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Detection of virulence genes of *staphylococcus aureus* and *candida albicans* with some immune paramrters responces in patients with Impetigo infections

A thesis

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بسم الله الرحمن الرحيم

﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ﴾

صدق الله العلي العظيم

(المجادلة: ١١)

Dedication

I dedicate the profit of my effort and work to the absent present in our hearts to the Imam of the era and time, Imam Mahdi, may God hasten his honorable reappearance.

To the one who gave me everything he possesses so that I may fulfill his hopes , to my father, may God prolong his life.

To the soul that gave me life to my mother, may God have mercy on.

To the one who was patient with everything, to the one who nurtured me with all care and was my support and whose prayers for success followed me step by step in my work, who sent in my soul determination and perseverance to my support in my loneliness and my candle in the darkness of my path to my beloved sister.

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Summary

A total of 120 clinical specimens were collected in the current study from patients attending the medical consultation department, dermatology unit at (Marjan Medical City) in Babylon Province , For the period from September 2022 to December 2022.

The current study divided into two part, First part deal with isolation and identification of bacteria and yeast causing agent by collecting of media swap of infecting area then culturing on differential culture media like (Mannitol Salt Agar, Sabouraud Dextrose Agar and Blood Agar base) and study of some virulence facter like (*ALS1* gene, *αINT1* gene, *Spa* gene, *Mce* gene) by molecular method through extracting genomic DNA for PCR technique aditionally to the antibiotic susceptibility test of the microbes .

The second part of this study highlight for estemenation of level TNF and IgE by Elisa technique, so collecting blood sample to seperate serum through centerfugation at 3500rpm for 10 min.

These clinical specimens distributed into Impetigo (74 cases) (61.7%) , Ecthyma (40cases) (33.3%) and Bullous (6 cases) (5.0%). 66.7% infection were in less than 20 years and 15.0% in age between (20-30) years. Both males and females have the same number of cases . For Impetigo, there are 37 cases in both genders, accounting for 30.8% of the total cases each. Ecthyma is reported in 18 cases among males (15%) and 20 cases among females (16.6%). Bullous impetigo is found in 5 cases (4.2%) for males and 3 cases (2.5%) for females. It was found that the most susceptible areas of the body to infection is the face (23.3%), foot (28.3 %), gluteus (6.7%), femoral (18.3%), fand (15.0%) fead(8.3%).

Pathogens were diagnosed using direct examination, culture on differential media, and biochemical tests. *Staphylococcus aureus* (18%), , *Staphylococcus epidermidis* (9%) , *Staphylococcus saprophyticus*(10 %) ,

Psedomonas. *aeruginosa* (7%) , *Streptococcus* (14%) , *Candida albican* (18%) *Candida species* (17%).

The results show that 40% of *Staphylococcus aureus* isolates were strong biofilm 24% were intermediate while 36% were weak formation of biofilm. the result shows the percentage intensity of strong Biofilm formation for *Candida albicans* were 52% strong , 20% intermediate, 16% weak and 12% negative biofilm formation.

Staphylococcus aureus isolate were tested against six different antibiotics sensitivity , The were sensitive to (Azithromycin, Gentamicin, Vancomycin, Levofoxacin), except with (Tetracyctine, Amoxicillin) that there are a non- significant relationship at p-value 0.05.

The isolates of *candida albicans* were tested against six different antifungal drugs: (Fluconazole, Ketoconazole, Nystatin, Amphotericin B, Itraconazole, and Clotrimazole) that there is a significant relationship between C. *albicans* and antifungal sensitivity at p-value 0.05.

In this research, a comprehensive analysis was conducted on 25 *Staphylococcus aureus* was to detect the presence of *mecA* and *spa* genes. all the clinical isolates showing a 100% (25 isolates) result of *mcA* gene while spa gene showed a positive result by 56% (14 isolates).

A comprehensive analysis was conducted on 22 *Candida albicans* . The aim was to detect the presence of *INT1* and *ALSI* genes. all the clinical isolates showing 81.8 %(18 a isolates) negative result of *INT1* gene while *ALSI* gene showed a positive result by 68.2% (15 isolates).

The study found that there is a highly significant difference in IgE levels among the groups under investigation (Impetigo, Ecthyma, and Control). The p-value of 0.000 means that the difference in IgE levels between Impetigo and the other groups is highly significant.

presents the evaluation of TNF (Tumor Necrosis Factor) levels in a study population, categorized into three groups: Impetigo, Ecthyma, and Control. the study suggests that there is a highly significant difference in TNF concentration between the impetigo group and the control group ($p \leq (0.05)$.

the correlation between two immunological markers, Tumor Necrosis Factor (TNF) and Immunoglobulin E (IgE), in three different groups within the study population (Impetigo, Ecthyma, and control).The correlation analysis suggests that there is no statistically significant correlation between TNF and IgE concentrations in any of the three groups studied

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List of Abbreviations

Abbreviation	Key
AD	Atopic Dermatitis
Agr	Accessory gene regulator
AIDS	Acquired Immunodeficiency Syndrome
AZM	Azithromycin
BHI	Brain Heart Infusion
BI	Bullous Impetigo
Clfs	Clumping Factors
CLSI	Clinical Laboratory Standard Institute
CNS	coagulase-Negative *Staphylococci*
CoPS	coagulase-positive *Staphylococci*
CP5	capsular polysaccharides type 5
CP8	capsular polysaccharides type 8
CRA	Congo Red Agar
CWA	cell wall-Anchored proteins
ECM	Extracellular Matrix
EG	Eosinophilic Gastritic
EPS	Extracellular polymeric substance
ETs	Exfoliative Toxins
FnBPA	Fibronectin-Binding protein A
FnBPB	Fibronectin-Binding protein B
GABHS	Group A Beta Haemolyic Streptococcus
GEN	Gentamicin
GI	gastrointestinal
GPI-CWPs	Glycosyl -Phosphatidylinositol-Cell Wall Proteins
Hla	Hemolysin Toxin
HysA	Hyaluronidases
ICUs	Intensive Care Unit

Ig	Immunoglobulin
LAB	Lactic Acid Bacteria
Mce	Mammalian Cell Entrey
MDR	Multi-Drug Resistance
MHA	Mueller-Hinton Agar
MRSA	Methicillin-Resistant *S. aureus*
MSA	Mannitol Salt Agar
MSCRAMM	Microbial Surface Component Recognizing Adhesive Matrix Molecules
MSSA	Methicillin-Sensitive *S. aureus*
NAC	Non-*albicans Candida*
NBI	Non-Bullous Impetigo
PBP	penicillin-Binding Protein
PBS	Phosphate Buffer Solution
PVL	Panton-Valentine Leukocidin
SAgS	Superantigens (SAgs)
SAPs	Secreted A*spar*tyl Sroteins
SCCmes	*Staphylococcal* Cassette Chromosome mec
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth Medium
SEIs	*Staphylococcal* Enterotoxin-like Toxins
SEs	*Staphylococcal* Enterotoxins
SEs	Enterotoxins
Spa	*Staphylococcal* protein A
SPR	solid phase receptacle
SSTIs	Skin and soft tissue infections
TNF	Tumor necrosis Factor
TNFR1	TNF receptor 1
TSST-1	Toxic Shock Syndrome Toxin-1
VISA	Vancomycin - Sensitive *S. aureus*

VRSA	Vancomycin - Resistant *S. aureus*
VWF	Von Willebrand Factor

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Chapter One
Introduction

1.1. Introduction

Skin infections are among the most common infections worldwide. They range in severity from minor, self-limiting, superficial infections to life-threatening diseases requiring all the resources of modern medicine (Olaniyi *et al.*, 2017). There are several type of the skin infections that affect the skin ,which impetigo, Ecthyma, Bullous impetigo, folliculitis, Boils and carbuncles and others (Sukumaran and Senanayake, 2016).

Impetigo is a highly contagious bacterial infection of the surface layer of the skin. Impetigo usually begins as erythematous macules on the skin, which rapidly develop into a thin, fluid-filled vesicle that becomes purulent, ruptures, and forms a thin, honey-colored crust (Bangert *et al.*, 2012; Miller *et al.*, 2022).

It is most prevalent in children aged 2-5 years old but can occur at any age. The peak incidence is during summer and fall. Bullous impetigo is more common in infants. Children younger than two account for 90% of cases of bullous impetigo. Impetigo accounts for approximately 10% of skin diseases in children. When considering all age groups, the incidence is the same in males and females. In adults, it is more common in men (Breyre and Frazee, 2018; Dayrit *et al.*, 2018; Sahu and Mishra, 2019).

Impetigo can be classified as either primary or secondary. Primary impetigo involves previously normal skin affected by direct bacterial invasion. Secondary impetigo involves infection forming at a previous skin wound site. There are three types of impetigo: non-bullous, and bullous and ecthyma , Nonbullous impetigo is most commonly caused

by *Staphlococcus aureus* which is responsible for 80% of cases. Bullous impetigo is caused almost exclusively by *S. aureus*. Sometimes a deep ulcerated infection may occur known as ecthyma, which is a complication of bullous impetigo (Nardi and Schaefer, 2017).

Most skin infections are caused by Gram-positive bacteria, most commonly *S .aureus* , which for more than 70% of all cases of skin and soft tissue infections (SSTIs) (Palit and Inamadar, 2010) the reason is that it has many virulence factors, such as its ability to secrete enzymes as an Coagulase enzyme, Haemolysin, superantigens , cytotoxins , protein A , Proteases and lipase (Moran *et al* ., 2019).

Biofilm is an important virulence factor in bacterial infections, estimated to be 80% of pathogenic bacteria involved in biofilm formation (Reffuveille *et al.,* 2017). Biofilm formation in *S. aureus* causes an increase in antibiotics resistance in chronic diseases, including osteomyelitis, endocarditis, and skin infection. The *S. aureus* biofilm structure is composed of polysaccharide, protein, and external DNA. Bacterial cells in the biofilm compose of resistant cells and exhibit antibiotic resistance (Parastan *et al.,* 2020).

Antibiotics since their discovery have a significant impact in reducing the rates of various infections for example Azthromycin, Gentamicin, Vancomycin, Tetarcycline and Levofloxacine, but the indiscriminate use of these antibiotics in treatment led to the emergence of antibiotic-resistant bacterial strains (Abdolmaleki *et al.,* 2019). The failure of antibiotics to kill the bacteria that cause skin diseases led to the resistance of these bacteria to antibiotics (Mascitti *et al.,* 2018) .

Candida is the most common cause skin infection, among the 200 known *Candida* species, only a few, including *C. tropicalis*, *C. parapsilosis*, and *C.* orthopsilosis, commonly found on healthy skin, can become pathogenic. *C. albicans* is the *Candida* species most often responsible for skin infections (Kühbacher *et al.,* 2017). Several physical and immunological factors influence *Candida* skin infections, which display a preference for occluded regions of skin where humidity and CO2 accumulate, and the skin is constantly experiencing friction. For example, such conditions can be found while infants are in diapers, where the combination of elevated pH and the presence of lipases and proteases from feces commonly leads to secondary *Candida* infections (Bonifaz *et al.,* 2016).

The use of Azoles as Fluconazole are used, leads to depletion of membrane ergosterols to inactivate the steric ATPase functions leading to impaired vacuolar acidification and ionotropic homeostasis (Zhang *et al.,* 2010). The fungal antigens class of polyenes and especially amphotericin B has long been considered the most effective fungal antibiotic administration. Amphotericin B is a fungicide, an external and internal antifungal drug with activity against *Candida*. and *Cryptococcus neoformans* and molds (Lemke *et al.,* 2005).

The immune response agenst skin infection like TNF and IgE . TNFα was named due to original research that in 1975 determined that TNFα causes hemorrhagic necrosis of tumors when found in high concentration . Later, it was discovered that TNFα was involved in a plethora of cellular processes and, more importantly, that it had paradoxical effects. It was initially described that TNFα was mainly produced by activated macrophages, monocytes, NK cells, T

lymphocytes, neutrophils and mast cells, but afterwards it was discovered that it was also expressed by non-immune cells like fibroblasts, endothelial cells, cardiac myocytes and neurons, among others (Falvo *et al* ., 2010). TNFα expression is induced transcriptionally by nuclear factor κB (NF-κB) , c-Jun, activator protein 1 (AP1) and nuclear factor associated with activated T lymphocytes (NFAT) (Mercogliano *et al* ., 2020).

Immunoglobulin E, like other immunoglobulins, is produced by B cells and plasma cells (usually) in response to an antigenic stimulus. The presence of interleukin (IL)-4 and IL-13 induce immunoglobulin class switching from other isotypes to IgE(Stone etal ., 2010).These 2 cytokines interact with receptors on the surface of B cells to initiate a signaling cascade mediated by Janus kinase 3 (JAK3) and signal transducer and activator of transcription 6 (STAT6).8 A second signal is required for class switching (Kelly and Grayson.,2016).

1.2 Aim of the Study:

Aims to evaluate the relationship between biofim formation of *Staphylococcus aureus* and *Candida albicans* with some virulance factor associated with antibiotic resistance, it was achieved by the following objectives :-

1- Isolation and identification of microbes causing skin infections, isolation and diagnosis of *Staphylococcus aureus* and *Candida* albicans by using cultural, biochemical characteristics.

2- Study of virulence factors for *Staphylococcus aureus* and *Candida* albicans .

3- Detection of the antimicrobial susceptibility of *Staphylococcus aureus* and *Candida* albicans for clinical isolates.

4- DNA extraction from clinical isolates (*Staphylococcus aureus* and *Candida* albicans) and molecular detection of risk gene by PCR technique.

5- Studying certain immunological parameters of patients with skin infections including IgE and TNF levels .

Chapter Two

Literature Review

2. Literature Review

2.1. Skin Infection

The skin is the largest organ of animal and human bodies, is the outermost and first line of defense against infectious diseases and is easily exposed to physical and chemical agents and different pathogens that cause a wide variety of infections and wounds (Mala *et al.*, 2021). The skin provides a remarkably good barrier against bacterial infections. Although many bacteria come in contact with or reside on the skin, they are normally unable to establish an infection. When bacterial skin infections do occur, they can range in size from a tiny spot to the entire body surface. They can range in seriousness as well, from harmless to life threatening (Van Wyk, 2016).

Generally, skin diseases are among the most frequently occurring illnesses in humans. Skin and subcutaneous disorders were the fourth leading cause of nonfatal disease burden worldwide in thy last decade (Hay *et al.*, 2014; Seth, 2017). The skin is an important physical barrier to the invasion of pathogens, it is colonized with a normal microbiome; the species of which vary depending on the location, and other factors such as the temperature, pH, the presence of moisture, sebum, salt and fatty acids(Lacey *et al* ., 2016). Skin and soft tissue infections (SSTIs) are clinical entities of variable presentation, etiology and severity that involve microbial invasion of the layers of the skin and underlying soft tissues. SSTIs range from mild infections, such as pyoderma, to serious life-threatening infections, such as necrotizing fasciitis. The minimum diagnostic criteria are erythema, edema and pain. The affected area may also become dysfunctional (eg, hands and legs) depending on the

severity of the infection. A patient's comorbidities (eg, diabetes mellitus and AIDS) can easily transform a normally mild infection into a rapidly advancing threat to life (Eron *et al.*, 2003).

2.2. Type of Bacterial Skin Infections

Bacterial skin infections represent one of the major healthcare issues affecting people worldwide (Tognetti *et al*., 2012). The skin microbiome is an ecosystem comprised of a multitude of microbial species interacting with their surroundings, including other microbes and host epithelial and immune cells (Laurice and Grice,2020). *Staphylococcus aureus* and *Staphylococcus epidermidis* may inhabit the human skin and other microbes (Skowron *et al*., 2021). Common infections of the skin such as impetigo and scabies represent a large burden of disease globally, being particularly prevalent in tropical and resource-limited settings (Taiaroa *et al*., 2021).

Microbial diversity is reduced in Atopic Dermatitis(AD) and inversely correlates with diseas e severity. Skin commensal microbes, including coagulase-negative *Staphylococci* (CoNS), may aid skin homeostasis and provide protection against *S. aureus*. Thus, the diminution of commensal skin microbiota with flares may promote *S. aureus* colonization and infection in AD. During flares of paediatric AD, both *Staphylococcus* epidermidis and *S. aureus* are increased, suggesting a compensatory role for S. epidermidis (Alexander *et al.*, 2020).

The impaired skin barrier in AD is characterized by reduced very-long-chain epidermal lipids, defective tight junctions, differentiation in protein deficiency (including from filaggrin loss-of-function mutations), enhanced protease activity and increased skin-surface pH. This impaired

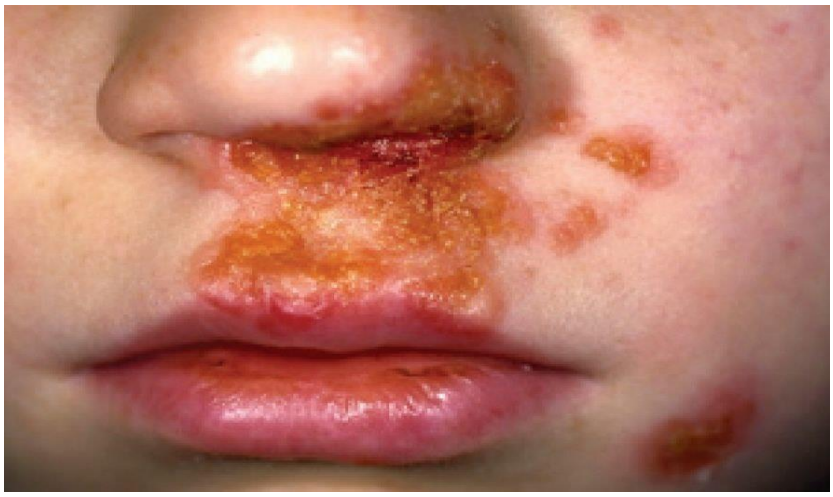
barrier provides a favourable environment for *S. aureus* colonization (Alexander *et al.,* 2020). The most common bacterial skin pathogens are *S. aureus* and *Streptococcus spp* (Scott, 2020).

The principal barrier against microbial invasion is the skin. It constantly interacts with the external environment and is colonized with a diverse population of microbes. The vast majority of colonizing flora consists of bacteria. To help organize the distribution of flora, one can divide the body into two halves at the waistline. The typical organisms that colonize the skin above the waist are usually Gram-positive species such as *Staphylococcus epidermidis* , *Corynebacterium* species, *S aureus* and *Streptococcus pyogenes. The* latter two species are particularly significant because they contribute to a majority of SSTIs. On the other hand, the typical organisms that colonize the skin below the waist are both Gram-positive and Gram-negative species. It is speculated that this difference is secondary to the proximity to the anorectal region. Enteric species, such as *Enterobacteriaceae* and *Enterococcus* species, gravitate to and colonize this area of the skin, the so-called 'fecal veneer' (Vincent and Coleman ,2008; Todar,2008).

2.2.1. Impetigo

Impetigo is a highly contagious, superficial bacterial skin infection that most commonly occurs in children 2 to 5 years of age typically because of damage to the cutaneous barrier(Cole and Gazewood, 2007 ; Davidson *et al.,* 2020). It is classified as a primary infection with direct bacterial invasion or a secondary infection (eg, in association with scabies or eczema) (D'Cunha *et al* .,2018; Nardi and Schaefer , 2017).

Impetigo clinically presents as a bullous or non-bullous type. Non bullous impetigo is the most common form of impetigo and is caused by *S. aureus* in 80% of cases and group A β-hemolytic *Streptococcus* alone or in combination with *S aureus* (Cole and Gazewood, 2007; Nardi and Schaefer, 2017).



2.2.2. Bullous Impetigo (BI)

Bullous impetigo is a common skin infection of early childhood, resulting from by *Staphylococcus aureus* exfoliative toxins. Due to compromised barrier function and immune dysregulation children (Mannschreck *et al.*, 2020). Bullous or blistering diseases are caused by a variety of traumatic, infectious, inflammatory, and autoimmune conditions (Horlings *et al.*, 2020). Bullous impetigo is almost exclusively caused by *S.aureus* (Cole and Gazewood, 2007; Hartman, 2014).

2.2.3. Ecthyma

Ecthyma is a form of ulcerative impetigo which erodes through epidermidis and dermis. Mostly, the crusts are found on the lesions, and when the crust is removed, there is a purulent ulcer at the bottom

(Karaaslan *et al.*, 2022). Ecthyma is often referred to as deep impetigo because it extends into the dermis. It begins with a small, pus-flled blister and red border, which eventually leaves a crusty ulcer underneath. Ecthyma is characterized by purulent, shallow ulcers with a punched-out appearancee. Overlying the ulcer is a thick‹ brown-black crust and surrounding erythema (Potter *et al*., 2016).

2.3. *Staphylococcus aureus*

Domain : Bacteria

Phlum : Bacillota

Class: bacilli

Order: Bacillales

Family: Staphylococcaeae

Genus: Staphylocceus

Species: aureus

Staphylococcus aureus is a nosocomial bacterium causing different infectious diseases, ranging from skin and soft tissue infections to more serious and life-threatening infections such as septicaemia. *S. aureus* forms a complex structure of extracellular polymeric biofilm that provides a fully secured and functional environment for the formation of microcolonies, their sustenance and recolonization of sessile cells after its dispersal (Muhammad *et al.*, 2021). In addition, it is belonged to the genus *Staphylococcus*, which is a genus of the family called Staphylococcaceae. *S. aureus* is one of the most significant human bacterial pathogens, causes some of the most severe infections in both

hospital and community settings. It causes a diverse range of clinical disease, with mortality from infection reported as high as 35% (Peleg *et al.*, 2012; Zhao., 2019).

Staphylococci organisms were first observed in human pyogenic lesions by Von Recklinghausen in 1871. Pasteur obtained liquid cultures of cocci from pus and produced abscesses by inoculating them into rabbits in 1880. Sir Alexander Ogston, a Scottish surgeon in 1880 also established conclusively the role of cocci as a causative agent of abscesses and other suppurative lesions in various animal species. He also gave the name *Staphylococcus* (Staphyle, in Greek means "bunch of grapes," Kokkos, means "berry") due to the typical occurrence of the cocci in grape like clusters in pus and in cultures. Ogston had noticed that non-virulent *Staphylococci* were also present on skin surfaces. The *S. aureus* is responsible for many infections ranging from relatively mild to life-threatening. Infection can be classified as a purulent or toxic-mediated disease. *S. aureus* can be recovered from almost any clinical specimen which is an important cause of nosocomial infections. And it is continuing to increase in importance as a community-acquired pathogen, drug resistance increases a concern with this common isolation (Mustapha *et al.*, 2014; Achmit *et al.*, 2021).

2.3.1 General Characteristics of *Staphylococcus aureus*

The *S. aureus* is a gram-positive organism with aerobic to facultative anaerobic lifestyle and colonizes skin, nose, and axillae of humans. *S. aureus* is a catalase-positive organism with most strains secreting coagulase and it ferments mannitol sugar to lactic acid. Testing for catalase is an important criterion to distinguish *Staphylococci* from

Streptococci and coagulase test for distinguishing *S. aureus* from *S epidermidis*. *S. aureus* is a non-spore forming coccus. It may be found singly, in pairs, in short chains, or in irregular clusters. The colonies are circular, smooth, and glistening. *S. aureus* is a major resident or transient colonizer of the skin and the mucosa of human and primates. These organisms occasionally live on domestic animals, although domestic animals are usually colonized by other species of *Staphylococci*. When *S.aureus* gains entry into the host, it causes variety of infection, from mild skin infection to life threatening invasive infections (Peacock, 2006; Reddy *et al.*, 2017).

This bacteria form round colonies and grows at an appropriate temperature of 37 C° although it may grow between 10 C° and 46 C°. The species is selective anaerobic with enhanced growth in the presence of CO2 and O2 (Talaro *et al.*, 2013). *S. aureus* is salt durable, which can thrive (grow) with 7.5% NaCl on mannitol salt agar also it is oxidase negative and positive to catalase and commonly produce extracellular polysaccharides capsule (Arumugam *et al.*, 2018).

2.3.2. Pathogenesis of *Staphylococcus aureus*

This bacteria can cause a wide variety of diseases ranging from mild skin and soft-tissue infections to severe systemic infections in human and animals (Wang *et al*., 2018) . Each year in the United States, about 400,000 patients in hospitals become infected with *S. aureus*, about 100,000 of whom die from complications caused by their infections (Haque *et al.*, 2018) , and is exhibits increasing virulence and resistance to various antibiotics, complicating prevention and treatment of infection (Holtfreter *et al.*, 2016).

It also has a great ability to cause opportunistic infections that vary from relatively simple skin infections to life-threatening systemic diseases for example**:** osteomyelitis, bacteremia, pneumonia, meningitis, and a variety of toxin-mediated diseases, especially when appropriate conditions are available for them, such as the presence of a defect in the immune defense, injuries to the skin, infection with other pathological organisms such as viruses, and the presence of chronic diseases such as cancers (Muñoz-Gallego *et al.,* 2020) .

The pathogenesis of *S. aureus* is caused by many virulence factors and their mechanisms into invasion and inflammation, which include colonization, synthesis of extracellular molecules, which promote adherence, and the ability to avoid host defenses; secreted virulence factors such as toxins (Al-Mebairik *et al.,* 2016). *S. aureus* causes several forms of human infections and syndromes, especially infections of the skin and soft tissue (Kobayashi *et al.,* 2015).

The *S. aureus* infection process involves five stages. They are

(1) colonization

(2) local infection

(3) systemic spread and/or sepsis

(4) metastatic infection

(5) toxinosis

The organism is in a carrier state in the anterior orifices and can remain so without causing infections for weeks or months (Farhadi *et al.*, 2019).

2.3.3 Virulence Factor of *Staphylococcus aureus*

To cause infection, a bacterium needs to first gain access to the host. This is preceded by attaching to the host cells or tissues. *S. aureus* has numerous surface proteins that promote attachment to host proteins such as laminin and fibronectin that form part of the extracellular matrix (Gordon *et al.*, 2021). The *S. aureus* expresses up to 25 different cell wall-anchored (CWA) proteins, which are covalently linked to the peptidoglycan layer by the enzyme sortase A and are primarily involved in adhesion and invasion of host cells and tissues, biofilm formation and immune evasion (Foster *et al.*, 2014). Several virulence factors are associated with the pathogenicity of *S. aureus* strains, including the production of extracellular enzymes, such as lipases, proteases, amylases, hyaluronidase, DNases, coagulase and lactamase; the production of several toxins and enterotoxins, such as β, G and δ haemolysins; and also the production of surface components, such as the capsule(Dinges *et al.*, 2000 ; Greig *et al.*, 2007).

2.3.3.1. Biofilm

A crucial biological tactic that enables bacteria to resist attacks from the host and other threats in a hostile environment to the production of three-dimensional structures termed ‘biofilms’ (Stoodley *et al.*, 2002; Kannappan *et al.*, 2020). *S. aureus* engages itself in the formation of biofilms on distinct surfaces and play significant roles in chronic infections in humans (Lister and Horswill, 2014). *S. aureus* prevails in biofilm formation on biotic (e.g., host tissue) or abiotic substrata, especially indwelling biomedical devices. To date, *S. aureus* has been reported on various biomedical materials, such as endotracheal tubes,

dental prostheses or implants, intravenous catheters, vascular prostheses, intrauterine devices or urinary catheters, cardiac pacemakers, contact lenses, prosthetic joints, and prosthetic heart valves (Pinto *et al.*, 2019).

To produce biofilms in a biological niche, the population of *S. aureus* follows a sequence of steps: (i) adhesion ; (ii) irreversible microcolonization; (iii) propagation of 3D biofilm; (iv) maturation; and (v) dispersion (Kostakioti *et al.*, 2013; Srinivasan *et al.*, 2021). Biofilm is a microbially community characterized by cells that are irreversibly attached to a substratum or to each other, embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription (Lamret *et al.*, 2020).

Bacterial biofilms are made up of a range of bacteria (polysaccharide, peptidoglycan, and DNA) and host (cell debris, coagulation products, and DNA) components and substances. The composition of biofilms varies according to the causative organism and/or host conditions. *S. aureus* in biofilms is highly resistant to antimicrobial activity as well as the actions of both the innate and adaptive immune defense systems, potentially leading to chronic infections and therapeutic failure (Lister and Horswill, 2014; Suresh *et al.*, 2019). *S. aureus* utilizes a number of strategies to impede antimicrobial killing and subvert the host immune system including, secreted proteases, surface factors, and biofilm development (Bhattacharya *et al.*, 2015; Ricciardi *et al.*, 2018).

Biofilm-associated *S. aureus* cells have been reported to have four different metabolic states, such as, they can either be growing

aerobically, can be fermentative, can be dormant, or can even be dead (Archer *et al.*, 2011). Besides the extracellular polymeric matrix that shelters the cells against antibiotics, the dormant and metabolically slow growing cells have also been reported to add to antimicrobial resistance (Lister and Horswill,2014). Moormeier and Bayles (2017) reported that *S. aureus* cells encased in a biofilm grow at different rates, such as, some cells grow at a faster rate as compared to other cells within the same biofilm. These cells are smaller and attain their normal size once released upon the dispersal of the biofilm.

2.3.3.2. Capsule

The *S.aureus* bacterium is surrounded by capsular polysaccharides. These capsular polysaccharides are important in the pathogenesis of *Staphylococcal* infection. There are 11 serotypes of capsular polysaccharides that have been identified, and a majority of strains express capsular polysaccharides type 5 (CP5) or 8 (CP8) (Visansirikul *et al.*, 2020). The capsule enhances *Staphylococcal* virulence by impeding phagocytosis, resulting in bacterial persistence in the bloodstream of infected hosts (Riordan and Lee, 2004).

2.3.3.3. Superantigens

Major classes of secreted virulence factors include superantigens Superantigens are commonly secreted by *S. aureus*; however, superantigen genes have rarely been detected in select coagulase-negative *Staphylococci*, including *Staphylococcus* epidermidis, *Staphylococcus* warneri, and *Staphylococcus* haemolyticus (Vasconcelos *et al* ., 2011 ; Pinheiro *et al* 2015). Superantigens play critical roles in the development and progression of disease (Bien *et al* ., 2011).

Superantigens include toxic shock syndrome toxin-1 (TSST-1), *Staphylococcal* enterotoxins (SEs), and *Staphylococcal* enterotoxin-like toxins (SEls) (Tuffs *et al.*, 2018).

2.3.3.4. Cell Surface Proteins

The surface of *S. aureus* is decorated with over 20 proteins that are covalently anchored to peptidoglycan by the action of sortase A. These cell wall anchored (CWA) proteins can be classified into several structural and functional groups (Foster,2023). Cell wall-anchored (CWA) proteins are characterized by the presence of a sorting signal at the C-terminus which is responsible for coupling the protein covalently to peptidoglycan. The surface of S. *aureus* is decorated with up to 24 CWA proteins. The precise number depends on the strain and the growth conditions. The repertoire of CWA proteins expressed by *S. aureus* is limited and many have evolved to perform important interactions with the host. They can be categorized into distinct structural and functional groups (Ponnuraj *et al.*, 2003; Foster *et al.*, 2014).

1-Staphylococcal Protein A (*Spa*)

Protein A (*Spa*) a key virulence factor involved in immune evasion and a potential vaccine antigen, is in 62% of isolates (Zukancic *et al.*, 2020). It is a conserved, multifunctional surface protein of *S. aureus*. Its N-terminus contains five tandemly linked triple-helical bundle domains, which are important for binding to IgG and other ligands, such as TNF receptor 1 (TNFR1) and Von Willebrand Factor (VWF) (Foster *et al.*, 2014).

The *Spa* is also a B cell superantigen that binds to the VH3+ immunoglobulins on the surface of B cell receptors, causing clonal expansion, which results in apoptosis (Lacey *et al.*, 2016). The *Spa* precursor has an N-terminal signal peptide that is cleaved prior to the secretion of the mature protein. Mature *Spa* has four to five highly conserved immunoglobulin (Ig) binding domains connected by short linkers at the N-terminus (Tam and Torres., 2019).

2-Fibronectin Binging Proteins

Fibronectin-Binding Protein A (FnBPA) of *S. aureus* is a microbial surface component recognizing adhesive matrix molecules and has been known as one of the most important virulence factors involved in the initiation step of *S. aureus* infection (Narita *et al.*, 2017)

The bacterium *S. aureus* strains express two CWA fibronectin (Fn)-binding adhesins FnBPA and FnBPB (Fn-binding proteins A and B), which are encoded by closely related genes (Speziale and Pietrocola., 2020). FnBPs mediate biofilm formation, including by clinically relevant resistant strain (Herman *et al.*, 2017).

***3-Staphylococcus aureus* Exotoxins**

large group of exotoxins are also secreted by *S. aureus*, including highly inflammatory cytolysins (mainly α, β, γ, and δ toxins) and superantigens (SAgs) such as enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1) (Li *et al.*, 2011).

4-Clumping Factor A (ClfA)

The ClfA is the major fibrinogen binding protein of *S. aureus*, and it binds to the C-terminal region of the fibrinogen γ-chain, this can result

in platelet aggregation or clumping of bacteria in plasma. ClfA is an important virulence factor of *S. aureus* and its contribution to pathogenesis has been demonstrated in several animal models of infection including endocarditis, arthritis, and sepsis. ClfA also appears to play an important role in skin infection (McAdow *et al.*, 2011; Kwiecinski *et al.*, 2014).

2.3.3.5. *Staphylococcus aureus* Enzyme

A major contribution to the success of *S. aureus* as a pathogen is the plethora of virulence factors that manipulate the host's innate and adaptive immune responses. Many of these immune modulating virulence factors are secreted toxins, cofactors for activating host zymogens, and exoenzymes. Secreted toxins, such as pore-forming toxins and superantigens are highly inflammatory and can cause leukocyte cell death by cytolysis and clonal deletion, respectively. Coagulases and staphylokinases are cofactors that hijack the host's coagulation system. Exoenzymes, including nucleases and proteases, cleave and inactivate various immune defense and surveillance molecules, such as complement factors, antimicrobial peptides, and surface receptors important for leukocyte chemotaxis. Additionally, some of these secreted toxins and exoenzymes can cause disruption of endothelial and epithelial barriers through cell lysis and cleavage of junction proteins(Tam and Torres., 2019).

A unique feature when examining the repertoire of *S. aureus* secreted virulence factors is the apparent functional redundancy exhibited by the majority of the toxins and exoenzymes. However, closer examination of each virulence factor revealed that each has unique

properties that have important functional consequences. (Tong *et al.*, 2015; Kayan and Victor, 2019). The most important enzyme include.

1-Coagulase Enzyme

Coagulase is an enzyme that is produced by some types of bacteria. The enzyme clots the plasma component of the blood. The only significant disease causing bacteria of humans that produces coagulase is *S. aureus.*

The blood clotting enzyme (Coagulase) plays a major role in the blood clotting process (Kmieciak and Szewczyk, 2019). *S. aureus* secretes two forms of coagulase enzyme, such as, bound coagulase and free coagulase. The coagulase enzyme, encoded by the *coa* gene, is an important virulence factor of *S .aureus* and can be used for typing of *S. aureus* isolates (Mahmoudi *et al.*, 2017).

2- Catalase Enzyme

Catalase is an enzyme present in the cells of aerobic (oxygen requiring) bacteria. In order to survive, *S. aureus* has many defense mechanisms such as catalase enzyme which facilitates cellular detoxification (Reiner, 2010).

3- Nucleases

Staphylococcal nuclease, also known as micrococcal DNase, is an enzyme produced by *S. aureus*. Ca+2 ions are required for nuclease activity, whereas other divalent cations are not. Thermonuclease is another name for *Staphylococcal* nuclease, which is named after its resistance to heat inactivation. Activity as an exonuclease and an endonuclease Contributes to the eluding of neutrophil extracellular traps

and the decomposition of host tissue into nutrients essential for bacterial growth (Berends *et al*., 2010).

Staphylococcal nuclease catalyzes the hydrolysis of both DNA and RNA at the 5’ position of the phosphodiester bond yielding a free 5’-hydroxyl group and a 3’-phosphate monoester. The pH optimum is between 8.6 and 10.3 and varies inversely with Ca+2 concentration, but at any pH rather high levels of Ca, typically (Hu *et al.*, 2012).

4- Proteases

The *S. aureus* produces a variety of secreted proteases that not only serve as virulence factors by cleaving *Staphylococcal* surface proteins, degradation of host tissue, and modulation of the host immune response but also aid in nutrient acquisition from the host. The proteases encoded by *S. aureus* include two cysteine proteases, staphopain A (ScpA) and staphopain B (SspB) (Kolar *et al.*, 2013; Pietrocola *et al.*, 2017).

Primary function of the extracellular proteases of *S. aureus* is to control the progression of infection by selectively modulating the stability of virulence factors (Gimza *etal.*, 2019)

5- Lipases

Lipase enzyme belongs to the hydrolases and is also known as fat splitting, glycerol ester hydrolase or triacylglycerol acylhydrolase. Lipase catalyzes the hydrolysis of triglycerides converting them to glycerol and fatty acids in an oil-water interface (Javed *et al.*, 2018).

This enzyme degrades lipid of the skin tissues which helps them in its spread Lipase degradation facilitates *S. aureus* to colonize the sebaceous glands (Chakraborty, 2005).

6- Hyaluronidases (HysA)

Bacterial hyaluronidases (known as hyaluronate lyases) are a class of enzymes that degrade hyaluronic acid (Hart *et al.*, 2013). Hyaluronidase enzyme is an extracellular enzyme that is considered to be an important virulence factor for *S. aureus*. Hyaluronidases have been found to be virulence factors that are involved in the invasion and penetration of tissues in many of Gram-positive bacteria. *S. aureus* is able to infect many tissues which contain high HA concentrations using HysA as a virulence factor (Abdelkader *et al.*, 2018).

2.3.3.6. *Staphylococcus aureus* Toxin

1- Hemolysins

The *S. aureus* encodes α-, β-, γ-, and δ-Hemolysins, which are mostly used to lyse erythrocytes by generating pores in host cell membranes or dissolving cell wall components and are regulated by the Accessory gene regulator (Agr). The Hla gene encodes a virulence factor that causes harm to a wide range of host cells, including epithelial cells, endothelial cells, erythrocytes, monocytes, and keratinocytes, as well as cell membrane damage and death (Divyakolu *et al.*, 2019). This toxin on its own is not considered toxic, it is its bonding capability and oligomerization into a heptameric structure on the host cell membrane that makes it dangerous (Oliveira *et al.*, 2018). The type of Hemolysins:

A- α-Hemolysin (Hla) toxin

Most *S. aureus* strains release this pore-forming toxin, which is predominantly made up of beta sheets, as a water-soluble monomer that targets red blood cells (Otto,2014).

B- Hemolysin-β (Sphingomyelinase C)

This toxin has shown to be highly hemolytic towards erythrocytes. The difference in susceptibility for erythrocytes may be due to the different sphingomyelin contents of these cells since the toxin is also known as sphingomyelinase. Sphingomyelinases are phosphoric diester hydrolases that cleave sphingomyelins, the most abundant sphingolipid in eukaryotic membrane (Oliveira *et al.,* 2018).

C- δ –Hemolysin

The δ-hemolysin not only lyses erythrocytes but also other mammalian and Binding of neutrophils and monocytes (Verdon *et al*, 2009; Zecconi and Scali, 2013).

2-Valentine Leucocidin

Panton-Valentine leukocidin (PVL) is a cytotoxin produced by some strains of Panton *Staphylococcus aureus.* These strains are responsible for primary skin infections and necrotizing pneumonia ((Duployez *et al.,* 2020).

3- Exfoliative Toxins (Ets)

Exfoliative toxins (ETs) are one of the important virulence factors that participating in *Staphylococcal* scalded skin (Mahmoudi *et al.*, 2020). Exfoliative toxins (ETs), also known as epidermolytic toxins, are

serine proteases that identify and hydrolyze desmosome proteins in the skin and are released by *S. aureus* (Bukowski *et al.*, 2010).

4-Enterotoxins

The *S. aureus* enterotoxins are a superfamily of secreted virulence factors that share structural and functional similarities and possess potent superantigenic activity causing disruptions in adaptive immunity (Fisher *et al.*, 2018). hese toxins are produced by 30% to 50% of *S. aureus* isolates (Schelin *ET AL.*, 2017).

2.3.3.7. Some *Staphylococcus aureus* Virulence Genes

Thirty-six common virulence genes of *S. aureus* were detected by PCR amplification, including 11 adhesion associated genes. (*bbp, clfA , clfB, can , ebps, eno, fib, fnbA, fnbB, icaA, and icaD*),12 enterotoxin genes *sea , seb , sec , sed, see, seg, seh, sei, sej , sem, sen*, and *seo*), and 13 other , virulence genes (*hla, hlb, hld, hlg, hlgv, lukM, lukED, pvl, psmα, tst, eta, etb and edin*) (Jiang *et al*., 2017).

1- *mec A* Gene

The *mec* gene complex contains one of two genes that have been identified as encoding methicillin resistance. The *mec* gene complex contains *mecA*, a gene that encodes an altered penicillin binding protein (PBP2a) in the vast majority of MRSA lineages (Ward *et al.*, 2016).

The *mecA* gene is a 2.1 kb exogenous DNA fragment carried on the *Staphylococcal* cassette chromosome mec (SCCmec), a mobile genetic element that inserts at site-specific positions on the *Staphylococcal* chromosome and is acquired through horizontal gene transfer (Piette and Verschraegen, 2009).The identification of the *mecA*

gene is the most reliable method for detecting methicillin-resistant strains of *S. aureus* (Becker *et al*., 2018).

2- *Spa* Gene

The *spa* gene is the most widely used for *S. aureus* typing, which is based on repeats of the hypervariable X region in the *spa* gene (Mayerhofer *et al.,* 2015). Protein A is a surface protein bound to the peptidoglycan of the *S. aureus* cell wall, *Spa* gene that encodes protein A is a virulence factor of *S. aureus* , which has polymorphism properties and can be used for strain typing, the gene that encodes for protein A (*spa*) is the most widely used marker for molecular typing because it contains polymorphic units. *Spa* genes are also a good choice to be able to identify and distinguish *Staphylococcus aureus* strain variability (Yunita *et al*., 2020).

The gene encoding this protein (*spa*) consists of two regions, one encodes the Fc- binding domain, and the other encodes X region. Virulence mediated by *spa* is not only by binding to the Fc of the Ig preventing normal phagocytosis, but also by binding to the Fab site of the B cell receptor blocking the production of antibody specific for *S. aureus* and provoking B cell death .This Fc-binding region, the so-called X-region of protein A gene (*spa*) (Keener *et al* ., 2017)**.**

2.3.4 Antibiotic Susceptibility in *Staphylococcus aureus*

Many antibiotics were given against *S. aureus* infection but eventually, the spread of Multi-Drug Resistance (MDR) from *S. aureus* began to appear (John *et al.,* 2019). Clinically relevant opportunistic pathogens like *S. aureus* have acquired resistance against multiple

antibiotics such as Penicillin, Methicillin, and Vancomycin. Methicillin-resistant *S. aureus* (MRSA) has become a major problem worldwide and is increasingly being detected in both hospitals and communities (Chambers and DeLeo, 2009).

In addition, they exhibit resistance to many antibiotics, with Methicillin-resistant *S. aureus* (MRSA) strains being resistant to most β-lactam antibiotics as well as may also be resistant to other classes of antibiotics such as Fluoroquinolones, Aminoglycosides and Tetracyclines; resistance has also been reported to last-resort drugs for resistant *S. aureus* infections such as Vancomycin, a Glycopeptide and inducible-Clindamycin-resistant *S. aureus* strains are increasingly reported (McGuinness *et al* ., 2017).

Infections caused by strains resistant to three or more classes of antimicrobial agents, also known as multidrug-resistant (MDR) strains, are increasingly difficult to treat (Abubakar and Sulaiman, 2018).

S. aureus is resistant to penicillin due to beta-lactamase production enzymes. There are two primary mechanisms of beta-lactamase resistance: the expression of a unique Penicillin-binding protein (PBP2a or PBP2) in the cell wall is encoded by the *mecA* gene responsible for the increased level Beta-lactam resistance, including penicillinase-resistant antibiotics such as methicillin. Second, expression of the betalactamase enzyme encoded by the blaZ gene hydrolyzed beta-lactamases such as Penicillin. Methicillin resistance confirms resistance, to all beta-lactams antibiotics such as Carbapenems and Cephalosporins. This also leads for greater resistance of *Staphylococcus* to other antibiotics such as Erythromycin, Tetracycline and intermediate strains

appear to be resistant Vancomycin (Thakurla and Lahon, 2013; Jafari-Sales *et al.*, 2018).

Vancomycin resistance was very clear observed, its transmission among the population and the rise of resistant strains was it has become a major threat globally (Mukherjee *et al.*, 2021). Vancomycin remains one of the first-line drugs for the treatment of MRSA infections. However, *S. aureus* isolates with complete resistance to vancomycin have emerged in recent years. Vancomycin-resistant *S. aureus* (VRSA) is mediated by a *vanA* gene cluster, which is transferred from Vancomycin-resistant enterococcus. Since the first *VRSA* isolate was recovered from Michigan, USA in 2002, 52 VRSA strains have been isolated worldwide (Cong *et al.*, 2020).

Another antibiotic is Azithromycin (AZM) is a broad-spectrum antibiotic that has been shown to be effective against gram-positive, gram-negative, and atypical bacteria (Ikemoto *et al*., 2020). It is widely used in clinical practice. It has been recognized that AZM exerts not only anti-bacterial activity but also anti-inflammatory effects, which are related to the NF-κB pathway (Steel *et al.*, 2012).

Tetracyclines are broad-spectrum antibiotics used in the treatment and prevention of bacterial infections and can be used for treatment of *S. aureus* caused infections such as skin and soft tissue infections (SSTIs) (Esposito *et al.*, 2009). Two main mechanisms of resistance against Tetracyclines have been identified in *S. aureus*: active efflux, which is mediated by plasmid encoded tetK and tetL genes and ribosomal protection that is encoded by chromosomal or transposonal tetM or tetO genes. It has been shown that *S. aureus* isolates harboring the tetK gene

are resistant to Tetracycline but not Minocycline while the tetM gene confers resistance to both of them (Khoramrooz *et al.,* 2017).

Gentamicin (GEN) belongs to the aminoglycoside family, which, aside from Spectinomycin, is the only ribosome inhibitor known to cause protein mistranslation (Li *et al.,* 2019). GEN inhibits the function of bacterial ribosomes, and results in bacterial death. In particular, GEN can bind 16S rRNA on the 30S subunit of bacterial ribosome, interfere with the connection between formyl-methionyl-tRNA and 30S rRNA, and block the synthesis of bacterial protein. Nevertheless, the repetitive usage usually causes high level of resistance, and compromises the therapeutic efficiency of GEN. Therefore, it is urgently needed to explore new strategies to treat bacteria and MDR bacteria. Gentamicin (GEN) was introduced in the 1970s to combat serious nosocomial infections, showing broad-spectrum antimicrobial activity and still being used in preclinical and clinical studies (Kashef and Hamblin, 2017).

Amoxicillin-resistant S*taphylococcus.* strains were designated by the Department of Medical Sciences, Ministry of Public Health, Thailand. Human *S.aureus* is more resistant to Amoxicillin than *S. aureus,* and *Staphylococcus aureus* was used as a positive contro (Siriwong *et al.,* 2016).

Levofloxacin is a Fluoroquinolone antibacterial agent with a broad spectrum of activity against Gram-positive and Gram-negative bacteria Levofloxacin is active against *Staphylococcus aureus,* The different classes of antibiotics are used to improve the treatment of MDR bacterial infections (Anderson and Perry, 2008).

2.4. *Candida* spp

The genus of *Candida* includes above 150 species (Neppelenbroek *et al.*, 2014), the taxonomy of *Candida* spp. is (Toylor, 2006):

Kingdom:Fungi
Phylum:Ascomycota
Subphylum:Ascomycotina
Class:Ascomycetes
Order:Saccharomycetales
Family:Saccharomycetaceae
Genus: *Candida*

The name *Candida* was suggested by the Dutch mycologist “Christine Berkhout” in 1923. The specific *epithet albicans* also comes from Latin word, albicare meaning"to whiten". These names refer to the commonly white form of *Candida* species when cultured (Barnett, 2004; Obladen,2012).The *Candida* is the yeast that a common commensal microorganism in human body, it has the ability to cause infection in the presence of supporting conditions for its growth or other risk factors such as reduced immune responses (Press *et al.*, 2014).

The *Candida* species are between the most common etiological agents of fungal infections in humans (Brown *et al.*, 2012). *Candida* species have different macroscopic and microscopic features. The macroscopic features of *Candida* clusters are cream to yellowish in color. The consistency of the colony differs from one species to another and can be dry, smooth, pale, shining, cloudy or wrinkled. Furthermore, microscopic features also differ among *Candida* species, while all

species produce blastoconidia, either individually or in a cluster. Only some *Candida* species are capable to form true hyphae and chlamydospores, whereas most species produce pseudohyphae (Eggimann *et al.*, 2003; Campbell and Johnson, 2013 and Pryszcz *et al.*, 2015).

More than 200 species of *Candida* have been described and around 65% of them are not pathogenic as they are unable to grow at normal human body temperature and the rest are found to be opportunistic human pathogens (Silva *et al.*, 2012).

However not all *Candida* are pathogenic due to many reasons. Firstly, the *Candida* is an opportunistic pathogen and a consistent part of the human microbiota. When immune function is suppressed, *Candida* spp, may create acute infectious process in shorter period than in the case of an external infection source. Secondly, within the genus *Candida*, there are many potentially pathogenic species that vary significantly in the specifics of the infectious process and may possess a wide spectrum of potential drug resistance. Thirdly, in the last few years, there has been an alarming increase in the formation and spread of new multi- and cross-resistant strains. This is usually due to the acquisition of resistance by the most frequent etiological agents of *Candida* diseases, such as *C. albicans*, but cases of acquired resistance in other *Candida* species have also been documented. For example, climate changes may create selective pressure for evolving fungal strains that are inherently more resistant to human body temperature, which has been shown for *C. auris* (Casadevall *et al.*, 2019). The relative rates of infection among all *Candida* infections, more than 90% of reported invasive infections are

associated with *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis* (Pfaller *et al.*, 2010).

2.4.1. *Candida albicans*

The first description of a yeast infection was of thrush, by Hippocrates in the fifth century B.C. Since its first microscopic detection in thrush swabs by Langenbeck, subsequently Berg and Gruby in 1839 (Ainsworth, 2002).

Candida albicans is the most common causative agent both of mucosal and systemic infection, and it is responsible for about 70% of fungal infections around the world (Morad *et al.*, 2018). It has been the leading cause of lifethreatening invasive infections for the past several decades. Despitetreatment, the mortality rate is close to 40%, especially in hospital conditions (Basmaciyan *et al.*, 2019; Chen *et al.*, 2020). *Candida albicans* is a diploid polymorphic yeast of human mucosal surfaces, a fungus that commonly found in the gastrointestinal (GI), respiratory, and urogenital tracts. It is generally commensal but able to turn into an opportunistic pathogen in immunocompromised or immuno-deficient individuals. It is the major species causing invasive candidiasis (46.3%) (Andes *et al.*, 2016).

One reason leading to overgrowth of *C. albicans* is its ability to respond to a myriad of environmental imbalances such as changes in nutrition, temperature, and pH (Pfaller and Diekema, 2007). Other important factors that increase the risk of *C. albicans* infection are prolonged treatment with broad spectrum antibiotics, various diseases such as diabetes, trauma, and other genetic disease or congenital

malformation (Dadar *et al.*, 2018).

2.4.2. *Candida glabrata*

Candida glabrata is often the second most common cause of *Candida* infections after *C. albicans*. *Candida glabrata* causing (24.4%) of invasive candidiasis (Andes *et al.*, 2016). *C. glabrata* infections can be mucosal or systemic and often occur in immunocompromised persons or people with diabetes (Fidel *et al*., 1999). In contrast to most *Candida* species, *C. glabrata* is not dimorphic and exists as small blastoconidia under all environmental conditions both as commensal and pathogenic (Dadar *et al.*, 2018).

Historically, *C. glabrata* strains were originally classified in the genus *Torulopsis* by Lodder and de Vries in 1938 due to its lack of filaments forms formation (Yarrow and Meyer,1978). However, in 1978 it was determined that the ability to form hyphae and/or pseudo hyphae was not a reliable distinguishing factor of members of genus *Candida* species, and it was proposed that *Torulopsis glabrata* should be classified in the genus *Candida*, due to its human pathogenicity (Banerjee *et al*.,1991). *C. glabrata* isolates are often associated with high resistance to the azole class of antifungal agents and less susceptibility to most other antifungal agents including amphotericin B (Vazquez *et al*., 1998).

2.4.3. *Candida parapsilosis*

The *C. parapsilosis* is often the third most prevalent cause of candidemia behind *C. albicans* and *C. glabrata* in (8.1%) (Andes *et al*., 2016). *C. parapsilosis* has increased in significance and prevalence over the past 2 decades. The infections are mainly associated with prosthetic

devices and catheters, especially in the nosocomial spread. Risk factors of *C. parapsilosis* infections include the hydrolytic enzymes secretion, prosthetics adhesion, and biofilm formation (Trofa *et al.*, 2008).

However, in patients under 13 years of age, *C. parapsilosis* is commonly the second most prevalent with an incidence rate of 30-40% and some studies focused on single centers have determined *C. parapsilosis* to be the most significant cause of candidemia in pediatric intensive care units (Pappas *et al.*, 2003; San Miguel *et al.*, 2005 and Lewis, 2009). The *C. parapsilosis* is more commonly found on skin and under fingernails than on mucosal surfaces. This makes *C. parapsilosis* more of an exogenous organism and allows it to spread easily to patients via the hands of healthcare workers (Weems,1992; Waggoner-Fountain *et al.*, 1996; Pfaller, 1996; Kuhn *et al.*, 2004 and Sarvikivi *et al.*, 2005).

2.4.4. *Candida tropicalis*

The *Candida tropicalis* is another prevalent pathogen in *Candida* species. In immunocompromised human patients, *C. tropicalis* isolates appeared to have increased virulence. Invasive *C. tropicalis* infections were found more frequently in acute leukemia or bone marrow transplants patients may indicate that polymorph nuclear leukocytes are the first defense line against of *C. tropicalis*. Overexpression of *ERG11* gene mutations in *C. tropicalis* likely causes resistance to azoles (Kothavade *et al.*, 2010).

Currently, *C. tropicalis* is one the most relevant non-*albicans* *Candida* species (Silva *et al.*, 2011; Cavalheiro and Teixeira,2018). This pathogen has been frequently detected in patients from intensive care

units, mainly those under prolonged catheterization and treatment with broad-spectrum antibiotics (Silva *et al.*, 2012). *C. tropicalis* is highly prevalent in tropical countries and responsible for elevated mortality rate due to candidiasis (Godoy *et al.*, 2003; Ann Chai *et al.*, 2010 and Chander *et al*., 2013).

2.4.5. *Candida krusei*

The *Candida krusei* was first discovered in 1839 by Langenbeck from a patient with typhus, 75 years later Castellani proposed the suggestion that *C. krusei* may cause disease in humans (Samaranayake *et al*.,1994).

Candida krusei has two basic morphological forms, yeast and pseudohyphae and both are often present simultaneously in growing cultures and not easily separated. C. *krusei* grows at a 37C° but can withstand temperature up to 45C°. C. *krusei* can grow in vitamin-free media even though most common *Candida* spp. require biotin or additional vitamin for growth. C. *krusei* ferments and assimilates glucose only as carbohydrate (Samaranayake and Samaranayake, 1994).

2.5. *Candidal intertrigo*

The *Candidal intertrigo* refers to superficial skin-fold infection caused by the *Candida* yeast. *Candidal intertrigo* is triggered by a combination of the factors such as the hot and damp environment of skin folds which is conducive to the growth of *Candida* species particularly *C.albicans*, Increased skin friction, Immunocompromise. Clinical features of *Candidal intertrigo Candidal* is classically presents as erythematous and macerated plaques with peripheral scaling. Affected areas may include the Skin folds below the breasts or under the

abdomen, Armpits, groin, Web spaces between the fingers or toes (erosio interdigitalis blastomycetica) (Gray, 2010; Kalra *et al.*, 2014; Tüzün *et al.*, 2015).

2.6. Virulence Factor of *Candida Spp*

The virulence factors provide immune stimulatory factors, activating dendric cells and promoting T cell infiltration and activation. Targeting virulence factors, can reduce the risk of resistance development in *Candida* infections (Staniszewska, 2020).

2.6.1 Adhesion and Biofilm Formation

The first event in *Candida* infection is its adherence of the organism to host and/or medical-device surfaces, often leading to the formation of biofilms .Thus, adhesion is an extremely important step in the infection process, and the extent of adhesion is dependent on microbial, host and abiotic surface properties, such as cell-surface hydrophobicity and cell-wall composition(Silva *et al* ., 2011). biofilms formed by *Candida* species causing superficial and systemic fungal infections in immune compromised patients. These infections are very difficult to treat due to the characteristics of these species: resistance to antifungal drugs, expression of virulence factors, and the ability to form biofilm (Cavalheiro and Teixeira ,2018).

Biofilm formation although being a process present in all the *Candida* differs significantly from species to species, and in the dependency of surface, host niche and other factors. Such as, *C. albicans* mature biofilms exhibit a more hetero genous structure, composed by blastophores and hyphae surrounded by an ECM of polysaccharide

material, The ECM provides structural scaffold for adhesion between cells and with different surfaces, and a barrier between the cells in the biofilm and the neighboring environment (Mitchell *et al.*, 2016). In the case of C. *glabrata*, the biofilm is exclusively composed by yeast form cells in a multilayer structure intimately packed or in clusters of cells (Silva *et al.*, 2009). In turn, C. *tropicalis* biofilm corresponds to a network of yeast, pseudohyphae, and hyphae, with intense hyphal budding (Bizerra *et al.*, 2008). While C. *parapsilosis* exhibits a biofilm formed by clusters of yeast cells adhered to the surface, with minimal ECM (Lattif *et al* 2010).

These differences highlight the complexity of the processes underlying biofilm formation and the difficulty to find a unique way to eradicate all *Candida* biofilms (Nett, 2016). The first crucial step of biofilm formation is adhesion. This process relies on several cell wall proteins, called adhesins, that promote the attachment to other cells, both too epithelial and too other microbial cells, or abiotic surfaces by binding to specific amino acid or sugar residues. Generally, adhesins are glycosyl-phosphatidylinositol-cell wall proteins (GPI-CWPs), comprising a GPI anchor, a serine/threonine domain and a carbohydrate or peptide binding domain (Verstrepen and Klis, 2006).

After adhesion, biofilm development continues through morphologic modifications, increase in cell number and production of EPS, influencing the final biofilm architecture. Formed biofilms are also dependent on the EPS that are produced, which give a gel-like hydrated three-dimensional structure to the biofilm where the cells become partially immobilized. EPS plays different roles such as defense against

phagocytosis, and biofilm integrity and prevention of drug diffusion. The production of EPS is dependent on the species and strain the carbon source and the rate of medium flow. Fluid flow rate also affects biofilm formation, affecting EPS production, among other factors (Cavalheiro and Teixeira, 2018).

2.6.2 Hemolytic Factor

Hemolytic facter production by pathogenic *Candida* species is considered an important attribute in promoting survival within the mammal host through the ability to assimilate iron from the hemoglobin-heme group (Furlaneto *et al.,* 2018).

2.6.3 Virulence Genes of *Candida spp*

1- *αINT1* genes

α*INT1* is a virulence factor that contributes to the ability of the pathogen to adhere to epithelial cells(Abdul-Lateef *et al*., 2015). αINT1 is a unique gene from Candida albicans; hence, it has been used for detection for phylogenetic analysis of *C. albicans* (Osman et al ., 2019).

αINT1 gene has been reported to affect epithelial adhesion , filamentous growth, and virulence (Abdul-Lateef et al ., 2015).

2-*ALS* gene

The *ALS* gene family of Candida albicans encodes large cell-surface glycoproteins that are implicated in the process of adhesion to host surfaces. *ALS* genes are also found in other Candida species that are isolated from cases of clinical disease .The *ALS* gene family consists of eight members (*ALS1-ALS7* and *ALS9*), with *ALS1* and *ALS3* being the most studied. Distinct members of the *ALS* family are expressed during

the yeast and hyphal morphologies of *C. albicans*. The *ALS* genes are one example of a gene family associated with pathogenicity mechanisms in *C. albicans* and other *Candida* species(Hoyer,2001 ; Romo and Kumamoto ,2020)..

2.6.4. Treatment and Resistance

Fungal cells, like human cells, are eukaryotic; both cell types are targeted by antifungal compounds, resulting in considerable side effects in patients and fewer available targets for drug action. Since the 1990s, there has been an increasing, but limited, discovery of antifungal agents (Sardi *et al.*, 2013; Paramythiotou *et al.*, 2014). Treatment of *Candida* infections is often ineffective due to the global problem of resistance of human pathogens to antifungal drugs (Berman and Krysan,2020).

In the last years, there has been a decrease in the number of patent applications filed in this area. Between 1990 and 1999, there were six U.S. patents granted evidence of the evolution of drug resistance and an increasing mortality rate. In general, the discovery and development of drugs is challenging. The global market for antifungal drugs was worth $14.6 billion in 2019 and it is estimated to grow to $17.6 billion by 2024 (Berman and Krysan, 2020). These include five antifungal drug classes available to date for the treatment of *Candida* infections, including Azoles, Echinocandins, Polyenes, Flucytosine and Allylamines (Perlin *et al.*, 2017). There are also new generation of biogenic drugs it is probiotics that can use in treatment of *Candida* (Hill *et al.*, 2014).

2.6.5. Antibiotic Susceptibility in *Candida* spp

Diagnosis of antifungal-resistant *Candida* infections is critical to the successful management of patients with these infections. Reports on resistance to antifungal agents are relatively rare (when compared to antibacterial agents) but became much more common with the introduction of additional classes of antifungal agents, particularly the Azoles (especially Fluconazole), which have been widely used against *Candida* infections (Arendrup and Patterson , 2017).

There are several classes of antifungal and anti-*Candida* agents, and the Azoles are one of the most common drugs. They include several compounds such as Fluconazole, Intraconazole, and Viriconazole, which have the ability to inhibit the biosynthesis of Ergosterol, an important component of the plasma membrane of fungal cells. Azole therapy is still the first choice in many diseases caused by *Candida* (Rewak-Soroczynska *et al.*, 2021).

2.7. Immunological Parameter

2.7.1. Immunoglobulin IgE

The IgE an immunoglobulin produced primarily by B-cells and plasma cells, is an important mediator of allergic disease. Elevated IgE is commonly seen in patients with atopic dermatitis, food allergy, and asthma (Ponsford *et al.*, 2018). IgE receptors can be divided into two types, high-affinity IgE receptors (FcεRI) and low-affinity IgE receptors (FcεRII). The biologic functions of IgE depend on the binding of two Cε3 domains to FcεRI and FcεRII located in several target cells (Froidure *et al.*, 2015; Cheng, 2021). IgE is the least abundant immunoglobulin and tightly regulated, and IgE-producing B cells are

rare. The cellular origin and evolution of IgE responses are poorly understood (Hoof *et al.*, 2020).

Higher markedly elevated serum IgE above 2000 IU/ml tends to be associated with severe atopy driven by mutations of the epithelial skin barrier or inborn errors of immunity (Ponsford *et al.*, 2018; Stadler *et al.*, 2021).

The original hyper-IgE syndrome is characterized by diminished inflammatory response, in combination with *S.aureus* skin abscess and pneumonia followed by pneumatocele formation (Minegishi, 2021).

2.7.2. Tumor necrosis factor (TNF)

Tumor necrosis factor (TNF) is a member of the large family of cytokines; not hormones, but important local signaling molecules that transmit information from one cell to another. Different cytokines convey different messages, but cytokines are key players in every important biological process, including immunity, inflammation, cell growth, migration, fibrosis, vascularization, etc (Udalova *et al.*, 2017).

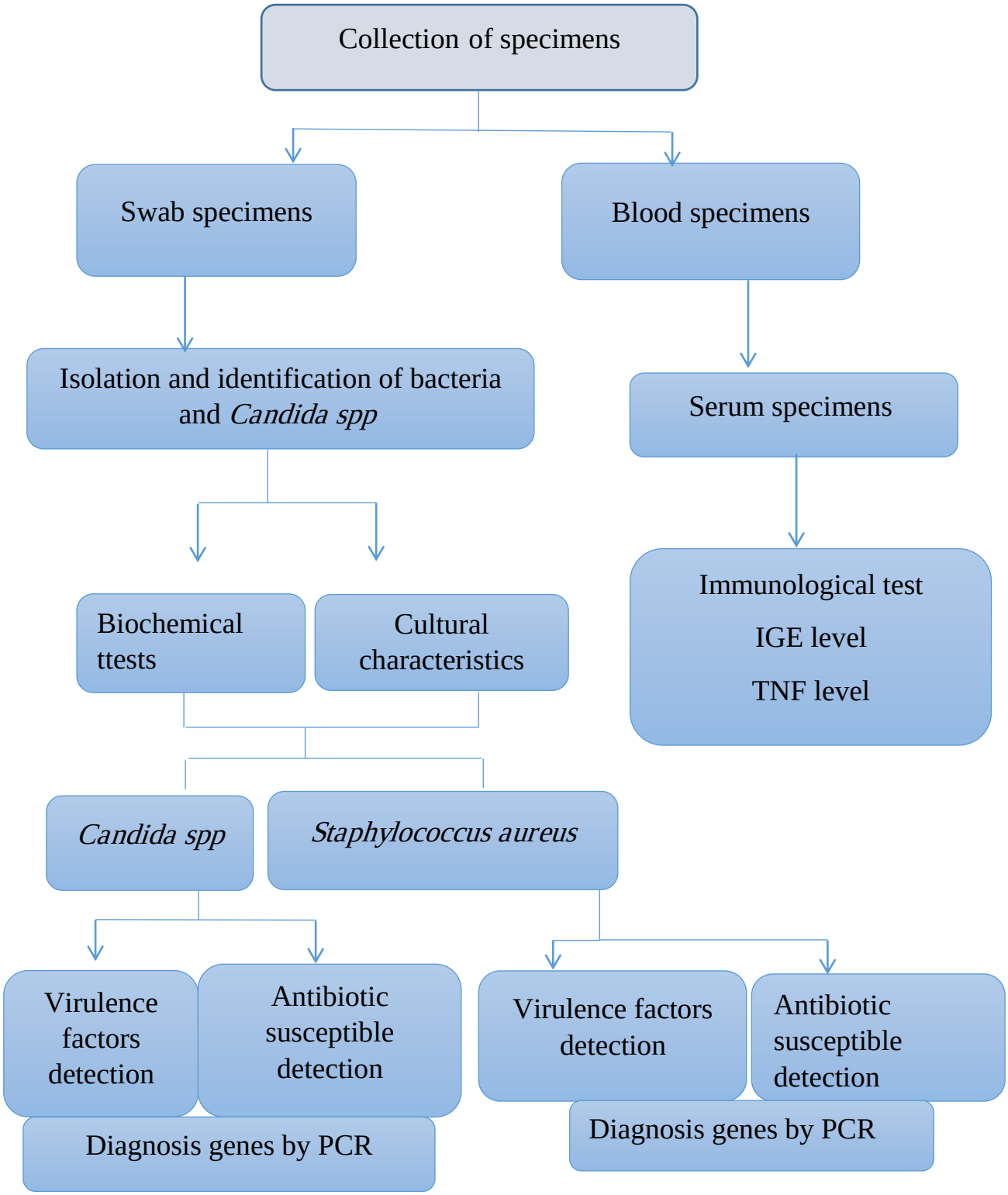
Biological activities attributed to TNF-α include: induction of pro-inflammatory cytokines such as interleukin-1 and interleukin-6, enhancement of leucocyte migration by increasing endothelial layer permeability and expression of adhesion molecules by endothelial cells and leucocytes, functional activation of neutrophils and eosinophils, and induction of acute-phase reactants and other liver proteins as well as tissue-degrading enzymes produced by synoviocytes and/or chondrocytes (Billmeier *et al*., 2016 ; Chaabo and Kirkham , 2015).

Structurally, TNF-α is a homotrimer protein consisting of 157 amino acids, mainly generated by activated macrophages, T-lymphocytes, and natural killer cells. It is functionally known to trigger a series of various inflammatory molecules, including other cytokines and chemokines. TNF-α binds to two different receptors, which initiate signal transduction pathways. These pathways lead to various cellular responses, including cell survival, differentiation, and proliferation. However, the excessive activation of TNF-α signaling is associated with chronic inflammation and can eventually lead to the development of pathological complications such as autoimmune diseases (Jang *et al.*, 2021).

Chapter Three
Materials and Methods

3. Materials and Methods:

3.1. Study scheme :this study was designed as a cross -sectional study as bellow



3.2. Materials

3.2.1. Instrumants and Equipment :The instrumants and equipment used in this study had been shown in Table (3-1).

Table (3-1): equipment used in present study .

NO	Equipment	Manufacturing company
1	Autoclave	Hariyama / Korea
2	Benzine burner	Iraq
3	Centrifuge	Labcco / Germany
4	Compound microscope	Olympus / Japan
5	Cool box	Tank / India
6	Cotton	Iraq
7	Disposable Gloves	TG Medical/ Malaysia
8	Disposable Syringes	Medico inject/ USA
9	Eppendorf tube1.5ml	Sigma /England
10	Ethanol 70%	Iraq
11	Flask (250-500) ml	Oxfords
12	Gel tube	Afco-Dispo (Jordan)
13	Incubator	Memmert/ Germany
14	Micro pipettes	Gillson instruments / France
15	petri dishes	Sterial (Jordan)
16	Plain tube	Afco-Dispo (Jordan)
17	Rack	Bioneer / Korea
18	Refrigerator	Concord / Korea
19	Sensitive balance	Denver / Swizer land
20	Slides	Japan
21	Standard loop	Labtech / India
22	Syringe	SUPER / China
23	Transport media Swab	China
24	Vortex mixture	Memmert/ Germany
25	Water distillatory	Gallenkamp /England
26	Water path	Gallen kamp / England
27	Yellow Tips	Afco / Jordon

3.2.2. Chemicals and Biological Materials:The chemical and materials used in this study had been shown in Table (3-2).

Table (3-2): - Chemicals and Biological Materials

NO.	Materials	Industrialization
1	Agar	Himedia/ India
2	Congo red dye	Himedia /India
3	Ethanol	BDH/ England
4	Glucose	BDA /England
5	Glycerol (C3H8O3)	Fluka/ Switzerland
6	Gram stain	Fluka/ Switzerland
7	Human blood	National Blood/ Bank (Iraq)
8	Human plasma	National Blood
9	Hydrogen peroxide H2O2	Fluka/ Switzerland
10	Normal Saline	Pioneer/Iraq
11	Phosphate buffer solution	Himedia /India
12	Proteinase k	Favorgen / Taiwan
13	Sucrose	Himedia /India
14	Tetracycline	Bionalyse /UK

3.2.3. Culture Media:The culture media used to conduct the experiments and tests in this study are listed in table (3-3).

Table (3-3): - Culture Media used in current study

No.	Media	Manufacture
1	Agar_Agar	Himedia / India
2	Blood Agar base	Himedia / India
3	Brain heart infusion agar	Oxoid / UK
4	Brain Heart Infusion Broth	Oxoid / UK
5	CHROM agar *Candida*	Liofilchem –Italy
6	Mackoncky Agar	Himedia / India
7	Mannitol Salt Agar	Himedia / India
8	Muller-Hinton Agar	Oxoid / UK
9	Nutrient Agar	Oxoid / UK
10	Nutrient broth	Oxoid / UK
11	Potato dextrose agar	Preparin laboratory
12	Sabouraud Dextrose Agar	Hi media-India
13	Sabouraud Dextrose Broth	Hi-media /India
14	Urea Agar Base	Himedia / India

3.2.4. Antibacterial and Antifungal Discs

The antibiotics discs presented were used for detecting the susceptibility of isolates according to the standard guideliues recommended by Clinical laboratory Standards Institute (CLSI).

Table (3-4): Type of Antibiotic Bacteria Used in this Study.

No.	Class	Antibiotic	Abbreviation	Con. disc / µg	Manufacture
1	β-Lactams antibiotic	Vancomycin	VA	30	Bioanalyse /Turkey
2		Amoxicillin	AMC	30	Bioanalyse /Turkey
3	Aminoglycosides	Gentamicin	CN	10	Bioanalyse /Turkey
4	Fluoroquinolones	Levofloxacin	LEV	5	Bioanalyse /Turkey
5	Macrolides	Azithromycin	AZM	15	Bioanalyse /Turkey
6	Medication	Tetracycline	TE	19	Bioanalyse /Turkey

Table (3-5): Type of Antibiotic Antifungal Used in this Study.

No.	Antifungal	Abbreviation	Dosage/ disc	Manufacture
1	Clotrimazole	CC	10 mcg	Hi-media /India
2	Fluconazole	FLC	10 mcg	Hi-media /India
3	Ketoconazole	KT	10 mcg	Hi-media /India
4	Itraconazole	IT	30 mcg	Hi-media /India
5	Amphotericin-B	AP	Mcg	Hi-media /India
6	Nystatin	NS	50 mcg	Hi-media /India

3.2.5. Immunological and Genetic material

Table (3-6): - List of Genetic Kits Used in current Study.

NO	Name	Company	Country
1	DNA extaction kit to extraction of Bacteria DNA	Favrogen	China
2	DNA extaction kit to extraction of *Candida* DNA	Favrogen	China

Table (3-7): - List of Immunological Kits Used in the Used in current study.

NO	Name	Company	Country
1	Human tumor necrosis facter (ELISA KIT)	BT LAB Bioassay technology laboratory	China
2	IgE Vidas	Biomerieux	France

Table (3-8): - Primers Used in the Current Study.

Primer	Sequence (5' → 3')	Size	Reference
ALS1	F:GACTAGTGAACCAACAAATACCAGA R: CCAGAAGAAACAGCAGGTGA	315bp	İnci *et al.*, 2013
αINT1	F: AAGCTCTGATACCTACACTAGCGA R: GTTAGGTCTAAAGTCGAAGTCATC	239bp	Shrief *et al.*, 2019
Spa	F: ATCTGGTGGCGTAACACCTG R: CGCTGCACCTAACGCTAATG	144-1392bp	Wichelhaus *et al.*, 2001
***mce A* gene**	F: TAGAAATGACTGACGTCCG R: TTGCGATCAATGTTACCGTAG	154bp	Geha *et al.*,1994

3.3. Patients and Specimens

3.3.1. Collection of Specimens:

Specimens were collected over a period of four months (from September 2022 to December 2022) total of (120) clinical specimens. Specimens were collected from patients attending the medical consultation department, dermatology unit at Marjan Hospital (Marjan Medical City) in Babylon Province. Specimens were taken from the patients under the supervision of the specialist doctor after the disease was diagnosed. Disease specimens were collected by medical swab, while blood specimens were collected in a Gel tube. Specimens were taken for different age groups, ranging from one year to 60 years. A questionnaire was used for each patient, as it contains some important information such as name, age, gender, antibiotics, and chronic diseases. The specimens were transferred to the college laboratory for the purpose of cultivation.

3.3.1.1. Inclusion Criteria:

The patients were enrolled in the current study Patients with bacterial skin infection were examined clinically and investigated in the laboratory; the diagnosis was established .

3.3.1.2. Exclusion criteria:

The patients were not enrolled in the current study if they have:

1.Solid organ transplant recipient (renal transplantation).

2.autoimmune skine disease and chronic disease patients.

3.3.1.3 .Ethical Approval:

The specimens of this study took the patient's approval for adult patients, precious and the Consent of the irrigation for young people in age as the

law and directives of the human rights organizations with adequate in formation in an ethical manner.

3.3.2. Specimens Culture:

Specimens were transferred in sterile transfer swabs and inoculated into selective culture medium of blood agar and mannitol salt agar using the direct inoculation method. Then they were incubated at 37°C for 14_18 hours (Cheesbrough, 2010). Growth was monitored before they were treated several times until pure isolates were obtained, after that a single pure isolated colony was transferred to Brain Heart Infusion broth for preservation and morphological evaluation by Gram staining and microscopy, and other biochemical tests that validate isolate identification.

3.3.3 Collection of Blood Specimens:

Three ml of blood were taken from patients with skin disease (Impetigo), then the blood was injected directly into a gel tube, and then placed in a centrifuge for five minutes, after which it was separated in an Eppendorf. The use of the cold box was transferred to transport specimens from the hospital and preserved at -20 C° for the purposes of later use in immunological tests.

3.4. Methods

3.4.1. Preparation of Reagent and Solutions

3.4.1.1. Gram Stain Solutions:

Gram staining is an important process by which bacteria can be identified, whether positive or negative bacteria. These solutions included four solutions: Crystal violate, Iodine, %96 ethanol, and safranin stain. It has been used to study the morphology and arrangement of cells, Gram- positive bacteria appear purple while Gram - negative

appear as pink/red. It has been done according to (Goldman and Lorrence, 2009**)**.

3.4.1.2. Phosphate Buffer Solution (PBS):

Dissolve 3.12 g in 90 ml of distilled water, add up to 100 ml with distilled water, and store until use.

3.4.1.3. Normal Saline Solution:

Normal saline was produced by adding 100 ml DW to 0.85g of sodium chloride NaCl until it completely dissolved (Freeman and Natanson, 2005).

3.4.1.4. MacFarland Standard Solution:

The microbial inoculum was standardized at a concentration of 0.5 MacFarland. MacFarland standards were used as a reference in microbiology to modify the turbidity of bacterial suspensions so that the number of bacteria remained within a defined range. Accuracy of the density of a prepared 0.5 MacFarland standard was checked by using a spectrophotometer. The absorbance of the wavelength of 625 nm should be between 0.08 and 0.1 (CLS1, 2020).

3.4.2. Preparation of Culture Media

3.4.2.1. Mannitol Salt Agar (MSA):

Mannitol salt agar was prepared according to the manufacturer 111 gm of suspension was dissolved in 1000 ml of distilled water. Sterilization by autoclaving, Distribute in Petri dishes and store at -4C° until use. Mannitol salt agar the plate is inoculated to isolate *Staphylococci*. (MSA) is both a selective and differential medium used in the isolation of *Staphylococci* (MacFaddin, 2000). It contains 7.5% sodium chloride and thus selects for those bacteria which can tolerate

high salt concentrations. MSA also distinguishes bacteria based on the ability to ferment the sugar mannitol, the only carbohydrate present in the medium (Shields and Tsang , 2006).

3.4.2.2. Blood Agar Base (BAB):

An enriched bacterial medium which encourages the growth of most types of bacteria. prepared as the manufacturer's instructions, 37.5 mg of blood agar base was applied to 1000 ml of distilled water and coated with cotton, then sterilized by autoclave After cooling to (45 - 50C°), add 5% blood to the volume of the prepare, mix until completely homogeneous , then pour into sterile petri dishes and harden until ready to use for culture (Forbes *et al* ., 2007).

3.4.2.3. Muller-Hinton Agar (MHA):

Prepared by mixing 38 gm in 1000 ml distilled water. It was used to test antibiotic resistance. The agar is non - selective and differential. It contains starch which is known to absorb toxins generated by bacteria (Forbes *et al.*, 2007).

3.4.2.4. MacCkonky Agar (MCA):

Prepared according to the manufacturer's instructions (5.1 grams of medium was suspended in 100ml of distilled water). Selective and differential media used to distinguish gram-negative bacteria and separate lactose-fermenting bacteria such as *E. coli*, *Klebsiella* spp., *Enterobacter* spp., which appear pink-colored, and non-lactose fermenting bacteria such as *Proteus* spp, *pseudomonas* spp, *Salmonella* spp and *Shigella* spp appearing in a pale or yellow colour (Carroll *et al.*, 2015).

3.4.2.5. Urease Agar Base (UAB):

It was prepared according to the manufacturer's instructions by dissolving 20 g of urea in 1000 mL of DW. This medium was used to test the bacteria's capacity to produce the urease enzyme (Brink, 2010).

3.4.2.6. Brain heart Infusion Broth (BHIB) :

Brain-heart infusion broth was made by dissolving 37gm in 1000 ml of distilled water. After that, 20% glycerol is added. It is used to preserve bacterial isolates for a specific period of time (Forbes *et al.*, 2007).

3.4.2.7. Brian Heart Infusion Agar (BHIA) :

Prepared according to the manufacturer was made dissolving 37gm in 1000 ml of distilled water. It was used to activate bacterial isolates as a growth medium and suspension for *Staphylococcus aureus.*

3.4.2.8. Nutrient Agar (NA) :

It is made by dissolving 13 gm in 1000 mL of distilled water according to the manufacturer. It contains the ideal nutrients for culturing a wide variety of bacteria.

3.4.2.9. Nutrient Broth (NB) :

It was prepared by dissolving 13 g of medium in 1000 ml of distilled water. This media was used to cultivate microorganisms and stimulate their growth medium (Macfaddin, 2000).

3.4.2.10. Motility Medium (Semi-Solid Medium) (MM) :

prepared by dissolving 1.3 gm of nutrient broth and 0.7 gm of agar-agar in 100 ml of distilled water. This medium is used to detect motility (Collee *et al.*, 1996).

3.4.2.11. Congo Red Agar (CRA) :

Prepared by mixing 37 g of brain heart broth in 1 L of distilled water, 50 g of sucrose, 0.8 g of congo red dye, and 10 g of agar. Then, the solution was autoclaved and distributed into 90 mm Petri dishes. Biofilm-producing bacteria were detected using these media. The biofilm-forming bacteria produced black colonies, while the non-forming strains produced red colonies after culture (Mariana *et al.*, 2009).

3.4.2.12. Sabouraud Dextrose Agar (SDA):

Sabouraud dextrose agar was prepared according to the manufacturer's instructions, by dissolving 65 gm in 1000 ml distilled water with added 250 mg of chloramphenicol that prevent growth of bacteria and sterilized by autoclave. Historically, it was used as a standard medium for isolating pathogenic fungi as well as for yeasts and is still widely used (Scognamiglio *et al.*, 2010).

3.4.2.13. Sabouraud Dextrose Broth Medium (SDB):

According to the manufacturer's instructions, this medium is prepared by dissolving 30 g in 1000 ml of distilled water. This method is used to test the ability of yeasts to form biofilms (Weerasekera *et al.*, 2016).

3.4.2.14. CHROM agar *Candida* Medium:

According to the manufacture's instruction, this medium is prepared by suspending 30 gm of CHROMagar *Candida* powder in 1000 ml of distilledwater, and then heated until it dissolved completely. This medium is used for preliminary identification types of *Candida* spp. (Horvath *et al.*, 2003).

3.4.2.15. Potato Dextrose Agar Medium:

According to the manufacture's instruction, this medium is prepared by suspending 39 gm of medium in 1000 ml of distilled water with adding 250 mg of chloramphenicol that prevent growth of yeasts and sterilized by autoclave. This medium is used to isolation of yeasts from swabs.

3.4.3. Sterilization Methods

3.4.3.1. Wet Heat Sterilization Methods (Autoclave):

All the culture media are sterilized using the autoclave device at a temperature of 121C° and a pressure of 15 lbs, as well as some of the tools used in the extraction such as the Eppendorf tube tips.

3.4.3.2. Sterilization by Formalin:

Sterilization by formalin is performed by adding 15 ml of formalin into Petri dish and left for (24-48) hours for sterilizing the incubator.

3.4.3.3. Sterilization by Heating and Alcohol Spirit:

Sterilization of the indoor of hood pang surfaces by alcohol while needle, tongs other steel tools is sterilized by heating.

3.4.4. Diagnosis of Isolates of *S. aureus*:

According to MacFaddin (2000), isolates were identified based on cell morphology and colony towards bacterial growth on media where they appear when cultivated as yellow colonies, in addition to many important biochemical tests. Biochemical assays were used to identify suspected Gram-positive bacterial isolates that are purple in color aggregated into disorganized spheres resembling a cluster of grapes when examined under a microscope, negative for oxidase, coagulation assays, and non-motile.

3.4.5. Gram Stain:

The staining process begins by placing a drop of distilled water on a clean glass slide, then a bacterial colony is transferred to the slide, then we install it on the Benzine burner, then we transfer the slide to a rack. After that we immerse the sample with dye Crystal Violet leave for 1 minute then wash in tap water to get rid of excess. Then we immerse the sample in iodine solution and let it stand for 1 minute, then it was washed with water. The slide is tilted at an angle of 45 degrees and decolorized with an ionic alcohol solution until the violet stops running and then washed off. Then we cover the sample with safranin dye and leave it for one to two minutes. was washed with water, get rid of the excess and dry gently. The sample is then ready for examination with an light microscope under an oil-immersed lens (Macfddin, 2000).

3.4.6. Biochemical Tests

3.4.6.1. Catalase Test:

A small amount of fresh bacterial growth was transferred with a sterile wooden stick onto the surface of a clean, dry glass slide. One drop 3% (H2O2) was added to the slide. The catalase detector is used to identify bacteria that have the ability to produce catalase enzyme. When gas bubbles form, this indicates a positive result (Macfaddin, 2000).

3.4.6.2. Coagulase Test:

This test is used for the detection of the ability of *S. aureus* to produce coagulase enzyme, which is an enzyme that causes clotting in plasma (Anyanwu and John, 2013). There are two methods that were used:

A- Tube Method:

A few colonies were transferred in diluted human plasma (plasma: saline 1: 5) in a tube. The tube was kept at 37 Cº and observed for clot after 1 to 4 hrs. or, next day according to (Tiwari *et al.*, 2008).

B- Slide Method:

The slide test is performed by preparing a suspension of bacterial into a drop of plasma on a slide. If bound coagulase is present on the bacterial cells, then the presence of plasma will cause the bacterial cells to clump (Katz, 2010).

3.4.6.3. Motility Test

This test was used to find out whether the bacteria are motile or non-motile. Semi-solid medium with active bacteria was stabbed with a sterile needle and then incubated for (24–48) hours at 37Cº. If the bacterial isolate is motile (positive result), the cloudy appearance around the stab will occur because of bacterial diffusion away from the stab, otherwise the isolate is considered non-motile (Woodland, 2004).

3.4.6.4. Urease Test:

Young bacterial colonies were transferred onto the surface of urea agar and incubated at 37 Cº. The result was read after 6 hours, 24 hours and every other day for 6 days. A mid-color change to violet-pink indicates a positive result (Macfaddin, 2000).

3.4.6.5. Oxidase test:

A small portion of the colony to be tested is placed by wooden stick on the oxidase test disk, we notice the color change after several seconds as the color change to blue or purple indicates a positive result (Forbes *et al.*, 2007).

3.4.6.6. CHROM agar *Candida* Test:

CHROM agar test is used for the presumptive identification of *Candida* species by production of different colors on this medium (*C. albicans*= green colonies , *C. tropical*= blue, *C. parapsilosis*= cream white, and *C. krusei*= rose-colored colonies with white edges, *C. glabrata* dark- pink to purple coloration) (Horvath *et al.*, 2003). This assay is performed by inoculating CHROM Agar *Candida* medium previously prepared from a *Candida* isolate culture grown on SDA for 24 h, then incubated for (24–48) h after which colony colors are observed (Paritpokee *et al.*, 2005).

3.4.7. Preservation of Bacterial Isolates:

3.4.7.1. Short Term Preservation:

The bacterial isolates were kept by transferring a single pure colony to nutrient agar slant in a covered tube, incubating overnight at 37Cº, and then storing at 4Cº for daily used (Vandepitte *et al.*, 2003).

3.4.7.2. Long Term Preservation:

New isolates were taken from mannitol salt agar culture cultured for 24 hour and placed in Brain Heart broth supplemented with 20% Glycerol and stored at -20Cº in a deep freezer until needed (Delgado *et al.*, 2020).

3.4.8. Preservation of *Candida* spp Isolates

3.4.8.1. Short Term Preservation:

Yeast isolates cultured on SDA medium containing antibacterial Tetracycline were purified after the initial isolation by transferring part of the colony of each yeast isolate and growing them in dishes containing SDA medium after that they were incubated at 25Cº for 48

hours and then kept at 4 degrees for daily use. Keeping in mind that it is renewed every period to ensure that the isolates remain in an active state.

3.4.8.2. Long Term Preservation:

Yeast isolates were maintained by transferring a pure isolated colony to PDA agar slanted in a covered tube, incubating for 48 hours at 29 Cº, and then storing at 4Cº.

3.4.9. Virulence Factors of Bacteria (*Staphylococcus aures*)

3.4.9.1. Hemolysis Test:

The susceptibility of bacteria to hemolysis was determined by inoculating the blood agar medium with bacterial isolates, then incubating for 28 hours at a temperature of 37Cº. A positive result is the formation of a clear halo around the development of the colony indicating decomposition . Blood agar media is a medium used to distinguish pathogenic bacteria based on their hemolytic power on red blood cells(Turista and Puspitasari , 2019).

3.4.9.2. Detection of Biofilm Formation of *Staphylococcus aureus*

3.4.9.2.1. Congo Red Agar (CRA) Method:

Cultivation of *S. aureus* strains on congo red agar was used to determine biofilm formation. the assessment criteria are based on visual study of the color of the colonies that grow on the agar (Hassan *et al.*, 2011; Liberto *et al.*, 2009). Where the bacterial colonies of *S. aureus* were cultured on congo red agar a medium and incubated at a temperature of 37 Cº for 18 to 24 hours, then the result was observed**.** Strains that generate tough, black colonies on Congo red plates are designated biofilm formation. Strains that generate smooth, red colonies,

on the other hand, are classified biofilm - negative (Cucarella *et al.*, 2001).

3.4.9.2.2. Tubes method:

S. aureus were cultured in tubes containing Brain Heart Infusion broth (BHI broth) and incubated for 2 days at 37Cº. The supernatant was then discarded, and the glass tube was stained with a crystal violet solution, washed three times with phosphate buffer solution, and dried.

The results were seen, as the tubes with a biofilm ring around the test tube wall appeared in a crystal blue-violet color, indicating that the bacteria formed the cell wall. (Ou *et al.*, 2020; Xie *et al.*, 2021).

3.4.10. Virulence Factors of Yeasts

3.4.10.1. Hemolysis Test:

The yeast's susceptibility to hemolysis is determined by culturing the yeasts on blood agar, then incubating them at ambient temperature for 37Cº to 48 hours and the positive result is the formation of a clear halo around the colony development (Sachin *et al.*, 2012).

3.4.10.2. Biofilm Formation Test:

Yeast isolate colonies are inoculated by the loop in to tubes to detect their ability to form the bio - membrane. The test tube contained containing 10 ml of Sabouraud dextrose broth fortified with 8 % glucose. The tubes are incubated at 35Cº for 48 hours. After the incubation period the medium was poured, and the tubes were dyed by Safranin dye. The stain is poured after 10 minutes. The tubes are rinsed with distilled water to remove excess stains. The results are recorded as negative result (-) positive result is weak (+), and (++) strong (Mohandas and Ballal, 2011).

3.4.11. Antimicrobial Susceptibility test

3.4.11.1. Antibacterial Tests:

The sensitivity test procedure was done according to (CLSI, 2020) as it is following steps:

1-Using a sterilized inoculating loop, four to five colonies of bacterial isolate were picked up from a fresh pure culture plate and homogenized in 5ml of sterile normal saline until the turbidity was nearly equal to the (1.5x 10^8) cell/ml, MacFarland turbidity level.

2-The sterile cotton swab was immersed into the bacterial suspension, and the excess fluid was removed by rotating the swab hard against the inside of the tube above the fluid level to remove the excess fluid. Was performed 3 times on the Muller hinton agar surface of the plate with the swab، rotary the plate between each line at 50 degrees. The inoculum had been allowed to dry with a lid in place for (5-10) minutes.

3- Using a sterile forceps, place the selected antimicrobial discs on the surface of the inoculation plate and gently push them into full contact with the agar. This step was repeated with all of the antimicrobial discs in the test, with them placed a similar distance apart.

4- The plates were subsequently incubated at 37C° and analyzed after (18-24) hours.

5-Inhibition zones were measured, and the zones' diameters were reported to the nearest millimeter using a metric ruler (Moreno *et al.*, 2006).

3.4.11.2. Antifungal Tests

They were applied on MHA as recommended by the Clinical Laboratory Standard Institute(CLSI) .

1-Using a sterilized inoculating loop, four to five colonies of isolate were picked up from a fresh SDA medium and homogenized in 5ml of sterile normal saline until the turbidity was nearly equal to the (1.5x 108) cell/ml, MacFarland turbidity level

2- A sterile cotton swab dipped in an in suspension, and then excess fluid is removed by rolling the swab on the inner surface of the tube above the surface of the liquid. The lines were placed on the surface of Mueller-Hinton agar (MHA). leaving the dish for (5- 15) minutes to allow the suspension to be absorbed and until the surface of the agar dries.

3- The tablets of the antifungals under study were placed at equal distances on the surface of the agar using sterile forceps and incubated at a 35-37Cº. After 2 days, was measured in mm for each antifungal agent using a metric ruler.

3.4.12. Molecular Study:

3.4.12.1. Bacterial DNA Extraction:

Using Favrogen DNA extraction kit protocol to extract Bacterial DNA.

1. Cultivation: *S. aureus* strains were incubated at 37Cº on nutrient broth medium 24 hours.

2. Sample preparation: Transfer 200ul of bacterial cell suspension for each *S. aureus* strain separately into 1.5 ml microcentrifuge tube. Add 20 of lysozyme enzyme then mix by pipetting. Incubate at 37Cº for 15 minutes.

3. 20 µl of Proteinase K was added to each tube in step 2, mixed well

by vortexing. Incubated at 60 Cº for 15 min, vortex 30 seconds every 5 minutes incubation.

4. Cell lysis: Add 200 µL of FATG buffer then mix by vortex. Incubate at 10Cº for 5 minutes, inverting the tube every 2 minutes.

5. 200ul of FATB buffer was added to the cells and re-suspend the cells by vortex, incubate at 70Cº for 10 minutes, inverting the tube every 3 minutes.

6. DNA binding: Add 200 µL of absolute ethanol to sample lysate and mix by vortex for 10 second. Place spin column in a 2 ml collection tube. Transfer all of the mixture to the Place spin column. Centrifuge at 13000 rpm for 1 minutes. Discard the flow- through, Place spin column back in the 2ml collection tube.

7. Washing: Add 400 µL of W1 buffer to the spin column. Centrifuge at 13000 rpm for 30 seconds then Discard the flow- through. Place spin column back in the 2ml collection tube.

8- Add 600 µL of washing buffer (make sure absolute ethanol was added) to the spin column.Centrifuge at 13000 rpm for 30 seconds then Discard the flow- through. Place spin column back in the 2ml collection tube. Centrifuge at 13000 rpm for 3minutes to dry the column.

9. Elusion: Transfer the dried spin column to clean 1.5 ml microcentrifuge tube. Add 75 µL of pre-heated elusion buffer (in step 3) to center of the column. Waite for 5 min to allow elusion buffer to be completely absorbed. Centrifuge at 13000 rpm for 30 seconds to elute purified DNA.Kept the purified DNA at-20Cº.

3.4.12.2. *Candida* DNA Extraction:

Using Favrogen DNA extraction kit protocol of human blood to extract *Candida* DNA.

1. Cultivation: *Candida* spp., were incubated at 28Cº on SDA 24h.

2. Sample preparation: Transfer tiny portion of *Candida* colony for each *Candida* ssp. separately to a1.5 ml microcentrifuge tube contained 150ul lysis cell. Add 20 of lyticase then mix by pipetting. Incubate at 37Cº for 15 minutes.

3. A total of 20 µl was added of Proteinase K (10 mg/ml) and mixed well by vortexing. Incubated at 60 Cº for 15 min, vortex 30 seconds every 5 minutes incubation.

4. Cell lysis: Add 200 µL of FATG buffer then mix by vortex. Incubate at 10Cº for 5 minutes, inverting the tube every 2 minutes.

5. 200ul of FATB buffer was added to the cells and re-suspend the cells by vortex, incubate at 70Cº for 10 minutes, inverting the tube every 3 minutes.

6. DNA binding: Add 200 µL of absolute ethanol to sample lysate and mix by vortex for 10 second. Place spin column in a 2 ml collection tube. Transfer all of the mixture to the Place spin column. Centrifuge at 13000 rpm for 1 minutes. Discard the flow- through, Place spin column back in the 2ml collection tube.

7. Washing: Add 400 µL of W1 buffer to the spin column. Centrifuge at 13000 rpm for 30 seconds then Discard the flow- through. Place spin column back in the 2ml collection tube.

8- Add 600 µL of washing buffer (make sure absolute ethanol was added) to the spin column.

Centrifuge at 13000 rpm for 30 seconds then Discard the flow- through. Place spin column back in the 2ml collection tube. Centrifuge at 13000 rpm for 3minutes to dry the column.

9. Elusion: Transfer the dried spin column to clean 1.5 ml microcentrifuge tube. Add 75 µL of pre-heated elusion buffer (in step 3) to center of the column. Waite for 5 min to allow elusion buffer to be completely absorbed. Centrifuge at 13000 rpm for 30 seconds to elute purified DNA. Kept the purified DNA at -20Cº.

3.4.12.3: Dissolving of Primers

All primer pairs used in this study were dissolved using Nuclease Free Water (Promega). Firstly, the primer stock tube prepared and then the working solution would prepare from primer stock tube. According to the instruction provided by primer manufacturer the Nuclease free Water were added to get 100 picomol/microliter concentration of primer stock solution. The working solution prepared from stock by dilution 10picomole/microliter primer stock solution with 90 microliter of Nuclease free Water to get 10 picomol/microliter (work solution).

3.4.12.4. PCR Mixture:

The PCR was achieved by using specific primer for gene and the contents of PCR mixture was shown in table (3-9).

Table (3-9): Contents of the Reaction Mixture (Promega) of PCR for virulence genes.

No.	Contents of reaction mixture	Volume Ml
1.	PCR PreMix	12
2.	Outer Forward primer	1.5
3.	Outer revers primer	1.5
4.	Patient DNA template	2
5.	Nuclease free Water (Promega)	8
Total volume		**25**

3.4.12.5. Thermal Cycling Conditions

The PCR reaction is shown in Table (3-10).

Tables (3-10): Thermal Cycling Conditions for Amplification Gene *αINT1* Gene and *ALS1* Gene under Interest

PCR condition for *αINT1* gene and *ALS1* gene			
Step Type	**Temperature C°**	**Time**	**Cycling**
Initial Denaturation	**95**	**3** min.	**1**
Denaturation	**95**	**30/** Sec.	**30**
Annealing	**53***/55**	**40** Sec.	
Extension	**72**	**40 Sec.**	
Final Extension	**72**	**3 min.**	**1**
Hold	**4**	**A**	**4**

Annealing temperature: *gene *ALS1*, ** *αINT1* gene.

Tables (3- 11): Thermal Cycling Conditions for Amplification Gene *mec* and *Spa* Gene Under Interest:

PCR condition for virulence *mec* gene and *Spa* gene			
Step Type	**Temperature C°**	**Time**	**Cycling**
Initial Denaturation	**95**	**3** min.	**1**
Denaturation	**95**	**30/** Sec.	**30**
Annealing	**54***/56**	**40** Sec.	
Extension	**72**	**40 Sec.**	
Final Extension	**72**	**3 min.**	**1**
Hold	**4**	**A**	**4**

Annealing temperature *gene mec and **_Spa_ genes.

3.4.12.6. Detection of Amplified Products by Agarose Gel Electrophoresis

The amplified products of PCR were separated by electrophoresis on a 1.5% agarose gel pre-stained with ethidium bromide. Agarose gel was prepared by dissolving 1.5g of agarose powder in 100 ml of 1X TBE buffer (pH:8) in flask then placed in microwave for 2minutes and waiting for cool to 50C° and adding about 0.5µl of ethidium bromide stain concentration (0.5mg/ml) (Sambrook and Russell, 2001).

The first well put the 4µl of DNA ladder (100-3000bp). The electric voltage 100 volt at 45min (Sambrook and Russell, 2001).

3.4.13. Immunological Tests

3.4.13.1. Immunoglobulin IgE Test

Assay of serum specific IgE. Serum collected and frozen at -20C° until use. The sera were simultaneously analyzed by the VIDAS Stallertest assays **.**IgE is detected directly with a combination of polyclonal and monoclonal anti-IgE (Fc) antibodies labeled with betagalactosidase to generate fluorescence**.** The VIDAS Stallertest assay combines a 2-step immunoenzymatic technique with fluorescent detection in the final step. Each single-dose test is comprised of a strip that contains the ready-to-use reagents and a disposable solid phase receptacle (SPR) onto which the allergens are coated. The allergen coated SPR serves as the solid phase for the capture of the antigen to be detected, as well as the pipettor for the assay. Reagents for the assay are available in sealed reagent strips. Conjugate is a polyclonal antibody linked to alkaline phosphatase. The glass cuvette of the reagent strip contains the substrate (4 methylumbelliferyl) and is used for the final reading. At the end of the assay, results are automatically computed by the VIDAS instrument by reference to a calibration curve stored in memory.

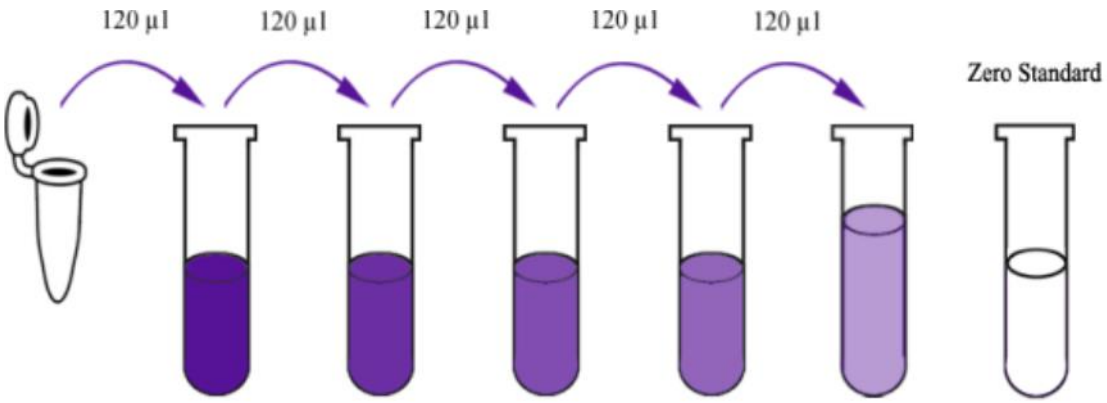
3.4.13.2. Reagent Preparation

1- All reagents should be brought to room temperature before use.
2- Standard Reconstitute the 120µl of the standard (960ng / L) with 120µl of standard diluent to generate a 480ng / L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (480ng / L) 1: 2 with standard diluent to produce240ng / L, 120ng / L, 60ng / L and30ng / L solutions. Standard

diluent serves as the zero standard (0 ng / L). Any remaining solution should be frozen at -20 C° and used within one month.

Table (3- 12) Dilution of Standard Solutions Suggested Are As Follows:

Standard stock	Standard No	Standard dilution
480ng / L	Standard No.5	120µl Original standard +120µl standard diluent
240 ng / L	Standard No.4	120µl standard No.5 +120µl standard diluent
120 ng / L	Standard No.3	120µl standard No.4 +120µl standard diluent
60 ng / L	Standard No.2	120µl standard No.3 +120µl standard diluent
30 ng / L	Standard No. 1	120µl standard No.2 +120µl standard diluent



Standard concentration	standard No.5	standard No.4	standard No.3	standard No.2	standard No.1
960 ng / L	480 ng / L	240 ng / L	120 ng / L	60 ng / L	30 ng / L

3- Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water i to yield 500 ml of 1x Wash Buffer. If

cryst*ALS* have formed in the concentrate, mix gently until the cryst*ALS* have completely dissolved.

3.4.13. 3. Human Tumor Necrosis Facter (TNF-α)
Assay Procedure

1. Prepare all reagents, standard solutions and specimens as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8C°.

3. Add 50 µl standard to standard well. Note: Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.

4. Add 40µl sample to sample wells and then add 10ul anti -TNF-α antibody to sample wells, then add 50ul streptavidin - HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37C°.

5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Add 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37 ° C in the dark.

7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.

8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

3.5. Statistical Analysis:

The Statistical Analysis System- SPSS program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of correlation coefficient between variables in this study.

Chapter Four

Results and Discussion

4. Results and Discussion

4.1. Demography of the Study Populations

A total 120 clinical specimens were collected in the current study from patients attending the medical consultation department, dermatology unit at (Marjan Medical City) in Babylon Province. 66.7% infection were in less than 20 years and 15.0% in age between 20-30 years , Table (4-1) show that less than two third (61.7%) of types of disease were impetigo ,Ecthyma (33.3%) and Bullous (5.0%).

Table 4-1: Frequency and Percentage of Types of Disease

		Frequency	Percent %
Types of disease	Impetigo	74	61.7
	Ecthyma	40	33.3
	Bullous	6	5.0
	Total	120	100.0

Bullous impetigo is a bacterial skin infection caused by *S.aureus* that results in the formation of large blisters called bullae, usually in areas with skin folds like the armpit, groin, between the fingers or toes, beneath the breast, and between the buttocks. It accounts for 30% of cases of impetigo, the other 70% being non-bullous impetigo (Hartman-Adams *et al* ., 2014) . Bullous impetigo is only caused by *S. aureus* and accounts for approximately 10% of cases, most often seen in infants (Russell *et al*., 2019) .

Impetigo is a highly contagious, superficial skin infection that most commonly affects children two to five years of age. The two types of impetigo are nonbullous impetigo (i.e., impetigo contagiosa) and bullous impetigo (Brown *et al*., 2003) . Ecthyma is a deep tissue form of

impetigo. It is characterized by crusted sores beneath which ulcers form with a "punched out" appearance and it is more common in children, older people and immunocompromised people or in conditions of poor hygiene and hot humid weather. Streptococcus pyogenes and Staphylococcus aureus are the bacteria responsible for ecthyma (Demircioglu and Oren, 2008).

The results in Table (4-2) and Figure (4.1) provides an overview of the distribution of different types of infections among different age groups, showing the distribution of different types of infection (Impetigo, Ecthyma, Bullous impetigo) among various age groups. The table also includes the number and percentage of cases for each type of infection within each age group. Age Group 1-10 Impetigo: 42 cases (35%), Ecthyma: 12 cases (10%) and Bullous impetigo: 4 cases (3.3%) in case of Age Group 11-20: Impetigo: 8 cases (6.7%) , Ecthyma: 8 cases (6.7%), Bullous impetigo: 0 cases (0%) while in Age Group 21-30: Impetigo: 6 cases (5%), Ecthyma: 6 cases (5%) and Bullous impetigo: 2 cases (1.7%).

Table 4-2: distribution of Infection Type According to Age Group

Age Group	Type of infection						Total
	Impetigo		Ecthyma		Bullous impetigo		
	No.	%	No.	%	No.	%	
1-10	42	35	12	10	4	3.3	58
11-20	8	6.7	8	6.7	0	0	16
21-30	6	5	6	5	2	1.7	14
31-40	12	10	4	3.3	0	0	16
41-50	4	3.3	8	6.7	0	0	12
51-60	2	1.7	2	1.7	0	0	4
Total	74	61.7	40	33.3	6	5	120

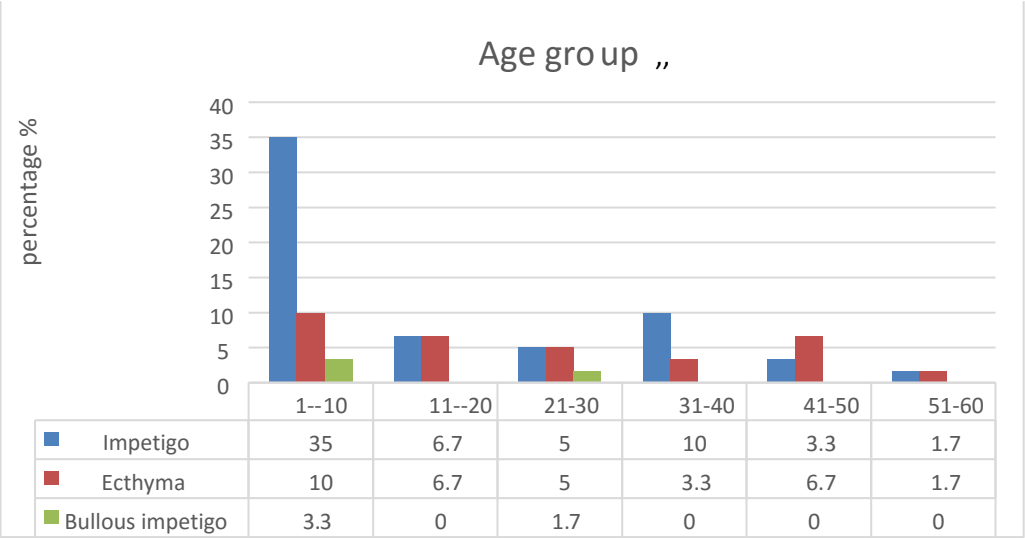


Figure 4.1: Distribution of Infection According to Age Group

There are three types of impetigo: non-bullous, bullous, and ecthyma. The most common form is non-bullous impetigo (NBI), also called *impetigo contagiosa*, accounting for almost 70% of cases (Cole and Gazewood, 2007).

Impetigo is the most common infection among all age groups, accounting for 61.7% of the total cases (74 out of 120). It is predominant in all age categories, with the highest number of cases in the (1-10) age group (42 cases). This finding suggests that Impetigo is a common skin infection that affects individuals across different age ranges, but it is more prevalent among children. The global prevalence of impetigo is estimated to be 11.2%, being 2.5-fold higher in children (12.3%) than adults (4.9%) (Hay *et al*.,2010).

In study done by Elisa *et al* (2022) found that only 6% of children in Italy have at least an episode of NBI, a lower prevalence than that reported in the international literature. Furthermore, our results show a significantly decreasing IR trend over time with the highest value in children (1–4) years old. Different from ours, all the above-mentioned studies considered a broad definition of impetigo, including the bullous

form, which seems to be more prevalent in children aged (2–16) years and could reflect a higher IR (Shallcross *et al*., 2013).

Ecthyma is the second most common type of infection, constituting 33.3% of the total cases (40 out of 120). It is distributed relatively evenly across the age groups, with a higher concentration in the 1-10(12 cases), 11-20 and 41-50 age groups (both with 8 cases each). This data suggests that while Ecthyma is not as prevalent as Impetigo, it affects individuals of various age groups. Bullous impetigo has the lowest number of cases, making up only 5% of the total (6 out of 120). It is primarily found in the 1-10 age group, with 4 cases reported. This indicates that Bullous impetigo is less common compared to the other two types of infection, and impetigo most commonly seen in children.

Table (4-3) show that the higher percentage (28.3%) of sample area were from foot Impetigo,then face percentage (23.3%), fermoral(18.3%), hand (15 %), head (8.3%) and gluteus (6.7%).

Table 4-3: Frequency and Percentage of Sample Area

		Frequency	Percent %
Specimens Area	Foot	34	28.3
	Face	28	23.3
	Gluteus	8	6.7
	Femoral	22	18.3
	Hand	18	15.0
	Head	10	8.3
	Total	120	100.0

A common superficial skin infection (Cole and Gazewood, 2007). The first signs of impetigo can usually be seen around the mouth and

nose in the form of an itchy reddish rash with small blisters. The blisters are filled with water or pus and burst easily. Once they have burst, yellowish crusts form. These fall off after some time without scarring. As well as on the face, impetigo can occur on the arms and legs (Galli *et al*.,2019). Impetigo is most commonly seen in children between the ages of 2 and 6 years old. This is attributed to several factors, including their underdeveloped immune systems, close contact with other children in daycare or school settings, and a higher likelihood of sustaining minor cuts or abrasions during play. However, impetigo can occur at any age, and adults are not immune to the infection (Koning *et al*., 2012).

It is a huge problem worldwide, affecting >2% of the global population (Kyu *et al*.,2018), and an estimated 162 million children (particularly those between the age of 2 and 5 years) suffer from impetigo at any one time (Bowen *et al*., 2015; Romani *et al*., 2015). In Australia, up to 49% of Aboriginal children living in remote communities are affected by impetigo at any given time (median prevalence of 44.5%) (Cole and Gazewood, 2007; Bowen *et al*., 2015).

Impetigo is a highly contagious bacterial skin infection that primarily affects children, but it can also occur in people of any age, including adults. The prevalence of impetigo varies across different age groups and genders (Sladden and Johnston, 2004).

Both males and females have the same number of cases for each type of infection. For Impetigo, there are 37 cases in both genders, accounting for 30.8% of the total cases each. Ecthyma is reported in 18 cases among males (15%) and 20 cases among females (16.6%). Bullous impetigo is found in 5 cases (4.2%) for males and 3 cases (2.5%) for females (Table 4-4 and Figure 4.2).

Table 4-4: Distribution of Infection Type According to Gender

Gender	Type of infection						Total No %	P value ($p \leq 0.05$)
	Impetigo		Ecthyma		Bullous impetigo			
	No.	%	No.	%	No.	%		
Male	37	30.8	18	15	5	4.2	60 (50%)	0.4 NS
Female	37	30.8	20	16.6	3	2.5	60 (50%)	0.9 NS
Total	74	61.6	38	31.6	8	6.7	120	
NS: non-significant deference by t-test under ($p \leq 0.05$)								

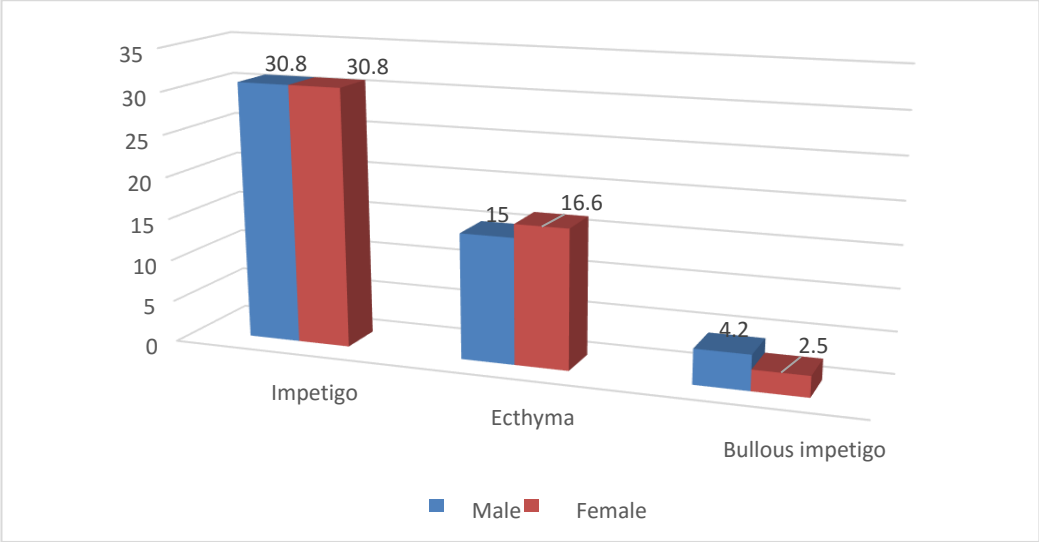


Figure 4.2: Percentage of Specimens Area in Study Population

The data analysis indicates that there is no significant difference in the distribution of infection types (Impetigo, Ecthyma, Bullous impetigo) between males and females. Both genders have similar numbers of cases for each type of infection, and the P-values suggest that any observed differences are likely due to chance rather than gender-related factors.

This finding may imply that the risk factors and exposure patterns leading to these skin infections do not vary significantly between males and females. Other factors such as age, geographical location, hygiene practices, or overall health status might play a more influential role in determining the prevalence of these infections.

4.2. Prevalence of Isolates among Different Clinical Specimens of skin Infections

Identification of all clinical specimens microorganisms was performed by classical microbiological methods for 120 specimens. There were 70% of specimens were positive (growth) and 30% were negative (no growth) as shown in (Figure 4.3).

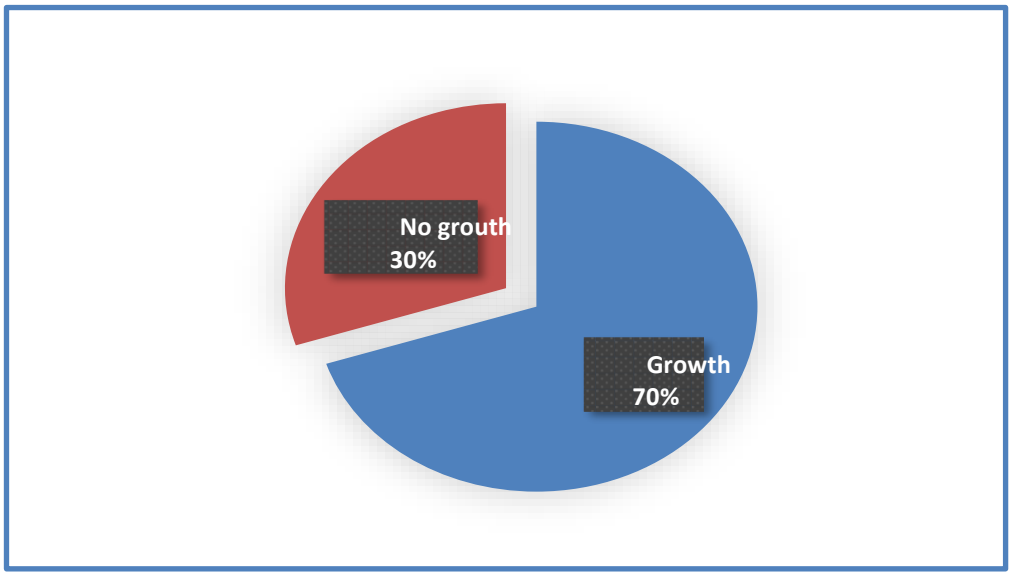


Figure 4.3: Percentage of Microbial Growth in Patients

S. aureus was 18%, *Streptococcus* 14%, *P .aeruginosa* 7%,*S. saprophyticus* 10%, *S. epidermis* 9% and *Candida albicans* 18% as shown in Figure (4.4).

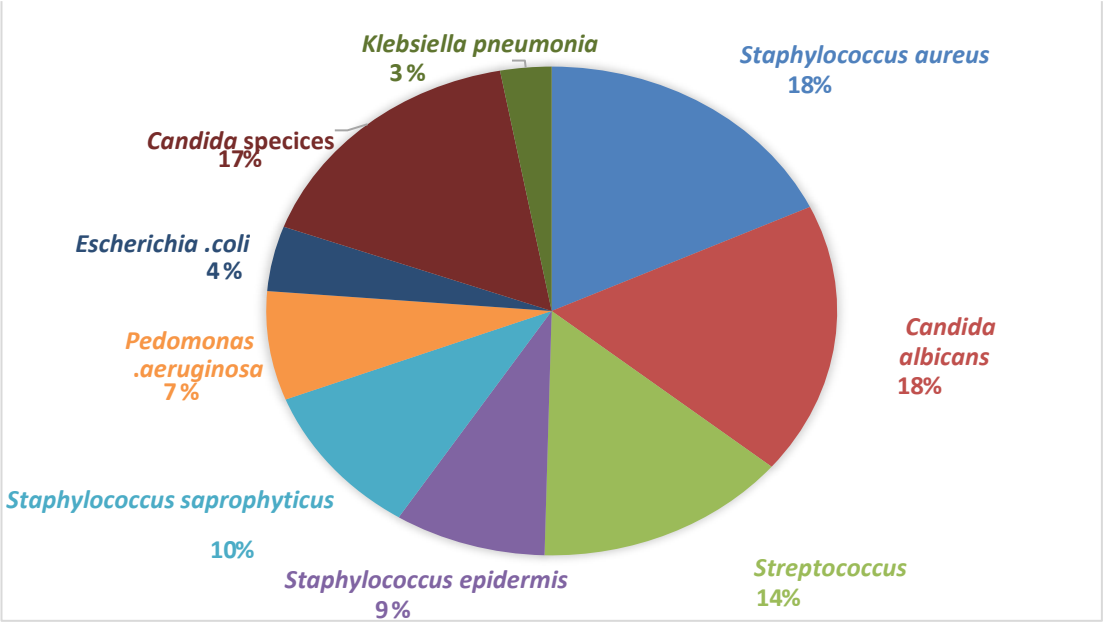


Figure 4.4: percentage of Microbial Isolate (n=9 from 70 %)

S. aureus is one of major pathogens in the skin and soft-tissue infections, which lead to abscess, cellulitis, folliculitis, and impetigo, resulting in a significant public health problem (Cho *et al*., 2011 ; Singer and Talan, 2014). Methicillin-resistant *S. aureus* could lead to complicated and difficult treatment of skin infection (Cho *et al*., 2011).Impetigo is an epidermal infection caused by *S. aureus*, *Streptococcus pyogenes*, or a combination of both. In northern countries *S. aureus* infections are predominant, representing 90% of the bacterial infection, whereas in developing countries *S. pyogenes* is reported to be predominant. Impetigo mainly affects children and predominates in underprivileged communities (Stanley *et al*., 2005; Bowen *et al*., 2014).

Staphylococcus aureus and *Streptococcus pyogenes,* either alone or together, are the most common causes of impetigo (Russell *et al*. ,2019). Historically, impetigo is caused by either group A β-

hemolytic *Streptococci* or *Staphylococcus aureus*. Currently, the most frequently isolated pathogen is *S. aureus*.

Methicillin-resistant *S aureus* (MRSA) (Gregory *et al*., 2005) and gentamicin-resistant *S aureus* strains have also been reported to cause impetigo (Kuniyuki *et al*., 2005). Impetigo is classified as either nonbullous (impetigo contagiosa) (about 74% of cases) (Cole and Gaze wood, 2004). Ecthyma associated with *S aureus* infection (Song *et al* ., 2015).

Coagulase negative *Staphylococci* are the most common organisms on the normal skin flora, with about 18 different species, and *S. epidermidis* being the most common of the resident staphylococci (Oumeish *et al*., 2000). *S. aureus* (coagulase positive) is often found in the skin, in a transient manner, in healthy children. Carriage status may occur in the nares in 35% of the population, in the perineum in 20%, in the axillae and interdigital regions in 5 to 10% (Ungprasert *et al*., 2013). The condition of staphylococcal nasal carriage was found in up to 62% of patients with impetigo (Sen *et al*., 2009).

Ecthyma can be caused by gram-positive organisms such as *Staphylococcus* and *Streptococcus* species (Kao *et al*., 2001 ; Reich *et al*., 2004).

Impetigo is an eczema observed in two bullous and non-bullous forms (Salah and Forgeman, 2015). *S. aureus* bacteria and Group A Beta Haemolytic *Streptococcus* (GABHS) are the most common causes of impetigo (Hartman-Adams *et al*., 2014).

In a review study of 167 cases of EG reported in the literature retrieved in PubMed, MEDLINE, and ScienceDirect between 1975 and 2013, the authors identified that in 73.6% of the total cases the agent

was *P. aeruginosa*, in 17.3% another bacterium, and in 9% the etiology was fungal. It is noteworthy that EG was a manifestation of sepsis in only 58.5% of the cases where the agent was *P. aeruginosa*. (Shallcross *et al*., 2013) .

4.3. Biochemical Test and Virulence Factors for Microorganisms

Biochemical tests are essential in microbiology to identify and differentiate various microorganisms based on their metabolic activities. Virulence factors are specific characteristics or molecules possessed by certain microorganisms that enable them to cause disease or infection. The result in Table (4.5) shows the biochemical test use for identification of *Candida albicans and S .aureus*. The most important virulence is the biofilm formation all *S .aureus* isolates were positive while 22 isolates of *Candida albicans* were positive.

Table 4-5: Biochemical Test for *Candida albicans* and *S. aureus*

Biochemical test	*Candida albicans*		Total
	Positive	Negative	
Hemolysis	25	0	25
Biofilm formation	22	3	25
	S .aureus		
Hemolysis	25	0	25
Congo agar (Biofilm)	25	0	25
Coagulase test	25	0	25
Catalase test	25	0	25
Oxidase test	0	25	25
Urease	25	0	25

The results in Figure (4.5) show that 40% of *S. aureus* isolates were strong biofilm 24% were intermediate while 36% were week formation of biofilm.

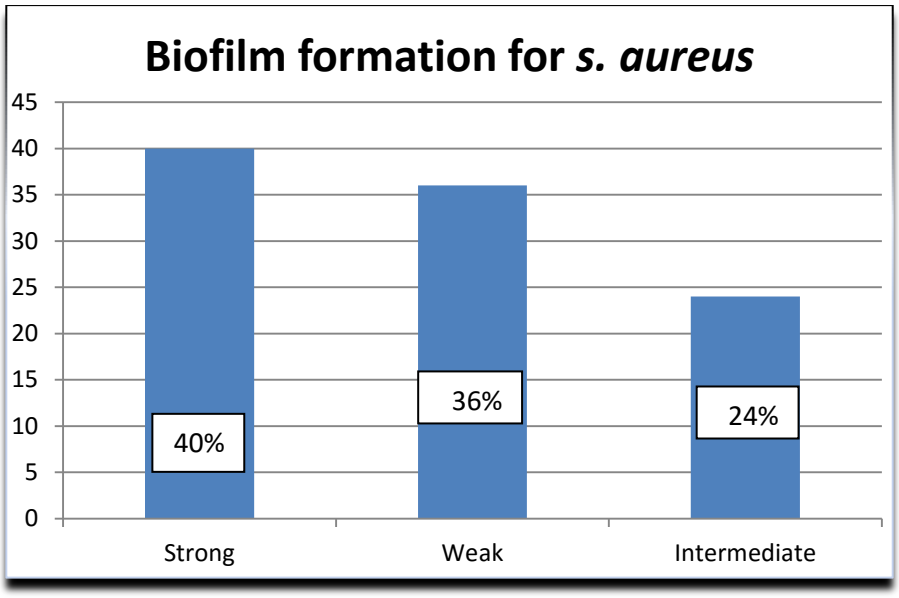


Figure 4.5: Percentage Intensity of Biofilm Formation for *S. aureus*

In Figure (4.6) the result shows the percentage intensity of Biofilm formation for *Candida albicans* were 52% strong, 20% intermediate, 16% week and 12% negative biofilm formation.

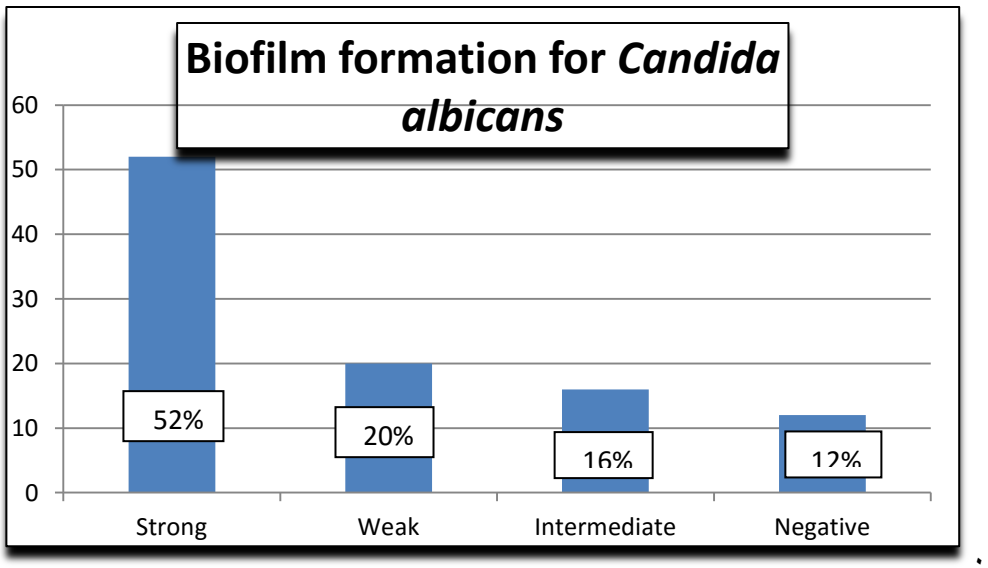


Figure 4.6: Percentage Intensity of Biofilm Formation for *Candida albicans*

Staphylococcus aureus can produce a multilayered biofilm embedded within a glycocalyx or slime layer with heterogeneous protein expression throughout (Nathan *et al*.,2011). The *S. aureus* responsible

for biofilm-associated infections can have different genetic backgrounds and, therefore, express a different spectrum of virulence factors during infection (Jarraud *et al*., 2002).

Candida species and strain type were shown to affect biofilm formation in vitro (Shin *et al*., 2002; Douglas, 2003; Hajjeh *et al*., 2004).

4.4. Antibiotics Susceptibility for *Microorganisms Isolates*

4.4.1. Antibacterial Susceptibility Test of *S. aureus* Isolates From Impetigo.

There is a significant relationship between *S. aureus* isolate and antibiotics sensitivity to (Azithromycin, Gentamicin, Vancomycin, Levofoxacin), except with (Tetracyctine, Amoxicillin) that there are a non- significant relationship at p-value 0.05, as shown in Table(4-6) and Figure (4.7).

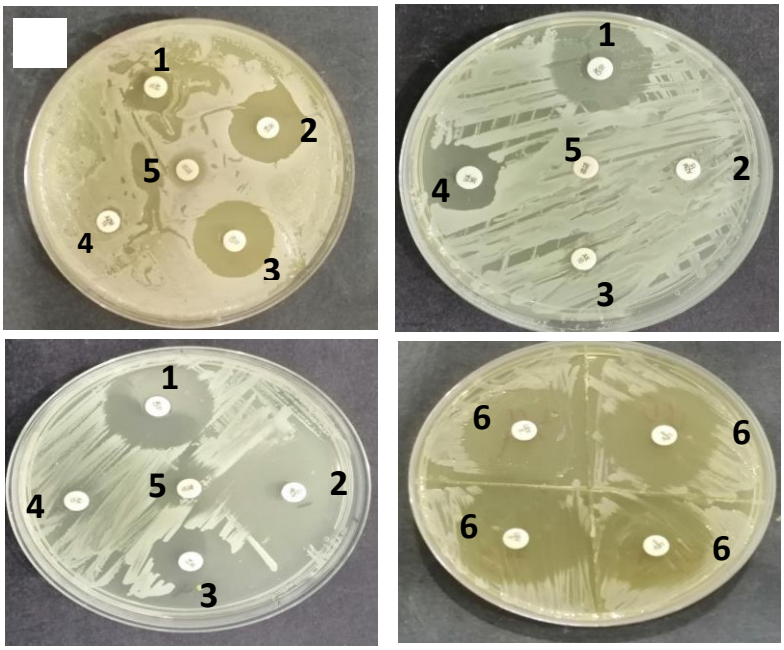


Figure (4.7):- Test for Identified of Sensitive and Resistance to Antibiotic in Clinical *S. aureus* Isolates.

(1- Amoxicillin 2- Vancomycin 3- Gentamicin 4- Azithromycin 5- Tetracyctine 6- Levofoxacin)

The results provided presents the distribution of *S. aureus* isolates in relation to their sensitivity to different antibiotics. Azithromycin: Out of 25 isolates tested, 20 (80%) were resistant and 5 (20%) were sensitive to Azithromycin. Gentamicin: Among the 25 isolates, 11 (44%) were resistant and 14 (56%) were sensitive to gentamicin. Tetracycline: For Tetracycline, 23(92%) isolates were resistant and only 2 (8%) were sensitive out of the 25 tested. The p-value associated with Tetracycline is 156 (N.S), indicating that the relationship between distribution and sensitivity is not statistically significant. Vancomycin: of the 25 isolates, 4 (16%) were resistant and 21 (84%) were sensitive to Vancomycin. Amoxicillin: Similarly, Amoxicillin showed a high resistance rate, with 24 (96%) isolates being resistant and only 1(4%) being sensitive out of the 25 tested. The p-value associated with Amoxicillin is156 (N.S), suggesting no significant relationship. Levofoxacin: Among the 25 isolates, 5 (20%) were resistant and 20 (80%) were sensitive to Levofoxacin. The p-values provided in the table indicate the statistical significance of the relationship between the distribution of *S. aureus* isolates and their sensitivity to each antibiotic. A p-value of .001 (H.S) for Gentamicin, Vancomycin, and Levofoxacin suggests a highly significant relationship between the distribution and sensitivity of isolates to these antibiotics.

Table 4-6: Distribution of *S. aureus* Isolate Relation to Antibiotics Sensitivity Test.

Antibiotics	Resist N (%)	Sensitive N (%)	Total for isolate NO.	*p-value
Azithromycin	20 (80)	5 (20)	25	
Gentamicin	11 (44)	14 (56)	25	.001 (H.S)
Tetracyctine	23 (92)	2 (8)	25	.156 (N.S)
Vancomycin	4 (16)	21 (84)	25	.001 (H.S)
Amoxicillin	24 (96)	1 (4)	25	.156 (N.S)
Levofoxacin	5 (20)	20 (80)	25	.001 (H.S)

*Chi-Square Tests, H.S= highly significance, N.S= no significance

There is a significant relationship between type of impetigo and antibiotics sensitivity to Amoxicillin, except with (Azithromycin, Gentamicin, Vancomycin, Tetracyctine, Levofoxacin) that there is a non-significant relationship at p-value 0.05,as shown in Table (4.6) Figure(4.8).

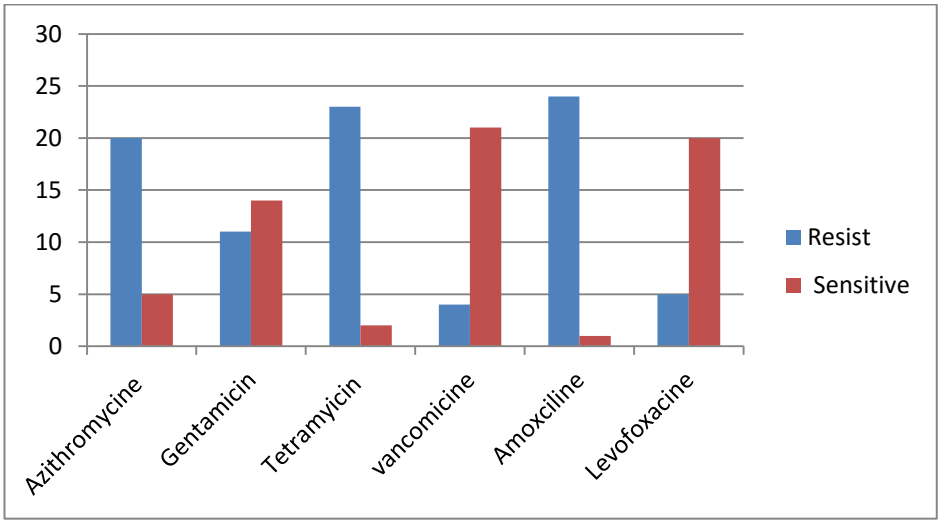


Figure 4.8:Distribution of *S. aureus* Isolate in Relation to Antibiotics Sensitivity Test.

This study supports the findings of Hossain *et al*., (2017) who found that *S. aureus* was completely sensitive to Amikacin, Imipenem, Meropenem, and Vancomycin. This is also in keeping with (Gitau *et al*., 2018) who showed that *S. aureus* had a high sensitivity to Imipenem (98%) and Vancomycin (95.1%). While the Gentamicin antibiotics in this study , does not consistent with (Yassin and Mahmood, 2021) they found *S.aureus* showed 100% susceptibility for Gentamicin respectively, and (Garoy *et al*., 2019) they showed low resistance rate (1.2%) for Gentamicin. The majority of *S. aureus* isolates were resistant to Amoxicillin-Clavulanic acid and Cefepime, according to the results this study supports the findings of (Verkaik *et al*., 2011) who observed that several strains of this bacterial species have developed resistance to certain antibiotics, including Methicillin and Amoxicillin. The target of β-lactam antibiotics is the transpeptidase moiety in Penicillin binding protein (PBP-2) (Foster *et al*., 2017)**.**

It is reported that 60% of coagulase-negative *staphylococci* (CNS) isolated from various specimens including central venous catheter tips, urine, and blood were highly resistant to Penicillin (90%), Ceftriaxone (40%), co-trimoxazole (60%), and Azithromycin (Azm) (60%) (Shrestha *et al*.2018). Many resistance genes of macrolides are parts of either transposon, plasmids, phages, or genomic islands and, as such, can easily transfer across species, strain, and genus boundaries (Feßler *et al*., 2018). The approach to treating *Staphylococcus* infections depends on the specific type of infection. In cases where the infection is caused by methicillin (oxacillin)-sensitive *Staphylococcus aureus* (MSSA), beta-lactamase antibiotics with potent activity against *S. aureus* are the preferred choice. Currently, there are only two β-lactam antibiotics, ceftobiprole and ceftaroline, that have demonstrated efficacy against the

most virulent strains of *S. aureus* (Talbot *et al*., 2019; De Oliveira *et al*., 2020).

Azithromycin is a commonly used antibiotic belonging to the macrolide class. While it is effective against a broad range of bacteria, its activity against *S . aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA), is somewhat limited (Parra-Ruiz *et al*., 2012).

The sub-inhibitory concentrations of Azithromycin could dose-dependently reduce the haemolytic activity and biofilm formation in *S. aureus* (Gui *et al*., 2014). More recent studies by (Parra-Ruiz *et al*., 2012)have shown that low concentrations of Clarithromycin could inhibit the biofilm formation of *S. aureus* .

Vancomycin, a glycopeptide produced by Amycolatopsis orientalis, has the ability to attach to the peptidoglycan side chains in the cell wall. It is employed in the treatment of invasive *staphylococcal* infections. When the cell wall is being formed, cross-linking takes place, and Vancomycin hinders this process. As a consequence, the cell wall becomes weaker, inhibiting growth and eventually leading to the death of the cells (Kiedrowski and Horswill, 2011).

Vancomycin-resistant strains of *S. aureus* can be classified into two types: intermediate-resistant and Vancomycin-resistant *Staphylococcus aureus* (VISA and VRSA, respectively). The genetic elements responsible for Vancomycin resistance are not present in VISA strains. However, during treatment with vancomycin, these bacteria can develop resistance due to selective pressure and mutations (Gardete and Tomasz, 2014). Most VISA strains are observed in patients who undergo prolonged Vancomycin regimens for the treatment of MRSA infections.

Gentamicin is not recommended as monotherapy for any infection caused by *S. aureus*. Rather gentamicin is used as an adjunct to agents with good efficacy against Gram-positive infections such as Vancomycin or nafcillin (Bisno *et al*., 1989).

In order to find the relationship between resistance to antibiotic and the degree of biofilm formation, Table (4-7) shows the type biofilm formation S. aureus and their sensitivity toward different antibiotic in vitro. Biofilms are communities of bacteria surrounded by a protective matrix that can form on surfaces, including within the human body. They are notoriously difficult to treat and can lead to persistent infections. In this study, the has been evaluated the impact of different antibiotics on the ability of *S. aureus* to form biofilms. Whether the antibiotic showed "Inhibition" or "Non-inhibition" of biofilm formation. The data is presented for three categories: "Strong," "Intermediate," and "Weak. The p-value indicates the statistical significance of the results. A p-value less than or equal to 0.05 ($p \leq 0.05$) is considered statistically significant, suggesting that the results are unlikely to be due to random chance, and "non-significant," indicating that there was no statistically significant difference observed between the groups (Inhibition vs. Non-inhibition) in terms of biofilm formation.

Azithromycin, Gentamicin, Tetracycline, Vancomycin, and Amoxicillin did not show a statistically significant difference in biofilm formation between the "Inhibition" and "Non-inhibition" groups ($p>0.05$). These antibiotics did not seem to have a substantial impact on biofilm formation by *S. aureus.*

Levofloxacin, on the other hand, showed a statistically significant difference in biofilm formation ($p=0.031$). It inhibited biofilm formation in a significant number of cases.

The study's main finding is that Levofloxacin demonstrated an inhibitory effect on biofilm formation by *S. aureus*, while the other antibiotics tested did not show a significant impact on biofilm formation. This information can be valuable for clinicians and researchers in choosing the appropriate antibiotics for the treatment of *S. aureus* infections, especially those associated with biofilm formation. However, it is essential to interpret the results cautiously and consider other factors, such as antibiotic resistance patterns, patient-specific conditions, and the infection site, before making clinical decisions.

S. aureus aggravate the antibiotic resistance and give rise to outrageous mortality and morbidity due to their proclivity to form biofilms in the wounds of the patients and on the medical devices implanted in the body (Neopane *et al*., 2018). The biofilm assists the pathogen to neutralize the action of antibiotics and to evade the host immune system resulting in persistent infections (Otto, 2018). biofilm is one of the major causes of amplifying antibiotic resistance, hence biofilm detection helps in investigating the severity of infection by *S. aureus* (El-Nagdy *et al*., 2020). Strong biofilm producing *S. aureus* isolates were more resistant to Cefoxitin (Neopane *et al*., 2018). Sedlacek and Walker (2007) They found that 90% or more of the bacteria with biofilms were resistant to Augmentin. Sun and associates who reported that the penetration of ciprofloxacin is significantly reduced through *S. aureus* biofilms (Sun *et al* .,2013). Stone *et al*. (2002) who concluded that Tetracycline has better penetration in biofilms. However, the effect of stronger biofilm formation in reducing the susceptibility of Tetracycline has also been reported by previous studies (Zhang *et al*.2018).

Table 4-7: Antibiotic Test Effect on *S . aureus* According to Intensity of Biofilm Formation

Antibiotic	Effect	Biofilm formation for *S. aureus*			Total	P value (p≤ 0.05)
		Strong	Interme diate	weak		
Azithromycin	Inhibition	2	0	3	5	.480 NS
	Non-inhibition	8	6	6	20	
Total		10	6	9	25	
Gentamicin	Inhibition	6	4	4	14	.280 NS
	Non-inhibition	4	2	5	11	
Total		10	6	9	25	
Tetracycline	Inhibition	2	0	0	2	.200 NS
	Non-inhibition	8	6	9	23	
Total		10	6	9	25	
Vancomycin	Inhibition	8	5	8	21	.600 NS
	Non-inhibition	2	1	1	4	
Total		10	6	9	25	
Amoxicillin	Inhibition	0	0	2	2	.200 NS
	Non-inhibition	10	6	7	23	
Total		10	6	9	25	
Levofloxaci n	Inhibition	10	6	6	22	.031*
	Non-inhibition	0	0	3	3	
Total		10	6	9	25	
***Significant difference under (p≤ 0.05) by Chi-seq. test. NS: non-significant**						

4.4.2. Antifungal Susceptibility Test of *Candida albicans* Isolate From Type of Impetigo.

The distribution of *C. albicans* isolates in relation to their sensitivity to various antifungal drugs, The isolates were tested against six different antifungal drugs: Fluconazole, Ketoconazole, Nystatin, Amphotericin B, Itraconazole, and Clotrimazole.The table showed number and percentage of isolates that were resistant and sensitive to each drug. that there is a significant relationship between C. *albicans* and antifungal sensitivity to (Fluconazol, Ketoconacol, Nystatin, Amphotercin B, Itraconazol, Clotrimazol) at p-value 0.05.

Fluconazole: Out of 25 isolates tested, 17 (68%) were resistant and 8 (32%) were sensitive to Fluconazole,

Ketoconazole: Among the 25 isolates, 21 (84%) were resistant and 4 (16%) were sensitive to ketoconazole.

Nystatin: Of the 25 isolates, 16 (64%) were resistant and 9(36%) were sensitive to Nystatin Amphotericin B: Similar to nystatin, 16 (64%) isolates being resistant and 9 (36%) being sensitive to amphotericin .

Itraconazole: Among the 25 isolates , 18 (72%) isolates were resistant and 7 (28%) were sensitive to Itraconazole. Clotrimazole: The majority of isolates, 21 (84%), were resistant, while only 4 (16%) were sensitive to Clotrimazole. as shown in Table (4-8) and Figure (4.9).

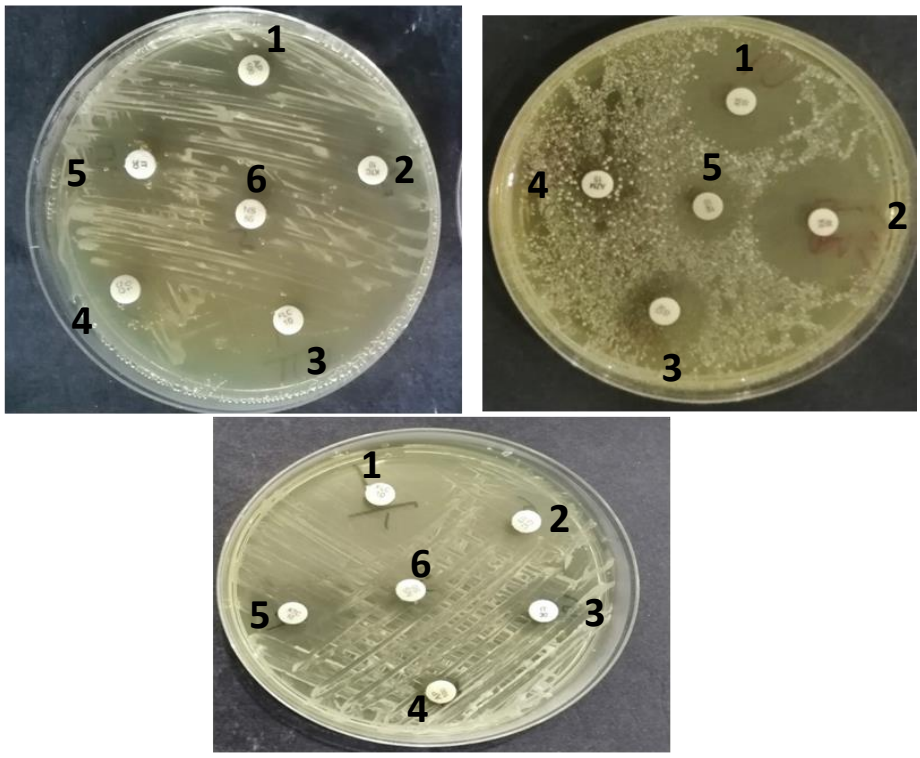


Figure 4.9: Test for Identified of Sensitive and Resistance to Antifungal in Clinical *C.albicans* Isolates.

(1- Amphotercin B 2- Ketoconacol 3 – Fluconazol 4 – Clotrimazol 5- Itraconazol 6- Nystatin, Itraconazol).

Table 4-8: Distribution of *C. albicans* in Relation to Antifungal Sensitivity Test.

Antibiotics	Resist N (%)	Sensitive N (%)	Total for isolate NO.	*p-value
Fluconazol	17(68)	8 (32)	25	.001 (H.S)
Ketoconazol	21 (84)	4 (16)	25	.001 (H.S)
Nystatin	16 (64)	9 (36)	25	.001 (H.S)
Amphotercin	16 (64)	9 (36)	25	.001 (H.S)
Itraconazol	18 (72)	7 (28)	25	.001 (H.S)
Clotrimazol	21 (84)	4 (16)	25	.001 (H.S)

***Chi-Square Tests, H. S= highly significance, N.S= no significance**

The p-values associated with each drug, indicated by "*p-value," suggest that there is a highly significant relationship between the distribution of *C.albicans* isolates and their sensitivity to each antifungal drug. The p-value of .001 (H.S) for all the drugs suggests a strong statistical association between the variables.

Multidrug-resistant (MDR) isolate was defined as an isolate non susceptible to one or more antifungal agent in at least two different drug classes (Arendrup *et al.*, 2017).

The mechanism of azole antifungals incorporates blocking the synthesis of ergosterol by inhibiting lanosterol 14α-demethylase. This enzyme has various potentials depending on the drug. For instance, posaconazole is significantly more potent than Itraconazole in inhibiting 14-alpha demethylase (Aydin *et al*., 2014). Fluconazole and Itraconazole are more cost-effective and less toxic drug forms of the azole family with excellent patient tolerance (Badiee *et al*. , 2009).

Ketoconazole is usually prescribed for fungal infections of the skin and mucous membranes (as a cream and shampoo), and its oral administration is limited due to risk of hepatic damage. The MIC90 for this agent in the current study was 0.125 µg/ml, indicating effectiveness against *C. albicans*. The resistance rates for this antifungal were reported to be 7% (Badiee *et al*.2009), 9.4% (Badiee and Alborzi,2011),34.9% (Mohamadi *et al*.2014).

The results in Table (4-9) presents the results of an experiment investigating the effect of different antibiotics on biofilm formation by *C. albicans*. Which is a common fungus that can form biofilms, which are complex structures that allow the organism to adhere to surfaces and become more resistant to treatment. In this study, evaluated the impact of various antibiotics on biofilm formating *C. albicans* in vitro.

Fluconazole, Nystatin, Itraconazole, and Clotrimazole did not show a statistically significant difference in biofilm formation between the "Inhibition" and "Non-inhibition" groups ($p>0.05$). These antibiotics did not seem to have a substantial impact on biofilm formation by *C. albicans*. While Ketoconazole and Amphotericin B, on the other hand, showed a statistically significant difference in biofilm formation ($p=0.040$).

They inhibited biofilm formation in a significant number of cases. The study's main finding is that Ketoconazole and Amphotericin B demonstrated an inhibitory effect on biofilm formation by *C. albicans*, while the other antibiotics tested did not show a significant impact on biofilm formation. This information can be valuable for clinicians and researchers in choosing the appropriate antifungal agents for the treatment of *C. albicans* infections, especially those associated with biofilm formation.

Table 4-9: Antibiotic Test Effect on *Candida albicans* According to Intensity of Biofilm Formation .

Antibiotic	Effect	Biofilm formation for *C. albicans*				Total	P value (p≤ 0.05)
		Strong	Intermediate	Week	negative		
Fluconazole	Inhibition	6	2	0	0	8	**.120 NS**
	Non-inhibition	7	3	4	3	17	
Total		13	5	4	3	25	
Ketoconacol	Inhibition	4	0	0	0	4	**.040 ***
	Non-inhibition	9	5	4	3	21	
Total		13	5	4	3	25	
Nystatin	Inhibition	6	1	2	0	9	**.120 NS**
	Non-inhibition	7	4	2	3	16	
Total		13	5	4	3	25	
Amphotercin B	Inhibition	7	2	0	0	9	**.040 ***
	Non-inhibition	6	3	4	3	16	
Total		13	5	4	3	25	
Itraconazol	Inhibition	5	2	0	0	7	**.080 NS**
	Non-inhibition	8	3	4	3	18	
Total		13	5	4	3	25	
Clotrimazol	Inhibition	3	0	1	0	4	**.240 NS**
	Non-inhibition	10	5	3	3	21	
Total		13	5	4	3	25	

***Significant difference under (p≤ 0.05) by Chi-seq. test. NS: non-significant**

Fungal biofilms differ from planktonic cells by their higher virulence, better adherence to mammalian tissue, and greater resistance to antifungals, especially Azoles (Uppuluri *et al*., 2018). Biofilms of *Candida albicans* are less susceptible to many antifungal drugs than are planktonic yeast cells (Perumal *et al* ., 2003).

Although all *Candida* species are potentially prone to form biofilms, a well-recognized fact in clinical settings, the formation of biofilms depends on several factors, involving both the microorganisms themselves and the host (Ramage *et al*., 2005). The biofilms and Fluconazole-resistant *C. albicans* were grown on polystyrene surfaces and showed the differences in the amount of cells in the initial inoculum necessary to establish the biofilm (Francisca *et al*., 2020).

4.5. Molecular Detection of Some Virulence Factors for Studied Microorganisms

By employing molecular techniques, such as polymerase chain reaction (PCR) or gene sequencing, researchers can identify specific genes or genetic markers associated with virulence factors in microorganisms. These virulence factors are molecules or structures that enable microorganisms to establish infection, evade the host immune response, and cause damage to host tissues. Examples of commonly studied virulence factors include toxins, adhesion factors, invasion factors, and immune evasion molecules (Nima *et al*., 2019).

The molecular detection of virulence factors provides several key advantages. Firstly, it allows for the accurate identification and characterization of pathogenic strains within a microbial population. This information is crucial for epidemiological studies, outbreak investigations, and understanding the diversity and evolution of

virulence factors. Secondly, it aids in the diagnosis of infectious diseases by confirming the presence of specific virulence factors in clinical specimens. This can help in guiding appropriate treatment strategies and monitoring the spread of virulent strains (Baba-Moussa *et al*., 2008).

4.5.1. Molecular Detection of Some Virulence Factors for *S. aureus*

Virulence factors of *S. aureus* are vital for the pathogenesis as well as for diagnosis of *S. aureus*. the molecular detection of virulence factors in *S. aureus* plays a critical role in unraveling the pathogenicity and virulence mechanisms of this bacterium. It helps in understanding the distribution, prevalence, and clinical relevance of specific virulence factors, aiding in diagnostics, epidemiological studies, and therapeutic interventions. Continuous research in this field is necessary to stay ahead of the evolving virulence factors in *S. aureus* and combat the associated infections effectively (Heilmann, 2011; Saei and Ahmad, 2011).

4.5.1.1. Molecular Detection of *mecA* Gene

In this research, a comprehensive analysis was conducted on 25 *S. aureus* isolates collected from skin infection (Impetigo). The aim was to detect the presence of *mecA* genes. Figure (4-10) visually represents the distribution of these genes in the isolates as percentages, with all the clinical isolates showing a 100% (25) occurrence.

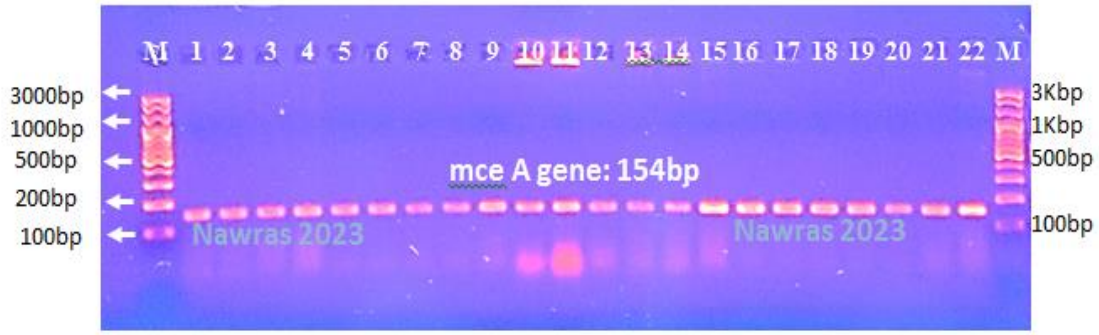


Figure 4.10: Amplification of *mceA* gene: : 2% Agarose gel electrophoresis analysis of PCR amplification products of *mecA* gene of 154 bp, extracted from *S. aureus* all *S.aureus* isolate possess *mceA* gene the electrophoresis was run at 70 volts. lane (L), DNA Molecular Size Marker (100 bp ladder) . Gene *mecA* produces positive results in all Lanes.

The present study involves identifying the specific genes responsible for the resistance to commonly used medications against *S. aureus* isolates in the country. One such gene is *mecA*, which plays a role in conferring Methicillin resistance in *S. aureus*. Methicillin resistance in *S. aureus* occurs when a Penicillin-binding protein, encoded by a chromosome, undergoes a mutation. This type of resistance can be transferred among *S. aureus* organisms through bacteriophages (Lakhundi *et al*., 2018).

MRSA is the major cause of nosocomial mortality and morbidity; it is commonly found in the community and hospital environment especially in the ICUs (Montesinos *et al*., 2002).

The findings from our study regarding clinical isolates align with the observations made by Yurtsever *et al*. (2020) and Jowad and Yousif (2013). They reported a 100% presence of the *mecA* gene in all of their isolates. However, our results differ from those of Alhamadani and

Tuwaij (2020) and Koosha *et al*. (2016), who found a *mecA* gene ratio of 80% and 87.3%, respectively, in *S. aureus* isolates.

4.5.1.2. Molecular Detection of *spa* Genes

A total of 25 *S. aureus* isolates were molecularly tested for the presence of *spa* genes after being collected from various skin infections. Gene frequency in the isolates under investigation as a percentage:56% (14) clinical isolates as shown in Figure (4-11).

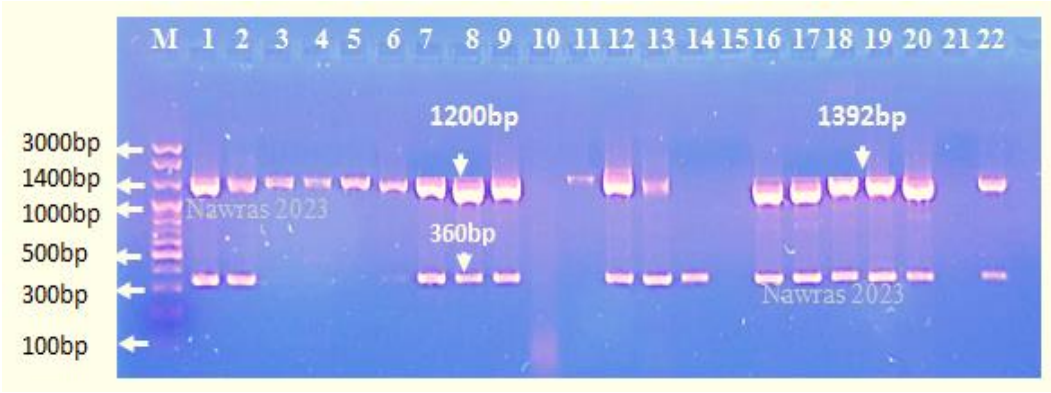


Figure 4.11: Electrophoresis diagram of Monoplex PCR generated products for extracted DNA of 25 *S. aureus* isolates Primer *spa* gene with Product (350bp). For 1.5 hours , the electrophoresis was run at 70 volts) Gene *spa* produces positive results in 14 isolates.

The *spa* protein, found on the surface of *S. aureus* bacteria, serves as both a virulence factor and a distinguishing marker for the bacterium. Utilizing molecular typing of this protein can help prevent epidemics, reduce the incidence of illnesses, and decrease the financial burden of nosocomial infections.

The results of clinical isolates are comparable to that of (Jowad and Yousif, 2013) which found that all *S. aureus* isolates possess *spa* genes. While this finding differs from that of (Rezashateri *et al* ., 2021) that showed the *spa* gene distribution was present in (82%) of the

specimens and (Kareem *et al* ., 2020 ; Ali *et al*., 2020) who showed that *spa* gene variation was detected in (63.5 %) of the isolates.

The study's main finding is that there is a highly significant difference in the PCR analysis of both the *mce A* and *Spa* genes in *S. aureus*. A high proportion of *S. aureus* isolates showed positive PCR analysis for these genes, indicating that they are actively being transcribed and may be involved in the biological processes of the bacterium , show in Table (4-10). *mce A* gene : out of the 25 *S. aureus* isolates analyzed, 25 isolates (100%) showed a positive PCR of the *Mce A* gene, while 0 isolates (0.0%) showed negative PCR of the *mce A*. *Spa* gene : Out of the 25 *S. aureus* isolates, 14 isolates (56.0%) showed a positive PCR analysis of the *Spa* gene, while 11 isolates (44.0%) showed negative PCR of the *spa* gene. Statistical significance: The p-values for both *Mce A* and Spa gene PCR are less than or equal to 0.05 ($P \leq 0.05$), denoted by the asterisk (*). This indicates that there is a highly significant difference in gene between the positive and negative groups for both genes.

Table 4-10: Expression of *(mce A* and *spa* gene) for *S. aureus*

Gene expression	*mce A* gene of *S .aureus*		*Spa* gene of *S .aureus*	
	Frequency	Percent	Frequency	Percent
Positive	25	100.0	14	56.0
Negative	0	0.0	11	44.0
Total	25	100.0	25	100.0
P ≤0.05	.001*		.003*	
*Highly significant difference under (P ≤ 0.05) by T-test				

These findings are crucial for understanding the virulence and pathogenicity of *S. aureus*, as these genes may play essential roles in the bacterium's ability to cause infections and evade the host's immune response. The rate of Methicillin resistance was 95.4% (Khairallah *et al*. 2020).

Many factors could contribute to the variation in the rate of resistance, such as the population studied, type of isolates, and prescription of certain antibiotics in different geographic areas. Two MRSA isolates were negative for the *mecA* gene; this could indicate that these MRSA isolates have a different mechanism for Methicillin resistance than through the *mecA* gene (Ba *et al*., 2014).

4.5.2. Molecular Detection of Some Virulence Factors for *Candida albicans*

Candida albicans is a type of yeast that can cause infections in humans, particularly in immunocompromised individuals or those with weakened immune systems. It is known to produce various virulence factors that contribute to its pathogenicity. Molecular detection methods have been developed to identify and study these virulence factors in *C. albicans*. The virulence factors play a role in adherence, haemolytic activity, phenotypic switching and production of hydrolytic enzymes (Megha *et al*., 2022).

Major steps of candidal infection are adhesion and formation of hyphae, which leads to tissue invasions (Galan-Ladero *et al*., 2013). Various techniques can be employed depending on the specific virulence factor and the research goals. These molecular detection methods provide valuable insights into the role of virulence factors in the pathogenicity of *C. albicans* and aid in the development of targeted

antifungal therapies. DNA extraction profile of 22 *Candida* isolates and presence of virulence genes: αINT1 and ALSA

4.5.2.1. Molecular detection of *αINT1* genes

The results in figure (4-12) show isolates: 2-4,6-8, 10-20, 22: αINT1 gene shown positive result (bands) 18 (81.8%) isolates were positive while 4 (18.2%) shown negative result .

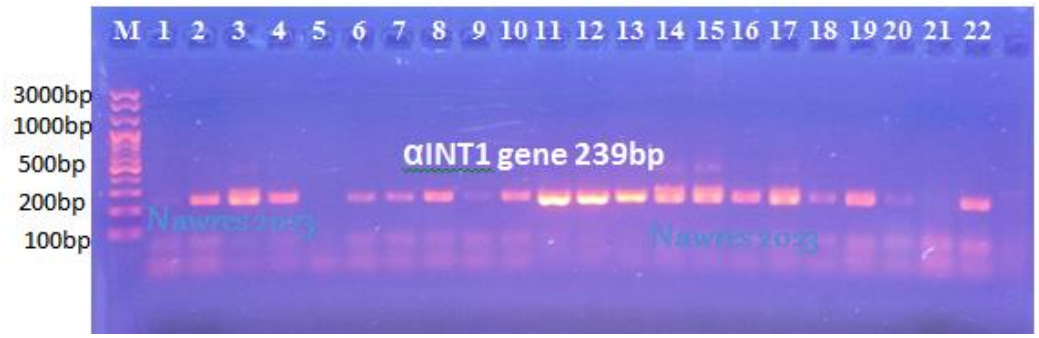


Figure 4.12: Illustrated of αINT1 genes in *Candida albicans* isolates shown positive PCR product of αINT1 gene= 239bp, isolates: 2-4,6-8, 10-20, 22: αINT1 gene shown positive result(bands). αINT1 gene shown negative result (no bands) in other *Candida albicans*). (M: Marker (100 bp for each step).

αINT1 is a unique gene from *C.albicans* ; hence, it has been used for detection interspecific phylogenetic analysis of *C. albicans* collected from human patients.

The **α**INT1 plays a critical role in *C. albicans* cell adhesion and virulence (Gale *et al*., 1998) and it was chosen for its uniqueness to C. albicans isolates (Abia-Bassey and Utsalo , 2006).

4.5.2.2. Molecular Detection of *ALS1 Gene* of *C. albicans*

The results in Figure (4-13) show 15 (68.2%) isolates were positive to *ALS1* gene while 7 (31.8%) isolates were negative.

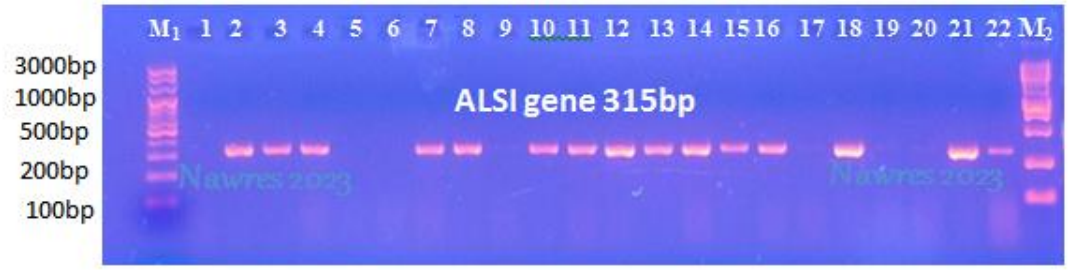


Figure 4.13: Illustrated of ALS genes in *Candida albicans* isolates shown positive PCR product of ALSI gene= 315bp, isolates: 2-4,7-8, 10-16,18,21-22: ALS1 gene shown positive result(bands). ALS1 gene shown negative result (no bands) in other *Candida albicans*). (M1: Marker (100 bp for each step. M2 Marker (250 bp for first step).

The *ALS1* gene and its protein product have been extensively studied in the context of *C. albicans* pathogenesis. Research has shown that *AlS1* is involved in the binding of *C. albicans* to human epithelial and endothelial cells, facilitating the formation of biofilms on both abiotic surfaces (such as medical devices) and host tissues. Biofilms provide protection to the fungus, making it more resistant to immune responses and antifungal treatments (Alves *et al*., 2014).

The *ALS1* was introduced as the major over-expressed gene in biofilm formation and adherence which in turn play an important role in drug inhibition to gain access to the fungi microcolonies (García-Sánchez *et al*., 2004; Dhamgaye *et al*., 2012; İnci *et al*., 2013). Similar findings by other investigators which show pathogenic *Candida* species established well-developed biofilm and are resistant to drug therapy and act as a source of reinfections. More studies are required to realize the exact mechanisms involved (Chandra *et al*., 2001; Kumamoto, 2005).

The Table (4-11) presents the results of the gene PCR analysis for two genes, *ALS* and *INT1*, in *C.albicans*. The analysis aims to determine whether these genes are (positive) or (negative) in the tested isolates. *ALS* gene were Positive: Out of the 22 *C. albicans* isolates analyzed, 15 isolates (68.2%) showed positive of the ALS gene and negative: 7 isolates (31.8%) showed negative of the ALS gene.

INT1 gene were Positive:18 out of 22 *C. albicans* isolates (81.8%) showed positive PCR analysis of the *INT1* gene and negative: 4 isolates (18.2%) of the *INT1* gene. The positive PCR analysis of the ALS gene is consistent with its known role in adhesion and colonization of host tissues. Similarly, the positive PCR analysis of the INT1 gene is in line with its function in hyphal development, which is crucial for *C. albicans* ability to invade host tissues and cause infections.

Cheng *et al*. (2005) evaluated the PCR analysis of *ALS* genes in *C. albicans* isolates using RT-PCR and showed a high frequency Nas *et al*. (2008) evaluated the PCR analysis of *ALS1* gene in *C. albicans* isolated from vaginal candidiasis and showed its PCR analysis in 69% of total isolates.

In a study by Shahla *et al*. (2016), 42 (79.5%) of isolates with an PCR analysis of at least one ALS gene were resistant to Fluconazole and 3 (5.5%) of them without *ALS* PCR analysis were absolutely sensitive to Fluconazole.

The presence of two functional copies of *INT1* appeared to facilitate *C.albicans* colonization ,suggesting that intestinal colonization may be another virulence factor associated with *INT1* and that the gene product may be an attractive target to control *C. albicans* intestinal colonization (Karen *et al*., 1999).

Table 4-11: Expression of (*ALS* and *INT1* gene) for *Candida albicans*

Gene expression	ALS gene of *C. albicans*		INT 1 gene of *C.albicans*	
	Frequency	Percent	Frequency	Percent
Positive	15	68.2	18	81.8
Negative	7	31.8	4	18.2
Total	22	100.0	22	100.0
P ≤0.05	.003*		.002*	
*Highly significant difference under (P ≤ 0.05) by T-test				

4.6. Relationship Of Virulance Factors To The Type Of Clinical IsolatOf S. aureus .

4.6.1. Distribution of *S.aureus* Biofilm Formation and Blood Hemolysis with *mce A* Gene and *Spa Gene*

Biofilm formation is a critical virulence factor for *S. aureus*, enabling the bacteria to adhere to surfaces, including medical devices and tissues, and resist host immune responses and antibiotic treatment. Positive PCR analysis : Out of the 25 *S. aureus* isolates analyzed and biofilm Formation: Among the isolates with positive *mceA* gene PCR analysis , 10 showed strong biofilm formation, 6 showed intermediate biofilm formation, and 9 showed weak biofilm formation.

Positive PCR analysis of *spa* gene: , 14 out of 25 *S. aureus* isolates played positive PCR analysis of the *spa* gene and biofilm formation , 5 showed strong biofilm formation, 3 showed intermediate biofilm formation, and 6 showed weak biofilm formation. Negative PCR analysis : Among the 11 isolates with no spa gene PCR analysis , 5 showed strong biofilm formation, 3 showed intermediate biofilm

formation, and 3 showed weak biofilm formation, as shows in Taple (4-12).

The results indicate that the PCR analysis of the *spa* gene *in S. aureus* is associated with a significant increase in biofilm formation. In contrast, the mceA gene's expression does not seem to have a statistically significant impact on biofilm formation.

Table 4-12: Relationship Between *S.aureus* Biofilm Formation and their Genes

Gene of S. Aureus	PCR analysis	Biofilm formation for *S. aureus*			Total	P value (p≤ 0.05)
		Strong	Interme diate	week		
MceA	Positive	10	6	9	25	.080 NS
	Negative	0	0	0	0	
Total		10	6	9	25	
Spa	Positive	5	3	6	14	.040 *
	Negative	5	3	3	11	
Total		10	6	9	25	
*Significant difference under (p≤ 0.05) by Chi-seq. test. NS: non-significa						nt

The *spa* gene encodes the *Staphylococcal* Protein A (*SpA*), which plays a crucial role in *S. aureus* virulence and immune evasion. *SpA* is known to bind to immunoglobulins and influence host immune responses. Its role in promoting biofilm formation its significance in *S. aureus* pathogenesis and its potential as a therapeutic target.

On the other hand, the *mceA* gene belongs to the Mce (Mammalian Cell Entry) protein family, involved in adhesion and invasion of host cells. While it did not show a statistically significant association with biofilm formation in this study, it might still play a role in other aspects of *S. aureus* pathogenicity.

In conclusion, the study provides valuable insights into the relationship between gene analysis and biofilm formation in *S. aureus.* The significant association of *spa* gene analysis with increased biofilm formation highlights its potential importance as a target for therapeutic interventions against *S. aureus* infections. However, further research is needed to elucidate the precise mechanisms and interplay of various genes contributing to biofilm formation in *S. aureus.*

Although it is particularly notorious for causing skin and soft-tissue infections, it has an ability to infect nearly every organ system in the human body, often with fatal consequences (Romero *et al*., 2021).

The distribution of *S. aureus* biofilm formation and blood hemolysis can be influenced by various factors, including the presence of specific genes such as *mce A* and *Spa* (Brady *et al*., 2007).

Biofilms are complex structures formed by bacteria, including *S. aureus*, that adhere to surfaces and are encased in a self-produced matrix. Biofilm formation plays a crucial role in the pathogenesis of *S. aureus* infections, as it provides protection against the host immune system and antimicrobial agents (Nathan *et al*., 2011).

The *mce A* gene, also known as the microbial surface component recognizing adhesive matrix molecules (MSCRAMM) gene, encodes a surface protein involved in adherence to host tissues and biofilm formation. The presence of the *mce A* gene can enhance the ability of *S.*

aureus to form biofilms, leading to increased virulence and resistance to treatment. The distribution of *mce A* gene among *S. aureus* strains can vary. Some strains may carry this gene, while others may lack it. The presence of *mce A* gene can contribute to differences in biofilm formation capacity among *S. aureus* isolates. However, it is important to note that biofilm formation is a multifactorial process influenced by several genes and environmental factors. *S. aureus* produces various virulence factors, including hemolysins, which are toxins that cause the destruction of red blood cells and contribute to the pathogenicity of the bacterium. Hemolysis is commonly assessed using blood agar plates, where *S. aureus* colonies can exhibit different hemolytic patterns. The MRSA nature of the isolates was confirmed by detecting the resistance against cefoxitin and through PCR by amplifying the *mecA* gene (Foster *et al*., 2020; Romero *et al*., 2021).

The method based on the resistance against Cefoxitin was found to exhibit good sensitivity and specificity in comparison to the PCR. All Cefoxitin-resistant *S. aureus*, nonetheless, were positive for the *mecA* gene. Indeed, finding the *mecA* gene is the major evidence for the detection of MRSA isolates, which has been endorsed by many workers (Mehndiratta *et al*., 2009; Habiband Qadir, 2022). MRSA strains that are community-associated have become a significant source of infection in people who have never visited the healthcare system. Our findings suggested the higher burden (62.92%) of the MRSA in contrast to the study conducted by Ahmed and Asrar (2014).

The *Spa* typing sequences the *S.aureus* -specific *staphylococcal protein A* (*spa*) gene which is one of the virulence factors on the surface of the organism preventing phagocytosis by the immune system (Votintseva *et al*., 2014).

The distribution of the *Spa* gene among *S. aureus* strains can also vary. Different *Spa* types have been identified, and certain types may be associated with specific lineages or clinical presentations. However, the presence or absence of the *Spa* gene alone does not determine the hemolytic activity of *S. aureus*. Other factors, such as the production of different hemolysins (such as, alpha, beta, or delta toxins), can contribute to the observed hemolytic patterns. The *Spa* gene (*Staphylococcal* protein A) is responsible for encoding a cell wall-associated protein that plays a role in immune evasion and host colonization. While Spa is not directly associated with hemolysis, it is one of the major surface proteins of *S. aureus* and can influence the interaction between the bacterium and host cells (O'Hara *et al*., 2016).

In study done by Ashika Singh *et al*. (2020) showed as *spa* typing was not done on all MRSA isolates and as MLST was only performed on a few selected isolates we could not confidently establish the circulating clones that are representative of entire surveillance population.

Table 4-13: The Distribution of *S.aureus* Biofilm Formation and Blood Hemolysis with *mce A* Gene and *Spa Gene*

Sample	Source of *S. aureus*	Biofilm	Blood hemolysis	*mce A* gene	*Spa* gene
1	Impetigo	Strong biofilm	Positive	Positive	positive
2	Impetigo	Strong biofilm	Positive	Positive	positive
3	Ecthyma	Weak biofilm	Positive	Positive	positive
4	Ecthyma	Weak biofilm	Positive	Positive	positive
5	Impetigo	Intermediate biofilm	Positive	Positive	positive
6	Impetigo	Weak biofilm	Positive	Positive	positive
7	Ecthyma	Weak biofilm	Positive	Positive	positive
8	Impetigo	Weak biofilm	Positive	Positive	positive
9	Impetigo	Strong biofilm	Positive	Positive	positive
10	Impetigo	Intermediate biofilm	Positive	Positive	positive
11	Impetigo	Strong biofilm	Positive	Positive	positive
12	Impetigo	Intermediate biofilm	Positive	Positive	positive
13	Impetigo	Weak biofilm	Positive	Positive	positive
14	Ecthyma	Strong biofilm	Positive	Positive	positive
15	Impetigo	Strong biofilm	Positive	Positive	Negative
16	Ecthyma	Strong biofilm	Positive	Positive	Negative
17	Ecthyma	Strong biofilm	Positive	Positive	Negative
18	Impetigo	Intermediate biofilm	Positive	Positive	Negative
19	Ecthyma	Strong biofilm	Positive	Positive	Negative
20	Ecthyma	Strong biofilm	Positive	Positive	Negative
21	Ecthyma	weak biofilm	Positive	Positive	Negative
22	Impetigo	Intermediate biofilm	Positive	Positive	Negative
23	Impetigo	weak biofilm	Positive	Positive	Negative
24	Ecthyma	Weak biofilm	Positive	Positive	Negative
25	Impetigo	Intermediate biofilm	Positive	Positive	Negative

4.6.2. Relationship Of Virulence Facter To The Type Of Clinical Isolat Of C. albicans.

The relationship between the PCR analysis of two genes, ALS and INT1, in *Candida albicans*, and their association with biofilm formation, a critical virulence factor for this fungal species. Positive PCR analysis : Out of the 25 *C.albicans* isolates analyzed, 15 isolates showed positive PCR analysis of the *ALS* gene. Negative PCR analysis : 7 isolates did not express the *ALS* gene. Biofilm Formation Among the isolates with positive ALS gene PCR analysis , 7 showed strong biofilm formation, 5 showed intermediate biofilm formation, and 3 showed weak biofilm formation.

Positive PCR analysis : 18 out of 25 *Candida albicans* isolates displayed positive PCR analysis of the *INT1* gene. Negative PCR analysis : 4 isolates did not express the *INT1* gene.

Biofilm Formation: Among the isolates with positive *INT1* gene PCR analysis , 10 exhibited strong biofilm formation, 5 showed intermediate biofilm formation, and 3 showed weak biofilm formation, as show in Table (4-14).

The results suggest that there is no statistically significant association between the PCR analysis of the *ALS* and *INT1* genes in *C.albicans* and their biofilm formation capability.

Table 4-14: Relationship Between *Candida albicans* Virulence and their Genes

Gene of Candida	Expression	Biofilm formation for *C. albicans*				Total	P value (p≤ 0.05)
		Strong	Intermediate	Weak	Negative		
ALS	Positive	7	5	3	0	15	.440NS
	Negative	6	0	1	3	10	
Total		13	5	4	3	25	
INT1	Positive	10	5	3	0	18	.440NS
	Negative	3	0	1	3	7	
Total		13	5	4	3	25	
NS:Non-Significant difference under (p≤ 0.05) by Chi-seq. test.							

The distribution of *C. albicans* biofilm formation and blood hemolysis can be influenced by specific genes such as *INT1* and *ALS*. *C.albicans* colonizes several tissues such as vaginal and oral epithelia, developing a biofilm that, in immunocompromised patients, can disseminate into the bloodstream and cause fatal systemic infections (Hawser *et al*., 1998, Fox and Nobile, 2013).

Biofilms play a significant role in the pathogenesis of *C. albicans* infections as they provide protection against the host immune system and antifungal treatments (Douglas, 2003).

The presence of the *INT1* gene can enhance the ability of *C. albicans* to form biofilms, leading to increased resistance within the host. However, it's worth noting that biofilm formation is a complex process influenced by various genetic and environmental factors (Fox and Nobile, 2012).

The *ALS* gene family comprises several members (*ALS1*, *ALS2*, *ALS3*, etc.), each encoding cell surface glycoproteins that play a role in adhesion, biofilm formation, and host colonization. These proteins can also contribute to the hemolytic activity of *C. albicans* (Hoyer *et al*., 2008).

The distribution of *ALS* genes among *C. albicans* strains can vary. Different strains may carry different combinations of *ALS* genes, which can result in variations in adhesion, biofilm formation, and hemolytic potential. The expression of specific *ALS* genes can be influenced by environmental factors, such as pH, temperature, and nutrient availability (Zhao *et al*., 2004: Murciano *et al*., 2012). The presence of specific genes, such as *INT1* and *ALS*, can contribute to the overall capacity of *C. albicans* to form biofilms and exhibit hemolytic activity. However, the interplay between multiple virulence factors and host-pathogen interactions ultimately determines the pathogenicity of *C. albicans* infections.

Table 4-15 : The Distribution of *C.albicans* Biofilm Formation and Blood Heamolysis with *INT1* Gene and *ALS Gene*

Sample	**Source of *S. aureus***	Biofilm	Blood hemolysis	INT1 gene	ALS gene
1	Impetigo	Strong biofilm	Positive	Positive	Positive
2	Impetigo	Intermediate biofilm	Positive	Positive	Positive
3	Ecthyma	Strong biofilm	Positive	Positive	Positive
4	Impetigo	Negative	Positive	Positive	Positive
5	Impetigo	Intermediate biofilm	Positive	Positive	Positive
6	Impetigo	Weak biofilm	Positive	Positive	Positive
7	Ecthyma	Intermediate biofilm	Positive	Positive	Positive
8	Impetigo	Strong biofilm	Positive	Positive	Positive
9	Impetigo	Negative	Negative	Positive	Positive
10	Ecthyma	Strong biofilm	Positive	Positive	Positive
11	Impetigo	Strong biofilm	Positive	Positive	Positive
12	Impetigo	Strong biofilm	Positive	Positive	Negative
13	Ecthyma	Strong biofilm	Positive	Positive	Positive
14	Ecthyma	Strong biofilm	Positive	Positive	Positive
15	Impetigo	Weak biofilm	Negative	Positive	Negative
16	Ecthyma	Strong biofilm	Positive	Positive	Positive
17	Ecthyma	Strong biofilm	Positive	Positive	Negative
18	Impetigo	Strong biofilm	Positive	Positive	Positive
19	Ecthyma	Intermediate biofilm	Positive	Positive	Positive
20	Impetigo	Intermediate biofilm	Positive	Positive	Positive
21	Ecthyma	Strong biofilm	Positive	Positive	Positive
22	Impetigo	Strong biofilm	Negative	Negative	Negative
23	Impetigo	Weak biofilm	Negative	Negative	Negative
24	Ecthyma	Weak biofilm	Negative	Negative	Negative
25	Impetigo	Negative	Negative	Negative	Negative

4.7. Relationship Between Types of Impetigo with IgE and Tumor Necrosis Factor alpha (αTNF) in Studied Groups

There is a significant difference between patient and control group in relation to IgE and αTNF test at p-value 0.05. the results indicate that there are notable variations in IgE levels between the different groups in this study population, and these differences are statistically significant, as shows in Table (4-16).

Table 4-16: Evaluation of IgE in Study Population

Population	N	Concentration of IgE kuI/1		P value (p≤0.05)
		Mean	Std. Deviation	
Impetigo	40	395.1192	62.57725	.001 *
Ecthyma	10	533.1580	134.42415	
Control	25	61.6804	5.68201	
Total	75	302.3781	42.61602	
***Highly significant difference under (p≤0.05) by one-way ANOVA**				

The study found that there is a highly significant difference in IgE levels among the groups under investigation (Impetigo, Ecthyma, and Control). The p-value of 0.001 (indicated by "*") for the Impetigo group means that the difference in IgE levels between Impetigo and the other groups is highly significant. While the Control group seems to have the lowest mean IgE concentration compared to the other two groups. The Impetigo group has the highest mean IgE concentration (395.1192 kuI/1) compared to the control group. Ecthyma group has an average IgE concentration of 533.1580 kuI/1, which is higher than both the Impetigo and Control groups.

Table (4-17) presents the evaluation of TNF (Tumor Necrosis Factor) levels in a study groups, categorized into three groups: Impetigo, Ecthyma, and Control.

Table 4-17: Evaluation of TNF in study population

Population	N	Concentration of TNF ng/l		P value (p≤0.05)
		Mean	Std. Deviation	
Impetigo	40	158.2645	12.96287	.001 *
Ecthyma	10	147.4740	29.81072	
Control	25	39.4916	4.78692	
Total	75	117.2348	10.25308	

*Highly significant difference under (p≤0.05) by one-way ANOVA

TNF is a pro-inflammatory cytokine that plays a crucial role in various physiological processes and immune responses. the study suggests that there is a highly significant difference in TNF concentration between the impetigo group and the control group ($p \leq 0.05$). However, there is not enough information to determine if the TNF concentrations in the ecthyma group are statistically different from the control group. Impetigo: The group of 40 individuals with impetigo had a mean TNF concentration of 158.2645 ng/l with a standard deviation of 12.96287. The p-value of .001 indicates that there is a highly significant difference in TNF concentration compared to the control group.

The group of 10 individuals with ecthyma had a mean TNF concentration of 147.4740 ng/l with a standard deviation of 29.81072. However, The p-value of .000 indicates that there is a highly significant difference in TNF concentration compared to the control group.

The control group consisting of 25 individuals had a mean TNF concentration of 39.4916 ng/l with a standard deviation of 4.78692.

During skin infection, a variety of immune cells and cytokines could coordinate host skin immunity (Stamatas *et al*., 2013; Papadogiannakis and Koutinas, 2015).

The *Staphylococcus aureus* bacteria play an important role in intensifying AD symptoms. The bacteria worsen the disease by producing super antigens and interleukins. They also cause severe infection by reproducing T lymphocytes (Wollenberg and Feichtner, 2014). MRSA was seen in the patient's culture and smear. Other observations included mild leukocytosis, increases in serum IgE (350), increase eosinophils levels (12%) in peripheral blood smear, and normal serum IgG levels. Impetigo infections should be studied since they are the most common among AD infants. Once the cause is identified, it can be removed. Due to the high immunoglobulin E levels (350), the disease was diagnosed as an IgE-mediated eczema which is the most common type of AD in 70% to 80% of the cases (Sugiyama *et al*., 2014; Salah and Faergemann, 2015).

Tumor necrosis factor-α is an early response cytokine that facilitates neutrophil infiltration into the lesion site and clears pathogens (Feldmann *et al*., 1996; Aufiero *et al*., 2007). The bacterial load was significantly higher in the *S. aureus*-induced experimental brain abscess in TNF mice, when compared with WT mice (Dadsetan *et al*., 2016).

In a study done by Chao *et al*., (2018) revealed that TNF-α was rapidly produced after *S. aureus* infection and involved in neutrophil recruitment into the skin lesion.

Table (4-18**)** represents the evaluation of immunological markers, specifically Immunoglobulin E (IgE) and Tumor Necrosis Factor (TNF), in a study groups. IgE is an antibody that plays a significant role in allergic responses, while TNF is a pro-inflammatory cytokine involved in various immune processes.

Table 4-18: Evaluation of Immunological Markers in Study Population

Population	N	Concentration of IgE kuI/1		Concentration of TNF ng/l	
		Mean	Std. Deviation	Mean	Std. Deviation
Impetigo	40	395.1192	62.57725	158.2645	12.96287
Ecthyma	10	533.1580	134.42415	147.4740	29.81072
Control	25	61.6804	5.68201	39.4916	4.78692
Total	75	302.3781	42.61602	117.2348	10.25308
P value (p≤0.05)		.001*		.001*	
***Highly significant difference under (p≤0.05) by one-way ANOVA**					

The group of 40 individuals with impetigo had a mean IgE concentration of 395.1192 kuI/1 with a standard deviation of 62.57725. They also had a mean TNF concentration of 158.2645 ng/l with a standard deviation of 12.96287. The p-values for both IgE and TNF are < 0.001 (indicated by .001*), indicating a highly significant difference compared to the control group. While the group of 10 individuals with ecthyma had a mean IgE concentration of 533.1580 kuI/1 with a standard deviation of 134.42415. Their mean TNF concentration was 147.4740 ng/l with a standard deviation of 29.81072. The p-values for both IgE and TNF are < 0.001, again indicating a highly significant difference compared to the control group. The control group consisting of 25 individuals had a mean IgE concentration of 61.6804 kuI/1 with a standard deviation of 5.68201. Their mean TNF concentration was 39.4916 ng/l with a standard deviation of 4.78692.

In summary, the table suggests that both IgE and TNF concentrations are significantly different between the impetigo and ecthyma groups compared to the control group ($p \leq 0.05$). The p-values indicate a highly significant difference in both immunological markers in these two conditions.

Table (4-19) shows the correlation between two immunological markers, Tumor Necrosis Factor (TNF) and Immunoglobulin E (IgE), in three different groups within the study population (Impetigo, Ecthyma, and control).

Table 4-19: Correlation Between Immunological Markers in Study Groups.

Correlations			
Type of infection			**TNF con. ng/l**
Impetigo	IgE con. UKI	Pearson Correlation	-.104-*
		Sig. (2-tailed)	.525
		N	40
Ecthyma	IgE con. UKI	Pearson Correlation	.375**
		Sig. (2-tailed)	.286
		N	10
Control	IgE con. UKI	Pearson Correlation	-.054-*
		Sig. (2-tailed)	.798
		N	25
***. Correlation is non-significant with an inverse relationship at the 0.05 level (2-tailed). ** Correlation is non-significant**			

Correlation analysis is used to understand the relationship between two variables and can help identify if there is any association between these markers. Impetigo: The Pearson correlation coefficient between TNF concentration and IgE concentration in the Impetigo group is -0.104. The p-value associated with this correlation is 0.525, which is greater than 0.05. Therefore, the correlation is considered non-significant

at the 0.05 level (2-tailed). The negative sign of the correlation coefficient (-0.104) indicates a weak inverse relationship between TNF and IgE concentrations in this group. Ecthyma: The Pearson correlation coefficient between TNF concentration and IgE concentration in the Ecthyma group is 0.375. The p-value associated with this correlation is 0.286, which is also greater than 0.05. Hence, the correlation is considered non-significant. The positive sign of the correlation coefficient (0.375) indicates a weak positive relationship between TNF and IgE concentrations in this group.

The correlation analysis suggests that there is no statistically significant correlation between TNF and IgE concentrations in any of the three groups studied. The correlations observed are either weak or close to zero, and the p-values indicate that these relationships are likely due to chance. It's important to note that non-significant correlations do not necessarily mean that there is no relationship between the variables; they just indicate that the relationship is not strong enough to be considered statistically significant in this particular sample.

Table (4-20) shows the concentration of Immunoglobulin E (IgE) in different patient groups based on the type of microbial infection they have. IgE is an important antibody involved in allergic responses and immune reactions against certain pathogens. the table indicates that there is a highly significant difference in IgE concentration between the microbial group and the control group. The results highlight the potential role of IgE in immune responses to different types of microbial infections. Further statistical analysis and study with a larger sample size could provide more insights into the relationships between IgE levels and specific microbial infections.

Table 4-20: Concentration of IgE Between Patients' Groups According to Type of Microbial Infection

type of microbial infection	N	Co. of IgE (UKI/1) Mean Std. Deviation	P *value* (p ≤ 0.05)
Stph .aureus	21	594.7057 ± 99.87244	.001 HS
Candida albicans	9	534.4989 ± 154.43620	
Stph .aureus* + *candida albicans	20	205.5260 ± 26.93961	
Control	25	61.6804 ± 5.68201	
Total	75	306.0245 ± 42.69347	
HS: highly significant difference compares to the control under (P ≤ 0.05) by One-way ANOVA table test			

The group of 21 patients with *Staphylococcus aureus* infection had a mean IgE concentration of 594.7057 UKI/1 with a standard deviation of 99.87244. The p-value of .000 (indicated as .000 HS) indicates a highly significant difference in IgE concentration compared to the control group. This suggests that patients with *Staphylococcus aureus* infection have significantly higher IgE levels than the control group . While the group of 9 patients with *Candida albicans* infection had a mean IgE concentration of 534.4989 UKI/1 with a standard deviation of 154.43620. The p-value of .000 (indicated as .000 HS) indicates a highly significant difference in IgE concentration compared to the control group . This suggests that patients with *Candida albicans* infection have significantly higher IgE levels than the control group.

Staphylococcus aureus + *Candida albicans* group: The group of 20 patients with both *Staphylococcus aureus* and *Candida albicans* infection had a mean IgE concentration of 205.5260 UKI/1 with a standard deviation of 26.93961. The p-value of .000 (indicated as .000 HS) indicates a highly significant difference in IgE concentration compared to the control group . This suggests that patients with *Staphylococcus aureus* +*Candida albicans* infection have significantly higher IgE levels than the control group .

The results in Table (4-21) shows the concentration of Tumor Necrosis Factor (TNF) in different patient groups based on the type of microbial infection they have. TNF is a pro-inflammatory cytokine that plays a crucial role in the immune response to infections and inflammation.

Table 4-21: Concentration of TNF Between Patients' Groups According to Type of Microbial Infection.

type of microbial infection	N	Co. of TNF (ng/l) Mean Std. Deviation	P *value* (p ≤ 0.05)
Stph .aureus	21	140.8990 ± 60.77725	.001 HS
Candida albicans	9	179.1556 ± 92.06056	
Stph .aureus* + *candida albicans	20	151.2220 ± 20.35160	
Control	25	39.4916 ± 4.78692	
Total	75	114.0826 ± 9.88924	
HS: highly significant difference compares to the control under (P ≤ 0.05) by One-way ANOVA table test			

The results indicates that there is a highly significant difference in TNF concentration between the microbial group and the control group.

The results suggest that patients with S.aureus infection may have elevated TNF levels, indicating an inflammatory response to the infection.

The p-value of .001 (indicated as .000 HS) indicates a highly significant difference in IgE concentration compared to the control group. This suggests that patients with *Staphylococcus aureus , Candida albicans and Staphylococcus aureus + Candida albicans* infection have significantly higher TNF levels than the control group .Further statistical analysis and study with a larger sample size could provide more insights into the relationships between TNF levels and specific microbial infections.

Table (4-22) shows the correlation between Tumor Necrosis Factor (TNF) and Immunoglobulin E (IgE) in different patient groups based on the type of microbial infection they have. Correlation analysis is used to understand the relationship between these two immunological markers in each group.

The correlation analysis suggests that there is no statistically significant correlation between TNF and IgE concentrations in any of the patient groups studied. The p-value associated with this correlation is greater than 0.05. Therefore, the correlation is considered non-significant at the 0.05 level .

The negative sign of the correlation coefficient indicates a weak inverse relationship between TNF and IgE concentrations in this group .The correlations observed are weak or close to zero, and the p-values indicate that these relationships are likely due to chance.

It's important to note that non-significant correlations do not necessarily mean that there is no relationship between the variables; they

just indicate that the relationship is not strong enough to be considered statistically significant in this particular sample and should be interpreted with caution. Further research with a larger sample size may be required to explore potential associations between TNF and IgE in these patient groups.

Table 4-22: Correlation between TNF and IgE in Patients Groups

Type of microbial infection			**TNF con. ng/l**
Stph .aureus	IgE con. UKI	Pearson Correlation	-.106- *
		Sig. (2-tailed)	.655
		N	20
Candida albicans	IgE con. UKI	Pearson Correlation	.110 **
		Sig. (2-tailed)	.778
		N	9
Stph .aureus* + *candida albicans	IgE con. UKI	Pearson Correlation	.313 **
		Sig. (2-tailed)	.179
		N	20
Control	IgE con. UKI	Pearson Correlation	-.054- *
		Sig. (2-tailed)	.798
		N	25
***Inverse (negative) non-significant correlation**			
** positive non-significant correlation**			

Conclusions and Recommendations

5.1 Conclusions

In light of the results interpretations and its discussion, the current study concludes that:

1- The study confirmed the presence of *S. aureus* and *C. albicans* in some clinical cases that caused impetigo disease .

2- The *S. aureus* isolates were resistant to most of the tested antibiotics sensitivity test (Azthromycin ,Tetracycline and Amoxicillin) except for vancomycin , levovoxacin and Gentamicin). While it was found that Candida albicans isolates were more resistant to antifungals Fluconazol, Nystatin, Amphotercin B ,Ketoconazol ,Itaconazol and Clotrimazol.

3- Most clinical isolates of *S . aureus* produce many virulence factors including biofilm, hemolysin, staphylococcus enzymes (Catalase, Coagulase, Urease).

4- *C . albicans* is capable of producing many virulence factors such as hemolysis and biofilm.

5-The study confirmed the presence of *Candida species* in clinical skin conditions such as *C. glabrata, C. albicans, C. krusei, C. tropicalis*.

6-The frequency of methicillin-resistant *S. aureus* (MRSA) in the community cases of pyoderma were comparatively higher than other *S. aureus* .

5.2 Recommendations

In light of the conclusions reached by the study, the researcher recommends the following:

1. Study of other virulence genes of *Staphylococcus aureus* and *candida albicans* isolated from skin infections and their prevalence.

2-Virulence factors of *S. aureus* detection should be carried out in all routine tests in Iraq.

3- Conducting a sensitivity test using other types of antibacterial and antifungal agents and other testing methods.

4-Determining the effect of other environmental factors on *Staphylococcus aureus* and *Candida albicans* isolates isolated from skin infections.

5- Spreading health awareness among the community and urging personal attention and limiting the spread impetigo infection.

6- Further research and studies are needed to understand the mechanisms of biofilm formation in Candida albicans and the potential of different antifungal drugs to inhibit biofilm formation effectively.

7-Further research is warranted to understand the specific functions and mechanisms of ALS and INT1 genes in Candida albicans and their implications for disease pathogenesis and potential therapeutic targets.

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Appendices

APPENDIX (A)

Tests

Appendix (A1)

Staphylococcus aureus ferment mannitol salt Agar

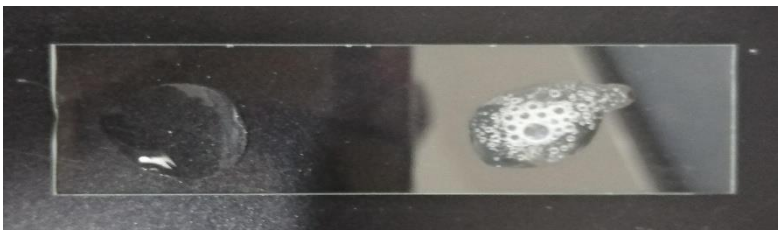
Appendix (A2)

hemolysis *Staphylococcus aureus* on blood agar

APPENDIX (A)

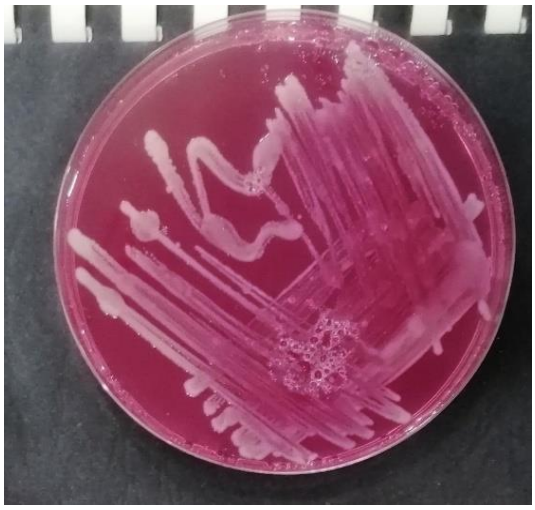
Appendix (A3)
Other biochemical tests for identification of of *S. aureus* isolates.

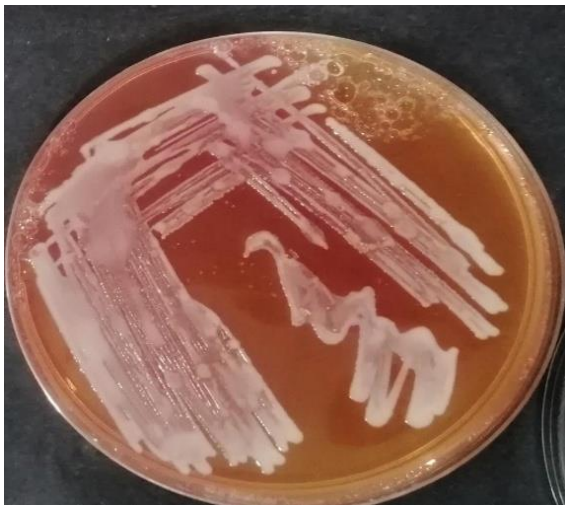
1- Catalase test of *Staphylococcus aureus*



Negative catalase **positive catalase**

2- : Urease Test of *Staphylococcus aureus*

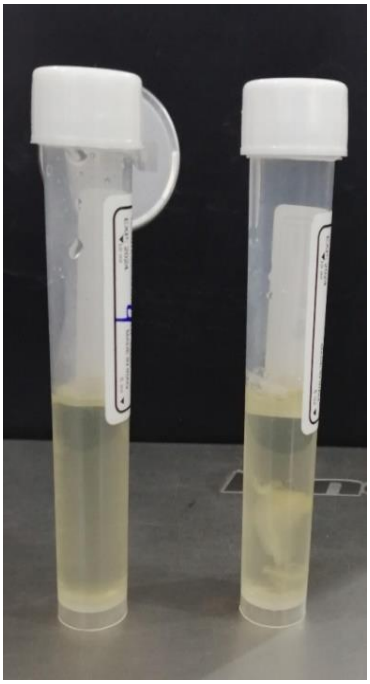




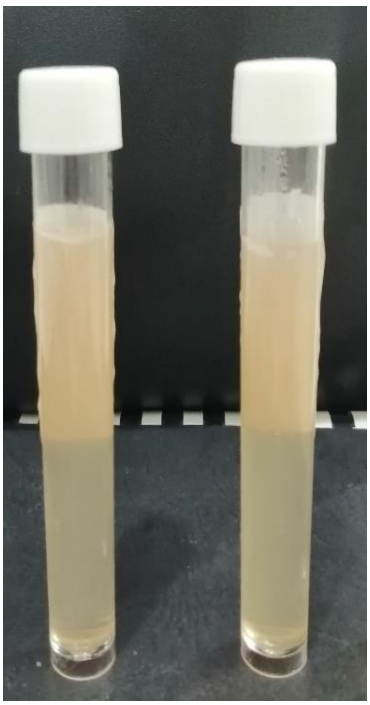
Positive **negative**

APPENDIX (A)

3- coagulase test of *Staphylococcus aureus*



4-: motility test of *Staphylococcus aureus*

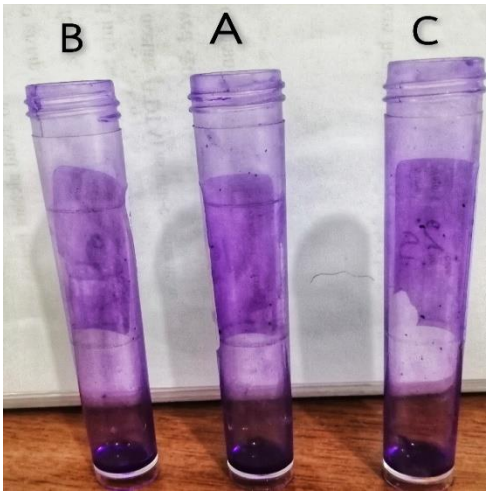


APPENDIX (A)

Appendix(A4)

biofilm test of *Staphylococcus aureus*

A _ congo agar B-Tub test

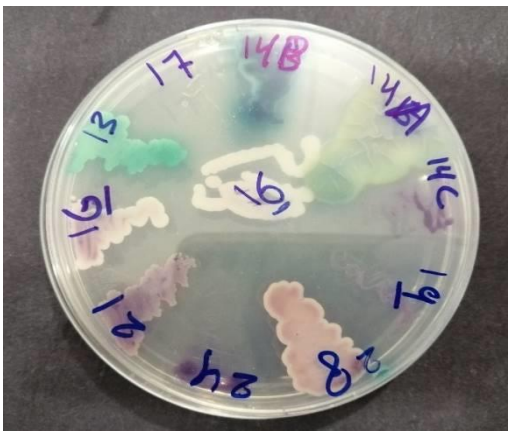


test

A_ strong biofilm **B**_ intermediat biofilm **C**_ weak biofilm

Appendix(B5)

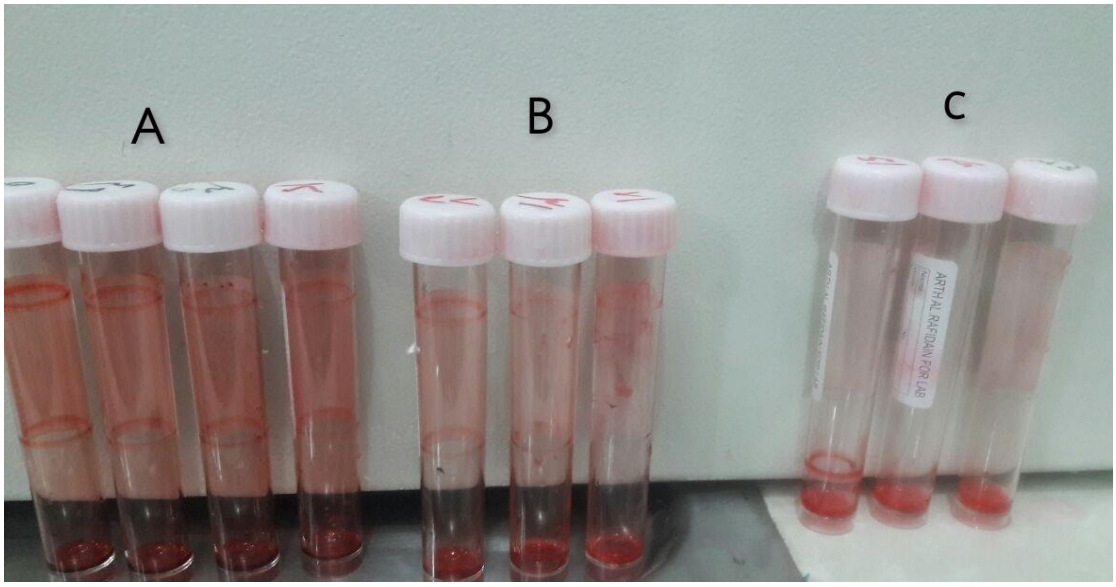
diagnosis candidiasis on cromaagar



APPENDIX (A)

Appendix(B6)

Biofilm test of *Candida*



A_ Strong biofilm B _ intermediat biofilm C_ weak biofilm

الخلاصة

تم جمع ١٢٠ عينة سريرية في الدراسة الحالية من المرضى الذين حضروا قسم الاستشارات الطبية ، وحدة الأمراض الجلدية في (مدينة مرجان الطبية) في محافظة بابل ، للفترة من سبتمبر ٢٠٢٢ إلى ديسمبر ٢٠٢٢.

تنقسم الدراسة الحالية إلى قسمين. يتناول الجزء الأول عزل وتعريف العامل البكتيري والعامل المسبب عن طريق جمع العينات من منطقة الإصابة ومن ثم زراعته على أوساط الزراعة التفاضلية مثل (أجار ملح المانيتول، أجار سابورو دكستروز، وأجار الدم). ودراسة بعض عوامل الضراوة مثل (جين ALS1، جين αINT1، جين سبا، جين Mce) بطريقة استخلاص الحمض النووي الجينومي PCR بالإضافة إلى اختبار حساسية الميكروبات للمضادات الحيوية.

الجزء الثاني من هذه الدراسة يسلط الضوء على تحديد مستوى TNF وIgE بتقنية Elisa، حيث تم جمع عينة الدم لفصل المصل بواسطة الطرد المركزي بسرعة ٣٥٠٠ دورة في الدقيقة لمدة ١٠ دقائق

تتوزع هذه العينات السريرية على القوباء القوباء (٧٤ حالة) (٦١.٧٪) ، الإكثيما (٤٠ حالة) (٣٣.٣٪) ، الفقاعات (٦ حالات) (٥.٠٪). (٦٦.٧٪)كانت نسبة الاصابة في المرضى التي تتراوح اعماهم اقل من ٢٠ سنة و(١٥.٠٪) في الاعمار بين ٢٠-٣٠ سنة. اظهرت النتائج لدى كل من الذكور والإناث نفس عدد الحالات. بالنسبة للقوباء ، هناك ٣٧ حالة في كلا الجنسين ، تمثل(٣٠.٨٪) من إجمالي الحالات لكل منهما. تم الإبلاغ عن إكثيما في ١٨ حالة بين الذكور (١٥٪) و ٢٠ حالة بين الإناث (١٦.٦٪). تم العثور على القوباء الفقاعية في ٥ حالات (٤.٢٪) للذكور و ٣ حالات (٢.٥٪) للإناث. وجد أن أكثر مناطق الجسم عرضة للإصابة هي الوجه (٢٣.٣٪) ، القدم (٢٨.٣٪) ، الألوية (٦.٧٪) ، الفخذ (١٨.٣٪) ، اليد (١٥.٠٪) الرأس (٨.٣٪).

تم تشخيص مسببات الأمراض بأستخدام الفحص المباشر ، والزراعة على الوسائط التفريقية ، والاختبارات البيوكيميائية. حيث ظهرت المكورات العنقودية الذهبية (١٨٪) ، المبيضات البيضاء (١٨٪) ، المكورات العنقودية البشروية (٩٪) ، المكورات العنقودية الرخامية (١٠٪) ، Psedomonas. الزنجارية (٧٪) ، العقدية (١٤٪).

أظهرت النتائج أن (٤٠٪)من عزلات المكورات العنقودية الذهبية كانت عبارة عن غشاء حيوي قوي و ٢٤٪ كانت متوسطة بينما(٣٦٪)كانت عبارة عن تكوين ضعيف للغشاء الحيوي. أظهرت النتائج أن النسبة المئوية لشدة تكوين الأغشية الحيوية القوية في المبيضات البيضاء كانت(٥٢٪) قوية و (٢٠٪) تكوين متوسط و(١٦٪) تكوين ضعيف و(١٢٪) كانت سلبية.

تم اختبار عزلة المكورات العنقودية الذهبية ضد ستة أنواع مختلفة من المضادات الحيوية كانت حساسة لكل من (أزيثروميسين ، جنتاميسين ، فانكومايسين ، ليفوفوكساسين) باستثناء (التتراسيكتين ، أموكسيسيلين) حيث توجد علاقة غير معنوية عند قيمة 0.05 pv.

تم اختبار عزلات المبيضات البيضاء ضد ستة عقاقير مختلفة مضادة للفطريات: (فلوكونازول ، كيتوكونازول ، نيستاتين ، أمفوتيريسين ب ، إيتراكونازول ، وكلوتريمازول) وظهرت أن هناك علاقة معنوية بين المبيضات البيضاء والحساسية المضادة للفطريات بقيمة 0.05 p

في هذا البحث تم إجراء تحليل شامل على ٢٥ عينة من بكتيريا المكورات العنقودية الذهبية.كان الهدف هو الكشف عن وجود جينات *mecA* و *Spa*. أظهرت جميع العزلات السريرية نتيجة (١٠٠٪)(٢٥ عزلة) لجين *mcA* بينما أظهر جين السبا نتيجة إيجابية بنسبة (٥٦٪) (١٤ عزلة).

تم إجراء تحليل شامل على٢٢ عينة من المبيضات البيضاء . كان الهدف هو الكشف عن وجود جينات INT1 و ALSI. أظهرت جميع العزلات السريرية أن (٨١.٨٪)(١٨ عزلة) نتيجة سلبية للجين INT1 بينما أظهر جين ALSI نتيجة إيجابية بنسبة(٦٨.٢ ٪) (١٥ عزلة)

وجدت الدراسة أن هناك فرقًا مهمًا للغاية في مستويات IgE بين المجموعات قيد الدراسة (القوباء ، الإكثيما ، والكونترول). تعني القيمة الاحتمالية البالغة 0.000 أن الاختلاف في مستويات IgE بين القوباء والمجموعات الأخرى كبير للغاية.

يقدم تقييم مستويات اختبار TNF في مجتمع الدراسة ، مقسمًا إلى ثلاث مجموعات: القوباء ، الإكثيما ، والكونترول ، تشير الدراسة إلى وجود فرق كبير في تركيز اختبار TNF بين مجموعة القوباء ومجموعة الكونترول بقيمة 0.05 p

بينت العلاقة بين اثنين من الاختبارات المناعية ، اختبار (TNF) والغلوبولين المناعي (IgE) ، في ثلاث مجموعات مختلفة ضمن مجتمع الدراسة (القوباء ، الإكثيما ، والكونترول) ، ويشير تحليل الارتباط إلى عدم وجود علاقة ذات دلالة إحصائية بين TNF وتركيزات IgE في أي من المجموعات الثلاث المدروسة .

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

الكشف عن جينات ضرارة المكورات العنقودية الذهبية والمبيضات البيضاء مع استجابات بعض المعايير المناعية لدى مرضى القوباء

رسالة مقدمة
الى مجلس كلية العلوم للبنات/ جامعة بابل
كجزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل
نورس بهاء منجي
بكالوريوس علوم حياة كلية العلوم للبنات/ جامعة بابل (٢٠٢٠)

بإشراف
أ. د. عبير فوزي مراد
د. امال مرزه حسن

٢٠٢٣ م
١٤٤٥ هـ