



The rise of forensic microbiology: unveiling the potential of microbiome in criminal investigations

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Using the special characteristics of microbial communities, the rapidly expanding science of forensic microbiology is able to provide important new information for criminal investigations. Examining its uses in postmortem interval (PMI) estimation, cause of death identification, and trace evidence analysis, this review explores the rapidly developing subject of forensic microbiology. Recent developments in sequencing technologies have transformed the way microbial communities are characterized, allowing forensic experts to interpret the microbial death clock and uncover latent traces at crime scenes. The potential for improving data analysis, creating reliable prediction models, and assisting in well-informed decision-making is enormous when artificial intelligence (AI) and machine learning (ML) are combined with forensic microbiology. However, in order to protect privacy and avoid discrimination, it is important to carefully navigate the ethical, legal, and social issues surrounding human microbiome research. A more thorough and data-driven approach to criminal investigations is made possible by forensic microbiology's ability to harness the power of the microbiome.

Keywords: *microbiome, forensic, trace evidence, machine learning, artificial intelligence, NGS, ethical considerations*

Introduction

In order to decipher the complexity of criminal cases, the discipline of forensic science is always changing and embracing new technology and approaches. Although forensic microbiology is a relatively new subdiscipline, it has already shown to be a significant tool for investigators, drawing insights from the special characteristics of microbial communities. This overview examines the growing field of forensic microbiology's uses in criminal investigations, emphasizing its utility in determining the cause of death, estimating postmortem intervals (PMI), and analysing traces of evidence. The microbiome, or diverse and dynamic microbial ecosystem, is present in the human body and is essential to both health and disease. Our knowledge of the microbiome has completely changed as a result of recent developments in sequencing technology, which have allowed scientists to learn more about the complex structure and functions of these microbial communities. Within the field of forensic science, these new insights have created fascinating new opportunities.

The emergence of forensic science

The goal of the broad and dynamic field of forensic sciences is to use the most advanced techniques to the resolution of legal issues. Forensic science comprises many subfields. Most people are familiar with toxicology, fingerprint comparison, DNA analysis, and ballistics, to mention a few. The discipline of forensic science known as forensic microbiology is very new. The first mainstream acknowledgment of forensic microbiology occurred in 2001 as a result of the *Bacillus anthracis* attacks on the US postal system. In previous studies on the issue, the only forensic

microbiological procedures particularly described were agar cultures for bacteria and fungi and PCR for specific species. The possibility of utilizing the succession of the microbiome to determine the postmortem interval has drawn the attention of forensic specialists in recent years due to the role that microorganisms play in postmortem decomposition and the predictable succession that they follow (Yuan et al., 2023). A few of the recently emerging areas of microbiology that are important to criminal and medicolegal investigations are monitoring the postmortem interval, identifying the cause of death, and analyzing traces of evidence. Recent advances in sequencing technology have allowed researchers to study microbial communities in cross-disciplinary settings and at a resolution never before possible (Carter et al., 2017b). Forensic microbiological investigations and other forensic investigations share a lot of processing similarities. They include investigating crime scenes, following protocols for the chain of custody, collecting, managing, and transporting evidence, as well as analyzing, interpreting, and presenting it in court. Apart from collecting and reviewing traditional forensic evidence, the forensic investigation will try to determine the causative agent and its origin, often in a way similar to an epidemiologic investigation. But attribution demands more detailed characterization (Budowle et al., 2005). Recent advancements in microbiology have led to a rise in interest in forensic microbiology research. Human cadavers that had been donated were employed as models in certain of these investigations, while nonhuman animal surrogates were used in others. A portion of these investigations focused on the microbial ecology involved in the decomposition of human remains. Decomposition research may be significant for forensics, but there are benefits and drawbacks to both approaches (Benbow & Pechal, 2017).

Microbial succession: the working of the microbial clock of death

Microbes can be used as concrete evidence in forensic science since they are ubiquitous and have predictable ecologies (Metcalf, 2019). When samples from various anatomical areas of bodies—also referred to as cadavers—are taken during routine death investigations, the resulting microbiome is known as the human postmortem microbiome (HPMM). In order to achieve important scientific discoveries in the domains of ecology, forensics, and medicine, high throughput gene sequencing is utilized to characterize and assess the microbial communities from various anatomical regions, such as the mouth, ears, and rectum [<https://hpmmdatabase.wixsite.com/hpmmdatabase/what-is-the-hpmm>]. Because microorganisms are both a natural part of the cadaver and a widespread presence in the environment, forensic methods have made extensive use of bacteriology and mycology as instruments. Actually, since the inception of the human microbiome research in 2007, knowledge of the myriads of microbial species that inhabit people has grown. A particular microorganism or group of them may provide clues as trace evidence from a forensic standpoint in a variety of situations (Who? What? When?), ranging from cause of death to PMI estimations (Cláudia-Ferreira et al., 2023). In the field of forensics, many personal circumstances, both during life and after death, can have an impact on the microbiomes of the three bodily regions that are not as well studied: the mouth cavity, skin, and vaginal cavity. Because many environmental factors have a considerably higher impact on the post-mortem microbiome, the microbiome changes after death (Ahannach et al., 2021). When the lungs, heart, bone marrow, and intestines break down in a well-regulated animal model, the anatomical and functional composition of the bacterial population changes (Burcham et al., 2019). Soil microbial communities play a major role in determining the rate of carrion degradation, which has important implications for our understanding of the ecology of carrion (Lauber et al., 2014). To determine the postmortem interval (PMI), or time since death, microbiological succession is used throughout the ecological process of decay. This microbial clock of death was constructed using a regression model that combined known PMIs with microbiome data from postmortem samples (skin swabs). A death investigation would collect a comparable sample type (skin swab, for example), profile the bacteria using DNA sequencing, and link the germs to a time point (the regression model). The practicality of this novel microbiome forensic method has been shown by recent research carried out by multiple independent scientific teams. However, in order to develop and implement novel forensic science techniques inside the legal system, obstacles related to technology, investigation, and law must be overcome (Metcalf, 2019). Certain bacteria may be a bioindicator or hidden indicator of the cause of death. In general, an autopsy revealing only one species of microbe in bodily fluids indicates a probable sickness during the patient's life, while a mixed profile indicates a postmortem invasion. This could be useful in finding the etiological agent of an infectious disease that was previously misdiagnosed, confirming the diagnosis of an antemortem infection, or identifying microbiological indicators for particular types of death (Roy et al., 2021).

Microbiome and postmortem analysis

Over the past ten years, the microbiome has demonstrated its potential utility as a tool for PMI estimation. There is a considerable temporal regularity to the microbial succession observed after death. Artificial intelligence (AI) technology has revolutionized forensic medicine in recent times by enabling the analysis of vast amounts of data, building prediction models, supporting decision-making, and more. Artificial intelligence (AI) and next-generation sequencing (NGS) can be used to enhance the dataset of microbial communities and give forensic practitioners comprehensive information on the inventory of specific ecosystems, community diversity quantifications, descriptions of their

ecological function, and even their application in legal medicine (Wang et al., 2022). One useful technique for ascertaining the cause of death is minimally intrusive autopsy. With the help of the thanatomicrobiome analysis, the post-mortem interval can be determined (Fernández-Rodríguez et al., 2019). Each deceased person's body is a unique complex microbial ecological system that varies widely due to dietary, lifestyle, and regional factors. It is important to note that a positive test for post-mortem microbiology (PMM) may suggest the presence of commensal microbes, PMBT, genuine infection, or contaminated sample. About 20% of normal autopsies when PMM has been performed are estimated to involve post-mortem contamination and PMBT. On the other hand, if consistent procedures are followed, post-mortem contamination can be less than 10% (Saegeman et al., 2021). It has been demonstrated that the use of machine learning (ML) techniques to the analysis of gut microbiome data can uncover latent patterns and accurately predict phenotypes. In order to identify microbial biomarkers for non-invasive illness risk assessment or the creation of therapies that target gut microbes, the gut microbiota can be analysed using machine learning. ML may also be used to classify patients according to their gut microbiota (Li et al., 2022).

Along with personally identifiable information, the study of the human microbiome may also provide other, unanticipated data. As such, human microbiome research still raises a number of ethical, legal, and social concerns while being highly regulated. The information contained in a person's microbiome can provide insight into their ethnic heritage, lineage, past exposures, and travel destinations. The subject's privacy rights may be violated if this information is utilized to identify them to law enforcement and homeland security organizations as persons of interest. Furthermore, human microbiome and balance are related. Certain microbes can indicate a person's vulnerability and inclination to particular clinical conditions, such as diabetes or obesity (correlation between intestinal microbiota and local or systemic infections; *S. aureus* colonization of the nasopharynx), which can lead to discrimination or stigmatization based on socioeconomic status. Ultimately, some social groups can consider the taking of faeces and vaginal samples, among other microbiological sample collection procedures, to be intrusive and inappropriate (Oliveira & Amorim, 2018).

Microbiological Evidences and Future Directions

When forensic science usually concentrates on certain technological developments to assess a single facet of the trace, we are distracted from comprehending the true nature, traits, and operation of the trace. Though there is always some discernible residue, a more thorough, formal investigation ought to result in a better understanding (Jaquet-Chiffelle & Casey, 2021). Unquestionable evidence of guilt may also be provided by microbiological remains found at the scene of the crime. For the identification of modern microorganisms, metagenome analysis is used, together with 16S rRNA gene amplicon-based sequencing for bacteria and ITS rRNA gene amplicon-based sequencing for fungi (Speruda et al., 2022). Because microorganisms can provide information on the location, cause, and duration since death, microbial DNA analysis has promise in the investigation of criminal cases and the advancement of justice due to the unique differences among individuals' bacteria. According to studies, biological and abiotic factors as well as the surrounding environment might have an impact on the final validation and subsequent analysis. This is an important phase of the forensic investigation because it plays a significant part in the court's decision. Law enforcement is equipped to address a variety of concerns in court because it follows the precise microbiological forensics program and can be utilized as a deterrence and means of attribution. The establishment of a big, trustworthy database, the development of more sensitive, repeatable, and precise procedures, and the appropriate amount of financing will all improve the forensic process in the judicial system as a whole (Yousefsaber et al., 2022).

Conclusion

Criminal investigations could be completely transformed by the rapidly developing area of forensic microbiology. Using the microbiome to their advantage, forensic experts can learn important lessons about cause of death, postmortem intervals, and trace evidence processing. The capabilities of forensic microbiology are further enhanced by the integration of machine learning (ML) and artificial intelligence (AI), which makes it possible to create reliable prediction models and support well-informed decision-making. Yet, in order to guarantee privacy protection and avoid prejudice, ethical, legal, and societal issues surrounding human microbiome research must be carefully considered. A more thorough and data-driven approach to solving criminal puzzles will surely result from the further evolution of forensic microbiology and its integration into the investigative scene.

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