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Microbial Profile in Burn Patients as Forensic Evidence

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[سورة طه, آية: 114]

صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

Certification

I certify this research entitled " **Microbial Profile in Burn Patients as Forensic Evidence** " was carried under our supervision at the College of Science, University of Babylon, as a partial my fulfillment of the requirements for the Degree of higher diploma in forensic evidences.

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Dedication

I dedicate this effort to …

The soul of my mother and father who have filled me with their blessings …

To my husband and children …

To everyone who contributed to my endeavor…

Eman Mahdi Shakir

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Summary

Burns are one of the most frequently seen injuries in hospitals and medical clinics, and they occur as a result of skin exposure to high heat for many reasons. The study included isolating skin microbes by taking a skin swab of the burned places of the skin for 59 of the burned people, ages 1-60 years, from Central Laboratory / Najaf for the period from 1/4/2021 to 31/7/2021 and on differential diagnostic media, and then they were diagnosed by biochemical methods and using several tests for diagnosis and confirmed by VITIK 2 compact system to identify the bacterial and fungal types that colonize burn wounds.

All patients were suffering from the second and third degrees of burns, and most of them were female, at a rate greater than male. As for the age group, the ages were 21-30 years, which is the highest percentage of burns under study compared to the rest of the age groups.

Forty bacterial isolates were diagnosed, including 17 isolates of *Pseudomona aeruginosa*, 12 isolates of *Acinetobacter baumannii*, 8 isolates of *Staplylococcus aureus*, and 3 isolates of *Klebsiella pneumonia*. Also, 24 isolates of two types of fungi were diagnosed: *Aspergillus nigar* (16 isolates) and *Candida albicans* (8 isolates).

List of Contents

Title		Page No.
Summary		
List of Contents		**I**
List of Tables		**V**
List of Figures		**VII**
List of Abbreviations		**VIII**
Series	**Subject**	**Page No.**
1.	**Introduction**	**1**
2.	**Literature Review**	**3**
2.1.	Microbial Forensics	3
2.2.	Biological and non- biological agents in microbial forensic	3
2.3.	Microbes in human body	4
2.3.1.	Microbes in skin	4
2.3.2.	Factors influence on human microbe	5
2.3.3.	Bacteria isolated from wound skin burns	5
2.3.3.1.	*Staphylococcus aureus* from patients with burns	6
2.3.3.2.	*Pseudomonas aeruginosa* with burns	7
2.3.4.	Fungi isolated from wound skin burns	7
2.3.5.	The skin, components and function	8
2.3.6.	Human skin micro biomes and their potential forensic application.	8
2.4.	Aid of microbial analysis in differentiation between humans	9
2.5.	Nosocomial infections associated with burns	10

List of contents

2.6.	Elements to Consider in Forensic Micro biome Analyses	11
2.6.1.	Transfer and Persistence of Micro biomes	11
2.6.2.	Collection and Storage of Samples	11
2.6.3.	Future Research and Recommendations, as well as Training and Interpretation	12
2.7.	Degree of burns	14
3.	**Materials and Methods**	**15**
3.1.	Materials	15
3.1.1.	Instruments	15
3.1.2.	Equipment	15
3.1.3.	The Biological and chemical substances	16
3.1.3.	The medium of culture	17
3.2.	The Methods	18
3.2.1.	Culture media preparation	18
3.2.1.1.	Transport Medium	18
3.2.1.2.	Blood agar (BA)	18
3.2.1.3.	MacConkey's Agar (M.A)	18
3.2.1.4.	Mannitol salt agar (MSA)	19
3.2.1.5.	Nutrient agar (N.A)	19
3.2.1.6.	Sabouraud's Dextrose Agar media	19
3.2.2.	Solutions and Reagents Preparation	19
3.2.2.1.	Preparation of Gram stain reagent	19
3.2.2.2.	The reagent Catalase	19
3.2.2.3.	The Oxidase reagent	20
3.2.2.4.	Urea solution	20

3.2.2.5.	Lactophenol cotton blue	20
3.3.	VITEK 2 COMPACT System	20
3.4.	Specimen collection and study design	20
3.4.1.	Specimen collection	20
3.4.2.	Experimental Design	21
3.4.3.	Microorganisms Isolation and diagnosis	21
3.4.4.	Isolation and Identification of Bacterial Isolates	21
3.4.5.	Isolation and Identification of Fungal Isolates	22
3.4.6.	Diagnosis of microbial under the microscope	22
3.4.6.1.	Diagnosis of bacteria under the microscope	22
3.4.6.2.	Diagnosis of fungi under the microscope	22
3.4.7.	Tests for identification	22
3.4.7.1.	Test for catalase	22
3.4.7.2.	Oxidase test	23
3.4.7.3.	Slide Coagulase test	23
3.4.7.4.	Urease test	23
3.5.	Isolation and Identification of Fungal Isolates	23
3.6.	Percentage distribution of fungal infections according to age groups	24
4.	**Results and discussion**	25
4.1.	Patients profile of peoples with burns injury	25
4.2.	Distribution of burns infections according to age groups	25
4.3	Gender disruption of burns patients	26
4.4	Age of gender disruption of burns patients	27

4.5	Degree of burns among patient states	28
4.6.	Isolation and identification of bacteria	29
4.6.1.	Cultural characteristics of *pseudomonas aeruginosa*	29
4.6.2.	Biochemical and VITEK 2 compact identification	30
4.6.3.	Cultural characteristics of *Acinetobacter bumannii*	30
4.6.4.	Biochemical and VITEK 2 compact identification of *Acinetobacter bumannii*	31
4.6.5.	Cultural characteristics of *Staplylococcus aureus*	32
4.6.6.	Biochemical, serological, and VITEK 2 compact identification of *Staplylococcus aureus*	33
4.6.7.	Cultural characteristics of *Klebsiella pneumonia*	34
4.6.8.	Biochemical and VITEK 2 compact identification of *Klebsiella pneumonia*	35
4.7.	Isolation and identification of fungi	35
4.7.1.	Cultural characteristics of *Candida albicans*	35
4.7.2.	Microscopic examination, and VITEK 2 compact identification for *Candida albicans*	36
4.7.3.	Cultural characteristics of *Aspergillus niger*	37
4.7.4.	Microscopic examination, and VITEK 2 compact identification for *Aspergillus niger*	37
4.8.	Prevalence of microbial infection in burns patients.	38
4.8.1.	Prevalence of bacterial infection in burns patients	38
4.8.2.	Prevalence of fungal infection in burns patients	39
4.9.	Distribution of bacterial infections according to age groups	41
4.10.	Distribution of fungal infections according to age groups	42

4.11.	Degree of burns, bacteria types among patient states	43
	Conclusions	45
	Recommendations	46
	References	47

List of Tables

Series	Title	Page No.
3-1	Instruments in this study	15
3-2	Instruments in this study	16
3-3	Chemical and biological materials	17
3-4	The culture media utilized in the research	18
4-1	Disruption of patients according to age and gender	27
4-2	Distribution of the burn patients according to degree of burn	28
4-3	Types of bacteria, number of sample and percentage (%) of bacteria isolates	39
4-4	Prevalence of fungal infection in skin burns patients	40
4-5	Percentage distribution of bacterial infections according to age groups	41
4-6	Percentage distribution of Fungal infections according to age groups	42
4-7	Degree of burns, bacteria types among patient states	43

List of Figures

Series	Title	Page No.
3-1	The study design for microbiological and biochemical identification of patients samples with burn	21
4-1	Range of ages of patients with burns	25
4-2	Percentage of the burn patients according gender	29
4-3	*Pseudomonas aeruginosa*; (A) *P. aeruginosa* on MacConkey agar with non–lactose, (B) *P. aeruginosa* on blood agar demonstrated hemolysis, (C) *P. aeruginosa* on nutrient agar with yellow-green pigment	30
4-4	(A) Oxidase test, (B) Catalase test	30
4-5	(A) *A. baumannii* on MacConky agar without fermented to lactose *A. baumannii* on blood ager without produce hemolysis	31
4-6	(A) *S. aureus* colonies on mannitol salt agar with the ability to fermentation of mannitol, (B) *S. aureus* colonies showed β- hemolysis zone on blood agar	33
4-7	(A) Positive result with coagulase test, (B) Negative coagulase reaction	34
4-8	*Klebsiella pneumonia* growth on MacConky agar after overnight	34
4-9	Morphology characteristic of *Candida albicans* on Sabouraud dextrose agar after 3 days	36
4-10	Microscopic of *Candida albicans*	36
4-11	A- Culture characteristic B- Microscopic morphology of *Aspergillus niger*	37
4-12	Bacterial isolates from skin burns patients	39
4-13	Fungal isolates from skin burns patients	41
4-14	Degree of burns, bacteria types among patient states	44

List of Abbreviations

Abbreviations	Meaning
A. baumannii	*Acinetobacter baumannii*
A. nigar	*Aspergillus niger*
BA	Blood agar base
BWEs	burn wound exudates
C. albicans	*Candida albicans*
CSI	Crime Scene Investigation
DAMPs	Damage – associated molecular patterns
DPT	Deep partial
E. coli	*Escherichia coli*
EDTA	Ethylene diamine traacetic acid
FST	FST
FT	full-thickness
G+ve	Gram positive
GI	gastrointestinal
GN	Gram negative
GP	Gram positive
G-ve	Gram negative
H_2O_2	hydrogen peroxide
ICUs	Intensive Care Units of burns
IFI	invasive fungal infection
K. pneumoniae	*Klebsiella pneumonia*
M.A	MacConkey's Agar
MRSA	methicillin-resistant *S. aureus*
MSA	Mannitol salt agar
N.A	Nutrient agar
NaCl	Sodium chloride
NHSN	National Healthcare Safety Network
NI	Nosocomial infections
P. aeruginosa	*Pseudomonas aeruginosa*
PVL	Panton-Valentine Leukocidin
S aureus	*Staphylococcus aureus*
SSSS	staphylococcal scalded skin syndrome
SSTIs	skin and soft tissue infections
SVM	support vector machine
TBSA	total body surface area
YST	Yeast

Chapter One

Introduction

Introduction

Forensic evidence has long been regarded as some of the most powerful evidence allowed and assessed in courtrooms (Schweitzer and Nuñez, 2018; Pearson *et al.*, 2018). It's critical that the general public understands forensic evidence by contrasting guilt and sentencing choices in criminal cases involving forensic vs eyewitness testimony evidence and analyzing if a Crime Scene Investigation *(CSI)* effect exists (Ling *et al.*, 2021).

Microbial forensics has been used to aid in the investigation and attribution of suspected or real biocrimes, illegal bioproliferation, bioterrorism, and biowarfare for over 20 years. Following in the footsteps of the United States (Murch & Budowle, 2020).

Microbiome research is a field that spans several disciplines, may contribute as criminal evidence. In forensic medicine, the use of skin microbiome profiling for person identification is a potential (Tozzo *et al.*, 2020).

DNA extracted from the skin can be unique to its host, allowing forensic biological evidence to be uniquely identified, forensic statistical tool estimates (FST) through skin microbiota profiling, FST coupled with support vector machine (SVM) may significantly enhance forensic Human identification (Jin *et al.*, 2021).

Burns are under appreciated injuries with high morbidity and death rates. Burn injuries, especially severe burns, trigger an immunological and inflammatory response, metabolic abnormalities, and distributive shock, all of which can be difficult to treat and lead to multiple organ failure. The loss of the skin's natural protective barrier is the most serious consequence of this malfunction (Corcione *et al.*, 2020).

The fact that the injury impacts not just the patient's physical health but also his or her mental health and quality of life is crucial (Jeschke *et al.*, 2020).

Invasive infection in burn victims has been linked to a number of resistant pathogens. Including; *Staphylococcus aureus*, *Pseudomonas*, vancomycin-resistant *Enterococcus*, *Acinetobacter*, *Aspergillus*, *Candida spp.*, and other yeast *Candida spp.* (Maurel *et al.*, 2020). *Staphylococcus spp*. were the most often isolated species (Chen *et al.*, 2020). The most common bacteria are *Pseudomonas sp.*, which have different levels of resistance to most of the medications examined (Forson *et al.*, 2017).

Ninety percent of patients with burns were colonized by fungus, with the majority of instances resulting in invasive fungal infection (IFI). especially for people who have long stay in the intensive care unit and who suffer from diabetes (Van Bang *et al.*, 2020).

Finally, Microbial forensics is an interdisciplinary field that brings together scientists, public health officials, the intelligence community, law enforcement, and policymakers.

Together, they form an integrated system that helps safe guard us from disease epidemics that occur naturally as well as acts of biological terrorism and biocrime (Lee *et al.*, 2020)

Aim of study

Detecting the microorganisms associated with burns that cause Skin inflammation as forensic evidence and comparing the number of selected and control samples.

Objectives

1-Collect swab of skin from patients with burn .

2-Detect and isolate different types of microorganism (bacteria & fungi)

Chapter Two

Literature Review

2. Literature Review

2.1. Microbial Forensics

Microbial forensics is the science of using scientific methods to analyze evidence from a bioterrorism assault, a bio crime, a hoax, or an unintentional release of a biological weapon or toxin, with the ultimate objective of attribution (Budowle *et al.,* 2003). The goal of microbiological evidence attribution is to identify a related source, offender, or group of persons to the greatest extent feasible.

Microbiology, genetics, bioinformatics, forensic science, immunology, population genetics, biochemistry, molecular biology, epidemiology, and other disciplines, as well as law enforcement, public health, policy, and intelligence groups, make up the microbial forensics field (Schmedes & Budowle, 2019).

2.2. Biological and non- biological agents in microbial forensic

Microbial forensic studies focus on the identification and characterization of biological and non-biological evidence. Bacteria, viruses, protozoa, fungus, and poisons are examples of biological agents.

Non - biological evidence, such as; growth media, additives, delivery devices, and so on, can be valuable in microbial forensics, giving investigation leads and assisting in determining manufacturing and distribution techniques (Velsko, 2011).

Although non-biological evidence analysis is an important element of microbial forensics, but studies focus on biological analytical methodologies .

2.3. Microbes in human body

The erroneous notion that the human body harbors microorganisms that exceed our somatic and germ cells by a factor of ten has sparked a surge in interest in defining human micro biota that is, all the bacteria that live in the human body in health and illness (Marchesi and Ravel, 2015), to get a better understanding of the interplay between human and microbial components, mostly bacteria but also fungi, viruses (Turnbaugh *et al.*, 2007).

The fact that different persons have vastly different micro biotas underlines the need of forensic views in determining what causes and governs this diversity in order to effectively employ microorganisms as forensic evidence (Budowle *et al.*, 2003).

2.3.1. Microbes in skin

The skin, as the largest organ of the human body, is a complex living ecosystem that harbors diverse microbial communities at different sites with unique niches, characterized by dry, moist, and sebaceous skin (Fitz-Gibbon *et al.*, 2013; Bashan *et al.*, 2016).

The taxonomic depth of the study determines the degree of skin micro biome diversity. While the phylum of bacteria may be used to identify just a few unique taxa among different persons, when identification is done at the genus, species, and strain population levels, with very specific diversity for each person, the discriminatory power becomes equivalent to a fingerprint (Gao *et al.*, 2007; Metcalf *et al.*, 2017; Oliveira *et al.*, 2018).

2.3.2. Factors influence on human microbe

Many variables influence the makeup of human microbial communities, including the environment, illness development, presence, or absence, behaviors, relationships, diet, and overall health condition (Williams and Gibson, 2019).

Furthermore, although ambient bacteria can affect the micro biomes of individuals who spend time in them, humans shed bacteria from their body surfaces into the surrounding environment, altering its bacterial makeup (Prussin *et al.*, 2015)

Many investigations have shown that human microbial signatures may be retrieved in a variety of indoor and outdoor environments, including homes, offices, healthcare facilities, schools, dorm rooms, toilets, and subways (Luongo *et al.*, 2017; Walker *et al.*, 2018).

2.3.3. Bacteria isolated from skin burns

Thermal damage breaks the epidermal barrier that ordinarily inhibits microbial invasion, making the burn a vulnerable location for opportunistic colonization by organisms of both endogenous and foreign origin. As a result, the burn is the most common source of sepsis in these individuals (Mooney and Gamelli, 1989).

Although burn surfaces are initially sterile after thermal damage, these wounds gradually get colonized by bacteria within the first 48 hours, gram-positive bacteria that survive the thermal shock, such as *Staphylococcus aureus*, which is found deep within sweat ducts and hair follicles, extensively populate the burn surface (Monafo and Freedman, 1987). The most common isolates from burns were *Staphylococcus aureus*, *Enterococcus spp*, *Pseudomonas aeruginosa*, and *E. coli* (Kaftandjieva *et al.*, 2021).

2.3.3.1. *Staphylococcus aureus* from patients with burns

Staphylococcus aureus is a gram-positive coccus. They are often in clusters that colonizes the nasal mucosa and skin of healthy individuals (Wertheim *et al.*, 2005) This organism can cause a wide range of diseases from skin or soft tissue infections to systemic and fatal diseases (Lowy, 1998; Tong et al., 2015).

Staphylococcal scalded skin syndrome (SSSS) is an illness characterised by red blistering skin that looks like a burn or scald, hence its name staphylococcal scalded skin syndrome. SSSS is caused by the release of two exotoxins (epidermolytic toxins A and B) from toxigenic strains of the bacteria *Staphylococcus aureus* (Mishra et al., 2016).

In burn centers, *S aureus* is still the most common source of infection (Chen *et al.*, 2012). In burn centers, the development and spread of methicillin-resistant *S. aureus* (MRSA) leads to poor outcomes such as prolonged hospitalization, bacteremia or sepsis, and even mortality, this necessitates further efforts in prevention and treatment (Issler-Fisher *et al.*, 2015).

Aside from antibiotic resistance, the development of various virulence factors is another key component that contributes to treatment failure in burns patients (Chen *et al.*, 2018).

When compared to PVL-negative bacteria, these Panton-Valentine Leukocidin (PVL)-producing *S. aureus* strains are linked with enhanced mortality (Holmes *et al.*, 2005). In burns patients, skin and soft tissue infections (SSTIs) are still considered the major cause (Chen *et al.*, 2017; Cen *et al.*, 2015).

2.3.3.2. *Pseudomonas aeruginosa* with burns

P. aeruginosa is a heterotrophic, motile, Gram-negative rod-shaped bacterium about 1–5 μm long and 0.5–1.0 μm wide. It is a facultative aerobe that grows via aerobic respiration and anaerobic respiration with nitrate as the terminal electron acceptor. It is a major cause of illness and death in humans with immunosuppressive and chronic conditions, and infections in these patients are difficult to treat due to a number of antibiotic resistance mechanisms and the organism's propensity to form multicellular biofilms (Diggle& Whiteley, 2020).

Burn wound sepsis is the leading cause of morbidity and mortality following a burn injury. Infections with well-known bacteria like *Pseudomonas aeruginosa*, patient recovery is hampered, and it can even be deadly.

The only pathogen capable of growing within burn wound exudates (BWEs) was *P. aeruginosa*. When compared to the levels found in regular laboratory media, expression of usual virulence factors such as pyocyanin and pyoverdine was even increased. (Gonzalez *et al.*, 2016).

2.3.4. Fungi isolated from wound skin burns

The due of the development of topical antimicrobials and the widespread use of broad-spectrum antibiotics, fungus colonization has become a growing concern. This has resulted in an increase in invasive fungal infection, which has been associated to a greater death rate independent of the severity of the burn, the presence of a coincidental inhalation injury, or the patient's age.

The development of opportunistic fungal infections is aided by extensive burnt regions, a weakened immune system, and antibiotic therapy (Lazarescu *et al.*, 2020).*Candida spp*. are the most common fungi

that colonize burn lesions, Although fungus such as *Aspergillus spp., Rhizopus spp., Penicillium spp., Mucor spp., Fusarium spp., Curvularia spp.,* and *Rhizomucor spp.,* can cause disease, may also cause colonization, and they can have a high invasive potential as well as a high death rate (Gallagher *et al.*, 2012).

2.3.5. The skin, component and function

The epidermis, which is made up of keratinocytes, is split into numerous layers. The basement membrane lies beneath the epidermis (also known as the dermo - epidermal junction). The epidermis is linked to the dermis by this thin, multilayered structure. The hypodermis, the layer beneath the dermis, is mostly made up of fat (Schlüter *et al.*, 2014).

With a surface area of $2m^2$, the skin and its appendages (hair, nails, and some glands) are the biggest organ in the human body (Hughes, 2001). The thickness of the skin varies from 0.1mm at its thinnest point (eyelids) to 1.5mm at its thickest point (palms of the hands and soles of the feet), accounting for 15% of the total adult body weight (Kolarsick *et al.*, 2011).

The skin serves three primary purposes; Protection, thermoregulation, sensation. It conducts numerous crucial and vital physiological activities inside this (Graham-Brown and Bourke, 2006).

2.3.6. Human skin micro biomes and their potential forensic application.

The microorganisms that dwell on and within a person, as well as those that reside in their immediate environment, make up the human microbiome. Using microbial profiles obtained from a human microbiome, microbial profiling may have forensic use in the

identification or connection of people with criminal actions (Neckovic *et al.*, 2020A).

Because of variables like as shedding propensities (e.g.; skin squames), human skin microbiomes have the potential to be used as trace evidence (Grice and Segre, 2011), differences in microbial community makeup due to body location (Perez *et al.*, 2016). In compared to human somatic cells, the sheer number of microbial cells, particularly bacterial cells (Sender *et al.*, 2016).

These bacteria that are linked with the skin are deposited within the body and can colonize constructed surroundings (Gibbons, 2016; Fujiyoshi *et al.*, 2017). This implies that human microbiomes may be easier to recover from crime scenes than DNA, perhaps giving more investigative clues.

2.4. Aid of microbial analysis in differentiation between humans

Microbial analysis has the potential to distinguish individuals based on the bacteria they carry, assisting in human identification while also giving alternative and sensitive targets for genetic research. Following contact, an unique bacterial signature can be transmitted to surfaces when a person touches an object (Budowle *et al.*, 2017).

'Forensic mycology' is the use of mycological evidence in criminal investigations and its testing in courts (Hawksworth and Wiltshire, 2011). Mycology can help with a range of forensic investigations, since they often deal with suicidal, accidental, and homicidal cases

A vast number of fungal and bacterial families interact in complicated ways, resulting in important behavioral alterations in microorganisms ranging from mutualism to antagonism (Deveau *et al.*, 2018)

Even using the same general methods used for human DNA research, the identification of these microbial remnants of human interaction allows for the connection of persons with items and locations (Fierer *et al.*, 2010; Lax *et al.*, 2015).

Furthermore, because a microbiome's makeup might provide information about a host's lifestyle (Phan *et al.*, 2020). Forensic objectives, such as identifying potential suspects who can be connected to a crime scene, microbiome-based analysis can be employed (Kreft *et al.*, 2018).

2.5. Nosocomial infections associated with burns

Nosocomial infections (NI) are localized or systemic diseases induced by an unfavorable response to the presence of an infectious pathogen or its toxins, according to the National Healthcare Safety Network (NHSN). Nosocomial infections are infections that emerge during a patient's stay in the hospital and are not present or incubating at the time of admission. It is a serious issue in health-care institutions, leading in lengthy hospital stays, high morbidity and death rates, and high expenditures (Stone *et al.*, 2002).

Burned individuals are particularly prone to infection because they have large open wound regions with necrotic tissue, patients with severe burns must also stay in high-risk critical care facilities for extended periods of time (Rastegar *et al.*, 2005). The size of the open wound determines the degree or amount of contamination (Lee *et al.*, 1990).

2.6. Elements to Consider in Forensic Micro biome Analyses

2.6.1. Transfer and Persistence of Micro biomes

Factors such as microbial changes between the period of deposition and the collection of human-associated microbiota are being investigated, may shed light on whether or not human microbiomes may be utilized to identify or connect a specific person.

Given that an individual's reference microbiome may be acquired at a much later point in an inquiry, this is very significant. The study of human microbiome persistence may be further complicated by naturally occurring temporal changes in community makeup (Flores *et al.*, 2014).

2.6.2. Collection and Storage of Samples

Microbial profiling becomes popular as a forensic tool, to stabilize the microbial populations on or inside the evidential objects, evidence gathered from a crime scene that has been transferred to a laboratory must be triaged correctly prior to inspection (Fonneløp *et al.*, 2016; Michelot *et al.*, 2017).

However, what may be a suitable technique for preserving forensic biological evidence for human DNA typing may not be good for preserving and/or stabilizing microbial ecosystems. The preservation of evidential objects in cold, dry settings, for example, may create ideal circumstances for some microorganisms to flourish while others become non-viable. Alternatively, as has been shown for the skin-associated bacteria Staphylococci, low temperatures can cause alterations in microbial development and structure (Onyango *et al.*, 2012).

Furthermore, aeration of swab containers or drying of cotton swabs may allow foreign microorganisms to enter the swab surface. While the

evidence packing may be a source of further microbial contamination (Weyrich *et al.,* 2019).

2.6.3. Future Research and Recommendations, as well as Training and Interpretation

Microbiological profiling were to be used in a forensic setting, The methodologies and processes used must first be validated and proven to be trustworthy; for microbial profiling to be validated, specific accrediting body requirements must be satisfied. It's also worth noting that accreditation authorities could create new criteria, specifically for microbiological profiling. While concerns of microbial profiling have been raised (Eisenhofer *et al.,* 2019).

Several suggestions have been made to bring microbiome research up to standard (Pollock *et al.,* 2018; Hornung *et al.,* 2019; Aagaard *et al.,* 2012; Wang and LêCao, 2019).

For the forensic use of microbiological profiling, there are no such specific requirements. It's worth mentioning, however, that microbiological forensics is a very new field (traditionally involving bioterrorism), following the events of 9/11 in the United States and the Bali bombings in 2002, it garnered a lot of attention, was adopted as a program in certain countries, with national testing bodies conducting conformity testing (Roffe and Robertson, 2011).

As a result, in the event that microbial profiling is used for forensic purposes in the future, to maintain high standards of admissibility of microbiological profiles as forensic evidence, authorities that set standards and certify laboratories must be involved (Sharma *et al.,* 2019).

Extraction and sequencing sensitivities must be addressed in future study in this area. specificity, repeatability of microbial profiles,

population frequencies of specific bacteria in human microbiomes, include simulated casework pertinent samples of known ground truth, as well as the effects of environmental variables (Neckovic *et al.,* 2020B).

Following that, forensic practitioners must be able to use them consistently, which may be tested by proficiency testing. It is critical that the training offered enables forensic practitioners to use bioinformatic approaches in accordance with established procedures to analyze and interpret data generated in the context of case-related issues (Navas-Molina *et al.,* 2013).

In terms of the sorts of studies used, the forensic value of microbiome-associated informatics tools would have to be limited, and thoroughly evaluated against mock case work samples and fake samples of known microbial compositions. These limitations would protect against any misidentifications, if the intended forensic use was to identify or associate persons with illegal activity (Goodrich *et al.,* 2014).

For the accurate identification of microbial species, forensic databases of microbial taxonomy may be required when analyzing microbiological data. Because taxonomic identification varies considerably depending on which database is used to analyze the data (Edgar, 2018).

To this aim, more study is required to establish the likelihood of human-associated microbial transmission. and, as a result, the accompanying probabilities for estimating the likelihood of microbial DNA evidence based on activity level hypotheses, one of which may be related to innocent microbial transmission. For example, innocent transfer might be seen of as the deposit of millions of bacterial cells from a

person's microbial cloud onto a constructed environment (Qian *et al.*, 2012).

Which could later be established as a crime scene, or the indirect transfer of one individual's microbiome to another individual through their presence within a shared space or handling of an item (Richardson *et al.*, 2019)

Given the lack of clear indications of mixed-source origins in a microbial profile (i.e., microbiota originating from two or more individuals), to properly conduct forensic investigations, profile deconvolution of possibly mixed-source signals would be an apparent, yet essential, difficulty (Shi and Horvath, 2005).

Random Forests classification algorithms and other techniques for deconvolution of mixed-source microbial profiles have been studied, in which the microbial similarity of samples is interrogated (Richardson *et al.*, 2019).

2.7. Degree of burns

Direct or indirect contact with heat, electric current, radiation, or chemical substances is a typical cause of burns. Burns can cause cell death, which necessitates hospitalization and is potentially deadly. Burns include three levels; First, Second, and Third degree.

In first degree burns only the outer layer of the skin is affected. They produce pain, swelling, and redness. but both the outer and underneath layers of skin are affected by second-degree burns. While burns of the third degree damage the skin's deep layers. Full-thickness burns are another name for them. They cause skin to become white or blackened, as well as burning. It's possible that the skin is numb (Schaefer and Szymanski, 2017).

Chapter Three

Materials

and

Methods

3. Materials and Methods

3.1. Materials

3.1.1. Instruments

The instruments, and manufacturing company employed in the current investigation, as listed in Table 3-1, :

Table (3-1): Instruments in this study

Apparatus	Manufacturer	State
AmScope40X-2500 Microscope with LCD Touchpad Screen	Optika	Italy
Analytical Balances	Sartorius AG	Germany
Autoclave	Hirayama	Japan
Bezel burner	Quinze Products	India
Class II Biological Safety Cabinet	Labgard	USA
Deep freezer	Samsung	Korea
DensiChek Plus	Biomeriux	France
Drying oven	Bio base	China
Incubator	CYAN	Belgium
Refrigeration unit	Kelon	Japan
Vitek 2 compact system	Biomeriux	France
Water bath	FALC BI	Italy
Water Distiller	K&K	Korea
With a hot plate, a magnetic stirrer	Wise stir	Belgium

3.1.2. Equipment

The following are the tools, and related equipment employed in the current investigation, as listed in Table 3-2

Table (3-2): Instruments in this study

Equipment, and tools	Manufacturer	State
Aluminum paper	Alhadaf	Jordon
Cotton	Baspinar,Gzinantep	Turkey
Guze swab	Sunvian	Japan
Inoculation loop	Lab-tech	Italy
Microscope Slides	Sunvian	China
Petri dishes (Disposable plastic)	Sunvian	China
Rack made of plastic	Sunvian	China
Rack made of steel	Citotest	China
Refrigeration unit	Kelon	Japan
Sterile swap stick	Sunvian	China
Syringes disposal	AYSE	Turkey
Transport box	Medical instrument company	USA
Tube in its natural state	Sunvian	China
Tubes screwed together	Sunvian	China
Wooden Applicator Stick	Sunvian	China

3.1.3. The Biological and chemical substances

Biological and chemical materials were used in current study

Table (3-3).

Table (3-3): Chemical and biological materials

Substances	Manufacturing company	orgin
Absolut alcohol	Sigma ,	Germany
Blood	Blood bank	Najaf
Crystal violet	Fluka	England
Dettol	Quinze Products	India
Grade water	Parenteral drugs	India
Gram stain	Himedia	India
Hydrogen peroxide 3%	Hardy diagnostics	USA
Lactophenol cotton blue	Quinze Products	India
Normal saline	Medical instrument company	USA
Oxidase	Hardy diagnostics	USA
Plasma-coagulase EDTA (Rabbit plasma)	MAST	USA
Safranine	Fluka	England
Sodium chloride (NaCl)	GCC	England
Urea solution	Himedia	India
VITEK 2 GN kit	Biomeriux	France
VITEK 2 GP kit	Biomeriux	France
VITIK 2 YST kit	Biomeriux	France

3.1.3. The medium of culture

Culture media were utilized for various purposes in a table (3-4).

Table (3-4): The culture media utilized in the research

The media	Manufacturing	The source
Blood agar base (BA)	Himedia	India
MacConkey's Agar (M.A)	Himedia	India
Mannitol salt agar (MSA)		Oxoid, England
Nutrient agar (N.A)	Himedia	India
Sabouraud's Dextrose Agar media	Oxoid	(UK)
Transport Medium	IVD products	China

3.2. The Methods

3.2.1. Culture media preparation

3.2.1.1. Transport Medium

This medium (IVD products, Chinia) was purchase of from stockpiles of medical supplies.

3.2.1.2. Blood agar (BA)

This medium was formulated according to the manufacturer's instructions, sterilized by autoclaving at 121ºC for 15min. after cooled to 50ºC, 5ml of sterile defibrinated blood was added for each 100ml of the medium, mixing well then poured in a sterile Petri-dish. This medium is appropriate for bacterial isolation, cultivation, and detection of a hemolytic kind and activity (Daka *et al.*, 2012).

3.2.1.3. MacConkey's Agar (M.A)

This medium was used in the detection of gram-negative bacterial isolates. According to manufacturer instruction, the preparation was conducted by suspending 51.5 g of medium powder in one liter of distilled water and heated to boiling until all powder was dissolved, then autoclaved for sterilization.

3.2.1.4. Mannitol salt agar (MSA)

MSA was used for the detection of *S. aureus* isolates as a selective and differential medium, the preparation performed by suspending 111 g of medium to 1000 ml of distilled water and mix well with heating until all medium was dissolved, then sterilization by autoclave.

3.2.1.5. Nutrient agar (N.A)

The preparation of this medium was like MSA medium but in a different amount of powder (28g in 1000 ml of D.W). used for preparing stock culture in screw tubes.

3.2.1.6. Sabouraud's Dextrose Agar media

This media was used in currant study by suspend 65 g of the medium in one liter of distilled water and heat with frequent agitation and boil for one minute to completely dissolved the medium. Then autoclaved at 121° C for 15 minutes. And after that cool to 45 to 50°C and pour into petri dishes or tubes for slants.

3.2.2. Solutions and Reagents Preparation

3.2.2.1. Preparation of Gram stain reagent

Gram stain involves four reagents: Crystal Violet stain, Lugol Iodine solution, Alcohol Acetone solution, and carbol fuchsin stain were used to detect if the bacteria were gram - positive or negative.

3.2.2.2. The reagent Catalase

This is a commercial product 3% hydrogen peroxide (H_2O_2), (Hardy diagnostics/USA), was used to detect the presence of bacterial catalase enzyme (MacFaddin, 2000).

3.2.2.3. The Oxidase reagent

This was prepared by dissolving 0.1 gm from N.N.N.N tetra methyl p-phenyl diamine- dihydrochoride in 10 ml distal waster. It was used to determine presence of Oxidase-cytochrome enzyme in bacteria (Shields & Cathcart, 2016).

3.2.2.4. Urea solution

This was prepared by adding 40 g urea to 100ml D.W. That was used for urease detection (Prescott *et al.*, 1996).

3.2.2.5. Lactophenol cotton Blue

Staining solution is made up of; distilled water 50ml added to cotton blue (Aniline Blue) 0.125g with phenol crystals ($C_6H_5O_4$) 50g, Glycerol 100mlm, Lactic acid (CH_3CHOH COOH) 50ml, and 70% ethanol.

3.3. VITEK 2 COMPACT System

A VITEK examination was conducted using the VITEK 2 COMPACT system to determine the genus of bacteria or fungi by used VITEK 2 GN kit, VITEK 2 GP kit, and VITEK 2 YST kit for identification of Gram negative, Gram positive and fungal isolates respectively. by taking a colony from the plate containing the bacterial or fungal growth and placeing it in a tube containing normal saline after mixing it well and calibrating it using Densi Chek Plus to obtain the required density. After about 15 minut results appeared.

3.4. Specimen collection and study design

3.4.1. Specimen collection

A total of 59 skin swabs were collected in a cross-sectional investigation from inpatient with burn attended to the burn center to laboratory public health central, samples taken from both genders irrespective to age.

The specimen was collected with sterile cotton swab and immediately incubated onto transport media. Then transported to the laboratory. The samples were collected along a period from 1 April 2021 to 31 July 2021.

3.4.2. Experimental Design

All steps that followed up for accomplishing in this study Figure (3-1)

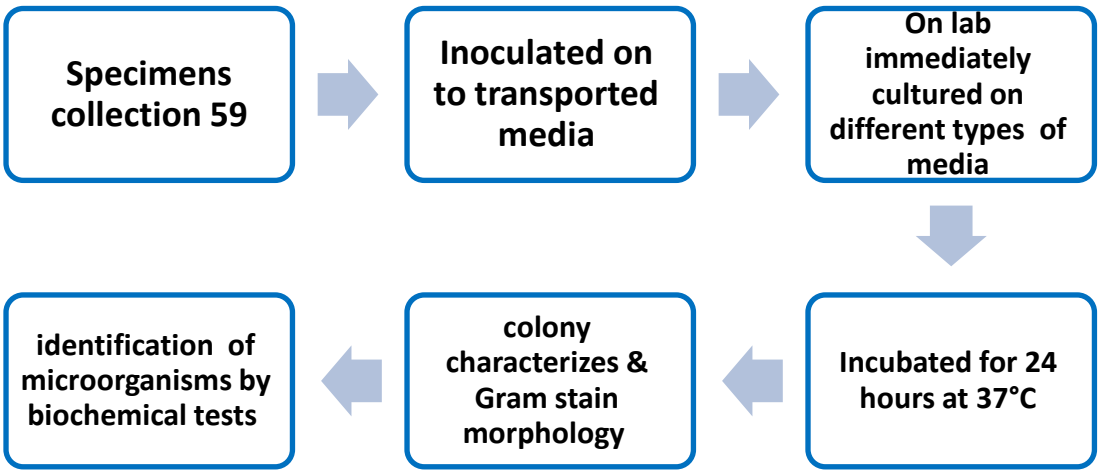

Figure (3-1) The study design for microbiological and biochemical identification of patients samples with burn

3.4.3. Microorganisms Isolation and diagnosis

Bacteria and fungi from burn injuery isolation and diagnosis and characterization were done according to Markey *et al.* (2013) including morphological characteristics of culture media and microscopy, as well as biochemical identification

3.4.4. Isolation and Identification of Bacterial Isolates

The burn swabs that collected with sterile cotton swabs and immediately inoculated in Cary Blair (IVD products, China) and transported to the laboratory public health central were streaked on

Nutrient broth, MacConkey's Agar, and blood agar base with human blood for first isolation and then subcultured on selective media such as; Mannitol salt agar

3.4.5. Isolation and Identification of Fungal Isolates

Burn swabs was streaked on Sabouraud's Dextrose Agar media for Fungi identification in burns.The streaked plates were then incubated at 37℃ under for 48 hrs in bacteria while fungi at 28℃ for 7 days. After the isolates were identified according to morphology (Sharma *et al.*, 2016).

3.4.6. Diagnosis of microbial under the microscope

3.4.6.1. Diagnosis of bacteria under the microscope

After staining the suspected colonies with Gram stain, microscopic observation was performed using an oil immersion lens to determine the shape, color, and arrangement of the bacterial colonies in order to validate the presence of gram positive and negative bacteria. in addition to the diagnosis of pigmented fungi with Lactophenol cotton blue.

3.4.6.2. Diagnosis of fungi under the microscope

Fungal colonies stained with lactophenol cotton blue techniques, after that obsereved under microscope using an oil immersion lens to determine the shape, color, and arrangement of fungal stracture like conidia, hypha, spores… etc.

3.4.7. Tests for bacterial identification

3.4.7.1. Test for catalase

The test was done by transfer of pure bacterial colony using wooden stick, onto dry, clean glass slide, then mixed with a drop of 3% hydrogen peroxide reagent if air bubbles are immediately developing, the test is regarded as positive (Reiner, 2013).

3.4.7.2. Oxidase test

Oxidase to determine presence of oxidase-cytochrome enzyme was carried out by using oxidase reagent through placed oxidase on filter paper. Then loopful of pure suspected colonies was spread over the paper with the aid of wooden stick, colonies should be from 24 hours old, the color should be change to purple within 30 seconds to be recorded as positive, any color appeared after this time was ignored (Espinosa *et al.*, 2011).

3.4.7.3. Slide Coagulase test

It was performed according to the coagulase test protocol of MacFaddin, (2000), using kit slide, one drop of EDTA-treated rabbit plasma was added and a proper amount of fresh bacterial suspension, and mixed well by a wooden stick, therefore the presence of bound coagulase proteins (clumping factor) on the bacterial cells will lead to clot formation.

3.4.7.4. Urease test

Urea slant was stabbed and streaked with frsh bacterial colony, and the tubes were incubated for 24-48 hr at 37 °C. The change of media agar color from yellow to pink indicates a positive result (Pan *et al.*, 2012).

3.5. 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’s dextrose agar and Potato dextrose agar, then incubated at 25 °C for 2-7 days (Forbes *et al.*, 2002).

3.6. 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:

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

Chapter Four

Results

and

discussion

4. Results and discussions

4.1. Patients profile of peoples with burns injury

The samples were 59 burns skin swabs. Samples were taken from people whose ages ranged from 1 to 60 years. Patients were of both genders, including 36 females and 23 males. Most of the patients were suffering from second and third degree burns in addition to bacterial and fungal infections.

4.2. Distribution of burns infections according to age groups

It is worth noting, according to the included age range from 1 year to 60 years with both male and female Figure (4-1) indicated , highest rate of burns by age was between to 21 – 30 years (47.5%), followed by age group 11-20 (27.5%), after that the age group 1-10 (22.5%), then the age group 31-40 (20%), while the least groups frequented were in age group 41-50 (17.5%), and age group 51-60 (12.5%). These results appeared a significant difference among age groups.

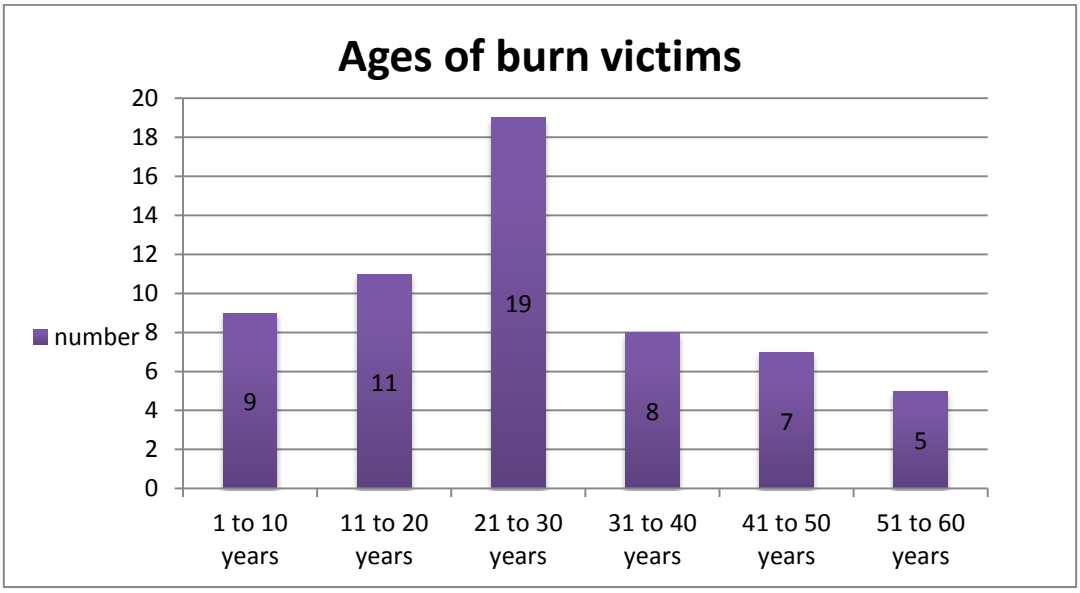

Figure (4-1): Range of ages of patients with burns

AL-Aali, (2016) recorded in this study age rates were similar to those of current study the majority of the burns patients were the age of 27-37 year, followed the 16-26 year, while the remaining were 5-15 year, 38-48 year, and 49-59 year were less frequency.

Another study gave results that are different from the current results, where children between the ages of 1 and 4 years had the highest rate of burns from emergency centers in South Africa's Western Cape

they were living in cramped housing conditions , perhaps due to time spent in the home (Blom *et al.*, 2016).

4.3. Gender disruption of burns patients

In the current study the burns were more spread in female at higher rates 36/59 (61%) compared to male 23/59 (38.9) Figure (4-2).

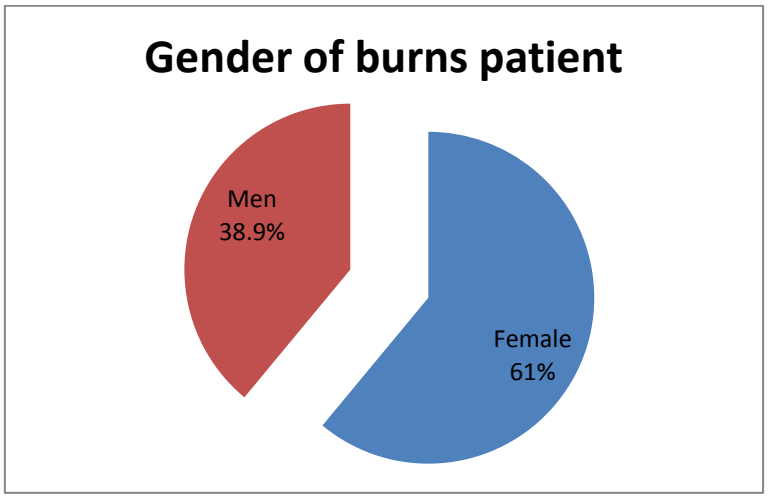

Figure (4-2): Percentage of the burn patients according gender

This is because of the large number of domestic violence against women and everything in life is difficult in Iraq and may be due to her works in home.

A according to previous studies self-immolation and self-burning are common forms of self-harm in the Eastern Mediterranean and South

and Central Asia. Young women make up the bulk of individuals who choose to self-harm in this way. Rasool and Payton, (2014) Used data from 100 young female survivors of suicidal attempts in the Kurdistan Region of Iraq, and reported the causes of this phenomenon that the period around first marriage is a time of particular trauma to women.

On the contrary, incidence of burns injury was seen more in males than in females from burn patients in southwest Iran and the majority of the burns were caused by flame injuries (Emami *et al.*, 2020). Also Blom *et al.*, (2016) reported among adults patients with burns, males had higher rates than females due to personal aggressiveness and suspected alcohol/other drug use more common on weekends, with more marked differences for hot liquid burns.

4.4. Age of gender disruption of burns patients

The ages of the burn victims ranged in both sexes from 1 to 60 as showed in table (4-1)

Table (4-1): Distribution of patients according to age and gender

Age / Gender	1- 10 (%)	11 - 20 (%)	21 - 30 (%)	31 – 40 (%)	41 – 50 (%)	51 – 60 (%)	Total number & Percentage
Female	6 (16.6)	8 (22.2)	11 (30.5)	5 (13.8)	3 (8.3)	3 (8.3)	36 (61%)
Male	3 (13)	3 (13)	8 (34.7)	3 (13)	4 (17.3)	2 (8.6)	23 (38.9%)
Total number	9	11	19	8	7	5	59
Percentage	15.2	18.6	32.2	13.5	11.8	8.4	100%

Perkins *et al*, (2020) reported according to the data from intensive care units of burn center's that provide specialist burn care in Australia and New Zealand the worse outcomes found in women are linked to their

age and injury patterns, and there was a link between gender and in-hospital mortality, but no link between gender and death time.

Another study on adult burn patients in a developing country demonstrated a higher proportion of deep burn injuries, number of surgeries, and longer hospital stay was recorded among the male group. while death rate was remarkably higher in the female group (Lam *et al.*, 2019). In addition, animal studies demonstrate that the sex hormones influence inflammatory response to injury (Horton *et al.*, 2004).

4.5. Degree of burns among patient states

All cases in the current research were with degrees of burns of the second and third degree. The second degree was (65%) and the third degree was (35%), there were a significant difference between control and the second degree of burns. Table (4-2).

Table (4-2): Distribution of the burn patients according to degree of burn

Degree	Thickness	Frequency	Percentage %	Mix of 2nd and 3rd degree Total = 4 (%)
Second	Superficial partial	26	65	4 (57.14)
Third	Deep partial	14	35	

A previous study in the Al-Fayhaa Burn Centre in Basra City recorded the percentage of total body surface area (TBSA) burned ranged from 1% to 100% (Al-Shamsi and Othman, 2017). Another study about Patients with burn injuries in Baskent University Ankara hospital adult emergency department was recorded several cases range between patients from burn area of less than 10 % to those with a percent burn area of more than 10% (Eser *et al*., 2016).

4.6. Isolation and identification of bacteria

4.6.1. Cultural characteristics of *Pseudomonas aeruginosa*

The pathogen *Pseudomonas aeruginosa* is an opportunistic pathogen that causes a variety of infections. Particularly in burn patients (Rostami *et al*., 2018). P*aeruginosa* produces at least 4 distinct pigments; pyocyanin- bluish-green, pyoverdin- yellow-green, give color for pus and gives a greenish color to the agar when combined with pyocyanin, pyorubin- red, and pyomelanin- black (Marrez et al., 2020).

In current study *Pseudomonas aeruginosa* isolated after 24 hours at 37° C was small colonies, on nutrient agar produced grape like odor with yellow-green pigment, to the agar resulted in combined with pyocyanin, also cultured on MacConkey and produced non–lactose fermenting colonies.

However on blood agar produced hemolysis. These results are similar to the isolation and diagnostic methods of *Pseudomonas aeruginosa* by Health protection agency (2015). All *Pseudomonas aeruginosa* isolates were showed in Figure (4-3).

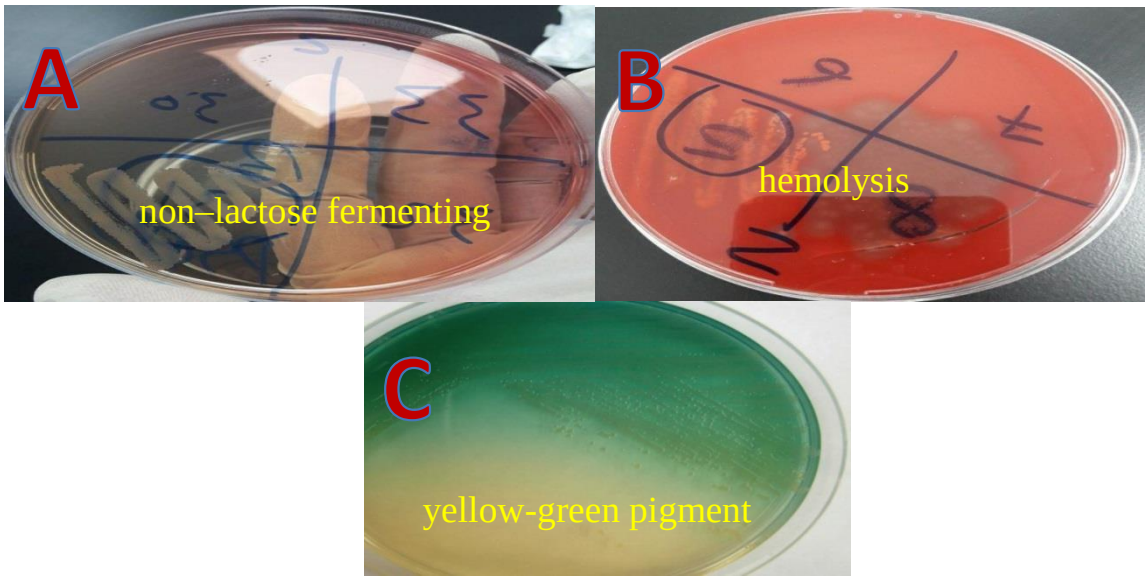

Figure (4-3) : *Pseudomonas aeruginosa*; (A) *P. aeruginosa* on MacConkey agar with non–lactose, (B) *P. aeruginosa* on blood agar demonstrated hemolysis, (C) *P. aeruginosa* on nutrient agar with yellow-green pigment (Pyoverdin).

4.6.2. Biochemical and VITEK 2 compact identification

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* with probability 97%. Previous study 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). Figure (4-4) showed biochemical test of *P. aeruginosa*.

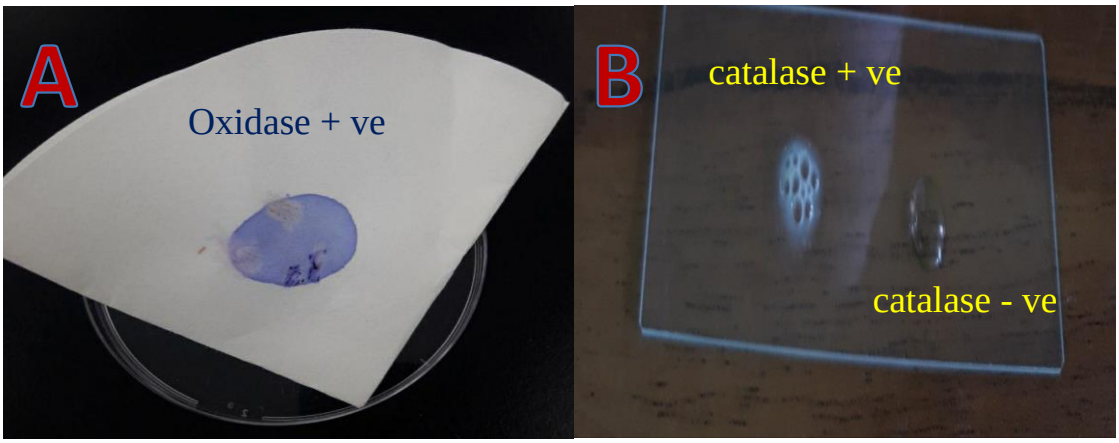

Figure (4-4) : (A) Oxidase test, (B) Catalase test

4.6.3. Cultural characteristics of *Acinetobacter baumannii*

Acinetobacter baumannii is a bacterial pathogen that is commonly linked with hospital-acquired illnesses. Septicemia, wound infections, pneumonia, and urinary tract infections are all caused by *Acinetobacter baumannii*, which is a major hazard to hospitalized patients (Centers for Disease Control and Prevention, 2019; Isler *et al.*, 2019).

The investigation demonstrated after overnight growth, *Acinetobacter baumannii* formed a complex streak on MacConky agar at

44° C and appeared small to pale colorless, round regular, not fermented to lactose. However MacConkey and blood agar plates were used to isolated *Acinetobacter* (Kumar *et al.*, 2020).

On blood ager *Acinetobacter baumannii* appeared in white or light gray and was convex in shape if it did not appear decomposition areas around the developing colonies due to their inability to produce hemolysin. Another research identified bacteria *Acinetobacter baumannii* from people in serious condition in the hospital by using several culture media in order to compared between them.

The result was blood agar detected (100%) and MacConkey agar detected (89%) of *A. baumannii* isolates (Ajao *et al.*, 2011). Figure (4-5) showed culture characteristics of *Acinetobacter baumannii*.

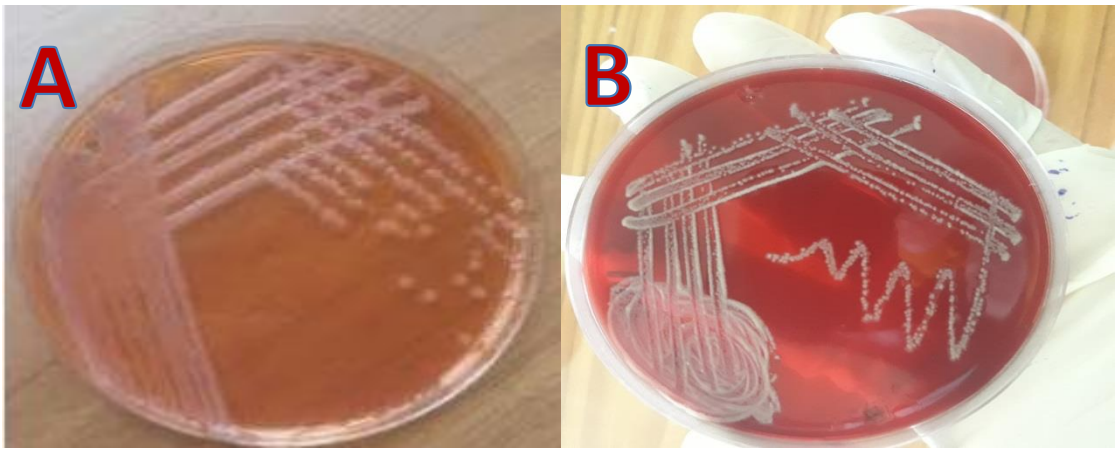

Figure (4-5) : (A) *A. baumannii* on MacConky agar without fermented to lactose *A. baumannii* on blood ager without produce hemolysis

4.6.4. Biochemical and VITEK 2 compact identification of *Acinetobacter baumannii*

A. baumannii appeared gram-negative Coccobacilli under microscope , but showed oxidase negative and catalase positive result, after that confirmed by VITEK 2 compact as a *A. baumannii* with probability 99%.

Among several studies *Acinetobacter*, as it is now defined, gram-negative bacteria that are strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, and oxidase-negative (Howard *et al.*, 2012). Previous study also performed VITEK 2 compact for identification of *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Bobenchik *et al.*, 2017).

4.6.5. Cultural characteristics of *Staplylococcus aureus*

S. aureus isolates from patient with burns were determined by using conventional standard bacteriological and biochemical tests. All the specimens cultured on blood agar medium supplemented with 5% of human blood at a temperature of 37^{o}C under aerobic condition for primary isolation, also selected by using selective media (MSA) for confirmation of the mannitol fermentation.

S. aureus colonies that were showed; large, round, opaque, and golden yellow in color. Furthermore, showed β- 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% sheep blood agar (BA), and incubated overnight at 37 ^{o}C (Amissah *et al.*, 2017)

Deep partial- (DPT) and full-thickness (FT) burn wounds colonized with *Staphylococcus aureus*. Damage-associated molecular patterns (DAMPs) changed dynamically as a result of *S. aureus* infection. These variations in DAMPs are thought to be linked to the severity of the burn and the bioburden of *S. aureus.* Overall, this model demonstrates *S. aureus'* evasiveness by suppressing the immune response, allowing it to thrive in the burn site (Weaver *et al.*, 2021).

S. aureus also cultured on Mannitol Salt Agar (MSA) isolated as to ferment mannitol and gram staining by Budiarso *et al.*, (2019). Figure (4-6) showed *S. aureus* isolated in the current study.

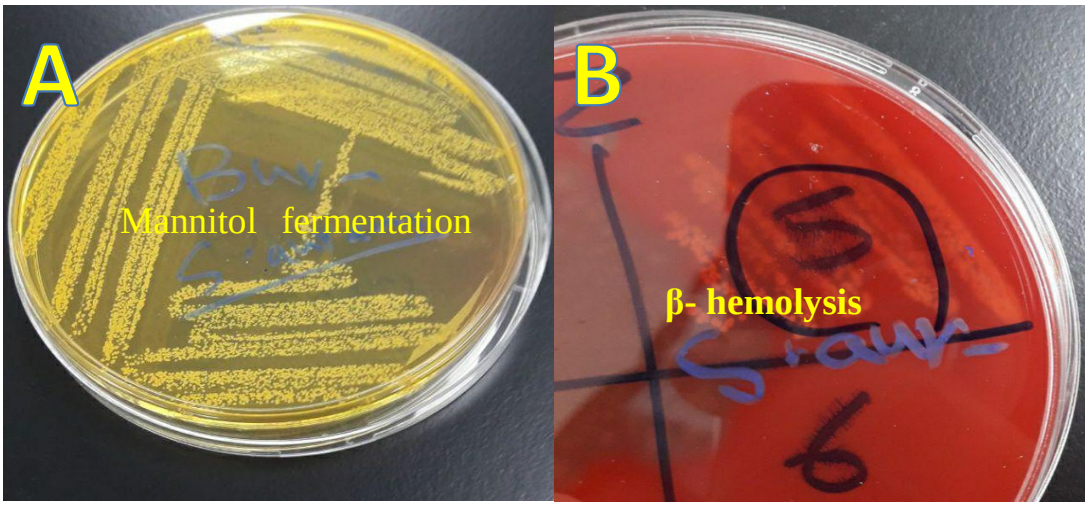

Figure (4-6): (A) *S. aureus* colonies on mannitol salt agar with the ability to fermentation of mannitol, (B) *S. aureus* colonies showed β-hemolysis zone on blood agar

4.6.6. Biochemical, serological, and VITEK 2 compact identification of *Staplylococcus aureus*

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 with probability 93%. 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).

Figure (4-7) showed positive and negative results with coagulase test utilized in our study to determine *S. aureus*.

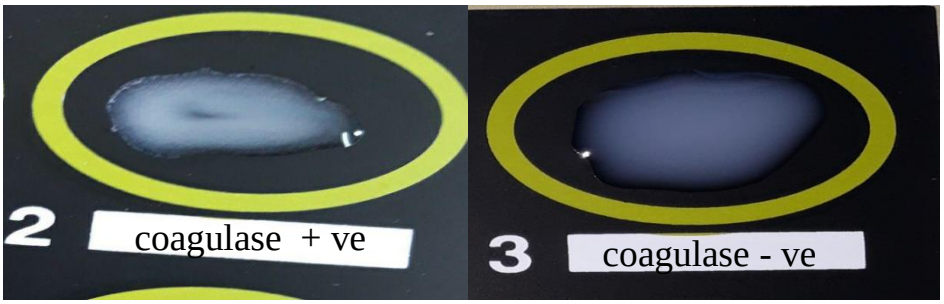

Figure (4-7): (A) Positive result with coagulase test, (B) Negative coagulase reaction.

4.6.7. Cultural characteristics of *Klebsiella pneumonia*

The most prevalent cause of hospital-acquired pneumonia nowadays is *K. pneumoniae* pneumonia which can enter the body through burn wounds during hospital treatment.

Human mucosal surfaces of the oropharynx and gastrointestinal (GI) tract are commonly colonized by *Klebsiella pneumonia*. Once within the body, the bacteria can exhibit significant levels of virulence and drug resistance (Paczosa and Mecsas, 2016)

Klebsiella pneumonia isolated in the current study on MacConky agar was rose pink colonies due to lactose fermentation, huge dome-shaped structure, mucoid, caused extracellular slime layer. Hassan,(2021) recorded in his investigation; *Klebsiella pneumoniae* colonies pink in color due to lactose fermentation, which resemble to our findings. *Klebsiella pneumoniae* growth as showed in Figure (4-8).

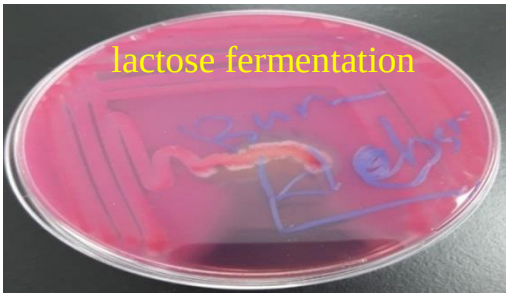

Figure (4-8) *Kebsiella pneumonia* growth on MacConky agar after overnight.

4.6.8. Biochemical and VITEK 2 compact identification *of Klebsiella pneumonia*

Klebsiella pneumonia was gram-negative, rods, short, fat, and straight. The biochemical test characteristics that were discovered were as follows: Catalase positive, oxidase negative, and urease positive. After that confirmed by VITEK 2 compact with probability 99%.

Another study involved confirmed a *Klebsiella pneumonia* isolates through gram stain and biochemical test like; Catalase and oxidase (Patel *et al.*, 2017). Registered study in India reported, the VITEK-2 Compact (Biomerieux) was used in the research of isolates obtained from various clinical samples a tertiary care hospital (Kaur *et al.*, 2019).

4.7. Isolation and identification of fungi

4.7.1. Cultural characteristics of *Candida albicans*

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 *et al.*, 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 & Cojandaraj, 2020) Figure (4-9) showed culture characteristic of *C. albicans* on Sabouraud dextrose agar.

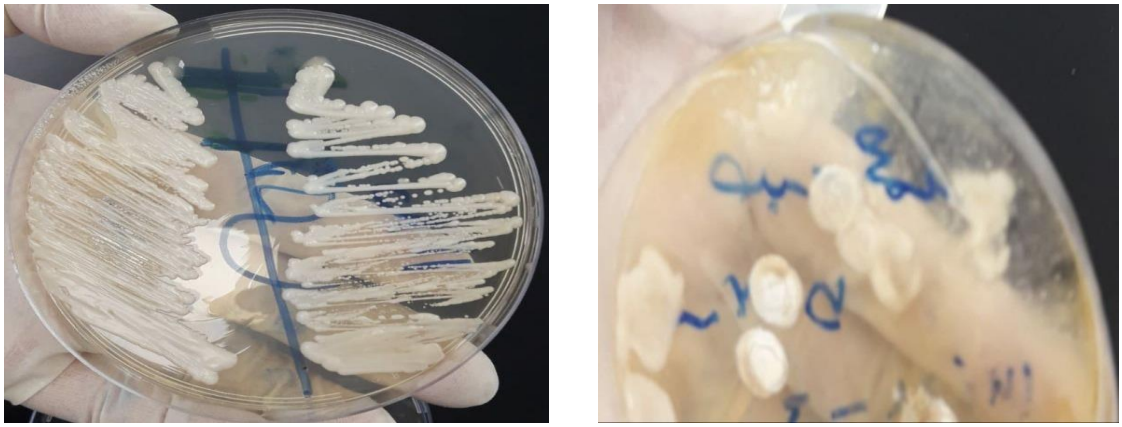

Figure (4-9): Morphology characteristic of *Candida albicans* on Sabouraud dextrose agar after 3 days

4.7.2. Microscopic examination, and VITEK 2 compact identification for *Candida albicans*

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 with probability 98%. Figure (4-10) showed microscopic morphology of *Candida albicans.*

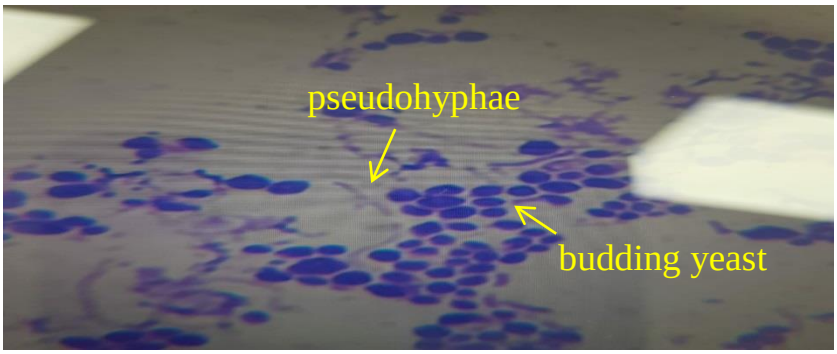

Figure (4-10): Microscopic of *Candida albicans*

The results were identical to what Zafar *et al.*, (2017) explained in the practical guide and the atlas for diagnosis of *C. albican* by

microscopic examination. Another study recorded similar to what was obtained from the current results (Sundaram & Murugan Navaneethakrishnan, 2016). On Sabouraud dextrose agar, clinical samples from various locations were grown and determined by the VITEK 2 compact system (Seyoum *et al.*, 2020).

4.7.3. Cultural characteristics of *Aspergillus niger*

Because of the angioinvasive nature of the illness, *Aspergillus* infection is linked with significantly higher mortality than other fungus in hospitalised patients with burns **(**Church *et al*., 2006**)**.

After cultured on Sabouraud dextrose agar current isolates characteristics; cottony appearance initially white to yellow and then with time turning black after incubated at 37°C for five days, Figure (4-11A)

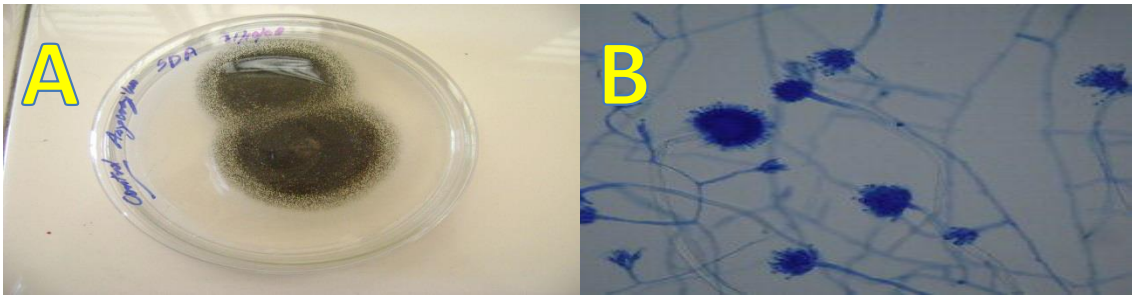

Figure (4-11) A- Culture characteristic B- Microscopic morphology of *Aspergillus niger*.

4.7.4. Microscopic examination, and VITEK 2 compact identification for *Aspergillus niger*

Then suspected colonies selected for staining with lactophenol cotton blue; was appeared in the form of septated fungal hyphae, Figure (4-11B). Also confirmed by the VITEK 2 compact system as *Aspergillus niger* with probability 97%.

4.8. Prevalence of microbial infection in burns patients.

Microbial infections are often associated with burn injuries, which affect high rates of morbidity and mortality in both developed and developing countries. As we mentioned earlier.

The overall prevalence of microbial isolated from burns skin swabs samples that confirmed by phenotypic and VITEK 2 compact system was 40/59 (67.7%) from isolates showed positive growth. The findings of the current study are consistent with those of Bourgi *et al*.**,** (2020) who found a prevalence rate of 55% of patients developed at least one infection during their stay in the hospital.

The prevalence of microbial infections very dynamic and varied according to geographic area, sampling and culturing methods, and other confiding factors. The most interesting finding that bacterial isolates was recovered in higher prevalence 40/59 (67.7%). While fungal isolates was 24/59 (40.6%). However Gram negative bacteria was the dominant among the other diagnosed microbial species.

Similar previous study reported Gram-negative bacteria were more prevalent (72.47%) than Gram-positive bacteria (47.62 %), with fungal isolates coming in third (4.59%) from burns skin swabs at a Tertiary Care Center in India (Mundhada *et al*., 2015).

4.8.1. Prevalence of bacterial infection in burns patients.

An interesting results in this data is that out of 59 burns swabs samples were 40/59 (67.7%) bacterial isolates, among this *pseudomonas aeruginosa* was highly prevalence 17/40 (42.5%), *Acinetobacter baumannii* was 12/40 (30%), *Staplylococcus aureus* was 8/40 (20%), and *klebsiella pneumonia* 3/40 (7.5%) . A showed in Table (4-3) and Figure (4-12).

Table (4-3): Types of bacteria, number of sample and percentage (%) of bacteria isolates.

Bacterial isolates	Frequency N= 40	Percentage %
Acinetobacter baumannii	12	12/40 (30)
Klebsiella pneumonia	3	3/40 (7.5)
Pseudomonas aeruginosa	17	17/40 (42.5)
Staplylococcus aureus	8	8/40 (20)
Total isolates	40	100 %

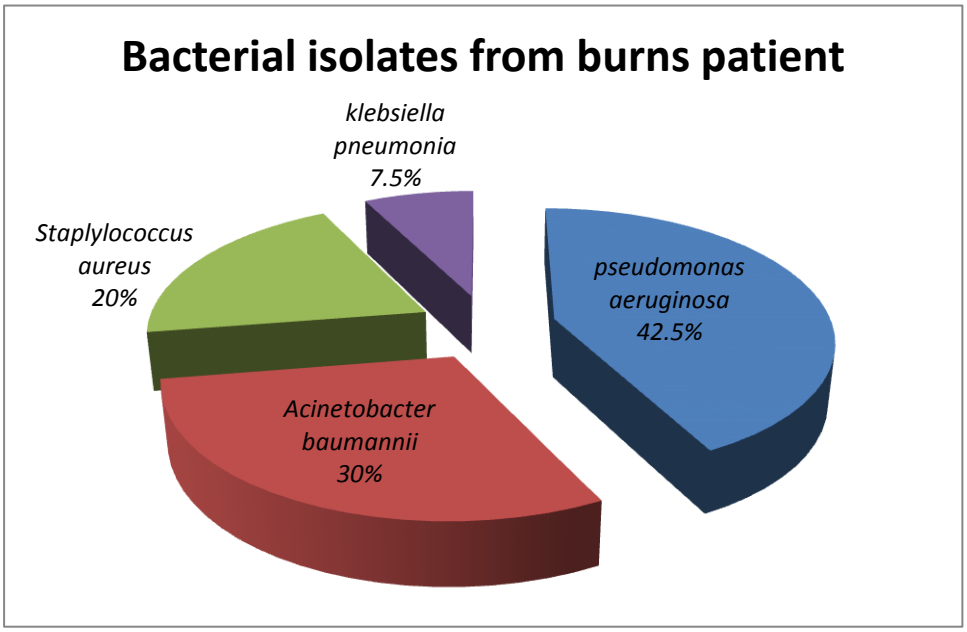

Figure (4-12) Bacterial isolates from skin burns patients

Our current results appear to be very close to what was obtained from Forson *et al.*, (2017) where their results were *Pseudomonas sp.* (30.2 %) and *Acinetobacter sp.* were the most often isolated species (20.9 %). and *Staphylococcus aureus* (2.3%) was the least commonly isolated.

The current findings are in agreement with D'Abbondanza and Shahrokhi (2021) whose reported that the most common pathogens in burns remain *Staphylococcus* and *Pseudomonas spp.*. While not in

agreement with the research submitted by Montazeri *et al.*, (2019) from burn hospital of Yazd in Iran Where dominant genus of G+ve bacteria was *Staphylococcus epidermidis* (62.5%), and the dominant genus of G-ve bacteria was *Citrobacter freundi* (11.5%).

Another study in Iraq included detection of *Staphylococcus aureus* isolates were found in (67%), collected from eight burn units in Baghdad throughout the examination (Abdulrahman *et al.*, 2020).

4.8.2. Prevalence of fungal infection in burns patients

Fungal infections have become very increasingly prevalent in Intensive Care Units of burns (ICUs). Out of 59 samples only 24 (40.6%) isolates was fungal isolates among this *Candida albicans* was 8/24 (33.3%), and *Aspergillus niger* was 16/24 (66.6%) as showed in Table (4-4) and Figure (4-13).

Previous study in Duhok city, Iraq, in demonstrated that the wound and burn fungal infection cases are relatively high with a variety of fungal pathogens included *Aspergillus nigar* and *Candida albicans* (Yassin and Oumeri 2020).

Table (4-4): Prevalence of fungal infection in skin burns patients

Type of fungi	Numbers of isolates	percentage (%) of isolates
Aspergillus niger	16	16 / 24 (66.6)
Candida albicans	8	8 / 24 (33.3)
Total isolates	24	100 %

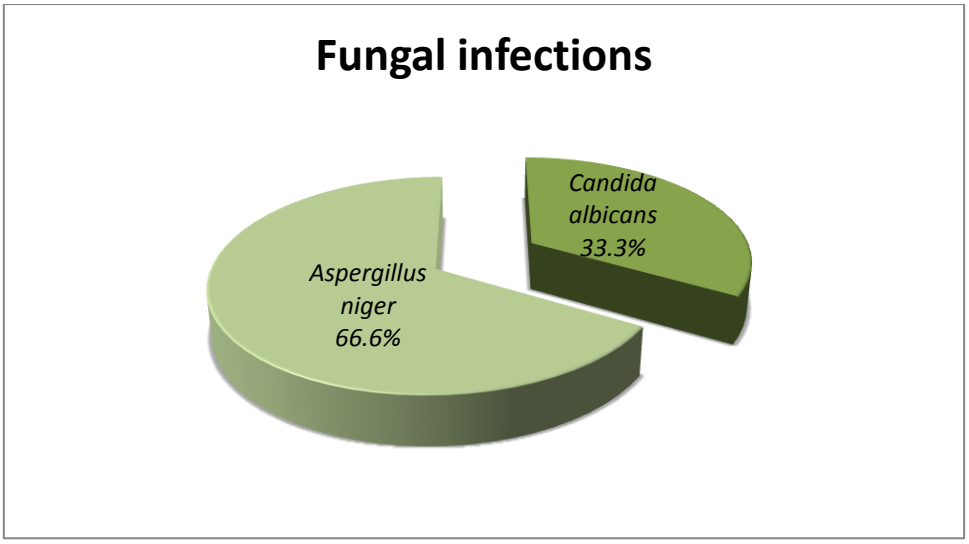

Figure (4-13): Fungal isolates from skin burns patients

4.9. Distribution of bacterial infections according to age groups

According to what was mentioned about our findings, burn injuries were the most in the ages between 21-30, in addition to that, Table (4-5) highlight about bacterial types isolated from each age groups. Four bacterial types recorded high rates were (90.9%), (84.2), (60%), and (50%) in age groups 11 – 20, 21 – 30, 41 – 50, 31 – 40, respectively. While recorded low rates in age groups 1- 10 and 51 – 60.

Table (4-5): Percentage distribution of bacterial infections according to age groups

Age / Bacteria	1- 10 Total= 9	11 - 20 Total= 11	21 - 30 Total= 19	31 – 40 Total= 8	41 – 50 Total= 7	51 – 60 Total=5	Total (%)
A.baumannii	2	3	5	1	1	0	12 (30)
K.pneumonia	1	0	2	0	0	0	3 (7.5)
P. aeruginosa	2	5	6	2	1	1	17 (42.5)
S. aureus	1	2	3	1	1	0	8 (20)
Percentage	44.4%	90.9%	84.2%	50%	60%	20%	100%

Another study agreed with our results from Burn Care Unit of a tertiary care hospital, Jharkhand, India reported bacterial infection was identified in 61.87% of the 16–30 and 31–45 year old age groups. In addition to that *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* were discovered to be the most common organisms in patients (Gupta *et al.*, 2019).

4.10. Distribution of fungal infections according to age groups

In research all fungal isolates was from age groups range from 1 year to 40 years with both male and female, highest rate of burns by age was between to 31-40 (75%), followed by age group1- 10 (66.6%), then the age group 21-30 (36.8%) and the age group 11 – 20 (27.2%). As showed in Table (4-6).

Table (4-6): Percentage distribution of Fungal infections according to age groups

Age / Fungi	1- 10 Total= 9	11 - 20 Total= 11	21 - 30 Total= 19	31 – 40 Total=8	40-50	51 – 60	Total = 24 (%)
Aspergillus niger	3	2	7	4	0	0	16 (66.6)
Candida albicans	3	1	2	2	0	0	8 (33.3)
Percentage	66.6%	27.2%	36.8%	75%	0%	0%	100%

Most of the patients' families were reticent about disclosing the reasons for the burning for security reasons and other family reasons. However, most of the burns male were a result of the nature of the their work and for female as a result of using the gas cooker and the oven during the preparation of both food and home bread, respectively.

4.11. Degree of burns, bacteria types among patient states

Also patient cases in our research were divided according to the types of bacteria associated with burns degrees as in the Table (4-7).

Table (4-7): Degree of burns, bacteria types among patient states

Degree	*P. aeruginosa* (%) Total =17	*A. baumannii* (%) Total =12	*S. aureus* (%) Total =8	*k. pneumonia* (%) Total =3
Second	12 (70)	8 (66.6)	5 (62)	1 (33.3)
Third	5 (29.4)	4 (33.3)	3 (37.5)	2 (66.6)

Generally, in current study there has been growth and high levels of bacteria; *Pseudomonas aeruginosa* (70%), *Acinetobacter baumannii* (66.6%), and *Staplylococcus aureus* (62%) in second – degree burns. These bacteria considered opportunistic bacterial pathogen primarily associated with hospital-acquired infections, While *Klebsiella pneumonia* recorded higher growth rates (66.6%) in third – degree burns, than in – second burns (33.3%), because it can display high degrees of virulence and antibiotic resistance. Figure (4-14) showed that .

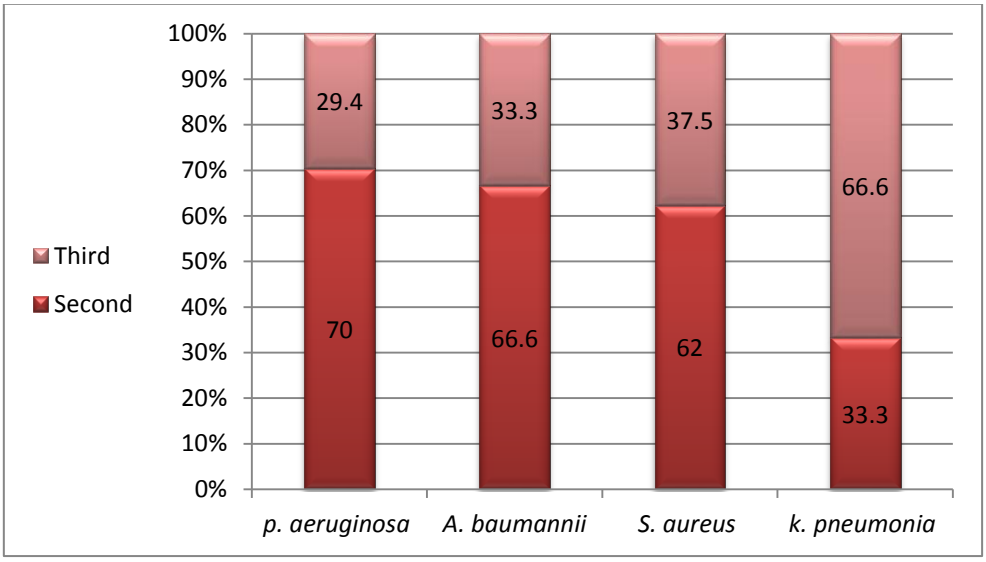

Figure (4-14): Degree of burns, bacteria types among patient states

Previous several studies recorded, each of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Staplylococcus aureus* can cause hospital-acquired infections (Howard *et al.*, 2012; Cornejo-Juárez *et al.*, 2015; Ruiz, 2020). There are a relationship between burns and bacterial infection, Significant thermal burns cause immunosuppression, which makes burn victims more susceptible to infection (Church *et al.*, 2006). Also Pre hospital treatment on the scene is critical for decreasing the severity of severe burn injuries and protected against bacterial infections particularly those of the second and third degrees (He *et al.*, 2021).

Conclusions

and

Recommendations

Conclusions

1- These results may help us to assume that such microbes can be used to identify the perpetrator or the identity of the unknown burnt person as a forensic evidence.

2- This study generally found a significant prevalence of bacterial types include *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staplylococcus aureus*, and *klebsiella pneumonia*. Also a significant prevalence of fungal types like; *Candida albicans* and *Aspergillus niger*.

3- All isolated microbes pose a danger to infected patients lying in the burn unit, which increases the possibility of delayed healing or may lead to death.

Recommendations

1- These results highlight the need to impose medical procedures of sterilization and decontamination to reduce any potential risks of contamination of burn wounds for people with burns and to benefit from skin microbes as criminal evidence.

2- Recommended the molecular study on bacterial and fungal infection in burn patient.

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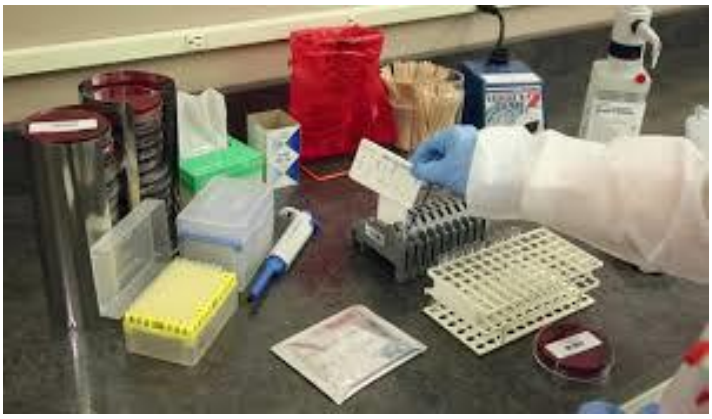
Appendixes

Appendixes

Appendixes (1): VITEK2compact system

Appendixes (2): An example of preparing VITEK2compact card



Appendixes (3): VITEK2compact card

Appendixes (4): An example of microbiology card report of selected organism: *Candida albicans* with their bio number.

bioMérieux Customer:
System #:

Laboratory Report

Printed Jun 14, 2020 10:02 CDT
Printed by: LabTech

Patient Name:
Isolate: 14621-3-1 (Qualified)

Patient ID:

Card Type: YST Bar Code: 2431067103422354 Testing Instrument: 0000148FF772 (Central lab)
Setup Technologist: Laboratory Technician(LabTech)

Bionumber: 4512544065327771
Organism Quantity:

Selected Organism: Candida albicans

Comments:	

Identification Information	**Card:**	YST	**Lot Number:** 2431067103	**Expires:**	Oct 28, 2020 13:00 CDT
	Completed:	Jun 14, 2020 04:53 CDT	**Status:** Final	**Analysis Time:**	17.77 hours
Organism Origin	VITEK 2				
Selected Organism	98% Probability		**Candida albicans**		
	Bionumber: 4512544065327771			**Confidence:**	Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					
Candida albicans	TyrA(17),				

Biochemical Details

3	LysA	-	4	IMLTa	-	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLYLa	+
13	TyrA	+	14	BNAG	-	15	ARBa	-	18	AMYa	-	19	dGALa	+	20	GENa	-
21	dGLUa	+	23	LACa	-	24	MAdGa	+	26	dCELa	-	27	GGT	-	28	dMALa	+
29	dRAFa	-	30	NAGA1	(-)	32	dMNEa	+	33	dMELa	-	34	dMLZa	-	38	ISBEa	-
39	IRHAa	-	40	XLTa	+	42	dSORa	+	44	SACa	+	45	URE	-	46	AGLU	+
47	dTURa	+	48	dTREa	+	49	NO3a	-	51	IARAa	-	52	dGATa	+	53	ESC	-
54	IGLTa	+	55	dXYLa	+	56	LATa	+	58	ACEa	+	59	CITa	+	60	GRTas	+
61	IPROa	+	62	2KGa	+	63	NAGa	+	64	dGNTa	+						

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 1

Appendixes (5): An example of microbiology card report of selected organism: *Klebsilla pneumonia* ssp. *pneumonia* with their bio number.

bioMérieux Customer:

Microbiology Chart Report

Printed Feb 15, 2020 11:12 CST

Patient Name:
Location:
Lab ID: 7621-2

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	4.07 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Klebsiella pneumoniae ssp pneumoniae** 6605734653564010		
ID Analysis Messages				

Susceptibility Information	**Analysis Time:** 9.18 hours			**Status:** Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Amikacin	32	I	Minocycline	8	I
Aztreonam	>= 64	R	Pefloxacin		
Cefepime	>= 64	R	Piperacillin	>= 128	R
Ceftazidime	>= 64	R	Piperacillin/Tazobactam	>= 128	R
Ciprofloxacin	>= 4	R	Rifampicin		
Colistin			Ticarcillin	>= 128	R
Gentamicin	8	I	Ticarcillin/Clavulanic Acid	>= 128	R
Imipenem	>= 16	R	Tobramycin	8	I
Meropenem	>= 16	R	Trimethoprim/Sulfamethoxazole	>= 320	R

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

AES Findings		
Confidence:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	CARBAPENEMASE (+ OR - ESBL),IMPERMEABILITY CARBA (+ESBL OR +HL AmpC)
	AMINOGLYCOSIDES	RESISTANT GEN TOB NET AMI

Appendixes (6): An example of microbiology card report of selected organism: *Staphylococcus aureus* with their bio number.

bioMérieux Customer: Microbiology Chart Report Printed Feb 15, 2020 11:02 CST

Patient Name: Patient ID:
Location: Physician:
Lab ID: 3621-4 Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.82 hours	**Status:** Final
Selected Organism	93% Probability **Bionumber:**	**Staphylococcus aureus** 010002073763221	
ID Analysis Messages			

Susceptibility Information	Analysis Time: 9.23 hours			Status: Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Cefoxitin Screen	POS	+	Teicoplanin	1	S
Benzylpenicillin	>= 0.5	R	Vancomycin	1	S
Oxacillin	>= 4	R	Tetracycline	>= 16	R
Gentamicin	>= 16	R	Tigecycline	<= 0.12	S
Tobramycin	>= 16	R	Fosfomycin		
Levofloxacin	4	R	Nitrofurantoin	32	S
Moxifloxacin	2	R	Fusidic Acid	>= 32	R
Inducible Clindamycin Resistance	NEG	-	Mupirocin		
Erythromycin	>= 8	R	Rifampicin	>= 32	R
Clindamycin	>= 8	R	Trimethoprim/Sulfamethoxazole	160	R
Linezolid	2	S			

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

AES Findings		
Confidence:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	MODIFICATION OF PBP (mecA)
	MACROLIDES/LINCOSAMIDES/STREPTOGRAMINS	MLSB+SA CONSTITUTIVE

Appendixes (7): An example of microbiology card report of selected organism: *Acinetobacter baumanni* with their bio number.

bioMérieux Customer:

Microbiology Chart Report

Printed Feb 15, 2020 11:05 CST

Patient Name:
Location:
Lab ID: 20621-2

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Acinetobacter baumannii

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.83 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Acinetobacter baumannii** 0241011103500212		
ID Analysis Messages				

Susceptibility Information	Analysis Time: 7.53 hours			Status: Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
ESBL			Imipenem	<= 0.25	S
Ampicillin			Amikacin		
Piperacillin/Tazobactam	<= 4	S	Gentamicin	<= 1	S
Cefazolin	>= 64	R	Ciprofloxacin	<= 0.25	S
Cefoxitin			Levofloxacin	<= 0.12	S
Ceftazidime	4	S	Tigecycline	<= 0.5	S
Ceftriaxone	8	S	Nitrofurantoin		
Cefepime	2	S	Trimethoprim/Sulfamethoxazole	<= 20	S
Ertapenem					

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

AES Findings	
Confidence:	Consistent

Appendixes (8): An example of microbiology card report of selected organism: *Aspergillus niger* with their bio number.

bioMérieux Customer:
System #:

Laboratory Report

Printed Jun 14, 2020 10:02 CDT
Printed by: LabTech

Patient Name:
Isolate: 14621-3-1 (Qualified)

Patient ID:

Card Type: YST Bar Code: 2431067103422354 Testing Instrument: 0000148FF772 (Central lab)
Setup Technologist: Laboratory Technician(LabTech)

Bionumber: 4512544065327771
Organism Quantity:

Selected Organism: Aspergillus niger

Comments:	

Identification Information	**Card:** MOL		**Lot Number:** 2431067103	**Expires:**	Oct 28, 2020 13:00 CDT
	Completed: Jun 14, 2020 04:53 CDT		**Status:** Final	**Analysis Time:**	17.77 hours
Organism Origin	VITEK 2				
Selected Organism	97 % Probability **Aspergillus niger**				
	Bionumber: 4512544065327771			**Confidence:**	Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					
Candida albicans	TyrA(17),				

Biochemical Details

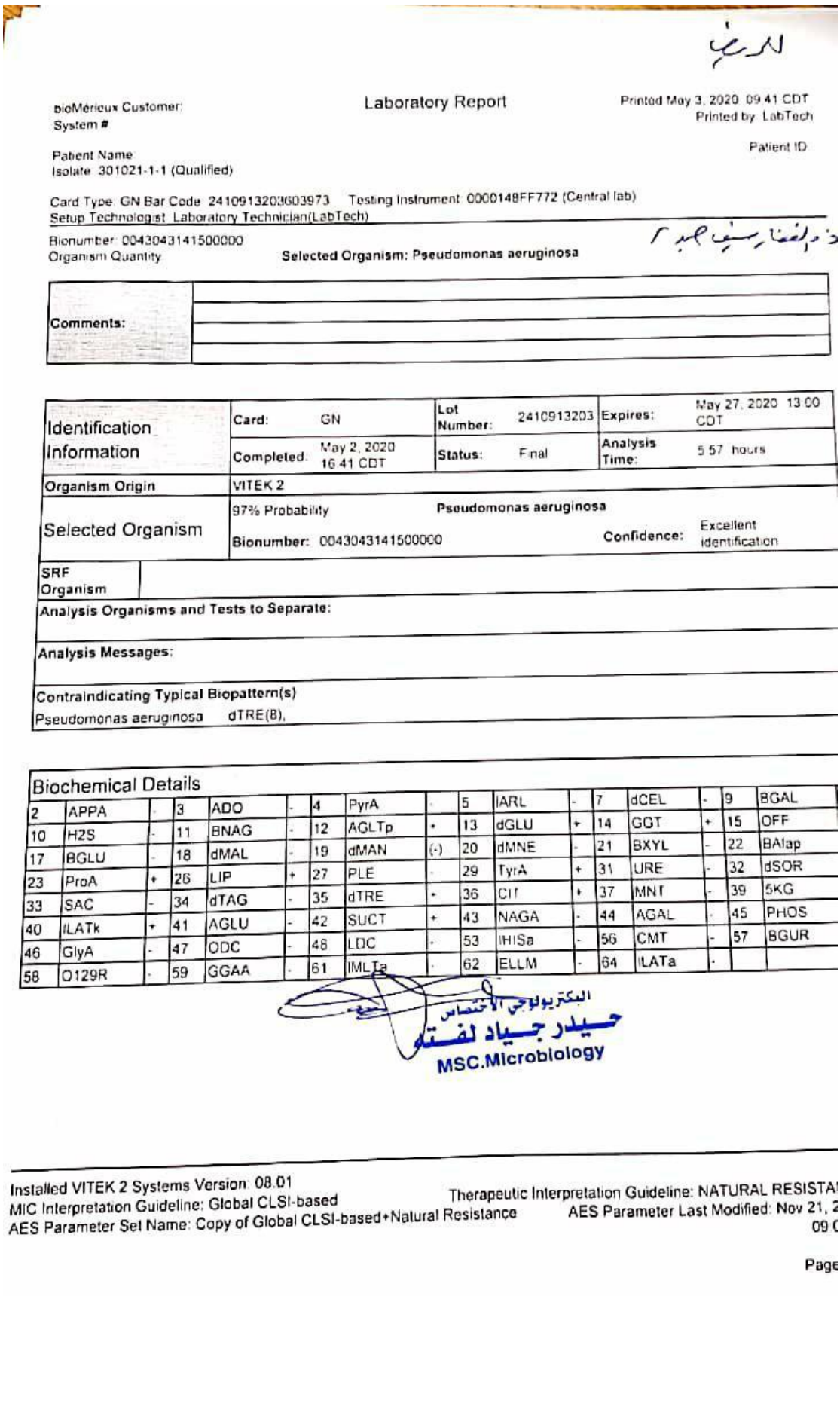
3	LysA	-	4	IMLTa	-	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLYLa	+
13	TyrA	+	14	BNAG	-	15	ARBa	-	18	AMYa	-	19	dGALa	+	20	GENa	-
21	dGLUa	+	23	LACa	-	24	MAdGa	+	26	dCELa	-	27	GGT	-	28	dMALa	+
29	dRAFa	-	30	NAGA1	(-)	32	dMNEa	+	33	dMELa	-	34	dMLZa	-	38	ISBEa	-
39	IRHAa	-	40	XLTa	+	42	dSORa	+	44	SACa	+	45	URE	-	46	AGLU	+
47	dTURa	+	48	dTREa	+	49	NO3a	-	51	IARAa	-	52	dGATa	+	53	ESC	-
54	IGLTa	+	55	dXYLa	+	56	LATa	+	58	ACEa	+	59	CITa	+	60	GRTas	+
61	IPROa	+	62	2KGa	+	63	NAGa	+	64	dGNTa	+						

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 1

Appendixes (9): An example of microbiology card report of selected organism: *Pseudomonas aeruginosa* with their bio number.



bioMérieux Customer:
System #

Laboratory Report

Printed May 3, 2020 09:41 CDT
Printed by LabTech

Patient Name
Isolate 301021-1-1 (Qualified)

Patient ID

Card Type: GN Bar Code: 2410913203603973 Testing Instrument: 0000148FF772 (Central lab)
Setup Technologist: Laboratory Technician(LabTech)

Bionumber: 0043043141500000
Organism Quantity

Selected Organism: Pseudomonas aeruginosa

Comments:	

Identification Information	**Card:**	GN	**Lot Number:**	2410913203	**Expires:**	May 27, 2020 13:00 CDT
	Completed:	May 2, 2020 16:41 CDT	**Status:**	Final	**Analysis Time:**	5:57 hours
Organism Origin	VITEK 2					
Selected Organism	97% Probability		**Pseudomonas aeruginosa**			
	Bionumber:	0043043141500000			**Confidence:**	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						
Pseudomonas aeruginosa dTRE(8),						

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL			
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF			
17	BGLU	-	18	dMAL	-	19	dMAN	(-)	20	dMNE	-	21	BXYL	-	22	BAlap			
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR			
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG			
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS			
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR			
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-					

البكتريولوجي الاختصاص
حيدر جياد لفته
MSC.Microbiology

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
AES Parameter Set Name: Copy of Global CLSI-based+Natural Resistance

Therapeutic Interpretation Guideline: NATURAL RESISTA
AES Parameter Last Modified: Nov 21, 2
09 (

Page

الخلاصة

تعد الحروق من أكثر الإصابات التي تتم معاينتها طبيا في المستشفيات والعيادات الطبية، وتحدث نتيجة تعرض الجلد للحرارة المرتفعة لأسباب عديدة . تضمنت الدراسة عزل الميكروبات الجلدية عن طريق اخذ المسحة الجلدية للأماكن المحروقة من الجلد لـ 59 من المصابين بحروق تتراوح أعمارهم من 1-60 سنة من المختبر المركزي / النجف الأشرف للفترة من 1/4/2021 ولغاية 31/7/2021 وعلى اوساط تشخيصية تفريقيه ومن ثم تم تشخيصها بالطرق البيوكيماوية واستخدام عدة اختبارات للتشخيص وتأكيدها بواسطة جهاز VITIK 2 compact system لمعرفة الانواع البكتيرية والفطرية التي تستعمر الجروح المحروقة.

كان جميع المرضى يعانون من درجات الحروق الثانية والثالثة وكان اغلبهم من النساء بمعدل يفوق ما هو عليه الحال بالرجال اما بالنسبة للفئة العمرية كانت الاعمار 21 – 30 سنة هي النسبة الاعلى من الاشخاص المحروقين قيد الدراسة مقارنة ببقية الفئات العمرية .

تم تشخيص 40 عزلة بكتيرية كان من بينها 17 عزلة من بكتريا *Pseudomona aeruginosa* و12 عزلة من بكتريا *Acinetobacter baumannii* و 8 عزلات كانت لبكتريا *Staplylococcus aureus* بينما كانت هناك 3 عزلات لبكتريا *klebsiella pneumonia*. كما تم تشخيص 24 عزلة فطرية كانت من نوعين من الفطريات هما: *Aspergillus nigar* وكان عدده 16 عزلة *Candida albicans* وعددها 8 عزلات.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم
قسم علوم الحياة

الاحياء المجهرية في مرضى الحروق كدليل جنائي

بحث مقدم إلى مجلس كلية العلوم / جامعة بابل كجزء من متطلبات نيل درجة الدبلوم العالي في العلوم / الأدلة الجنائية

من قبل
ايمان مهدي شاكر رزوقي
بكالوريوس علوم حياة / جامعة الكوفة
(1998-1997)

بأشراف
أ.د. ابتهال معز عبد المهدي الحسيني

1443هـ 2021 م