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Forensic Microbiology: NGS, Evidence, Challenges

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Introduction

The field of forensic microbiology has seen significant advancements, particularly through the integration of Next-Generation Sequencing (NGS) and comprehensive microbiome analysis. This evolution presents substantial opportunities to utilize microbial evidence, such as samples from skin, soil, or the gut, to establish connections between suspects and crime scenes, or even to pinpoint specific geographical origins. However, realizing this potential in legal settings demands overcoming considerable challenges in standardizing methodologies and interpreting complex microbial data [1].

Microbial source tracking (MST) offers a powerful tool for environmental forensic investigations. By carefully analyzing microbial communities, researchers can effectively identify pollution sources, monitor the spread of pathogens, and forge clear links between environmental samples and specific contamination events. Both established analytical methods and innovative genomic approaches are crucial here, showing great promise for accurately attributing environmental contamination and determining liability [2].

Analyzing the soil microbiome presents a unique avenue for forensic science, particularly in determining the geographical origin of samples. This technique relies on various sequencing methods, sophisticated bioinformatics tools, and statistical analyses to characterize the distinct microbial communities found in soil. These communities often possess unique geographical signatures that can serve as crucial evidence to link individuals or objects to particular locations. Despite its promise, the approach faces hurdles like environmental variability and the nuanced interpretation of data [3].

The study of the postmortem microbiome is an expanding area with significant implications for estimating the Postmortem Interval (PMI). After death, microbial communities undergo predictable changes within various tissues and environments, making them valuable biological indicators. Detailed methodologies for microbiome profiling are being developed to leverage these changes, though the establishment of standardized models for accurate PMI estimation across diverse forensic contexts remains a complex challenge [4].

Microbial forensics plays a vital, strategic role in national security, particularly in efforts to counteract biological threats such as bioterrorism. This involves deploying advanced techniques to swiftly identify pathogens, ascertain their precise origin, and meticulously track their dissemination. The need for rapid and accurate analysis is paramount, as is the development of scientifically rigorous methods that can withstand legal scrutiny. Translating these complex scientific findings into actionable intelligence poses an ongoing and critical challenge [5].

A practical guide highlights the application of Next-Generation Sequencing (NGS) in forensic microbiome analysis, covering the entire operational workflow. This spans from meticulous sample collection and Deoxyribonucleic Acid (DNA) extraction to advanced sequencing, bioinformatics processing, and thoughtful data interpretation. Emphasizing data quality and reproducibility is essential for presenting microbial evidence effectively in court. Further considerations include adapting the workflow for different sample types and addressing specific investigative questions [6].

The human skin microbiome holds considerable forensic potential for individual identification and source attribution. Systematic reviews reveal how the unique microbial composition of an individual's skin can link them to specific objects or locations. Research explores factors influencing the stability and transferability of these microbial communities. This area shows immense promise, yet it requires addressing several methodological challenges to solidify its use as reliable forensic evidence [7].

The application of environmental Deoxyribonucleic Acid (eDNA) in microbial forensics is rapidly gaining traction, offering new possibilities for source tracking and identifying microbial signatures in a wide array of samples, even when viable organisms are absent. The methodologies for eDNA collection and subsequent analysis are crucial for tracing contamination events or establishing connections between environmental samples and their specific origins. While powerful, this approach also comes with current limitations and paves the way for future research avenues aimed at optimizing eDNA-based forensic investigations [8].

Microbiome analysis of various forensic body fluids, including saliva, semen, and vaginal fluid, is emerging as a valuable tool for personal identification. The distinct microbial signatures within these fluids can generate important investigative leads, aiding in differentiating individuals or establishing links to crime scenes. Despite its promising potential, limitations persist, and standardization challenges must be overcome to accurately interpret body fluid microbiome profiles in real-world forensic casework [9].

The complex relationship between microbial communities and human decomposition is increasingly important for forensic taphonomy and estimating the time since death. Sequential changes in microbial populations on and within human remains serve as crucial indicators in forensic investigations. Researchers are developing various methods to analyze these decomposition microbiomes, though applying these intricate ecological dynamics accurately to diverse, real-world forensic cases presents ongoing challenges [10].

Description

Forensic microbiology is revolutionizing criminal investigations by integrating advanced techniques like Next-Generation Sequencing (NGS) and comprehensive microbiome analysis to leverage microbial evidence effectively [1]. This involves analyzing diverse microbial communities—from skin, soil, and gut samples—to establish crucial links between individuals and crime scenes or specific geographical locations [1]. Beyond personal identification, microbial source tracking (MST) provides a powerful framework for environmental forensic investigations, enabling the identification of pollution sources, monitoring pathogen dissemination, and establishing definitive connections between environmental samples and particular events [2]. Both established and emerging genomic approaches are vital in attributing environmental contamination and liability, demonstrating the broad applicability of microbial forensics [2].

The analysis of specific microbial environments offers unique forensic insights. For instance, soil microbiome analysis plays a significant role in determining the geographical origin of samples, utilizing various sequencing techniques, bioinformatics tools, and statistical methods to characterize distinct microbial communities with unique geographical signatures [3]. Similarly, the human skin microbiome holds considerable potential for individual identification and source attribution, as its unique microbial composition can link individuals to objects or locations [7]. Research in this area examines factors influencing the stability and transferability of these skin microbial communities, highlighting both their promise and inherent methodological challenges [7]. Furthermore, microbiome analysis extends to various forensic body fluids—such as saliva, semen, and vaginal fluid—providing unique microbial signatures that can generate investigative leads for personal identification and link individuals to crime scenes [9]. While promising, this area also confronts limitations and demands standardization for reliable interpretation in forensic casework [9].

Estimating the time since death, or the Postmortem Interval (PMI), is another critical application where microbial forensics offers novel approaches. The postmortem microbiome undergoes predictable successional changes in various tissues and environments after death, making these microbial communities valuable bioindicators [4]. Methodologies for microbiome profiling are actively being developed to refine PMI estimation, though establishing standardized models for diverse forensic contexts remains a significant hurdle [4]. Parallel to this, the intricate relationship between microbial communities and human decomposition is explored in forensic taphonomy. Changes in microbial populations on and within human remains serve as crucial indicators in forensic investigations, with various analytical methods addressing the complex ecological dynamics involved, despite the inherent challenges in applying these to real-world cases [10].

The operational aspects of forensic microbiome analysis emphasize the need for robust methodological pipelines. A practical guide outlines the entire workflow for Next-Generation Sequencing (NGS) applications, covering sample collection, Deoxyribonucleic Acid (DNA) extraction, sequencing, bioinformatics, and data interpretation [6]. The focus here is on ensuring high data quality and reproducibility, which are absolutely crucial for the admissibility of microbial evidence in court [6]. Environmental Deoxyribonucleic Acid (eDNA) further broadens the scope of microbial forensics, enabling source tracking and the identification of microbial signatures in diverse samples, even without requiring viable organisms [8]. This utility is particularly significant for tracing contamination events or linking environmental samples to specific origins, although ongoing research aims to optimize eDNA-based investigations and address current limitations [8]. Crucially, microbial forensics also plays a strategic role in national security by countering biological threats like bioterrorism. This involves identifying pathogens, determining their origin, and tracking their spread through advanced techniques, emphasizing the need for rapid, accurate analysis and robust scientific methods for legal scrutiny and actionable intelligence [5].

Conclusion

Forensic microbiology is rapidly advancing, leveraging Next-Generation Sequencing (NGS) and microbiome analysis to enhance criminal investigations. Microbial evidence from diverse sources like skin, soil, gut, and postmortem samples can link suspects to crime scenes or specific geographic locations. This field extends to environmental forensics, where microbial source tracking helps identify pollution origins and trace pathogen spread, attributing environmental contamination. Furthermore, it plays a critical role in national security by identifying pathogens and tracking biological threats like bioterrorism. Methodologically, a practical workflow for NGS applications, including sample handling, Deoxyribonucleic Acid (DNA) extraction, bioinformatics, and data interpretation, ensures the quality and reproducibility of microbial evidence for legal contexts. The analysis of postmortem microbiomes offers insights into estimating the Postmortem Interval (PMI), while unique microbial signatures in body fluids contribute to personal identification. Environmental Deoxyribonucleic Acid (eDNA) provides new avenues for source tracking even without viable organisms. Despite significant promise, challenges remain in standardizing methods, interpreting complex data, accounting for environmental variability, and translating scientific findings into actionable intelligence, all crucial for the legal acceptance of microbial evidence.

Acknowledgement

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Conflict of Interest

None.

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