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Review Article:

Forensic Microbiology: A Comprehensive Review

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Abstract: Forensic microbiology merges microbiology and forensic science to support legal inquiries. It centers on the identification and analysis of microbial communities linked to human remains, environments, or objects. Its uses include estimating postmortem intervals, detecting bioterrorism, and identifying suspects. The advancement of molecular biology techniques, such as metagenomics and next-generation sequencing, has enhanced the importance of forensic microbiology, particularly in challenging cases involving skeletal or heavily decomposed remains [1]. Microbial signatures are unique to individuals and environments, allowing their use as forensic trace evidence[2]. These signatures are shaped by ecological, geographic, and physiological factors, offering specificity in linking evidence to people or places[3]. This review presents a comprehensive overview of methodologies, applications, and significant developments in forensic microbiology, while also discussing limitations and future directions (4).

Key words: microbiology, fungus, virus, forensic, trace.

Introduction: The convergence of microbiology with forensic science has given rise to forensic microbiology, a field that seeks to extract legal information from microbial evidence. It addresses challenges in criminal investigations by identifying microbial patterns that develop on or around human remains, in environments, and on forensic evidences [5]. Unlike conventional forensic tools, which often rely on physical or chemical markers, forensic microbiology taps into the dynamic diversity of microorganisms. The human body, for instance, harbours a vast array of microbes that undergo predictable changes following death [6]. These successional shifts allow investigators to estimate the time since death or detect body relocation [7]. One of the earliest high-profile applications of this discipline was during the investigation of the **2001 anthrax attacks in the United States**, where microbial fingerprinting successfully traced the origin of Bacillus anthracis [8]. The incorporation of high-throughput sequencing and computational tools has further enhanced microbial profiling, making it a powerful tool for forensic science[9].

Reviews: The evolution of forensic microbiology is underpinned by numerous studies that established its value in criminal investigations. Some of the studies mentioned in Table 1 [Please refer to Table 1 here] Collectively, these studies validate the forensic potential of microbial evidence, which can be employed for PMI estimation, personal identification, and bio-criminal investigations.

Postmortem Microbial Succession

Microbial succession following death refers to the orderly and predictable changes in microbial populations as decomposition progresses. This phenomenon is now recognized as a biological clock for estimating PMI. In the early "fresh" stage, the body is colonized primarily by aerobic bacteria from the skin, gut, and surrounding environment[20]. As decomposition enters the bloated and active decay phases, anaerobic bacteria [19] like **Clostridium spp.** Become dominant due to the oxygen-depleted environment[21]. Studies have systematically catalogued these microbial transitions using metagenomic analyses of body sites such as the mouth, rectum, and skin [22].

Environmental factors like ambient temperature, humidity, and soil contact affect microbial succession, yet core patterns remain consistent across different bodies and settings [23]. Recent advancements include the use of machine learning to model and predict these microbial shifts with high accuracy, as demonstrated by Javan et al. [24]. When microbial data is combined with entomological evidence and chemical profiling, investigators gain a more robust estimate of PMI[25]. Thus, microbial succession has become a critical element in postmortem investigations.

Soil and Environmental Microbiomes in Forensics

Soil microbiomes, due to their spatial specificity, are a valuable tool for geographic origin estimation in forensic investigations. When a body decomposes, it releases nutrients and microbes into the soil, altering the local microbial ecology[13]. These changes are site-specific and can help determine the PMI or indicate if a corpse has been relocated [11]. Comparing microbial DNA from crime scenes with that found on a suspect's shoes or tools can provide evidence of presence or contact[15].

The identification of microbial DNA from soil using techniques like **16S rRNA** gene sequencing has proven effective in matching crime scene soils with samples from specific regions[23]. In addition to terrestrial ecosystems, aquatic microbiomes can indicate whether drowning occurred in a specific water body, while airborne microbial communities can help track exposure or geographic origin in certain bio-crime scenarios [20]. Though environmental variability remains a challenge, methodological consistency and advanced analytical tools ensure forensic reliability[19]. The expanding scope of environmental microbiomes is enhancing their use in global forensic practices.

Human Microbiome as Forensic Evidence: The human microbiome, comprising microbial communities on and inside the body, offers a unique biological fingerprint for forensic investigations. Shaped by diet, hygiene, environment, and genetics, an individual’s microbiome is remarkably specific, allowing for personal identification through microbial profiling [6]. Skin microbiota can be transferred to touched objects and persist long after contact, making them valuable as microbial trace evidence[5].

Research has shown that these microbial signatures can survive even after conventional cleaning methods, retaining forensic value[15]. The oral and gut microbiomes are also being explored for identity verification, especially in cases where DNA is compromised due to heat, moisture, or degradation[14]. In infectious disease-related deaths, microbiome analysis may assist in determining the cause of death[9]. Although legal challenges such as reproducibility and evidentiary standards persist, the field is supported by large reference datasets like those from the Human Microbiome Project[16]. Thus, human microbiomes are increasingly accepted as individualized forensic markers.

Forensic Applications in Bioterrorism and Biocrime: Microbial forensics has become an indispensable tool in identifying and investigating acts of bioterrorism and crimes. The 2001 anthrax attacks in the United States marked a defining moment for the field, where scientists traced the Bacillus anthracis strain back to a specific laboratory using genome sequencing and MLVA (Multiple-Locus Variable-Number Tandem Repeat Analysis) [8,9]. These techniques established microbial forensics as essential for national security.

The field also plays a crucial role in distinguishing between natural outbreaks and deliberate releases of pathogens, particularly in cases involving emerging infectious diseases[17]. To support rapid identification, forensic labs now maintain databases of pathogenic microbial genomes and agent libraries[25]. Programs like the FBI's BioWatch and the European Union’s Microbiome Forensic Network reflect the global initiative toward biothreat preparedness[12]. The integration of epidemiology, genomics, and forensics ensures a robust response to crimes, highlighting the strategic importance of forensic microbiology in maintaining public health and safety.

Discussion: The findings from diverse global research affirm forensic microbiology as a transformative field within forensic science. Microbial evidence extends the investigative toolkit by enabling PMI estimation, corpse tracking, and even suspect identification in scenarios where traditional evidence is degraded or unavailable[6,13,15]. However, challenges remain. These include environmental variability, difficulties in contamination control, and the current absence of universally accepted forensic microbial protocols[18,23]. Despite these issues, advances in sequencing technologies and the integration of artificial intelligence have significantly enhanced accuracy and usability[24].

Importantly, courts are beginning to recognize microbial evidence, although its admissibility often hinges on expert testimony and validation studies[17]. Interdisciplinary collaborations among forensic pathologists, microbiologists, bioinformaticians, and legal experts are critical for refining standards and ensuring robust application. As scientific and legal frameworks continue to evolve, forensic microbiology is expected to become a core component of death investigations and biosecurity responses[10,25].

Conclusion: Forensic microbiology represents a rapidly expanding frontier in forensic science, offering precise and often non-invasive solutions to complex legal cases. By leveraging the diversity and specificity of microbial communities, investigators can estimate PMIs, link individuals to crime scenes, and respond effectively to bioterrorism threats. The integration of metagenomics, machine learning, and large-scale databases has significantly elevated the discipline's capabilities. Although challenges such as legal admissibility and environmental variability persist, ongoing innovations and international cooperation are addressing these hurdles. As forensic microbiology matures, it promises to redefine the standards of evidence in modern criminal justice systems, ensuring more accurate and holistic approaches to solving crime.

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Table 1

S.no.	Author(s)	Year	Key Contribution	Forensic Relevance
1	Budowle et al[8].	2001	Established foundational principles of microbial forensics post-anthrax attacks.	Defined protocols for bio-agent detection, evidence preservation, and forensic use.
2	Metcalf et al[2].	2013	Introduced the "microbial clock" concept using DNA sequencing during decomposition.	Enabled accurate postmortem interval (PMI) estimation through microbial succession.
3	Hyde et al[7].	N/A	Investigated bacterial community shifts from onset to end of the bloat stage.	Reinforced consistency in microbial succession patterns relevant for PMI.
4	Fierer et al[3].	2010	Demonstrated individual-specific skin microbiota persistence on surfaces.	Supported suspect-object associations via microbial trace evidence.
5	Turnbaugh et al[6]. (Human Microbiome Project)	2007	Provided a comprehensive dataset of healthy human microbiota.	Created baseline references for differentiating postmortem or pathological changes.
6	Cobaugh et al[11].	2015	Analyzed soil microbial shifts beneath decomposing corpses.	Aided PMI estimation and detection of corpse relocation.
7	Pechal et al[24].	2014	Tracked reproducible evolution of cadaver microbiomes.	Highlighted predictable microbial changes as forensic markers.
8	Javan et al[24].	2016	Applied machine learning to microbiome data for enhancing PMI predictions.	Improved accuracy and applicability of microbial forensics in criminal investigations.