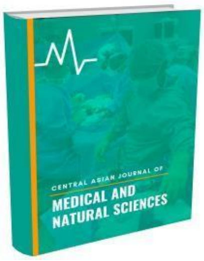

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Microbiological Forensics: New Possibilities in Criminal Investigations

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Abstract: Forensic evidence is the keystone of criminal investigation, providing objective scientific evidence to substantiate the identification or exclusion of crimes within a court. One of the emerging trends in the area is the new addition of a new sub-discipline called microbial forensics, founded on the examination of the microscopic microbial populations of the individual or crime scene. These microbe populations are a unique biological trace which can serve as new evidence in criminal offenses, especially if other evidence is lost or admissible. The sources of microbials evidence are also varied and vary from blood, skin, and hair to biological tissues, soil, surfaces, and crime equipment. It is characterized by its variability and biological intricacy and shows various environmental and personal effects. Techniques of analyzing this evidence include microbial culture, polymerase chain reaction (PCR), and high-throughput gene sequencing (NGS), along with the use of nanotechnology and artificial intelligence in analyzing big data and improving result accuracy. Microbial evidence can be used in identifying individuals, tracking environment or biological crimes, linking evidence to criminals, and determining the time of death with accuracy using monitoring of changes in microbial populations at the time of death. Despite being of immense potential, it also comes with problems that it encounters, such as the problem of sampling accurately, environmental contamination of microbial data, and low legal acceptance, with ongoing development, there is little doubt that microbial forensics will be an essential tool of use in criminal investigations, more so in reference database construction, addition to standard genetic evidence, and bolstering legislative frameworks for use in courts.

Keywords: Microbial Forensics, Microbial Fingerprinting, Gene Analysis, Artificial Intelligence, Genome Sequencing

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1. Introduction

Forensic evidence is also one of the foundation pillars of criminal investigation since it itself directly helps to unveil the truth and find objective scientific evidences that establish the fact of whether or not a crime has occurred in a court of justice [1], [2].

Forensic evidence is founded on the recovery and analysis of physical and biological information regarding suspects or crime scenes with the intention of linking the suspected individuals to the cases under investigation [3], [4]. Decades have witnessed forensic evidence increase substantially, and the type of evidence available to be used has varied more to include sophisticated biological elements as DNA, fingerprints, and tissue marks, and sophisticated imaging and analyzing devices. A new forensic line of evidence has been established in recent years by the name forensic microbiology, relying on the examination and analysis of microscopical microbial communities in various environments and their association with the investigated people, objects, or sites [5], [6], [7].

This line relies on employing the specific microbial diversity deposited by people or other organisms onto their environments. These microbial communities are a specific biological fingerprint that can be applied as novel forensic evidence in criminal investigations [8]. Microbiological forensic science is the application of understanding regarding the amount and types of microbes, i.e., fungi, bacteria, and viruses, as an attempt to uncover criminal problems by linking individuals, items, or places through their microbial fingerprint. Microbiological forensic science is new, and it breaks new grounds in the provision of more discriminative and sensitive evidence, especially where traditional evidence is not available or insufficient [9], [10].

This evidence is most applicable in the context of the obstacles to modern criminal investigations because the crimes are becoming more advanced and the techniques involved in perpetrating them are more diverse.

Microbiological evidence plays a significant role in the area of offering additional corroboration to legal proceedings. It makes it possible for one to properly identify people, follow the source of evidence, and estimate the time of events, e.g., the time of death, thus allowing investigators to properly reconstruct events of a crime [11]. The rising trend in this direction is based on the unprecedented advance in microbial analysis technologies, primarily high-resolution gene sequencing technologies known as next-generation sequencing (NGS), which have provided the precise identification of the members of the microbial community at lower costs compared to traditional methods [12].

Along with this, the concept of the personal microbiome came into being, implying that each human being has his or her own specialized group of microbes which live on and within his or her skin and body. What this implies is that the microbial groups are presented as an individual's fingerprint that identifies him or her from the rest of humankind. Microbiological forensic science was used in recent years to identify a person from the microbial signature that a person leaves behind on a surface or object [13]. The possibility of using changes in postmortem microbial communities in the estimation of postmortem interval, which remains one of the grand challenges of forensic science, has also been demonstrated. It is a new and important contribution to criminal chronology that assists investigators in finding the sequence of events. In addition, recent advances in big data analysis, artificial intelligence, and machine learning techniques have also contributed to increasing the validity and reliability of results obtained from microbiological data to process and analyze more effectively large volumes of complex data and utilize them in criminal cases [14], [15]. This promises a lot for building predictive models in order to assist with forecasting specific criminal crimes through microbial biological evidence.

With all these advances, the moment is now ripe to make research and development in the field of microbiological forensics a priority, and to accelerate convergence among the disciplines of microbiology, genetic sequencing technologies, and forensic data science, to provide improved and more accurate tools for investigators to allow them to be able to present strong and valid scientific evidence to the courts, which results in the pursuit of justice in an extended and professionalized manner [16], [17], [18].

Microbiological Forensic Theory

Microbiological forensics is a relatively new forensic science discipline that handles sampling, analysis, and analysis of microbes on an individual, in the crime scene environment, or on crime scene equipment.

It produces advanced biological information based on the vast diversity of microbes, like bacteria, fungi, viruses, algae, and many other microbes. Microbes are everywhere on the surface, on the ground, and in the body of living things. They react swiftly to their environmental surroundings and hence become individual and unique "biosignatures."

Each individual possesses its own unique microbial community, forming a biosignature that is able to leave an impression on the surfaces or machinery that they come into contact with, offering new avenues of possibility for their utilization in linking evidence to individuals or locations [19].

Types of Samples That Are Currently Available to Be Tested in Microbiological Forensic Evidence

The source of the microbiological samples that can be analyzed in forensic evidence has broadened exponentially. They are no longer limited to traditional biological samples such as blood or tissue samples but have now come to include:

Blood: Blood may, in its usual application, be utilized to carry microbes that reflect the immediate environment or status of an individual and hence contribute additional evidence towards a person or his circumstances [20].

Human skin and hair: These contain specific microbial populations that are distinct in each human individual. These are very useful in making contact or presence in a crime scene since the microbial impression left by a person on surfaces can be utilized for their identification.

Biological tissue: Irrespective of whether they are cadaver-sourced or sourced from animals, microbial flora in them may provide crucial information about the time of death based on a study of microbial post-mortem change over time.

Soil: An important biological reservoir of site-specific microbial communities, composition of which can be researched to trace evidence back to a given site, or even trace an individual's travel through soil microbial transmission [21], [22].

Surfaces and equipment: Either from weapons and apparel or from crime scene sample and clothing, surfaces take up microbes from individuals who handled them, which can be analyzed to rule in or rule out people.

Scratches and other biological samples: These hold biodiversity that is indicative of the environment or individuals present in a crime scene and may be subjected to analysis to yield detailed information on conditions or activities.

The Difference Between Microbiological and Traditional Forensic Evidence

While there may be areas of overlap between disciplines, there are inherent differences which distinguish microbiological evidence from traditional forensic evidence, for instance, fingerprints or human DNA typing:

The biological and environmental background of the evidence: Traditional evidence relies on the identification of an individual by stable molecular or mechanical features, while microbiological evidence relies on microbial communities that are dynamically controlled and subject to personal and extraneous influences [23].

The unique multilayered fingerprint: The traditional fingerprints, similar to fingerprints, provide a good mechanical signature, whereas microbial fingerprints provide an evolved biological signature with plenty of environmental connections like the past history of exposure, lifestyle, and even changes in health.

Complementarity: Microbiological evidence should not replace the traditional evidence but provide a complement that might be invaluable where traditional evidence does not exist, is indistinct, or is nonexistent.

Applications of Microbiological Evidence in Crime Investigation

Practical utilization of microbiological evidence has opened up new horizons to forensic science in which the following main applications are brought to the forefront:

a. Identification of the presence of an individual at the crime scene by identifying their own individual microbial profile [24].
b. Identification of the chronology of criminal activity by tracking changes in the population of microbes on corpses or tangible objects.

c. Recognition of crime objects or tools by direct observation of microbes carried.
d. Identification of environments or locations by microbial imprints on soil or organic matter.

These capabilities render microbiological forensics a valuable support tool augmenting and complementing current criminal investigations.

2. Methodology

Methods Employed in Microbiological Forensics

Analytical and diagnostic methods are the pillars of this new science. Microbial forensic science uses a variety of methods and instruments to harvest, identify, and quantify microbial community prints in an attempt to link evidence to crime scenes, suspects, or victims in a valid way. The ordinary procedure often consists of three main steps: (1) sampling and maintenance, (2) detection of microbial communities, and (3) advanced data analysis using nanotechnology and artificial intelligence.

a. Microbiological Sample Sampling and Storage

Microbiological sampling is the most significant microbial evidence-based investigation process. Collection should be carried out with the assistance of aseptic procedures by employing sterile tools such as cotton swabs, DNA-free tubes, and heat- and moisture-resistant products. Samples may be collected from dirty surfaces, soil, clothing, weapons, human skin, or even environment air at the crime scene. To maintain microbial community integrity and prevent degradation of DNA or protein denaturation, ultra-low temperatures from -20°C to -80°C are used to store the samples. In addition, stabilization solutions like RNA Later or metagenomic stabilizers are used for stabilization of biomolecules in native form until laboratory processing.

b. Microbial Identification and Analytical Techniques include

- Classical Culture: Culture refers to a traditional method towards the isolation of bacteria or fungi that can be grown in laboratory media. It's a limitation as more than 90% of environmental microbes are unable to be cultured under normal conditions.
- Polymerase Chain Reaction (PCR): PCR revolutionized microbial forensics by offering for the amplification of a particular DNA or RNA sequence. Its modifications such as quantitative PCR (qPCR) allow for quantitation, and reverse-transcription PCR (RT-PCR) is best suited for RNA virus detection.
- Next-Generation Sequencing (NGS): Next-generation sequencing technology enables deep profiling of uncultivated microbial communities to expose subtle differences that may be correlated with individual or geographic origin.
- Spectrometry: Approaches like mass spectrometry (MS) and Fourier-transform infrared spectroscopy (FTIR) probe microbial proteins or organic molecules and generate chemical fingerprints that are used in identification and classification.

c. Artificial Intelligence and Nanotechnology Incorporated into Data Analysis include

- Nanotechnology: It enhances forensic identification by biosensors made of nanomaterials (gold or silver nanoparticles) that amplify biological signals from trace amounts of microbial material, increasing test sensitivity. DNA purification and spectroscopic interrogation of intricate samples is facilitated with nanomaterials.
- Artificial Intelligence (AI) and Machine Learning (ML): The vast amounts of data produced by NGS and metagenomic profiling demand stable computational technologies. AI and ML programs classify microorganisms, identify microbial patterns, and correlate microbial signatures with specific environments or suspects. They are invaluable especially when traditional forensic procedures yield very little information. For instance, deep learning techniques have been applied to quantify temporal changes in the body's microbiome during the decomposition process to

help establish time of death and construct a new forensic clock that competes with traditional approaches.

Collectively, these technologies constitute a paradigm shift in forensic science wherein it is possible to generate highly accurate and reproducible microbial evidence for use in legal proceedings.

3. Results and Discussion

Applications of Microbiological Forensic Evidence in Investigations

As microbiological testing advances, microbiological evidence is being the focal point to support criminal investigations with an array of new applications emerging:

a. Identification of Individuals (Microbial Fingerprinting)

Each person possesses a unique microbial signature that depends on genetic makeup, lifestyle, diet, environment, and ongoing contact. Science attests to the fact that communities of bacteria in the skin, oral pharyngeal flora, and the gut flora are different for each person [25]. This evidence can be matched by forensic experts through the collection of microbial samples from touched objects of a suspect or his/her personal objects and cross-matching the same with reference profiles to link individuals to crime scenes, particularly when traditional fingerprint or DNA evidence is not available [26].

b. Tracing the Origin of Environmental or Biological Crimes

In crimes of environmental contamination such as the dumping of industrial wastewater or water contamination, microbial fingerprints in water or soil can trace sources of contamination by comparing bacterial populations against areas to be targeted or against factories. Similarly, in crimes of a biological nature such as the intentional dissemination of pathogens, microbial genome sequencing can follow infection sources and help in biosecurity and disease control processes.

c. Tracing Suspects from Evidence with Microbial Analysis

Microbial traces remaining on weapons, attire, or devices are indirect proof that can incriminate offenders with crime devices. Microbial traces on cars, enclosed areas, and personal devices carry the unique microbial imprint of users, making it easier to track in complex forensic analysis [27].

d. Postmortem Microbiome Analysis

One of its principal applications is determination of the postmortem interval (PMI) by following understandable, sequential microbial population changes during the course of decomposition a process termed as "microbial clock". The technique will also presumably provide more reliable estimates of time of death than traditional forensic techniques under unfavorable circumstances like delayed decomposition and environmental contamination [28]. PMI estimation is also aided by soil microbiome analysis below dead bodies.

e. Biological Toxin and Biocrime Analysis

Forensic analysis using microbiology plays a central part in the examination of agents used in biological attacks or poisonings, such as Bacillus anthracis (anthrax) or Clostridium botulinum. Microbial species can be typed, source identification made, genetic alteration analyzed, and suspected enzyme or toxin characterized [29]. The information is crucial in characterizing attack mechanisms, suspect identification, and intent determination behind the act.

Case Studies and Practical Examples

Microbiological data have increasingly been utilized in forensic science, with various studies showing its usability:

- Microbial Fingerprinting: Fierer et al provided evidence for the relationship of microbes on individuals' hands to those on their keyboard, illustrating that microbial fingerprints are viable for the identification of individuals and linking them to certain surfaces.

- Estimation of typical time of death: Metcalf et al. employed microbial community variability within a deceased to estimate accurate post mortem intervals, providing valuable information to use in homicide convictions.
- Identification of environmental crime: Newton et al. employed microbial profiling of buried samples of contaminated water to trace criminal dumping of waste to factories, resulting in successful convictions.
- Biological Crime Investigation: During the 2001 U.S. anthrax attack, microbial DNA from mailed envelopes was compared to a lab strain, further narrowing the suspects.

Challenges and Limitations

While promising, microbiological forensics is full of challenges:

- Sample Collection and Handling: Microbial populations are highly susceptible to environmental conditions. Suboptimal collection or delay can result in contamination or degradation, rendering evidence questionable.
- Overlapping Environment: Microbial signatures overlapping in different locations (e.g., public transport and crime scenes) can be possible, making causality hard to establish.
- Legal Recognition: The courts are not likely to accept evidence from microbiology presently due to its novelty, the absence of precedents, and the fact that legal professionals are not familiar with it.
- Expertise: It is an inter-disciplinary analysis that requires expertise in microbiology, molecular biology, and advanced data analysis without trained personnel of conventional forensic labs.

The future for microbiological forensics is promising with robust drivers including:

- Genetic Forensics Coupling: Merging microbial data and human DNA profiles (multi-omics) to obtain complete environmental and personal mapping of evidence.
- Artificial Intelligence and Automation: AI-facilitated analysis enables quick and accurate microbial identification, suspect profiling, and reconstruction of crimes.
- Reference Database Development: Global efforts concentrate on building comprehensive microbial community databases as benchmarks for forensic comparison and geographic sourcing.

Applications in Biosecurity: Microbiological forensics plays a pivotal role in bioterrorism threat monitoring and pathogen incidents, such as during the COVID-19 pandemic.

4. Conclusion

Microbiological forensic science evidence is a leading crime investigation technology, offering a precise and innovative addition—or in the case of some evidence, substitute for traditional forensic technique. Microorganisms leave in-depth biological evidence of individual interactions between organism and environment, enabling identification, association of evidence, and temporal analysis of crime activity. Integration of advanced technologies such as NGS, PCR, spectroscopic methods with nanotechnology and AI has revolutionized analysis and interpretation of microbial evidence. This extends forensic potential beyond fingerprints and human DNA to include unique microbial fingerprints on humans and the surroundings. But more research is required to further explain microbial community function and the way they interact with the environment. More applied work and case studies will better increase collection, analysis, and interpretation strength and standardization. Regulatory models must also evolve to recognize microbiological evidence validity and ethical constraints. This is going to require close collaboration between scientists, forensic experts, legislators, and judges to position microbial forensics within the legal system.

In general terms, microbiological forensic evidence could be the cornerstone of future criminal cases, and research, infrastructure, and policy funding will enable a revolution jump to a more science-oriented and just system.

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