

Republic of Iraq  
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# **Microbial Study on Burn Victims Blood Samples as Forensic Evidence**

A Research

Submitted to Council of the College of Science /  
University of Babylon in Partial Fulfillment to the  
Requirements for the Degree of High Diploma in Science  
/Forensic Evidence

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**2021 A. D**

**1443 H.D**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(وَمِنْ آيَاتِهِ أَنْ خَلَقَكُمْ مِنْ تُرَابٍ ثُمَّ إِذَا أَنْتُمْ

بَشَرٌ تَنْشُرُونَ)

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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# CERTIFICATION

I certify that research is prepared under my supervision at the Department of Biotechnology, College of Sciences, University of Babylon, as partial requirements for the Degree of Higher Diploma in Forensic Evidence.

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## *Dedication*

*To my creators and commissioner God,  
who teach humans and guide them to  
knowledge*

*To the prophet and messenger  
Muhammad peace be upon him and his  
family*

*To my father the great heart to the source  
of patience and hope*

*To my lovely mother who made me what  
I am now*

*There no words enough to thanks you*

*To my husband who support and  
encourage me along my study.*

*To my children flowers of my life*

*To my brother who gave me a lot support*

**Zahraa**

# *Acknowledgments*

*Praise be on Allah and peace and blessings be upon his Messenger the Prophet Muhammad peace be upon him and his family and who followed him until the Day of religion.*

*I would like to thank my advisor prof. **Dr. Ibtihal Muiz***

***Abd Mahdi Al Hussaini***

*, University of Babylon for his supervision of the construction of this scientific effort that has been a great asset for me.*

*My Thanks are extended to the University of Babylon  
College of Science  
Department of Biology*

*And the staff in Center of burns in the AI-Hussein Medical City Hospital in Karbala for helping and guiding me.*

**Zahraa**

## Summary

Burns injury is a major problem in many areas of the world. Thermal injury destroys the skin barrier that normally prevents invasion by microorganisms . There are many situations requiring medicolegal investigation in both living cases and cases that have died as a result of burn trauma.

Fourteen blood samples was included in this study, Thirty of which were collected from burn patients and ten samples were collected from nonburn individuals in four months from April to August 2021. This study was carried out at Center of burns in the AI-Hussein Medical City Hospital in Karbala.

The results showed that the age and gender in the patients groups. The age is considered the one of the risk factor especially among the youngest group. The higher in the age groups 25-34 years. While demonstrated that the (60%) of burn injuries that occurs among females than males (40%) (16 female and 14 male).

The blood burn samples showed positive cultures was founded 14 (46%) , that positive bacterial cultures in 12 (40.%) of burned patients, 2 (6.6%). of them was of single growth, the other 16 (53.%) also the control negative bacterial cultures.

Moreover the predominance of gram negative bacteria is clear from the high frequency of *Pseudomonas. aeruginosa* at percentage (23.3%), while the *Staphalococcus. aureus* 16% percentage. On the other hand the fungi *Candida. albicans* showed 6.6% percentage in the burn patients.

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### List of Abbreviations

Abbreviation	Meaning
<b>AAFS</b>	American Academy of Forensic Sciences
<b>AIDS</b>	Acquired immunodeficiency syndrome
<b>ASP</b>	Aspergillosis

<b>BSI</b>	Blood stream infection
<b>CF</b>	Cystic fibrosis
<b>CoNs</b>	Coagulase negative staphylococcus
<b>COPD</b>	Chronic obstructive pulmonary diseases
<b>Fe<sup>2+</sup></b>	Ferrous
<b>FFI</b>	Filamentous fungal infection
<b>FUS</b>	Fusariosis
<b>Hb</b>	Hemoglobin
<b>HCWs</b>	Healthcare workers
<b>Hhb</b>	Human hemoglobin
<b>HIV</b>	Human immunodeficiency
<b>IV</b>	Intra venous
<b>LMIC</b>	Low and middle income countries
<b>MITS</b>	Minimally invade tissue sample
<b>MMC</b>	Mucormycosis
<b>MSCRAMMs</b>	Microbial surface component recognizing adhesive matrix molecules
<b>RBCs</b>	Red blood corpuscles
<b>TBSA</b>	Total body surface area
<b>WBCs</b>	White blood cells

*Chapter One*  
*Introduction*

## 1.1 Introduction

Forensic science is a multidisciplinary subject, which encompasses many different branches of science, and medicine, as exemplified by the 11 sections of the American Academy of Forensic Sciences (AAFS) with a current membership of over 6500: anthropology, criminalistics, digital and multimedia sciences, engineering and applied sciences, general, jurisprudence, odontology, pathology/biology, psychiatry and behavioral sciences (Jones, 2021).

There are many situations requiring medicolegal investigation in both living cases and cases that have died as a result of burn trauma. In Turkey, it is mandatory for a forensic examination and autopsy to be performed in all cases of death occurring as a result of burns. There must also be an investigation by the judicial authorities into surviving cases of burns trauma with judicial notification made by the physicians (workplace accidents, suspected neglect or abuse). The burn traumas that are subject to a legal process may occur in various forms such as domestic accidents, occupational accidents, or negligence or abuse of the elderly or children. The history obtained during the medical examination is very important for the evaluation of abuse. (Aydogdu, *et al*, 2021).

Burns jury is a major problem in many areas of the world. It has been estimated that 75% of all deaths following thermal injuries are related to infection. Thermal injury destroys the skin barrier that normally prevents invasion by microorganisms, making the burn wound the most frequent origin of sepsis in these patients (Vindenes, and Bjerknes, 1995).

Correspondingly, it has been suggested that bacteria derived from the skin and oral-microbiomes could also diffuse into the blood when the barriers between these environments and the circulatory system are compromised (Iwai 2009). Pathogens of specific concern in the burn population include strains of *P. aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia* and methicillin resistant *S. aureus*, *Enterobacteriaceae* in burn units (Lachiewicz *et al.*, 2017)

## 1.2 The aim of study

This study was aimed to detecting the Microorganisms associated with burns that cause blood poisoning as forensic evidence and comparing the number of selected and control samples.

### Objectives of study

- 1-Collect blood samples from patients with burn .
- 2-Detection the microorganism of studied population blood .
- 3-Compare between bacterial and fungal isolate with burn cases .

# *Chapter Two*

*Literature review*

## 2.Literature review

### 2.1 Forensic science:

Burns are defined as acute injury of the skin or other organic tissues caused mainly by thermal or other trauma. Burn trauma is known to occur because of hot or cold materials, chemicals, electricity, or radioactive rays. The grades of thermal damage were investigated by Moritz and Henriques in 1947. They reported that even heat of 44°C may cause damage when applied for a long time. The clinical consequences of thermal trauma range from simple burns to life-threatening and fatal injuries. It has been reported that approximately 300,000 persons per year die as a result of burn trauma. Independent of mortality (Aydogdu, *et al.*, 2021).

In some cases, minimally invasive assay (MIA) includes the procurement of tissue samples, also known as Minimally Invasive Tissue Sampling (MITS), using fine needles to collect small amounts of tissue from key organs and body fluids. Currently, MIA is increasingly carried out as a complement or replacement for the traditional complete autopsy, mostly in low- and middle-income countries (LMIC) (Palmiere, *et al.* 2015).

Sample contamination during collection of peripheral blood is most commonly due to the introduction of coagulase-negative staphylococci from the skin. In general, a mixed bacterial growth likely represents sample contamination whilst single isolates are generally interpreted to represent true positives (Dargère, *et al.* 2018)

## 2.2 Burn Wound Infections:

Burn injuries, caused by thermal, electricity, friction, chemical agents, radiation, etc., are a common global health problem and present an enormous medical and financial burden. Burn-initiated pathophysiological events can raise the risk of disfigurement, functional impairment and serious complications by increasing vascular permeability and fibrinolysis, changing in extravasation of plasma proteins and platelet aggregation. According to the World Health Organization, ~180 000 deaths worldwide annually are related to burn injuries, and 11 million people worldwide were burned severely enough to require medical treatment. Generally, epidermal burns and superficial partial-thickness burns tend to heal within 2 or 3 weeks with moderate treatment, whereas severe burns, including deep dermal burns and full-thickness burns, often require an extended period for healing . In deep dermal burns, wounds may remain open for a few weeks, which provide an entry point for pathogens and microorganisms invading. Consequently, biofilms formed, thereby delaying wound closure by triggering systemic inflammatory and immunological responses . Apart from the hypertrophic or keloid scars formation, biofilms can exacerbate wound infection and lead to life-threatening complications, such as sepsis, respiratory failure and multi-organ failure. Therefore, simultaneous inhibition of biofilm formation and promotion of wound healing is paramount to deep dermal burns treatment (**Yang, et al, 2021**).

Burns are consider one of the leading causes of accidental injuries and death worldwide. Although most burn patients seeking medical advice do not require hospitalization, severe burn injuries can lead to significant morbidity and mortality. Several risk factors may contribute to increasing mortality among adult burn patients, including total body surface area (TBSA) affected, old age, inhalation injury and infection. Burn patients are

more prone to developing invasive infections secondary to the breakdown of the natural skin barrier, which may be serious enough to progress to sepsis or septic shock (Ellithy, *et al.*, 2021).

Burn wound infections can lead to scarring, bacteremia, sepsis, and multi-organ dysfunction, contributing to 75% mortality in burn patients. Hence these studies are important for providing adequate and effective treatment of the cases thereby decrease the morbidity and mortality. Burns provides a suitable site for bacterial multiplication and are more persistent richer sources of infection than surgical wounds, mainly because of the larger area involved and longer duration of patient stay in the hospital. Burn injuries affect the skin to a large extent. Burns are caused by scalds (hot liquids), heat, radiation, open flame, electricity and hot water bottles. The occurrence of nosocomial burn infections depends on several factors such as the burn severity, immune status, prolonged stay, invasive procedures and overcrowding leading to cross infections. The exposed skin following thermal injury is prone to infection and can be contaminated with resistant organisms serving as a source of prolonged infection affecting other burn patients. In every hospital set up, the incidence and organisms isolated will be different. The infecting microorganism may belong to aerobic as most commonly isolated aerobic microorganism include *Staphylococcus aureus*, Coagulase-negative staphylococci (CoNS), *Enterococci*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter species*, *Proteus mirabilis*, *Candida albicans* and *Acinetobacter*. *Pseudomonas aeruginosa* has emerged as a predominant member of the burn wound flora and in the absence of topical therapy is cultured from the burn injuries of 70% patients by the third week (Sharma, and Sharma. 2021).

### 2.3 Sources of organisms:

Sources of organisms may be endogenous (the patient's own normal flora) or exogenous (the environment and from health care personnel). Organisms associated with infection in burn patients include gram-positive, gram-negative, and yeast or fungal organisms. The distribution of organisms changes over time in the individual patient, however, and such changes can be ameliorated with appropriate management of the burn wound and the patient (**Ramzy et al.**, 1998)

The typical burn wound is initially colonized predominantly with gram positive organisms, which are fairly quickly replaced by antibiotic-susceptible gram-negative organisms, usually within a week of the burn injury. If wound closure is delayed and the patient becomes infected, requiring treatment with broad-spectrum antibiotics, these flora may be replaced by yeasts, fungi, and antibiotic-resistant bacteria. Organisms of particular concern include methicillin-resistant *S. aureus*, enterococci, group A B-hemolytic *streptococcus*, and coagulase-negative *staphylococci* (**Rutala, et al.**, 1983). Risk factors identified in patients colonized with drug-resistant organisms include prior use of third-generation cephalosporins and antibiotics active against anaerobes, a critically ill patient with severe underlying disease or immunosuppression, and a prolonged hospital stay. Gram-negative organisms have long been known to cause serious infection in burn patients and have been reported to be associated with a 50% increase in predicted mortality for patients with bacteremia compared with those without bacteremia (**Mason, et al**, 1986). Fungal organisms, especially *Candida* (yeast) species and molds like *Aspergillus*, *Mucor*, and *Rhizopus*, have been associated with serious infections in burn patients (**Becker, et al**, 1991). *Candida* colonization seems to be primarily from endogenous sources, whereas molds are

ubiquitous in the environment and can be found in air handling and ventilation systems, plants, and soil (Spebar and Lindberg, 1979).

## 2.4 Modes of Transmission of Microorganism:

Modes of transmission include contact, droplet, and airborne spread. In burn patients the primary mode is direct or indirect contact, either by the hands of the personnel caring for the patient or from contact with inappropriately decontaminated equipment. Burn patients are unique in their susceptibility to colonization from organisms in the environment and in their propensity to disperse organisms into the surrounding environment(Sharma, 2007).

In general, the larger the burn injury, the greater the volume of organisms that are dispersed into the environment from the patient. In almost all cases the colonized patient is thought to be a major reservoir for the epidemic strain. Other important sources include contaminated hydrotherapy equipment; common treatment areas; and contaminated equipment, such as mattresses, which seem to pose unique risks of cross-contamination in the burn care environment Risks associated with care of the burn wound, such as hydrotherapy and common treatment rooms, are related to the use of water sources that are frequently contaminated by gram-negative organisms intrinsically, and may also be contaminated by organisms from other patients. This aquatic environment is difficult to decontaminate because of continuous re-inoculation of organisms from the patients' wound flora and because of the organisms' ability to form a protective glycocalyx in water pipes, drains, and other areas, making them resistant to the actions of disinfectants. Adequate decontamination of this equipment (eg, tanks, plinths, shower tables, straps) is difficult to achieve between patients using this equipment on a daily basis and monitoring

techniques are insufficient to provide timely detection of contamination . The two areas most likely to become contaminated when caring for the burn patient are the hands and gowns of the personnel, because the surfaces (eg, beds, side rails, tables, equipment) are often heavily contaminated with organisms from the patient. Likewise, all equipment used on the patient (eg, blood pressure cuffs, thermometers, wheelchairs, IV pumps) is also heavily contaminated and the same may be transmitted to other patients if strict barriers are not maintained and appropriate decontamination not performed. A single cause is uncommon in a burn unit outbreak; in almost all instances, multiple factors contribute to occurrence and perpetuation of infecting organisms (**Sharma,2007**).

### **2.5 Sources of infection:**

There are many factors that have been postulated as reasons that favour intense colonization and infection in this population; such as the impaired function of both the cellular and humoral immune systems, the devitalisation of tissues, the loss of the protective skin barrier, the translocation of intestinal microbial flora, multiple surgical procedures and the use of invasive monitoring devices. After survival from the acute phase of a burn injury, septic complications become the first cause of mortality among severe burns patients. Bloodstream infections (BSI) are among the most prevalent and problematic infectious complications (**Sousa, et al, 2018**)

## 2.6 Bacterial infection of Blood :

### 1- *Staphylococcus aureus*:

*Staphylococcus aureus* is a Gram-positive bacterium capable of infecting nearly all host tissues, causing severe morbidity and mortality. Widespread antimicrobial resistance has emerged among *S. aureus* clinical isolates, which are now the most frequent causes of nosocomial infection among drug-resistant pathogens. *S. aureus* produces an array of virulence factors that enhance *in vivo* fitness by liberating nutrients from the host or evading host immune responses . Staphylococcal virulence factors have been identified as viable therapeutic targets for treatment, as they contribute to disease pathogenesis, tissue injury, and treatment failure. Anti-virulence strategies, or treatments targeting virulence without direct toxicity to the inciting pathogen, show promise as an adjunctive therapy to traditional antimicrobials (**Ford, et al., 2021**). catheters as a strong predictor of subsequent *S. aureus* bacteremia (SAB), even in the absence of clinical signs of systemic infection. Bacteremia was effectively prevented by timely antibiotic therapy. We conducted this study to corroborate the validity of these findings in non-university hospitals (**Hetem, et al., 2011**).

It is an important pathogen with a variety of virulence factors that can cause life threatening infections . The bacteria is on endogenous flora or contact with contaminated environmental surfaces, the hands of healthcare workers (HCWs) and the air . Nasal carriage of *S. aureus* plays a critical role in the development of *S. aureus* infection. Biofilm production of *S. aureus* plays an important role in adherence and colonization of microorganisms on mammary epithelium cells and resistance to antibiotics or evasion from host immunological response. The intracellular adhesion

(ica) operon is necessary for the control of biofilm production. Among ica locus, the icaA and icaD genes are more important than other genes. The other virulence factors involved in adherence to the host tissue are microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) which contains molecules called collagen-binding protein (Cna), elastin binding protein (EbpS), fibronectin-binding proteins (FnbA and FnbB), laminin-binding protein (Eno) and fibrinogen binding protein (Fib) ( **Sedaghat, et al.,** 2021).

## 2- *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* is a ubiquitous Gram-negative bacterium found in soil and surfaces of aqueous environments. Because of its metabolic versatility and high intrinsic resistance to antimicrobials, *P. aeruginosa* efficiently adapts and thrives in a wide variety of natural and artificial settings, including in-hospital facilities and patient devices. Although rarely infecting healthy individuals, *P. aeruginosa* is a leading and notorious opportunistic pathogen, especially in immunocompromised patients with defective immune defenses, including chronic neutropenia and defects of neutrophil function, cancers, human immunodeficiency (HIV) acquired immunodeficiency syndrome (AIDS), and diabetes mellitus. *P. aeruginosa* is best known to chronically colonize and infect the lung of people with cystic fibrosis (CF) and advanced stages of chronic obstructive pulmonary disease (COPD) (**Morin, et al.,** 2021).

Also, *P. aeruginosa* is responsible for approximately 4% total cases of hospital-acquired bloodstream infections, and was the third leading cause among Gram-negative pathogens. Poor antibiotic stewardship in the past few decades has led to emergence and spread of multidrug-resistant strains. Effective treatment options are increasingly scarce; consequently, *P.*

*aeruginosa* infections are associated with high morbidity and mortality (Morin, *et al*, 2021).

It is a Gram-negative bacterium and one of the greatest threats to patients with severe partial- and full-thickness burn injuries. It is the most important human pathogen in the *Pseudomonadaceae* family and the second leading cause of burn infections. The extreme use of antibiotics has increased the antibiotic resistance of *Pseudomonas* species worldwide. Multidrug-resistant (MDR) *P. aeruginosa* infection is a serious problem in treating hospitalized patients with burns. Most pathogenic bacteria must attach to the target cell surface, leading to biofilm formation in various environments. Biofilm formation is one of the causes of antibiotic resistance and failure to treat bacterial infections (Abootaleb, *et al*, 2021).

### **2.7 Diagnosis of isolated bacteria:**

Primary diagnosis of isolated bacteria was mainly depending on the culture characters such as colony morphology, color, and blood hemolysis. Type of Gram stain was also performed after staining with Gram stain to differentiate between Gram positive bacteria (taken violet color from crystal violet stain) and Gram negative bacteria (taken a pink color from Safranin stain), also this Gram stain useful to determine the shape, size and arrangement of bacteria cells (Kayser, and De Knijff, 2011).

## 2.8 Fungal infection:

The most common fungal pathogen causing infections in burn patients (**Branski et al.**, 2009). Furthermore it is the fourth most frequent pathogen in blood cultures in ICU patients (**Wisplinghoff, et al**, 2004).

Mold infections are rare and uncommon but seem to have become more frequent in burn patients over the last decades. In general they are associated with higher risk for morbidity and mortality and are associated with poor prognosis. (**Rutala, et al**, 1983)

1- **Aspergillus species** are the most common non-Candida pathogens, with an incidence reported of approximately 14%–17% in patients who are severely burned. Furthermore, they are correlated more closely with lethal infections, resulting in high morbidity and mortality rates of up to 33% ( **Ballard, J. et al.**, 2008). A retrospective single-cohort study reviewed 1,849 burned patients with a positive filamentous culture. Mold infection was present in 1.7% (1.2% for aspergillosis (ASP), 0.5% for mucormycosis (MMC), and 0.2% for fusariosis (FUS). This study highlighted that most were cutaneous and occurred in patients with mean total body surface area (TBSA) of 55% and mean full thickness burn area of 45%, which was higher than in burned patients without filamentous fungal infection (FFI). Of the FFI, infection caused by *Aspergillus* was more lethal (30%) and seems to be more serious than other FFI( **Ballard, et al**, 2008).

**2-Cutaneous mucormycosis**, in particular zygomycetes, shows an increase in infection rate that can lead to a wide spectrum of diseases. In contrast to *Candida*, which is part of the healthy human skin flora, zygomycetes are soil fungi. Zygomycetes typically are parasites of plants, insects, and small animals and in general, are rare and uncommon. In burn

patients they are likely to occur only in those patients who are severely immunocompromised (**Schaal, et al.**, 2015). They are associated with a high mortality rate of 54%, which has not improved in the last decades, as reported in the review by Ledgard et al. [**Ledgard,et al.**, 2008).

**3-Candida** plays a major role in fungal burn wound infections, there is a recognized increasing prevalence of non-*Candida albicans* spp., such as *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, and *Candida glabrata*. They are associated with higher mortality rates and a decreased susceptibility to the widely used fluconazole and other antifungal agents (**Katz, et al.**,2014).The wide use of fluconazole and its impact on the epidemiology of invasive *Candida* has been described, as studies reported the emergence of *Candida krusei* (**Girmenia,et al.**, 1996).

## **2.9 Risk Factors of fungi infections:**

There exist multiple risk factors for invasive fungal infections in burns. The infections usually occur after the second week of thermal injury. Immunodeficiency in the patient who is severely burned has the most impact on development of fungal infections. Also inhalation injury, renal dysfunction, and replacement therapy as well as gastrointestinal complications are major risk factors. Furthermore, broad-spectrum antibiotic treatment and being on a ventilator for longer than 48 hours increase susceptibility to fungal infections. The single most important risk factor for fungal infections however, is the burn wound itself (**Norbury, et al.**, 2016) .

## 2.10 Diagnosis of fungal infections:

The diagnosis of fungal infections is particularly difficult, especially in the early phase of an infection because they can mimic early low-grade bacterial infections. Several different culture media are necessary to identify the specific genome. Nevertheless, it requires several weeks to obtain laboratory confirmation, which may result in delay of adequate treatment. Furthermore, laboratory tests may have limited impact. Despite the low sensitivity of 50%–70%, obtaining fungal cultures is still the gold standard and identifying the exact fungal strain causing the burn wound infection has become increasingly important. Similar to treating bacterial infections after resistance testing (Norbury, *et al*, 2016).

### a- Germination tube test:

This procedure is used for presumptive identification of *Candida* species and is also known as the germ tube test. The culture of *Candida* species is treated with normal human pooled serum or sheep or rabbit or fetal calf serum and incubated at 37°C for 2-4 hours. A drop of suspension is examined on the slide under a microscope. The germ tubes are seen as long as tube-like projections extending from the yeast cells. There is no constriction at the point of attachment to the yeast cell as seen in the case of Pseudohyphae. The demonstration of the germ tube is known as the Reynolds-Braude phenomenon (Raghunath, *et al*, 2014).

This is a rapid method for identifying and differentiating *C. albicans* from other *Candida* spp. Buds and pseudohyphae can be distinguished from germ tubes by the constricted attachment at the point of origin. Germ tubes don't show constriction at the point of origin (Makwana, *et al*, 2012).

**b- Principle of Germ Tube Test:**

Germ tube is a short outgrowth, non-septate germinating hypha. It is  $\frac{1}{2}$  the width and 3 – 4 times the length of the cell from which it arises. When *Candida* species are incubated in serum at 37°C for 2-4 hours and producing short, slender, tube-like structures called germ tubes. The formation of this germ tube is associated with increased synthesis of protein and ribonucleic acid and is observed in *Candida albicans* (Moya, *et al.*, 2018).

*Chapter Three*  
*Materials and*  
*Methods*

### 3. Materials and Methods

#### 3.1. Materials

##### 3.1.1. Apparatuses and Equipment's

Apparatus and instruments of this study are included in table (3-1 ) and table ( 3-2).

**Table (3-1): Apparatuses and instruments used in the study**

No.	Apparatus	Company Origin	Origin
1	Autoclave	Hirayama	Japan
2	Biological safety cabinet	Labtech	Korea
3	Bunsen Burner	Bunsen Burner Amal	Amal
4	Digital balance	Denver	German
5	Incubator	Termaks	Denemark
6	Micropipette	Slamed	Germany
7	Microscope	Olympus	Phelepin
8	Oven	Steri-dent	USA
9	Refrigerator	Refrigerator memmert Korea	Korea
10	Turbidity meter	BioMerieux	France
11	VITEK2 compact	BioMerieux	France
12	Vortex	Stuart	USA

**Table (3-2): Equipment used in the study**

No.	Equipment	Company Origin	Origin
1	Plain tube	ArthAL-Rafidin	China
2	Glass slides	Supertek	India
3	Gloves	Top-Glove	Malaysia
4	Inoculating loop	Ambala	India
5	Microscope cover glass	Supertek	India
6	Filter paper	United	India
7	Cylinders	Marienfeld	Germany
8	Pyrex conical flask	Marienfeld	Germany
9	Petri dish	PlastLab	Lebanon
10	Syringe 5 ml	MEDI	China
11	Cotton	Alsalama	Iraq
12	Mask	medical	Turkey
13	Rack	Rack	Lebanon
14	Disposable loop	Ambala	India
15	Can tube	Marienfeld	Germany
16	Yellow and blue tips	PlastLab	Lebanon

### 3.1.2. Chemical and biological materials

Different types of chemical and biological materials were used in current study (table 3-3).

**Table (3-3): Chemical and biological materials used in the study.**

No.	Chemical and biological material	Company	Origin
1	Ethanol (70 )	Joudtol	Iraq
2	Gram stain	JouriLabs	Sorachin SwitzerLand
3	H <sub>2</sub> O <sub>2</sub>	Hydrox	USA

### 3.1.3 Culture Media

All of cultured media that already prepared we purchased as mention in table (3-4).

**Table (3-4): Agars culture media used in the laboratory experiments**

No.	Media	Manufacturing company	Origin
1	Brain heart infusion Agar	Himedia	India
2	Blood Base Agar	Neogen	USA
3	MacConkey Agar	Neogen	USA
4	Sabouraud dextrose agar	Panreac	Spain
5	Potato dextrose agar	Panreac	Spain

## 3.2. Methods

### 3.2.1. Agars Culture Media used in the Study :

#### 3.2.1.1 Blood Agar

was prepared about 40gm of the medium is added to 1 Liter of distilled water. Sterilization of culture media and solutions are achieved by autoclaving at 121 C /1 Pascal for 15 minutes. Then the medium cooled at 40 – 45 C , To this add 5% sterile defibrinated blood and mix well. The medium is then poured into sterile petri plate. This medium used to growth the bacteria and differentiation between type of bacteria according to type of hemolysis (**Niederstebruch, et al.,** 2017).

#### 3.2.1.2 MacConkey Agar

Prepared 53.49 grams of medium in 1000 ml distilled water Heat to boiling to dissolve the medium completely Sterilize by autoclaving at 121°C for 15 minutes. Cool to 45-50°C. Mix well before pouring into sterile Petri plates. MacConkey agar is used for the isolation of gram-negative enteric bacteria. It is used in the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae and the genus *Pseudomonas* (**Karunakaran, et al.,** 2011).

#### 3.2.1.3 Brain Heart Infusion Agar

Brain Heart Infusion Agar is a solid medium recommended for the cultivation of fastidious pathogenic bacteria, yeasts and molds. Suspend 57 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at pressure (121°C) for 15 minutes

Mix well before pouring. This medium used to cultivated bacteria to get heavy growth (**Martin**, 2004).

#### **3.2.1.4 Sabouraud Dextrose Agar:**

Medium was prepared according to the manufacturer's instructions, by dissolving 65 gm from Sabourauds Dextrose Agar in 1000 ml. of distilled water , sterilized by autoclave a temperature of 120 °c for 20 minutes and then cooled to 45°c ,after that adding chloramphenicol in concentration of 250 mg /l used the agar for the purpose of isolating and diagnosing fungus (**Al-Ebady and Al-Mohana**. 2010)

#### **3.2.1.5 Potato dextrose agar medium (PDA)**

PDA used for the purpose of isolating and diagnosing fungi. Medium was prepared according to the producer's instructions, by dissolving 39 gm of the agar in 1000 ml of distilled water , sterilized by autoclave a temperature of 121°c for 20 minutes and then cooled to 45°c , after that adding chloramphenicol in concentration of 250 mg /l (**Burtelow, Merker and Baron**. 2009)

### **3.3 Isolation and Identification of bacterial Isolates:**

A total of 40 blood samples were collected involved in a case control study. They were divided into 4 main groups including 10 patients with full thickness burns , 10 with superficial partial thickness , 10 with superficial and 10 controls. Age range from 22 - 45 years (18 males and 22 females). Subjects of this study were enrolled during admitted as inpatients in the Center of burns in the AI-Hussein Medical City Hospital in Karbala province from April to August 2021.

### 3.3.1. Blood culture:

Blood Culture was done in triplicates and as following. About 0.5ml of blood were inoculated in 10ml Brain heart infusion broth tubes then incubated at 37°C for two weeks or until growth appear. Thereafter, 0.5ml of culture broth was spread on blood agar media and incubated at 37°C for week. Cultured on Successive cultures were sub Sabouraud dextrose agars purification.

#### 3.3.1.1 Diagnosis of isolated bacteria and fungi:

##### 3.3.1.1.1 Primary diagnosis of isolated bacteria

Primary diagnosis of isolated bacteria was mainly depending on the culture characters such as colony morphology, color, and blood hemolysis. Type of Gram stain was also performed after staining with Gram stain to differentiate between Gram positive bacteria (taken violet color from crystal violet stain) and Gram negative bacteria (taken a pink color from Safranin stain), also this Gram stain useful to determine the shape, size and arrangement of bacteria cells (**Habib, et al., 2015**)

##### 3.3.1.1.2. Biochemical test:

**Catalase test:** The catalase test is a biochemical test for aerobic organisms used to detect production of catalase enzyme that act in breakdown hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to O<sub>2</sub> and H<sub>2</sub>O. A small amount of isolated bacteria was taken by a wood stick and put on a microscope slide, and then a drop of 3% H<sub>2</sub>O<sub>2</sub> was added on the bacterial elements. Bubble observation meaning a positive results (**Reiner, 2010**).

**Coagulase test:** The coagulase test is mainly used to differentiate *Staphylococcus aureus* from *Staphylococcus epidermidis* and other coagulase – negative species. The coagulase test can be performed using two different procedures: slide and tube tests. The slide test is simple and gives results within 10 seconds, but it can give false negative. The tube test is the definitive test and need up to 24 hours to complete. Several colonies from culture of *Staphylococcus spp.* was mixed with 0.5 ml of human plasma in sterile tube. Tube was incubated at 35-37 °C in ambient air for 4 hours. The visible clot will indicate the positive result, while negative result indicated by non-clotting appearance. If the result is negative after 4 hours, the tube must be incubated again at room temperature overnight and check it for clot formation (**Katz, 2010**).

**Oxidase test:** The oxidase test was used for identifying Gram negative bacteria included *Pseudomonas spp.* that has the ability to produce cytochrome oxidase enzyme. A small amount of 24 hours growth of isolated bacteria was picked and streak on a small piece of filter paper, then 1 or 2 drops of 1% oxidase reagent was added on the organism smear to observe color changes. Positive result was indicated by color changes to dark purple within 5 to 10 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes (**Shields and Cathcart, 2010**).

#### **3.3.1.1.3 Confirmatory identification:**

Complete diagnosis of isolated bacteria was performed using VITEK2 compact system. The instrument is an automated microbial identification system depending on many biochemical tests using a special Card.

### 3.4 Isolation and Identification of Fungal Isolates

The specimens were transported by screw-capped cups to the fungi and mycotoxin laboratory in Babylon university and each specimen was inoculated using direct method of inoculation by streaking on two general media namely Sabouraud dextrose agar and Potato dextrose agar, then incubated at 25 °C for 2-7 days (**Forbes et al.**, 2007).

### 3.5 Percentage distribution of fungal infections according to age groups

Percentage distribution of fungal was calculated according to the age group (Pitt *et al.*, 2009). by:

$$\text{Percentage distribution} = \frac{\text{Number of each fungal species in one age group}}{\text{Total number of this fungal species in all age group}} * 100\%$$

*Chapter Four*  
*Results and*  
*Discussion*

## 4. Results and Discussion

### 4.1. Isolate and diagnose the burn patients depending of age and gender

A total of (30) Burn patients' blood and (10) unburun individuals was included in this study. It was that (20%) cases was noticed among age (20 – 24), (22.5%) cases among age(25 – 34), also (15%) found in the age (35-39).( 17.5% , 2.5%) it founded in (40 – 49) years. Regarding to the age and gender in the patients groups the females more than male the results was shown as in the table (4-1).

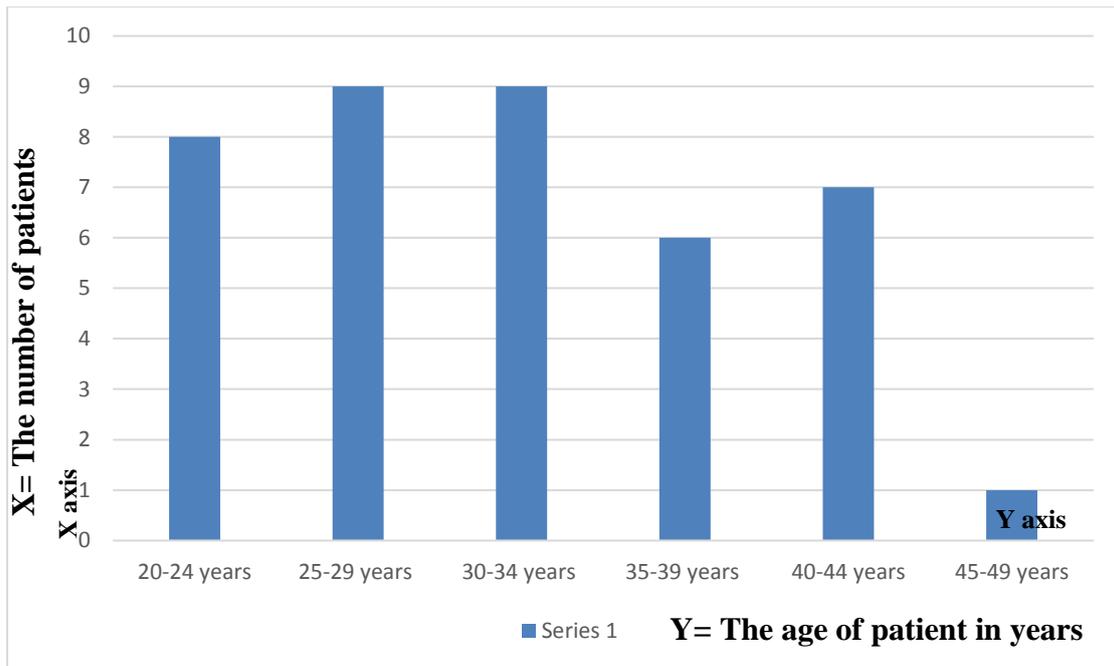
The age is considered the one of the risk factor especially among the youngest group. In this study the mortality was higher in the age groups 15-25 years. A study from Iran by **Hosseini et al.** reports the highest frequency of burns (53.5%) occurs to the age groups 16-25-year-old. An Indian proposes the highest incidence of the burns was 47.01% in the 21-30-year age group. This could be due to different economic and psychological situations in different countries, especially among countries with instability due to wars. Moreover, the mortality was higher among housewives, because of household equipment and standing ( **Hosseini et al.**, 2007). An Indian Lal. (2012), female burns patients are housewives and this is because of poverty and customs in which countries force women to work at home ( **Lal et al.**, 2012).

However, there are many studies that not agree with current study. The burn center in Surabaya admitted mostly patients aged 25–65 years, in India 47.5% of patients were young adults, and in USA 42% patients were aged 21–49 years (**Wardhana et al.**, 2017).

**Table (4-1) Distribution of study population according to age and gender**

Age Groups	Burn samples		control	
	Male	Female	Male	Female
20-24	3	3	1	1
25-29	4	3	1	1
30-34	4	3	1	1
35-39	1	3	1	1
40-44	2	3	0	1
45-49	0	1	0	1
Total	30		10	

In the table (4-1) the result showed (60%) cases were noticed among females and (40%) cases among males.



**Figure (4- 1 ) The age distribution in burn victims in this study show that the age between (25 – 34) is more associated with the burns when compared with the other age of the patients.**

These results demonstrated that the flame were responsible for (36%) of burn injuries that occurs among females than males (24%). The results of this study agree with (**Rajupt et al., 2008**) who observed that the domestic burn injuries caused by flame were common among females (60%) more than males (40%). This result may be due to that the women do all cooking for the family and have higher incidence of domestic burns as mentioned (**Nega and Lindtjorn, 2002**). In addition, the pattern of clothing of the females when they are doing the domestic work made the female more exposed to burn (**Ahuja and Bhattacharya, 2004**).

Additionally, flames are considered as the major cause of injuries than scald. This may be attributed to the accidents of home generators, kerosene stoves, lanterns and portable kerosene heaters. (**Alghalibi et al., 2011**) found that burns by flame are most common (69.5%) in severely

burned patients, while (Attia *et al.*, 1997) found that flame injury represented the most common cause of burn injuries (66.8%) and tended to affect females more than males. Moreover, they clarified that kerosene stoves were the most common source of flame injury probably due to more use of kerosene stove and kerosene lamp by the people of low socioeconomic status especially in rural area.

De Macedo *et al.* (2003) who stated that the flame burn was the predominant cause of burn amongst patients who had sepsis due to the fact that this agent produces deeper and more extensive lesions than other agents leading to more colonization of burn wound and then to sepsis and death.

#### 4.2. Distribution Samples according to the type of burns

In the table (4.2) show the classification of the patients into three groups according to the degree of burns, there was 10 at percentage 33.3% of patients with superficial. According to superficial partial thickness and full thickness there was 10 patients at percentage 33.3% of each types of burns.

burns are confined to the outer portion of the dermal layer and cause moderate edema but are unlikely to cause scarring. Deep-partial-thickness burns extend into the reticular layer of the dermis and heal slowly, are likely to scar, and may require skin grafts. Finally, full-thickness burns extend through the full depth of the dermal layer into the subcutaneous tissue and require extensive medical treatment (Merz *et al* 2010)

First responders or treating physicians will often describe burns by degree and surface area, but it should be recognized that these are visual categorizations (Abraham *et al.*, 2018).

**Table (4.2 ) Distribution Samples according to the type of burns**

Types of burn	Frequency	Percent %
Superficial burn	10	33.3
Partial burn	10	33.3
Thickness burn	10	33.3
Total	30	100

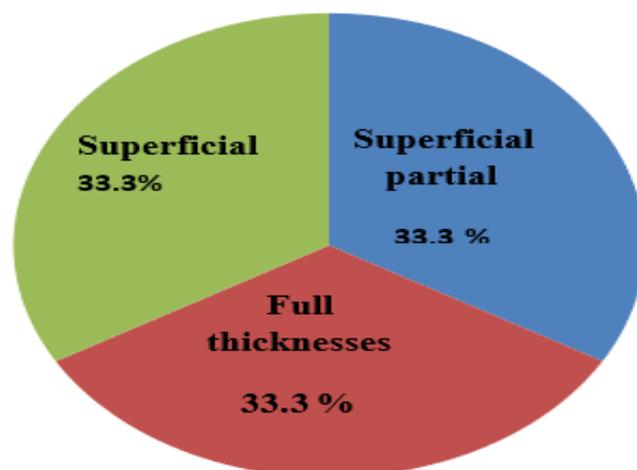
**distirdution of the burns types**

Figure (4-2) :Show the distribution of types of burn in the patients groups , 33.3% is a full thicknesses burns , 33.3% in superficial and 33.3% in superficial partial group.

#### **4.3.Qualitative assessment of bacterial and fungal growth in the culture:**

The results of the blood culture table (4.3), results showed that positive bacterial cultures were found in 10 (33.3%) and of burned patients.

This result is higher than that obtained by (De Macedo *et al.*, 2003) in Brazilia who showed that (19.4%) of burned patients developed proven sepsis. In this study, the high rate of positive blood culture may be attributed to the high level of nosocomial infections acquired from medical devices in burn wards, the crowding of wards with burned patients and the unavailability of recent techniques in the sterilization of burn wards.

The burn samples showed positive cultures in 14 (46.6%) the other 26 (53.3%) were negative bacterial cultures table (4.3). These findings reflect the higher percentage of bacterial contamination of the burn unit which explains the higher percentages of positivity of blood cultures found in this study.

**Table(4.3) Qualitative assessment of bacterial and fungal growth in the culture**

Sample	Positive culture ( %)	Negative culture (%)
Control	0 (0%)	10 (100%)
Burn	14 (46.6%)	16 (53.3%)

#### **4.4 Types of bacteria, number of sample and percentage (%) of bacteria isolates.**

In table (4-4) the results showed that bacteria was found among the patients groups, the percentage of bacteria in blood and burn cultures

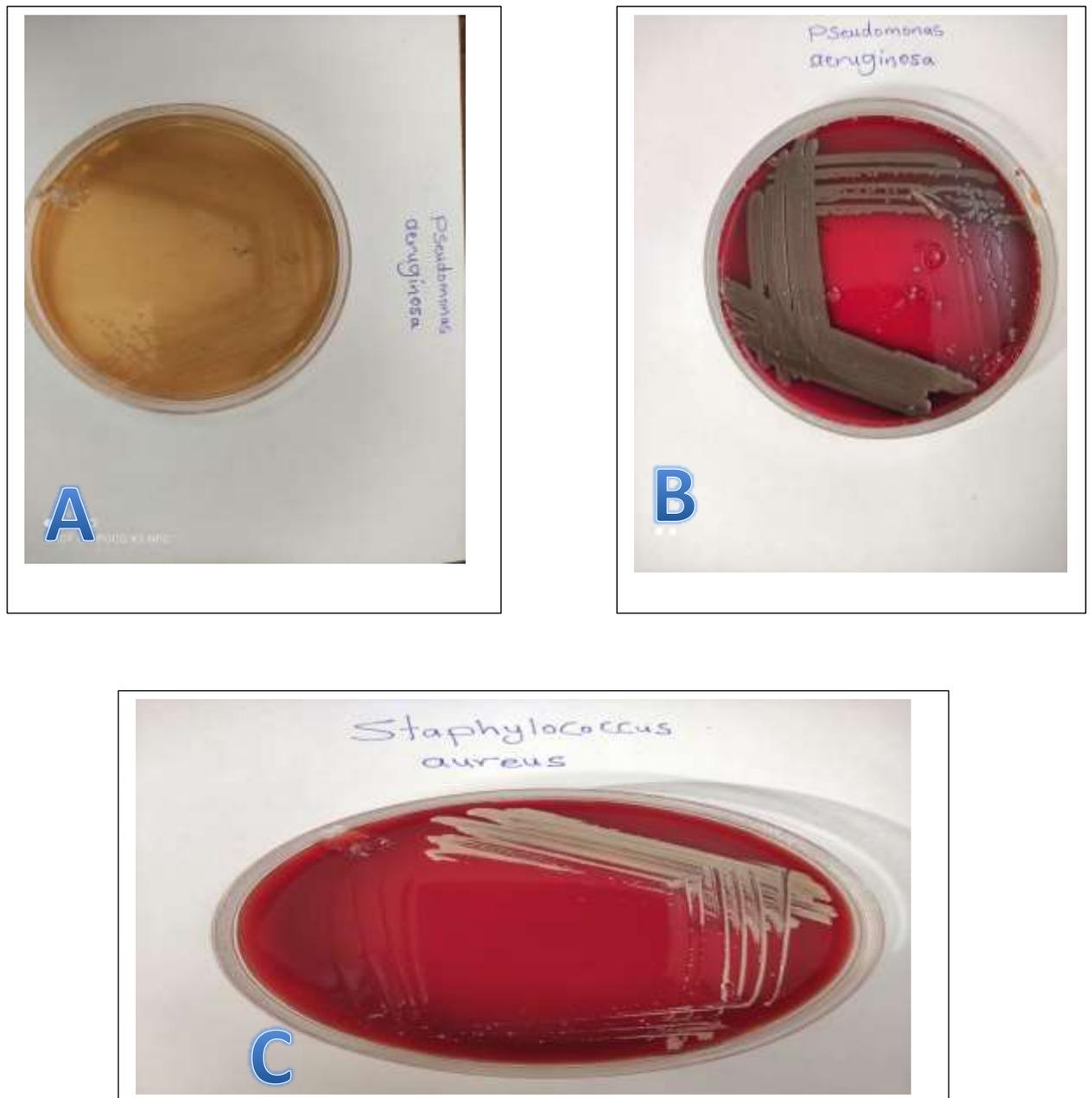
respectively *P.aeruginosa* at percentage 23.3% ,while *S.aureus* 16.6% and *C.albicans* at percentage 6.6%.

It is clear from the total number of isolates that gram negative bacteria are more frequent than gram positive type. This agrees with **Anuradha et al.**, (2008) who found that gram negative bacteria represent about (80.7%) of micro-organisms that cause burn wound infection and that this type of bacteria has assumed a primary lethal role among the cases of burn wound infection and septicemia (**Anuradha, et al.**, 2008)

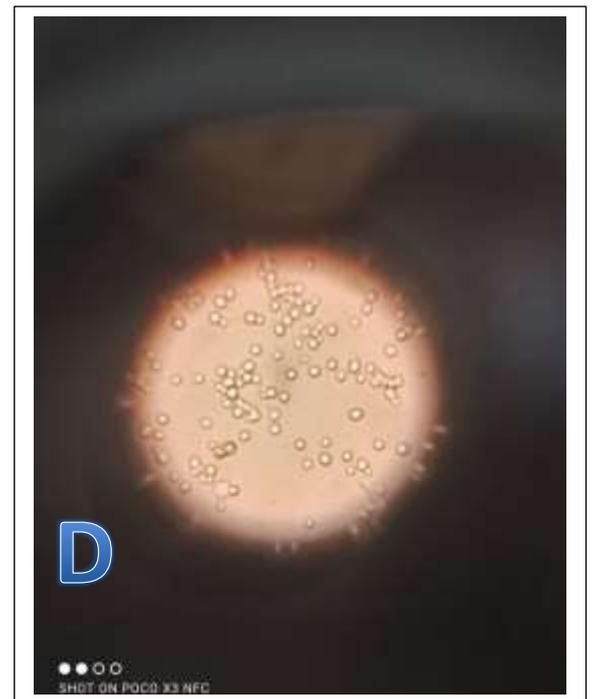
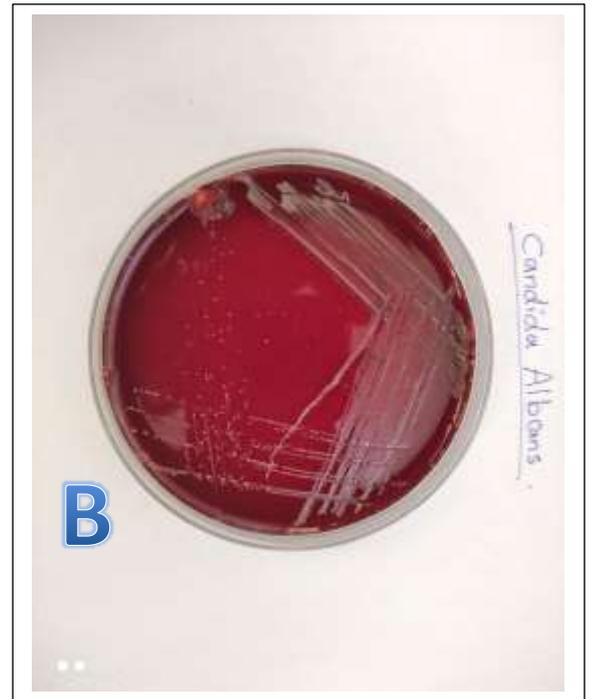
The predominance of gram negative bacteria is clear from the high frequency of *P.aeruginosa* in each source of the cultures. This agrees with (**Alireza and Enayat**, 2007) who found the most predominant bacterial isolate from burn patients was *P. aeruginosa* followed by *S. aureus*, these results are also in accordance with other studies (**Agnihotri,et al.**, 2004). The reasons for this high prevalence may be due to factors associated with the acquisition of nosocomial pathogens in patients with recurrent long term hospitalization complicating illnesses, prior administration of antimicrobial agents and the immunosuppressive effects of burn trauma (**Yotis**, 2005).

In this study the most gram positive bacteria isolated from the blood are *S. aureus*. The percentage as in table (4-4). These results were approximately near with that of (**De Macedo et al.**, 2003) who showed that the most common bacteria isolated from blood culture were *S.aureus* should be considered as an important pathogen for sepsis in burns. The organism, being ubiquitous in a hospital environment, and burn wounds being the ideal medium for its multiplication, it is hardly surprising that these bacteria would be the cause of 20.7% of septic episodes (**De Macedo et al.**, 2003).

Regarding to the *Candida* species occupied the bottom of the list of isolates in our series, as they were recovered at a frequency of 3.6%. In other reports, they accounted for 9.7% of all organisms grown. *Candida* species are harmless saprophytes as long as they colonize the burn wound, but as invaders of viable subeschar tissue or the blood stream, they are dangerous pathogens with a mortality rate exceeding 90% .Fortunately, Candidal invasion of viable tissue is a relatively rare event (**Nasser. *et al.*, 2003**).



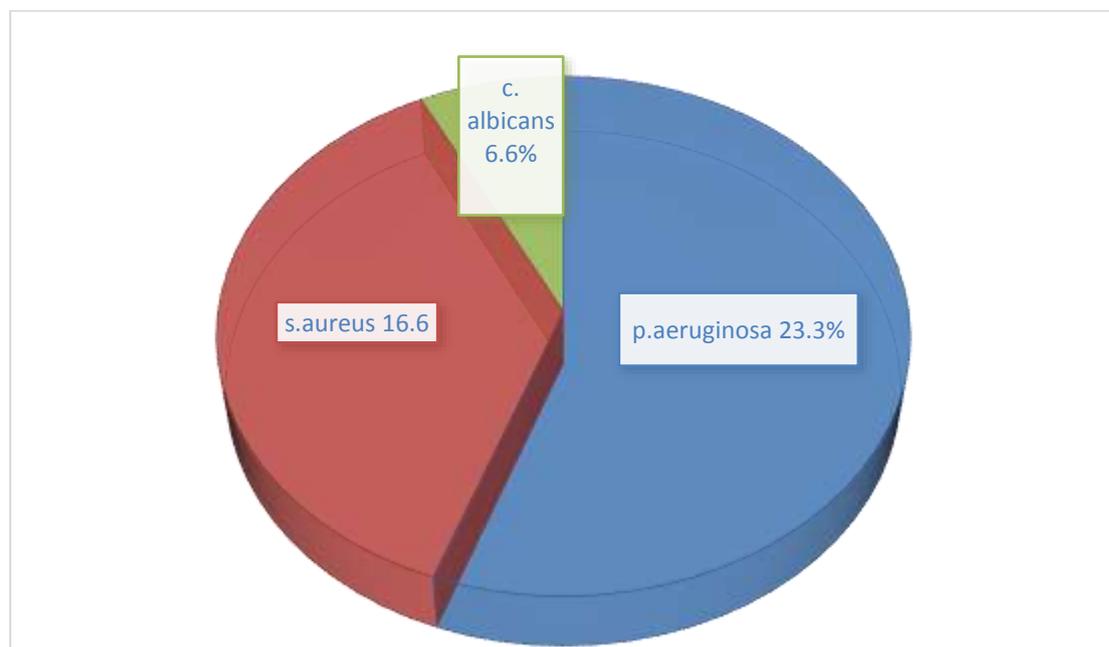
**Figure (4-3) : Characters *P. aeruginosa* and *S. aureus*; (A) *P. aeruginosa* on MacConkey agar with non-lactose, (B) *P. aeruginosa* on blood agar demonstrated hemolysis, (C) *S. aureus* on blood agar demonstrated hemolysis.**



**Figure (4-2):** Showed characteristic of *Candida albicans*; (A and C) *Candida albicans* on Sabouraud dextrose agar, (B) *C. albicans* on blood agar, (D) *Candida albicans* under the light microscopy.

**Table (4-4): The bacterial and fungal isolates from blood burns patients.**

Samples	-ve culture NO (%)	+ve culture No (%)		
		<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>Candida albicans</i>
Control	10 (100%)	0	0	0
Burn	16 (53%)	5 (16.6%)	7(23.3%)	2(6.6%)



**Figure (4-5): show the bacterial and fungal isolates from blood burns patients**

The predominant bacteria in the burn was *P. aeruginosa* at percentage **23.3%**, followed by *S. aureus* at percentage **16.6%**, while the *Candida albicans* at percentage **6.6%**.

*Pseudomonas aeruginosa* appeared gram-negative rods under microscope, also showed oxidase and catalase positive result, after that confirmed by VITEK 2 compact as a *P. aeruginosa*. Previous studies reported the gold standard is VITEKVR –MS for the quick and reliable identification of *Pseudomonas*-associated nosocomial infections in critically sick patients admitted to the intensive care unit (**Moehario et al., 2021**). Moreover, microscopic inspection, biochemical testing, and the VITEK-2 compact system were used to identify *P. aeruginosa* bacteria, from a variety of sources in Baghdad hospitals (**Al-fridawy et al., 2020**)

*S. aureus* colonies that were showed; large, round, opaque, and golden yellow in color. Furthermore, showed  $\beta$ - hemolysis zone on blood agar, and the ability to fermentation of mannitol on mannitol salt agar.

Furthermore the current isolation method looks similar implicated swabs from patients and healthcare workers in a tertiary medical center's burn unit in Ghana were streaked on 5% blood agar (BA), and incubated overnight at 37 °C (**Amissah et al., 2017**)

After cultured *S. aureus* isolates subjected to gram's staining, catalase, oxidase, and further slide coagulase test. Totally isolates were classified as gram-positive, Small spherical, cluster like grapes, oxidase negative, and catalase-positive, also isolates showed positive results with a slide coagulase test as an identification diagnosis and confirmed with VITEK 2 compact. A Coagulase test was used to confirm the diagnosis of *S. aureus* by (**MacFaddin, 2000; Mayar Hezam, 2019**).

A prior research used the VITEK-2 small GP colorimetric identification card (BioMérieux, France) was used to identify *S. aureus* isolates, from burn victims in a regional burn hospital of Southeastern China (Chen *et al.*, 2018).

Burn wounds are particularly vulnerable to fungal colonization and infection. The growth of opportunistic fungi like *Candida albicans* is aided by a large wound area, reduced local immunity, and broad-spectrum antibiotic treatment, which can lead to candidiasis invasive (von Muller , 2020).

Isolated cultured was carried out aerobically at 28°C for 2 to 5 days on Sabouraud dextrose agar, with cycloheximide (specific for *C. albicans*). *Candida albicans* colonies were whitish, shiny and convex, are 4 to 5mm in diameter after incubation for 3 days. Several previous studies have documented that on Sabouraud dextrose agar at 25°C, colony shape of *C. albicans* might range from white to creamy, soft, and wrinkled. Dry, wrinkled variants are possible (Chow *et al.*, 2008; Milazzo *et al.*, 2014; Koundal and Cojandaraj, 2020) Figure (4-2) showed culture characteristic of *C. albicans* on Sabouraud dextrose agar.

Staining for *Candida albicans* was conducted by lactophenol cotton blue with growth colonies after 3 days. Under microscope appeared ovoid to subspherical budding yeast cells, also *C. albicans* produces tubular outgrowths (germ tubes) as opposed to elongation of a budding yeast (pseudohyphae), in addition to that more confirmed identification was carried on VITEK 2 compact system.

*Conclusion  
and  
recommendation*

## Conclusion and Recommendation

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### Conclusions:

1. Burn injury is more frequent in female than male patients. Generally, burn injury because of flaming is more frequent than scald injury.
2. Gram negative bacteria are more predominant as causative agents for burn infections than the Gram positive type, and it was seen that *P. aeruginosa* was found predominant among Gram negative bacteria whereas, *S. aureus* among Gram positive bacteria.

### **Recommendations:**

1. More attention should be directed to the burn unit as an etiology for burn wound infections.
2. Further investigation on the other virulence factors associated with burn wound infection such as quorum sensing and phospholipase C.
3. Molecular techniques should be used to identify the microorganism associated with burn wound infection like bacteria and other causative organisms such as fungi and viruses.

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الحروق هي المشكلة الرئيسية في العديد من مناطق العالم. الحروق تدمر الجلد الذي يمنع عادة الغزو من قبل الكائنات الحية الدقيقة. وهناك حالات كثيرة تتطلب إجراء تحقيق طبي في كل من الحالات الحية والحالات التي ماتت نتيجة لصدمة الحروق.

تم تضمين اربعين عينة دم في هذه الدراسة، تم جمع ثلاثين عينة من مرضى الحروق والذين قسموا الى ثلاث مجاميع اعتمادا على درجة الحرق وكذلك تم جمع عشر عينات من أفراد غير المحترقين في أربعة أشهر من نيسان إلى اب 2021. أجريت هذه الدراسة في مركز الحروق في مستشفى مدينة الحسين الطبية مركز الحروق في كربلاء.

وقد أظهرت النتائج أن العمر والجنس في مجموعات هي عوامل خطورة حيث ان النساء وخاصة بين الفئات العمرية 25-34 سنة هي اكثر نسبة عرضة للحروق من الفئات العمرية الاخرى . في حين أظهرت أن (60%) من إصابات الحروق التي تحدث بين الإناث بينما نسبة الذكور (40%) (16 أنثى و 14 من الذكور).

وكما أظهرت 14 عينات الدم نتيجة موجبة وبنسبة (46%) ، حيث قسمت الى زراعات البكتيرية الإيجابية بأكثر من نمو واحد في 12 اشخاص وبنسبة (40%) من المرضى المحترقين و 2 بنسبة (6.6%). منهم من نمو واحد، و 28 الأخرى (53%) كانت الزراعات البكتيرية السلبية.

وبالإضافة على ذلك فإن غلبة البكتيريا هي *Pseudomonas. aeruginosa* في النسبة المئوية (17.5%)، في حين أن *Staphylococcus. aureus* ظهرت بنسبة 12.5%. من ناحية أخرى أظهرت الفطريات

*Candida. albicans* ونسبة (6.6%) في مرضى الحروق.



جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل/ كلية العلوم

قسم علوم الحياة

# دراسة مايكروبية لعينات دم ضحايا الحروق كدليل جنائي

بحث مقدم الى

مجلس كلية العلوم – جامعة بابل

كجزء من متطلبات نيل درجة الدبلوم العالي في العلوم/ أدلة جنائية

من قبل

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