Forensic Microbiomics: Exploring the Human Microbiome as a Tool for Forensic Identification

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Abstract : Forensic microbiomics has emerged as a transformative field that extends beyond traditional human identification methods by leveraging the unique microbial communities associated with the human body. Every individual harbors a distinct microbiome shaped by genetics, lifestyle, environment, and physiology, offering a novel biological signature that persists across multiple body sites and personal objects. This paper provides a comprehensive examination of the human microbiome's forensic relevance, detailing the principles of microbial fingerprinting, the diversity of microbial evidence, and the mechanisms of microbial transfer and persistence on surfaces. Analytical advancements such as 16S rRNA sequencing, shotgun metagenomics, nanopore technologies, and machine-learning-based classification models are discussed in relation to their ability to enhance microbial profiling accuracy and reliability. Case studies demonstrate current applications in identifying users of personal devices, estimating postmortem intervals, tracing environmental contact, and supporting microbial forensic investigations in bioterrorism events. Despite its promise, forensic microbiomics faces challenges including environmental variability, contamination risks, lack of standardized protocols, computational complexity, and significant ethical and privacy concerns regarding the use of microbiome data. The paper concludes by emphasizing the need for validation studies, global reference databases, legal frameworks, and interdisciplinary collaboration to ensure the responsible and effective integration of microbiomic evidence into modern forensic practice.

Keywords: Forensic microbiomics; Microbial fingerprinting; Human microbiome; Postmortem interval (PMI); Microbial forensics.

1. INTRODUCTION

For a long time, forensic examinations have depended on biological and physical traits that are distinctive to every single person. Forensics as a science began with fingerprinting and blood groupings, progressing to the first DNA profiling, the great revolutionary technique of the time. With a high level of precision, DNA identification is one of the outstanding, advanced, and accepted techniques to tie a suspect to a certain crime scene. It is a technique that changed the nature of criminal investigations and legal action [1]. Forensic science continued to operate with biological materials despite the development of distinctive biological and physical traits for each individual, the increasing complexity of intertwined cases, and the need for biological trace materials to be more resilient and informative. Continued development and refinement in the field of forensic science has led to the incorporation of the research field of forensic microbiomes and the interdisciplinary science of forensic biological individuality (Figure 1).

The human body is home to countless microorganisms, while the collection of these microorganisms is called the human microbiome. They inhabit various locations of the body, including the skin, mouth, gut, and respiratory tract. These microorganisms are structured and form a complex ecosystem, which is associated to the physiology, lifestyle, and environment of the individual [2]. Microbiomics is the study of microbiomes, and uses genomics, sequencing technology, and computational analysis to study the microbial communities in terms of their composition and functional roles. Due to the rapid advancement of technology in the past decade, the ability to conduct research using high throughput sequencing has expanded the study of microbial ecosystems greatly. The microbial ecosystems' variability is at the core of forensic microbial ecosystem studies.

Forensic microbiology is an emerging science that received international prominence following the *Bacillus anthracis* attacks via the United States mail service in 2001 [3]. Microbial forensics and microbiomics are terms that are often used interchangeably, but they are two very different disciplines. Traditionally, microbial forensics is concerned with the identification, tracking, and attribution of microorganisms that are used in biological attacks, biocrimes, or inadvertently released pathogens [4]. It is concerned with pathogenic microbes that are environmental and/or microbial traceability in relation to security and public health. On the other hand, forensic microbiomics has a focus on human-associated microbial communities. It is not concerned with the tracking of pathogens, but with the microbial signatures that people leave on objects, surfaces, and in bodily fluids. This new discipline operates on the premise that each individual has a microbial profile that is unique to them and can be isolated, analyzed, and matched to a singular individual in the same way that a profile can be created with DNA or fingerprints. No method has better accuracy than DNA analysis, but even so, it possesses inherent flaws. In every line of work, even the forensic ones, DNA strands can get damaged by extreme heat, humidity, ultraviolet radiation, and microbes. It can also be the case that there is not enough biological material to work with. Other times the biological material is handled and analyzed but it is deficient, rendering it ineffective [5]. Touch DNA is infamous for its ineffectiveness un highly contaminated environments. When just minimal biological evidence is present at the crime scene, standard DNA profiling is sometimes ineffective. These instances exemplify the superiority of alternative technologies employed to accomplish tasks when DNA-related technologies falter. The hypothesis that the microbiome can be used as a forensic tool comes from the fact that people lose microorganisms in every place they proceed. Those microorganisms can build unique "microbial fingerprints" corresponding to the microbial comunidades that inhabit the individual human's body. Research has found that personal belongings, including cell phones, computers, and clothes, develop microbial comunidades that are akin to their owner's. Microbial communities are unlike DNA in that they contain a wealth of insight that can be altered by someone's habits, surroundings, health and activities [6]. Because of this, microbial communities may provide clues that can be used in the analysis of a criminal event. In this sense, microbial communities may augment traditional methods used in forensic science and improve the analysis of the evidence.

Forensic science continues to enhance and diversify its methodologies, with microbial analysis being a pertinent and enduring technique. Microbial environmental stress is able to survive in extreme conditions and environmental circumstances making this method useful in cases in which traditional biomarkers fail [7]. Microbial evidence is relevent in providing information other than just DNA, such as death, environmental context, and material handled. As the other fields advance such as medicine, bioinformatics, and genomics, forensic science also has alignment in these fields. Forensic microbiomics will be able to continue to improve investigative methods as it is gaining strong recognition in the scientific community and microbiomics will provide evidence that is contextually more complex and diverse.

2. Overview of the Human Microbiome

The human microbiome is comprised of up to 100 trillion microorganisms that inhabit our bodies. While many of these microbes are bacterial, archaea, and protozoa, viruses, and fungi also contribute to what many researchers characterize as the microbial second genome. Each microbial ecosystem is tailored to the specific physiological characteristics of the various regions of the body and the microbiome as a whole is impacted by what body part the microorganisms inhabit [8]. For instance, moisture, surface, and environmental influences determine the composition of the skin microbiome, which, and is predominantly comprised of bacterial microbe species such as Staphylococcus and Corynebacterium. The mouth contains a very diverse range of microorganisms and a complex microbial community influenced by carbohydrates, the glycoprotein and zymogen contents of the saliva, and mouth cleansing practices [9]. The microbiome of the gut is the most densely populated and is not only the most functionally important, but also contributes to one's digestion, metabolism, immunity, and health overall. It is mostly comprised of the Firmicutes and Bacteroidetes which affects the overall health of the individual. The vaginal microbiome, which is also less diverse and dominanted by the bacterial microbe Lacobacillus, is important to protect infections and maintain an acidic pH. This feature of the vaginal microbiome also makes it important in sexual assault investigations. These site-specific microbial signatures are relevant to forensics as every niche has its microbial traces, still recoverable on surfaces or objects, and remains unique.

Many internal and external factors shape the diversity and structure of the human microbiomes, including but not limited to, genetics, age, diet, hygiene, hormonal cycles, geography, climate, and even profession [10]. Differences among genetically identical organisms, such as monozygotic twins, are striking and helpful to highlight the individuality of these microbial ecosystems. Such variation enriches the idea of microbial individuality, central to forensic microbiomics. The variation between different people - interpersonal variation, is almost always more prominent than the intra-personal variation - the differences of the same individual over time. Microbial ecosystems are known to change overtime, and can possibly be driven by factors such as environment, lifestyle, or health. However, core microbial constituents remain, and are even likely to remain salient over time, leading to the characterisation of such microbes as good forensically relevant identifiers.

The forensic significance of microbiome analysis is influenced by temporal consistency and microbial dynamics. Microbiomes are not static; however, changes in microbiomes follow certain trends, and some microbial species linger long enough to connect individuals to items or places [11]. Additionally, the time shifts that occur in dead hosts' microbial communities offer potential to determine the postmortem interval (PMI), as the microbial shifts that occur with decomposition follow distinct stages that can be observed forensically. Therefore, the core microbiota's stability, combined with the orderly changes in microbiomes over time, support the conclusion that microbiomes are a multifaceted asset to forensic science.

Microbiome analysis has developed thanks to improvements in sequencing technologies. Of these improvements, 16s rRNA sequencing is still the most common, as it is able to classify bacteria on different taxonomical levels due to its sequencing of the more conserved regions of ribosomal genes [12]. On the other hand, shotgun metagenomic sequencing offers higher resolution for its sequencing of all the DNA within a given sample, allowing identification down to the species level as well as profiling of different functional genes. For profiling the microbial community, metatranscriptomics helps to identify the microbes that were active during sampling by sequencing the RNA rather than the DNA, thus revealing the transcriptionally active genes. Together, these sequencing technologies with the help of advanced bioinformatics algorithms allow the forensic analysis of microbial evidences.

3. The Concept of Microbial Fingerprinting

Microbial fingerprinting is possible because all humans have a unique profile of microorganisms present in and around them. Microbial fingerprinting is the identification and comparison of people using the unique profiles of the distinct microbiome communities present in the individuals [12]. These microbial communities are the result of diverse profiles of interaction among genes, foods consumed, and lifestyle including the social, environmental, and sanitary habits, and routines of the individual. The basis of microbial fingerprinting is founded upon the phenomenon through which people shed, and subsequently deposit microorganisms into their environments, leaving microbial footprints upon all the materials, objects, and surfaces, including clothing and electronics, they use. Over time, these microbial footprints build up onto the surfaces they have been deposited upon, and intellectually identifiable structures are formed which, with the use of modern microbial profiling and computational pattern recognition technologies, can be linked back to the original human source. In comparison to other forensic markers such as DNA and fingerprints in which the information and materials used to make the identification are static, microbial fingerprints are unique in documenting a history of the original source, and as a result offer extraordinary potential in forensic science.

The personalized powdered proteins customers receive are an example of the concept of individual specific signatures. The composition and abundance of microbial taxa within the microbiome surround a spectrum of individual specific signatures, despite anyone sharing a common microbiome. The fact that people within the same household, and thus the same environment, have completely identifiable microbiomes is counterintuitive. For example, the abundance and composition of a moisture microbiome is likely to be more at a statically significant difference from the neighboring individual of the same household than a person is from a completely different household, even if the individuals have similar occupations and personal activities [13]. This uniqueness to the microbiome composition is the ability to identify an individual. The robustness of microbiome signatures from different sites of the same body enables the value of variations of forensic microbial evidence to provide more than one site for the individual identification.

Microbial evidence can include skin-associated microbes and microbes from oral and salivary microbiomes; gut and fecal microbiota; environmental microorganisms; and microbes associated with various objects. Skin-associated microorganisms are one of the most easily accessed and sampled forms of microbial evidence, as skin microbiota are regularly shed via touching surfaces [14]. Saliva, including oral microbes, constitutes another high-biomass source of microbial evidence that can be recovered easily and analyzed from multiple drinking cups, bite-marked vessels, and other discarded containers. Gut or fecal microbiomes are linked to particular hosts and are present in cases involving defecation or other contamination scenarios. Microbial traces in soil, on shoes, and debris in fabric, as well as high-microbe residue on frequently-used surfaces, may reflect the person's microbial biota or a specific contact with their environment. These diverse sources allow microbes to be recovered for evidence forensic analyses even when traditional biomaterials are lacking or compromised, overcoming other typical challenges in forensic biology.

Persistence of microbes during human interaction with objects and environments is one of the most crucial aspects explaining the phenomenon of microbial fingerprinting. People touching surfaces and objects leave behind very small amounts of skin debris, oils, sweat, and microbes. Some environmental conditions, such as humidity, temperature, and composition of the surface, influence how long the microbes will persist [15]. For instance, microbes deposited on soft or textile surfaces stay longer than those on smooth metallic surfaces. It has been shown that the microbial communities on keyboards, door handles, bedding, and mobile phones are stable and reflect the identity of the current or past users. Additionally, transfer of microbes can happen without touching surfaces directly, e.g. via aerial transmission of skin microbes or respiratory droplets. Such patterns of transfer and persistence can be used as a basis for detecting unique microbial signatures and is useful in forensic applications.

Microbial fingerprinting has great potential for individual identification based on object-user contact. Evidence indicates that mobile phones, keyboards, mice, and clothing all harbor the skin-associated microbes of their users. For instance, one study found that microbiomes of smartphones and their owners could be paired with over 80% accuracy, demonstrating the active and consistent transfer of skin microbes to frequently used objects. Other personal items, such as keyboards, wallets, eyeglasses, and wristwatches, also contain microbial communities that demonstrate these relics have been closely used. In the absence of identifiable smudged or wiped fingerprints, this type of microbial evidence could be used to complement or substitute existing DNA evidence. For these reasons, microbial fingerprinting is an important tool for digital forensics, object-user attribution, and burglary detection.

In considering the strengths and weaknesses of DNA fingerprinting and microbial fingerprinting, it is vital to keep in mind the fact that, as of now, DNA profiling is the 'gold standard' in human identification, as it is the most specific, reliable, and reproducible method that is also accepted in a court of law. Still, in certain forensic contexts, the fragile nature of the environmental DNA (eDNA) that can be lost due to heat, sunlight, and moisture might limit its usefulness [16]. In contrast, microbial communities (microbiota) are more robust. The microbial DNA can even be extracted in circumstances where human DNA is either missing or in insufficient quantities. Moreover, microbiological evidence can furnish significant contextual insights that DNA evidence alone cannot, including details about an individual's health, lifestyle, or the precise place they last occupied. On the other hand, the precision and standardization of microbial fingerprinting (Figure 2) is still behind DNA profiling, and the flexible nature of microbial communities can lead to the sort of variability that requires more caution in interpretation. In this sense, although microbial fingerprinting is indeed promising, it is most useful as a complement to DNA evidence, rather than as a substitute.

4. Sources of Forensic Microbiomics Evidence

The entrepreneurial potential of microbiomics stems from the ability to collect microbial signatures from different human-associated niches. Each niche has its distinctive benefits and drawbacks. One of the most frequently encountered niches in forensic microbiomics is the human skin and contact microbiome. Microbial skin communities are found to be frequently and easily shed from the epidermal layer to the skin surface and shed onto objects passengers and users during skin contact and interaction [1]. Microbial skin sleating, or shedding during contact, is evidenced through friction and contact pressure. Research has shown that people can be differentially and individually profiled from the microbial communities shed onto keyboards, computer mice, mobile phones, and similar objects that are frequently and personally used or passed around. Microbial residues on these surfaces can be left from previous users for hours and even days, which makes these surfaces highly exploitable for forensic microbiomics in the absence of fingerprints, touch DNA or other users. Studies involving the forensic sample transport microbiota have shown that the skin microbiome and the user of the forensic sample transportation systems, can be matched, connecting the forensic microbiomic identifier to the transport systems touch microbiome for forensic identifier to the transport systems touch microbiome. The salivary and oral microbiome also provide a unique form of forensic microbial evidence. Microbial evidence from saliva is easily recovered and analyzed from bite marks, cigarette butts, chewing gum, bottle rims, and eating utensils, given the high microbial biomass in saliva [2]. The unique microbial community structures and functions in the oral cavity, shaped by eating habits, oral hygiene practices, and overall health, can create a distinct oral microbiome signature that varies with individuals and even population clusters. In forensic odontology, the microbial analysis of bite marks can assist in making complement morphological comparisons and help in properly attributing victims and objects to the suspects. Also, saliva found on weapons, articles of clothing, or other items recovered from a crime scene can help provide suspect leads, especially when other forms of DNA evidence are of poor quality or when there is a minimal amount of DNA evidence present.

Microbiomes of the gastrointestinal system, especially of the feces, offer a plethora of valuable forensic data, especially during the investigation of sexual assaults and homicides, or environmental contaminations. The gut microbiome is unique to each individual and remains unique over extended periods of time. This uniqueness enables gut microbiomes to be viable forensic identifiers. When feces is present at a crime scene, the assessment of its microbiome can establish a positive ID of the feces' contributor, and incriminate or exonerate a suspect, or provide the microbiome profile of the individual and valuable data on their dietary and health status [5]. Microbiomes of the gastrointestinal system and the dead body can also provide data for forensic investigations. Microbial translocation, and other processes of decomposition, enable estimation of the PMI. The gut microbiota continues to display predictable changes during postmortem, which can be utilized for forensic analysis.

The microbiomes within the vagina and the male reproductive tract are particularly useful for ethical investigations of sexual assault. Vaginal microbiomes, which are usually dominated by lactobacillus species, vary from person to person and may assist in the identification of contributors when traditional DNA evidence is compromised or mixed. Likewise, certain microbial taxa within the seminal microbiome can provide additional evidence for identifying male contributors [6]. Still, the specificities of the reproductive microbiomes pose ethical questions related to the sensitive data the microbiomes can provide. The variables of informed consent, privacy, and stigma that can arise from intimate microbiome data in forensic studies are issues that must be handled with great care.

One of the fastest growing areas of forensic microbiomics is the study of the postmortem microbiome, or thanatomicrobiome. After death, a body goes through a number of microbial successions brought on by the breakdown of soft tissues, the removal of immune activity, and the spread of anaerobic bacteria from the viscera. This microbial synthesis occurs through fairly predictable intervals that help researchers estimate the PMI with even greater accuracy. The microbiome analyses of the body's internal organs and cavities, or the soil under a body undergoing decomposition, can provide forensic pathologists with extremely useful data, especially when traditional PMI estimation techniques, like entomological or body cooling methods, fail [7]. The research focusing on the thanatomicrobiome continues to grow and refine our understanding of the processes of decomposition and provides even greater accuracy in the estimations of time of death.

5. Analytical Techniques and Bioinformatics Approaches

Microbial data critical to forensic analysis is only supported by strong methodologies across disciplines such as advanced bioinformatics. Microbial identification includes recovery and measurement, to avoid sample loss caused by injuries, while sediment layers are preserved without intrusion during forensics investigations. Microbial remnants such as decomposing bodies, soils, or surfaces, require nylon or cellulose compressed swabs, as well as sterile, non-corrosive gloves, and DNA-free containers [8]. Immediate sample stabilization is essential for the prevention of loss of DNA or other chemical traces from target microorganisms. Relying on the unique signatures will require thorough methodological approaches to ensure the preservation of integrity required for transport. Microbial communities can rapidly shift, especially at a crime scene with multiple people or environmental debris. The fading evidence of contaminants shifts the reliability of the evidence, and case solving skills are higher with more complex evidence.

Following the collection of the samples, the next important step is the extraction of the DNA, which is a process that requires more attention for low-biomass samples as opposed to the more routine, higher-biomass microbiome studies. Microbial forensic samples, as the name implies, usually have only trace amounts of DNA Extraction protocols must include highly sensitive kits capable of lysing both Gram-positive and Gram-negative organisms while minimizing DNA loss [10]. Environmental samples such as soil, fabric, and bodily fluids, can have a lot of secondary inhibitors that require careful sample purification to remove contaminants, and that purification step requires attention to ensure a minimal loss of sample yield. The microbial fingerprinting that can be performed is only as good as the extraction method that is used, and for these reasons, reliable extraction methods that have been optimized for low-biomass and degraded samples need to be developed.

Once DNA is isolated, forensic practitioners take advantage of a variety of DNA sequencing technologies, each with particular strengths. 16S rRNA gene sequencing is the dominant method of taxonomic profiling of bacteria, as it amplifies phylogenetically conserved regions that target specific microbial species [11]. It is relatively cheap and thus ideal for large comparative studies, albeit without functional resolution. Conversely, shotgun metagenomic sequencing interrogates all of the DNA in the sample and allows for the identification of microbial species and strains, functional genes, and metabolic pathways. It is a more detailed, albeit more computationally intensive approach. The third and most significant type is Nanopore sequencing, which facilitates real-time, portable, and thus field-friendly (including the crime scene or other remote locations) sequencing. Long reads that improve resolution to the species level and the speedy generation of results make nanopore sequencing particularly appealing to forensic practitioners.

The products of the sequencing technologies are in the form of raw sequences, which are subsequently analyzed by bioinformatics applications. These applications also allow for the classification of microbial communities, quantify the diversity, and determine the specific signatures. Rather, researchers, in the past, applied the Operational Taxonomic Units (OTUs) approach, which allowed for the grouping of sequences into clusters by employing the similarity threshold of 97%, which was the most accepted threshold. But as accuracy improved, the ASV (Amplicon Sequence Variants) approach was developed and implemented to determine individual sequence variants, But ASVs resolve exact sequence variants and do not involve clustering, which offers more accuracy [12]. Long, contiguous sequences can also be assembled from shotgun metagenomic datasets for functional purpose, that is, for the purpose of reconstructing the genomes of specific microbes or for the discovery of certain biomarkers. These applications are the core of microbial fingerprinting as they create biological sense from the raw sequences that were produced.

Initially, diversity measures, techniques, and community analysis are employed to examine the structure and composition of microbial communities. Measures of alpha diversity, such as the Shannon and Simpson indices, quantify microbial richness within a single sample; however, metrics of beta diversity (e.g., Bray-Curtis and UniFrac distances) are used to quantify and compare microbial communities among individuals or surfaces. Such comparisons are fundamental to determining whether a microbial trace from a crime scene shares a closer resemblance to a suspect's microbiome than to random background samples. PCoA (Principal Coordinate Analysis) and NMDS (Non-metric Multidimensional Scaling) are visualization tools to analyze patterns, cluster designations, and samples, and also discriminate between different organisms and environments. Microbial forensics has benefitted in the past few years from advances in machine learning and artificial intelligence, particularly for the purposes of pattern recognition and classification. Microbial differences that are subtle in nature can be detected and predictive models developed for the purposes of determining individual identity, object-user attribution, and even the geographic origin of a sample using random forest, support vector machine, convolutional neural networks, and deep learning models [13]. Microbial succession patterns in postmortem samples also improve accuracy in determining the PMI. With the growth of datasets, the microbial fingerprints in forensic investigations will largely depend on machine learning.

Lastly, the analytical workflow is founded on dependable reference databases for taxonomic and functional annotation. Systems like QIIME (Quantitative Insights Into Microbial Ecology), MG-RAST (Metagenomic Rapid Annotation using Subsystem Technology), Kraken, MetaPhlAn (Metagenomic Phylogenetic Analysis), and the SILVA rRNA gene databases offer tailored microbial sequences which support precision in identification [1]. These databases are still expanding through global input, however, there are still issues pertaining to completeness, geographic representation, and strain-level resolution. These databases need to be enhanced in order to standardize microbiome-centric forensic studies.

6. Current Forensic Applications and Case Studies

Evidence of practical forensic microbiomics has been advancing quickly due to several studies focused on discerning how microbial communities differ between individuals, how they persist on surfaces, and how they change over time. Research has evidenced that individuals leave identifiable microbial signatures on their environments, and these signatures can be detected with high accuracy [2]. Even in experimental settings where environmental control has been emphasized, it has been shown that microbial communities harbor temporal information concerning how long a given object was in use, or how long a decomposition event had been taking place.

Besides other, less relevant applications, there is no doubt that the analysis of microbiome traces on personal devices like phones, laptops, and keyboards, is the most developed one. These devices acquire and retain stable microbial communities that are characteristic of the device owner [5]. Forensic microbiome studies have demonstrated that biomedical keyboards can be microbiome profiled, and the users of the keyboards correctly identified with over 70-80% accuracy. Moreover, mobile phones contain and preserve rich microbial communities due to their constant skin contact and exposure to the surrounding environment, making them significant forensic materials. Microbial evidence becomes especially useful when fingerprints or DNA have been absent or intentionally omitted, as it opens avenues for identifying individuals or reconstructing the interactions that have taken place between users and objects.

Another important area of application is the estimation of the PMI based on microbiomes. During postmortem microbial succession, there are phases of predictable events where aerobic and anaerobic bacteria colonize the tissues, decompose, and interact with the other microbes in the environment. Through the sequencing of the microbial populations in the organs, the skin, and the surrounding soil, forensic scientists have developed models to estimate the time since death [6]. These methods aid traditional PMI estimates and are particularly helpful in the absence of entomological evidence or when the environmental conditions make it difficult to visualize and the body undergoes physical changes. The forensic microbiomes have also extended to tracing soil and environmental microbiomes where it is possible to link individuals or items to particular places. Soil microbiomes differ from one place to another, and this helps to determine the geographic origin of soil found on shoes, clothes, or vehicles. Microbial ecosystems found in water, artificial and natural enclosed spaces, and agriculture also aid in providing geographic information that helps to reconstruct a crime scene [7]. In a more specific area, the analysis of microbiomes has also been combined with microbial forensics in cases of bioterrorism. When there is a malicious use of biological agents like Bacillus anthracis (anthrax), microbial genomics can determine the strain, the phylogenetic history, and the source of the agent. With advanced sequencing technologies, legal authorities can attribute biological threats by matching the genomes of pathogens to reference genomic databases and tracing the pathogens by microbial genomics for specific threats. There continues to be difficulty with reproducibility and admissibility of evidence from legal forensic microbiomics, notwithstanding the increasing potential in this area [8]. Absence of uniformity in sampling, sequencing, and analysis continues to produce variability in outcomes. Microbiome evidence is still regarded as inadmissible in court. For establishing reproducibility, Robinson validation studies will be required to allow microbial evidence to be used in court.

7. Challenges and Limitations

Despite the advances being made in the field of microbial forensics, there are numerous obstacles that continue to impede the field's progress. One of the most impactful of these obstacles is the environmental and temporal variability that characterizes most microbial communities. Microbial communities are variable at the level of the community, population, and species to changes in humidity, temperature, pH, and UV light, and this variability is made worse by the rapid growth of certain microbial organisms. To the untrained eye, this phenomenon could be interpreted to be a unique environmental signature to an individual, yet in reality, it is an individual microbial pattern that changes with diet, mode of living, and environment, making it limited and unstable identifier.

Another major issue is sample loss due to degradation and contamination. Microbial samples are small and dynamic and are easily contaminated by the sample collector, a bystander in the area, or microorganisms that are already in the surrounding environment. A sample's degradation can occur relatively quickly, especially if it is stored in an environment that is high in temperature, has prolonged UV exposure, and is barren of moisture. Furthermore, there is minimal availability of standardized collection protocols. With no agreed upon workflows, it is impossible to achieve meaningful and valuable collaborations between laboratories, which ultimately leads to a loss of reproducibility. A further challenge in the field is the reliance on high computing resources, the need for advanced and sophisticated skilled expertise, as well as the use of custom specified devices. However, high inter-individual similarity and misclassification can occur, especially in individuals with analogous patterns of cohabitation and shared living spaces. Microbial forensic signatures, while not always unique enough to meet forensic matching thresholds. To conclude, ethical, privacy, and legal issues arise from the boundless nature of microbial data, which can expose information regarding people's health, behaviors, and personal surroundings. Creating accurate databases also has several challenges, including the need for diverse, representative data, and long-term sustained effort.

8. Future Prospects and Research Directions

In spite of the present constraints, the integration of multi-omics (i.e. proteomics, metabolomics, transcriptomics, genomics) in forensic microbiomics will result in more complex and comprehensive forensic signatures. Integrated datasets will provide better estimations in PMI, individual pinpointing, and in environmental sampled tracing. Deep learning and AI (Artificial Intelligence) will analyze complex datasets in multidimensional forensics. Advanced neural networks will identify and classify microbes with greater accuracy from subtle patterns which is a limitation of classical statistical approaches. An encompassing goal is the creation of global microbial reference databases with precise geographic, ethnic, and environmental diversity. This will facilitate precise microbial attribution and the corroboration of worldwide crime. There is a need for standardization for greater legal acceptance which will integrate validated methodologies, pathway accreditations, and promote judicial training. Cold case analysis and digital forensics, tracing of environmental crime, identification of missing persons, and international security efforts will benefit from the expanded future applications. Collaboratively, microbial forensics can be established as a legally recognized global scientific and legal discipline.

9. Ethical, Legal, and Social Implications (ELSI)

Including microbiome analysis in forensic science raises ethical, legal, and social concerns that must be resolved in order to be done responsibly. The human microbiome contains personal and sensitive information, such as health and lifestyle records, places where a person has lived, and how they behave. This information is almost as sensitive as genetic data, and even less is done to protect it. The crucial issue here is that subjects dont have to be aware that microbial remnants left on surfaces could be a means of uncovering their identity or traced to them as part of a crime scene. Consequently, the data concerning these microbiomes must be regulated to minimize the risk of classification, data sharing, or data analysis discrimination. Moreover, the possibility of misclassification or overinterpretation of obtained microbiomes raises concern and should be addressed in future regulations to ensure that there is a clear, transparent, and comprehensive consideration of methodologies, as well as that the data obtained contains information, if left unattended, could infringe an individual's privacy. Defining regulation is key to cutting down the potential that forensic microbiomics will infringe privacy or civil liberties and will serve to the aid of scientific advancement.

10. Conclusion

The enormous potential of forensic microbiomics has to advance the contemporary forensic science. Throughout this thesis, we have explored the enormous potential of offering unique microbial signatures on an individual level, enhancing and extending the utility of DNA evidence, as well as presenting novel applications such as estimating the postmortem interval and tracing the environment. As is the case of any novel field, microbial forensic science also has its weaknesses that involve high degrees of variability, contamination, and lack of standardization as well as ethical issues. The potential incorporation of microbial evidence into the forensic science system entails the need for the completion of validation studies and the creation of reference standard systems. The emergence of forensic science microbiomics is indeed a radical departure from the sole reliance on DNA forensics and has the potential to open new frontiers in science. However, the rise of forensic microbiomics also calls for exceptional custodianship of ethics in order to prevent misuse and provide socially beneficial outcomes.

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