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University of Babylon

College of Science

Department of Biology

# Potential Use of Microbial Profile as Forensic Evidence

A Research

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

**By**

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**2021 A.D** **1443 H.D**

**بسم الله الرحمن الرحيم**

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

صدق الله العلي العظيم

**(سورة المجادلة: الآية١١)**

# *Certification*

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

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Data: / /2021

In view of available recommendation, I forward this research for debate by the Examination Committee.

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Name: Assistant Professor Dr. Adi Jassim Abd AL-Razzaq

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# *Committee Certification*

We, the examining committee, certify that we have read the research entitled **(Potential Use of Microbial Profile as Forensic Evidence**) and examined the student (Kadeem Abdullah Kadeem ) in its contents at / /2021, and that in our opinion it is accepted as a research for the degree of **High Diploma in Forensic Evidence** with excellence estimation.

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# *Dedication*

In honor of my father

In honor of my mother, the wellspring of all my affection and kindness... devotion and generosity

To my brothers and sisters...

So here it is, the product of my labor

**Kadeem**

# *Acknowledgments*

*Let me begin by thanking ( Prof. Dr. Mohammed Abdullah Jebor for suggesting the study topic and for providing me with follow-up and guidance... For my sake, may God reward her.*

*Thank you very much the Deanship of Science College, in particular with Professor Inas Muhammad Al-Rubai, the Head of the Biology Department, Assistant Professor Adi Jassim Abdul Razzaq, as well as all of the renowned academics, sincerely thank them for their time and consideration.*

**Kadeem**

## Summary

Microbial profiling is an emerging area of interest in forensic science, now included in the expanded definition of "microbial forensics," tasked largely with investigating the possibility of identifying individuals, or relating them to items and/or environments through microbial characterization analysis. (such as bacteria, fungi, and viruses) inside their microbiomes. The study lasted one month, from 23/7 to 22/8/2021. This study was carried out in the College of Science at the University of Babylon. 150 swabs were collected from 50 healthy individuals aged 20–40 years. 50 swabs were taken from the palm of the hand, 50 swabs were taken from the phone screen, and 50 swabs from the computer keyboard. all samples were collected in sterile swabs. All samples were cultured on media (Blood Agar, maconky agar, as well as Sabouraud dextrose agar). The bacteria were diagnosed with the Api 20 and Vitk 2 devices. Results No growth in Macunkey, as well as Sabouraud dextrose agar. In most of the samples, Staph epidermes bacteria grew on them. Three samples gave unique patterns in that one sample did not produce growth on the three media for all smears taken from three surfaces, and the other samples grew on the smears taken from the three surfaces with unique patterns. Where the same bacteria grew on all three surfaces, the bacteria that grew was Aerococcus Viridans. Other sample also produced a special pattern in which both epidermal staphylococcus and Staphylococcus aureus grew.

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# CHAPTER ONE

## *INTRODUCTION*

# 1. Introduction

The microbiome is often defined as the total genetic content of a microbial community, but the term may also be used to refer to the physical microbial community within a given environment . The human microbiome, more specifically, refers to the microbial communities that are human-associated, living both on and within individuals and immediately surrounding them . Research has revealed that human microbiomes are highly individualised ; human gut microbial compositions, for example, may reveal microbiota that are intrinsically linked to factors such as an individual's diet , lifestyle and biogeography . Profiling of human microbiomes has increased over recent years, particularly within the health sciences, due to a combination of the decreasing costs associated with sequencing, and the accessibility of bioinformatics protocol knowledge and software (Neckovic, A H van Oorschot, *et al*. 2020).

Microbial forensic research has led to the development, expansion, and evolution of new technologies, tools, and analytical capabilities bolstering our collective preparedness and response. The scientific bases, advances, applications, interpretations, and lessons learned by those who have been intimately involved in the early years of microbial forensics need to be documented and transferred to the next generation of scientists and decision makers. An understanding of the microbial forensic field is essential to determining what evidence is collected, what proper and safe methods of collection and preservation to employ, how the evidence is analyzed, what the significance of a result is, and what is supportive in identifying a perpetrator for prosecution. Thus, the burgeoning field of microbial forensics should be accompanied by a concomitant development of educational infrastructure and resources targeted at the next generation of practitioners, as well as diverse elements for the policy, research, and law enforcement communities (Lee *et al*. 2020).

Although forensic, investigative, and diagnostic microbiology have distinct goals, the techniques, technology, and approaches to dealing with a material are still the same. In forensics, the specimen's "chain of custody" is critical for ensuring that a specimen (evidence) stays uncompromised and that the results may be utilized as evidence in criminal proceedings. The necessity of specimen identification and connection to a specific patient is important in investigative and diagnostic microbiology. Critical specimens are required for individuals with mild to severe infections (i.e., blood cultures, cerebral spinal fluid) (Blondeau *et al*., 2019).

Research has shown the microbiome is individualised, and that personal microbial signatures can be recovered from surfaces such as phones, shoes and fabrics(Neckovic, van Oorschot, *et al*. 2020).

The aim of current study is to determine the identity of individuals by detecting bacteria that grow on their skin and items that have been touched.

The objectives of this study are :

1.Swab preparation from individuals and their item.

2. Culturing of the samples.

3. Diagnosis bacteria isolates by API 20 and several biochemical tests.

# CHAPTER TWO

## *REVIEW OF LITERATURES*

# 2. Review of Literatures

## 2.1. Important Microbe in Forensic

Microbial profiling is an emerging area of interest within forensic science, now included in the expanded definition for 'microbial forensics' , which is largely tasked with researching the potential to identify individuals, or to associate them to items and/or environments through the analysis of distinguishing microbial features (i.e., bacteria, fungi or viruses) within their microbiomes. Recent studies have revealed that individuals demonstrate highly personalised microbial signatures within their microbiomes ; these microbial signatures are thought to have potential forensic utility, to identify or associate an individual with a criminal activity (Williams and Gibson 2019; Kodama *et al*. 2019; Neckovic, A H van Oorschot, *et al*. 2020).

The human microbiome encompasses the fungi, bacteria and viruses that live on, within, and immediately surrounding the body. Microbiomes have potential utility in forensic science as an evidentiary tool to link or exclude persons of interest associated with criminal activities. Research has shown the microbiome is individualised, and that personal microbial signatures can be recovered from surfaces such as phones, shoes and fabrics. Before the human microbiome may be used as an investigative tool, further research is required to investigate the utility and potential limitations surrounding microbial profiling. This includes the detectability of microbial transfer between individuals or items, the associated risks (such as contamination events) and the applicability of microbial profiling for forensic purposes (Neckovic, van Oorschot, *et al*. 2020).

Recent advances in genetic data generation, through massive parallel sequencing (MPS), storage and analysis have fostered significant progresses in

microbial forensics (or forensic microbiology). Initial applications in circumstances of biocrime, bioterrorism and epidemiology are now accompanied by the prospect of using microorganisms (i) as ancillary evidence in criminal cases; (ii) to clarify causes of death (e.g., drownings, toxicology, hospital-acquired infections, sudden infant death and shaken baby syndromes); (iii) to assist human identification (skin, hair and body fluid microbiomes); (iv) for geolocation (soil microbiome); and (v) to estimate postmortem interval (thanatomicrobiome and epinecrotic microbial community). When compared with classical microbiological methods, MPS offers a diverse range of advantages and alternative possibilities. However, prior to its implementation in the forensic context, critical efforts concerning the elaboration of standards and guidelines consolidated by the creation of robust and comprehensive reference databases must be undertaken (Oliveira and Amorim 2018).

The human skin microbiome is comprised of diverse communities of bacterial, eukaryotic, and viral taxa and contributes millions of additional genes to the repertoire of human genes, affecting human metabolism and immune response. Numerous genetic and environmental factors influence the microbiome composition and as such contribute to individual-specific microbial signatures which may be exploited for forensic applications. Previous studies have demonstrated the potential to associate skin microbial profiles collected from touched items to their individual owner, mainly using unsupervised methods from samples collected over short time intervals (Neckovic, van Oorschot, *et al*. 2020; Schmedes *et al*. 2018).

The human microbiome contributes significantly to the genetic content of the human body. Genetic and environmental factors help shape the microbiome, and as such, the microbiome can be unique to an individual. Previous studies have demonstrated the potential to use microbiome profiling for forensic applications; however, a method has yet to identify stable features of skin microbiomes that

produce high classification accuracies for samples collected over reasonably long time intervals (Schmedes, Woerner, and Budowle 2017).

## 2.2. Structure and Function of the Human Skin Microbiome

An abundant and diverse collection of bacteria, fungi, and viruses inhabits the human skin. These microorganisms vary between individuals and between different sites on the skin. The factors responsible for the unique variability of the skin microbiome are only partly understood, but results suggest that host genetic and environmental influences play a major role. Today, the steady accumulation of data describing the skin microbiome, combined with experiments designed to test the biological functions of surface microbes, has provided new insights into links between human physiology and skin microbiota (Schommer and Gallo 2013).

Human skin is the first line of defense against pathogens, while simultaneously harboring a diverse milieu of commensals,including bacteria, fungi, and viruses. These symbiotic organ-isms play essential roles in lipid metabolism, colonization resis-tance to transient organisms, and education of the immune system Previous studies have shown strong site specificity microbial community composition and function: the physiologic characteristics of a skin site, including pH, tempera-ture, moisture, sebum content, and topography, shape the local microbial community (Costello *et al*. 2009; Findley *et al*. 2013; Grice *et al*. 2009).

In addition to skin biogeography, as determined by physiological factors, such as oily, wet, or dry traits, it is also likely that individual distinguishing traits contribute to the dynamics of the skin's microbial community over time. Microbial species, including bacteria, fungi, and viruses, are distinct between individuals. Monitoring district dynamics is meaningful because these internal influences may exacerbate disease severity (Peleg, Hogan, and Mylonakis 2010).

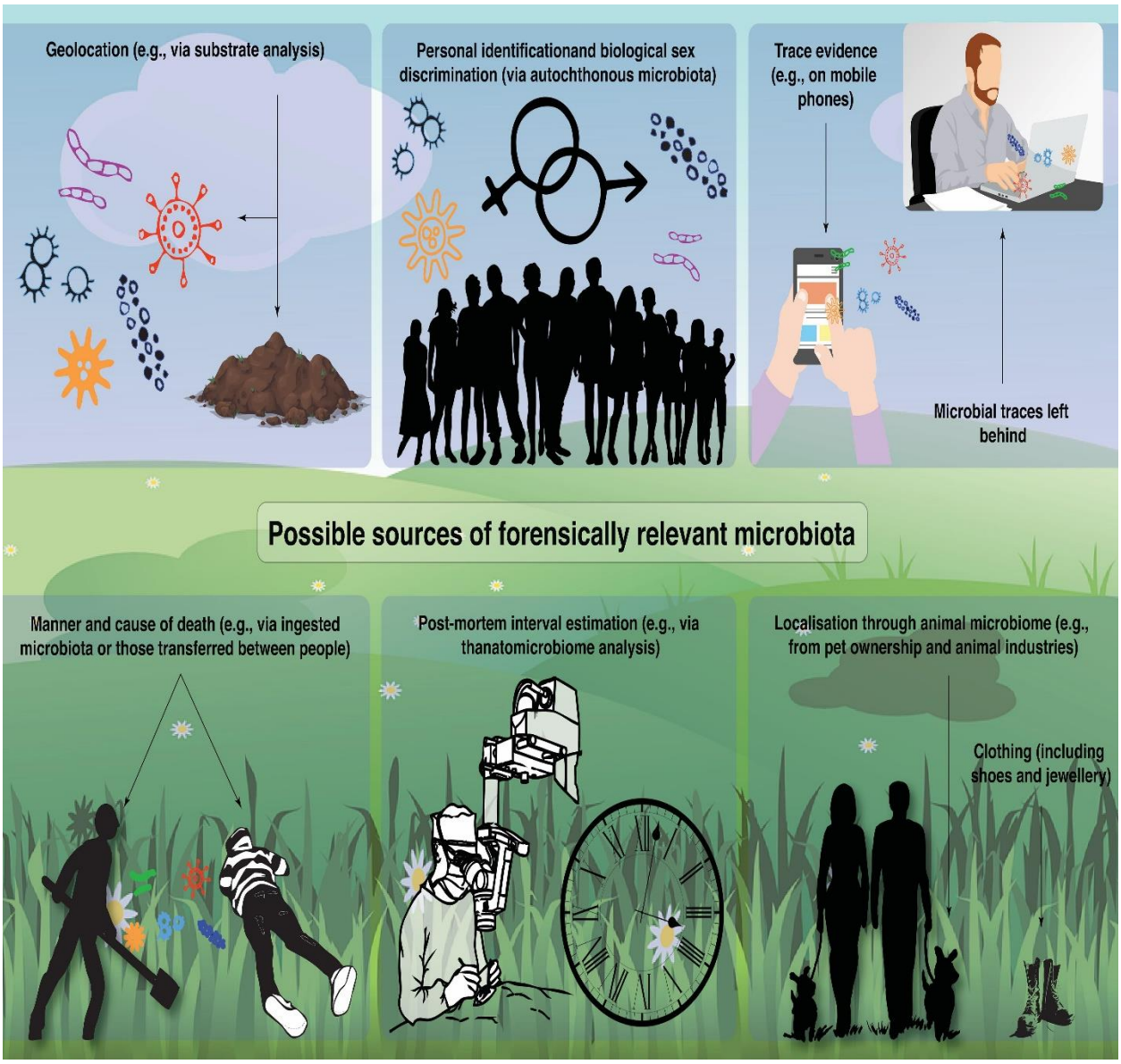



Figure 2- 1 A summary of possible sources of forensically relevant microbiota identified (Robinson *et al.*, 2021).

## 2.3. Microbial Profiling Overview

Microbial communities, whether sampled from human or environmental sources, are often retrieved through swabbing, commonly using cotton swabs . Following this, the DNA of both non-specific targets and the microbial communities is extracted using either standard or optimised DNA extraction protocols, through the use of commercially available DNA isolation kits. The samples then undergo

amplicon library preparation, involving ligation of primers and PCR amplification, aimed at targeting specific genetic markers within microbial communities, such as the 16S rRNA for bacterial communities (Neckovic, A H van Oorschot, *et al*. 2020).

Following the addition of sample indices (barcodes) and amplification, amplicon libraries are multiplexed (pooled) and the region(s) of interest are sequenced, typically on Illumina platforms [54]. Sequenced reads must then be assigned to each individual sample (demultiplexed) before further computational analyses are carried out. Several bioinformatics pipelines exist for microbiome data processing, refining raw sequences into informative visualisations; two of the most commonly used are Quantitative Insights Into Microbial Ecology (QIIME, or more recently, QIIME2 ) and Mothur (Bolyen *et al*. 2019; Schloss *et al*. 2009).

Microbial sequences may first be quality filtered using Deblur or the Divisive Amplicon Denoising Algorithm (DADA2) to infer amplicon sequence variants (ASVs). Taxonomic analyses may then be achieved by mapping these variants using a Naïve Bayes classifier, against commonly used reference databases including Greengenes and SILVA . Typically, analyses involving the microbiomes of individuals will include assessments on α (within) and β (between) diversity analyses, using a variety of distance metrics including Unweighted Unifrac (Callahan *et al*. 2016; Lozupone *et al*. 2011).

Metrics such as Unweighted Unifrac focus on the presence/absence of distinguishing features (e.g., ASVs) within samples and are useful for distinguishing microbial communities between samples using numeric distances. This information may be visually interrogated through Principal Coordinates Analysis (PCoA) , whereby samples, and their distances relative to each other, are arranged within a 2D or 3D space; samples that appear to cluster are considered to be more similar in their microbial compositions than samples that are further apart (Borcard and

Legendre 2002).

## 2.4. Microbiome Transfer and Persistence

In the past few years, intensive work has been carried out to characterize environmental microbiome, particularly in urban environments and transit systems. These studies have demonstrated that unique community profiles may exist in certain areas of a city (Afshinnekoo et al., 2015; Rosenfeld et al., 2016), as well as "molecular echoes" of environmental events, and even a forensic capacity for geospatial microbiomic data In the following, we focus on two leading aspects of geolocalization.(Robinson *et al.*, 2021) .

Different Spatial Dimensions and the Power of Machine Learning The growing interest in sampling and predicting environmental microbiome profiles at different spatial scales and orientations (e.g., between households, cities, states, and altitudes) to provide information on the location and provenance of people and objects resulted in the development of a multitude of approaches. Chase et al. (2016) identified the three cities where nine offices were located with 85% accuracy based on analyzing office microbiome samples using sampling plates, and although this study suffers from a small sample size (n = 3 per office), it demonstrates potential with further refinement. Lax et al. (2014) analyzed samples from household occupants (n = 1625 from 18 participants in 10 houses) and their built environments. The authors matched feet microbiome samples to the house with 82.9% accuracy—a relatively low degree of accuracy from an evidentiary perspective but demonstrating the potential of such methods for fine-scale biolocalization. Walker and Datta (2019) analyzed whole-genome sequenced microbiota sampled from 12 cities in seven different countries as part of the 2018 CAMDA MetaSUB Forensic Challenge. The CAMDA dataset (n = 30) included three mystery samples. The authors applied machine learning techniques to identify the geographical provenance

of the microbiome samples. Up to 90% of the samples were correctly classified, demonstrating the potential of machine learning applications to biogeography, although further evidence is necessary to employ these applications in an evidentiary context (Robinson *et al.*, 2021).

Human microbiomes may be transferred between cohabitating couples, family members and their pets , students who share dormitory rooms and via direct and indirect mechanisms between non-cohabitating individuals' hands . Through the shedding of one's skin-associated microbiota, which is collectively referred to as the 'microbial cloud' of an individual, human microbiomes may also be indirectly deposited into built environments (Neckovic, A H van Oorschot, *et al*. 2020).

Previous study has assessed the direct transfer of human microbiomes, linking individuals to personal effects , office equipment , fabrics , and shared spaces/surfaces within homes (e.g., kitchen counters) , the persistence of a transferred microbiome over time is yet to be comprehensively understood. Some indicators within these previous studies are also somewhat contradictory, suggesting that microbial signatures may either persist on items within office or home environments over a short period of time , or decay rapidly from the surfaces once the environment is no longer occupied (Neckovic, A H van Oorschot, *et al*. 2020).

This inconsistency may be due to how different surface types affect the persistence of microbiomes, including surfaces commonly encountered in a crime scene. However, there is limited information surrounding the persistence of microbiomes on different surface types, including the impact of additional factors such as pH, light, humidity/temperature and nutrient availability. Studying such factors, including microbial shifts between the time of deposition and collection of human-associated microbiota, may provide insight into the capacity for which human microbiomes may or may not be used to identify/associate an individual. This

is particularly important, given that an individual's reference microbiome may be collected at a much later stage in an investigation (Flores *et al*. 2014).

## 2.5. Typing and strain identification

Differences among microbes have to be assessed to determine whether strains are from the same source or lineage or from a different origin. The accuracy and precision will depend on the typing method, expected mutation rates, and other characteristics of the organism. In court scientists may need to quantify the reliability of a relationship among strains determined using molecular phylogenetic analyses. This will establish the probability of association to a certain source of infection . Techniques for forensic microbiology can be very similar to those being used for phylogenetic and epidemiological investigations e.g. for food-borne outbreaks. Molecular-epidemiological tools used for genotyping are most promising and have been applied in the past to elucidate the origin of biological agents. Especially whole genome sequencing and bioinformatic tools for comparison of genomes are potent tools, but technical complexity and costs are still prohibitive for routine application (Tomaso and Neubauer 2011).

### 2.5.1. Normal Flora as Evidence

The incorrect belief that the human body hosts microbes that outnumber our somatic and germ cells by a factor of ten has sparked interest in characterizing human microbiota, or all the bacteria that live in the human body in health or disease, in order to better understand the balance between human and microbial components, primarily bacteria but also fungi, viruses, and protists, in order to better understand the balance between human and microbial components, primarily bacteria but also fungi, (Marchesi and Ravel 2015) .

However, it has recently been demonstrated that the microbial cells that infiltrate the human body (i.e., microbiota) are at least as numerous as our somatic cells, with a more realistic bacteria-to-human cell ratio of about 1.3. Given that the human body is home to 500–1000 distinct species of bacteria, each with a genome containing thousands of genes, the overall DNA content of microorganisms inhabiting our bodies, known as the microbiome, clearly provides considerably more genetic variety than the human genome..(Gilbert *et al*. 2018) .

The fact that different people have substantially varied microbiota underlines the need of forensic perspectives in understanding what causes this variety and what regulates it in order to utilize microbes as forensic evidence appropriately. As a result, while first defined as the "discipline of applying scientific methods to examine data from a bioterrorism assault, bio-crime, hoax, or inadvertent discharge of a biological weapon or toxin, with attribution as the ultimate goal,"(Budowle *et al*. 2003) .

Recent years have seen rapid advancements in molecular sequencing and computational techniques. Massive parallel sequencing (MPS) technology, also known as next-generation sequencing (NGS) or high-throughput sequencing (HTS), boosted the amount of sequencing data available for forensic inquiry substantially.

Concurrently, new technologies reduced not just the analytical costs associated with the generation of sequencing data, but also the time necessary to conduct the analysis. Using NGS to sequence total DNA extracts from any sample enables the sequencing of an entire microorganism's 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 multidisciplinary issue with a variety of applications and techniques for inquiry, as well as numerous computational approaches and models. Indeed, the advent of metagenomics permits the characterization of hundreds of thousands of microorganisms that form an individual's microbial community, even if cultivating these microbial species in vitro is difficult or impossible.

Researchers have focused on the possibility of determinants as it has been established that microbiota variation across persons is larger than within the same individual and that humans have customized microbiomes with a high degree of interpersonal variance.(Williams and Gibson 2019).

The degree of skin microbiome diversity is determined by the study's taxonomic depth. As a result, while the phylum of bacteria allows for the identification of only a few distinct taxa among different people, when identification is achieved at the genus, species, and strain population levels, with extremely specific diversity for each person, the discriminatory power becomes similar to a fingerprint.(Costello *et al*. 2009) .

The environment, development, the presence or absence of diseases, habits, relationships, diet, and general health status all have an influence on the composition of human microbial communities. Furthermore, although ambient bacteria can impact the microbiomes of people who spend time there, humans shed bacteria from their skin into the surrounding environment, changing its bacterial makeup. Many studies have demonstrated that human microbial signatures may be recovered in a wide range of indoor and outdoor settings, including residences, companies, healthcare facilities, schools, dormitory rooms, toilets, and underground places.(Costello *et al*. 2009; Tozzo *et al*. 2020).

### 2.5.2. Microbiome Data

Microbiome studies generally focus on bacteria, but microbes are found in all three domains of life: bacteria, Archaea, and Eukarya, Additionally, viruses are sometimes considered part of the microbiome. Molecular methods can be used to characterize microbial communities by targeting regions of DNA that are taxonomically informative (amplicon sequencing) for different portions of the tree of life or by sequencing all gene fragments in a sample (shotgun metagenomics). Communities of bacteria and Archaea can be characterized by amplifying and sequencing 16S rRNA genes. Similarly, the 18S rRNA gene can be amplified and sequenced to survey microbial eukaryotes represented in a sample, and the taxonomic resolution of fungal taxa can be improved by sequencing an internal transcribed spacer (ITS) region (Metcalf *et al*. 2016).

Studies that include taxonomic data from multiple domains have revealed important trophic ecological interactions . Ampliconbased sequencing is an efficient and inexpensive way to characterize microbial communities for thousands of samples. Shotgun sequencing of all DNA fragments in a sample provides not only a snapshot of taxonomic relative abundance, similar to amplicon-based methods, but also functional gene data, strain-level taxonomy , and the assembly of genomes or partial genomes (Nielsen *et al*. 2014)(Nielsen *et al*. 2014; Luo *et al*. 2015).

However, the cost is higher than that of amplicon-based methods, and data sets can be overwhelmed by the most abundant taxa, whether the DNA is bacterial or from the host. As costs decline, technical advances for preparing and separating microbial DNA molecules from host molecules improve, and computational tools become more standardized and efficient, shotgun metagenomics will likely become more commonly used (Metcalf *et al*. 2017).

## 2.6. Personal Identification

A growing body of evidence suggests that human individuals may be uniquely identified based on stable autochthonous (i.e., native to a given environment) microbial profiles. This could have a substantial impact on forensic science—for example, in situations where the investigator cannot retrieve sufficient amounts of human DNA (i.e., from human somatic and germ cells). Yet it is unknown whether the variation in microbial communities between people is sufficient to identify individuals within large populations uniquely or stable enough to place them over time.

To answer some of these questions, Franzosa et al. (2015) tested different body site-specific microbial profiles and attempted to match them with 25–105 microbiome profiles during the person's first and second visits to the sampling site. The authors reported that these profiles were useful in distinguishing individuals at the initial sampling time point and that 30% of the individuals were still uniquely identified several months later. In this study, gut microbiome samples were used to pinpoint 80% of individuals (n = 120) up to a year later. These results are encouraging—particularly in shorter timescales—however, they still suffer from relatively high variability. As such, greater improvements, e.g., in methods and sampling effort, will be needed before such approaches can be useful in a forensic setting.

High resolution melting analysis that targeted the 16S rRNA gene from oral swab samples have also been used to demonstrate its potential in distinguishing between individuals (Wang et al., 2019), albeit with a very small sample size in this study (n = 5). Schmedes et al. (2018) demonstrated accurate identification of individuals (n = 12) based on skin swab samples from different body sites (n = 14). They achieved 97% accuracy by sampling shirts and 96% accuracy using palm

samples based on 1-nearest neighbor classification on nucleotide diversity of the bacterial genome. In another recent study, the researchers utilized a similar approach to identify individuals (n = 51). They analyzed microbiome samples collected from three different body sites—the manubrium (i.e., the upper-most segment of the sternum), the palmar surface of the hand, and the ball of the foot (Woerner et al., 2019).

## 2.7. Microbiome Evidence in the Criminal Justice System

Microbiomes have yet to be admitted as evidence of PMI, grave location, or links to objects or locations. Admitting microbiome evidence would require an investigator or a lawyer (prosecution or defense) to conclude that the microbial evidence in question is relevant and reliable. The evidence would then be subject to a hearing (known as a Frye hearing or a Daubert hearing) to establish admissibility. Therefore, the reliability of microbiome tools for forensic science must be better researched and error rates established using quantitative machine-learning methods. Although the research thus far is promising, there are several issues with microbiome science that must be addressed before it can be established as a consistent component of forensic investigations. Importantly, police departments have already expressed interest in these tools, as discussed above. This is the first step toward admissibility: establishing awareness. Yet, several other key hurdles exist, including incorporating the cost, training, and equipment in the crime laboratory workflow. Given the current backlog for analyzing human DNA evidence, the addition of microbiome techniques to a crime laboratory could be resource prohibitive, although rapidly declining costs in sequencing and compute power may resolve this issue. Furthermore, a validation strategy for these techniques must be identified so that an acceptable protocol and error rate can be established. These issues are not insurmountable, but they are crucial and must be addressed in the near future (Metcalf *et al*. 2017).

# CHAPTER THREE

## *MATERIALS AND METHODS*

# 3. Materials and Methods

## 3.1. Materials

### 3.1.1 Instrument and Equipment

The following instruments and equipment that used in the study are showed in table (3-1)

Table 3- 1 Equipment and their origin.

| NO. | Type of equipment | Company/Origin |
|---|---|---|
| 1. | APi 20 staph | Biomerienx / France |
| 2. | Api 20 strep | Biomerienx / France |
| 3. | Autoclave | Haramaya/ Japan |
| 4. | Centrifuge | Hettich/German |
| 5. | Disposable Tips | Gilson/France |
| 6. | Micropipette Set (20- 200µl) | Dragon MED/USA |
| 7. | Plain Tubes (10 ml) | Dolphi medical/Jordan |
| 8. | Sensitive Balance | Sartorius/U.K |
| 9. | Timer | Chania |
| 10. | Vitek 2 | Biomerienx / France |
| 11. | Vitek card | Biomerienx / France |

### 3.1.2. Chemical and their Suppliers

Chemical's materials that were used showed table (3-2)

Table 3- 2 Chemicals that used in the study.

| No. | Chemicals | Company/origin |
|---|---|---|
| 1. | Distil water | Iraq |
| 2. | Gram stain | Kemadia /Iraq |
| 3. | Hydrogene peroxide 20% | Ameya/U.A.E |

### 3.1.3 Media

Media that were used in a study showed in table (3-3).

Table 3- 3 Media which were used in the study.

| No. | Name of Media | Company/origin |
|---|---|---|
| 1. | Blood agar | Neogen/USA |
| 2. | Maconky agar | Neogen/USA |
| 3. | Monitol agar | Neogen/USA |
| 4. | Sabouraund dextrose agar | Lioflichem/ Italia |

## 3.2. Methods

### 3.2.1. Study Design

Steps of study plan were showed in figure (3-1).

Steps of study plan were showed in figure (3-1).

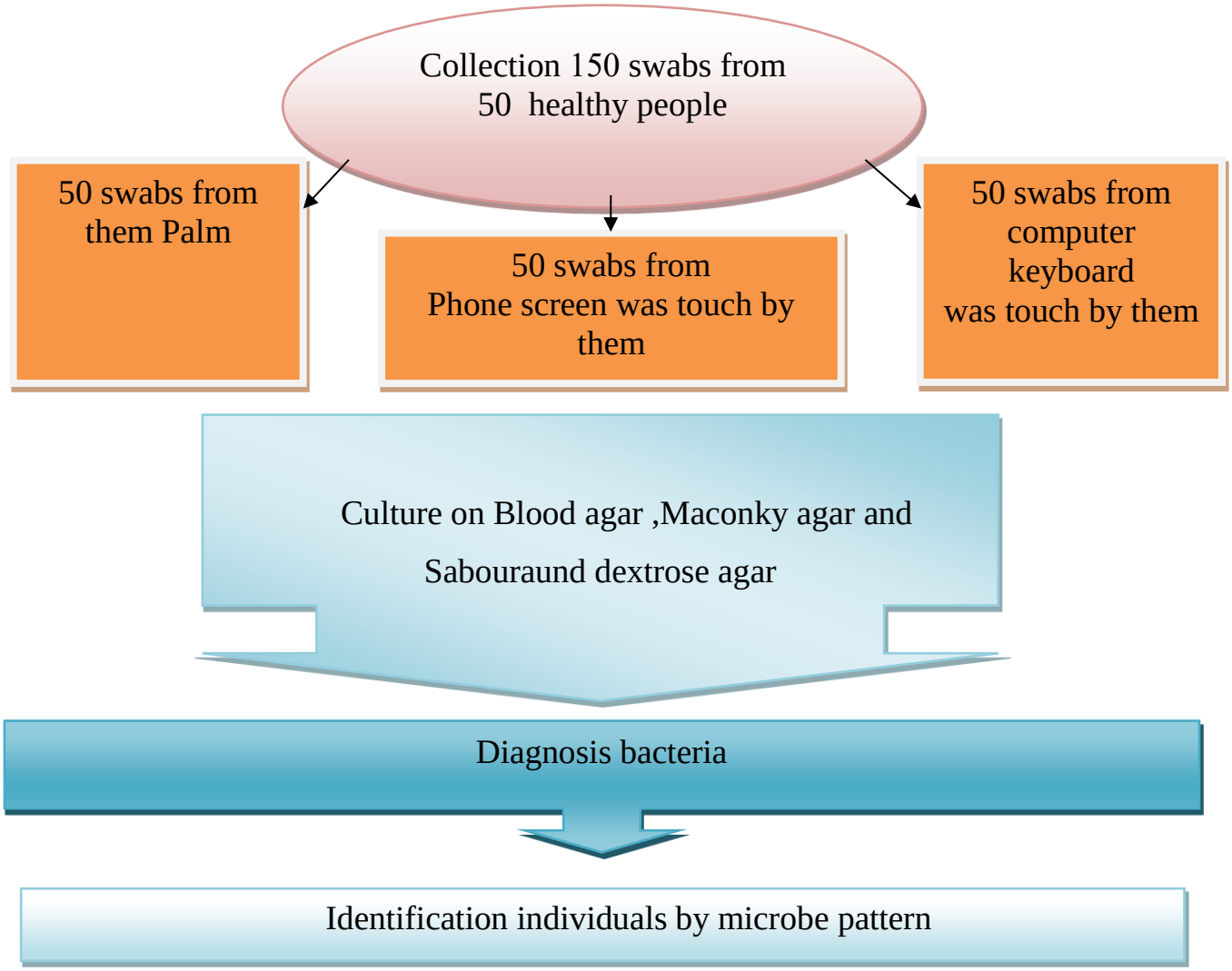



Figure 3- 1 Experiment design of the main steps involved of the research plan.

### 3.2.2. Sample Collection

Collection 150 swabs from 50 healthy people aged between (20-40) years. swabs samples were taken by trained clinic nurses working for Main Blood Bank in Al-Najaf Al-Ashraf city / AL- Hakem Teaching hospital. 50 swabs from the palm of the hand, 50 swabs from Phone screen were touched by them and 50 swabs from the computer keyboard were touched by them. All samples were collected in sterile swabs. Study continued five months from 23/3 to 22/8/2021. This study was carried out at the Faculty of Science, University of Babylon.

### 3.2.3. Control Samples

Negative control was all experiment components excepting swabs, that were replaced with distilled water. Positive control was all experiment components excepting swabs, that were replaced with known bacteria (*E. Coli )* .

### 3.2.4. Samples Culture

All samples were collected by sterile swabs. The culture media were prepared according to the manufacturer's instructions, where three types of culture media were prepared, namely Macunkey, Blood Agar, as well as Sabouraud dextrose agar.

After preparing the media, they were placed in Petri dishes and numbered with the same sample numbers, then they were transferred with the samples to the hood for the culture process.

Then the sample was mixed, and then the sterile loop by flame then dipped in the with the sample, and the cover of the culture medium was also opened, then a smear was made for the media through the loop in the form of a zigzag, then the dish was closed and placed upside down in the incubator for 24 hours at 37 C .

### 3.2.5. Diagnosis Microbe

1. The samples were cultured on the three mentioned culture media

2. After incubating for 24 hours, a gram stain test was performed

3. Microscopic examination colony shape and dye color

4. We grow the samples that appeared in the microscopic examination in the form of clusters on the mannitol salting agar, because it is an optional differential medium for the staph bacteria.

5. The samples that showed growth on the mannitol salting agar are examined by means of the API 20 staph assay

6. The samples that appeared in the microscopic examination in the form of clusters and did not grow on the saline the mannitol salting agar are examined by means of the APi 20 strip assay.

7. Finally, all samples are checked on the Vitec 2

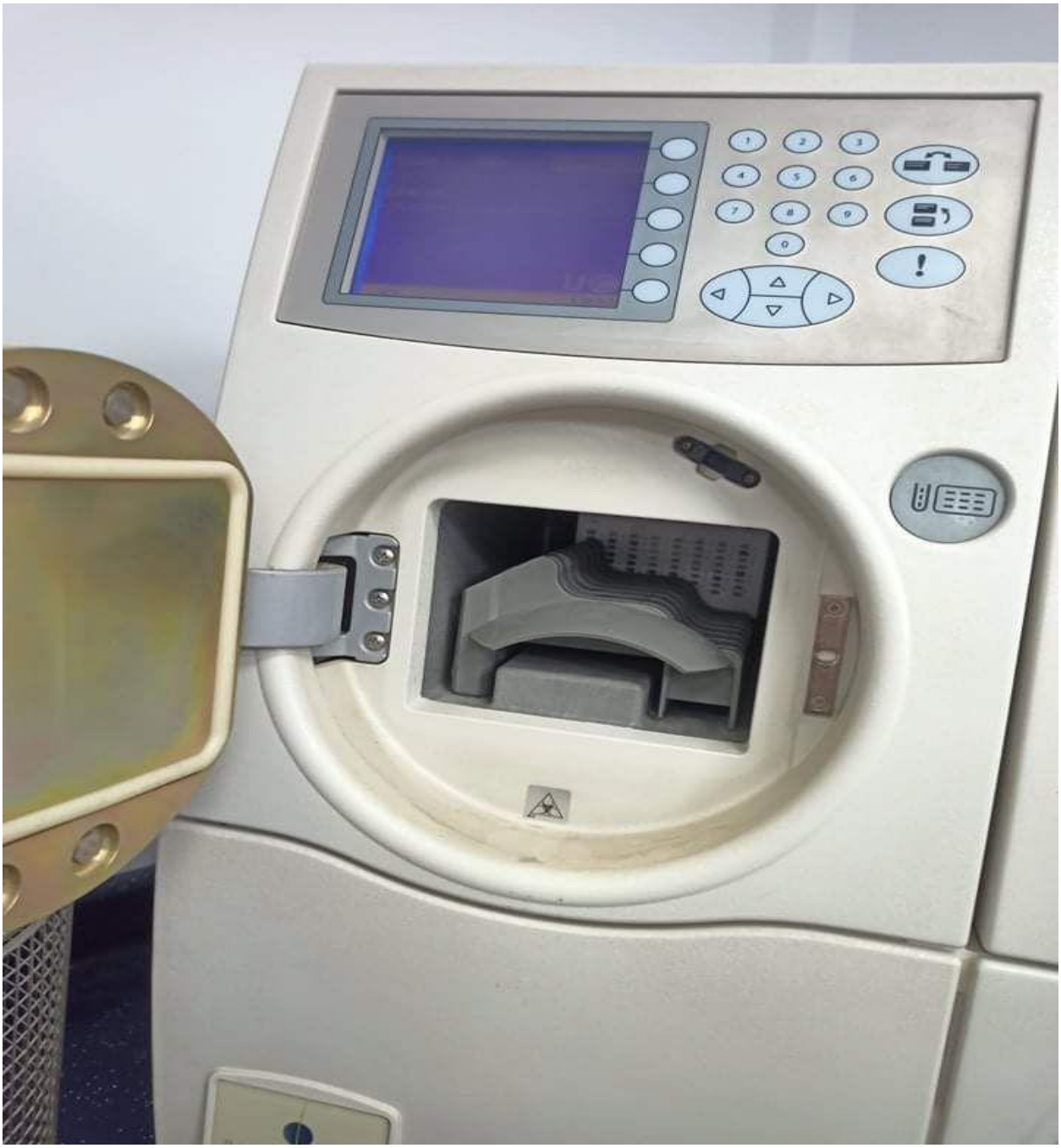


Figure 3- 2 Vitc 2 instrument that use as confirmatory diagnosis bacteria in current study.

# CHAPTER FOUR

## *RESULTS AND DISCUSSION*

# 4. Results and Discussion

## 4.1. Results

### 4.1.1. Culture of Samples

The total 150 samples that were collected from 50 persons, every person 3 swabs( palm swabs , phone screen swabs and computer keyboard). Samples were culture on three types of culture media ( Macunkey, Blood Agar, as well as Sabouraud dextrose agar). After incubated all samples that culture on Macunkey and Sabouraud dextrose agar have no growth table 4-1,2, and figure 4-1 .

Table 4- 1 Bacteria species and count it samples on every surface

| **Computer keyboard** | **Palm Swabs** | **Phone screen Swabs** | **Count of Sample NO.** |
|---|---|---|---|
| *Aerococcus Viridans* | *Aerococcus Viridans* | *Aerococcus Viridans* | 1 |
| *E. Coli* | *E. Coli* | *E. Coli* | 1 |
| No Growth | No Growth | No Growth | 2 |
| *Staph . Epidermidis* | *Staph . Epidermidis* | *Staph . Epidermidis* | 47 |
| *staph aureus+ Staph . Epidermidis* | *staph aureus+ Staph . Epidermidis* | *staph aureus+ Staph . Epidermidis* | 1 |

### 4.1.2. Bacterial Diagnosis

After the isolation and purification of the bacterial colonies, they were diagnosed by the methods mentioned in Chapter Three, in current study most of bacteria that growth were staph Epidermidis in most of the samples taken from the three surfaces (the palm of the hand, the phone screen and the keyboard of a personal computer). All result were similar, except for three people whose results had unique patterns It is the same on all three surfaces

Whereas, sample 13 did not produce growth on the three media for all swabs that taken from three surfaces, and sample 3 also grew on the swabs taken from the three surfaces with unique patterns. It is the same on all three surfaces, the bacteria that growth was *Aerococcus Viridans*. Also sample 17 produced a special pattern where both *staph Epidermidis* and *staph aureus* grew.

Figure 4- 1 Bacteria growth on manitol agar .

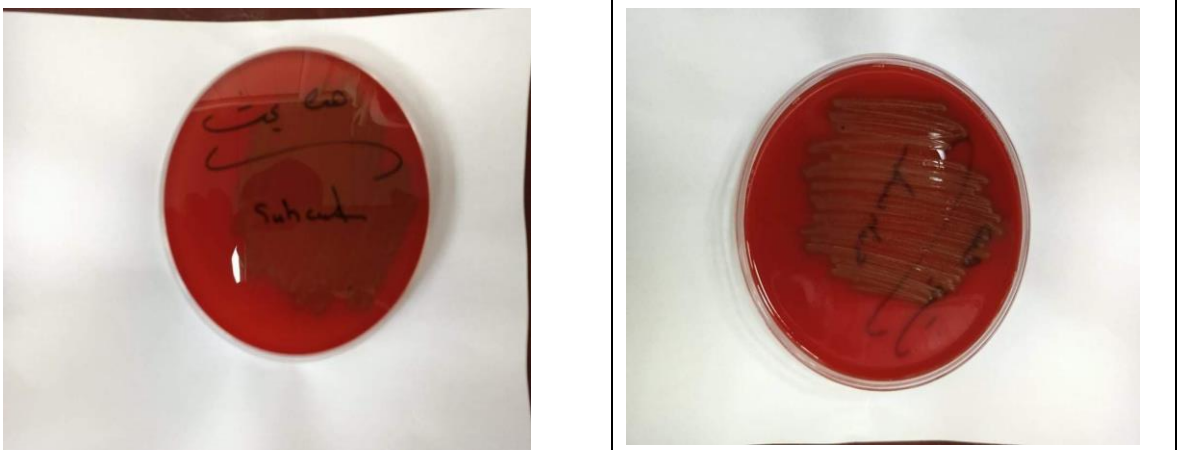
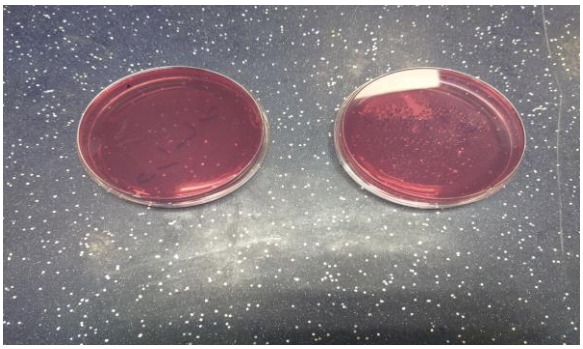


Figure 4- 2 Bacteria growth on blood agar.



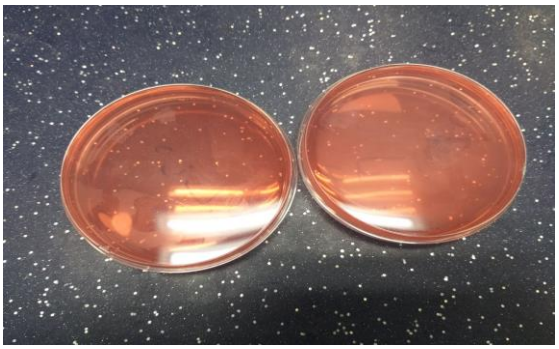


Figure 4- 3manitol and Maconkey agar

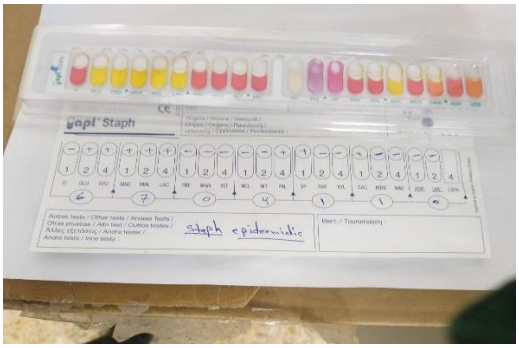




Figure 4- 4bacteria diagnosis by API staph 20

Table 4- 2 The sample number and bacteria that growth that taken by swabs from three surface were touched by individuals.

| Palm Swabs<br>Bacteria growth<br>(Replicate ) | Phone screen Swabs<br>Bacteria growth<br>(Replicate ) | Computer keyboard Swabs<br>Bacteria growth<br>(Replicate ) |
|---|---|---|
| *Staph . Epidermidis*<br>(47) | *Staph . Epidermidis*<br>(47) | *Staph . Epidermidis*<br>(47) |
| *Aerococcus Viridans*<br>(1) | *Aerococcus Viridans*<br>(1) | *Aerococcus Viridans*<br>(1) |
| *staph aureus+ Staph . Epidermidis*<br>(1) | *staph aureus+ Staph . Epidermidis*<br>(1) | *staph aureus+ Staph . Epidermidis*<br>(1) |
| No growth<br>(2) | No growth<br>(2) | No growth<br>(2) |

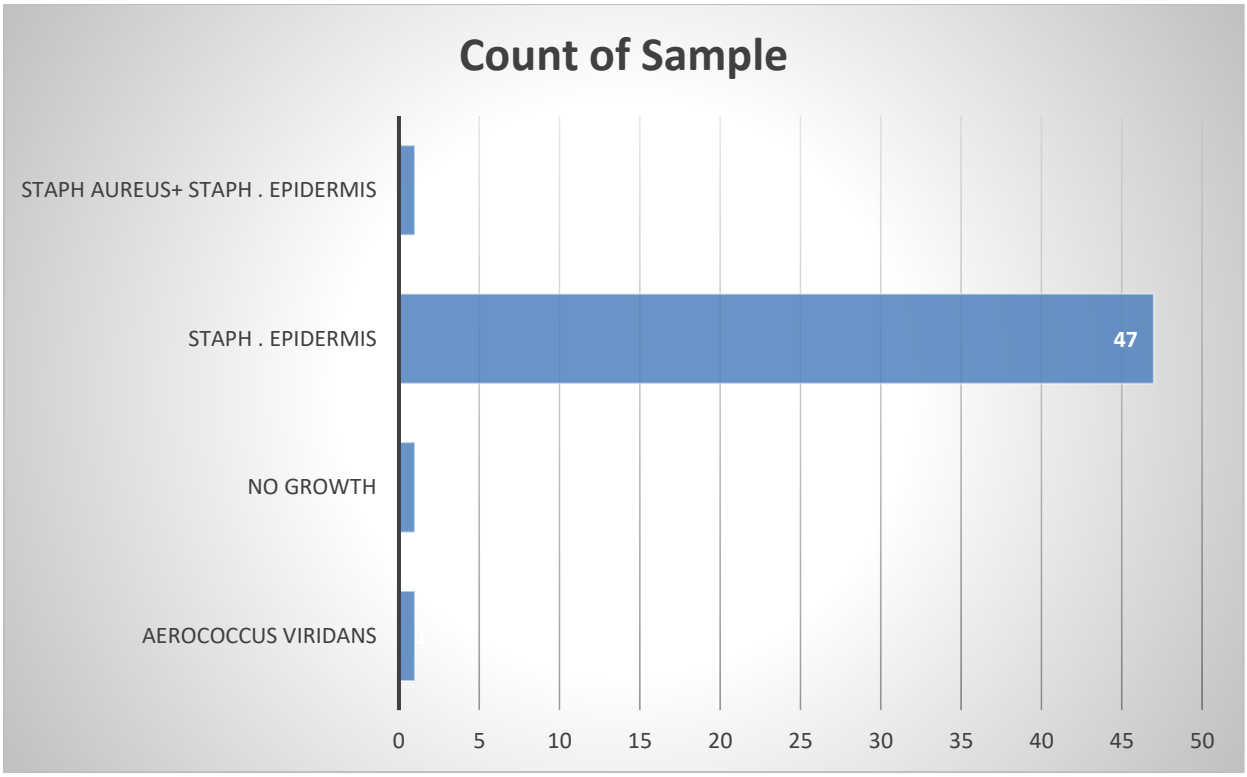



Figure 4- 5 Show bacteria species and their number in samples

## 4.2. Discussion

### 4.2.1. Bacteria Growth

150 swabs each were taken from 50 people, resulting in 150 total samples. On three different types of culture medium, samples were cultured ( Macunkey, Blood Agar, as well as Sabouraud dextrose agar). No growth occurs after a period of incubation in all samples cultured on Macunkey and Sabouraud dextrose agar.

No growth in macconkey because most normal that is usually found in humans skin is positive gram stain that is agreed with (Cogen *et al*. 2008).

Also no growth in Sabouraud dextrose agar that is due to the harsh physical landscape of skin, particularly the desiccated, nutrient-poor, acidic environment that is agreed with previous study (Byrd *et al*. 2018).

### 4.2.2.Bacteral Diagnosis

Using the techniques described in Chapter 3 to isolate and purify the bacterial colonies, they were identified as staph epidermidis in majority of the samples collected from the three surfaces (the palm of the hand, the phone screen and the keyboard of a personal computer), because staph epidermidis is most bacteria as human skin normal flora that is referred by previous study (Otto 2009).

From table (4-1) notes the presence of bacterial isolates present in the majority of people, such as normal flora, and there are also unique bacterial isolates in some people, and this serves forensic science.

Each person's results were comparable, with the exception of three persons whose results showed distinct patterns. On all three surfaces, it's the same, that is agreed with previous study In 2010, Fierer *et al*. (Fierer *et al*. 2010) firstly demonstrated the potential of microbiome analysis for forensic identification. They showed how the analysis of the skin microbiome could be used to link an individual

to an object they touched, illustrating that it is possible to link touched surfaces to individuals who touched the objects by comparing the bacterial communities yielded by an individual's skin and an object's surface. In particular, they performed three interrelated studies: - the first one (the "keyboard study") aimed to compare bacterial communities on the keys of three personal computers to the communities yielded by the fingertips of keyboard owners; - the second one (the "storage study") concerned the evaluation of long-term temporal stability of bacteria yielded by skin on swabs, which were stored at -20 ◦C or left under typical indoor environmental conditions (about 20 ◦C) for up to 14 days; - the last study (the "computer mouse study") analyzed the possibility of linking objects to specific individuals by comparing bacterial communities found on their computer mice against a database filled with information derived from the hand of the owner and from more than 270 hands that never touched the mouse.

Whereas, sample 13 did not produce growth on the three media for all swabs that taken from three surfaces, and sample No. 3 also grew on the swabs taken from the three surfaces with unique patterns. It is the same on all three surfaces, the bacteria that growth was *Aerococcus Viridans.* Also sample No. 17 produced a special pattern where both *staph Epidermidis* and *staph aureus* grew. that is agreed with (Morgan and Huttenhower 2012; Schmedes *et al*. 2017) .

# CONCLUSIONS

# AND

# RECOMMENDATIONS

# Conclusions and Recommendations

## Conclusions

1. Positive bacteria were more present on human skin than negative bacteria
2. Fungi do not spontaneously grow, so they are considered few on the skin of healthy people
3. The microbial profile can be used to differentiate between people, as well as to prove the identity of the dead bodies
4. Staph . Epidermidis most bacteria that found on human skin .
5. Fungi not found on human skin as normal flora .
6. Gram negative bacteria also rare found on human skin as normal flora .
7. Forensic situations in which microbiome-based analysis is used to reliably distinguish between persons is a promising tool that is especially beneficial when other identification approaches are unable to offer relevant information .

## Recommendations

1. It is foreseeable that microbiome-based evidence could contribute to forensic investigations in the future.
2. Make related microbiome-based research in a forensics context and greater exploration of fungal and viral communities may also lead to an important enhancement in the forensic toolkit in the future.

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## الخلاصة

يعد التنميط الميكروبي مجالًا ناشئًا للاهتمام في علم الطب الشرعي ، وهو مدرج الآن في التعريف الموسع لـ "الطب الشرعي الميكروبي" ، والمكلف إلى حد كبير بالتحقيق في إمكانية تحديد الأفراد ، أو ربطهم بالعناصر و / أو البيئات من خلال تحليل الخصائص الميكروبية. (مثل البكتيريا والفطريات والفيروسات) داخل الميكروبيومات الخاصة بهم. استمرت الدراسة شهرا من 7/23 حتى 2021/8/22. نفذت هذه الدراسة في كلية العلوم بجامعة بابل. تم جمع 150 مسحة من 50 فردًا سليمًا تتراوح أعمارهم بين 20-40 عامًا. تم أخذ 50 مسحة من راحة اليد و 50 مسحة من شاشة الهاتف و 50 مسحة من لوحة مفاتيح الكمبيوتر. حيث تم جمع العينات في أوقات مختلفة ، تم جمع جميع العينات في مسحات معقمة. تمت زراعة جميع العينات على الأوساط ( Blood Agar، maconky agar ، وكذلك Sabouraud dextrose agar). تم تشخيص البكتيريا بجهازي Api 20 و Vitk 2. النتائج: لا يوجد نمو في Macunkey ، وكذلك Sabouraud سكر العنب أجار. في معظم العينات ، نمت بكتيريا البشرة العنقودية عليها. أعطت ثلاث عينات أنماطًا فريدة من نوعها ، حيث لم تنتج عينة واحدة نموًا على الوسائط الثلاثة لجميع اللطاخات المأخوذة من الأسطح الثلاثة ، ونمت العينات الأخرى على المسحات المأخوذة من الأسطح الثلاثة بأنماط فريدة. حيث نمت البكتيريا نفسها على الأسطح الثلاثة ، كانت البكتيريا التي نمت هي Aerococcus Viridans. أنتجت عينة أخرى أيضًا نمطًا خاصًا نما فيه كل من المكورات العنقودية الجلدية والمكورات العنقودية الذهبية.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
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# إمكانية استخدام معلومات الكائنات المجهرية في التحري عن الأدلة الجنائية

**بحث مقدم الى**

**مجلس كلية العلوم – جامعة بابل**

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

## من قبل

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**٢٠٢١ م** **١٤٤٣**