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THE USE OF MICROBIOME FORENSICS: IDENTIFYING PEOPLE BY THEIR BACTERIA

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Abstract

Microbiome forensics is an emerging field in forensic science that utilizes the unique microbial communities associated with individuals as a novel form of trace evidence. Unlike traditional forensic methods such as fingerprinting or DNA profiling, microbiome forensics examines the personalized "microbial fingerprint" left behind on objects and surfaces through human contact. These microbial communities, shaped by genetics, environment, diet, and lifestyle, exhibit both individuality and temporal stability, making them viable for personal identification and investigative leads. Initially propelled by bioterrorism cases such as the 2001 anthrax attacks, microbiome analysis has since expanded to a wider range of criminal and civil applications. The technique primarily relies on high-throughput DNA sequencing methods like 16S rRNA amplicon sequencing and shotgun metagenomics, followed by advanced bioinformatic analysis. This approach offers advantages over traditional methods, including greater persistence on surfaces, resilience to environmental degradation, and potential to yield contextual information about individuals. However, the field also faces significant challenges such as lack of standardized protocols, dynamic nature of the microbiome, risks of contamination, and ethical concerns regarding privacy. Future directions include integrating microbiome data with traditional forensic techniques, expanding global microbial databases, and applying machine learning to enhance identification accuracy and inference. With continued research and the development of legal and scientific standards, microbiome forensics holds significant promise in reshaping investigative practices and achieving more comprehensive and equitable justice outcomes.

Key words: *Microbiome forensics, Human microbiome, Microbial fingerprint, Forensic science, Trace evidence.*

Introduction:

In the field of forensic science, a new method called microbiome forensics is becoming more popular. This approach uses the tiny living communities found on and in our bodies as a new form of evidence. Typically, forensic experts depend on clues like fingerprints, blood, hair, and DNA to identify who was involved in a crime or to figure out what happened. However, with new advancements, scientists have discovered that every person has a unique collection of microbes, similar to a microbial fingerprint. This unique fingerprint can be left behind on surfaces and things we touch, such as doorknobs, clothes, or electronic devices. These microbial traces can help identify people, especially when other types of evidence are unavailable, damaged, or not enough.

The human microbiome includes bacteria, viruses, fungi, archaea, and protozoa on different parts of our bodies. These microbial communities are shaped by our genetics, diet, lifestyle, health, and the environment, making

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them unique for each individual. The skin microbiome is particularly interesting for forensic scientists because these microbes can remain on surfaces for a long time and survive environmental changes, which helps in determining recent human contact.

Microbiome analysis gained attention after the 2001 anthrax attacks, initially focusing on bioterrorism detection. Nowadays, it's also used in cases of burglary, assault, and solving old, unsolved cases. By using advanced DNA sequencing like Next-Generation Sequencing (NGS), scientists can identify and compare these microbial communities on people and objects. This makes microbiome forensics a promising addition or even an alternative to traditional forensic techniques, especially when usual biological evidence is missing or compromised.

This explanation introduces the basic concepts of microbiome forensics, explores how it is applied in investigations, highlights its benefits and current challenges, and considers its potential role in the future of forensic science.

Imagine a scenario where the key to solving a crime lies not in the familiar swirls of a fingerprint or the unique sequence of DNA, but in the microscopic world of bacteria left behind on a seemingly innocuous object like a doorknob. This concept introduces the burgeoning field of microbiome forensics, a discipline that focuses on the study of human-associated microbial communities as a novel form of forensic evidence.[1633] Once primarily confined to investigations of bioterrorism, the application of microbiome analysis has expanded significantly, now encompassing a broader range of criminal and civil cases.[1634] At its core, microbiome forensics is a scientific endeavor dedicated to characterizing microbiological evidence with the aim of generating investigative leads and ultimately attributing actions in various legal contexts.[1635] The

impetus for this field's growth was notably amplified by the anthrax attacks of 2001, which highlighted the forensic significance of microbial agents. The underlying principle that makes this field so promising is the understanding that each human being harbors a unique and complex community of microorganisms, a personalized microbial "fingerprint" that distinguishes them from every other individual. While an aspect of your identity, this microbial signature is actively released into the environment. It is now among everything we touch - and anything ostensibly on its surface - too.[1636] Your microbiome, rich with bacterial, fungal, archael, and viral organisms, is an essential component of nearly every human biological function, such as digestion and the establishment of your immune system. Scientific studies have clearly shown that personal microbial populations have enough distinctiveness to routinely and fairly accurately identify an individual for long periods of time. When considering that the microbes that are the most unique to an individual are often the most stable over time, it seems like a fair assumption that the contributing part of this signature is permanent. This article discusses the potential of microbiome forensics to emerge as a new tool in criminal investigations. We will present some basic principles and ideas about microbiome forensics, some of its capabilities to disrupt the investigative status quo, the limitations and barriers to be aware of, and what this growing and evolving field might look like in the future.

What is the Human Microbiome?

The human microbiome is the overall set of all microorganisms, such as bacteria, fungi, viruses, archaea, and some protozoa, which inhabit the surface or inside tissues and biological fluids of the human body. The

[1633] Jun Zhang, Application of Microbiome in Forensics, https://pmc.ncbi.nlm.nih.gov/articles/PMC10372919/
[1634] forensic-microbiome-invisible-traces-we-leave-behind https://nij.ojp.gov/topics/articles/
[1635] Forensic Applications of Microbiomics: A Review by Robinson at el.2021, https://www.frontiersin.org/articles/10.3389/fmicb.2020.608101/full
[1636] Sarah E. Schmedes, Forensic Human Identification Using Skin Microbiomes, https://journals.asm.org/doi/full/10.1128/aem.01672-17

microorganisms inhabit various anatomical locations and develop unique and complex ecosystems specific to the environment at each location. These locations include the gastrointestinal tract (or gut), the skin, the oral cavity, the respiratory tract, and the urogenital tract. The sheer number of these microbial residents is staggering, with estimates that the number of non-human cells within the human body is equal to, or even surpasses, the number of human cells. In addition, the total genome of these microbes encompasses many more genes than the whole human genome. Varying areas of the body offer disparate ecological niches that harbor distinct microbial communities in tune with moisture content, temperature, and the availability of nutrients. For example, oily zones like the scalp and back accommodate different microbes from dry zones on the forearms or wet conditions such as the mouth and nostrils. The gut microbiome is the largest and functionally most important microbial environment, and it is responsible for several essential functions including digestion, metabolism, immune system development, and even mental health. The oral microbiome, the body's second largest microbial population, is the portal through which microorganisms enter the digestive tract and is also essential to ensure oral health and systemic wellness.

In forensic investigations, the skin microbiome has emerged as particularly relevant. As the outermost layer of the body, the skin remains constantly in contact with the outside world, and therefore the easy transfer of skin-related bacteria onto surfaces after coming into physical contact is a high probability occurrence. These bacteria on the skin have an impressive capacity to survive on touched surfaces for prolonged periods because of their intrinsic resistance to different environmental stresses, such as changes in moisture and temperature, and UV radiation exposure. Scientific studies have repeatedly shown that microbial communities living on human skin are specific to an individual and can be used to identify personal identity effectively. In addition, these skin bacterial communities have a significant level of temporal stability, with the variation in community composition between individuals typically greater than the variation seen within the same individual over time.

Variation in microbial populations between individuals is not random but is influenced by a multifactorial interplay of various components, such as an individual's genetics, his/her environment, his/her dietary pattern, and his/her way of life. Though the genetic make-up of a person can contribute to their microbiome, especially during early development, environmental circumstances, such as geographical location, climate, urbanization, and contact with varying microbial sources, have a pronounced impact on setting up and maintaining such microbial ecosystems. Diet itself plays an essential role by impacting the availability of nutrients that feed microbial growth and, therefore, promote the expansion of certain microbial species according to their metabolic repertoire. Lifestyle aspects like exercise, stress, sleep habits, and hygiene routines can also trigger changes in the composition and diversity of a person's microbial community. Even medication, particularly antibiotics, can cause dramatic and sometimes long-term changes to the microbiome. This intricate combination of genetic predispositions and environmental influences results in the highly personalized nature of the human microbiome, making it a potential indicator not only of an individual's identity but also of certain aspects of their lifestyle and environmental exposures.

III. Microbiome Forensics in Action

The use of microbiome analysis within forensic environments comprises a sequence of steps starting from the careful extraction of microbial specimens from crime scenes or persons. Swabbing, using sterile swabs, is the most used technique in this case where microbial material from surfaces such as skin, attire, and subject items is scraped off. Following collection, samples are subjected to DNA extraction

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whereby the microorganism's genetic material is extracted from them. The purified DNA is then analyzed by high-throughput DNA sequencing methods, mainly Next-Generation Sequencing (NGS), which enables the rapid and unbiased identification of the DNA sequences of all microorganisms in a sample.

Two primary NGS methods are widely applied in microbiome forensics: amplicon sequencing and shotgun metagenomic sequencing. Amplicon sequencing focuses on target marker genes like the 16S rRNA gene for bacteria via PCR amplification and is therefore a cost-efficient technique for the profiling of microbial communities and detection of taxonomic structure. Shotgun metagenomic sequencing, however, sequences the entirety of DNA fragments in a sample and gives a more comprehensive picture of the microbial community, including functional data and strain-level resolution, even though it tends to be more resource-hungry. The huge volume of sequencing data produced is then processed with advanced bioinformatics software and matched against large microbial databases to determine the types and relative abundance of microorganisms present.

A number of studies have already shown the potential of microbiome analysis to identify individuals based on the bacteria they deposit on objects. For instance, studies have confirmed that bacterial populations on computer mouse and keyboards demonstrate a close match with their owner's skin microbiome, proposing that human users leave a special microbial "print" behind these devices. Analogously, the microbial prints could be recovered on commonly used surfaces such as smart phones and trace back to owners. Experimental research has also examined the transfer of skin microbiomes onto clothing and their longevity, which suggests future applications in forensic investigation in discarded clothing-related cases.[1637]

A groundbreaking study at the University of Colorado at Boulder explored the validity of determining computer users through the bacteria on their keyboards. The study showed that the bacterial DNA on individual keys closely correlated with the bacterial DNA prints on the fingertips of the keyboard owners with a validity of about 70 to 90 percent. A subsequent test including keyboard mice further corroborated these findings, displaying a notable similarity between the bacteria on the mouse and the palm bacteria of its owner. The accuracy rate in the user question being 95% notwithstanding, the results of the study, albeit marginally lower, nevertheless presented strong early evidence for the viability of microbiome analysis for personal identification from touched items.

Microbiome forensics is being found most helpful in particular kinds of crime scenes. Indoor settings, in which microbial populations are more fixed, are ideally suited for microbiome forensics.[1638] Crime scenes that include physical contact, which allow direct transfer of microbial communities from person to person, also offer optimum situations for analyzing microbiomes.[1639] Most importantly, microbiome forensics provides an excellent alternative in instances where conventional forensic evidence like fingerprints or DNA is either missing, destroyed, or not sufficient for use in identification.[1640] Such crime offenses include robberies, murders, rape, and burglaries. In addition, microbiome analysis can even be used to identify an object that was touched by a deceased person before they died.

IV. Advantages Over Traditional Forensics:

Microbiome forensics has a number of strong benefits over conventional forensic techniques like fingerprinting and DNA testing. One major

[1637] https://www.ishinews.com/emerging-methods-of-human-microbiome-analysis-and-their-forensic-applications/

[1638] Hye-Won Cho Yong-Bin Eom, Forensic Analysis of Human Microbiome in Skin and Body Fluids Based on Geographic Location, https://www.frontiersin.org/journals/cellular-and-infection-microbiology/articles/10.3389/fcimb.2021.695191/full

[1639] Microbial Fingerprints: Exploring the Microbiome's Contribution to Forensic Evidence, https://www.azolifesciences.com/authors/benedette-cuffari

[1640] https://nij.ojp.gov/topics/articles/forensic-microbiome-invisible-traces-we-leave-behind

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advantage is that it can potentially yield investigative leads in situations where no identifiable fingerprints or adequate quantities of good-quality human DNA are obtained.[1641] In most cases, bacterial DNA, which tends to be more prevalent on touched surfaces than human DNA, is more resistant to degradation by the environment and is therefore a more consistent target for analysis.[1642] This is especially useful in trace evidence situations where recovery of recoverable human DNA is unreliable.

In addition, microbial evidence proved to be more resilient than DNA in some environments. Skin microbiota, for instance, can survive different environmental challenges and can be detected on surfaces over long periods, sometimes up to two weeks under normal indoor settings. Such long survival time can prove vital in the investigation process when the evidence is not promptly found.

Another benefit is that a person's microbiome, though transferable, is naturally more stable and individualized than other forms of forensic evidence. The individual makeup of an individual's microbial community is an extremely precise identifier.

Lastly, microbiome analysis has the promise to yield rich context clues over and above simple identification. The makeup of a person's microbiome is shaped by diet, lifestyle, health, and

geographical location.This could possibly reveal information about an individual's habits, health issues, and even recent whereabouts.

V. Navigating the Limitations and Challenges of Microbiome Forensics.

Microbiome forensics holds promise in solving crimes, but it also faces many significant challenges that must be addressed to make it widely useful and reliable in criminal investigations. A major issue is the absence of standard methods for collecting, processing, and analyzing samples.[1643] This lack of consistency leads to different results from various studies and labs, making it difficult to compare and trust findings.[1644] It is essential to develop and adopt standard procedures for each step, from sample collection to data interpretation, to ensure microbiome evidence is both reliable and legally admissible.

The human microbiome, a collection of tiny organisms living in us, is dynamic and changes frequently[1645]. These microbes respond to environmental factors like temperature, humidity, and seasons. Additionally, a person's microbiome can change over time due to antibiotic use, illness, or changes in diet and lifestyle. Even minor daily fluctuations can alter these microbial communities. When using microbiome data in crime investigations, it's crucial to account for these variations to accurately interpret the data.

Contamination during sample collection and processing poses another challenge.[1646] Microbial samples are prone to contamination from the environment or other individuals, which can skew results and lead to incorrect conclusions. It is difficult to distinguish which microbial traces are relevant to a crime scene and which come from background environmental sources. Moreover, analyzing microbiome data generates large amounts of information, requiring advanced tools and specialized knowledge to interpret correctly. There's also a risk of overinterpreting data, leading to false positives with serious legal implications.

[1641] **Lorenzo Franceschetti,** University of Milan https://www.researchgate.net/publication/379724957_Exploring_the_role_of_the_human_microbiome_in_forensic_identification_opportunities_and_challenges

[1642] Jun Zhang, Application of Microbiome in Forensics, https://pmc.ncbi.nlm.nih.gov/articles/PMC10372919/

[1643] Meghna Swayambhu , Rolf Kümmerli, Natasha Arora , Microbiome-Based Stain Analyses in Crime Scenes, https://journals.asm.org/doi/10.1128/aem.01325-22

[1644] Suman Singh and Jiya Agarwal, Review on Forensic Analysis of Microbiota in Human, https://www.forensicscijournal.com/fulltext/jfsr/jfsr-aid1060.php

[1645] Jun Zhang , Wenli Liu , Halimureti Simayijiang, **Application of Microbiome in Forensics , https://academic.oup.com/gpb/article/21/1/97/7274175?login=false**

[1646] Eva Thiel, MSc, **Forensic Applications of the Microbiome, https://www.azolifesciences.com/article/Forensic-Applications-of-the-Microbiome.aspx**

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The use of microbiome data in forensics also involves important legal and ethical questions.[1647] Human microbiomes contain sensitive information about a person's lifestyle, health, and medical history, raising privacy concerns and potential misuse of genetic data. Issues such as data ownership and obtaining informed consent for using microbiome data in investigations must be thoughtfully addressed.

VI. The Future Landscape of Microbiome Forensics.

Looking to the future, microbiome forensics has the potential to revolutionize crime investigations. A key way it can help is by clearing people who have been wrongly accused and solving old cases that have gone cold. Sometimes, traditional DNA evidence breaks down over time and becomes unusable, but microbial evidence can last longer, allowing us to revisit old cases. Microbiome analysis can provide extra information, supporting or sometimes disproving the findings from traditional DNA tests.[1648]

Artificial Intelligence (AI) and machine learning (ML) will likely become more important in microbiome forensics.[1649] These technologies excel at handling the large and complex data sets that come from microbiome research. They can help identify individuals, guess where someone originates from, and estimate how long a person has been deceased, which is known as the postmortem interval. Additionally, AI can analyze multiple types of data together, such as microbiome, gene activity (transcriptomics), and protein data (proteomics), for richer forensic insights. Machine learning can also make predictions about characteristics like gender and diet from microbiome data.

The growth of global microbiome databases is crucial for advancing this field. These databases act as vital resources, allowing comparisons of microbiome data from crime scenes against known samples to draw conclusions about sample origins and identities. The Forensic Microbiome Database (FMD) is one such example, offering tools for visualizing, comparing, and predicting locations of human microbiomes from 16S rRNA data. Continuous updates with diverse data from different places and communities worldwide are essential for keeping these databases relevant and useful.

In the future, forensic science might see a blend of microbiome data with traditional methods. This integration can provide a fuller understanding of evidence, strengthening investigations. Microbial markers can support standard forensic tests or even serve as an alternative when conventional methods fall short.

To ensure microbiome forensics is accepted in legal settings, it needs to demonstrate strong scientific reliability and legal admissibility.[1650] This means ongoing research is necessary to confirm the reliability and validity of these methods through thorough testing. Developing and adopting standard protocols for microbiome analysis steps in forensic scenarios is also crucial for gaining legal acceptance. Moreover, educating the public about microbiome forensics will be vital to gaining acceptance from juries and the legal field.

VII. Conclusion

Microbiome forensics is a growing tool in solving crimes. It uses the unique bacteria on people's bodies to identify them and find clues. This idea of a personal microbial fingerprint, combined with better DNA reading technologies and data analysis, offers new ways to work, especially when older methods aren't enough. The field has potential to improve how we solve crimes, help with cold cases, and clear people who are

[1647] Edmond Locard (1877-1966), https://nij.ojp.gov/topics/articles/forensic-microbiome-invisible-traces-we-leave-behind

[1648] Trason Lasley, Forensic Micr ensic Microbiome E obiome Evidence: F vidence: Fourth Amendment Applications th Amendment Applications and Court Acceptance, https://scholarship.law.edu/cgi/viewcontent.cgi?article=1146&context=jlt

[1649] Evolution of Diagnostic and Forensic Microbiology in the Era of Artificial Intelligence Anwita Mishra , Salman Khan , Arghya Das , Bharat C. Das, https://www.cureus.com/articles/181410-evolution-of-diagnostic-and-forensic-microbiology-in-the-era-of-artificial-intelligence.pdf

[1650] MATTHIESEN, WICKERT & LEHRER, S.C. https://www.mwl-law.com/wp-content/uploads/2018/02/ADMISSIBILITY-OF-EXPERT-TESTIMONY.pdf

wrongly accused. However, some problems need fixing. There are no standard rules yet, bacteria can change due to the environment or time, contamination risks exist, and understanding the data can be tough. Continued research and creating best practices are necessary to overcome these issues. Using artificial intelligence and building large global databases of microbiome data are important for making the most of this field. When combined with traditional methods, microbiome forensics can strengthen investigations and lead to fairer legal outcomes. As research moves forward and problems are addressed, microbiome forensics is set to become a valuable tool for forensic scientists.