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A Comparative Molecular Study of Two Staphylococcus species as Tracing Evidence of Crime Detection

A Research

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

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بسم الله الرحمن الرحيم

﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ﴾

صدق الله العلي العظيم

(سورة المجادلة: الآية١١)

Supervisor's Certification

I, certify that this research entitled" **A Comparative Molecular Study of Two Staphylococcus species as Tracing Evidence of Crime Detection**" was prepared by "Alaa Kamil Oleiwi Hussein " under my supervision at the Department of Biology/ College of Science/University of Babylon as a partial fulfillment of the requirements for the Degree of Diploma in Criminal Evidence

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Date: / / 2021

Dedication

To the cleanest two hearts in my life

To those of them who have gained the power of love without limits ..... My father and my mother

To the homeland we are looking for, and we yearn to see it one day as we wish it safe and upright

To all those whose spring butterflies dance to those who open the anemones and yasin ..... to the martyrs

Alaa

2021

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Alaa

2021

Summary

One hundred samples were collected to investigate the bacteria of *Staphylococcus aureus* and *Staphylococcus epidermidis*, Samples were collected during the study period from Marjan Teaching Hospital in Babylon for the period from May 1, 2021 to August 30, 2021.

The samples were distributed as follows (30 samples were collected from 5 families From the province of Babylon, where 15 samples were collected in the first week, and the collection process was repeated from the same families after two weeks for confirmation the results and determine the possibility of isolation *S. aureus* it as a bacterial fingerprint for these families, 35 samples of wounds and 35 samples of burns were collected were collected from patients admitted to Marjan Teaching Hospital) it was found that 60 isolates belong to staphylococcus bacteria distributed as follows (40 isolates belong to *Staphylococcus aureus* and 20 belong to *Staphylococcus epidermidis*) .

The bacteria were diagnosed based on phenotypic, culture and biochemical characteristics and the study showed that bacteria isolated from pathological conditions have the ability to produce some virulence factors such as extracellular enzymes such as coagulase enzyme, catalase, heamolysine and urease at 100%, while isolated from normal flora (from families), which is considered a natural flora, produced cagulase enzymes by 70% and 100% of the rest of the enzymes including catalase, hemolysin and urease .

That *S. epidermidis* bacteria produced catalase and urease by 100% in all pathogenic and normal samples, and all isolates of *S. epidermidis* were negative for the enzyme coagulase *S. epidermidis* isolated from healthy individuals (of the families) produced hemolysin enzyme in 34%,

66% of wounds and 50% of burns.18 isolates of *S. aureus* bacteria (pathogenic and normal flora) were selected depending on their ability to produce virulence factors to investigate some virulence genes.

Molecular study of some virulence genes of *S. aureus*, which are the *coa* gene, which encodes the production of coagulase enzyme, and the *hly* gene, which encodes for the production of the heamlysine enzyme, showed the presence of the genes in all selected clinical isolates, while the *coa* gene was not present in 6 isolates of the normal flora (from the families).

The study showed by tracking the results, it was found that the natural bacteria isolated from healthy people differ from pathogenic bacteria and can be inherent to each person and can act as a bacterial fingerprint for people to help track the crime .

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CHAPTER ONE

INTRODUCTION

1. Introduction

Humans contain more bacterial cells in their body than they do of human cells . Bacterial transfers appear to be more practical to examine on objects compared with DNA as DNA leaves only a small amount of residue, if any. Although circumstances like the CSI effect tend to influence the public into believing that DNA is readily available, testable and reliable but this is often not the case, as DNA transfer would be quite minimal on object transfer compared with microorganisms. The environments are not sterile nor are humans or animals; As such, bacterial (perhaps even yeast and fungal) transfer occurs regularly (Blondeau *et al*. 2019) .

In recent years, researchers have found ways to use molecular biology and genetics to highlight the forensic value of microorganisms. Recent scientific studies have shown that the gut microbiota has incredible diversity, which may be related to discovering one's race, place of origin, and even personal identity. Though bacteria may be found on different parts of the body, their abundance and location vary from one part of the body to another. Their abundance and location may also serve as an indicator for whether stains were produced by saliva or vaginal fluid (Park et al. 2017).

The concept of the human microbiome explains the connection between people and microorganisms. Human skin is home to a diversity of microorganisms since it has a significant contact with the outside world. According to (Park *et al*. 2017).

It resembles a human fingertip According to the research, if endogenous skin microbiota/strains are recovered from physical fingerprints and have regionally restricted DNA diversity, these microorganisms can be transmitted to

the touched items and may provide forensically valuable information about the individuals who contacted them, for example, about where they originate. Proper postmortem assessment of alcohol intake may be challenging due to the microbes that may cause havoc on the evaluation. Because microbial forensics is linked with the discovery of bacteria used for biological terrorism, it's used mostly in instances when people are intentionally exposed to such bacteria. Human finger DNA left behind on touched items may contain forensically important information about the person whose fingerprints are on the item and who handled it, which may be helpful in human host conclusions. Positives include: (Tims *et al*. 2009) .

Studies show that the human body contains ten times more bacteria than human cells (HMP). Many different kinds of microbial communities reside in different organs of the body, both for reasons of human health and sickness. Despite scientific research establishing the critical role of microbial communities in the live body, little is known about the possible alterations to the microbial community that occur after death, which is why so many scientists are researching the composition of the thanatomicrobiome and what it might be used for in the forensic sector. (Ventura Spagnolo *et al*. 2019)

Staphylococcus aureus is a key component in forensic investigations because it produces enterotoxins, poisons which are often found in food poisoning. Hospital reviewers who suffer from skin lesions are a critical health issue. A member of the bacterial genus staphylococcus is one of the most common pathogens in the environment and is naturally present on human skin and is related to a broad variety of skin disorders. This *S. aureus* is very pathogenic because of its morbidity, which is the virulence (i.e. the degree of pathogenicity) it produces(Ibrahim et al. 2019).

Either an important internal source or an external source must be contributing to the skin invasion of *S. aureus*. The development of antibiotic-resistant strains of *S.aureus* in the clinical setting is a worldwide issue. This strain of Staphylococcus produces penicillinase, which is an enzyme that reduces the effectiveness of penicillin. *S. aureus* has a family of mobile genetic components known as the Staphylococcal cassette chromosome mec (SCCmec).

The human infections caused by *S. aureus* strains are quite common. They are very virulent, and have numerous virulence factors, so they may cause illness even in healthy hosts. The coagulase enzyme generated by *Staphylococcus aureus* is responsible for clotting plasma. Enzymatically active, the prothrombin-enzyme combination attaches to fibrin; together, they become enzymatically active and start fibrin polymerization. The enzyme may deposit fibrin on the surface of *S. aureus*, possibly making it difficult for the bacterium to be phagocytosed and/or phagocytosis would be inhibited. as a consequence of variations in sequence in the 3′ variable area, the 3′ variable region of the coagulase gene amplificons exhibit a wide degree of polymorphism, leading to a high degree of discriminatory power that may be further discriminated by restriction digestion (Zango *et al*. 2019).

A novel screening method for shed skin cells by detecting *Staphylococcus epidermidis* (*S. epidermidis*), which is a resident bacterium on skin, was developed. *Staphylococcus epidermidis* was detected using real-time PCR. *Staphylococcus epidermidis* was detected in all 20 human skin surface samples(Nakanishi et al. 2016).

1.1. Aim of Study

The aim of study a comparison between bacteria isolated from infections with bacteria isolated from hosts as a bacterial fingerprint to find the differences that help in determining the bacterial fingerprint to investigate the crime by object of study :

1. Isolation of pathogenic and normal flora .
2. Isolation and identification of staphylococcal bacteria .
3. Investigation of virulence factors .
4. Detection and Gene analysis .

CHAPTER TWO

REVIEW OF LITERATURE

2. Review of Literatures

2.1. Microbial Forensics

Microbial forensics is a branch of science concerned with the investigation of evidence from a bioterrorism attack, biocrime, or an accidental release of microorganisms or toxins. The use of biological threats is still in its early phases, and the discovery of a suitable set of instruments for identification and attribution of biothreat incidents is still in the early stages as well (Tucker and Koblentz, 2009).

Using biological weapons exposes one's own life, as well as the lives of others around them, to risk. Because there will be scientific, political, and media-based debate surrounding any investigation into a possible use of a biological weapon, it is probable that the role that scientific information or evidence may play in such an investigation will be significant. (Tucker and Koblentz, 2009).

The above considerations support the conclusion that promoting microbial forensics awareness and bolstering our microbial forensics ability help to inform our knowledge of biothreat events, and strengthened international partnerships will benefit our microbial forensics skills. Another aim would be to facilitate an agreed-upon knowledge of the scientific underpinnings of microbial forensics analysis, and its inherent strengths and weaknesses. (Committee on Science Needs for Microbial Forensics: Development of an International Roadmap for Microbial Forensics) have developed an initial international roadmap (Lee et al. 2020).

Regardless of the distinction between these subdisciplines, the objectives and procedures used for handling materials remain the same. Forensic science

is especially concerned with the "chain of custody" of a specimen (a piece of evidence) to ensure that it remains uncontaminated and the findings may be used as evidence in criminal proceedings. Because of the need of identifying specimens and associating them with patients, in both investigational and diagnostic microbiology, the importance of specimen identification and identification of the patient's relationship is critical. Since people with moderate to severe illnesses (e.g., blood cultures, cerebral spinal fluid) must have critical specimens, it is important to always have a backup sample of your own blood or spinal fluid (Blondeau *et al*., 2019).

Better microbiological collecting techniques, quickly developing bioinformatics approaches, and advances in genome sequencing technology have all driven the development of microbiomics and metagenomics. Human bodies include a broad variety of microbial populations, who continuously interact with and influence their surrounding environment. Environmental and human microbial profiles may be helpful in forensics because they may provide information about these interactions. "Robinson *et al*., (Robinson *et al*., 2020).

The recent developments in the speed and cost of sequencing-based genetic data generation, storage, and analysis has driven significant advances in microbial forensics (or forensic microbiology). In the case of biocrime, bioterrorism, and epidemiology, early application in these disciplines have now been augmented by the ability to use microbes as evidence in criminal investigations (thanatomicrobiome and epinecrotic microbial community). MPS offers a multitude of advantages and different alternatives, in contrast to conventional microbiological methods. Before forensics uses can begin, a great deal of work will have to be done to establish standards and norms and then

support it with a large and well-indexed reference database(Schmedes et al. 2016).

It clear that different people have microbiomes that are greatly disparate, underscoring the need of seeing the microbiomes as forensic evidence. Due to the prior research showing that the microbiota variations across individuals are larger than those found inside the same individual, investigations have concentrated on whether these differences have the ability to be utilized to distinguish people. Studies suggest that the skin microbiome has great potential for treatment. This is because skin, the biggest organ in the human body, has a high degree of interpersonal variety .(Tozzo *et al* 2020).

Poisons and diseases are used in bioterrorism, as they are transformed into bioweapons. In order to be as little as possible, pathogens and toxins are employed. It spreads quickly, and a treatment or medication is hard to find. Within a few minutes, pathogens may multiply significantly, inflicting severe harm. Other microorganisms present in food and water are taken into consideration as well. Computer-based networks must be created in order to track the harmful germs' progress in real time. The pathogen is identified, and many different methods are used to find the microbe, discover its features, and prevent the disease from spreading. To paraphrase: (Clark and Pazdernik, 2016)

The newly formed SWGMGF (Scientific Working Group on Microbial Genetics and Forensics) comprises representatives from different federal agencies, all of whom are serving in the role of working group members. Bioterrorism and biocrime problems are addressed by bringing together professional people and other groups. To ensure that the rules are set and clear for gathering physical evidence and for gathering information regarding the

person(s), place(s), procedure(s), equipment, and/or time in which the unlawful activity occurred. Quality assurance is the program's primary emphasis.(Budowle *et al* 2005)

2.1.1. Forensic and Normal Flora

However, it has recently been proven that the microbial cells that invade the human body (i.e., microbiota) are at least as plentiful as our somatic cells, with a more realistic estimated bacteria-to-human cell ratio of around 1.3. Given that the human body is home to 500–1000 different species of bacteria, and that each bacterial strain has a genome containing thousands of genes, it is clear that the total DNA content of microbes inhabiting our bodies, known as the microbiome, provides far more genetic diversity than the human genome.(Gilbert *et al*., 2018) .

The fact that different persons have vastly diverse microbiota emphasizes the need of forensic views in understanding what causes this diversity and what governs it in order to properly employ microorganisms as forensic evidence. As a result, while first described as the "discipline of using scientific methods for the examination of data from a bioterrorism assault, bio-crime, hoax, or unintentional release of a biological weapon or toxin, with attribution as the ultimate goal,"(Budowle *et al*., 2003) .

IRapid improvements in molecular sequencing and computational methods have occurred in recent years. Massive parallel sequencing (MPS) technology, also known as next-generation sequencing (NGS) or high-throughput sequencing (HTS), significantly increased the amount of sequencing data accessible for forensic investigation. Simultaneously, these technologies lowered not just the analytical expenses involved with the creation of sequencing data, but also the time required to complete the analysis. Using

NGS to sequence total DNA extracts from any sample allows for the sequencing of a given microorganism's entire genome as well as the examination of entire communities of microbes, with the possibility of rapidly and efficiently identifying all different bacterial taxa and strains, providing an overview of the resident microbial population.(Kuiper, 2016) .

Microbiome research is a highly interdisciplinary topic with several applications and methodologies for investigation, incorporating various computational approaches and models. Indeed, the advent of metagenomics enables the characterisation of hundreds of thousands of microorganisms that comprise an individual's microbial community, even if these microbial species are difficult or impossible to cultivate in vitro. Since it has been demonstrated that microbiota variation between people is greater than within the same individual, and that humans have customized microbiomes with a high degree of interpersonal variety, researchers have focused on the potential of determinants. (Williams and Gibson, 2019).

Many variables impact the makeup of human microbial communities, including environment, development, the presence or absence of illnesses, behaviors, relationships, diet, and overall health condition. Furthermore, although ambient bacteria can affect the microbiomes of individuals who spend time there, humans shed bacteria from their body surface into the surrounding environment, altering its bacterial makeup. Many investigations have shown that human microbial signatures may be retrieved in a variety of indoor and outdoor environments, including homes, businesses, healthcare institutions, schools, dormitory rooms, toilets, and subterranean spaces.(Costello *et al*., 2009; Tozzo *et al*., 2020).

2.1.2. Forensic Bacteria and Injury

The rise of microbiomics and metagenomics has been driven by advances in genomic sequencing technology, improved microbial sampling methods, and fast-evolving approaches in bioinformatics. Humans are a host to diverse microbial communities in and on their bodies, which continuously interact with and alter the surrounding environments. Since information relating to these interactions can be extracted by analyzing human and environmental microbial profiles, they have the potential to be relevant to forensics For more than a century, microbiology has played a minor part in forensic science. The sequencing of amplified viral DNA was used to support a case claiming transmission of Human Immunodeficiency. The advent of PCR-mediated genotyping of bacteria was regarded as a valuable prospective tool in forensics—for example, due to the pace of technical breakthroughs at the time, it was predicted that forensic science will soon be a significant field for the application of PCR-mediated genotyping..(Robinson *et al*., 2020).

A forensic biologist is someone who examines organisms or cells of organisms that have been linked to criminal behavior. Many creatures, including insects, bacteria, plants, and fungus, can be used as evidence because they indicate the time of an occurrence or link a specific person to an object or a location. The use of genetics to identify these species is quite widespread. In many cases, forensic practitioners in these fields have a broad applicability for their subject, as well as a diverse variety of career prospects (e.g., casework, research, and teaching)(Wells and Stevens, 2008).

2.1.3. Forensic Bacteria and Burn

Burns are one of the most visible manifestations of trauma. Patients suffering from severe heat injury require immediate targeted care to prevent

morbidity and death. Burn injuries are known to have a high fatality rate. Burn injuries can be accidental, suicidal, or even homicidal in character and can be caused by a number of thermal, electrical, and mechanical items. Because the investigation and study are restricted to finding patterns and causes of burns(Stevens *et al*., 2014).

2.1.4. *Staphylococcus aureus* and Forensic

Scientific classification

Domain: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Staphylococcaceae

Genus: Staphylococcus

Species: *S. aureus*

Binomial name *Staphylococcus aureus* Rosenbach 1884

Staphylococcus aureus is the most common microorganism isolated in iatrogenic injection therapy-related septicaemia among a broad spectrum of microorganisms causing nosocomial bloodstream infections, including Gram-positive bacteria, Gram-negative bacilli, and fungi, and staphylococcal septicaemia is more frequently fatal than septicaemia caused by other bacteria. Such deaths from *Staphylococcus aureus* septicaemia have been recorded relatively seldom in the forensic medical literature.(Tsokos and Püschel, 1999)

.

" A typing procedure based on polymorphism of the coagulase gene (coa) was used to discriminate *Staphylococcus aureus* isolated from Minas Gerais dairy cows with mastitis. Amplification of the gene from the 64 *S. aureus* isolates produced 27 different polymerase chain reaction (PCR) products; 60 isolates showed only 1 amplicon, and 4 showed 2 amplicons. The isolates were grouped into 49 types by analyzing the restriction fragment length polymorphism (RFLP) of the coa gene; the 10 most common types accounted for 39% of the isolates "(Rodrigues da Silva and da Silva 2005).

Microbes may wreak havoc on the postmortem evaluation of alcohol consumption, posing difficulties for forensic investigators. (Baranowski *et al*., 2008). Microbial forensics, on the other hand, is frequently connected with the identification of extremely dangerous microorganisms to which humans are purposefully exposed in situations of biological terrorism. Human fingertip microflora left behind on touched things at crime scenes, on the other hand, may possibly include forensically significant information beneficial for human host inferences accessible via microbial DNA fingerprinting of physical fingerprint (Enserink and Ferber, 2003).

For example, if endogenous microbial skin species/strains with a regionally restricted distribution can be recovered from touched objects using microbial DNA analysis, the geographic origin of the human host individual may be identified indirectly. In suspect-less forensic situations where the evidentiary DNA sample does not match either a suspect's DNA profile or any in a criminal DNA database, information regarding the geographic location of origin may be significant. Geographic information generated from crime scene samples is intended to decrease the possible pool of suspects in such situations by allowing police investigations to focus on certain groups of persons, namely

those from a given geographic location. Numerous human genetic markers, primarily at the continental level, have been proposed for determining human genetic ancestry. (Lao *et al*., 2006) .

However, direct ancestry inference based on human genetic markers is far from ideal at the moment, raising the question of whether microbial DNA can be utilized to augment human DNA markers in trustworthy ancestry reconstruction of unknown people. Helicobacter pylori, a stomach harmful bacterium, has recently been proven to have coevolved with its human host.(Linz *et al*., 2007).

The interactions of skin microorganisms with the human host, as well as the microbial inhabitants, are still little known. The majority of what we know about skin microbiota comes from cultivation-based investigations, however molecular fingerprinting approaches have lately been used. If a similar relationship exists between humans and their skin microbiota, as has been observed for *H. pylori*, new methods for determining human geographic origin based on DNA analysis of fingertip microflora could be developed, with interesting new applications to molecular analyses of physical fingerprints left at crime scenes.(Gao *et al*., 2007; Grice *et al*., 2008).

2.1.5. *Staphylococcus aureus* Pathogenicity

Staphylococcus aureus is a highly successful pathogen that colonizes ~30% of the population asymptomatically, but it is also capable of causing infections ranging from mild skin and soft tissue infections to invasive infections, such as sepsis and pneumonia . When *S. aureus* infects the host, it produces many virulence factors that promote the manipulation of the host’s immune responses while ensuring bacterial survival(Tam and Torres 2019).

These virulence factors include secreted toxins (exotoxins), which represent approximately 10% of the total secretome . While there are over 40 known exotoxins produced by these bacteria, many of them have similar functions and have high structural similarities. Closer examination of these seemingly redundant exotoxins revealed that each has unique properties. Exotoxins fall into three broad groups based on their known functions: cytotoxins, superantigens (SAgs), and cytotoxic enzymes . Cytotoxins act on the host cell membranes, resulting in lysis of target cells and inflammation. Superantigens mediate massive cytokine production and trigger T and B cell proliferation. Secreted cytotoxic enzymes damage mammalian cells. Collectively, these exotoxins modulate the host immune system and are critical for *S. aureus* infections (Tam and Torres 2019).

Because it produces toxins like enterotoxins, *Staphylococcus aureus* is highly significant in forensic science. Skin lesions affect a large number of hospital reviewers and are considered a serious health issue. *S.aureus* is one of the most common viruses found in most environments, as well as on human skin, and is linked to a variety of skin disorders. The hazard posed by this *S. aureus* is its morbidity, which is a virulence factor. *S.aureus* skin invasion is caused by either an internal or external source. . The emergence of strains of *S.aureus* resistant to antibiotics such as MRSA is a global problem in clinical medicine . Staphylococcal resistance to penicillin is mediated by producing penicillinase. Staphylococcal cassette chromosome mec (SCCmec) is a family of mobile genetic elements of *S. aureus* (Investigation of mec A and (tst-1) Ge...) .

2.1.6. *Staphylococcus epidermidis* and Forensic

Scientific classification

Domain: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Staphylococcaceae

Genus: Staphylococcus

Species: *S. epidermidiste*

Staphylococcus epidermidis (Winslow & Winslow 1908) Evans 1916

When it comes to criminal investigations, DNA typing is a must. Because of advancements in the sensitivity of DNA typing, shed skin cells known as touch samples have recently become a tar- get for crime investigations. Prior to DNA typing, forensic scientists in a laboratory conduct a screening test to identify a body fluid, such as blood, sperm, or urine, in order to determine the origin of DNA.

Microorganisms are ubiquitous on all terrestrial and aquatic environments. Microbes have played an important role in medicine, fermentation, and food industry for millennia but have not generally been exploited in forensic medicine. However, molecular biology tools such as DNA fingerprinting, whole genome sequencing, and microarray analysis have significantly advanced the field of microbial forensics over the last two decades . Massively parallel sequencing (MPS) was applied to the routine analysis of microbial

forensic evidence (. MPS, which is also known as next-generation sequencing (NGS), enables the detection of low levels of microorganisms and unknown pathogens even in mixed samples. With the reduction in sequencing costs and the continued development of bioinformatics analysis via increased throughput, MPS has been used to characterize the microbial community for forensic applications (Cho and Eom 2021).

This way of recognizing skin is quite precise. However, when an examiner needs to analyze samples in large quantities, this approach may not be available in all investigations owing to the employment of rigorous methods such as RNase contamination prevention and cDNA synthesis performance. On the other hand, new approaches for identifying bodily fluids that use molecular biology-based techniques to detect indigenous bacteria have recently been described. (Nakanishi *et al*. 2009; Fleming and Harbison 2010).

Because they identify bacterium genes, these techniques, which rely on DNA extraction, need the same procedure as DNA typing. As a result, the development of a screening approach for identifying indigenous bacteria on the skin is expected to speed up the identification procedure. The human skin's microbial flora consists of 103–104 bacterial cells per square centimeter, with a maximum of 106 bacterial cells per square centimeter. (Nakanishi *et al*. 2016).

Staphylococcus epidermidis (*S. epidermidis*) are resident bacteria found in large quantities on the hands that are the leading cause of catheter-associated sepsis and implant infections (Larson and Daniels 1998)

2.1.7. *Staphylococcus epidermidis* pathogenesis

Although nosocomial infections by Staphylococcus *epidermidis* have gained much attention, this skin-colonizing bacterium has apparently evolved

not to cause disease, but to maintain the commonly benign relationship with its host. Accordingly, S. *epidermidis* does not produce aggressive virulence determinants. Rather, factors that normally sustain the commensal lifestyle of S. epidermidis seem to give rise to additional benefits during infection. Furthermore, we are beginning to comprehend the roles of S. *epidermidis* in balancing the epithelial microflora and serving as a reservoir of resistance genes (Otto 2009).

2.1.8. Hemolysin gene and Coagulase gene

Hemolysins of staphylococci are categorized into four different types, including alpha (α), beta (β), gamma (γ), and delta (δ). The α-toxin is encoded by the hla and acts as a pore-forming cytotoxin (PFT) which actives against a wide array of human cells. Pathogenicity of this toxin is due to hemolytic, dermonecrotic, and neurotoxic effects . β-toxin which encoded by the hlb gene is known as Mg2+ -dependent sphingomyelinase. Incubation at temperatures below 10 °C intensifies the cytolytic activity of β-toxin; thus, it is often referred to as the 'hot–cold' hemolysin .The hld gene encodes a 26 amino acid peptide, which is referred to as delta (δ) hemolysin. This toxin with its detergent function degrades erythrocytes. The delta toxin may cause intestinal diseases that can vary from acute diarrhea to severe enteritis . Because the association between antimicrobial resistance and virulence factors of CoNS isolates had never been done before as well as in a similar work in staphylococcus aureus strains observed a significant relationship between antibiotic resistance and hemolysin genes (Nasaj *et al*. 2020).

Coagulase-negative staphylococci (CoNS) are considered as opportunistic pathogens that cause a variety of infections, particularly among immunocompromised, long-term hospitalized patients, preterm infants and

in patients with indwelling or different implant polymer bodies [. Among various CoNS species, Staphylococcus epidermidis, Staphylococcus haemolyticus, and Staphylococcus saprophyticus have been confirmed to be responsible agents for the vast majority of nosocomial infections . Treatment of CoNS infections has become more complicated, as many isolates in hospitals show high rates of resistance to multiple antimicrobial agents of clinical relevance also a reservoir for resistance genes that can be transmitted to other pathogens . About 80–90% of CoNS isolates associated with nosocomial infections are methicillin-resistant coagulase-negative Staphylococci . Most antibiotic resistance genes are carried on a plasmid and more often found in methicillin-resistant . CoNS are capable of producing several toxins and enzymes characteristically associated with Staphylococcus aureussuch as hemolysins, which are responsible for the invasion of host cells a (Nasaj et al. 2020).

Human infections are frequently caused by *S. aureus* strains. They contain a plethora of virulence factors that allow them to cause illness even in healthy hosts. *S. aureus* produces coagulase, an enzyme that clots plasma. It binds to prothrombin, and the two become enzymatically active, causing fibrin polymerization to begin. The enzyme may deposit fibrin on the surface of *S. aureus*, either preventing phagocytosis or destroying it within phagocytic cells. Coagulase gene amplificons are highly polymorphic due to variations in sequence at the 3′ variable region, resulting in strong discriminating power that may be further discriminated by restriction enzyme digestion.(Ibrahim *et al*. 2019).

CHAPTER THREE

MATERIALS AND METHOD

3. Materials and Methods

3.1. Materials

3.1.1. Equipment and Apparatuses

Different Equipment and Apparatuses were used through the study from different manufactured company as shown in table (3.1) .

Table 3- 1 Equipment and Apparatuses used in the study.

No.	Equipment and Apparatuses	Manufactured Company (Origin)
1.	Autoclave	Webeco-GmBH (Germany)
2.	Centrifuge	Meter PJ600 (Japan)
3.	Electric Oven	Gallenkamp (England)
4.	Epindorf tube	Heidolph MR-Heat Standard (China)
5.	Hot Plate	Heidolph MR-Heat Standard (China)
6.	Incubator	Gallenkamp (England)
7.	Light Microscope	Olympus (Japan)
8.	PH Meter	Hoeleze and Cheluis K.G.(Germany)
9.	Refrigerators	Vestal (Turkey)
10.	Sensitive Balance	Sartorious (USA)
11.	Separating Venal (500, 1000) ml	Separating Venal (500, 1000) ml
12.	Water Path	Gallenkamp (England)
13.	Flask (250, 500, 1000) ml	Afco Dispo (China)
14.	Loop	Afco Dispo (China)
15.	Para film	BDH (England)
16.	Petri Dish	Afco Dispo (China)
17.	Plane Tube	Afco Dispo (China)
18.	Cylander (250, 500, 1000) ml	Afco Dispo (China)
19.	Syringes	Afco Dispo (China)

3.1.2. Biological Media and Chemicals.

3.1.2.1. Biological Media

The following biological media was used in the study in table (3-2)

Table 3- 2 Cultural Media were used in the Study (Redy to use)

No.	Media	Company/Origin
1.	Blood Agar	BDH/England
2.	Mannitol Salt Agar	BDH/England
3.	Nutrient agar	BDH/England
4.	Nutrient broth	BDH/England
5.	Luria broth	Himedia (India)

3.1.2.2. Chemical Materials Used in the Study:

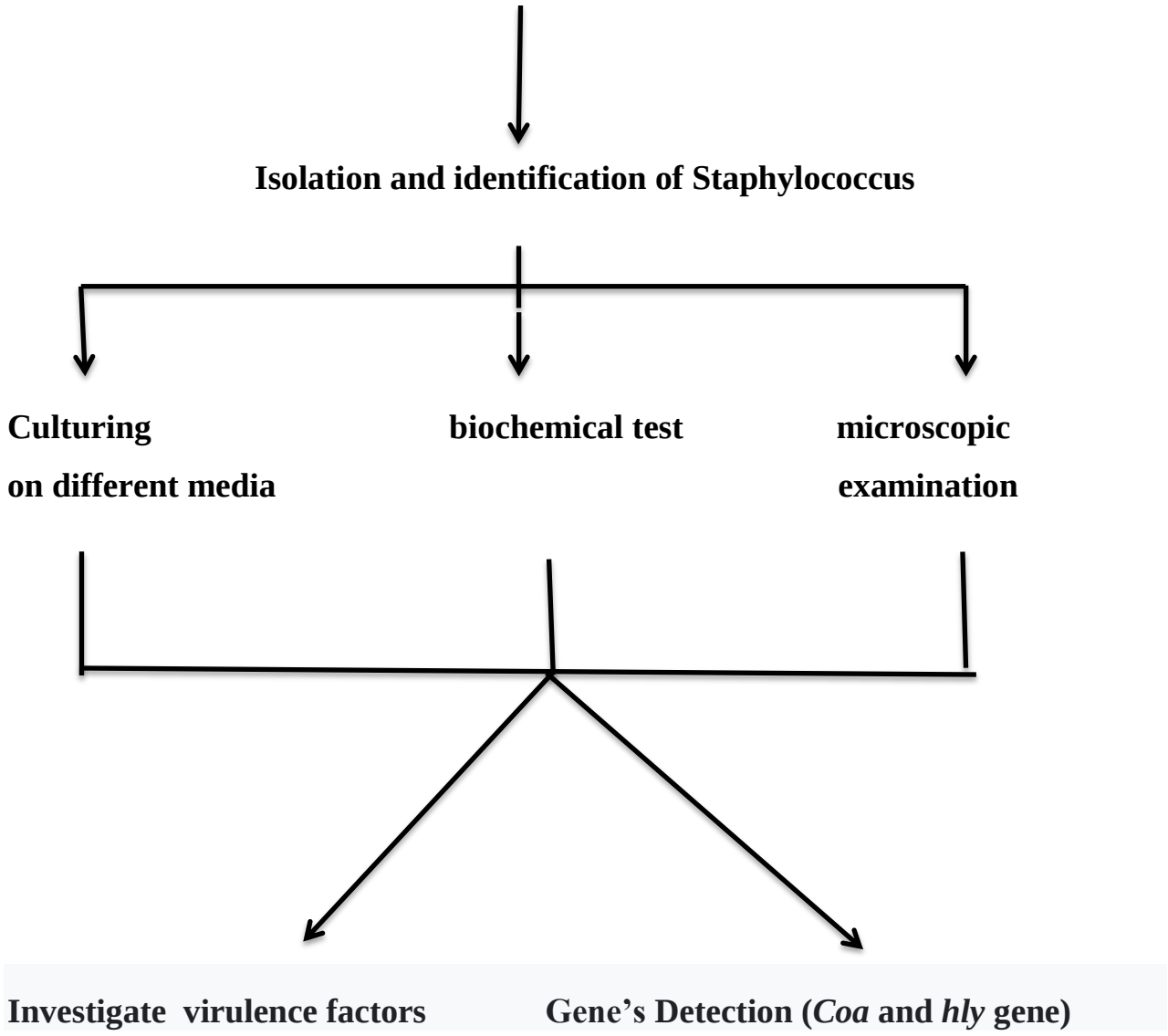
Through the study we used some chemical material as clarified in table (3-3).

Table 3- 3 Chemical Material and Manufactured Companies

Chemical Materials	Company/Origin
Absolute methanol	BDH/England
Agar	Difco (USA)
Agarose gel	Norgen Biot (USA)
DNA extraction Kit	Bioneer (Korea)
DNA ladder	Bioneer (Korea)
DNA loading buffer	Fermentas (Germany)
Ethylene diamine tetra acetic acid (EDTA)	Sigma/ USA
Ethidium bromide	BDH (England)
Ethanol	Sigma/ USA
Glycerol	BDH (England)
Gram stain	Sigma (England)
Master Mix Kit primers	Bioneer (Korea)
Primers	Bioneer (Korea)
TBE	BDH (England)
TE buffer	Sigma/ USA

3.2. Methods

3.2.1. Study Design



3.2.2. Samples Collection

One hundred samples were collected to investigate the staphylococcus bacteria, Samples were collected during the study period from Marjan Teaching Hospital in Babylon for the period from May 1, 2021 to August 30, 2021 distributed as follows (Collect swabs from the palm of the hand 30 samples from a group of families as a bacterial fingerprint, 15 samples were collected in the first week and repeated in the second week to confirm the result, 35 samples for wounds and 35 samples for burns).

3.2.3. Preparation of culture media

3.2.3.1. Blood Agar (BA)

An enrichment media in which most bacteria grow and it is considered a differentiating media between some species Hemolytic bacteria and Used according to the Manufactured Company (Akond, 2016; Al-Hulu 2010), (Collee *et al* 1996).

3.2.3.2. Mannitol salt agar

Differentiated media between species Staphylococcus and *Staphylococcus aureus* which ferments mannitol with its effect on the color of the Red Phenol reagent They give yellow color while other staph does not ferment mannitol Used according to the Manufactured Company.(Al-Hulu, ,2011; Ali, 2014), (Collee *et al* 1996).

3.2.3.3. Nutrient broth

A suitable media for the growth and activation of bacteria and was prepared according to the manufacturer company (Al-Hulu, ,2011; Ali, 2014), (Collee *et al* 1996).

3.2.3.4. Nutrient agar

A suitable media for the growth and Preservation of bacteria and was prepared according to the manufacturer(Akond, 2016), (Collee *et al* 1996).

3.2.4. Gram Stain

It includes of four solutions which are :-

1- Crystal Violet Dye

2- iodine

3- alcohol 70%

4- Safranin Dye

3.2.5. Bacterial Diagnosis

Bacteria were cultured on a medium of blood agar, the plates were incubated in the incubator for 24 hours at a 37c.On the next day afler incubation period, the developing colonies on the plate were counted by surface counting method and to identify the shape of colonies and their decomposition and diagnose colonies based on the morphological characteristics, then the characteristics of the bacterial cells were studied under the microscope after staining by gram stain to distinguish it(Collee *et al* 1996) .

3.2.6. Biochemical tests

3.2.6.1. Catalase Test*:*

When gram-positive bacteria appeared, a catalase test was performed on them to find out aerobic gram-positive bacteria, by taking from a colony of gram-positive bacteria and distribute it on the slide and add to it a drop of catalase, and if bubbles appear, they are positive for the test, and if it does not appear, it is negative for the test(Collee *et al* 1996) .

3.2.6.2. Coagulase Test

Then a blood plasma test was performed on her, where a drop of distilled water was placed on the Slide and by Loop a bacterial colony was taken and placed on the slide and then homogenized colony with distilled water, after that we add a drop of blood plasma and note if Thrombosis occurred or did not occur(Collee *et al* 1996) .

3.2.6.3. Urease Test

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide**.** Christensen's urea agar is used to detect urease activity in a variety of microorganisms **.** urease production is indicated by a bright pink (fuchsia) color on the slant that may extend into the butt after 1-6 hours of incubation. The culture medium will remain a yellowish color if the organism is urease negative. For Stuart's urea broth, urease production is indicated by a bright pink (fuchsia) color throughout the broth(Collee *et al* 1996) .

3.2.6.4. H_2O_2

It was used to test for catalase to distinguish Aerobic bacteria pneumatic. A drop of 3% H_2O_2 is added to the dropper on one side of the swab of bacteria colony on the glass slide and the results were observed:

1-If bubbles appear, they are positive for the catalase test (+).

2- If no bubbles appear, they are negative for the catalase test (-).

3.2.7. Cultivation of bacteria on differential medium:

To ensure that the positive bacteria were cultured on the manthol differential medium, the dishes were incubated For 24 hours at a temperature of 37 C, after which bacterial growth was observed on the colored medium Yellow as well as the color of the middle changed(Collee *et al* 1996) .

3.2.8. Molecular Study

PCR assay was performed to detection important virulence factors genes (*coa & hly*) genomic DNA was extracted directly from bacteria and using genomic DNA extraction kit as described by manufactured company.

3.2.9. Protocol of Bacterial DNA Extraction

Genomic DNA was extracted from *S. aureus* isolated by using Genomic DNA extraction kit , as in the following steps:

1- One ml of fresh activated bacteria (24-48)hr incubation period on brain heart infusion broth) was transfer to 1.5 eppendorf tube and centrifuged at 10000 rpm for one minute then discard the supernatant .

2- A 200 µl of FATG buffer was added to the tube , suspended the tube by shaking vigorously by vortex, then incubate at room temperature for 10 minutes. The tube was inverted every 3 minutes through incubation period

3- The resulting homogenous cell suspension was incubated for 10 min at 70 C° and vortex for 10 sec. every 3 min until the sample lysate is clear.

4-DNA was extracted from the homogenous suspension by the adding 200 µl of absolute ethanol and then transfer to the FABG column and put inside 2 ml collection tube

5- Centrifuge 5 min at 14000 rpm and the flow through was discard.

6- The FABG column was placed in a new 2 ml collection tube and 400 µl of W1 was added and centrifuge 14000 rpm for 30 sec. and the flow through was discard

7- The column was replaced in 2 ml collection tube , 750 µl of wash buffer was added and centrifuge 14000 rpm for 30 sec. and the flow through was discard.

8- The FABG column was dried by further centrifugation at 14000 rpm for 3 min. over a new 2 ml collection tube to remove any residual ethanol solution.

9- The dried FABG column was then transferred to a new 1.5 ml microcentrifuge tube 100 ml of preheated elution buffer was added directly to the center of FABG column membrane and let stand for 2-3 min .

10- DNA was harvested by centrifugation at 14000 rpm for 1 min and the eluted genomic DNA was stored at -20 C° until be used.

3.2.10. Polymerase Chain Reaction

All primers were used in this study were illustrated in table (3-4) (Abdul-Kareem *et al* 2015).

Table 3- 4 Sequences of nitrogenous bases for specific primer

No.	Gene	primer sequence	Expected gene size	the manufacture company
1	*Coa*	5'ATA GAG ATG CTG GTA CAG G3' 5'GCT TCC GAT TGT TCG ATG C3'	440-1400 bp	Alpha DNA
2	*hIy*	F:GGTTTAGCCTGGCCTT R:CATCACGAACTCGTTC	534 bp	Alpha DNA

3.2.11. Primer Pairs Preparation

All primer pairs used in this study were dissolved using nuclease free water, firstly the primer stock prepared as 100 pmol and then the working primer would prepared from primer stock tube . According to the instruction provided by manufacture (Bioneer / Korea) nuclease free water was added to get 100 Pmol/ µl as a stock solution . And then making dilution to get 10 Pmol/ µl as working solution .

3.2.12. The PCR Mixture

Amplification of DNA was carried out in a final volume of 25 µl reaction mixture as illustrated in Table (3-5).

Table 3- 5 Content of PCR reaction mixture

No	Content of PCR Reaction Mixture	Volume
1-	Master mix	12.5 µl
2-	Forward primer	2 µl
3-	Reverse primer	2 µl
4-	Template DNA	5 µl
5-	Nuclease free water	3.5 µl
Total volume		25 µl

3.2.13. Thermal Cycles Condition

Conventional PCR was used to amplify the target DNA using specific primers. It include three consecutive steps that repeated for specific number of cycles to get amplified PCR product , all thermal cycling condition was listed in table

Table 3- 6 Thermal cycling conditions

Genes	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No of cycles
coa	**95C° / 10 min**	**95C° / 30 sec**	**55 C° /1min**	**72 C° /1min**	**72 C° /5min**	**35**
Hly	**95C° / 10 min**	**95C° / 30 sec**	**55 C° /1min**	**72 C° /1min**	**72 C° /5min**	**35**

3.2.14. PCR Product Analysis

Agarose gel electrophoresis analyzed the PCR products as in the following steps:

1- A 1.5 % agarose gel was prepared by using 1x TBE buffer and dissolving in Macrowave , after that left to cool to 50 C° .

2- A 5 µl of red safe stain was added into agarose gel solution and mix well

3- Agarose gel solution was poured in to the tray after fixing the comb in the proper position, then left to solidify at room temperature.

4- The comb was removed gently from the tray and then PCR product was loaded in the comb well and also 10 µl of DNA marker (100 bp ladder) was loaded in first lane.

5- The gel tray was fixed in the electrophoresis chamber and filled by 0.5X TBE buffer , and electric was performed at 100 volts and 70 AM for one hour.

6- After finishing of electrophoresis , PCR product was visualization by using UV transilluminator .

CHAPTER FOUR

RESULTS AND DISCUSSION

4. Results and Discussion

4.1. Samples Collection

One hundred samples were collected to investigate the staphylococcal bacteria distributed as follows (Collect swabs from the palm of the hand 30 samples from 5 families as bacterial fingerprint were collected during two weeks, 15 samples in the first week and repeated after two weeks to confirm the result, 35 samples from wounds and 35 from burns. 60 samples of staphylococcus were obtained distributed as follows (40 isolates of *Staphylococcus aureus* and 20 isolates of *Staphylococcus epidermidis*) as show in table (4-1). Diagnosed based on phenotypic characteristics and biochemical tests.

Table 4- 1 Bacterial distribution throughout the study population

Type of sample	*S. aureus*	%	*S. epidermidis*	%	Total
Normal flora	20	50%	15	75%	35
Wound	15	37.5%	3	15%	18
Burns	5	12.5%	2	10%	7
Total	40	100%	20	100%	60

4.2. Diagnosis of *Staphylococcus sp.*

To Diagnosis and identification of *Staphylococcus spp*. All samples culturing on media used in this study included: Nutrient Agar, difference will be due to different factors such as, Blood agar medium, Nutrient broth and Mannitol salt agar medium . Use a MSA to distinguish between *S. aureus* and *S. epidermidis* Where bacteria *S. aureus* ferment mannitol sugar (Ryan and Ray ,2004) as show in figure (4-1)

All media were prepared according to the manufacturer's specification and sterilized at 121°C I bar for 15 mint.

Staphylococcal colonies obtained from selective media were subjected to gram staining as show in figure (4-1) . Purification was done by several sub-culturing on corresponding media.

Staphylococcal appears as staphylococci (grape-like clusters) when viewed through a microscope as in figure (4-2) and has large, round colonies, often with hemolysis, when grown on blood agar plates as in figure (4-3) *(*Ryan and Ray ,2004).

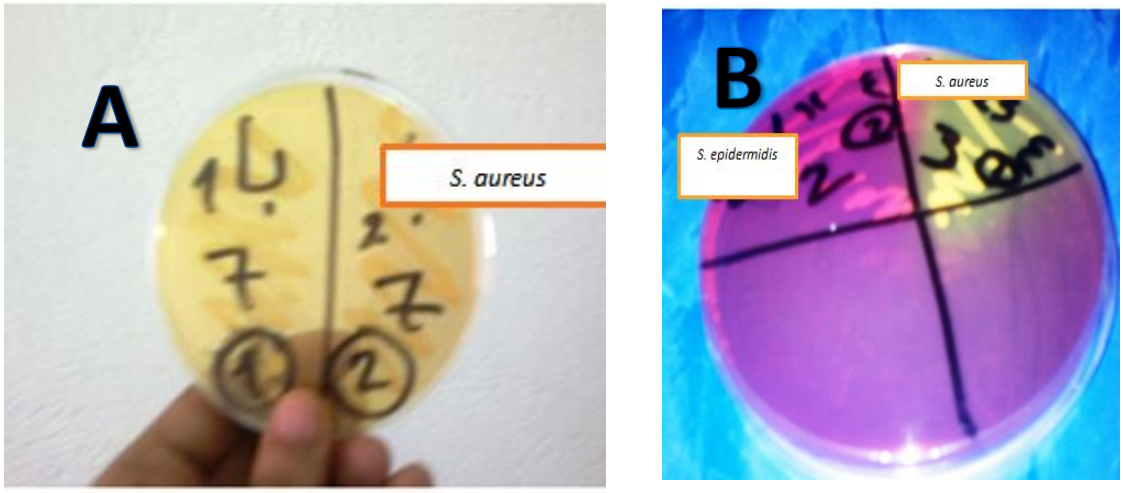


Figure 4- 1 Bacterial growth on MSA medium

A/ *S. aureus* isolated from clinical samples

B. / *S.aureus* & *S. epideermidis* isolated from normal flora

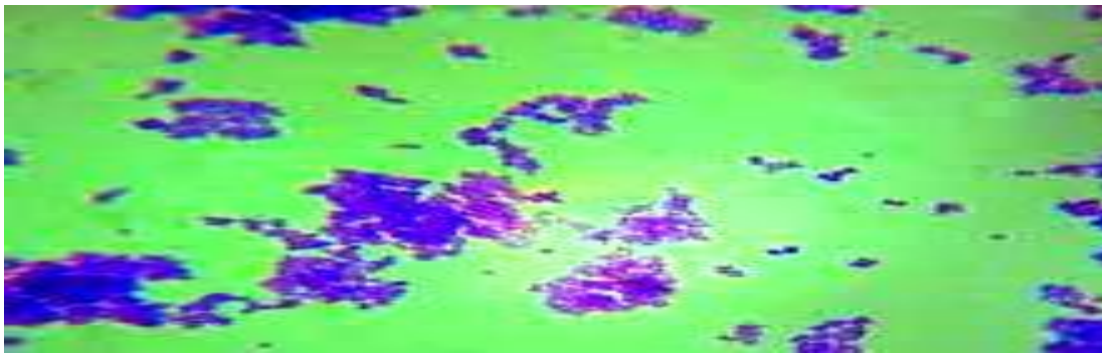
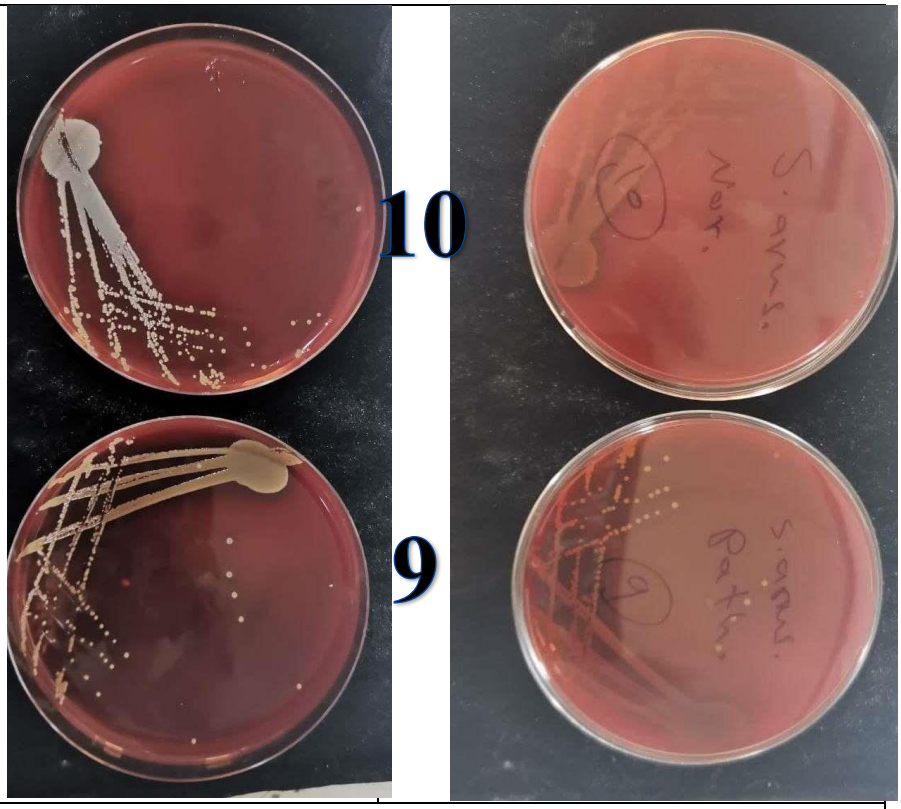


Figure 4- 2 Gram stained staphylococcal bacteria



No.9 / from clinical samples

No. 10 / from normal flora

Figure 4- 3 Staphylococcal bacteria on the media of blood agar

In figure (4-3) the growth of bacteria on the blood medium, and the hemolysis is noted, and the comparison between the hemolysis in the clinical samples and the natural sample can note a small difference in the decomposition and this is due to the difference in the environment in which the bacteria are located(Schenck *et al* , 2016) .

In humans, *S. aureus* can be present in the upper respiratory tract, gut mucosa, and skin as a member of the normal microbiota (Wollina , 2017). However, because *S. aureus* can cause disease under certain host and environmental conditions, it is characterized as a pathobiont (Otto , 2010).

Staphylococcus are catalase-positive (meaning it can produce the enzyme catalase). Catalase converts hydrogen peroxide to water and oxygen. Catalase-activity tests are sometimes used to distinguish staphylococci from enterococci and streptococci. Previously, *S. aureus* was differentiated from other staphylococci by the coagulase test. However, not all *S. aureus* strains are coagulase-positive(Varrone ,2014; Matthews, 1997) and incorrect species identification can impact effective treatment and control measures.

The pure cultures were incubated at 37°C for 24-48 hrs, then stored at 4°C in refrigerator

4.3. Identification of Staphylococci Isolates:

Staphylococcus spp. Were identified based on coagulase test (slide test) and as well as biochemical tests for example Urease test as described by (Harrigan, 1998) and (Barrow and Gelthan, 1993) were carried out.

4.4. Investigation of the Production of Staphylococci for a Group of Extracellular Enzymes

Some laboratory media was used to investigate the ability of *Staphylococcus aureus* to produce some extracellular enzymes as virulence factors. *S. aureus* isolates from clinical samples were productive at 100% of each catalase , Coagulase. Urease and haemolysin – ß while *S. aureus* isolates from families as normal flora samples were productive at 100% of each catalase Urease and haemolysin – ß but 70% (14 isolates) were had ability to produce coagulase as show in table (4-2) . while *S. epidermidis* were productive at 100% of each catalase , Urease and 50% for haemolysin only as showed in table (4-3).

Table 4- 2 The ability of *Staphylococcus aureus* to produce some extracellular enzymes as virulence factors.

Type of samples	No.	Urease test%	Coagulase test %	Catalase test %	Hemolysis test %
Normal flora	20	100%	70%	100%	100%
Wounds	15	100%	100%	100%	100%
Burns	5	100%	100%	100%	100%

Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. In the laboratory, it is used to distinguish between different types of Staphylococcus isolates. Importantly, *S. aureus* is generally coagulase-positive, meaning that a positive coagulase test would indicate the presence of *S. aureus* or any of the other 11 coagulase-positive Staphylococci(Becker *et al*., 2014)

A negative coagulase test would instead show the presence of coagulase-negative organisms such as *S. epidermidis* or *S. saprophyticus*. However, it is now known that not all *S. aureus* are coagulase-positive(Ryan & Ray , 2004; González-Martín *et al* 2020). Whereas coagulase-positive Staphylococci are usually pathogenic, coagulase-negative Staphylococci are more often associated with opportunistic infection(Ortora *et al* ,2013).

Table 4- 3 The ability of *Staphylococcus epidermidis* to produce some extracellular enzymes as virulence factors.

Type of samples	No.	Urease test%	Coagulase test %	Catalase test %	Hemolysistest %
Normal flora	15	100%	0	100%	34%
Wounds	3	100%	0	100%	66%
Burns	2	100%	0	100%	50%

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. Christensen's urea agar is used to detect urease activity in a variety of microorganisms **.** urease production is indicated by a bright pink (fuchsia) color on the slant that may extend into the butt after 1-6 hours of incubation. The culture medium will remain a yellowish color if the organism is urease negative. For Stuart's urea broth, urease production is indicated by a bright pink (fuchsia) color throughout the broth(Collee *et al* 1996) .

4.5. Correlation between Bacterial Production of Enzymes and Bacterial Fingerprint

In Table 4-2, we noticed that 30% of the *S. aureus* isolated from the normal flora (from the hosts) did not produce coagolase, while all pathogenic samples produced the enzyme (100%), and this result was obtained after repeating the examination after two weeks in the same samples and from the same people, which means that these bacteria are a bacterial fingerprint of these people, and their inability to produce this enzyme can be relied upon as a distinguishing mark as showed in figure (4-4).

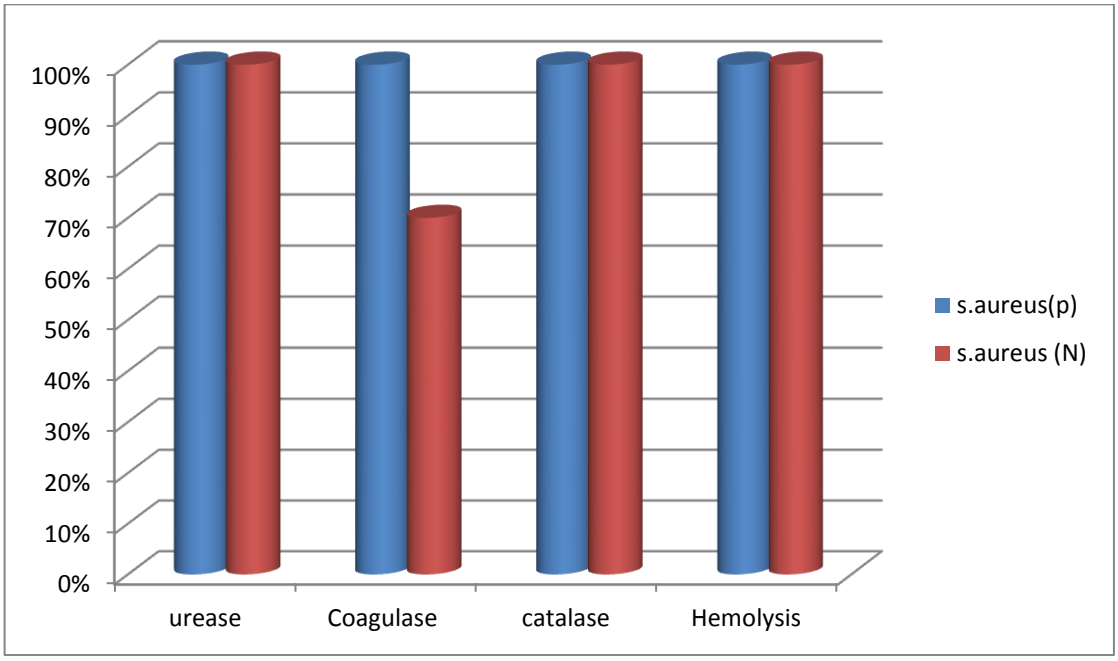


Figure 4- 4 Enzyme production by *S. aureus* (N) and *S. aureus* (P)

***p= pathogen.**

***N= Normal flora**

In Table 4-3, we noticed that 66% of *the S. epidermidis* isolated from the normal flora (from the hosts) did not produce heamolysin, while all pathogenic samples produced the enzyme in higher percent , and this result was obtained

after repeating the examination after two weeks in the same samples and from the same people, which means that these bacteria are a bacterial fingerprint of these people, and their inability to produce this enzyme can be relied upon as a distinguishing mark as showed in figure (4-5).

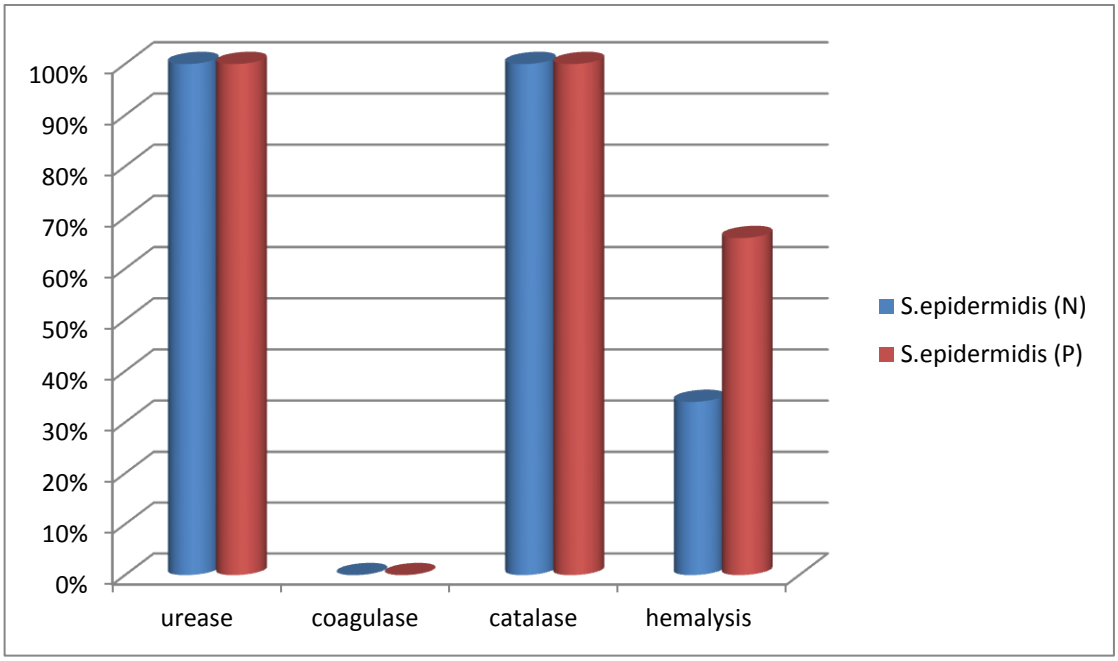


Figure 4- 5 Enzyme production by *S. epidermidis* (N) and *S. epidermidis* (P)

***p= pathogen.**

***N= Normal flora**

Every individual harbors a large number of microorganisms. The human microbiome refers to the total microorganisms found in and on the human body **.**Also bacterial species in the skin have diverse and unique composition between individuals. So-Yeon Lee *et al* in (2015) thought that a bacterial fingerprint obtained from surfaces including computer keyboards aids forensic individual identification in case of evidence deficiency through it study Microbial Forensic Analysis of Bacterial Fingerprint by Sequence Comparison of 16S rRNA Gene.

4.6. Molecular Study.

4.6.1. Molecular Detection of *Coa gene and Hly gene*

Eighteen *s. aureus* isolates(10 isolated from normal flora as families sample 5 from them negative for caogulasr test while other 5 isolates were positive for coagulase and 8 isolates from clinical samples while we take 8 isolate nagetive to heamolysis test and 10 were positive) were selected based on the Its ability to produce coagulase and heamolysine enzyme as virulence factors to evaluate the presence of genes encoding the enzymes coagulas(coa) and hIy(haemolysin) by chain polymerase PCR (reaction) showed the presence of the hIy gene in all studied isolates, while the coa gene was present in all 8 clinical samle but present in 6 families Isolation only as showed in figuer (4-5) and (4-6).

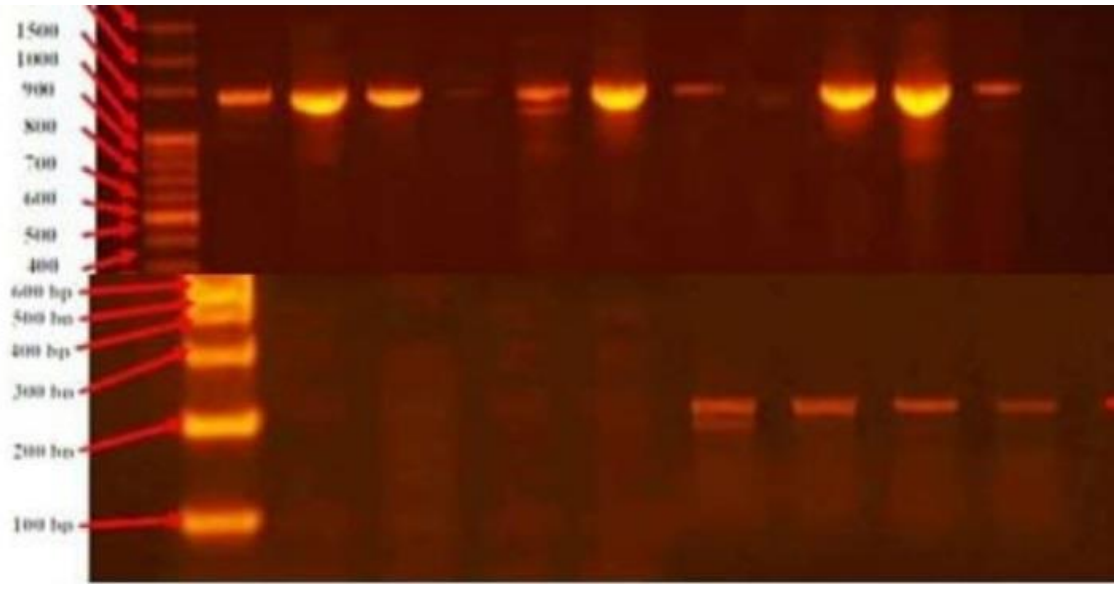


Figure 4- 6 PCR results of DNA isolates of *S, aureus*. bacteria isolated from infections of wounds, burns and families sample by using specialized primers of *coa genes* on acarose at a concentration (1.5%) at (60) volts for a period of time.*(* Two hours).

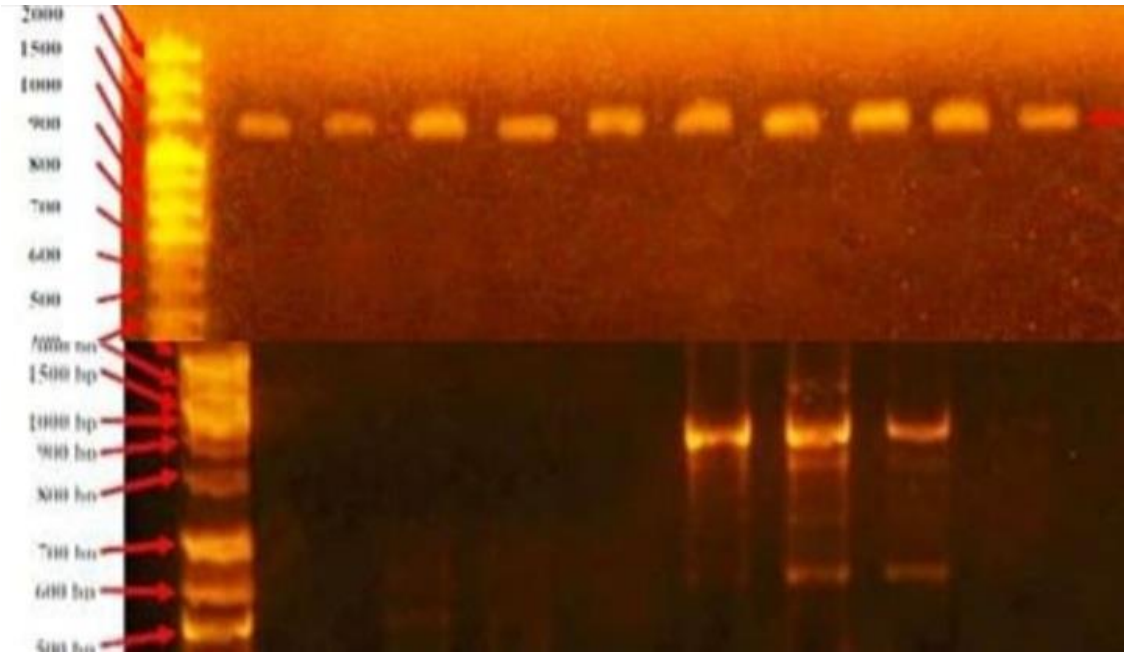


Figure 4- 7 PCR results of DNA isolates of *S, aureus*. bacteria isolated from infections of wounds, burns and sample from families by using specialized primers of *hIy genes* on acarose at a concentration (1.5%) at (60) volts for a period of time.*(* Two hours)

The results of the coa gene were consistent with what was found by Clark *et al* (1993) where they stated that the majority of *S. aureus* isolates are producing this enzyme because it is a working factor virulence in the pathogenicity of this bacterium, and the results of the study also converged with what was mentioned by Cuny *et al* 2015 where they used gene coagulase as a virulence factor for methismine-resistant Staphylococcus aureus, and de Freitas *et al* (2013)indicated that most strains of *Staphylococcus aureus* are *S.aureus* was the producer of the enzyme coagulase as a significant virulence factor in the pathogenicity of this bacterium with a rate of 39%. The ratio of gene spread to the different source and number of isolates .

The results of the current study for the presence of the hIy gene which clear in figure (4-6)came relatively close to what was found by Ben *et al* (2008), as it was found that the pathogenicity of these bacteria is due to Productively, the virulence factor of haemolysine enzyme, where it was mentioned that the proportion of (18.81)% are genes encoding production gene hla and (18.18)\ Any genes encoding hIb and also the results of the current study coincided with what was found by Budd *et al* (2015) where they stated that haemolysin-α is more A frequency of haemolysin-, at a rate of (4.43)%, and the results of the current study also converged with what was found by(Buzzola *et al* 2007).

4.6.2. Correlation between Bacterial Production of Enzymes and Bacterial Fingerprint through Gene Analysis

Among the results obtained during the study, represented in the study of the ability of *S. aureus* bacteria to produce extracellular enzymes that consider as virulence factors such as coagulase enzyme, and from Table (4- 2) which showed that there are some bacterial isolates from family samples did not produce these enzymes and after repeating the examination many times.

The presence of the genes responsible for the production of these enzymes was examined, and it was found that some of these isolates, which were negative for the coagulase assay, do not possess this gene, which helps to link the existence of a relationship between bacteria and the people isolated from them, which helps in the diagnosis and identification of bacterial fingerprint for these families.

In table (4-4) showed the present of *coa* gene through the positives coagulase while the negative coagulase test isolates didn't have *coa* gene

Table 4- 4 Distribution of *coa gene* and *hly gene* among *S.aureus* isolates

S. aureus isolates (No.18)			
	Positive coagulase test		Negative coagulase test
Total	13		5
	P(8)	N(5)	N(5)
coa gene	+	+ (4) -(1)	-
hly geng	+	+	+

The fact that different people host radically different microbiota highlights forensic perspectives in understanding what leads to this variation and what regulates it, in order to effectively use microbes as forensic evidence Therefore, while, at first, microbial forensics was defined as the "discipline of applying scientific methods for the analysis of evidence from a bioterrorism attack, bio-crime, hoax, or inadvertent release of a biological agent or toxin, with attribution as the ultimate goal" (Budowle *et al* , 2003) subsequently, an expanded definition was proposed as the "discipline of characterizing microbiological evidence to develop investigative leads in criminal and civil cases(Schmedes *et al* , 2016).

CONCLUSIONS

AND

RECOMMENDATIONS

Conclusions and Recommendations

Conclusions

- Not all *S. aureus* isolated from healthy persons produce coagulase enzyme.
- Not all *S. aureus* isolated from healthy persons have *coa* genes that encode for the coagulase.
- all pathogenic *S. aureus* isolated from have *coa* genes that encode for the coagulase
- all *S. epidermidis* isolated from healthy persons have *hly* gene which coded to heamolysine enzyme

Recommendations

- Inclusion of the largest number of families in the study
- Study the 16s rDNA for families bacteria
- Study gene expression for *coa* gene

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الخلاصة

تم جمع 100 عينة للتحري عن بكتريا المكورات العنقودية الذهبيه والبشروية حيث تم جمع العينات خلال فترة الدراسة في مستشفى مرجان التعليمي في بابل للفترة من 1 مايو 2021 إلى 30 أغسطس 2021. تم توزيع العينات على النحو التالي (30 عينة جمعت من 5 عوائل في محافظة بابل حيث جمعت 15 عينة في الاسبوع الاول وكررت عملية الجمع من نفس العوائل بعد اسبوعين للتأكد من النتيجة وتحديد امكانية عزلها كبصمة بكتريه لهذه العوائل تم جمع 35 عينة من الجروح و35 عينة من الحروق جمعت من مرضى راقدين في مستشفى مرجان التعليمي) وجد ان 60 عزل تعود لبكتريا المكورات العنقودية موزعة بالشكل الاتي (40 عزلة تعود للمكورات العنقودية الذهبيه و20 تعود للمكورات العنقودية البشرويه) شخصت البكتريا اعتماد على الصفات المظهرية والمزرعية والاختبارات الكيموحيوية . بينت الدراسة ان بكتريا المكورات العنقودية الذهبيه المعزولة من الحالات المرضية لها القدرة على انتاج بعض عوامل الضراوة كالانزيمات الخارج خلويه مثل انزيم الكوكيوليز والكتاليز والهيمولايسين و اليوريز بنسبة 100% بينما كانت المكورات العنقودية الذهبيه المعزولة من العوائل والتي تعتبر فلورا طبيعية منتجة لإنزيم الكوكيوليز بنسبة 70% و100% لباقي الانزيمات المتضمنه الكاتليز والهيمولايسين واليوريز وان بكتريا المكورات العنقودية البشروية منتجة لانزيم الكتاليز واليوريز بنسبة 100% في جميع العينات الممرضة والطبيعية وكانت جميع عزلات المكورات العنقودية البشرويه سالبة لانزيم الكوكيوليز . انتجت المكورات العنقودية البشروية المعزولة من الاشخاص السلمين (من العوائل) انزيم الهيمولايسين بنسبة 34% وبنسبة 66% من الجروح و50% من الحروق تم اختيار 18 عزلة من بكتريا المكورات العنقودية الذهبيه (الممرضة والفلورا الطبيعية) اعتمادا عل قابليتها على انتاج عوامل الضراوة للتحري عن بعض جينات الضراوة. بينت الدراسة الجزيئية لبعض جينات الضراوة لبكتريا المكورات العنقودية الذهبيه وهي جين *coa* الذي يشفر انتاج انزيم الكوكيوليز و جين *hly* الذي يشفر انتاج انزيم الهيمولايسين وجود الجينات في كل العزلات السريرية التي تم اختيارها بينما لم يتواجد جين *coa* في 6 عزلات من العزلات الفلورا الطبيعية (من العوائل) . ومن خلال تتبع النتائج وجد ان البكتريا الطبيعية المعزولة من الاشخاص السليمين تختلف عن البكتريا الممرضة ويمكن ان تكون ملازمه لكل شخص وممكن ان تكون كبصمة بكترية للأشخاص وتساعد في تتبع الجريمه .

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

دراسة جزيئية مقارنة بين نوعين من بكتريا المكورات العنقودية كدليل تتبع لكشف الجريمة

بحث مقدم الى

مجلس كلية العلوم – جامعة بابل

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

من قبل

علاء كامل عليوي حسين

(بكالوريوس علوم حياة، جامعة القادسية، ٢٠١٧)

إشراف

استاذ مساعد دكتور يازي عبدالله جاسم بربيد

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