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Characterization and Forensic Evaluation of Skin Microbiome Profiles in Sudanese Cadavers

A Proposal Submitted for Fulfillment of PhD Degree in Microbiology

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CHAPTER ONE

1. Introduction

1.1 Background

The human skin, recognized as the largest organ of the body, functions as a highly specialized and dynamic interface between the internal physiological environment and the external surroundings. It serves as a multifaceted barrier mechanical, chemical, and immunological protecting the body against physical injury, pathogenic invasion, and harmful ultraviolet radiation [1,2]. Structurally, the skin is composed of three principal layers: the epidermis, which forms the outer keratinized barrier responsible for water retention and protection; the dermis, a connective tissue matrix rich in vasculature, neural elements, and adnexal structures such as hair follicles and glands; and the hypodermis, consisting primarily of adipose tissue that provides thermal insulation and energy storage [3].

Importantly, the physicochemical characteristics of the skin, including pH, temperature, hydration, and sebum production, vary considerably across anatomical regions. These microenvironmental differences create distinct ecological niches that shape the composition and distribution of microbial communities colonizing the skin surface [4].

In recent decades, the concept of the microbiome has emerged as a central paradigm in biomedical sciences. The microbiome encompasses the entire assemblage of microorganisms, including bacteria, fungi, viruses, and archaea, along with their collective genomes and metabolic activities within a defined environment [5].

The skin microbiome, in particular, exhibits remarkable diversity, spatial heterogeneity, and inter-individual variability. Each individual harbors a unique microbial signature or “microbial fingerprint,” influenced by a complex interplay of intrinsic factors such as genetics, age, sex, and immune status, as well as extrinsic determinants including geographic location, climate, hygiene practices, occupation, and lifestyle [6,7].

Unlike the relatively stable gut microbiome, the skin microbiome is highly dynamic and responsive to environmental interactions, rendering it a valuable indicator of both biological identity and recent environmental exposure [8].

Consequently, microbiome research has expanded beyond clinical applications to encompass fields such as personalized medicine, environmental science, and increasingly, forensic science.

The integration of microbiome analysis into forensic science represents a transformative advancement, offering a novel class of biological evidence that complements conventional human DNA profiling [9]. The forensic potential of the skin microbiome is multifaceted. Firstly, its high degree of inter-individual variability supports its application in individualization, whereby microbial signatures may function as biological barcodes capable of linking individuals to objects or surfaces they have contacted [10].

Secondly, the post-mortem evolution of microbial communities follows relatively predictable successional patterns during decomposition. These temporal dynamics provide the basis for estimating the post-mortem interval (PMI), particularly in advanced stages of decomposition where traditional forensic methods become less reliable [11,12]. Thirdly, the microbiome may assist in inferring an individual's geographic origin or recent movements [13]. Collectively, these applications highlight the potential of microbiome analysis as a complementary approach in modern forensic investigations.

Advancements in microbial analysis methodologies have played a critical role in enabling these applications. Traditional culture-dependent techniques, which rely on the growth of microorganisms on selective media followed by phenotypic and biochemical characterization, have historically been the cornerstone of microbiological investigation. However, these approaches are inherently limited, as they capture only a small fraction, often less than 1%, of the total microbial diversity present in a given sample [14].

The advent of culture-independent molecular techniques has revolutionized the field, enabling comprehensive and unbiased characterization of microbial communities. Among these, 16S ribosomal RNA (rRNA) gene sequencing remains a widely used approach for bacterial identification [15]. More advanced techniques, such as shotgun metagenomic sequencing, allow for the simultaneous analysis of all genetic material within a sample, providing both taxonomic and functional insights, including metabolic pathways, virulence determinants, and antimicrobial resistance genes [16]. These methodologies are further enhanced by next-generation sequencing (NGS) platforms, which facilitate high-throughput, rapid, and cost-effective sequencing [17].

Despite these significant advancements, there remains a notable lack of region-specific microbiome data, particularly in underrepresented regions such as Sudan. The Sudanese environment is characterized by unique climatic conditions, predominantly hot and arid, as well as substantial ethnic and cultural diversity, all of which are likely to influence the structure and dynamics of the skin microbiome.

This gap in knowledge presents both a challenge and an opportunity for forensic science. Establishing a comprehensive reference database of skin microbiome profiles within the Sudanese population is essential for enabling accurate interpretation of microbiome-based evidence [18]. Such a database should account for geographic variation, environmental factors, and population heterogeneity.

Furthermore, the application of skin microbiome profiling in forensic practice in Sudan holds considerable potential across multiple domains. These include the use of microbiome signatures as trace evidence to associate individuals with crime scenes or objects, the development of predictive models for post-mortem interval estimation under local environmental conditions, and the utilization of microbiome-derived complementary evidence in cases where human DNA is degraded or insufficient.

Additionally, integrating microbiome analysis with advanced statistical and computational approaches such as machine learning and network analysis may further enhance its accuracy and reliability in forensic contexts [19].

In conclusion, the skin microbiome represents a rapidly evolving frontier in forensic science, offering innovative approaches to human identification, post-mortem interval estimation, and geographic inference. However, its successful implementation requires region-specific validation, standardized methodologies, and robust reference datasets. This study aims to address these gaps by characterizing and evaluating the forensic applicability of skin microbiome profiles in Sudanese cadavers, thereby contributing to the advancement of forensic microbiology in both regional and global contexts.

1.2 Problem Statement

1.2.1 Research Questions

Building upon the identified gaps and informed by current advances in forensic microbiome research, this study seeks to address the following key research questions:

1.What is the taxonomic composition and diversity of the skin microbiome in Sudanese cadavers, and how does it vary across anatomically distinct skin sites?

2.To what extent do demographic variables such as age, sex, and geographic origin contribute to variability in skin microbiome profiles among Sudanese individuals?

3.How does the structure and composition of the skin microbiome change over time following death, and can these changes be quantitatively modeled to estimate the post-mortem interval (PMI) under Sudanese environmental conditions?

4.What is the comparative performance of culture-dependent versus culture-independent (NGS-based) methodologies in profiling the skin microbiome for forensic purposes?

1.2.2 Hypotheses

In alignment with the study objectives and existing literature, the following hypotheses are proposed:

Null Hypothesis (H0)

There are no statistically significant differences in the composition or diversity of the skin microbiome in Sudanese cadavers compared with those reported in other geographic regions, and microbial profiles do not exhibit meaningful associations with post-mortem interval or individual identity.

Alternative Hypotheses

H1: Geographic and Environmental Differentiation Hypothesis

Sudanese cadavers harbor distinct skin microbiome profiles characterized by differential abundance of key bacterial taxa such as Corynebacterium, Cutibacterium, and Staphylococcus, reflecting the influence of local environmental conditions, climate, and host-specific factors.

H2: Microbial Succession Hypothesis

The skin microbiome undergoes a statistically significant and temporally structured succession following death, with predictable shifts in microbial community composition that correlate with post-mortem interval and can be modeled for PMI estimation under Sudanese conditions.

H3: Forensic Individualization Hypothesis

The skin microbiome exhibits sufficient inter-individual variability to function as a complementary forensic tool for individual identification, with next-generation sequencing (NGS)-based approaches demonstrating superior discriminatory power and sensitivity compared with traditional culture-based methods.

1.3 Justification

The importance of forensic microbiome research is increasingly recognized as a promising tool in forensic investigations. However, significant gaps remain, particularly in underrepresented regions such as Sudan. The justification for this study can be summarized across three main dimensions:

Scientific:

Current studies lack accurate data on the composition and diversity of the skin microbiome among Sudanese populations. Existing research is concentrated in specific regions worldwide, limiting the generalizability of findings. This study aims to generate baseline data that reflect local environmental and biological characteristics.

Applied (Forensic):

Forensic practices in Sudan rely primarily on conventional methods, which may be limited in cases of advanced decomposition. The microbiome offers a promising complementary tool, particularly for post-mortem interval (PMI) estimation and trace evidence analysis.

Contextual and Environmental:

Sudan is characterized by unique climatic conditions, including high temperatures and arid environments, that influence the microbiome and its post-mortem dynamics. Most existing models have been developed under temperate conditions, highlighting the need for locally adapted models.

Accordingly, there is a clear need to develop microbiome-based forensic tools and models tailored to the Sudanese environment.

1.4 Objectives

1.4.1 General Objective

This study aims to characterize the microbiome in human cadavers in Sudan and to critically evaluate its forensic applicability as a complementary tool for individual identification and post-mortem interval (PMI) estimation under local environmental conditions.

1.4.2 Specific Objectives

1.To isolate and characterize the predominant aerobic and facultative anaerobic bacterial communities colonizing selected anatomical regions (retroauricular crease, palm, and axilla) of Sudanese cadavers using conventional culture-dependent microbiological techniques, thereby providing a phenotypic overview of cultivable taxa.

2.To determine the taxonomic composition and microbial diversity of the skin microbiome using high-throughput next-generation sequencing (NGS) targeting the 16S rRNA gene, enabling comprehensive culture-independent microbial profiling.

3.To compare the resolution, sensitivity, and forensic applicability of culture-dependent and NGS-based methods, with particular emphasis on their ability to discriminate between individuals and detect low-abundance taxa.

4.To assess the temporal dynamics of the skin microbiome in relation to post-mortem interval (PMI) by analyzing successional changes in microbial community structure under Sudanese climatic conditions, and to evaluate their potential for PMI prediction.

5.To develop a preliminary reference database of skin microbiome profiles derived from Sudanese cadavers, which may serve as a foundational resource for future forensic investigations and population-specific comparative analyses.

CHAPTER TWO

2. Literature Review

A growing body of research has demonstrated that the human skin microbiome is highly structured and site-specific, with microbial composition largely determined by the physicochemical properties of different anatomical regions.

Metcalf et al. (2013) This seminal murine study introduced the concept of the "microbial clock" for PMI estimation. The authors showed that microbial succession during decomposition follows a predictable temporal pattern, enabling PMI estimation. This work laid the foundation for using post-mortem microbial communities as potential forensic indicators.

Pechal et al. (2015) An analysis of human cadavers across multiple anatomical sites revealed predictable post-mortem shifts in the microbiome. Specific taxa, particularly Ignatzschineria, became dominant during advanced decomposition stages. This study provided important evidence of structured, site-specific microbial changes relevant to PMI estimation.

Javan et al. (2016) A comprehensive review of forensic microbiome research highlighted the potential of microbial succession as a forensic indicator. The authors emphasized the possible utility of post-mortem microbial communities in PMI estimation while also recognizing the influence of environmental variability.

DeBruyn and Hauther (2017) Investigating gut microbial translocation in human cadavers, the study demonstrated that intestinal bacteria migrate into surrounding tissues in a predictable, time-dependent manner. This highlighted the potential of post-mortem microbial changes as biological indicators of decomposition dynamics.

Carter et al. (2018) This study explored environmental influences such as temperature, humidity, and soil type on decomposition and microbial community structure. Results showed that these variables significantly affect both decomposition rate and microbial composition, underscoring the need to incorporate environmental conditions into PMI models.

Ying et al. (2019) By comparing populations in tropical and temperate regions, this study revealed substantial differences in skin microbiome diversity and composition driven by climatic factors. Findings emphasized the importance of considering environmental and climatic determinants in forensic applications.

Massawi et al. (2022) A systematic review of forensic microbiology research highlighted challenges associated with hot climates. The authors reported that high ambient temperatures accelerate decomposition and alter microbial dynamics, potentially affecting PMI estimation. The review stressed the need for climate-specific forensic tools, particularly in regions such as the Middle East and North Africa.

Suzuki et al. (2024) Using a multi-omics approach that integrated metagenomics and metabolomics, this study demonstrated the potential of advanced integrative analyses to enhance the precision and reliability of microbiome-based forensic applications.

García et al. (2026) This study developed an AI-driven platform for real-time forensic microbiome analysis, demonstrating the potential of artificial intelligence in forensic science to improve the speed and accuracy of PMI estimation and microbial evidence interpretation.

There is notable global progress in this field; however, there are currently no well-documented studies on the human skin microbiome in Sudan or the broader Sudanese region that comprehensively examine its role in decomposition and post-mortem interval (PMI) estimation.

Sudan is characterized by unique environmental and demographic features, including high temperatures, arid conditions, seasonal variation, and a diverse population. These factors are likely to influence both the composition of the skin microbiome and the dynamics of post-mortem microbial activity.

CHAPTER THREE

Materials and Methods

3.1 Study Area

Approximately 50–120 samples will be collected from cadavers found in forensic mortuaries, hospitals, and crime scenes across five Sudanese states: Northern State, Khartoum State, Gezira State, Kassala State, and North Kordofan State.

3.2 Study Design

Cross-sectional forensic microbiome study conducted on cadaver cases.

3.3 Study Population

Samples will be collected from human cadavers and associated environmental sources, obtained from morgues, hospitals, and crime scenes within Sudan during the study period.

3.4 Sample Size and Sampling Strategy

A stratified sampling approach will be used to ensure representation across different post-mortem interval (PMI) categories and anatomical sampling sites.

Based on pilot observations and comparable forensic microbiomes studies, a total sample size ranging from 50 – 120 cadavers will be targeted. This range is considered sufficient to achieve approximately 80% statistical power for detecting significant difference in alpha diversity indices and other microbial parameters across study groups.

The sample size will be calculated using the following formula:

$n = Z^2 p(1-p) / d^2$

Where:

- n = required sample size
- Z = standard normal deviate (1.96 at a 95% confidence level)
- p = expected prevalence (estimated proportion, taken as 0.5)
- d = desired precision, taken as 0.05

Due to the relatively low frequency of cadaver cases and the irregular availability of samples in forensic mortuaries, crime scene, and hospitals across Sudan, the proposed sample size rang (50 – 120) cadavers is considered both realistic and methodologically appropriate. This rang reflects practical and logistical constraints associated with sample collection challenges, and variability in access to forensic facilities. (39,40)

Moreover, this sample size is consistent with exploratory studies in forensic microbiome research, where relatively small to moderate sizes (typically between 30 and 100 cases) have been shown to yield meaningful preliminary insights into microbial diversity and forensic patterns [39,40,42].

3.5 Sampling Method:

A convenience sampling method will be used to select cadavers admitted to participating mortuaries, identified at crime scenes, or received at participating hospitals, will be selected according to predefined inclusion and exclusion criteria.

3.6Inclusion Criteria

This study will include adult cadavers aged ≥18 years of both sexes with a known or estimated post-mortem interval.

Cases are selected from crime scenes, hospitals, and morgue (mortuary) facilities, provided that the cause of death is non-infectious (trauma or cardiovascular disease) and bodies are maintained under standard mortuary conditions.

Samples are collected from accessible and intact anatomical sites suitable for microbiome analysis.

3.6Exclusion Criteria

Cadavers will be excluded if they exhibit advanced decomposition that prevents reliable sampling, or if they have been exposed to chemical preservatives or embalming.

Cause with evidence of systemic infection at the time of death are also excluded to avoid pathological microbial interference. Additionally, cadavers that have

undergone prior autopsy before sampling are excluded to prevent contamination and alteration of native microbiome.

3.7 Sample Collection

Skin swab samples will be collected from predefined anatomical sites. Environmental and object-surface samples may also be collected when appropriate. Sterile cotton swabs will be used following standard forensic evidence-collection procedures.

The proposed sampling sites will include:

Palmar surface of the hand, Forearms, Forehead, and Soil.

Each swab will be placed in sterile transport tubes and stored at –20°c until laboratory processing.

3.8 Sampling Procedure:

Sterile rayon or nylon-flocked swabs pre-moistened with phosphate-buffered saline (PBS) containing 0.1% Tween-20 will be used for sample collection.

Each sampling site, covering an area of approximately 4 cm^2, will be swabbed using a firm zig-zag motion for approximately 30 seconds.

Duplicate swabs will be collected from each sampling site:

• One swab will be used for culture-dependent analysis and stored at 4°C until processing within 24 – 48 hours.

• The second swab will be used for molecular analysis and stored at −80°C in DNA/RNA Shield.

Each sample will be labeled using a unique cadaver identification code, sampling site, date, and relevant environmental information.

3.9 Data Collection:

A standardized data collection form will be used to record:

- Cadaver code, Age, and Sex
- Cause of death
- Estimated PMI in hours/days
- Date and time of sampling

- Anatomical sampling site
- Ambient temperature, and humidity
- Known antibiotic use prior to death

3.10 Culture-Dependent Analysis

Culture-dependent analysis will be performed using appropriate microbiological culture media, including:

- Blood agar
- MacConkey agar
- Mannitol salt agar
- Chocolate agar
- Anaerobic culture using a GasPak system

Samples will be incubated at 37°C for 24 –72 hours under appropriate aerobic and anaerobic conditions.

Bacterial identification will be performed based on:

- Colony morphology, Gram staining, Catalase test, Oxidase test, Coagulase test.
- Biochemical identification using Analytical profile systems (API)

3.11 Culture-Independent Molecular Analysis

3.11.1 DNA Extraction

Microbial DNA will be extracted from the collected samples using the DNeasy PowerSoil Pro Kit (Qiagen) according to the manufacturer's instructions.

3.11.2 16S rRNA Gene Amplification

- The V3–V4 region of the bacterial 16S rRNA gene will be amplified using universal primers 341F and 805R with Illumina adapters.
- PCR reactions will be performed in triplicate, and the resulting products will be pooled for downstream analysis.

3.11.3 Library Preparation and Sequencing

- PCR products will be purified using AMPure XP beads.

- Libraries will be prepared using the Nextera XT kit and subjected to paired-end sequencing (2 × 300 bp) using the Illumina MiSeq platform.

3.11.4 Bioinformatics Analysis

- Sequence data will undergo quality filtering and chimera removal using the DADA2 pipeline.
- Taxonomic assignment will be performed using reference databases such as SILVA version 138 or Greengenes.

Microbial diversity will be assessed using:

- Shannon diversity index, Simpson diversity index, Chao1 richness index

Beta diversity will be evaluated using:

- Principal Coordinates Analysis (PCoA)with Weighted / UniFrac, Unweighted UniFrac, and Bray– Curti's dissimilarity

Differential abundance analysis will be performed using DESeq2 or ANCOM-BC.

3.12 Statistical Analysis

Statistical analysis will be performed using SPSS version 31 or later and R statistical software.

R packages including phyloseq, vegan, and ggplot2 will be used for microbiome data analysis and visualization.

Descriptive statistics will include:

- Means and standard deviations for continuous variables
- Frequencies and percentages for categorical variables
- Continuous variables will be compared using analysis of variance (ANOVA) or the Kruskal–Wallis test, as appropriate.
- Categorical variables will be analyzed using the chi-square test or Fisher's exact test.
- Spearman correlation analysis will be used to assess associations between PMI, microbial diversity, and individual taxa.

- Predictive models, including Random Forest or other machine-learning approaches, will be used to estimate PMI and anatomical sampling sites.
- Cross-validation will be applied to evaluate the predictive performance of the models.

3.13 Ethical Considerations:

The study will be conducted in accordance with the Council for International Organizations of Medical Sciences (CIOMS) 2016 guidelines and the Declaration of Helsinki.

3.13.1 Regulatory Approvals

Ethical and regulatory approval will be obtained from:

1.The National Research Ethics Committee, Federal Ministry of Health, Sudan.

2. The Ministries of Health of the respective participating states.

3. Dongola University.

4. General directorate of forensic evidence - Sudanese police forces.

3.13.2 Consent and Authorization

Written consent will be obtained from the legally authorized representative or next of kin after providing adequate information regarding the study procedures, potential risks, and expected benefits.

Legal authorization will also be obtained from the investigating prosecutor, in accordance with the Sudanese Criminal Procedure Code and applicable regulations governing the collection of samples for research purposes.

3.14 Data Privacy and Confidentiality

All samples and study records will be identified using unique alphanumeric codes rather than personal identifiers.

A separate, password-protected master list linking the study codes to identifying information will be maintained and accessible only to the principal investigator.

Genomic sequence data will be handled using controlled-access databases and appropriate data-protection procedures.

4.study Timeline (36 month):

The study will be conducted over a 36 – month period, following a structured and phased approach to ensure systematic progress, data integrity, and high-quality scientific output.

Phase	Duration	Activities
Phase 1	Months 1– 3	Ethical approval, training, and procurement
Phase 2	Months 4 –16	Sample collection from mortuaries and crime scenes
Phase 3	Months 6 – 24	Laboratory analysis, culture, DNA extraction, and NGS
Phase 4	Months 18 – 30	Data analysis, bioinformatics, and statistical modeling
Phase 5	Months 30 – 36	Thesis writing, manuscript preparation, and publication

5.Estemated Budget

The estimated budget for this study reflects the costs associated with laboratory analyses, sequencing, personnel, transportation, fieldwork, logistics, data analysis, and dissemination.

Budget item	description	Estimated cost (Sp)
Consumable	Culture media, sterile swab, pipette tips, gloves, cryovial, and molecular biology reagent including DNA extraction kits (e.g., powerSoil pro kit)	600,000 – 800,000
Sequencing services	16S rRNA amplicon sequencing (V3 – V4 region) for approximately 300 samples using Illumina MiSeq plat form	1500,000 – 2500,000
Bioinformatics resource	Computational infrastructure, software tools, and potential cloud computing services	500,000 – 1000,000
Field work and transportation	Travil to mortuaries, fuel, vehicle rental, and field assistance.	1000,000 – 1500,000
Personnel	Research assistants and laboratory technical support	200,000 – 400,000
Dissemination cost	Thesis printing, binding, publication, fees (open-access journals), and conference participation.	500,000 – 750,000
Contingency	Unforeseen expenses and equipment maintenance.	200,000 – 300,000
Total estimated budget		**4500,000 – 7250,000**

QUESTIONNAIRE

Characterization and Forensic Evaluation of Skin Microbiome Profiles in Sudanese Cadavers

Instructions:

This questionnaire is designed to collect relevant data for cadaveric skin microbiome analysis. Data should be collected by trained forensic personnel in accordance with ethical and biosafety standards.

Section 1: Cadaver Identification

1. Unique Case ID: _____
2. Date of Sampling: / /
3. State: Khartoum ☐ Northern ☐ Al-Jazirah ☐
   North Kordofan ☐ Kassala ☐ White Nile ☐
4. Location of Body: Crime Scene ☐ Mortuary ☐ Hospital ☐
5. Cause of Death (if known): _____
6. Sex: Male ☐ Female ☐ Unknown ☐
7. Approximate Age: _____
8. Post-Mortem Interval (PMI) in Hours: _____
9. Condition of the Body: Intact ☐ Decomposed ☐ Burned ☐
   Other_____
10. Sample Collection Site:
    Forehead ☐ Hands (palms) ☐ Neck ☐ Feet ☐ Other: _____
11. Sampling Method: Sterile swab ☐ Other: _____
12. Number of Swabs: _____
13. Transport Medium Use: _____
14. Time from Collection to Laboratory Processing (hours):

_____

Section 2: Environmental and Scene Data

15. Environment:

1- Urban ☐ 2- Rural ☐ 3- Agricultural ☐ 4- Desert ☐ 5- Semi-arid ☐

16. Surface Contact:

1- Soil ☐ 2- Concrete ☐ 3- Furniture ☐ 4- Water ☐ Other: _____

17. Sunlight Exposure:

1- Yes ☐

2- No ☐

18. Weather Conditions:

1- Hot ☐ 2- Cold ☐ 3- Humid ☐

4- Dry ☐ 5- Rainy ☐ 6- Dusty ☐

19. Body Storage Conditions:

1- Refrigerated ☐

2- Room Temperature ☐

3- Other: _____

20. Other Relevant Information:

_____

_____

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