (Yoon and Na, 2019).

1.2.3.5. Inhalation burns

The American Burn Association has stated that inhalation injuries are the leading cause of burn morbidity and mortality (Hendrickson *et al*., 2019). Three etiologies primarily cause respiratory tract burns: thermal injury, chemical exposure, and smoke inhalation. Thermal damage is usually limited to the supraglottis due to reflexive supraglottic and glottal closure. Chemical exposure more commonly affects the lower part of the respiratory tract, causing an inflammatory response, rupture of the respiratory mucosa, and ultimately mucosal sloughing. Smoke inhalation is the most common cause of laryngeal burns. (Tracy, Shehan, and Grillone, 2020).

1.2.3.6. Cold burn

Cold burn is a severe health condition that is manifested in situations where an individual is exposed to extreme cold conditions. Touching materials such as ice packs, or just being exposed to a low wind speed in air temperature below 15 °C can result in a freeze-burn. The manifestations vary from a self-healing superficial burn (frostnip) on skin to conditions as severe as gangrenous necrosis, leading to auto-amputation of the affected limb (Gupta, A., Soni, R., and Ganguli, M. 2021). Cold burns differ from thermal burns in both severity and time course due to the mechanism of cellular injury, and furthermore, vasoconstriction, endothelial injury and thromboembolism promote vascular insufficiency and ischaemia, synergistically contributing to tissue destruction. Finally, thawing of tissues generates oedema and loss of endothelial integrity (Barry *et al*., 2023).

1.2.4. Evaluation of burn severity

1.2.4.1. Wound depth

In the early 1950s, Jackson described two degrees of burn depth based on the appearance of skin after injury-partial-thickness skin loss and full-thickness skin loss and observed that the existence or lack of a sufficient amount of live epithelial components to regenerate the region indicated the

distinction between these two depths. Today, a structural-anatomical classification system is used to identify the depth of burns, ranging from minor epidermal injuries to full-thickness burns (Karim, Shaum and Gibson, 2020).

Classifying a burn into a wide category is necessary to determine the best course of treatment (Volety and Jeeva, 2022). Burns are classified into four degrees based on the depth of the burn (Cook *et al*., 2022).

1.2.4.1.1. First-degree burn

First-degree burns are localized to the epidermal layer and are painful, erythematous, dry, and blanching. These burns do not blister and take 3 to 6 days to heal (Lanham *et al*., (2020). First-degree burns are often uncomfortable but are self-limiting and generally don't require medical intervention unless there are additional complications, such as dehydration, or they encompass a large surface area (Miller, N. 2023)

1**.2.4.1.2. Second-degree burn**

Second-degree burns are referred to as partial-thickness burn because they extend into the dermis, which cause complete damage to the epidermis and a portion of the dermis. Second-degree burn injuries may expose intact sensory nerve endings, making them extremely sensitive and painful to touch. Re-epithelialization is contingent on the degree of dermal degradation and the quantity of injured skin appendages (Cook *et al*., 2022). Most second-degree burns heal without surgical intervention (Blome-Eberwein *et al*., 2021).

1.2.4.1.3. Third-degree burn

Third-degree burns affect the entire thickness, comprising the epidermis, dermis, hypodermis, all cutaneous annexes, and, in some cases, even the adipose tissue (Ocon *et al*., 2019). These severe injuries are mostly painless. Third-degree burns cause the skin to become leathery, and they often accompany other burns of varying degrees, leading to the observation

of a wide range of colors, including pale white and brown. Some cases result in charred skin (He, McCarthy, and Camci, 2021). Third-degree burns remain the most common reason for flame burns (Durdu *et al*., 2022).

1.2.4.1.4. Four-degree burn

The term "fourth-degree burn" is not commonly used in literature because it is typically associated with fatal injuries. These injuries occur mostly after high-voltage electrical burns or severe fire burns (Sahin *et al*., 2012). Fourth-degree burns characterized by a complete carbonization of tissues. Its range covers the full thickness of the skin, sometimes also with muscles, fascias and bones frequently result in amputation or severe functional disability (Zdanowski, Radziszewski, and Gorgone, 2019).

1.2.4.2. Total body surface area

To ensure appropriate treatment, the percentage of the burned body surface area to the total body surface area is a critical parameter. An inaccurate burn evaluation may lead to incorrect medical decisions, which can have severe consequences for patients. These include, for example, fluid aggregation caused by burn edema and overresuscitation. (Giretzlehner, Ganitzer and Haller, 2021). Several long-standing methods exist to estimate burn size to include the Rule of Nines, Lund-Browder Chart, and Palmar method; all of which remain in clinical use today (Carrougher and Pham, 2024).

An elementary method of estimating the extent of a burn is to use the patient’s hand as a reference for approximately 1% of the body surface according to the Rule of Palm. As a hand actually represents only about 0.7% of the body surface in females and 0.8% in males, the method overestimates TBSA (Holm *et al*., 2021).

The rule of nines divides the body into different sections and assign each section a percentage. The head is worth 9%, each arm through the hand is worth 9%, the torso is worth 36%, each leg is worth 18%, and the genitalia

are worth 1%. This rule also applies to children; however, due to variations in body ratios, the computation of surface area percentage differs (Miller, 2023).

Currently, the most extensively used and accurate chart for calculating the area of the whole body damaged by a burn injury is the Lund and Browder chart. The challenge of doing mathematical calculations based on the percentages assigned to different body parts that have only partially burned makes it difficult to utilize charts to compute burn percentages. Having to do mental calculations is often tiresome, particularly in an emergency (Murari and Singh, 2019).

Due to intensive medical research, it has been possible to develop three-dimensional computer-based systems that consider patients' body characteristics and allow a very realistic burn size assessment Although traditional paper-based documentation is still used in practice, it no longer meets modern requirements. Instead, adequate documentation is ensured by electronic documentation systems. These models do not take into consideration physical defects, sex, age, body mass, or other pertinent variables (Giretzlehner, Ganitzer and Haller, 2021).

Three-dimensional model achieved through software of the same name, which is a thorough and accurate burn-treatment documentation schema, facilitated by three-dimensional digital models tracked over time. Systemic errors can be avoided by modifying these models according to sex, height, weight, and body shape. Superimposing photos of the burned areas on the model prevents individual error and can be combined with methods of burn-depth evaluation. The program includes automatic encoding of diagnostic and therapeutic procedures. The model resolution is 1 cm2 and finer, allowing for the documentation of even small scars' locations and extents, thereby facilitating the registration of long-term results. Computer-aided

methods can tremendously reduce these errors, but incorrect input of burn areas remains a problem (Haller *et al*., 2009)

1.2.5. Risk factor for burn injury

There are several factors that increase an individual's risk of burn injuries (Spiwak, Sareen and Logsetty, 2022).

1.2.5.1. Age-related factors

1.2.5.1.1. The elderly

The world's population is increasing in age, as the proportion and numbers of elderly individuals in nearly every country are growing. World Population Prospects 2022 predicts a significant increase in the global population aged 65 and older, from 10% in 2022 to 16% in 2050 (Wu, Xi, and Xie, 2023). The elderly experience higher mortality rates and poorer outcomes compared to younger burn survivors with similar injuries (Harats *et al*., 2019). The occurrence of burn injuries among older people has been attributed to decreasing physical strength, impaired protective mechanisms, poor vision, the existence of multiple co-morbidities, and decreased reaction time (Bayuo and Botcgway, 2017).

1.2.5.1.2. Child under five years

Compared to other age groups, burn injuries are more common in young children less than age of five (Van Zoonen *et al*., 2022). The majority of burn injuries among children are caused by accidents at home (Lami and Al Naser, 2019). Risk factors for a higher prevalence in the pediatric population include inadequate supervision, crowded housing, low socioeconomic status, and low education levels (Elrod *et al*., 2019). Other risk factors linked to cooking burn injuries included not having a separate kitchen in the home (Chauhan, Aundhakar and Patil, 2023).

1.2.5.2. Sex-related factor

On a global scale, females have higher rates of deaths due to burn injuries resulting from unsafe cooking environments, especially in low- and

middle-income countries (Spiwak, Sareen, and Logsetty, 2022). Males are also at high risk for burn injuries chiefly due to males are responsible for most of the duties outside the home, which increases the risks of burn accidents occupation-related injuries (Al Laham, Elmanama and Tayh, 2013).

1.2.5.3. Socioeconomic factor

Individual-level socioeconomic disparities impact burn-related incidence, severity, and outcomes (Mason *et al*., 2023). People's living conditions, lifestyles, and cultures greatly influence the risk of burns, partially explaining the global differences in burn-related injury rates and distribution, both between and within countries (Bolm *et al*., 2016). Burns have remained a major health problem in most developing countries, which has increased mortality and morbidity among the population (Gatea, Nedjat, and Yekaninejad, 2019).

1.2.5.4. Comorbidities

Peripheral neuropathy, epilepsy, and other mental and physical impairments are comorbidities associated with an increased incidence of burn injuries. These comorbidities are common predisposing factors for burn injuries. Epilepsy in particular is a common cause of fires, severe burns, and burn-related fatalities, as those suffering from the condition may fall into an open fire or onto a cook stove (Lee, 2023). Despite a decrease in burn injuries due to seizures, such injuries still result in significant mortality and morbidity. Since these patients need to take their medication as prescribed, controlling them remains difficult (Johari, Mohammadi, and Dastgerdi, 2019).

1.2.5.5. Workplace burns

Occupational burns are among the important causes of work-related injuries (Basaran, and Ozlu, 2020). There are several reasons for workplace burn injuries, including thermal, electrical, and ultraviolet sunlight exposure

(Toolaroud, S., *et al*. (2023). Burn accidents in the workplace are mostly caused by the lack to use safety equipment and the presence of inadequate equipment (Bagheri *et al*., 2022).

1.2.6. Pathogenesis of burn

A burn injury can be classified into three zones: the coagulation zone, which is the most damaged area in the centre; the zone of stasis, also known as the zone of ischaemia; and the hyperaemia zone (Jeschke *et al*., 2020). The zone of coagulation represents the area of necrosis with irreversible tissue damage incurred at the time of injury, the stasis area surrounds the coagulation zone and is moderately damaged with vascular transudate, elevated vasoconstricting factors, as well as local inflammatory reactions, resulting in impaired tissue perfusion. Depending on the wound environment, the zone may recover or progress to necrosis. The zone of hyperaemia has dilated vessels caused by inflammation. It is characterised by increased blood flow to healthy tissues without much risk of necrosis, unless there is severe sepsis or prolonged hypoperfusion (Zwierełło *et al*., 2023)

Burning, oedema, and inflammation are caused by a variety of factors. Patients typically experience generalized oedema when their burns exceed 30% of their total burned surface area. The heat can directly damage vessels and increase permeability. Increased capillary permeability to proteins is one of these changes. Equally important changes include the presence of an initial profound negative interstitial pressure sucking fluid into the tissue and a marked increase in interstitial space compliance. Heat alters proteins, which activate complement, resulting in histamine release and subsequent increases in vessel permeability, thrombosis and coagulation system activation. This leads to the release of serotonin (vasoconstriction) and bradykinin (increased permeability). The alteration or destruction of membrane phospholipids triggers the arachidonic acid cascade, resulting in

the release of leukotrienes (which increase permeability and neutrophil recruitment), thromboxane A2 (vasoconstriction), prostacyclin (a vasodilator), and prostaglandins (increased dilation and constriction) (Roth and Hughes, 2015).

The increased permeability of capillaries and the resulting plasma leak persist for 48 hours and reach their maximum in the first 8 hours. The capillaries either return to normal permeability within 48 hours or undergo thrombosis, rendering them unavailable for circulation. This loss of plasma is the cause of hypovolemic shock. During the acute phase, thrombosed vessels beneath the burned skin lose red blood cells. Therefore, the loss of blood increases with the depth of the burn. Heat reduces the lifespan of the red blood cells in circulation, leading to early hemolysis. Extensive burns also cause bone marrow depression, which leads to anemia (Tiwari, 2012).

Severe burn often have systemic effects that extend beyond the injury's immediate area. Severe burns trigger a systemic inflammatory response. Multiple organs are involved. The circulatory system changes hemodynamics; the respiratory system increases breathing rates; the endocrine system causes hypermetabolism and hyperglycemic states; and the immunological system changes its function (Burgess *et al*., 2022). Multiple organ dysfunction syndrome is common in severe burn patients, leading to poor outcomes. Altered organ function in two or more organ systems causes multiple organ dysfunction syndromes in acutely ill patients with severe trauma, burn, shock, and infection (Seada and Younis, 2020).

Severe organ dysfunction attributed to the host's disordered response to infection" defines sepsis, one of the most common burn complications, and is the primary cause of death in burn patients (Zhang *et al*., 2021). Sepsis involves more than systemic inflammation. It affects many organ functions. On the cellular and molecular levels, the pathogenesis of sepsis is extremely complex, including an imbalance in the inflammatory response, immune

dysfunction, mitochondrial damage, coagulopathy, neuroendocrine immune network abnormalities, endoplasmic reticulum stress and other pathophysiological processes (Huang, Cai and Su, 2019).

1.2.7. Role of bacterial infection in the burns wound

Burn injuries may cause serious damage to the human skin, impairing one of the primary defenses against infection. For patients with burn wounds, infection is the main cause of death. Even among patients' survivors, infections can cause long-term harm and are notoriously difficult to treat, with longer hospital stays and delayed healing (Maslova *et al*., 2021).

Hospital-acquired infections are defined as infections that occur within 48 hours after admission to the hospital or staying in a healthcare facility (Khammarnia *et al*., (2021). Hospitals are the primary medical facilities for infection acquisition. Lack of financial funding, poor infrastructure and administration, incorrect use of antimicrobials, and an absence of educated personnel are major challenges for successful infection control in low-income countries's hospitals (Raka, 2010).

Hospital-acquired infections can be classified into two categories depending on the source of the pathogen: endogenous infections and exogenous infections (Custovic, Smajlovic, and Dzafic, 2020). Infection is endogenously acquired from body's flora. Bacteria are found on the skin and mouth, in the nose, gastrointestinal tract, throat, and in the female genital tract. Exogenous sources of infection may be either animate or inanimate. People, including patients and hospital personnel, release a large number of bacteria into the environment via their skin, as well as through their oral and nasal secretions, when they sneeze, speak, and other body movements (Nayek, S. 2019).

Patients with burn wounds have different bacterial types depending on the injury's location and timing. Gram-positive bacteria are the predominant

microbes shortly after burning. After that, Gram-negative bacteria like *P. aeruginosa* and *Acinetobacter* species invade the burn site (Alturki, 2021).

Many cutaneous microorganisms are part of the skin's microbiota. *Staphylococcus epidermidis*, for example, can enter the wound, colonize it, and cause infection. *Staphylococcus aureus* is common in burn wounds. This bacterium has numerous virulent factors and colonizes the nasopharyngeal cavities. (Markiewicz-Gospodarek *et al*., 2022). Also, the Gram-positive microbes in the inside of sweat ducts and hair follicles can withstand the heat of the original injury., and unless topical antibiotics are used, within 48 hours, these bacteria colonize the wounds extensively after injury (Nasser., Mabrouk, and Maher, 2003).

Burn wound infection may involve a number of dynamic pathophysiological mechanisms, such as microbial colonization, biofilm formation, and invasive burn wound infection. Eschar (avascular necrotic tissue) results from deep partial- and full-thickness burns, which provide a protein-rich environment for bacteria colonization and growth (Zhang *et al*., 2021).

1.2.8. *Pseudomonas aeruginosa*

1.2.8.1. *Pseudomonas aeruginosa* general characteristics

Pseudomonas aeruginosa, belonging to the Pseudomonadaceae family, is Gram-negative, rod-shaped, motile (with a single polar flagellum), aerobic, non-spore forming, oxidase and catalase positive (Urgancı *et al*.,2022). It grows well at 37 °C, but it can survive in broad temperatures ranging from 4–42 °C. It is an important soil bacterium that is capable of breaking down polycyclic aromatic hydrocarbons but is often also detected in water-reservoirs polluted by animals and humans, such as sewage and sinks inside and outside of hospitals (Diggle and Whiteley, 2020)

Pseudomonas' simple growth requirements and nutritional versatility enable its broad environmental distribution. They are capable of using many

organic compounds as sources of carbon and nitrogen, and some strains can even grow in distilled water by using trace nutrients (Murray, Rosenthal, and Pfaller 2020). This bacterium is not fermentative and, in aerobic conditions, utilizes the glycolytic pathway to breakdown glucose, with oxygen serving as the final recipient of electrons. But nitrogen can function as an electron acceptor in anaerobic environments (De Sousa, *et al*., 2021).

1.2.8.2. Virulence factor of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is able to adapt to the adverse environment in hosts by secreting a variety of virulence factors, which contribute to successful infection and causing disease (Qin *et al*., 2022). The virulence of *P. aeruginosa* is characterized by the accumulation of six biologically competent attributes that initiate a multistep progression of the disease. Included them are colonization factors for hosts and motility of bacteria, the formation of biofilms, the production of damaging enzymes, siderophores that chelate iron, and toxin (Chadha, Harjai, and Chhibber, 2022).

1.2.8.2.1. Pili

The surface of *P. aeruginosa* contains long hair-like proteinaceous protrusion known as pili (Shanmugasundarasamy, Govindarajan, and Kandaswamy, 2022). Pili play versatile roles in bacterial physiology, including adhesion and host cell invasion, DNA and protein secretion and uptake, biofilm formation, and cell motility (Lukaszczyk, Pradhan, and Remaut, 2019).

1.2.8.2.2. Flagella

Pseudomonas aeruginosa has a single polar flagellum to swim in liquid and swarm across solid surfaces. Flagellum-dependent motility is essential for *P. aeruginosa* in establishing acute and chronic bacterial infections and evading host immune systems. Flagellum-dependent swimming motility enables the rapid dissemination of *P. aeruginosa* from the initial infection

site of burn patients to cause potentially life-threatening systemic infections (Xin *et al*., 2019)

1.2.8.2.3. Toxins

Bacterial toxins are thought to play a role in delayed wound healing in critically colonized and infected wounds. Endotoxins are released from Gram-negative bacteria when they are lysed by host phagocytic cells during an immune response, or by antimicrobial agents, potentially leading to a detrimental effect on the host tissues. Endotoxins can affect all aspects of the wound healing process, leading to delayed healing and contributing to wound chronicity. Release of endotoxins by bacteria can also have serious systemic effects (for example, septic shock) that can lead to high levels of patient mortality (Rippon, Westgate, and Rogers, 2022).

One of the most important virulence factors produced by *P.aeruginosa's* is Exotoxin A. It appears to play a role in both local and systemic *P. aeruginosa* disease processes. Exotoxin A It possesses necrotizing activity thus thought to aid in the colonization of the bacteria (Sekhi, 2022).

1.2.8.2.4. Enzymes

The degradation of elastin is accomplished by a synergistic action of two elastases, namely LasA (serine protease) and LasB (zinc metalloprotease). In acute infections, these enzymes can also break down complement components and stop neutrophils from moving and working properly, which can cause the infection to spread even more and damage tissues. Alkaline proteases, like elastases, facilitate the spread and destruction of tissue by *P. aeruginosa*. Additionally, it interferes with the host's immunological response. Phospholipase C is a heat-labile hemolysin that degrades lipids and lecithin, facilitating tissue destruction (Murray *et al*., 2020).

1.2.8.2.5. Type 3 secretion system

Type 3 secretion systems (T3SS) are particularly relevant among the virulence factors, being one of the most important in *P. aeruginosa*. It is a complicated "molecular syringe" that can insert different effectors into host cells. This can break down cell machinery, change immune responses, and make it easier for bacteria to survive (Horna and Ruiz, 2021).

1.2.8.2.6. Biofilms

Autogenic extracellular polymeric substances primarily compose the structure known as biofilm. These compounds serve as structures that attach bacteria on surfaces together, shielding them from external pressures. As a result, biofilm enables the bacteria to establish colonies and survive for extended periods of time (Thi, Wibowo, and Rehm, 2020). The complex architecture of the *P. aeruginosa* biofilm enhances the harmful nature of this microbe, resulting in treatment inefficacy, avoiding the immune system and developing persistent infections that are difficult to eradicate (Tuon *et al*., 2022).

1.2.8.3. Pathogenesis of *Pseudomonas aeruginosa* in burn patients

Chronic and acute infections caused by *P. aeruginosa* act a significant challenge to human health, especially in hospital settings. It has two antagonistic pathogenic strategies that parallel two different lifestyles: free-living cells are predominantly cytotoxic and induce an acute inflammatory reaction, while biofilm-forming communities cause refractory chronic infections (Valentini *et al*., 2018) Acute infections often spread rapidly and can cause tissue damage and sepsis with high mortality rates; chronic infections can persist for weeks, months, or years in the face of intensive clinical intervention (Turner *et al*., 2014).

Pseudomonas aeruginosa causes infections and diseases, particularly in immune-compromised patients, as well as many hospital-acquired infections. Given that *P. aeruginosa* is an opportunistic pathogen (Azam and

Khan, 2019). It exhibits increased pathogenicity due to the presence of a wide range of virulence factors, including both cell-associated and extracellular factors. Some of these factors enhance bacterial invasion, while others promote colonization (Rocha *et al*., 2019).

pseudomonas aeruginosa infections usually start as a localized, superficial lesion with a typical characteristic yellow or green color and a malodorous fruity smell, which may become an invasive infection termed "ecthyma gangrenosum," causing blue-purplish "punched-out" lesions in the skin *P. aeruginosa* can subsequently spread into deeper tissues rapidly to cause sepsis (Roy *et al*., 2024).

1.2.8.4. Diagnosis of *Pseudomonas aeruginosa*

The use of microscopy to observe thin Gram-negative rods arranged singly and in pairs is suggestive of *Pseudomonas*; however, it is not definite because *Burkholderia* and other *Pseudomonads* have a similar appearance. Because *pseudomonas* has simple nutrition requirements, the bacteria are readily recovered on blood agar and MacConkey agar. *P. aeruginosa* requires aerobic incubation. Identification Colony morphology, odor, and rapid biochemical tests (for example, a positive oxidase reaction) are sufficient for the early identification of these bacteria. These bacteria produce green pigmentation caused by the production of blue (pyocyanin) and yellow-green (pyoverdin) pigments and a characteristic sweet, grapelike odor (Murray *et al*., 2020).

The culture medium Cetrimide Agar Base selectively isolates and identifies *P. aeruginosa*. Cetrimide is a quaternary ammonium that inhibits a large number of bacteria, including those of the genus *Pseudomonas*, other than *P. aeruginosa* (Bonnet *et al*., 2020). VITEK 2 system is the next generation of the best method to identify bacteria and represents advanced colorimetric technology (Al-Saffar and Jarallah, 2019).

1.2.8.5. Treatment of *Pseudomonas aeruginosa*

Antibiotics belonging to the β-lactam class, including Carbapenems, Monobactams, Cephalosporins, and Penicillins, are highly effective in treating infections caused by *P. aeruginosa*. However, many isolates of these bacteria are resistant to β-lactams, complicating therapy and leading to poor patient outcomes (Glen and Lamont, 2021).

Newer agents such as Ceftazidime-Avibactam, Ceftolozane-Tazobactam, Imipenem-Relebactam, and Cefiderocol are useful for treating MDR-*P. aeruginosa* infections. Pathogens resistant to first-line antipseudomonal-lactams can benefit from these agents, which offer improved efficacy and less toxicity compared to aminoglycosides and polymyxins (Blomquist and Nix, 2021).

Antibiotic resistance is a worldwide problem. Isolation in some countries is multi-drug-resistant (resistant to three or more classes of antimicrobials), extensively-drug-resistant (resistant to all but one or two classes), or even pan-drug-resistant (resistant to all available classes) (Meletis and Bagkeri, 2013). *Pseudomonas aeruginosa* can rapidly develop resistance to wide-spectrum antibiotics (Hasan, Najati, and Abass, 2019).

Generally, the major mechanisms of *P. aeruginosa* used to counter antibiotic attack can be classified into intrinsic, acquired and adaptive resistance. The intrinsic resistance of *P. aeruginosa* includes low outer membrane permeability, expression of efflux pumps that expel antibiotics out of the cell and the production of antibiotic-inactivating enzymes. The acquired resistance of *P. aeruginosa* can be achieved by either horizontal transfer of resistance genes or mutational changes (Pang *et al*., 2019)

1.2.9. *Monocyte chemoattractant protein-1* gene polymorphism

1.2.9.1. *Monocyte chemoattractant protein-1*gene define

Monocyte Chemoattractant Protein-1(*MCP-1*) is one of several cytokine genes clustered on chromosome 17 (He, Yao, and Li, 2023). In

1989 witnessed the birth of *MCP-1* into the light of scientific investigation at the National Cancer Institute, Maryland, USA. This protein was initially identified in the conditioned media of the human myelomonocytic cell line as the monocyte chemotactic factor. It was further named monocyte chemotactic and activating factor, which was found to be rapidly produced in normal human dermal fibroblasts (Panee, 2012).

Chemokine (C-C motif) ligand 2, another name for *monocyte chemoattractant protein-1*, is an inflammatory mediator. It binds to CCR2, which is its receptor for increasing calcium flow and starting chemotactic activity (Zhu *et al*., 2021). It is produced by a variety of cell types in response to different signals such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interferon-gamma (Gupta, Chaturvedi, and Jain, 2013).

1.2.9.2. Role of *Monocyte chemoattractant protein-1*gene polymorphism in burns wound patients

Chemokines are small proteins that belong to the cytokine family. Their role is to control the migration of cells to sites of tissue damage or infection by binding appropriate receptors, thereby modulating the movement and function of target cells, especially leukocytes (Harvanová, Duranková, and Bernasovská, 2023).

Monocyte chemoattractant protein-1 is one of the most important chemokines that regulates the migration and infiltration of macrophages and monocytes throughout the immune response (Zhang, and Luo, 2019). Monocyte migration from the circulatory system across the vascular endothelium is critical for both regular immunological tissue surveillance and inflammation response (Mohammadi *et al*., 2019).

In the pathophysiology of various illnesses, *MCP-1* is essential and hence *MCP-1* inhibition may have beneficial effects in such conditions (Yadav, Saini and Arora, 2010). Clinical studies have demonstrated that elevated systemic levels of *MCP-1* correlate with mortality in burn patients.

Hence, *MCP-1* is a myeloid-associated factor that is linked to worse clinical outcomes after thermal injury (Eitas *et al*., 2017; Schaffrick, 2023).

Monocyte chemoattractant protein-1 is the most potent monocyte chemoattractant and inter-individual differences in its expression level have been associated with genetic variants mapping to the cis-regulatory regions of the gene. An A to G polymorphism in the *MCP-1* enhancer region at position 2578 (rs1024611; A>G) was found in most studies to be associated with higher serum *MCP-1* levels and increased susceptibility to a variety of diseases, such as HIV-1-associated neurological disorders, tuberculosis, and atherosclerosis (Pham *et al*., 2012).

The genetic variation in *MCP-1* has been seen in multiple diseases and varies with ethnicity. A certain *MCP-1* gene polymorphism may affect a particular ethnic group but not act in the same manner in another (Singh, Anshita, and Ravichandiran, 2021). In addition, *MCP-1* was more commonly observed in septic shock patients than in those without shock, in non-survivors compared to survivors, and also in patients with fulminant meningococcal sepsis compared to those with mild meningococcal sepsis (Vermont *et al*., 2006).

Chapter Two

Subjects, Materials and Methods

2. Subjects, Materials and Methods

2.1. Subjects

The sample size includes (83) participants as the patient group with burn wounds. A burn swab was collected from each patient for bacterial culture. Also, 2 ml of blood were collected in an EDTA tube to determine the *MCP-1* gene polymorphism of 35 burn patients with bacterial infection. On the other hand, 2 ml of blood samples were collected in an EDTA tube from 35 individuals as a healthy control group for detection the *MCP-1* gene polymorphism by using the amplification refractory mutational system (ARMS). The samples were taken from both sexes (44) males and (39) females, whose ages ranged from (1-60) years old, attending the burn unit of Imam Al-Hussein Medical City, Karbala /Iraq during the period extended from August (2023) to December (2023). Case information sheets involving age, sex, and others were carried out for each patient.

Inclusion criteria include:

All patients with burn wound infections were diagnosed based on clinical symptoms and other investigations.

Exclusion criteria include:

Patients who have wounds other than a burn, autoimmune diseases, cardiovascular disease, asthma, septicemia, pneumonia, cancer, and others.

2.1.1. Ethical approval

The study protocol was sent to the relevant ethical committee in the health directorate. Also, verbal approval was taken from all the patients or any family members, before taking the sample. During sample collection, health measures and safety taken.

2.1.2 Study design

Case- control study.

2.1.2.1. Study Design Scheme

The design of study was illustrated in Figure (2-1).

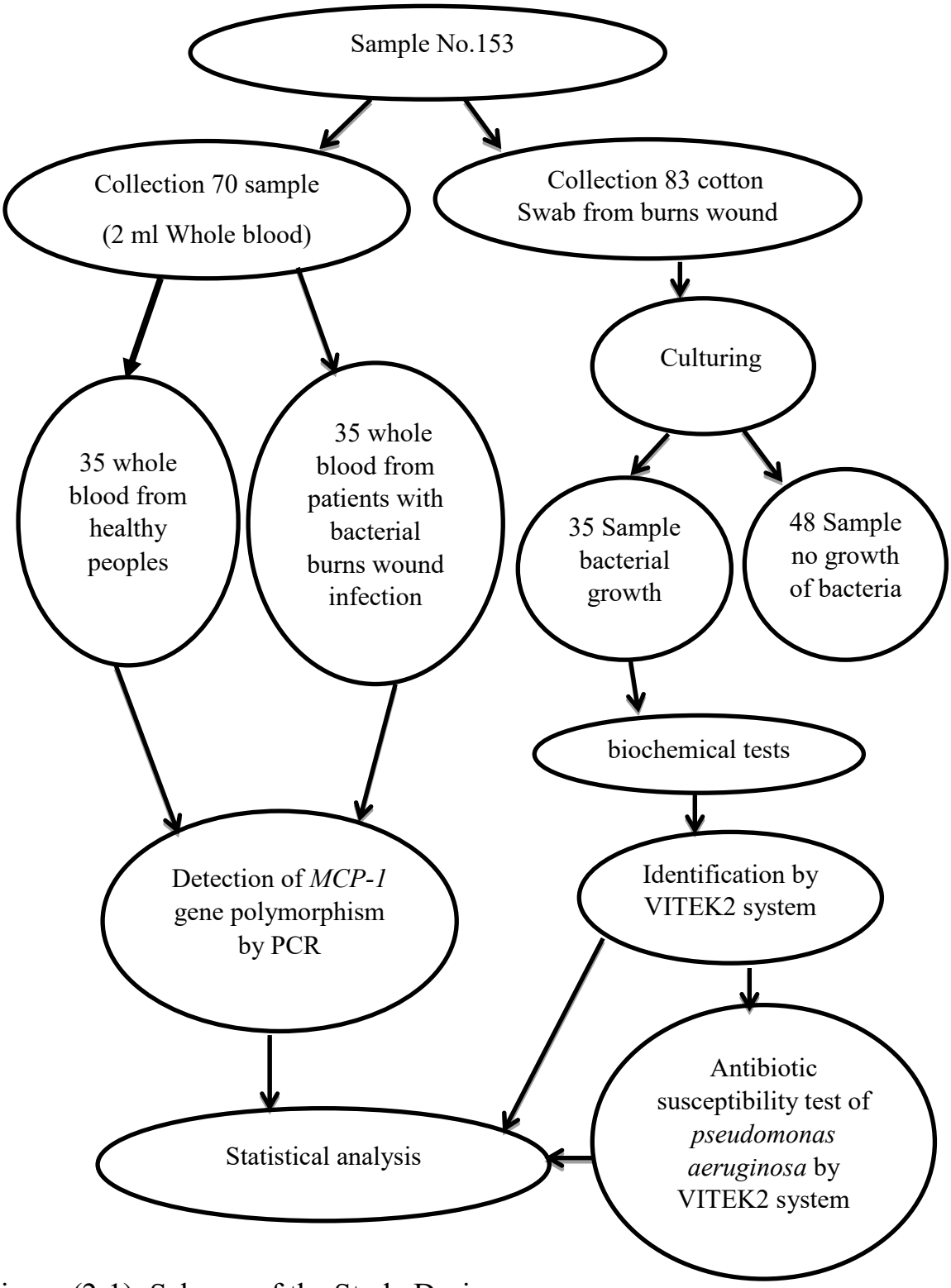

Figure (2-1): Scheme of the Study Design.

2.2. Materials

2.2.1. Laboratory Equipment and instruments

The equipment and instruments used in this study were displayed in Table (2-1).

Table (2-1): Equipment and instruments with their manufacturing company and country of origin.

Names of laboratory Equipment and instruments	Company/ Origin
Autoclave	Hirayama /Japan
Benzene burner	Amal /Turkey
Centrifuge	Hitachi/Japan
Compound light microscope	Medline /England
Conventional PCR system	Biobase/China
Cooling centrifuge	Biobase/Cina
Cotton	Almodawa/Iraq
Deep freeze refrigerator	Concord /Lebanon
DensiChek	Biomerieux / France
Eppendrof tubes	Geneaid/korea
EDTA tube	Zhongfan medical/china
Filter paper	Himedia /India
Gel electrophoresis	Biobase/China
Glass cylinders (250,500 and 1000 ml)	Isolab/Germany
Glass Flasks (250,500.1000 ml)	Isolab/Germany
Gloves	Marco Medical SDN/Malaysia
Incubator	Memmert (Germany)

Inoculation Loop sterile	Himedia (India)
Microbiologic safety cabinet	Nuve (turkey)
microcentrifuge tube	Geneaid/Korea
Micropipette (different sizes)	Eppendorf/ Germany
Micropipette tips (different size)	Human/ Germany
Microwave oven	Samsung /Korea
Petri dishes	Himedia/India
PH-meter	WTW (Germany)
Plane tube (10 ml)	AFCO(Jordan)
Refrigerator	Concord /Lebanon
Sensitive electrical balance	WTW (Germany)
Slide and cover slide	Super star/India
Sterile cotton swab	AFCO/Jordan
Syringes 3 ml	DMK/China
UV Transilluminator	Biobase/China
Vortex mixer	IKA (Germany)
Water distiller	GFL(Germany)

2.2.2. The culture media

The culture media used in this study demonstrated in Table (2-2).

Table (2-2) The cultures media used in this study with their manufacturing company and country of origin.

Name of culture media	**Company/ Origin**
Blood agar	Liofilchem (Italy)
MacConkey agar	
Mannitol salt agar	
Nutrient Agar	

2.2.3. Chemical and biological materials used in the study

Chemical and biological materials used in this study listed in Table (2-3).

Table (2-3). Chemical and biological materials are used with their manufacturing company and country of origin.

Name of material	Company/ Origin
Absolute Ethanol	Bioneer/ Korea
Agarose	Conda/Aspain
Ethanol 70%	MIRNIA/Iraq
Ethidium bromide	Bioneer/ Korea
Gram stain kit	Himedia/India
Human blood	Blood bank/Karbala
Hydrogen peroxide	UN/Germany
Normal saline (0.9%)	Choueifa/ Lebanon
Oil immersion	BDH/ England
Oxidase	Himedia (India)
Povidone iodine	Onion/Australia

2.2.4. VITEK2 kits that were used in this study

The VITEK2 kit for this study is found in Table (2-4).

Table (2-4) VITEK2 kits that were used in this study with their manufacturing company and country of origin.

Kits	Company /origin
ID- GN Card	Biomerieux / France
ID- GP Card	
AST-GN222	

2.2.5. Antibiotics groups for *Pseudomonas aeruginosa*

A list of Antibiotics to the contents of the AST-GN222 card used during this study for susceptibility testing by the VITEK2 compact system as shows in Table (2-5).

Table (2-5). Content Antibiotic cards for AST-GN222 that used in this study by VITEK2 compact system according to the manufactures company**.**

NO	Antibiotics	Disk Symbols	Concentration (µg/mL)	Company /origin
1	Amikacin	AN	8,16,64	Biomerieux / France
2	Cefepime	FEP	2,8,16,32	
3	Ceftazidime	CAZ	1,2,8,32	
4	Ciprofloxacin	CIP	0.5,2,4	
5	Colistin	CS	4,16.32	
6	Gentamicin	GM	4,16,32	
7	Imipenem	IPM	1,2.6.12	
8	Meropenem	MEM	0.5,2,6,12	
9	Piperacillin	PIP	4,16,32,64	
10	Ticarcillin	TIC	16,32,64	
11	Ticarcillin/clavulanic acid	TCC	8/2,32/2,64/2	
12	Tobramycin	TM	8,16,64	

2.2.6. DNA Amplifications Materials

2.2.6.1 DNA Extraction kit

Kit for the DNA extraction that was used in this study is found in Tables (2-6).

Table (2-6) DNA extraction kits that were used in this study with their manufacturing company and country of origin.

DNA Extraction Kit	Company /origin
Proteinase k	Geneaid/Korea
GB Buffer 40 ml	
W1 Buffer 45 ml	
Wash Buffer 45 ml	
Elution Buffer 30 ml	
GD Colum100 pcs	
2ml collection tubes 100 pcs	

2.2.6.2. Master Mix

The contents of the master mix used in PCR are found in Table (2-7).

Table (2-7) The contents of the master mix used in PCR with their manufacturing company and country of origin.

PCR Master Mix composition	Company /origin
Taq DNA polymerase	Bioneer/korea
dNTPs (dATP, dCTP, dGTP, dTTP)	
Reaction buffer with 1.5 mM MgCl2	
Stabilizer and tracking dye	

2.2.6.3. PCR Materials

The content of PCR materials was listed in Table (2-8).

Table (2-8) The PCR materials.

PCR Material	Company /origin
Master mix	Bioneer/korea
100–1500 bp DNA ladder	Bioneer/ Korea
Free nuclease water	Bromeca /USA
TBE (Tris-Borate EDTA) buffer	MarLiJu/ Korea

2.2.6.4 Primer

Primer design according to nucleotide sequence gene bank website by using primer blast https://www.ncbi.nlm.nih.gov/tools/primer-blast/. The following primers were used in this study to identify the *MCP-1* gene polymorphism listed in Table (2-9).

Table (2-9) primers sequences of *MCP-1* gene polymorphism used in this study.

Primer name	Primer sequences (5'_3')	Product size(bp)
Forward outer primer (FO)	5′-TAACTGAGGATTCTGGACAG-3′	379
Reverse outer primer (RO)	5′-TTATCTGATAAAGCCACAATC-3′	255
Forward inner primer (FI) (A allele)	5′-GTGGGAGGCAGACAGATA-3′	255
Reverse inner primer (RI) (G allele)	5′-AGAAAGTCTTCTGGAAAGTTAC-3′	379

2.3. Methods

2.3.1. Sample collection

2.3.1.1. Bacteriological sampling collection

The burn swabs were collected from 83 burn wound patients using sterile swabs. The swabs were sent to the laboratory as soon as possible.

2.3.1.2. Blood sample collection

Two ml of blood samples were collected in an EDTA tube from 35 burn patients with bacterial infections and from 35 individuals as a healthy control group for the detection of the *MCP-1* gene polymorphism. The tubes were stored in deep freeze until assayed for detection of the *MCP-1* gene polymorphism.

2.3.2. Sterilizing method

2.3.2.1. Ethanol 70%

The outer surface of the workbench and some study tools were treated with 70% ethanol (Harrigan and McCance, 2014).

2.3.2.2. Autoclave (moist heating)

The culture medium was sterilized using moist heat sterilization at a temperature of 121°C and under pressure of 1.5 bar for 20 minutes (Harrigan and McCance, 2014).

2.3.2.3. Oven (dry heating)

The glass wares were sterilized using dry heat in an electric oven at 160 °C for two hours (Harrigan and McCance, 2014).

2.3.3. Preparation of media

In this study, the culture media grade in the Table (2-2) was used

2.3.3.1. Blood Agar

Prepare this culture medium according to the manufacturer's instructions (Liofilchem/Italy) by dissolving 40.0 gm of Blood Agar in 1000 ml of D.W. The medium was heated until completely dissolved, and then sterilized by autoclave at 121 °C for 20 minutes, cooled to 45 °C, and

5% of fresh human blood was added. It was used as an enrichment medium for the bacterial isolates and to determine their ability to hemolyze RBCs (MacFadden, 2000).

2.3.3.2. MacConkey Agar

To prepare this medium, dissolve 51.5 gm of agar in 1 liter of D.W. This medium is selective for Gram-negative bacteria and differential for lactose-fermenting and lactose-nonfermenting bacteria (MacFadden, 2000).

2.3.3.3 Mannitol Salt Agar

Depending on the manufacturer, it prepared by dissolving 111 gm of powder in a liter of D.W. This type of media is selective for the isolation of staphylococci (MacFadden, 2000).

2.3.3.4. Nutrient Agar

It prepared depending on manufacturing company by dissolving 28.0 gm of powder in a liter of D.W. It has been used to cultivate, activate, and isolate bacteria, when it is required (MacFaddin, 2000).

2.3.4. Preparation of solutions and indicators

Ready-made reagents and solutions were used, such as Gram stain and normal saline, while in-stand reagents were prepared as needed, as follows

2.3.4.1. Oxidase reagent

According to the manufacturer's company, the oxidase reagent was prepared at a concentration of 1% by dissolving 0.1 gm of tetramethyl-p-phenylenediamine dihydrochloride in 10 ml of distilled water. The oxidase test is useful in identifying microorganisms that may manufacture the cytochrome oxidase enzyme. The test distinguishes between the oxidase-positive Pseudomonacea and the oxidase-negative Enterobacteriacea groups (Green, and Goldman, 2021).

2.3.4.2. Catalase reagent

According to the manufacturer's company, the catalase reagent was prepared at a concentration of 3% by adding 1 ml of 30% hydrogen peroxide to 9 ml of distilled water. It has been used to detect the ability of bacteria to produce the catalase enzyme (Green, and Goldman, 2021).

2.3.4.3. Tris-Borate-EDTA Buffer

This solution was prepared according to the manufacturing company (MarLiJu/Korea) by dilution of the solution from 10× to 1x by adding 100 ml of tris-borate-EDTA buffer (10×TBE) prepared by MarLiJu/Korea to 900 ml of sterile distilled water and kept at room temperature (Sambrook and Russell, 2001).

2.3.5. Specimens culturing

The burn wound swabs were streaked on MacConkey agar, blood agar and then incubated for 48 hours at 37°C under aerobic conditions. After that, the plates were examined for growth the next day, and then a pure colony for all bacterial types was prepared (Dubey and Maheshwari, 2023).

2.3.6. Identification of bacterial isolation

2.3.6.1. Macroscopic characteristics

All bacterial isolates were identified according to the general cultural characteristics (color, shape, and size) of the colony and their effects on media such as blood hemolysis, lactose fermentation, and their ability to ferment mannitol (Mahon and Lehman, 2022).

2.3.6.2. Microscopic examination

A small amount of a bacterial colony was spread on a clean slide with a drop of normal saline, fixed by a flame, stained with Gram stain, and then examined using an oil immersion (Atlas, 2010).

2.3.6.3. Biochemical test

2.3.6.3.1. Catalase test

The test was done on a slide by mixing a colony of bacteria with a few drops of 3% H2O2 and looking for bubble formation within 10 seconds. The hydrogen peroxide will be neutralized by the catalase enzyme generated by these bacteria, and bubbles will form, indicating a positive test (Benson, 2002)

2.3.6.3.2. Oxidase test

The test was carried out by impregnating a filter paper with 1% tetramethyl-p-phenylenediamine dihydrochloride, which acts as an artificial electron donor, and drying it. The bacterial colonies are smeared on a paper strip, and the color change is checked after 10 seconds (Benson, 2002)

2.3.6.3.3. Coagulase test (tube method)

The tube test was performed by adding the bacteria to plasma in a test tube. Coagulation of the plasma (including any thickening or formation of fibrin threads) within 24 hours indicates a positive reaction. The plasma is typically examined for clotting (without shaking) after about 4 hours because it is possible for coagulation to take place early and revert to liquid within 24 hours (Benson, 2002)

2.3.6.4. Identification of bacteria by VITEK2 compact system

The automated system VITEK-2, manufactured by BioMerieux in France, is used to determine the identity and susceptibility of bacterial isolates. It relies on biochemical reactions between the bacterial isolates suspended in their solutions and the media in the VITEK-2 Identification Cards (Pincus, 2006). The next step is the identification of bacteria according to the instructions provided by the manufacturer.

1. A single pure colony of bacterial isolate had been suspended in 3 ml of normal physiological saline in a sterile tube.

2. The turbidity of the bacterial suspension had been measured with the DensiChek VITEK device; the turbidity is 5.0–0.63.

3. The tubes had been placed in their own racks after the addition of a VITEK 2 cassette to each tube examination, depending on the diagnostic Gram stain.

4. The rack containing the tubes and the cassette had been transferred to the system, putted in the first field of fillers (filler), which automatically populates the cassete with bacterial suspension, and after finishing the process, the end signal had been delivered from the device.

5. The second field reader (reader) had been transferred to it, the first of which was to cut the tapes, and the offer order (burden) is a digital signal that had been held. The rack holding the tube moves from the device to provide the data for each sample on a computer connected to the VITEK2 compact system.

6.The taps had been left for 24 hours at 37 °C, then the results had been read for the diagnosis of bacteria. The findings were reported by the manufacturer as 96% to 100%, excellent identification; 93% to 95%, very good identification; 89% to 92%, good identification; 85% to 88%, acceptable identification; and on the other hand, no identification in another Isolates.

2.3.7. Genotyping Assay by Amplification-Refractory Mutation System (ARMS) Method

For detecting known point mutations was first described by Newton *et al.* (1989). The amplification-refractory mutation system (ARMS) is a simple procedure to find any variation that involves minor decreases or changes to a single base. The basic foundation of ARMS is on the use of PCR primers that are unique to a certain sequence. These tests only allow for the amplification of DNA when the target allele is present in the sample.

The absence or presence of PCR product following an ARMS reaction acts as a diagnostic indicator for the presence or absence of the target allele. (Little, 1995). In a single-tube PCR, Tetra-ARMS-PCR amplifies both the wild-type and mutant alleles in addition to a control segment. A non-allele-specific control amplicon is obtained by amplifying the area surrounding the locus of interest using two common (outer) primers. The simultaneous amplification of the wild-type and mutant amplicons is possible by combining common primers with two allele-specific (inner) primers that have opposite orientations. The specificity of allele-specific primers is conferred by the match of the terminal 3'nucleotide with either the wild-type or the mutant allele, and it is enhanced by the introduction of a deliberate mismatch near the primer 3'end. The two allele-specific amplicons have different lengths and can be easily separated by standard gel electrophoresis because the mutation is asymmetrically located with respect to the common primers (Galmozzi *et al.*, 2011).

2.3.7.1. DNA Extraction Procedure

The DNA extraction included the following steps

1-The blood was collected in EDTA tubes.

2-A volume of 200 µl of whole blood had been transferred to a 1.5 ml microcentrifuge tube.

3- A volume of 20 µl of Proteinase K had been added to the sample tube and gently mixed.

4-The mixture had been placed in incubation at 60 °C for 10 minutes. During incubation, the tube was inverted every 3 minutes.

5- A volume of 200 µl of GB buffer had been added to the sample and mixed by vortex for 10 seconds.

6- The tube was incubated for at least 10 minutes at 70°C to ensure that the sample lysate had been cleared. During incubation, the tube was inverted every 3 minutes.

7- A volume of 200 µl of absolute ethanol had been added and immediately mixed by shaking vigorously for 10 seconds.

8-Carefully apply the mixture from step 7 to the GD Column (in a 2 ml collection tube), close the cap, and centrifuge at 14,000 rpm for 1 minute. Discard the filtrate and place the column in a new 2 ml collection tube.

9- A volume of 400µl of wash1 buffer had been added to the GD column. The centrifugation process was achieved at 14000 rpm for 30 seconds.

10- The flow-through had been discarded, and the GD column was placed back in the GD column.

9- A volume of 600 µl of washing buffer (ethanol added) had been added to the GD column and centrifuged at 14000 rpm for 30 seconds.

10-The flow-through had been discarded, and the GD column was placed back into the GD column.

11-The centrifugation had been done at 14000 rpm for 1 minute to dry the column matrix.

12-The dried GD column had been transferred to a clean 1.5 microcentrifuge tube.

13-A volume of 100 µl of pre-heated elution buffer had been added to the center of the column matrix and left to stand for at least 3 minutes to ensure the elution buffer was absorbed by the matrix.

14-The centrifugation process had been achieved at 14000 rpm for 30 seconds to elute the purified DNA. Eluted DNA was stored at -20 °C until used for PCR.

2.3.7.2. Primer preparation

The primers were prepared according to manufacturer instructions to form a stock solution with a concentration of 100 pmol/µl by dissolving the lyophilized primers with deionized D.W. Then the working solution was prepared by dissolved 25µl of 100 pmol/µl with 225 µl of deionized distilled water to form 250 µl of 10 pmol/µl.

2.3.7.3. Polymerase Chain Reaction (PCR) Mixture

Polymerase Chain Reaction Mixture were list in Table (2-10).

Table (2-10) Polymerase Chain Reaction Mixture.

PCR Mixture	Volume (µl)
Master mix	3.5
Forward outer primer	1
Reverse outer primer	1
Forward inner primer (A allele)	1
Reverse inner primer (G allele)	1
Nuclease free water	12.5
template DNA	5
Total volume	25 **(**µl)

The components of the PCR mixture were placed in the PCR tubes prepared with the kit, then the tubes were carefully mixed with the vortex device for 5 seconds, after which the tubes were transferred to the PCR thermal cycle device to conduct thermal cycles according to the optimal conditions for DNA replication.

2.3.7.4. Polymerase Chain Reaction Conditions by amplification-refractory mutation system (ARMS) method

To amplify a target DNA, specific primer pairs and the conventional-PCR were used. the amplification-refractory mutation system (ARMS) method used for producing a PCR product. Denaturation, annealing, and elongation were the three stages that comprise the process typical and they repeated cycle after cycle (amplicon). The conditions for the PCR were listed in Table (2-11).

Table (2-11): PCR Conditions for Amplification of the *MCP-1* Gene.

Step	Temperature	Time	Number of cycles
Initial Denaturation	95 °C	5 Minutes	1 cycle
Denaturation	95°C	30 Seconds	29 cycles
Annealing	58°C	58 Second	
Extension	72°C	60 Seconds	
Final Extension	72°C	5 Minutes	1 cycle
Hold	4 °C	∞	

2.3.7.5. Agarose Gel Electrophoresis

This process was carried out according to (Lodish, Berk, and Matsudaira, 2004). Successful PCR amplification was confirmed by agarose gel electrophoresis. Agarose gel was prepared by mixing 100 ml of previously prepared TBE buffer with 1 gm of agarose powder. (final concentration was 1 X and pH 8 after adding 900 ml of D.W. to 100 ml of TBE buffer l0X). After heating the mixture until it became clear, it was allowed to cool to 50°C then 0.5 µg/ml of ethidium bromide was added. The agarose was placed in a stabilized gel tray previously, set with a comb fixed to the end, and the tray of gel sealed at both ends. For 30 minutes, the agarose was left to solidify in the room temperature. Comb and seal were carefully removed from tray. The PCR product was loaded into the comb-made wells. After that, a ladder of DNA markers (five microliters) was added to one well in agarose gel well then, 1 X TBE buffer was added to the electrophoresis chamber until the gel tray was completely filled. The current of electricity was 60 volts for 35 minutes. Ultraviolet transilluminator was used for the observation of DNA bands.

2.3.8. Statistical analysis

Statistical analyses were performed using SPSS statistical package for Social Sciences (version 20.0 for windows, SPSS, Chicago, IL, USA). Quantitative data are represented as mean, standard deviation and range. Qualitative data are represented as count and percentage. ANOVA test (analysis of variance) was used to test the difference between multiple groups. Chi-square test was used to test the relation of qualitative data. *P value of* <0.05 was considered statistically significant.

Chapter Three

Results

3. Results

3.1. Sex, age, and weight characteristics of the patients and control groups

The patients consisted of 44 (53.0%) males and 39 (47.0%) females, whereas in control, 15 (42.9%) were males and 20 (57.1%) were females. There were non significant differences (*P*=0.420) between study groups regarding sex. The age of the patients ranged from (1-60) years, while the age of the controls ranged from (1-52) years, and they were classified into six groups. The results revealed that burn cases were more frequent in age < 11 years 37 (44.6%) than other age groups, and there were non significant differences *(P*=0.575) between the control and patient groups.

According to the weight of the patients, 78 (94.0%) of them were of normal weight, and 5 (6.0%) of them were overweight, while in the control group, all of them were of normal weight 35 (100%). There were non significant differences (*p*=0.320) when compared between patients and the control group, as shown in Table (3-1).

Table (3-1): Distribution of sex, age, and weight characteristics of the studied groups

Variables		Group				P value
		Patients (N=83)		Control (N=35)		
		Count	%	Count	%	
sex	Male	44	53.0%	15	42.9%	0.420
	Female	39	47.0%	20	57.1%	
	Total	83	100%	35	100%	
Age (year)	<11	37	44.6%	12	34.3%	0.575
	11-20	11	13.3%	6	17.1%	
	21-30	8	9.6%	3	8.6%	
	31-40	13	15.7%	6	17.1%	
	41-50	8	9.6%	7	20.0%	
	51+	6	7.2%	1	2.9%	
	Total	83	100%	35	100%	
Weight	Normal wt.	78	94.0%	35	100.0%	0.320
	Over wt.	5	6.0%	0	0.0%	
	Total	83	100%	35	100%	

***p value* is non significant (chi-square test)**

3.2. Clinical characteristics in patients' group

In analyzing the results concerning burn types, it was clear that patients with scalding burns had 44 (53.0%), followed by flame burns at 34 (41.0%), and then electrical burns at 5 (6.0%). About the patients with inhalation injuries, it was found that 80 patients (96.4%) had non-inhalation injuries, whereas only 3 patients (3.6%) had inhalation injuries. However, the number of patients with in-hospital mortality was 9 (10.8%), while 74 (89.2%)

patients were healed. For the patients with mechanical ventilation, it was found that 80 patients (96.4%) had non mechanical ventilation, whereas only 3 patients (3.6 %) had mechanical ventilation. Also, total body surface area (TBSA) ranges from (4.00- 75.00%) with a mean (17.05) and SD (14.02). Also mean length of hospital stays ranges from (2.00-21.00) days with a mean (9.31) and SD (4.36), as found in Tables 3-2.

Table (3-2): Distribution of clinical characteristics in cases group

Variables		Count	%
Burn types	Scalding	44	53.0%
	Flame	34	41.0%
	Electrical	5	6.0%
	Total	83	100
Inhalation injury	-ve	80	96.4%
	+ve	3	3.6%
	Total	83	100%
In-hospital mortality	-ve	74	89.2%
	+ve	9	10.8%
	Total	83	100%
Mechanical Ventilation	-ve	80	96.4%
	+ve	3	3.6%
	Total	83	100%
		Mean (SD)*	Range
Total body surface area (TBSA)		17.05 (14.02)	4.00-75.00
Length of hospital stay		9.31 (4.36)	2.00-21.00

***SD: Standard deviation**

3.3. Severity of burn in patients' group

The degrees of burn severity as illustrated in Table (3.3) show that the second degree constituted the most common of cases 55 (66.3%), while the third-degree burn constituted 21 (25.3%), and the second +third degree constituted only 7 (8.4%), as presented in Table (3-3).

Table (3-3): Degrees of burn severity in patients

Variables		Count	%
Burn severity	Second degree	55	66.3%
	Third degree	21	25.3%
	Second + third degree	7	8.4%
	Total	83	100%

3.4. Bacterial culture results in patients' group

A total of 83 swab specimens were collected from patients with suspected burn wound infection; among them, 28 (33.735%) of specimens were found to be with Gram-negative bacteria, 7 (8.434%) with Gram-positive bacteria, and 48 (57.831%) with negative culture., as demonstrated in Table (3-4)

Table (3-4): Bacterial culture results in cases group

Variables		Count	%
Bacterial Culture	Gram –negative	28	33.735%
	Gram +positive	7	8.434%
	No growth	48	57.831%
	Total	83	100%

3.5. Types of bacterial isolates in patients

Culturing investigation depending on morphological, biochemical and VITEK2 system results showed that *Pseudomonas aeruginosa* was the commonest isolate (31.43%), then *Klebsiella pneumoniae* (20.00), followed by *Escherichia coli* (14.29%), *Acinetobacter baumannii* (8.57), then *Staphylococcus aureus*, *Staphylococcus haemolyticus* and *Coagulase-negative staphylococcus* (5.71%) for each isolale, whereas *Enterobacter aerogenes*, *Serratia liquefaciens*, and *Staphylococcus epidermidis* represented the lowest isolated bacteria, and each of them was found only in one sample with a percentage equal to (2.86%), as shown in Figure 3-1.

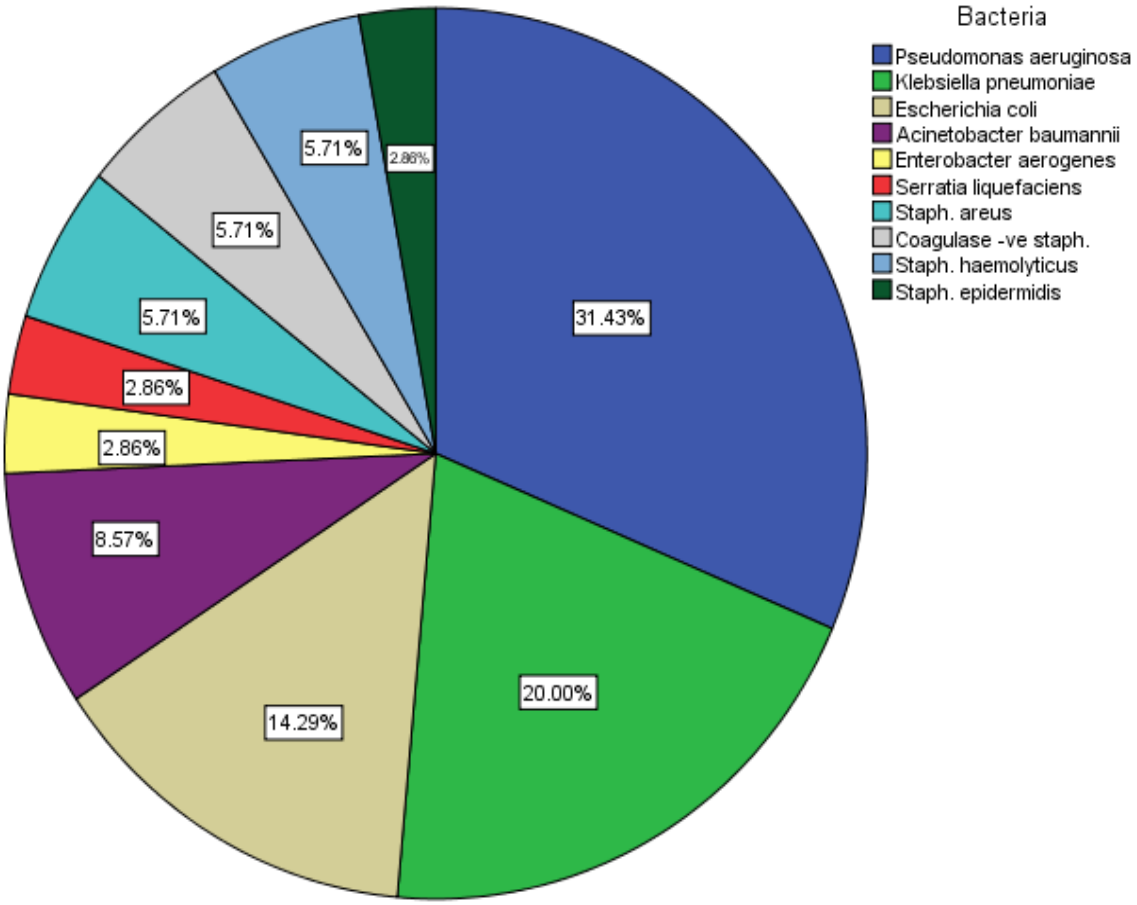

Figure (3-1): Distribution of bacterial isolates in patients swabs

3.6. Association of bacterial isolates to burn severity in patients with bacterial infection

Table (3-5) showed that patients with second-degree burns had more positive cultures (28) isolates compared to the positive cultures 3 and 4 isolated from patients with third-degree and second+ third-degree burns, respectively, (28) isolates in the second-degree burn patients were divided into 9 types of bacteria, *P. aeruginosa* and *Klebsiella pneumoniae* were 6 (21.429%) for each isolate, than *Escherichia coli* was 5 (17.857%), *Acinetobacter baumannii* was 3 (10.714%), *Staphylococcus aureus*, *Coagulase-negative staphylococcus* and *Staphylococcus haemolyticus* were 2 (7.143%) for each isolate, while *Serratia liquefaciens* and *Staphylococcus epidermidis* were only 1(3.571%) for each isolate. The isolates with third-degree burns were *Pseudomonas aeruginosa* 2 (66.7%) and *Enterobacter aerogenes* was 1 (33.3%), only while the isolates with second+ third-degree burns were *Pseudomonas aeruginosa* 3 (75.0%) and *Klebsiella pneumoniae* was 1 (25.0%). There were non significant differences (P= 0.342) between bacterial isolates and burn severity, as clarified in Table (3-5).

Table (3-5): The relation of bacterial isolates to burn severity in patients with bacterial infection

Types of bacteria	Burn severity						*P value*
	Degree 2		Degree 3		Degree 2+3		
	Count	%	Count	%	Count	%	
Psudomonas aeruginosa	6	21.429%	2	66.7%	3	75.0%	0.342*
Klebsiella pneumoniae	6	21.429%	0	0.0%	1	25.0%	
Escherichia coli	5	17.857%	0	0.0%	0	0.0%	
Acinetobacter baumannii	3	10.714%	0	0.0%	0	0.0%	
Enterobacter aerogenes	0	0.0%	1	33.3%	0	0.0%	
Serratia liquefaciens	1	3.571%	0	0.0%	0	0.0%	
Staphylococcus aureus	2	7.143%	0	0.0%	0	0.0%	
Coagulase-negative Staphylococcus	2	7.143%	0	0.0%	0	0.0%	
Staphylococcus haemolyticus	2	7.143%	0	0.0%	0	0.0%	
Staphylococcus epidermidis	1	3.571%	0	0.0%	0	0.0%	
Total	28	100%	3	100%	4	100%	

**** p value* is non significant (chi-square test)**

3.7. Association of bacterial isolates to sex in patients with bacterial infection

In the present study, it was observed that the number of *P. aeruginosa* were higher in males 6 (37.5%) compared to females 5 (26.32%), the number of *Klebsiella pneumoniae* were higher in females 4 (21.05%) compared to males 3 (18.75%), while the number of *Escherichia*

coli were higher in males 3 (18.75%) compared to females 2 (10.53%). The number of *Acinetobacter baumannii* in females were 2 (10.53%), while in males it was 1 (6.25%). Also, the number of *Coagulase-negative staph*ylococcus in females were 2 (10.53%), while in males it was 0 (0.0%). The equal numbers of *Staphylococcus aureus* and *Stapylococcus haemolyticus* in both males and females were 1(6.25%) and 1 (5.26%), respectively. Also, the equal number of *Enterobacter aerogenes* and *Serratia liquefaciens* in females was 1 (5.26%), while in males it was 0 (0.0%), and the number of *Staphylococcus epidermidis* in males was 1 (6.25%), while in females it was 0 (0.0%). There were non significant differences (p=0.707) concerning the relation of bacterial isolates to sex in patients with bacterial infection, as found in Table (3-6).

Table 3-6: The relation of bacterial isolates to sex in patients with bacterial infection

Type of bacteria	Sex				*P* value
	Male		Female		
	Count	%	Count	%	
Pseudomonas aeruginosa	6	37.5%	5	26.32%	0.707*
Klebsiella pneumoniae	3	18.75%	4	21.05%	
Escherichia coli	3	18.75%	2	10.53%	
Acinetobacter baumannii	1	6.25%	2	10.53%	
Enterobacter aerogenes	0	0.0%	1	5.26%	
Serratia liquefaciens	0	0.0%	1	5.26%	
Staphylococcus aureus	1	6.25%	1	5.26%	
Coagulase-negative Staphylococcus	0	0.0%	2	10.53%	
Staphylococcus haemolyticus	1	6.25%	1	5.26%	
Staphylococcus epidermidis	1	6.25%	0	0.0%	
Total	16	100%	19	100%	

**** p value* is non significant (chi-square test)**

3.8. The relation of bacterial isolates to age in patients with bacterial infections

Pseudomonas aeruginosa isolate in ages of patients ranged from (2.00-52.00) years with a mean value (27.00), while the *Klebsiella pneumonia*e isolate in ages of patients ranged from (1.00-45.00) years with a mean value (22.86). Also, the *Escherichia coli* isolate in ages of patients ranged from (1.00-50.00) years with a mean value (19.40), whereas the *Acinetobacter baumannii* isolate in ages of patients ranged from 3.00–46.00 years with a

mean value (26.67). The *Enterobacter aerogenes* isolate in the ages of patients ranged from 14.00-14.00 years with a mean value (14.00) and *Serratia liquefaciens* isolate in the ages of patients ranged from 6.00-6.00 years with a mean value (6.00). However, the *Staphylococcus aureus* isolates in the ages of patients ranged from 5.00-10.00 years, with a mean value (7.50). Also, the *Coagulase-negative Staphylococcus* isolate in ages of patients ranged from (3.00-3.00) years with a mean value (3.00), while, the *Staphylococcus haemolyticus* isolate in ages of patients ranged from (33.00–52.00) years with a mean value (42.50). The *Staphylococcus epidermidis* isolate in the ages of patients ranged from 50.00-50.00 years, with a mean value (50.00). There were non significant differences (p=0.357) regarding the relation of bacterial isolates to ages in patients with bacterial infection, as presented in Table (3-7).

Table 3-7: The relation of bacterial isolates to age in patients with bacterial infection

Age(year)

Type of bacteria	**Mean**	**SD**	**Minimum**	**Maximum**	***P value***
Pseudomonas aeruginosa	27.00	15.96	2.00	52.00	0.357*
Klebsiella pneumoniae	22.86	19.18	1.00	45.00	
Escherichia coli	19.40	23.66	1.00	50.00	
Acinetobacter baumannii	26.67	21.83	3.00	46.00	
Enterobacter aerogenes	14.00	0.00	14.00	14.00	
Serratia liquefaciens	6.00	0.00	6.00	6.00	
Staphylococcus aureus	7.50	3.54	5.00	10.00	
Coagulase-negative Staphylococcus	3.00	0.00	3.00	3.00	
Staphylococcus haemolyticus	42.50	13.44	33.00	52.00	
Staphylococcus epidermidis	50.00	0.00	50.00	50.00	

**** p value* is non significant (ANOVA test)**

3.9. PCR-Based Detection of SNP

3.9.1. Evaluation of genomic DNA extract quality and integrity

The quality and integrity were checked by agarose gel electrophoresis before performing the PCR reaction, as shown in Figure (3-2). All DNA extracts showed a single bright band.

3.9.2. *Monocyte chemoattractant protein-1* Gene Amplification

DNA extracts from each patient with a bacterial burn infection of the wound and a healthy control group were amplified to identify the gene segment that may have a single nucleotide polymorphism within the *MCP-1* gene region. The polymerase chain reaction was carried out under optimal conditions using a specific primer; the PCR result was then electrophoresis on an agarose gel at a 1% concentration. The results showed a single distinct band with a molecular size of the G allele (379 bp) and the A allele (255 bp). The amplicon's size was determined by comparing it to the 100 bp DNA ladder, as shown in Figure (3-3).

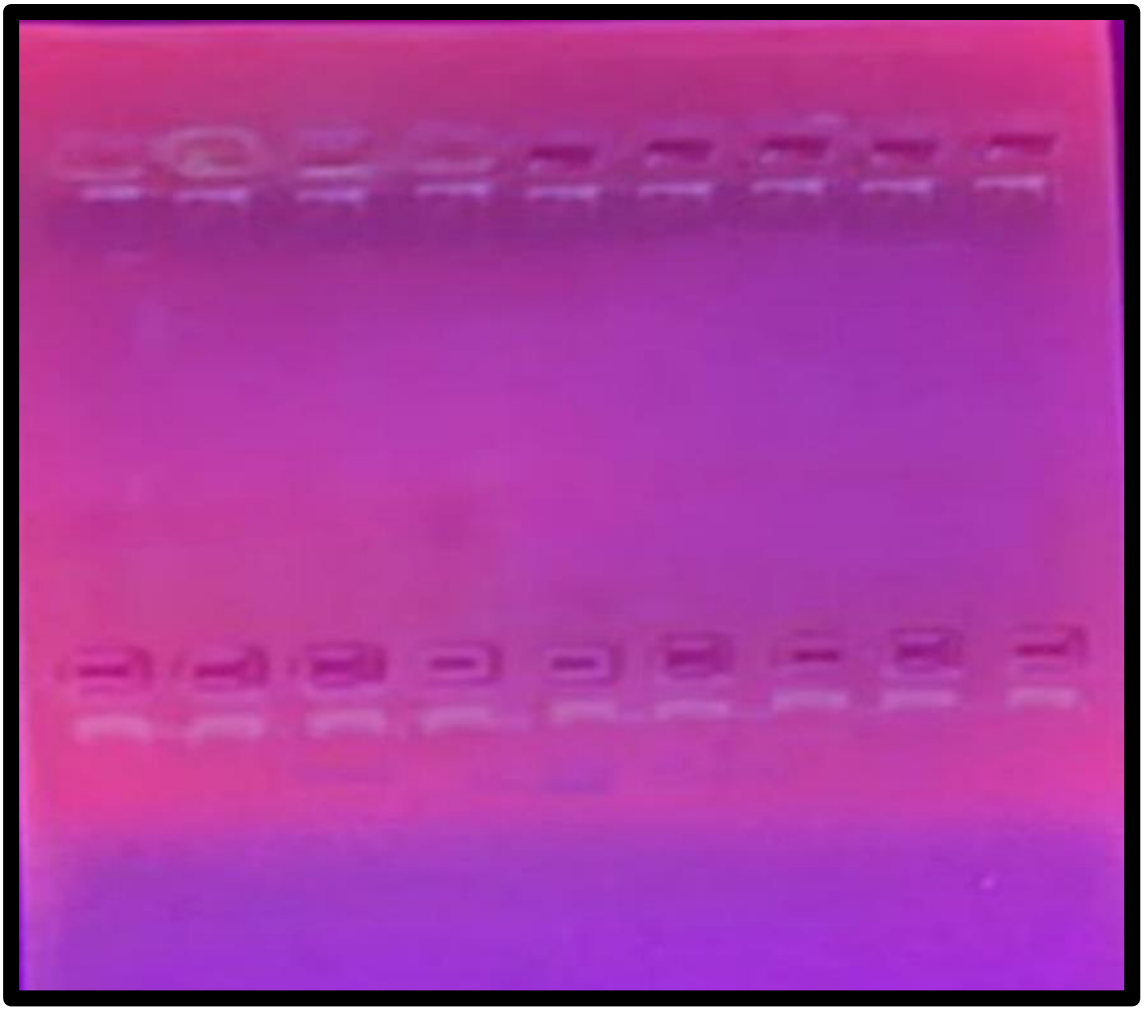

Figure (3-2): Evaluation of DNA Extract Quality and Integrity. 1 % Agarose Gel Electrophoresis of Genomic DNA in 60 Volts at 15 Minutes.

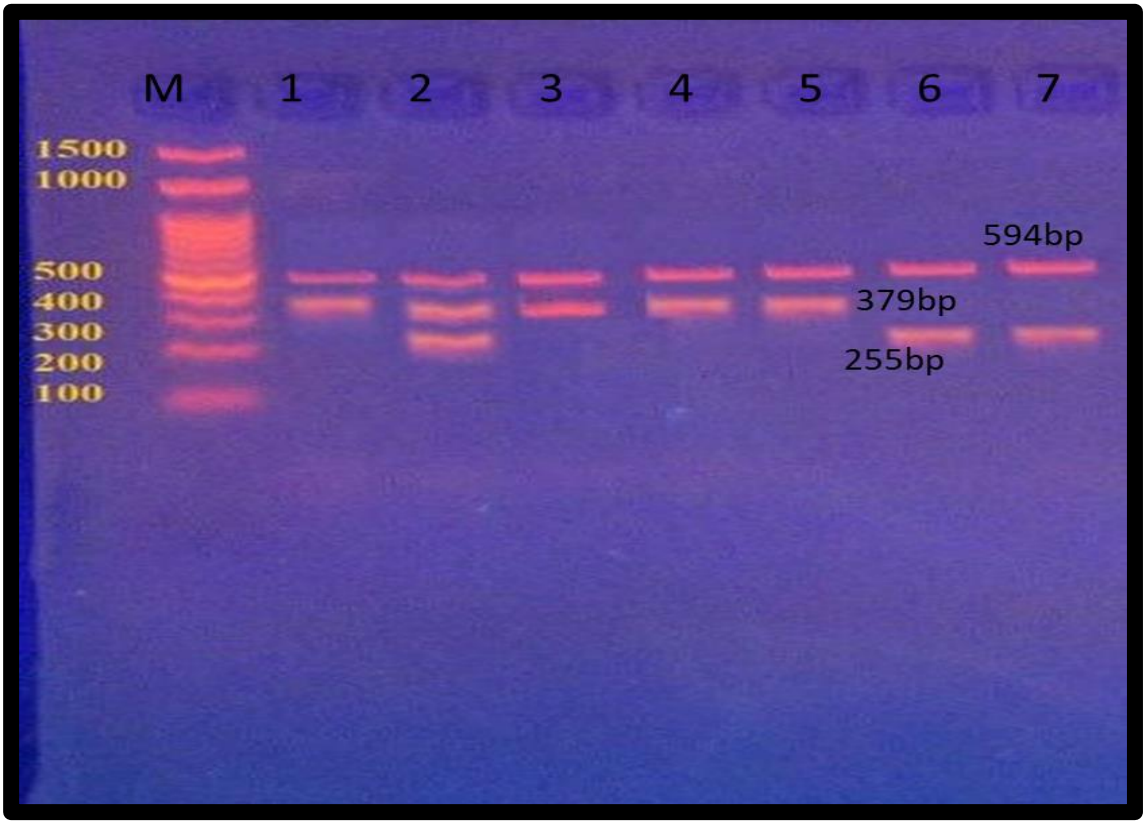

Figure (3-3): Gel Electrophoresis for PCR Product of the SNP (–2581 A/G *MCP-1* Gene with DNA Ladder 100bp (M) on Agarose Gel 1 % in 60 Volts, 35 Minutes and Detection of the Result by UV Documentation System. The size of product is 594 bp–control, 379 bp—G allele and 255 bp—A allele.

3.9.3. Detection of *MCP-1*Gene (rs1024611) Polymorphism in Patients and Control by conventional PCR

Three distinct genotypes of a genetic polymorphism (rs1024611) in the *MCP-1* genome have been identified: GG, AA, and AG. in burn wound infection patients as well as controls. The Patients showed an elevated AA genotype compared to the control (40.0% vs. 8.6%, respectively), the AG gene was discovered to be in higher rates in control than patients (17.1% vs. 14.3%, respectively), and The GG gene was found to be more prevalent in controls compared to patients (74.3% vs. 45.7%, respectively). There were a significant difference in the genotype with the group studied (P=0.008). The "A" allele had a greater frequency in patients than the control group (47.2% vs. 17.2%, respectively), whereas the "G" allele increased in control in

contrast to patients (82.8% vs. 52.8%, respectively), as shown in Table 3–8. There were a significant difference in alleles between the patients and the control groups (*P=0.001*)

Table (3- 8): Genotype and allele frequency distribution of *MCP-1* gene polymorphism in patients and control groups.

Variables		Group				*P value*
		Patients		Control		
		Count	%	Count	%	
MCP-1 genotype	A/A	14	40.0%	3	8.6%	0.008*
	A/G	5	14.3%	6	17.1%	
	G/G	16	45.7%	26	74.3%	
	Total	35	100%	35	100%	
MCP-1 allele	A	33	47.2%	12	17.2%	0.001*
	G	37	52.8%	58	82.8%	
Total		70	1005	70	100%	

**** p value* is significant (chi-square test)**

3.10. Genotype and allele frequency distribution of *MCP-1* gene polymorphism according to severity groups in patients with bacterial infection

According to the frequency distribution of *MCP-1* gene polymorphism to burn severity in patients with bacterial infection. The AA genotype was found to be at a higher frequency in patients with second degree 13 (46.4%) while with third degree and second +third were 0 (0.0%), 1 (25.0%) respectively, the AG genotype was found to be at a higher frequency in patients with second+third degree 1(25.0%) while with second and third

degree were 4 (14.3%), 0 (0.0%), respectively, the GG genotype was found to be at a higher frequency in patients with third degree 3 (100%), while with second and second+third degree were 11 (39.3%), 2 (50.0%) respectively. There was non significant difference between the genotype and the burn severity (*P*=0.323). The "A" allele was elevated in patients with second degree 30 (53.6%) compared with third and second +third degree were 0 (0.0%) and 3 (37.5%), respectively. The "G" allele was elevated in patient with a third degree of 6 (100%) compared with those with a second degree and second+third degree were 26 (46.4%), 5 (62.5%) respectively. There was a significant difference in the alleles with burn severity (*P*=0.037), as shown in the Table (3-9).

Table (3-9): Genotype and allele frequency distribution of *MCP-1* gene polymorphism according to severity groups in patients with bacterial infection

Variables		Burn severity						*P value*
		Degree 2		Degree 3		Degree 2+3		
		Count	%	Count	%	Count	%	
MCP-1 genotype	A/A	13	46.4%	0	0.0%	1	25.0%	0.323*
	A/G	4	14.3%	0	0.0%	1	25.0%	
	G/G	11	39.3%	3	100.0%	2	50.0%	
Total		28	100%	3	100%	4	100%	
MCP-1 allele	A	30	53.6%	0	0.0%	3	37.5%	0.037**
	G	26	46.4%	6	100.0%	5	62.5%	
Total		56	100%	6	100%	8	100%	

**** p value* is non significant, *** p value* is significant (chi-square test)**

3.11. Relation of *MCP-1* genotypes according to bacterial culture results

Table (3-10) showed that patients with the GG genotype had more positive cultures (16) isolates compared to the positive cultures 14 isolated from patients with the AA genotype and 5 isolated from patients with the AG genotype. The number of *P. aeruginosa* from the bacterial isolate was higher in patients with AG genotype 2 (40.0%) compared to AA and GG genotypes 3 (21.43%), 6 (37.5%) respectively. Also, the number of *Klebsiella pneumoniae* was higher in patients with GG genotype 4 (25.0%) compared to AA and AG genotypes, which were 2 (14.28%) and 1 (20.0%), respectively; however, the number of *Escherichia coli* were 3 (21.43%), 2 (12.5%), and 0 (0.0%), respectively, in patients with AA, GG, and AG genotypes. In addition, the numbers of *Acinetobacter baumannii* in patients with AA, AG, and GG genotypes were 1 (7.14%), ,1 (20.0%) and 1 (6.25%) respectively; however, the numbers of *Enterobacter aerogenes* and *Staphylococcus epidermidis* in patients with GG genotypes were equal to 1 (6.25%) for each isolate, while in AA and AG were 0 (0.0%). About the number of *Coagulase-negative Staphylococcus* and *Staphylococcus haemolyticus* in patients with AA, genotypes were 2 (14.29%) for each isolate, while AG and GG were 0 (0.0%). Regarding the number of *Staphylococcus aureus* in patients with AA, GG and AG genotypes, they were 1 (7.14%), 1 (6.25%), and 0 (0.0%), respectively. Whereas, the number of *Serratia liquefaciens* in patients with AG genotype was 1 (20.0%), while in patients with AA and GG genotypes were 0 (0.0%). There was non significant difference (P=0.453) according to the relationship between bacterial culture results and *MCP-1* genotype.

Table (3-10): Distribution of *MCP-1* genotype according to bacterial types results.

Types of bacteria	*MCP-1* genotype						*P value*
	A/A		A/G		G/G		
	Count	%	Count	%	Count	%	
P. aeruginosa	3	21.43%	2	40.0%	6	37.5%	0.453*
K. pneumoniae	2	14.28%	1	20.0%	4	25.0%	
Escherichia coli	3	21.43%	0	.0%	2	12.5%	
Acinetobacter baumannii	1	7.14%	1	20.0%	1	6.25%	
Enterobacter aerogenes	0	0.0%	0	.0%	1	6.25%	
Serratia liquefaciens	0	0.0%	1	20.0%	0	.0%	
Staphylococcus aureus	1	7.14%	0	0.0%	1	6.25%	
Coagulase-negative Staphylococcus	2	14.29%	0	0.0%	0	.0%	
S. haemolyticus	2	14.29%	0	0.0%	0	.0%	
S. epidermidis	0	0.0%	0	0.0%	1	6.25%	
Total	14	100%	5	100%	16	100%	

**** p value* is non significant (chi-square test)**

3.12. Antibiotic susceptibility test for *Pseudomonas aeruginosa*

In vitro susceptibility of *P. aeruginosa* isolates was determined with minimal inhibitory concentration by VITEK2 system. The antibiotic sensitivity test was performed on 11 burn wound isolates. The results showed

that these isolates were found to be resistant to Ticracillin, Ticracillin clavulanic acid, Piperacillin, Ceftazidime, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Tobromycin, and Ciprofloxacin (100%), while the results showed susceptibility (100%) for Colistin only, as shown in Figure (3-4).

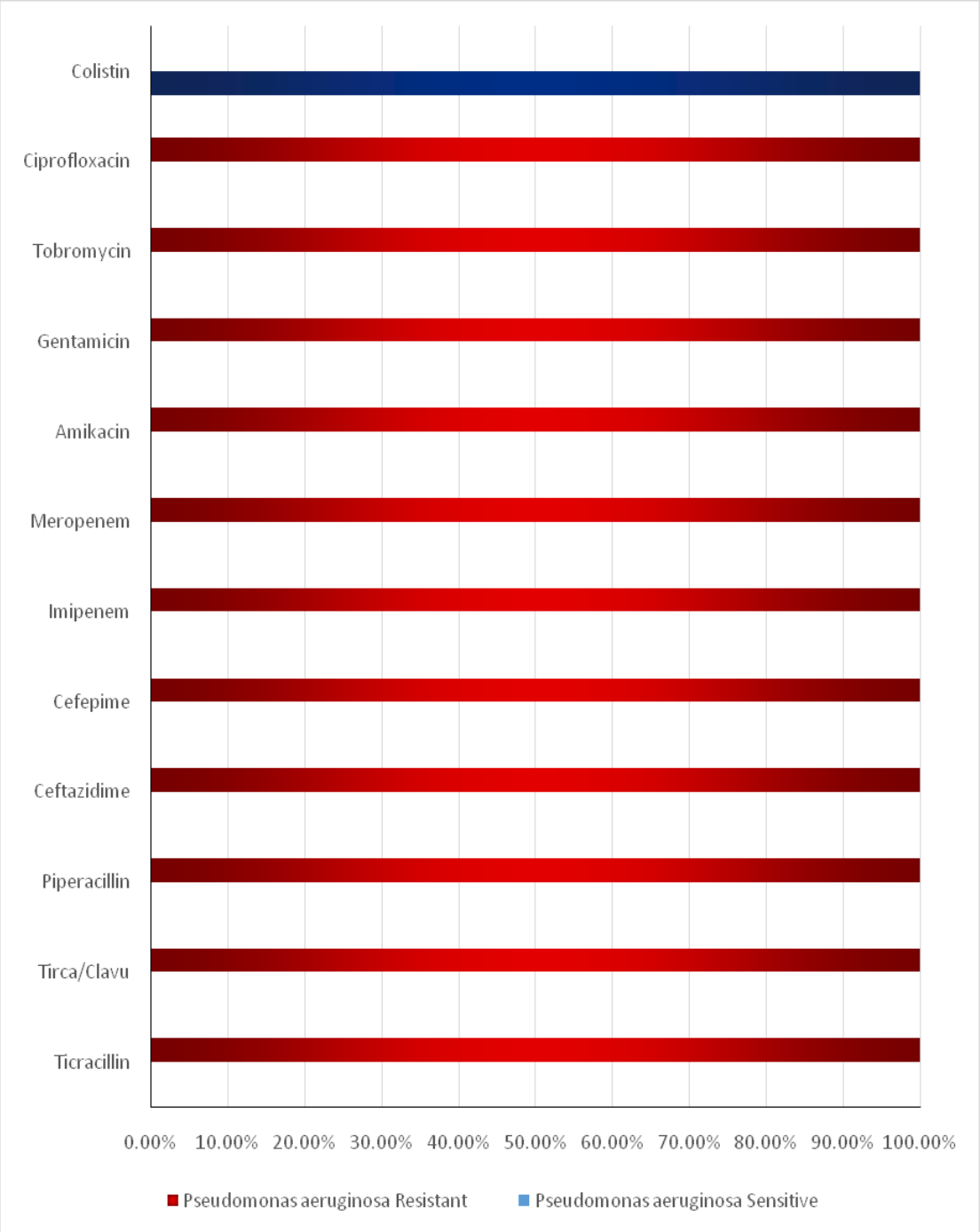

Figure 3-4 antibiotic susceptibility profile of *p. aeruginosa* isolate

Chapter Four

Discussion

4.Discussion

4.1. Distribution of sex, age, and weight characteristics of the patients and control groups

Burn injuries are a significant cause of both mortality and morbidity, leaving the patient with lifelong physical, psychological and emotional disabilities (Abdilkarim, 2022).

In this study, there were non significant differences (*P*= 0.420) between study groups regarding sex, as found in Table 3-1. This finding was similar to the study accomplished by Matsuura *et al.* (2019) who showed non significant differences regarding sex between the patients with burns and the controls. Additionally, in the present study, it was observed that the number of patients with burn wounds was higher in males compared to females, these results were in line with the results of Hasan and Al-Humairi (2022) who found that the number of significant burn wounds was higher in males (53.4%) than in females (46.6%). This result was also in agreement with other studies that showed that males have a higher frequency of burn wounds than females (Obaid, Baiee, and Ismail, 2020; Mulatu *et al.,* 2022). On the contrary a study conducted by Almutlaq *et al*. (2020) found that the highest incidence of skin burn cases was recorded among females (80%) compared to males (20%), The increased risk of burns in women may be attributed to their propensity for cooking, the use of stoves that are unsafe and can catch fire, as well as self-inflicted or interpersonal violence.

There were non significant differences (*P*=0.575) between study groups regarding age. This finding was similar to the study by Matsuura *et al.* (2019), who showed non significant differences regarding age between the patients with burns and the controls. Additionally, it was observed that the number of patients with burn wounds was higher in ages less than 11 years, these results were in line with the study in Babylon conducted by Obaid and Baiee (2022), who found that the number of significant burn wounds in

children of age 1–9 years constituted 35.8% of burning people. This may be attributed to that children tend to be more active and thus more exposed to stoves, fire places, hot kitchen appliances, and hot liquid.

According to the weight of the patients, there were non significant differences (p=0.320) when compared between the patients and the control group. On the contrary, a study result was conducted by Aghaei *et al*, (2018), who found that the body mass index had a positive and significant relationship (p <0.0004), which indicates that by increasing the body mass index, the chance of burning increased. It was decided that the risk of burns in obese patients was higher than that of normal people.

4.2. The clinical characteristics of the burn patients' group

Table (3-2) showed that from the results concerning burn types, it was clear that the most common cause of burns in burn patients was scalding burns (53.0%), followed by flame burns (41.0%), and leas frequency was electrical burns (6.0%). This study was in the same line as the study in Al-Hilla City by Hasan and Al-Humairi (2022), who showed that 43.9% of patients had scald burn, followed by flame burn at 34.2% and electrical burn at only 1.4%. Also, this study agreed with other studies, such as those by Tibebu *et al*. (2021); Mulatu *et al*. (2022), which found that scald burn was the major cause, followed by flame burn. This may be explained based on the fact that hot liquids are of high importance at home and are most frequently used in many life situations. The current study did not agree with the study in Duhok and Erbil by Qader, Solmaz, and Merza (2020), which demonstrated that burns from a flame were the most common cause of burn wounds, representing 55.5% of the patients, whereas scalding was the second most common cause of burn wounds, representing 37.7% of the patients. Also, a study by Polse, Khalid, and Mero (2023) found that flame burn had the highest rate (38.18%), followed by scald burn (15.45%). The differences between these results may be due to the fact that most flame injuries occur

at home and that most women in society perform daily household tasks like cooking and heating in areas of the kitchen where there is a greater danger of flame burns.

The current study found that only 3.6% of patients had an inhalation injury. It is less than the study in China conducted by Yu *et al*. (2016), which showed that 6.7% of patients had inhalation injuries. This result was also less than a study in Saudi Arabia by Al-Mutairi *et al.* (2023), who found that (9.9%) of patients had inhalation injuries. The variety could be due to population distribution, burn severity, and causes of burns.

However, in Table 3-2, the frequency of in-hospital mortality was 10.8%, compared to 89.2% of the patients who healed. This study is in line with study conducted by Kirschbaum-Rubin *et al*., (2021) who found that (9%.) of the patients were dead. On the contrary, a study result in western Uganda conducted by Baraka *et al*. (2023), who found that (4.7%) of the patients were dead. Also, a study result in Basra by Al-Shamsi and Othman (2017), which showed that the outcome of accidents was 79% of patients cured and discharged and 21% dead. The mortalities are different, which is possibly due to the difference in care, treatment, and following the standards. Goodarzi *et al.* (2014) suggested that the high mortality rate of burns can be due to inappropriate routine resistance to bacteria and septicemia. The presence of large TBSA% burns and inhalation injuries were predicting factors for mortality linked to burn injuries (Ismaeil *et al*., 2020).

Also in this study, the patients were placed on mechanical ventilation; it was found that only 3 patients (3.6%) had mechanical ventilation, as shown in Table (3-2). In contrast to other studies by Pirat *et al*. (2010) and Ismaeil *et al.* (2020), which revealed that 21% and 20% of the patients required mechanical ventilation respectively. The increasing severity of inhalation injuries has been consistently shown to be associated with mechanical ventilation.

However, in this study, total body surface area (TBSA) had a mean value (17.05) and SD (14.02) as showed in Table (3-2). The present study was consistent with a study accomplished by Mulatu *et al*. (2022), who found a total body surface area with a mean value (15.49%) and a standard deviation (13.78%). on contrast a study by Harish *et al*. (2019) who showed that the mean TBSA was 1.9 ± 2.1%.

Also, in this study, length of hospital stay had a mean value (9.31) and SD (4.36), as clarified in Table 3-2. It was in the same line with study conducted by Baraka *et al*. (2023), who found that the mean length of hospital stay was 9 days with a standard deviation of (5.7), the minimum was 3 days and the maximum was 28. The current study is inconsistent with the study conducted by Sari *et al*., (2024) who reported that the mean length of hospital stay was 10.29 ± 9.59 (minimum 1–maximum 74) days. The differences noted in the length of hospital stay in comparison to those seen in other studies are possibly because of the differences in the characteristics of the study participants, age, depth, size of burn, and other burn-specific characteristics may be affecting the length of hospital stay.

4.3. The degrees of burn severity in the patients group

The results of the current study in Tables (3–3) showed that the highest percentage of burn degrees was in patients with second-degree burns (66.3%), followed by third-degree burns (25.3%), and the lowest percentage was in second+third degree burns with a percentage of 8.4%. This study agreed with a study in Cameroon conducted by Forbinake *et al*. (2020), who found that almost half (48.9) of all burns were 2nd degree. Also, a study by Babakir-Mina, (2017) showed that second-degree burns were the majority (72.6%), while third and mixed-degree burns were (27.4%). The current study differs from a study in Sulaimanyah accomplished by Abdilkarim (2022), who found (51 %) of burn patients had second+third degree burns, while 38% were second and 11% were third degree burns. The longer burn

exposure times increase the possibility of prolonged skin-to-burn contact, which raises the degree of burn.

4.4. Bacterial culture results in patients

Gram-negative bacteria were the most frequently encountered bacteria in the present study (33.735%) compared to Gram-positive bacteria (8.434%), as demonstrated in Table (3-4). This study agreed with a study accomplished by Tchakal-Mesbahi, Abdouni, and Metref (2021) who revealed that Gram-negative bacteria were the most common bacteria isolated from burn wound swabs (68.95%), followed by Gram positive bacteria (28.62%). Also, the current study result is compatible with the results of other studies by Jobayer *et al*. (2021); Gomersall *et al*., (2023) and Abdilkarim (2022) which found that the Gram-negative bacterial isolate was more than Gram-positive bacteria. The results of the present study differed from study of Alam *et al.* (2021), who revealed that the presence of Gram-positive and Gram-negative bacterial isolates were 68.8% and 66.0%, respectively. Another study in Turkey was accomplished by Durgun and Yiğit (2023), who found that the most common bacterial isolates were Gram-positive bacteria (70.55%), followed by Gram-negative bacteria (28.68%). The variability in study populations and sample sizes amongst studies could be the reason for this unevenness.

A negative culture does not exclude a burn wound infection. Ganatra and Ganatra (2007) suggested that the reasons for "no growth" in burn wounds should be looked at with the possible causes of dry surface swabs in burn centers. Data from dry swabs are qualitative. In addition to being a low-cost method, it cannot distinguish between superficial contamination and deeper wound infection. Moreover, this subeschar space is not sampled by surface swab cultures, where the growth of microorganisms takes place before they invade the underlying living tissues. Even quantified swab

cultures from the wound surface could contain false positives or false negatives due to erroneous counts.

4.5. Distribution of bacterial isolates in patients

In this study, the most common bacterial isolate from burn wounds was *P. aeruginosa* (31.43%), as shown in Figure (3-1). This study was in the same line as the study in Maysan by Hateet (2021), who found that *Pseudomonas aeruginosa* was the most common pathogen (20%), also other studies by Alwaeli (2021)., Ellithy *et al*. (2021) and Fatema *et al*. (2021), whose found that *P. aeruginosa* was the highest pathogen recovered from burn swabs. The remarkably high prevalence of *P. aeruginosa* in burn wounds may be due to the fact that the organism grows in a moist environment, and *P. aeruginosa* is known for its ability to resist killing by a variety of antimicrobials. In addition, the minimal nutritional requirements of *P. aeruginosa*, including its ability to grow in distilled water and its tolerance to a wide variety of physical conditions, contribute to its ecological success and ultimately to its role as an effective opportunistic pathogen (Ahmed, Al-Ghanimi and Abboud, 2014). Also, the pathogenicity of *P. aeruginosa* is mediated by its ability to create a wide range of virulence factors, which is bolstered by its inherent resilience to environmental stresses and xenobiotics, including antibiotics, antiseptics, and heavy metals. Based on this data, it has been established that these characteristics facilitate the pathogen's ability to achieve efficient invasion, colonization, and persistence within the host organism (Hateet, 2021). The results of the current study differed from the results reported in Baghdad by Al-Azzawi and Alkalifawi (2023), who showed that the predominant bacteria was *Staphylococcus aureus* (25%*).* Another study by Thomas, Arora, and Arora, (2022) who found that most common bacterial isolate was *Klebsiella pneumoniae* (27.43%) while Study conducted by Sheeba, Prathyusha, and Anila, (2024) showed that the most common bacterial isolate from burn wound

was *Escherichia coli* (23%). This difference could be attributed to differences in age, distribution of populations and data analysis period.

However, the current study showed that the highest isolate rate was for *P. aeruginosa* bacteria with an isolate rate of (31.43%), followed by *Klebsiella pneumonia* with an isolated percentage of (20.00%) and the percentage of infection with *Escherichia coli* bacteria with an isolate rate of (14.29%), as clarified in Figure (3-1). This result was in agreement with a study by Kanagapriya *et al*., (2015), who found the percentage of isolates of *P. aeruginosa, K. pneumonia* and *E. coli* was 28%, 20%, and 8%, respectively. Also, a study by Jasim and Hussein, (2023) found that the most frequent isolate was *P. aeruginosa* (31.37%), followed by *K. pneumonia* (17.64%) and then *E coli* (15.68%), unlike a previous study in Kirkuk city by Al-Byti *et al.* (2019), who found an increase in the rate of isolation of *Staphylococcus aureus* (33.33%), followed by *P. aeruginosa* (31.81%), and *E. coli* (15.15%). The reason may be due to the number of samples that were included in the study or because, 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.

4.6. The relation of bacterial isolates to burn severity in patients' group

Tables (3-5) showed that patients with second-degree burns had more positive cultures isolated compared to the positive cultures isolated from patients with second+third degree and third-degree. The number of *P. aeruginosa and Klebsiella pneumonia*e from bacterial isolates were higher in patients with second+third degrees of burns compared to those with second and third degree of burns. Whereas, the number of *Escherichia coli*, *Staphylococcus aureus,Coagulase-negative staphylococcus*, *Staphylococcus epidermidis, Acinetobacter baumannii, Serratia liquefaciens, and Staphylococcus haemolyticus* were higher in patients with second-degree of

burn compared with third and secod+third degree of burn. *Enterobacter aerogenes* was higher in patients with the third-degree compared with other degrees of burn. A study by Moghadam *et al*., (2022) showed that patients with second-degree burns *P. aeruginosa, Klebsiella pneumonia*e and *Staphylococcus aureus* were isolated from 22.2%, 0%, and 11.1%, respectively, from burns. In addition, a study by Mohammed, (2022) showed that isolation of bacteria in patients with second-degree burns were *Enterobacter sp*, *P. aerugionsa* and *Klebseilla sp*. were (33%), (22%) and (12%) respectively.

4.7. The relation of bacterial isolates to sex in patients with bacterial infection

Table (3-6) showed that there were non significant differences (P=0.707) between males and females. The *P. aeruginosa* had a higher frequency in males (37.5%) compared to females (26.32%). This study was in the same line with a study by Qader, Solama, and Merza (2021), whose showed that *P. aeruginosa* isolated from males, accounting (60.4 %) whereas (39.6%) of them were isolated from females. However, a study by Al-Azzawi and Alkalifawi, (2023) whose found that (11.66%) of isolates were males and 5% of *P. aeruginosa* were females. The current study is incompatible with a study in Duhok City by Polse *et al*. (2023), who found that (26.36%) were isolated from males and (38.18%) from females. There were statistically non-significant ($P > 0.05$) differences between both sexes. Also, the present study is in contrast to a study in Kirkuk City by Hasan, Najati, and Abass (2019), which found that *P. aeruginosa* was more prevalent among females than males (61.1%) and (38.9%), respectively.

On the other hand, in Table (3-6) the frequency of *Klebsiella pneumonia*e were higher in females (21.05%) compared to males (18.75%). This study, with the same line as the study in Al-Najaf city by Oda Alquraishi and Al-Fatlawi, (2020), showed that *Klebsiella pneumonia*e was higher in

females (60%) compared to males (40%). Also, the current study was in the same line with a study in Egypt by Ahmed *et al*. (2023), who found that females were higher (12,50%) compared to males (11.40%). On contrast, the study in Iran by Vaziri *et al*., (2020) demonstrated that *K. pneumonia* isolates showed a prevalence of (57.9%) and (42.1%) for males and females, respectively.

However, in this study, the frequency of *Escherichia coli* was higher in males (18.75%) compared to females (10.53%), as illustrated in Table (3-6). This study agreed with a study by Ahmed *et al*. (2023), who found that *E coli* isolates were (8%) in males and (3,10%) in females. In contrast, a study in Kirkuk City by Magthab, (2023) who found that most of the patients infected with *E. coli* were females (71.43%) and males (28.57%). These discrepancies may be related to variations in the bacterial culture method and the different geographical settings of these studies.

4.8. The relation of bacterial isolates to age in patients with bacterial infection

In the present study, there was non significant difference (*P=0.357*) between bacterial isolates and ages in patients with bacterial infection. This study is in the same line as a study conducted by Gupta, Naik, and Singh (2019), who reported non significant differences in bacterial infection with age (*P*= 0.212). *P. aeruginosa* isolates in patients with mean age (27.00) and a SD (15.96) as shown in Table (3-7). This study agreed with the study in Basra by Jalil, Abdul-Hussien, and Al-Hmudi (2017), who found that *P. aeruginosa* isolates had a mean age of 21.3 and a SD of 15.8 years; also, a study by Coetzee, Rode, and Kahn (2013) found that *P. aeruginosa* isolates had a mean age of 3 years. On the other hand, the current study revealed that *Klebsiella pneumonia* isolates patients with mean age (22.86) and SD (19.18). Other study by López-Camacho *et al*. (2014) who showed that *Klebsiella pneumonia* isolate had a mean and SD of 54.9+16.8, also, a study

by Vaziri *et al*. (2020) who reported that *Klebsiella pneumonia* isolate had a mean age of participants was (36.11) and SD (48.42). However, a study by Melake *et al*. (2016) showed that *Klebsiella pneumonia* isolates with mean value (54.32) and SD (19.12). However, the present study found the *Escherichia coli* isolate in patients with mean age (19.40) and a standard deviation (23.66). Other study by Loan and Viet, (2023) showed that *Escherichia coli* isolate had a mean age 69.93.

4.9. The detection of *MCP-1* gene (rs1024611) polymorphism in patients and control by conventional PCR

Two distinct areas located in the 5'-flanking domain of the *MCP-1* genes control the *MCP-1* transcription (Ueda *et al*., 1994). The -2518 A/G (rs1024611) promoter polymorphisms affect the distal regulating region, which is upstream from the transcriptional starting site (1.9–2.7 kb) (Ping, Jones, and Boss, 1996). It is considered a strong candidate for a genetic vulnerability to a range of inflammatory disorders, such as sepsis (He *et al*., 2017) and spontaneous bacterial peritonitis (Hassen *et al*., 2022)

This study was our knowledge to explore the clinical relevance of a specific *MCP-1* gene promoter polymorphism, rs1024611 (-2518 A>G), for burn wound infection in the Iraqi population. This study revealed an elevated AA genotype in patients compared to controls. However, the AG and GG genes were shown to be elevated in control compared to patients with a significant difference (P=0.008). The "A" allele showed an increase in patients compared with control, while the "G" allele increased in control when compared to patients with significant difference (P=0.001), as shown in Table (3-8). Rovin, Lu, and Saxena, (1999) originally reported this A-2518G polymorphism in *MCP-1*. Subsequently, numerous studies have linked this polymorphism to various diseases. For example, a study by Li *et al. (*2022) demonstrated the connection between the onset and progression of type 2 diabetes (T2DM) with sepsis and the rs1024611 polymorphism in

the *MCP-1* promoter region. Their findings demonstrated that the T2DM with sepsis group had a considerably greater ratio of G allele frequency and rs1024611 AG/GG genotype frequency than the control group. However, study in China by He *et al*. (2017) showed that the frequency of the rs1024611 AG/GG genotyping within the septic group was significantly higher than in the control group (*P*= 0.0001). The frequencies of the rs1024611 G alleles were more prevalent in sepsis patients relative to control (*P= 0.0004);* a study in Egypt by Hassen *et al.* (2022), who found there were statistically highly significant variations between the tested groups (spontaneous bacterial peritonitis and control groups) among *MCP-1* (-2518A/G) polymorphisms and statistically significant variations concerning alleles. Ascitic patients with the AG genotypes had a 5.24-fold increased risk of spontaneous bacterial peritonitis compared to those with the GG genotypes. Ascitic patients with the G allele were less likely to develop spontaneous bacterial infections. However, a study by Kim *et al*. (2012) found that between Behçet's disease patients and the control, there were no statistically significant variations in allele (G versus. A, with *p*= 0.845) or genotype associated with the -2518 SNP in the *MCP-1* gene (GG versus. GA versus. AA, *p* = 0.916). No clinical features were related to genotypes having the -2518 polymorphism for *MCP-1*. Also, in a study by Khan, Murthykumar, and Ganapathy (2023), they found that there was no statistically significant difference in the prevalence of the *MCP-1* genotype compared to the control and periodontal groups. The AG and AA polymorphisms weren't more prevalent in the periodontitis group than in the group with healthy controls. The frequency of both A and G alleles remained the same in the periodontitis groups as in the healthy control group. These findings show that *MCP-1* may not be a periodontal biomarker.

4.10. Genotype and allele frequency distribution of *MCP-1* gene polymorphism according to severity groups in patients with bacterial infection

According to the frequency distribution of *MCP-1* gene polymorphism to burn severity in patients with bacterial infection. Patients with a second-degree showed a greater frequency of the AA gene compared to those with other severity degrees. While AG genotypes were shown to be present at greater prevalence in patients with second+ third degree, the GG genotype was identified as occurring at a greater occurrence in patients with third degree., with non significant difference (*P=0.323*). Also, the "A" variant frequency increased in patients second degree compared with the G allele, which was higher in the third degree with a significant difference (*P*=0.037), as shown in the Table (3-9). Other studies, such as a study by He *et al*. (2017), showed that those with mild sepsis had genotype distributions based on the rs1024611 polymorphism that were found to be significantly distinct from those who had septic shock (*P*= 0.010) and severe sepsis (*P* = 0.0005). The subgroups for septic shock and severe sepsis showed dominant frequencies of the rs1024611 G alleles, compared to the mild septic group. This observation suggests that the rs1024611 A>G allele contributes to the progression from mild sepsis to severe sepsis/septic shock. Also study by Li *et al*. (2022) found that the occurrence of the rs1024611G allele within the type 2 diabetes. with septic shock was substantially higher than in the type 2 diabetes group. The rs1024611G allele, with the general sepsis group (*P* = 0.02), might have an influence on the development of type 2 diabetes, from sepsis in generalized sepsis to septic shock after infection. Patients with T2DM who had sepsis with GA/GG genotypes had significantly higher acute physiological and chronic health evaluation II scores compared to those with AA genotypes (*P* <0.05). However, a study by Kim *et al*. (2012) revealed that in Behçet's disease in people who had moderate to severe lesions, the

incidence of either the GA or AA genotype was considerably higher than in those with the GG genotype (*p= 0.044* and *p= 0.038*, respectively). The AA genotype had greater total severity ratings compared to the GG and GA genotypes. (*p= 0.039* and *p= 0.003*, respectively). Furthermore, patients who had a GA or AA genotype scored higher compared to those with the GG genotype ($p = 0.041$).

4.11. Relation of *MCP-1* genotypes according to bacterial culture results

Table (3–10) showed that patients with the GG genotype had more positive culture isolates compared to the positive cultures isolated from patients with the AA and AG genotypes. The number of *P. aeruginosa*, *Acinetobacter baumannii* and *Serratia liquefaciens* in the bacterial isolate was higher in patients with the AG genotype compared to the AA and GG genotypes. Whereas, number of *Escherichia coli*, *Staphylococcus aureus, Coagulase-negative Staphylococcus* and *Staphylococcus haemolyticus* were higher in patients with the AA genotype compared with the AG and GG genotypes. *Klebsiella pneumonia*e, *Enterobacter aerogenes* and *Staphylococcus epidermidis* were higher in patients with GG genotype compared with other genotypes. There was non significant difference (*P=0.453*) according to bacterial culture results for *MCP-1* genotypes. A study in India about spontaneous bacterial peritonitis by Murthy *et al*, (2021) showed that the patients with AG/GG genotypes had a higher yield of positive ascitic fluid culture positivity (20%) than those with the AA genotype (7.7%), the Gram-negative organisms were predominant in both groups, and Gram-positive organisms were found only in patients with AG/GG genotypes in this study. In patients with AG/GG and AA genotypes, the numbers of *Escherichia coli* were 5 and 2, respectively, also numbers of *Klebsiella pneumoniae* were 3 and 1 respectively and number of Coagulase-negative staphylococci were 2 and 0, respectively.

4.12. The antibiotic susceptibility test for *Pseudomonas aeruginosa*

In this study, 100% of isolates were susceptible to Colistin, as shown in Table (3-11). This result was consistent with other studies by AL-Fridawy, Al-Daraghi, and Alkhafaji, (2020); Barzelighi *et al*. (2020), Kumar *et al*. (2020); Raheem and Hussein (2022) whose found that (100%) of the isolates were susceptible to Colistin. A study by Cai *et al.* (2018) who demonstrated the efficacy of this antibiotic in treating infections brought on by multidrug-resistant *P. aeruginosa*. Bassetti *et al.* (2019) suggested that the use of polymyxins (Colistin) should be adjusted for dosage and indications in order to minimize side effects, increase efficacy, and stop additional polymyxin resistance from developing.

On the other hand, the results of the present study showed that 100% of the isolates were resistant to Gentamicin, Tobramycin and Amikacin compatible with a study by Barzelighi *et al.* (2020), who found that 100% of the isolates were resistant to Gentamicin and Tobramycin. Also, the present results were much higher than those of the study by Mohamed, Mohamed and Afifi, (2022) whose demonstrated that *P. aeruginosa* resistant to the Aminoglycoside group by (66.7%), (74.4%) and (74.4%) to Amikacin, Gentamycin and Tobramycin, respectively. *P. aeruginosa* frequently develops resistance to aminoglycosides through the acquisition of aminoglycoside-modifying enzyme genes as well as mutations in the *parRS, fusA1, mexZ, and armZ* genes (Atassi *et al*., 2023).

However, 100% of the isolates were resistant to Ceftazidime and Cefepime. This study was in the same line with the previous studies carried out by Al-Janahi *et al*, (2020); Alkhudhairy and Azeez (2022), and Jasim and Hussein (2023), which showed an excessive rate of resistance to Ceftazidime and Cefepime reported in (100%) of *P arruginosa* isolates. The present results were much higher than those reported in Bakistan by Ismail and Altaai

(2021), who found that the *P. aeruginosa* isolates displayed resistance to Cefepime (77.55%) and Ceftazidime (48.96%).

Also in this study, 100% of the isolates were resistant to Imepenem and Meropenem. The present results were much higher than those reported in Baghdad by Jasim and Hussein (2023), who showed that Imipenem and Meropenem resistance were (52.08%) and (79.18%) respectively.

In addition, in this study, 100% of the isolates were resistant to Ticracillin, Piperacillin, and Ticarcillin/Clavulanic. This study was in the same line as a study by Attiah, Majeed, and Mohammed (2021), who reported that the highest MIC of the antibiotics was (90%) for Ticarcillin, (96%) for Ticarcillin/Clavulanic acid, and (96%) for Piperacillin.

Furthermore, in this study, 100% of the isolates were resistant to Ciprofloxacin. This study agreed with a study by Barzelighi *et al.* (2020), who found that about (98.5%) of *P. aeruginosa* isolates were resistant to ciprofloxacin. *P. aeruginosa* resistance to fluoroquinolones, including Ciprofloxacin, is a result of efflux pump effects or chromosomal mutations (Nabilou, Babaeekhou, and Ghane, 2021). Patients are exposed to various antibiotics due to the elevated prevalence rate of *P. aeruginosa*, which results in the development of multidrug resistance (Ullah *et al.,* 2023).

Conclusions and Recommendations

Conclusions:

1- The present study showed that the highest risk groups were males and ages less than 11 years. The main burn etiology was hot liquid (scalds). Second-degree burns were prevalent in burn patients.

2- It was observed that *P. aeruginosa* was the dominant and most prevalent type in burn wound patients compared to other bacterial species. The antibiotic colistin may be an effective choice of drug in cases of resistance of *P. aeruginosa* to polymyxins, a class of antibiotics.

3- The result of this study has shown that in patients, the AA genotype was higher than in controls. Therefore, the *MCP-1* gene (rs1024611) polymorphism may have a protective role in burn wound bacterial infection.

Recommendations:

The current study recommends the following.

1- A drug combination regimen must be achieved due to resistance to multiple agents.

2-For a better understanding of the role and association of the *MCP-1*gene (rs1024611) polymorphism in burn wound infection, more research is needed with larger sample size and different population.

3-Other studies are required to examine the association between other SNPs of the *MCP-1* gene and other genes such as *CCL7* and *CCL11* polymorphism with burn wound infection.

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Appendices

Appendix I: Burn wound patients' questionnaires

1- Age years

2- Sex ……. - Male ……... - Female

3- Type of burn

- Flame Burn (FB)……
- Scalding burn (SB) ……
- Electrical burn (EB)…….
- Chemical burn (CB)…….

4- Burn severity

- Degree 1…….
- Degree 2…….
- Degree 3…….
- Degree 4 ……

5- % TBSA (total body surface area) ……

6- Inhalational Injury ……

7- In-Hospital Mortality ……

8- Mechanical Ventilation ……...

9- HAI (healthcare associated infection)- …….

10- Length of Hospital Stay …….

11- Weight

- Normal weight……
- Overweight…….
- Obese ……

Appendix II: Healthy control questionnaires

1- Age years

2- Sex ……. - Male ……... - Female

3- Weight

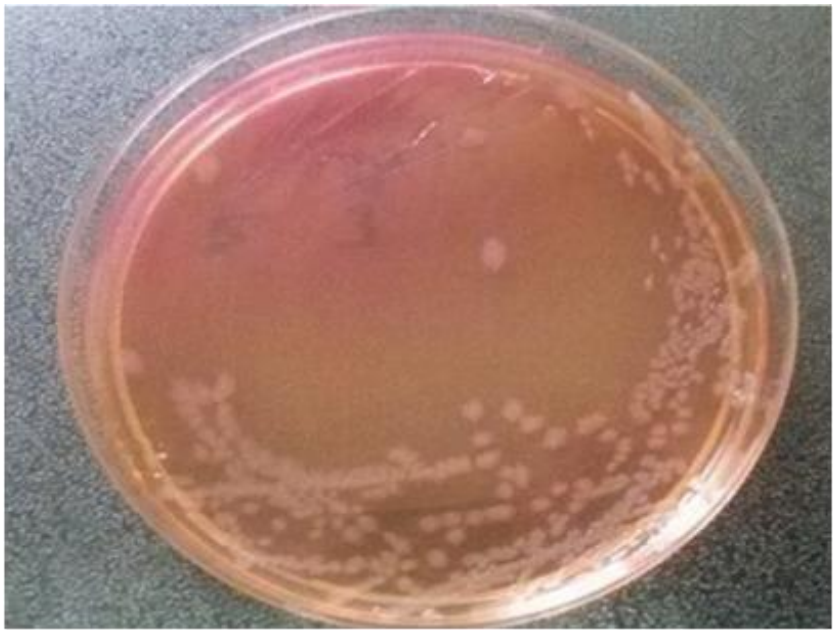
- Normal weight…….
- Overweight…….
- Obese ……

Appendix III: VITEK2 System

Appendix IV: Conventional PCR

Appendix V: Bacterial growth on MacConkey Agar.

a. *P. aeruginosa*, **b**. *E. coli* **c**. *K. pneumoniae*



a

b

c

الخلاصه

عدوى جروح الحروق هي واحدة من أكثر أنواع العدوى البكتيرية انتشارا في جميع انحاءالعالم . سبب هذه الالتهابات في الغالب هي بكتريا الزائفة الزنجارية. يرتبط *Monocyte chemoattractant protein-1* ، وهو عامل مرتبط بالنخاع ، بنتائج سريرية أسوأ بعد الإصابة الحرارية. تحتوي منطقة الجين *Monocyte chemoattractant protein-1* على تعدد الانماط الوراثيه عند (-2518 A > G) rs1024611 ، والذي يؤثر على تعبير *Monocyte chemoattractant protein-1* ويرتبط بعدد من الأمراض الالتهابية.

شملت هذه الدراسه 83 مسحة من كلا الجنسين (44) ذكرا و (39) أنثى راقدين في ردهة الحروق في مدينة الإمام الحسين الطبية ، كربلاء / العراق خلال الفترة الممتدة من أغسطس (2023) إلى ديسمبر (2023). تراوحت اعمارهم بين (1-60) سنة. بالإضافة إلى ذلك تم جمع 70 عينة دم في أنبوب Ethylene diamine tetra acetic acid كان 35 منها من المرضى المصابين بالتهابات بكتيريه في الجلد و 35 اشخاص اصحاء تراوحت أعمارهم بين (1-52) سنة. تم استخدام عينات الدم للكشف عن تعدد الأشكال الجيني *Monocyte chemoattractant protein-1*. تم زرع جميع المسحات البكتيرية على مختلف الاوساط الزرعيه التشخيصيه وكذلك تم عمل الفحوصات مجهرية وكيميائية حيوية .وباستخدام نظام VITEK2 تم تأكيد من تشخيص هذه الانواع البكتيرية وكذلك اختبار الحساسية للمضادات الحيوية لبكتيريا a*Pseudomonas aeruginos*. كما وتم قياس تعدد الانماط الجيني *Monocyte chemoattractant protein--1* عن طريق تضخيم نظام الطفرات المقاومة للحرارة Amplification refractory mutation system بواسطة تقنية تفاعل البلمرة المتسلسل.

أظهرت النتائج أن نسبة الذكورالمصابين بحروق الجلد بلغت 53.0٪ ، بينما كانت نسبة الإناث 47.0٪. بينما في مجموعه السيطرة (42.9٪) ذكور و (57.1٪) إناث. لم تكن هناك فروق ذات دلالة إحصائية ($P = 0.420$) بين مجاميع الدراسة فيما يتعلق بالجنس. وكان أعلى معدل للإصابة في الفئة العمرية <11 سنة مقارنة مع الفئات العمرية الأخرى ولم تكن هناك فروق ذات دلالة إحصائية ($P = 0.575$) بين المرضى والاصحاء فيما يتعلق بالعمر. و سجلت الدراسه أعلى نسبة لإصابات الحروق ناتجة عن الحروق بالسوائل بنسبة 53.0٪، تليها الحروق بالنار بنسبة 41.0٪، وكانت أقل نسبة حروق الكهرباء (6.0٪). وفيما يتعلق بدرجات الحروق، كانت الحروق من الدرجة الثانية أكثر

الحالات (66.3٪)، بينما الحروق من الدرجة الثالثة كانت (25.3٪) وحروق من الدرجه الثانية + الدرجة الثالثة شكلت فقط (8.4٪). كما أظهرت نتائج الدراسة أن *الزائفة الزنجارية* المعزوله من جروح الحروق هي البكتريا الأكثر شيوعا (31.43٪) ، ثم الكلبسيلا الرئوية (20.0٪) ، تليها الإشريكية القولونية (14.29٪). أظهرت النتائج أيضا معدلات مقاومة عالية للزائفة الزنجارية لجميع المضادات الحيوية (100٪) ، باستثناء ال Colistin كانت الحساسية (100٪) لهذا المضاد الحيوي.

امـا حول تعـدد الانمـاط الجيني لجين *Monocyte chemoattractant protein-1* rs1024611 ، أظهرت الدراســة الحالية أن النمط الجيني AA كان مرتفعا في المرضـــى مقارنة بمجموعة الســيطرة (40.0٪ مقابل 8.6٪ ، على التوالي) ، بينما تم العثور على النمط الجيني AG بنسـبة أعلى في مجموعة السيطرة مقارنة بالمرضـى (17.1٪ مقابل 14.3٪ ، على التوالي) ، كما وجد أن النمط الجيني GG في مجموعة السـيطره أعلى مقارنة بالمرضـى (74.3٪ مقابل 45.7٪ ،على التوالي). كانت هناك فروق ذات دلالة إحصائية في الأنماط الجينية (p=0.008) بين المرضى والمجموعة السيطره. كما وجد ان الأليل "A" في المرضى اعلى مقارنة بمجموعة السيطرة (47.2٪ مقابل 17.2٪ ، على التوالي) ، في حين كان الأليل "G" مرتفعا في مجمو عة الســيطرة مقارنة بالمرضـــى (82.8٪ مقابل 52.8٪ ، على التوالي) ، كانت هناك فروق ذات دلالة إحصـــائية (P = 0.001) بينهما.

أظهرت نتيجة هذه الدراسة أنه في المرضى ، كان النمط الجيني AA أعلى منه في السيطرة. لذلك ، قد يكون لتعدد انماط الجين *Monocyte chemoattractant protein-1* rs1024611 دور وقائي في العدوى البكتيرية لجرح الحروق. بالإضـــافة إلى ذلك ، *فإن* الزائفة الزنجارية هي البكتيريا الأكثر انتشارا في المرضى المصابين بحروق الجلد.

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فرع الأحياء المجهرية

العلاقة بين *تعدد الأشكال الجيني MCP-1* Chemokine وشدة الاصابه بالعدوى البكتيرية الهوائية لدى مرضى الحروق

رسالة

مقدمة الى مجلس كلية الطب/جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الاحياء المجهرية الطبية

من قبل الطالب

حسن فاضل خضير مطر

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