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# **Future of Forensic Microbiology**

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# Abstract

This review dives into the emerging field of forensic microbiology, highlighting its potential to revolutionize criminal investigations. The Human Postmortem Microbiome (HPMM) and its predictable postmortem succession, offering a novel approach to Postmortem Interval (PMI) estimation is explored. Bioindicators from specific pathogens can shed light on cause of death, while metagenomics and AI tools further refine microbial evidence analysis. However, ethical and legal challenges surrounding privacy and discrimination must be addressed before widespread adoption. By overcoming these hurdles, forensic microbiology promises to unlock invaluable hidden evidence, paving the way for a more just and accurate legal system.

Keywords: Human postmortem microbiome; Microbial succession; Postmortem interval; Bioindicators; Metagenomics; Post-mortem bacterial translocation

# Introduction

The forensic sciences are a wide and evolving subject that aims to use the most cutting-edge methods to solve legal problems [1]. There are numerous subfields of forensic science. To name a few, most people are aware of ballistics, toxicology, DNA analysis, and fingerprint comparison. Forensic microbiology is a relatively recent field of forensic science [2]. In 2001, the Bacillus anthracis attacks on the US postal system led to the first widespread recognition of forensic microbiology. Agar cultures for bacteria and fungus paired with PCR for specific species were the only forensic microbiological procedures specifically mentioned in earlier works on the subject. In the last several years, forensic experts have started to investigate the viability of using the succession of the microbiome to infer the postmortem interval since microorganisms play a role in postmortem decomposition and have a succession that follows a predictable pattern [3]. Postmortem alterations, determining cause of death, measuring postmortem interval, and trace evidence analysis are some of the newly developing fields of microbiology that are significant to medicolegal and criminal investigations. Researchers can now explore microbial communities with unprecedented resolution and in cross-disciplinary settings because to recent advancements in sequencing technology [4]. Processing-wise, forensic microbiological investigations are very similar to other forensic investigations. They involve the examination of crime scenes, chain of custody procedures, the gathering, handling, and shipment of evidence, its analysis, its interpretation, and its presentation in court. In addition to gathering and examining conventional forensic evidence, the forensic investigation will make an effort to identify the causal agent and its etiology, frequently in a manner akin to an epidemiologic inquiry. However, a higher level of characterization is required for attribution [5]. An increase in interest in forensic microbiology research is a result of recent microbiological advances. In some of these studies, nonhuman animal surrogates were utilized as models, while in other cases, donated human cadavers were used. Some of these studies concentrated on the decomposing microbial ecology of human remains. Decomposition research has potential importance to forensics, but both methodologies have advantages and disadvantages [6].

### Forensic Microbiology

Since microbes are common and have predictable ecologies, they have the potential to be employed as tangible evidence in forensic science [7]. The Human Postmortem Microbiome (HPMM) is the microbiome of an individual that has been identified from various anatomical sites of bodies (also known as cadavers) that have been sampled during regular death investigations. High throughput gene sequencing is used to describe and evaluate the microbial communities from different anatomical sites, such as the mouth, ears, and rectum, in order to make key scientific discoveries in the fields of ecology, forensics, and medicine [https://hpmmdatabase.wixsite.com/ hpmmdatabase/what-is-the-hpmm]. Bacteriology and mycology have been utilized as tools for a wide variety of forensic procedures because of the pervasive presence of microorganisms in the

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environment and inherent to the cadaver. In reality, understanding of the countless microbial species that live inside of humans has continued to expand ever since the human microbiome project was established in 2007. From a forensic perspective, a specific or collection of microorganisms might offer hints as trace evidence in several scenarios (Who? What? When?), from personal identity to cause of death or PMI estimates [8]. In forensics, the microbiomes of the three understudied bodily locations (oral cavity, skin, and vaginal cavity) can be affected by a variety of personal circumstances both during life and after death. The microbiome changes after death because a variety of environmental influences have a far greater impact on the post-mortem microbiome [9]. In a well-regulated mouse model, changes in the anatomical and functional makeup of the bacterial population occur as the lungs, heart, bone marrow, and intestines decompose [10]. The pace of carrion decay is significantly influenced by soil microbial populations, and this has crucial consequences for our knowledge of carrion ecology [11]. Microbiological succession throughout the ecological process of decomposition to calculate the Postmortem Interval (PMI), or time since death. By integrating microbiome data obtained from postmortem samples (such as skin swabs) with known PMIs, a regression model was created to create this microbial clock of death. In a death inquiry, a similar sample type (such a skin swab) would be gathered, the bacteria profiled using DNA sequencing, and the microbes would be matched to a moment on the clock (i.e., the regression model). Recent studies conducted by numerous independent scientific teams have demonstrated the viability of this new microbiome forensic technique. But there are technical, investigative, and legal barriers that must be surmounted in order to create and introduce new forensic science methods into the justice system [12]. A hidden indicator or bioindicator for the cause of death may be certain bacteria. In general, if we find only one microbial species in body fluids after autopsy, it is likely that the person had an illness while they were alive, whereas a mixed profile suggests a postmortem invasion. This may be helpful in verifying the diagnosis of an antemortem infection, locating the etiological agent of an infectious disease that was misdiagnosed earlier, or locating microbiological markers for specific forms of death [13].

# Futuristic View of Forensic Microbiology

The microbiome has been proven to be a potentially useful tool for PMI estimate in the past ten years. Postmortem microbial succession exhibits a certain temporal regularity. Through the analysis of massive data, the creation of prediction models, the aiding of decision-making, etc., Artificial Intelligence (AI) technologies have recently shed fresh light on forensic medicine. Next-Generation Sequencing (NGS) and AI techniques can be used to improve the dataset of microbial communities and provide forensic practitioners with detailed data on the inventory of particular ecosystems, quantifications of community diversity, descriptions of their ecological function, and even their use in legal medicine [14]. Minimally invasive autopsies are one effective method for determining the cause of death. The postmortem interval can be estimated with the aid of the thanatomicrobiome analysis [15]. Due to dietary, lifestyle, and geographic circumstances, the deceased human body is a complex microbial ecological system that differs greatly from person to person. It is crucial to emphasize that a positive Post-Mortem Microbiology (PMM) test could indicate a true infection, sample contamination, commensal organisms, or Post-Mortem Bacterial Translocation (PMBT). According to estimates, postmortem contamination and PMBT occur about 20% of the time in typical autopsy where PMM has been done. However, postmortem contamination can be kept to less than 10% when consistent processes are followed [16]. The study of gut microbiome data using Machine Learning (ML) algorithms has proven successful in revealing hidden patterns and making precise phenotype predictions. The gut microbiota can be analyzed using ML to find microbial biomarkers for non-invasive disease risk assessment or the development of gut microbe-targeted therapeutics. We may also categorize patients based on their gut microbiota using ML [17].

The study of the human microbiome may reveal additional, unexpected data in addition to personal identification. Because of this, and in spite of being heavily regulated, human microbiome research still presents a number of ethical, legal, and social issues. Microbiomes may reveal details about a person's ancestry, ethnic background, and previous exposures as well as the nations and places they have travelled to. This information may be used to identify the subject as a person of interest to homeland security and law enforcement agencies, violating the privacy rights of the individual. Additionally, the health and equilibrium of humans are linked to their microbiotas. The presence of specific microbes may reveal an individual's susceptibility and predisposition to specific clinical conditions like obesity or diabetes (correlation between intestinal microbiota and local or systemic infections; colonization of the nasopharynx by S. aureus), contributing to socioeconomic stigmatization or discrimination. In the end, certain societal subgroups may view the collection of microbiological samples (such as the taking of vaginal and stool samples) as intrusive and unacceptable [18-21].

# Conclusion

We are diverted from understanding the trace's true nature, characteristics, and functioning since forensic science typically focuses on specialized technology advancements to evaluate a single aspect of the trace. Despite the fact that the trace is always somewhat perceptible, a more comprehensive, formal study should lead to improved comprehension. The microbiological remains discovered at the scene of the crime can also offer unmistakable proof of guilt. Modern microorganism identification relies on metagenome analysis, 16S rRNA gene amplicon-based sequencing for bacteria, and ITS rRNA gene amplicon-based sequencing for fungi. The ability of microorganisms to provide evidence such the geolocation, cause, and time since death makes microbial DNA analysis potentially useful in resolving crimes and promoting justice because of how distinctively different each person's microbes are. Studies have shown that the environment, together with biological and abiotic elements, would influence the subsequent analysis and the final validation, which is a crucial stage in the forensic inquiry because of its crucial role in the court decision. Law enforcement has the infrastructure for attribution and deterrent (such as adhering to the exact microbiological forensics program) so that it can be used in court to handle various issues. The entire forensic process in the legal system will be enhanced with the development of more repeatable, sensitive, and accurate methodologies, the creation of a large, reliable database, and the allocation of the proper amount of funding.

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