

Role of Microbial Forensic in Homicide Investigation: A Review

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Abstract

Microbial forensics, positioned at the intersection of forensic sciences, legal inquiry and microbiology, has emerged as a transformative discipline in modern homicide investigation. This review explores the growing utility of microbiological evidences in cases where conventional forensic methods may fall short due to deterioration, contamination, or the absence of human DNA. The study focus on the microbial communities, particular in the epinecrotic and thanatomicrobiome in determining postmortem interval (PMI), geolocation of a crime and trace suspect-victim contact. Advanced molecular techniques such as 16S rRNA gene sequencing, metagenomic profiling, and quantitative (qPCR) enables the detection of microbial traces in bodily fluids, hair, fingerprints, and decomposing tissues. The review further highlights the forensic applications of microbial signatures in analyzing body movement, victim and suspect identification, and crime scene reconstruction. Limitations regarding the sample collection, environmental variability, and legal admissibility are critically assessed. Moreover, the integration of the artificial intelligence and machine learning is discussed as a promising avenue for enhancing the interpretation of microbiological data. The study focuses on the needs of standardization, interdisciplinary collaboration, and the development of robust creation of forensic microbial databases to harness the full potential of microbial forensics in solving complex homicide cases.

Keywords: Homicide, Thanatomicrobiome, Postmortem interval (PMI), Forensic sciences

1. Introduction

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A subfield of epidemiology known as "microbial forensics" focuses on defining microorganisms and determining their strain rather than just their species. Finding the specific details required to identify a microbe that might be used as evidence in court was the goal (Atlas, 2004). In actuality, the results and analysis of microbiological forensics can be used to definitively identify the source of a particular biological incident when combined with other types of direct and indirect evidence. In the context of investigations, prosecutions, and attributions, microbial forensics assumes particular significance. It can help identify the contributing elements to the incident and absolve falsely accused people, groups, or nations of any responsibility. Microbial forensic evidence must be assessed through a number of non-scientific frameworks and perspectives, including religion, medicine, law, and culture, before it can be used in policymaking or legal adjudication procedures (Murch et al., 2020).

Comparison studies, which compare evidence gathered from a crime scene with evidence gathered elsewhere, are a major tool used by forensic experts. Locard's exchange principle, which asserts that material is transferred from one object to another when two come into contact, serves as the foundation for these comparisons. Furthermore, among other things, forensic microbiologists can employ trace evidence to identify individuals at crime scenes, look into bioterrorism incidents, and ascertain the reason and timing of death (Aggarwal et al., 2014). In court, the results of microbiological forensic studies are frequently used to prove or disprove an accusation. Thus, to guarantee optimal sensitivity and clarity, thorough and unbiased testing is essential. To obtain precise and objective results, impartial mechanisms must also be in place (Castro et al., 2022). The microbiome and thanatomicrobiome, which are colonies of microorganisms that reside both within and outside of a decomposing corpse, are studied by researchers in the field of forensic microbiology. Particularly in the calculation of the post-mortem interval (PMI), shifts in the species makeup of these communities offer important insights about the crime (Speruda et al., 2022).

The deliberate and illegal killing of one person by another is known as homicide, and it remains a major issue in the criminal justice system. In homicide investigations, meticulous forensic evidence gathering and analysis are essential (McEwen & Regoeczi, 2015). With classifications that take into account the complexities of human behavior and purpose, homicide is a complicated legal and social matter. According to Luebering (2010), legal systems seek to strike a balance between the pursuit of justice and an awareness of the circumstances and driving forces behind each homicide case.

2. Microbial Communities and their role in Forensic studies

The human body, soil, water, and decomposing matter are just a few of the numerous populations of microorganisms known as microbial communities, or microbiomes. These groups are important to forensic science, especially when it comes to postmortem examinations. The term "thanatomicrobiome," which describes the microbial communities linked to internal organs after death, is one of the most researched microbiomes in forensics. For example, because of their anaerobic metabolism and tissue-degrading enzymes, bacteria like *Clostridium* spp. dominate the thanatomicrobiome and play a crucial role in the breakdown process (Javan et al., 2016). The epinecrotic microbiome, which is made up of microorganisms on the surface of decaying remains, is

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another significant microbial community. These include organisms of *Bacillus*, *Pseudomonas*, and *Staphylococcus* that can be used to estimate the postmortem interval since they change reliably over time (PMI) (Pechal et al., 2014).

The microbiome of an individual varies from one section of the body to another and can reveal personal traits. This uniqueness gave rise to the idea of a "microbial fingerprint," which may be able to connect a suspect to an item or crime scene. Propionibacterium and Corynebacterium-dominated skin microbiota, for instance, can be extracted from surfaces and compared for forensic identification (Fierer et al., 2010). Using bacteria from leftover skin or hair on objects, microbial community variations can be utilized for forensic identification (Kim et al., 2020).

Microbial communities vary by host health, race, and body place, according to research on the human microbiome *Corynebacterium*, *Staphylococcus*, and Propionibacterium are discovered on the skin; *Clostridium* and Mycobacterium species are found in the stomach; *Lactobacillus* and *Haemophilus* species are found in the oral cavity. Every country has its own hand microbiota. Zhang et al., (2023) claim that while Americans have *Streptococcus* and *Propionibacterium* and Koreans and Japanese have less of either, Canadians carry *Bacillus*, *Streptococcus*, and Propionibacterium.

Direct contact between people or surfaces during a homicide can spread skin microbiomes, which are dominated by germs including *Propionibacterium acnes*, *Corynebacterium* spp., and *Staphylococcus epidermidis*. This transfer can assist link a suspect to a weapon or crime scene, or it can help establish physical interactions between the victim and the attacker (Fierer et al., 2010). Similar to a microbial fingerprint, investigators have occasionally been able to link skin microbial DNA obtained from objects or surfaces to certain people. In forensic settings, the oral microbiome which includes a varied community of bacteria like *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Streptococcus mitis* can also be important. For example, microbial DNA left on the victim or in the mouth cavity after bites, saliva, or smothering can be examined for both microbial and human components. This is especially helpful when there is insufficient or damaged human DNA. Based on microbial infiltration and inflammation, oral microbial profiles can also be used to differentiate between antemortem and postmortem wounds. In breakdown and PMI assessment, the gut microbiome which includes bacteria such as *Bacteroides*, *Escherichia coli*, and *Clostridium* spp. is crucial. The process by which gut bacteria move to nearby tissues and organs after death can be monitored to establish the exact moment of death. The predictable progression of these bacteria can assist in determining the time of death in homicide instances where the body is found after a delay. (Javan et al., 2016). In homicide-related sexual assault instances, vaginal and rectal microbiomes might be important. According to the Human Microbiome Project Consortium (2012), the vaginal microbiome, which is normally dominated by *Lactobacillus* species, can be disturbed and replaced with foreign microbial DNA, possibly indicating trauma or sexual intercourse.

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Table 1:- Application of forensic sample

APPLICATION	DESCRIPTION
PMI Estimation	Microbial succession on decomposing remains can provide time-of-death estimates
Trace Evidence	Microbiome signatures can serve as biological trace evidence
Geolocation	Microbial fingerprints from soil or water can link a body or object to a geographic location
Personal	Individual-specific microbiomes may eventually support personal identification in forensic cases

3. Microbial Evidences in Postmortem Estimation

Determining an agent's microbial signature is a primary use of forensic microbiology that is also employed in outbreak investigations. Similar to a fingerprint, this can enable tracking back to the original source. When the body is decaying, forensic microbiology also becomes relevant. It can be helpful in estimating the time of death, which might range from two to four days to as long as twenty-five days. This also holds true for fatalities that take place in less than optimum circumstances, including extremely hot or cold weather. In Blondeau et al., 2019 before samples arrive at the lab, they must be identified, collected, handled, and preserved to prevent interfering with later tests. Maintaining signatures in the sample after it is taken from the crime scene is a challenge. In addition to supplying sufficient material for microbiological forensic analysis, evidence collecting protocols should be designed to preserve conventional forensic evidence, such as hair, fibers, fingerprints, and human DNA, if at all possible. Trace proteins, nucleic acids, and other signatures are difficult to extract and purify from materials. Although there are several instruments and processes, such as swabs and high-efficiency particulate air (HEPA) vacuum filters, very few have undergone thorough validation, particularly in relation to extraction efficiency (Walters et al., 2005).

3.1 Hair - Hairs are frequently found during a crime investigation and have been shown to be a significant source of DNA that aids in determining the extent of the crime. The hair bulb, which is made up of living cells that produce the keratin shaft, is one type of hair. DNA is present in the bulb cells. The suspect and the weapon and the murder scene may be linked with the use of this DNA. Additionally, if it is discovered on the corpse of the accused or deceased, it offers compelling proof.

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The primary responsibility of a crime scene investigator is to conduct a comprehensive examination of the scene and gather all relevant evidence in order to solve the crime. While doing the autopsy, medical officials conducting the postmortem also have the crucial responsibility of gathering all evidence found on or within the deceased's body. In order to solve any crime case, it is crucial that evidence be properly gathered, preserved, and submitted on time. Sample deterioration and the loss of crucial evidence could result from noncompliance with any of these requirements. Furthermore, it could provide difficulties while establishing the case in court. An individual's gender and genetic information can be obtained by DNA profiling of the evidence gathered. It is possible to determine whether hair has been chopped or pulled out in a particular way by examining its morphology. A single hair's DNA content is frequently below the ideal level needed for nuclear DNA markers. (Mahajan et al.,2019)

3.2 Fingerprint - Because fingerprints may be obtained from a wide range of evidence types, including weapons handles, equipment gathered in criminal cases, and things that don't appear to be stained, they can be extremely valuable for forensic biology. The quality of the generated STR profiles is ultimately impacted by the wide variations in the quantity and quality of DNA extracted from fingerprints. When fingerprint samples have varied STR characteristics as a result of handling different people, additional challenges may occur. After employing a tried-and-true method for PCR, DNA extraction (using an enzyme that functions at high temperatures), and sample collection (swabbing with 5% Triton X-100), amplification With the ultimate goal of creating valuable profiles ($\geq 50\%$ complete), a thorough analysis was conducted to gain a better understanding of the difficulties associated with fingerprint samples. Investigations into the effects of time on deposited fingerprints showed that, even while profile quality declined, samples may still yield complete STR profiles after 40 days of room temperature storage. We discovered a little higher quality from the non-dominant hand when comparing the STR profiles of the dominant and non-dominant hands, albeit this difference was not always noticeable. Fingerprints appear to be more affected by substrates. Tests on typical substrates used in homes and workplaces, such as glass, plastic, and paper, revealed that glass produced the greatest results, followed by plastic and paper, while virtually no profiles were found from a Quarter dollar. (Ostojic & Wurmbach., 2017).

3.3 Body fluids: Blood, menstrual blood, semen, saliva, vaginal secretions, and skin are among the body fluids and tissues which are of relevance to forensic research. Any test for their location and identification should be sensitive, simple to apply at the crime scene or in the lab, unique to the bodily fluid and, ideally, to the species (human), and nondestructive so that DNA profiling can be used for further study. Tests must be able to function properly on a range of substrates since bodily fluids can be deposited on a number of surfaces. With the advent of new multiplexes, DNA profiling has grown more sensitive, and complementing body fluid identification technologies are required. (Harbison & Fleming, 2016).

Recent research has proposed the human microbiome as a practical forensic technique for goals including body site classification, body fluid identification, and post-mortem interval (PMI)

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computation. Especially well researched has been the human oral microbiota. Earlier studies reveal that behind the gut, the microbiota of the mouth cavity is the second-biggest and most diverse bacterial population in the human body. Saliva samples are commonly used in criminal cases. According to reports, the saliva microbiome may be used to determine a person's identity and geographic location (Lan et al., 2025). Using presumptive and/or confirmatory tests based on chemical, enzymatic, immunological, spectroscopic, or microscopic techniques, forensic body fluid/tissue identification is carried out. Since many of these techniques have shortcomings in terms of sensitivity, specificity, or both, forensic researchers are now investigating new strategies based on markers unique to human tissue, such as mRNA, microRNA (miRNA), or methylation indicators. Because of their widespread presence at human body sites and their distribution patterns, bacterial markers have been proposed as a possible new technique for body site identification in recent years. Although there is variance across various body parts and individuals, bacteria are thought to outnumber human cells generally and are present throughout the human body. However, these figures imply that even in cases when human DNA or RNA is damaged or has low copy numbers, bacterial DNA from bodily places may still be detected. (Dobay et al., 2019).

4. Microbial Forensic at Crime Scene Investigation

In forensic science, microbes are increasingly being used as useful tools, especially in crime scene investigations (CSI). Finding and examining microbes on people, at crime scenes, or on associated artifacts is the focus of microbial forensics. These bacteria aid in the reconstruction of events, the establishment of timeframes, the identification of individuals, and the connection of suspects to scenes.

4.1. Crime Scene Documentation and Sample Collection

Thorough sample collection and recording are the first steps in microbiological forensic investigation. To preserve microbiological evidence, investigators gather swabs from a variety of places, including skin, clothing, weapons, door handles, and soil. To avoid contamination, sterile swabs, gloves, and transport media are utilized. To enable comparison and control, samples are usually taken from the crime scene as well as the victim. For instance, contact may be indicated if the microbiological signatures on a suspect's hands and a weapon match (Fierer et al., 2010).

4.2. Sample Preservation and Transport

Since microbial communities can be impacted by environmental variables including temperature, humidity, and time, proper preservation is essential for microbiological evidence. To preserve the integrity of microbial DNA for further analysis, samples are kept in environments that inhibit microbial growth or degradation, frequently in cold-chain systems or preservation buffers (Burcham et al., 2019).

4.3. DNA Extraction and High-Throughput Sequencing

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Standardized procedures are used to harvest microbial DNA once it is in the laboratory. High-throughput sequencing techniques like metagenomic sequencing or 16S rRNA gene sequencing next help to identify and characterize the microbial populations present. Categorizing and differentiating among several kinds of bacteria, the 16S rRNA gene a conserved area of bacterial DNA is used. Furthermore giving a more thorough view of microbial presence, metagenomic techniques allow the detection of viruses, fungi, and archaea in addition to bacteria (Metcalf et al., 2013). A preliminary screening for the presence of biological material usually precedes the analysis of biological evidence. Chemical analysis, immunological assays, protein catalytic activity testing, spectroscopic techniques, and microscopy are frequently used to identify bodily fluids. Tissue-specific DNA methylation, proteomics and microbial analysis & mRNA profiling are examples of new techniques. Studies have demonstrated that the relative degradation patterns of particular RNA transcripts may be used to assess the age of blood stains, and that mRNA degradation levels vary dramatically among samples of different ages (Salzmann et al., 2021). At crime scenes, it's critical to separate microbial DNA from other biological evidence, particularly in situations involving sexual assault. Next-generation sequencing is used by forensic analysts to distinguish between samples and examine the vaginal microbiome. Body fluids can be identified using mRNA, miRNA, and epigenetic markers such as GpC island methylation. Although there are particular indicators for fluids like blood and semen, these techniques may not be very specific. Although miRNA stabilizes samples, it might not accurately identify a particular fluid. The accuracy of forensic results is increased when various methods are combined (Gouello et al., 2021).

4.4 Microbial Profiling and Comparative Analysis

Microbial profiles are produced by researchers using bioinformatics techniques to characterize the variety and quantity of microorganisms present in each sample. To find similarities or clear differences, these profiles are compared across several samples, such as those from the crime scene, victim, suspect, and environment. Evidence of touch or presence at the scene may be supported when microbial profiles match, such as when the skin microbiota of an object and a victim is identical (Lax et al., 2015). Conventional immunological and enzymatic techniques frequently lack sensitivity and specificity and demand a lot of sample material. Even while using endogenous biomarkers, like DNA methylation and microRNA, has potential for discriminating biological materials, there are still many obstacles to overcome, especially when dealing with low template or differentiating between vaginal and saliva. Additionally, human cells are frequently found at crime scenes in very small amounts or may not be detectable due to the development of counter-detection tactics used by criminals, which reduces the efficacy of human cell-based detection approaches. On the other hand, microbes have demonstrated their significant utility in human health research and are progressively becoming biomarkers in forensic medicine due to the ongoing developments in high-throughput sequencing technologies.(Lei et al.,2025).

4.5. Postmortem Interval (PMI) Estimation

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Microbial succession, or the anticipated change in microbial communities after death, can be used to determine the postmortem interval. At different stages of decomposition, some bacteria—including *Bacteroides* species, Enterobacteriaceae, and *Clostridium* species—predominate. According to Pechal et al. (2014) and Javan et al. (2016), these microbial shifts can be traced over time and act as a biological clock to calculate the time since death. Some of the traditional methods for determining the TOD (late and early PMI) include temperature-based processes, electrical stimulation, rigor mortis, ocular changes, decomposition, histology, as well as entomology, environmental, and related data. However, novel approaches for early PMI assessment have showed promise thanks to modern forensic science tools including pH measurements and RNA or DNA degradation assays. By using degradation principles that rely on the amount of time that has passed since the TOD in biological markers, these components are examined to ascertain the precise PMI. Basic methods including spectroscopy, immunohistochemistry, electrophoresis, and flow cytometry have become indispensable instruments for determining PMI, which is used to assess the time since death. The amalgamation or combination of several approaches is applied to each method separately in order to minimize the margin of error introduced by a single technique. In this age of forensic intelligence, the efficacy and adaptability of artificial intelligence and machine learning have made them an important tool for evaluating PMI (Singh et al., 2025).

4.6. Geolocation and Trace Evidence

Because climate, rainfall, altitude, soil, and environmental energy sources all affect the composition of microbiota, knowing which bacteria are unique to a given area could help associate a person or object with that location (Haarkötter et al., 2021). Analyzing environmental trace evidence is a useful method for forensic investigations, especially when attempting to profile an unidentified place of forensic interest or compare and exclude materials from crime scenes and suspects. Numerous environmental markers, including as pollen, fungus, diatoms, microbial communities, and soil quartz grains, have been regarded as trace evidence. Environmental material analysis is regularly documented in casework and is becoming more and more prevalent in empirical research, with an emphasis on persistent dynamics and evidential transmission. The development of strong frameworks necessary to enable forensic interpretations within the criminal justice system depends on such experimental methodologies (Stevens & Scott, 2025). Soil microbiomes are distinct to particular habitats and are extremely localized. It may be possible to identify a person at a crime scene if dirt from their shoes or clothes matches the soil microbiota there. Environmental microbiomes have been used to test this strategy, which may be used to connect people to certain regions (Truong et al., 2017).

4.7. Courtroom Interpretation and Validation

Microbial forensic evidence must satisfy legal requirements for scientific relevance and dependability in order to be admitted in court. This covers expert interpretation, reproducibility of outcomes, and procedure validation. Creating reliable protocols and standard operating procedures

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is a continuous endeavour because the area of microbiological forensics is still in its infancy (Budowle et al., 2008).

5. Microbial Profiling in Forensics

In forensic science, microbial profiling examines the microbial communities connected to crime scenes, victims, or evidence using molecular techniques. In addition to providing vital information like the time since death and links between people and places, this enables forensic investigators to identify bacteria and fungi.

Targeting a conserved gene in bacterial DNA with hypervariable sections, 16S rRNA gene sequencing is one of the most used techniques for taxonomic identification down to the genus or species level. This method can help with postmortem interval (PMI) estimation and is notably useful in forensic microbiology for defining bacterial populations involved in decomposition, particularly the thanatomicrobiome (Pechal et al., 2014). Certain gene areas are amplified by 16S rRNA sequencing (Marcos-Zambrano et al., 2023).

For fungal communities, researchers frequently use Internal Transcribed Spacer (ITS) sequencing, which looks for varied areas of fungal DNA. This technique can help with understanding environmental context or contamination and is helpful in forensic circumstances involving soil or surfaces where fungus are common. Although ITS and 16S sequencing are both very useful for taxonomic profiling, they are only applicable to fungi and bacteria, respectively. All of the DNA in a sample can be sequenced using the thorough method known as metagenomic shotgun sequencing. This gives an Overview of the microbial community by enabling the detection of bacteria, fungi, viruses, and archaea in addition to the functional genes they contain. In complicated forensic samples with significant microbial diversity, such soil, decaying tissue, or interior habitats, metagenomics is very helpful.

Quantitative PCR (qPCR) is used when it is required to quantify particular microbial species. This technique is very helpful for identifying recognized microbial indicators in decomposition or contamination investigations and enables the focused amplification and quantification of particular microbial DNA sequences. qPCR is perfect for tracking microbial loads in bodily fluids or tissues during forensic investigations since it is quick, sensitive, and specific (Finley et al., 2015).

Past use of T-RFLP in forensic microbiology studies involves breaking down PCR-amplified microbial DNA with restriction enzymes and measuring the lengths of the resulting fragments. This technique offers a quick summary of community diversity and has been used to track microbial changes throughout decomposition, although being less accurate than sequencing.

In forensic microbiology, microarray-based profiling is useful, especially when examining environmental samples or identifying pathogens linked to bioterrorism. DNA microarrays enable the simultaneous and high-throughput identification of numerous taxa by using particular probes set on

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a chip to detect hybridization with microbial DNA from a sample. Its use is restricted to known organisms found in the probe database, though (Wilson et al., 2002).

Next-generation sequencing technology has the potential to completely transform forensic microbiology by giving researchers the ability to determine the genetic makeup of microorganisms with previously unheard-of speed, accuracy, and depth (Oliveira et al. 2024). This makes it invaluable for a variety of applications. The study of the human gut microbiota has been transformed by next-generation sequencing (NGS), which makes it possible to identify bacteria with previously unheard-of precision. Rapid sequencing of genomes and cost-effective data generation provide opportunities for clinical translation (Shahrokh, S. et al., 2020).

6. Microbial Traces in Decomposition

Numerous biotic and abiotic elements, including weather, season, scavengers, and bacteria, influence decomposition, which sustains a diverse spectrum of creatures and adds to the ecosystem's distinctiveness. The necrobiome, a collection of interacting and interrelated species that includes bacteria, fungus, vertebrates, and invertebrate scavengers, supports the entire decomposition process (R Wang et al., 2021). Since microorganisms are ubiquitous and take part in all stages of decomposition, they are crucial to the research of death. Microbial communities facilitate the breakdown of organic materials by aiding in putrefaction, bone diagenesis, and translocation. (D. O. Carter, 2020) The term "Thanatomicrobiome" describes the post-mortality microbial community, which is essential to comprehending the phases and chronologies of decomposition. The postmortem interval can be inferred from changes in these microbial populations. Forensic investigators can ascertain the time of death and associated conditions by examining microbial traces. The precision of these estimations is increased by sophisticated methods like as gene sequencing, underscoring the significance of microbiology in forensic research (Bucheli et al., 2020). The body changes in a number of ways after death, and these changes can be used to calculate the period since death. Determining the post-mortem interval (PMI) is essential in forensic science in order to look into illegal conduct and assess the legal ramifications. It has been suggested that PMI can be estimated from changes in the bacterial community during putrefaction. The body's natural defence deteriorate with death, allowing microbes from the microbiome to proliferate and invade previously unreachable regions. The microbial populations undergo a predictable series of changes as the body breaks down. These communities are analysed in the field of study known as thanatomicrobiome (Zapico, S. C., & Adserias-Garriga, J., 2022). A model known as the "microbial clock of death" was created utilizing microbiome information gathered from postmortem samples with known postmortem intervals (PMIs), like a skin swab. . A similar sample, such a skin swab, would be gathered for a death investigation, and DNA sequencing would be used to examine the bacteria. The regression model would then match the resulting microbial profile to a point on the microbial clock (Metcalfe, J.L., 2019).

Dead animals are recycled by a wide variety of decomposers, who use them as nutrients. Fresh, Active Decay (Bloating and Rupture), and Advanced Decay are the three stages of decomposition that are recognized by forensic experts. Numerous biological changes are linked to the decomposition

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process, including changes in the biochemistry of the soil surrounding the body, the growth rate of blow fly larvae, and the succession of insects. These changes can be used to estimate the post-mortem interval (PMI) However, it is important to note that no single method can be successful in all situations (Gonzalez et al., 2013). Microbial communities change during decomposition, providing a guide for PMI inference from non-boiled cadavers. However, temperature affects microbial communities, and the succession of communities may differ during decomposition of boiled cadavers (Yan et al., 2023).

7. Microbial Evidences in Identification of Victims and Suspects

In forensic science, microbial evidence is becoming more and more popular for identifying suspects and victims. Microbial signatures from the various microorganisms in and around us provide distinct and dependable patterns, in contrast to conventional identifiers like fingerprints or DNA. These can be used to establish presence at a crime scene, trace contact, and confirm identity. Two categories of evidence exist: 1. Direct evidence: Confirms a fact without additional research. For instance, comments made by victims or eyewitnesses may contain errors. 2. It is necessary to identify and compare circumstantial evidence to a control sample. Although it is more objective than direct evidence, it still needs to be handled carefully to prevent loss, contamination, or destruction. It can be biological (like DNA or botanical components) or physical (like fingerprints or shoe impressions) (Magalhães, 2015).

The examination of postmortem microbial communities is one of the primary uses of microbiological evidence in victim identification. Microbial succession after death occurs in a largely predictable order, with particular bacterial species predominating at different phases of decomposition. As the body breaks down, for instance, Clostridium species, Bacteroides species, and Proteobacteria often multiply, especially in the gastrointestinal tract. The postmortem interval (PMI), which is essential for identifying unidentified victims and reconstructing the chronology of death, can be estimated using this change in microbial communities, sometimes referred to as the thanatomicrobiome (Javan et al., 2016). Furthermore, the epinecrotic microbial population, which grows on the skin and interacts with the environment after death, offers hints regarding the location, movement, or exposure to the environment of the deceased. Forensic specialists can ascertain whether a victim was transported postmortem by looking at the microbial community on the body's surface, which may indicate environmental changes if the body is moved after death (Metcalf et al., 2016).

Using microbial trace evidence, microbial forensics also makes it possible to identify or rule out suspects. Every person has a distinct microbiological signature, especially on their skin, which can be passed from one person to another or to items or surfaces through physical contact. The potential of microbial "fingerprints" for individual identification has been demonstrated by research that has shown that frequently touched objects, such as cell phones, keyboards, or door handles, retain microbial communities that closely resemble those found on the person's skin (Fierer et al., 2010). Skin bacterial communities recovered from keyboards highly accurately matched their users.

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Environmental linkage and geolocation can benefit from microbial evidence. The presence of dirt or dust particles on a suspect's clothing or car can be strongly suggested if they match the microbiome at a crime scene because soil and ecological microbiomes are highly localized. Regional variations in soil microbiomes have been demonstrated by studies, underscoring its potential use in forensic geolocation (Truong et al., 2017). Individualizing microbiomes for the purpose of identifying suspects is another exciting use. Research is still being conducted to determine whether microbiome profiles are stable enough to function as distinct identities, much like fingerprints. According to longitudinal research, some individual characteristics of the microbiome stay comparatively constant and discernible across months or years, even while the microbiome itself changes over time (Franzosa et al., 2015).

During forensic investigations, it can be difficult to determine whether suspects are connected to homicides. Although biological materials such as blood, semen, and saliva are frequently collected, it is not always possible to get high-quality DNA (Karadayı, 2021).

Molecular Approach

A molecular approach called random amplification of polymorphic DNA (RAPD) uses short, single primers of any sequence to amplify random genomic DNA segments. It is used to identify genetic polymorphisms and doesn't require any prior knowledge of the DNA sequence. RAPD has been used in microbiological forensics to differentiate between various strains or isolates of bacteria or fungi, and it can assist in forensics by identifying individuals based on their distinct DNA profiles. (William's et al., 1990).

An extremely sensitive DNA fingerprinting method for identifying genetic variation throughout the entire genome is amplified fragment length polymorphism (AFLP). This technique involves ligating particular adapters to the sticky ends of the DNA fragments after genomic DNA has been initially broken down by restriction enzymes. Primers corresponding to the adapters and neighboring sequences are then used in PCR to selectively amplify these fragments. Gel or capillary electrophoresis is used to separate the resultant amplified fragments, giving each sample a distinct banding pattern (Vos, et al., 1995).

By examining differences in the length of terminal restriction fragments of amplified genes, usually the 16S rRNA gene in bacteria, terminal restriction fragment length polymorphism (TRFLP) is a molecular approach used to profile microbial communities. PCR amplification of microbial DNA with fluorescently labeled primers is the first step in the procedure. Following their digestion by restriction enzymes, the PCR results yield DNA fragments of various lengths. Capillary electrophoresis is used to separate and detect these fragments, producing a distinct fingerprint of the microbial population based on fragment sizes (Forney et al., 1997).

PCR amplification of the 16S rRNA gene is followed by restriction enzyme digestion in amplified ribosomal DNA restriction analysis (ARDRA). To create a pattern that represents the organization of

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the microbial community, the resultant DNA fragments are separated using gel electrophoresis. It is helpful for determining dominating taxa and comparing bacterial diversity (Kaplan & Kitts 2003).

The intergenic spacer region (ISR), which is located between the 16S and 23S rRNA genes and varies in length and sequence among various microbial species, is the focus of ribosomal intergenic spacer analysis (RISA). A community fingerprint is created by electrophoresing the amplified ISR fragments (Ranjard, & Richaume, 2001). Regions of microbial DNA (often the 16S-23S ISR) where natural length differences among species produce distinct profiles are amplified by length heterogeneity-polymerase chain reaction (LH-PCR). Capillary electrophoresis is used to examine the PCR results, enabling quick and sensitive comparison of microbial communities (Suzuki & Giovannoni, 1996). PCR-amplified DNA fragments are separated using denaturing gradient gel electrophoresis (DGGE), which is based on how each sequence melts in a denaturing gradient gel. A banded pattern that symbolizes microbial variety is produced when DNA fragments with distinct sequences cease migrating at various locations as it partially denatures (Muyzer et al., 1993).

8. Challenges in Microbial Forensics

To prevent contaminating subsequent assays after leaving the crime scene, samples must be properly identified, collected, handled, and preserved (Budowle et al., 2005). The first responder should put their personal safety first and have a solid grasp of the kinds of microorganisms involved while reacting to a suspected biological attack. Finding the causative agent's source and figuring out which people or groups might have been impacted is the next important step (Sharma & Bansod, 2019).

- Inaccurate results may arise from contamination at any point, from collection to analysis. It may be the result of handling, the environment, or other samples. DNA profiles may be incorrectly attributed or banned as a result.
- Environmental elements such as heat, moisture, and sunshine can break down DNA samples, producing incomplete profiles that are challenging to interpret and may compromise the accuracy of the results.
- Because numerous people's DNA may be present in forensic situations, it can be challenging to distinguish between individual DNA profiles.
- To preserve integrity, DNA must be handled and stored properly. Protocols to recover human nDNA from evidential materials can be impacted by inappropriate handling or storage. As part of these procedures, things must be sealed in the proper bags or containers and kept dry and refrigerated until analysis. Drying swabs and aerating containers minimize microbial degradation. Protocols for preserving forensic biological evidence for human DNA typing may not work for stabilizing microbial communities (Durdle et al., 2020).

9. Future Direction in Microbial Forensics

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In order to identify people, look into bioterrorism situations, and determine the cause of death, forensic microbiology analyzes evidence. It draws insights from a variety of fields, including epidemiology, computer science, forensics, bioinformatics, microbial genomes, phylogenetics, and classical microbiology. Our understanding of the variety of bacteria that live in and on humans has significantly increased because to next-generation DNA sequencing. We can determine contributing species and estimate bacterial diversity in human microbiomes using 16S ribosome sequencing (Nelson, 2021).

For consistency and comparability, future studies should concentrate on standardizing procedures, creating a forensic microbial bank, and discovering stable biomarkers within the dominating Bacillota and Pseudomonadota phyla. Unique microbiological signatures for robust databases can be created by identifying trustworthy postmortem interval indicators among certain bacterial species/strains during body decomposition. Microorganisms can be used to supplement standard forensic testing; however, in order to fill in knowledge gaps and follow Locard's notion of trace evidence, thorough studies and machine learning models are needed (Freitas, 2023).

The discipline of microbiological forensics has been strengthened by recent developments in research and development that have produced new techniques, technologies, and analytical capabilities. To provide the next generation of scientists the abilities they need, it is equally crucial to offer education and training in this area. Enhancing first responders' awareness and readiness through education and training is essential for maintaining the integrity of the evidence and guaranteeing public safety. Additionally, it can increase the number of qualified microbial forensics practitioners and educate the public and decision-makers about the potential and constraints of microbiological forensics (Keim et al., 2020).

There are special uses for microorganisms in forensic identification. The growth of metagenomics and forensic microbiology has been aided by cost savings and sequencing advancements. Artificial intelligence has demonstrated its distinct worth in forensics and its capacity to answer pertinent queries (Liu et al., 2022). New technologies are used in forensic labs to improve the precision and sensitivity of DNA results. To satisfy increasing demands for higher throughput, they have automated sample preparation and data interpretation. Short Tandem Repeat (STR) typing is still the predominant method in forensic DNA analysis, even though other genetic markers are employed for certain purposes (Butler, 2015).

Although NGS technology offers a wealth of genetic data for examining microbial communities, the enormous amount of data produced necessitates an efficient analysis technique that can delve deeper. In order to develop an efficient "time fingerprint" mathematical model, AI can build thorough assessment models that analyze and mine large data, assess weights, and spot trends (Zhao et al., 2022). By making it possible to sequence billions of DNA fragments in parallel, NGS systems such as Oxford Nanopore, Pacific Biosciences, and Illumina have completely changed the field of genomics. New understandings of microbial diversity, gene expression, genetic variation, and epigenetic changes have resulted from this. NGS has aided in the understanding of intricate biological processes

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such tumor heterogeneity and development, the identification of disease-causing variations, and the discovery of therapeutic targets (Malonia et al., 2023).

A. Faster results - NGS provides a quicker turnaround time than traditional microbial methods, like culture, allowing for the high-throughput analysis of multiple samples, which is crucial for forensic investigations and allows for quicker and better-informed decision-making in response to possible public health threats (Dinis-Oliveira et al., 2024).

B. Greater information content and sensitivity Since next-generation sequencing makes it possible to detect and provide comprehensive information (such as genetic composition, virulence factors, and antibiotic resistance) on a particular pathogen, it makes it easier to do in-depth genomic analysis of microbial strains. These characteristics provide crucial information about their possible significance, modes of transmission, and similarity to other strains, enabling more precise epidemiological studies (Dinis-Oliveira et al., 2024).

C. Microorganisms can be detected in complex samples or at trace amounts thanks to next-generation sequencing. This high sensitivity makes it possible to identify pathogens, which is especially useful when there are traces of microbial DNA present (such as in degraded or mixed samples). Additionally, NGS is applicable to a wide range of sample types that are obtained in different ways and undergo different processes (such as formalin-fixed paraffin-embedded tissues, swabs, blood, and environmental samples). This adaptability is helpful in forensic contexts where sample types may differ.

This approach provides a more realistic perspective of the microbial population and enables the simultaneous detection of several pathogens that may interact during disease by identifying a wide range of microorganisms (such as bacteria, viruses, and fungi) in a single sequencing run. Also, NGS allows distinguishing between closely related microbial strains, an important aspect in determining pathogens' source and transmission pathways in environmental contamination, disease outbreaks, or bioterrorism/biocrime attacks (Dinis-Oliveira et al., 2024).

10. Conclusion

Using the pervasiveness of microbes to provide insight into events that occur before, during, and following death, microbial forensics has become a potent adjunct to conventional investigative methods. Researchers can produce reliable postmortem interval estimates with previously unheard-of accuracy by examining the distinct succession and makeup of microbial communities—the epinecrotic microbiome that thrives on surface tissues and the thanatomicrobiome that colonizes interior organs. Even when environmental factors add complexity, these biological clocks which are based on repeatable patterns of community turnover provide vital temporal anchors in homicide investigations, aiding in the reduction of time frames of death.

Microbial signatures function as unique "microbial fingerprints" on items and surroundings, going beyond temporal quantification. Communities whose taxonomic and functional characteristics reflect

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their provenance can be found in soil, textile fibers, and latent fingerprints. Therefore, associative relationships that go beyond the boundaries of human DNA evidence can be established through comparative analysis between crime scene samples and those collected from suspects, particularly in situations when human genetic material is absent or destroyed. By capturing both community structure and metabolic potential, high-throughput sequencing platforms from targeted amplicon approaches (16S rRNA and ITS) to comprehensive shotgun metagenomics allow researchers to expand the scope of the evidence to include environmental context, pathogen detection, and toxin exposure.

Forensic sensitivity is further increased by combining digital and quantitative PCR, which enables the precise measurement of important microbial markers even in samples with little biomass or heavy contamination. These quantitative data, when combined with machine learning classifiers trained on carefully selected reference databases, enable probabilistic evaluations that are effectively conveyed in court. Rapid screening of massive datasets is made possible by artificial intelligence-driven pattern identification, which highlights unusual community compositions that demand further investigation. When combined, these molecular and computational methods provide a complex framework for evidence that can be adjusted to meet the legal requirements of reproducibility and dependability while still being sensitive to small biological indications.

The field has important obstacles that need to be overcome in order to guarantee the microbiological data's evidential integrity. To reduce exogenous contamination and maintain in vivo community structures, sample collecting procedures from swabbing methods to storage conditions must be standardized. To resolve methodological differences in DNA extraction, library preparation, and bioinformatic workflows, cross-laboratory validation studies are necessary. Environmental variables can complicate postmortem interval estimations and geolocation analyses by accelerating microbial succession or introducing allochthonous species. These variables include temperature changes, humidity, and substrate composition.

Interdisciplinary cooperation will be essential to the success of microbiological forensics as it moves from research labs to normal casework. In order to maintain scientific rigor and legal admissibility, microbiologists, bioinformaticians, legal scholars, and law enforcement must unite around uniform standards, accreditation procedures, and training programs. Microbial forensics will establish itself as a transformative tool in homicide investigations one that deepens our understanding of death, advances the pursuit of justice, and ultimately serves the larger goals of public safety and truth through concerted efforts to improve methodologies, validate findings, and communicate complex microbial data in courtroom settings.

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