

ORIGINAL RESEARCH

Forensic Analysis of Human Microbiome in Skin and Body Fluids

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ABSTRACT

Background: The study was conducted for Forensic Analysis of Human Microbiome in Skin and Body Fluids. **Material and methods:** The present study was conducted for Forensic Analysis of Human Microbiome in Skin and Body Fluids. A total of 100 subjects were evaluated. Inclusion criteria for the present study included subjects who came for routine checkup. Complete demographic and medical details of all the patients was obtained. A Performa was made and complete clinical details of all the patients was obtained. Skin and body fluid samples were obtained and were cultured. Predominant bacterial organism was isolated and recorded. All the results were subjected to statistical analysis using SPSS software. **Results:** A total of 100 subjects were evaluated. Mean age of the subjects was 51.8 years. Majority proportion were males. In the skin samples, dominant bacteria were *Corynebacterium*, *Staphylococcus* and *Bacillus*. In the saliva sample, dominant bacteria were *Streptococcus*, *Veillonella*, *Prevotella*, *Porphyromonas* while in the genital swab, *Bifidobacterium* was the dominant bacteria. Significant results were obtained while correlating bacterial profile among males and females. **Conclusion:** Skin and body fluids could be employed in assisting forensic analysis.

Keywords: Microbiome, Forensic

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INTRODUCTION

Microorganisms are found in abundance across all terrestrial and aquatic ecosystems. For centuries, microbes have been integral to fields such as medicine, fermentation, and the food industry; however, their potential has largely remained untapped in the realm of forensic medicine.¹⁻⁵ Recent advancements in molecular biology techniques, including DNA fingerprinting, whole genome sequencing, and microarray analysis, have propelled the discipline of microbial forensics forward significantly in the past twenty years.⁶⁻⁸

The implementation of massively parallel sequencing (MPS), also referred to as next-generation sequencing (NGS), has become a standard approach for analyzing microbial forensic evidence.^{9,10} This technology allows for the identification of low concentrations of microorganisms and previously unrecognized pathogens, even within complex sample mixtures. As

sequencing costs have decreased and bioinformatics capabilities have improved through enhanced throughput, MPS has increasingly been utilized to delineate microbial communities for forensic purposes.¹¹

Forensic investigations can be significantly enhanced through the analysis of microbiome data derived from suspects, victims, and environmental samples. This microbiome information has the potential to serve as valuable evidence for human identification, the detection of bodily fluids, and the geographical tracing of individuals.¹²⁻¹⁵

The human microbiome can be sourced from various locations, including skin and bodily fluids, as well as from environments such as residences, dormitories, classrooms, workplaces, restrooms, and personal items like footwear, keyboards, textiles, and mobile devices. Samples of body fluids and skin swabs from different populations exhibit distinct characteristics.

This review outlines various forensic indicator sites, including skin, vaginal secretions, fecal matter, and saliva from populations across different geographical regions.¹⁶

Sequencing analyses of microbiomes from these diverse sources have revealed that bacterial DNA can reflect individual lifestyles and behavioral tendencies. Consequently, microbiomes can yield geographic, ethnic, or lifestyle insights that forensic scientists can utilize to trace the origins of unidentified samples in criminal cases.^{17,18}

Furthermore, evidence indicates that integrating microbiology, molecular biology, and forensic science may facilitate the identification of both suspects and victims through human microbiome samples collected at crime scenes, particularly in instances where conventional human DNA sources are inadequate.¹⁹⁻²²

In recent years, significant progress has been made in the fields of molecular sequencing and computational methodologies. The advent of massive parallel sequencing (MPS) technology, commonly known as next-generation sequencing (NGS) or high-throughput sequencing (HTS), has dramatically enhanced the volume of sequencing data available for forensic investigations.²³

Concurrently, these advancements have led to a reduction in both the analytical costs associated with generating sequencing data and the time required for

analysis. Employing NGS to sequence total DNA extracts from various samples facilitates the comprehensive sequencing of an organism's entire genome, as well as the analysis of entire microbial communities. This capability allows for the rapid and efficient identification of diverse bacterial taxa and strains, providing a comprehensive overview of the resident microbial population. Research into the microbiome represents a highly interdisciplinary domain, encompassing a broad spectrum of applications and methodologies for its investigation, which includes various computational strategies and models.^{24,25,26} This study was conducted for Forensic Analysis of Human Microbiome in Skin and Body Fluids.

MATERIAL AND METHODS

RESULTS

A total of 100 subjects were evaluated. Mean age of the subjects was 51.8 years. Majority proportion were males. In the skin samples, dominant bacteria were *Corynebacterium*, *Staphylococcus* and *Bacillus*. In the saliva sample, dominant bacteria were *Streptococcus*, *Veillonella*, *Prevotella*, *Porphyromonas* while in the genital swab, *Bifidobacterium* was the dominant bacteria. Significant results were obtained while correlating bacterial profile among males and females.

Table 1: Demographic data

Variable	Number	Percentage
Mean age (years)		51.8
Males	66	66
Females	34	34

Table 2: Distribution of dominant bacteria

Area	Dominant bacteria
Skin	<i>Corynebacterium</i> , <i>Staphylococcus</i> , <i>Bacillus</i>
Saliva	<i>Streptococcus</i> , <i>Veillonella</i> , <i>Prevotella</i> , <i>Porphyromonas</i>
Genital swab	<i>Bifidobacterium</i>

Table 3: Sexual dimorphism in terms of dominant bacteria

Area	R ² - value	p-value
Skin	0.12	0.12
Saliva	0.25	0.25
Genital swab	2.81	0.00*

*: Significant

DISCUSSION

Trace evidence is typically characterized as the residual indicators of a past event or action associated with a particular agent.²⁷ The examination of diverse materials found at a crime scene plays a crucial role in legal proceedings, as it can uncover the presence of individuals or their connections to objects or locations. Such indicators of criminal activity are generally categorized into three primary domains: digital engineering, physics and chemistry, and human identification. Alphonse Bertillon, a notable French forensic scientist, was pioneering in his application of

microorganisms as trace evidence, successfully identifying their source locations. His research involved the analysis of approximately 500,000 microbes per cubic meter and 88,000 microbes across various sites in Paris.²⁸ This study was conducted for Forensic Analysis of Human Microbiome in Skin and Body Fluids.

A total of 100 subjects were evaluated. Mean age of the subjects was 51.8 years. Majority proportion were males. In the skin samples, dominant bacteria were *Corynebacterium*, *Staphylococcus* and *Bacillus*. In the saliva sample, dominant bacteria were *Streptococcus*,

Veillonella, Prevotella, Porphyromonas while in the genital swab, Bifidobacterium was the dominant bacteria. Significant results were obtained while correlating bacterial profile among males and females. Nagasawa S et al developed a method for determining the geographical origin of unidentified cadavers by determining the genotype of Helicobacter pylori, which is latent in one-half of the world's population. In the first stage, DNA was extracted from samplings at 5 points in the gastric mucosa of 177 individuals randomly selected from cadavers undergoing medico-legal autopsy. 16S-rDNA of H. pylori DNA was detected by polymerase chain reaction (PCR) in 101 cadavers (57.0%); by sex, 74 of 123 (60.1%) males and 28 of 54 (46.4%) females were positive. There were no significant differences in H. pylori detection rate among the 5 sampling points of the gastric mucosa, cause of death, or age. In the second stage, amplified fragments of H. pylori vacA regions s and m from 17 individuals with the following ethnic backgrounds were sequenced: Japanese, 10; Chinese, 2; South Korean, 1; Taiwanese, 1; Thai, 1; Afghan, 1; and Filipino, 1. A phylogenetic tree constructed with these and 28 previously reported H. pylori strain sequences revealed 3 major gene clusters consisting of East Asian type I (Japanese, South Korean and Chinese), Western type II, and Southeast Asia type III. The Taiwanese and Filipino samples deviated from the clusters type III to which they typically belong. The ultimate aim of the present study was to develop a more accurate method of determining of geographic origin of unidentified cadavers through the combination of the present method with other, virus-based methods. H. pylori DNA was detected from over half of the cadavers tested and vacA genotypes showed specificity to geographical origin. Therefore, these results suggest that the H. pylori genome provides valuable additional information for tracing the geographical origin of unidentified cadavers.²⁹ Old J. B et al demonstrated that RSID-Saliva is accurate, reproducible, and highly sensitive for human saliva; RSID-Saliva detects less than 1 microL of saliva. The sensitivity of RSID-Saliva allows investigators to sample a fraction of a questioned stain while retaining the majority for DNA-STR analysis. They demonstrated that RSID-Saliva identifies saliva from a variety of materials (e.g., cans, bottles, envelopes, and cigarette-butts) and it does not cross-react with blood, semen, urine, or vaginal fluid. RSID-Saliva is a useful forensic test for determining which evidentiary items contain saliva and thus may yield a DNA profile.³⁰

CONCLUSION

Skin and body fluids could be employed in assisting forensic analysis.

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