

REVIEW ARTICLE

From Sequencing to Prediction: Leveraging Next Generation Sequencing & Machine Learning in Microbiome-Based Postmortem Interval Estimation

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ABSTRACT

Optimal determination of the postmortem interval (PMI) is one of the cornerstones of forensic analysis but oftentimes the dependence and variability of the conventional methods like entomology, histology and biochemical techniques most commonly experience sudden limitations since they are sensitive to the environment. New also cites the human microbiome as an active and quantifiable state of an after death postmortem clock, and post mortal microbial succession is an excellent clock. The integration of Next-Generation Sequencing (NGS) technologies and Machine Learning (ML) algorithms, though, is revolutionizing the field and is now able to profile microorganisms at high resolution and model and predict PMI with high accuracy. NGS technologies such as 16S rRNA sequencing, shotgun metagenomics and whole-genome sequencing can potentially lead organizations to find various types of microbial communities which were not used before with higher accuracy. None of them are capable, however, of bulking up these enormous data sets with superior computing methods. Machine learning algorithms like rule-based systems Rain Forest, the Support Vector Machines and deep learning systems have shown

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the potential to recognize patterns of microbial succession regimes and translate them into useful prediction of PMI. The answer to this challenge lies in switched NGS and ML: with sequencing, the biological signal is provided, and predictive models are utilized in order to refine the temporal resolution and minimize the margin of error. Even with such progress, there are more challenging problems. In this instance variability between settings, the absence of standard procedures, small databases of forensic microbiomes, and its potential legal inadmissibility all pose hurdles to its application on a large scale. The development of portable sequencing machines, global forensic databases on microbes, and the explicable AI to suit the needs of the judiciary are the way forward. The article is a review of the current conditions of microbiome-based PMI estimation with a focus on highlighting how both NGS and ML can be valuable in developing an effective and standardized postmortem clock in forensic scientists.

KEYWORDS

- Postmortem Interval (PMI) Estimation • Forensic Microbiome • Thanatomiobiome
- Next-Generation Sequencing (NGS) • Machine Learning Models

INTRODUCTION

One of the fundamental issues of forensics science has always been the estimation of the period between death and its discovery. Proper determination of the time of death not only aids in determining the chronological order of events surrounding a death, it is also an important component of justice in terms of the proceedings of a criminal court.¹ Historical methods, such as forensic entomology, biochemical analysis of body fluids or histopathological changes in tissues have been the prime methods employed over the decades. Concurrent with being useful, flaws like environmental dependency, geographical variation, and limited ranges of accuracy normally undercut such methods. In response, forensic scientists have redirected their attention to sharper, more molecular scale techniques in which higher accuracy and accuracy description of PMI can be attained. Study of the human microbiome has been one of the brighter spots among these newer strategies. The correlation, like that of the thanatomiobiome, of the microbial communities, which are sequentially deployed during the highs and lows of the successional processes, implies that the evolution dynamics in microbial life rise and fall in a predictable manner over time.^{2,3} These fluctuations can be used as the biological markers so that they can be compared to the postmortem clock. By following the microbial succession in current use in significant body locations like the mouth cavity, the gut, skin and even to the soil surrounding the body, forensic experts

are coming up with new perspectives into how much further the PMI estimation can be optimized beyond the intravenous methods. With the integration of Next-Generation Sequencing (NGS), this discipline has moved at a rapid rate with the possibility of obtaining a complete profiling of a microbial community⁴. Microbial signatures can now be identified with heretofore unimaginable levels of resolution thanks to the high-throughput methods of 16S rRNA sequencing, shotgun and metagenomics sequencing, and whole-genome sequencing. The complexity and scale of NGS data however render meaningful interpretation of such data using sophisticated computational platforms.⁵

Here, Machine Learning (ML) has emerged as a game-changer. Random Forest classifiers, Support Vector machines and deep learning models are ML algorithms that can identify subtle relationships between microbial succession data and make predictions about PMI. With the real biological accuracy of NGS and predictive accuracy of ML, scientists are one step closer to establishing a reliable, overarching, and admissible system to determine the time since death. This paper addresses the intersection of sequencing technology and computational model in forensic microbiome science. It addresses innovation in NGS-driven profiling, the significance of ML within predictive PMI systems and lays out challenges, obstacles and how the field can develop. All these developments can be regarded as a key breakthrough in the case of forensic science, as

they offer the potential to make PMI estimation younger, more accurate, more standardized and technologically advanced.

LITERATURE REVIEW

Forensic entomology, biochemical and other histological evidence establishment has been in the forefront of classic approaches to the estimation of postmortem interval (PMI) since decades. Forensic entomology based on insect colonization has been employed widely but very critically depending on climatic conditions, geographical distribution and insect activity, particularly in such instances, which take place indoors. Similarly, biochemical and histological methods, including vitreous humour potassium level, algor mortis, rigor mortis and autolysis of tissues, are useful though time-constrained techniques, age-dependent, disease-dependent, and environmental. It is against these constraints that there has been a need to seek molecular-level markers that will provide a more precise and reliable result.⁶⁻⁸ The examination of the human microbiome has become a promising alternative with the idea of the thanatomicrobiome, or microbial communities/communities whose succession is predicted to follow death. It has been found that bacterial niches like oral cavity, gut, skin and surrounding soil vary according to time dependent changes with some taxa prevailing in particular stages of the decomposition process. An example is that in the early stages of PMI, Proteobacteria tend to become very abundant, whilst Firmicutes and other taxa become predominant later in the process.⁹ These microbially modified changes, serving as a post-mortem time keeping clock, have advantages over application of traditional methods of investigation because the information presented are not as dependent on external environmental factors. Yet, host genotype, geographical diversity, and environmental diversity continue to be an influence. Next-Generation Sequencing (NGS) technology has improved the pace of investigation in microbiome research in criminology through offering in-depth profiling of the microbial community. Such approaches such as 16S rRNA sequencing, shotgun metagenomics, and whole-genome sequencing permit an extensive exploration of the abundance, diversity, and functionality of the microbes.¹⁰ Various pilot studies have

demonstrated that the succession of discrete microbes found in chromatograms of NGS are linked with complete decomposition timelines and the finding is enlightening as far as PMI estimation is concerned. But the benefit of NGS is that it can perceive unculturable and has the capability to provide statistically dense, high-resolution data, which is inversely compared to spectral constraints of other forensic processes, but its widespread systemic application has the issue of costly and skilled bioinformatical image computing, although it is still technically challenging. Machine learning (ML) has emerged as a critical tool that needs to be employed to interpret the vast amounts of data generated by NGS. Random Forests, Support Vector machineries and deep learning models among other algorithms have been implemented successfully in classifying the organization of microbial succession and forecasting PMI with greater accuracy¹¹. In forensic practice, it has been argued in case studies that prediction windows of a few hours or a number of days are possible by using computational models. However, these issues affect the reproducibility and generalizability since there are concerns about overfitting, smaller data sets and the availability of worse standardized databases of the forensic microbiomes. The combination of NGS with ML is a synergy that can be used to estimate PMI. Many common stages in a typical workflow include sequencing, quality control and preprocessing, microbial signature extraction features, and using predictions through ML predictive modelling. Joining efforts has proved to be much more accurate than microbiome analysis or traditional ones. In addition, new technology including portable sequencing systems (e.g., Oxford Nanopore MinION) combined with cloud-based ML systems has a chance of providing near real-time PMI estimation on crime scenes. Then there may come the introduction of universal predictive models that can be applied universally across populations and a variety of settings (the establishment of world microbial databases).¹² Comparative investigations indicate that traditional techniques can be complemented by microbiome-based PMI estimation and in certain instances it can actually perform better than the conventional techniques. Although the evidence recorded based on entomology is useful in subsequent stages of decomposition, the microbial

analysis gives a blanket and range of stages of decomposition which are both initial and sequential. Likewise, microbial succession is not restricted by the close time scales that biochemical and histological techniques present related to PMI estimations. Microbial methods, entomological and chemical methods have become hybrid in the array of microbial, entomological and chemical methodologists now being considered highly signifier. The adoption of microbiome-based PMI tools in the forensic setting is plagued by a number of challenges, despite its potential. Unsalability of the microbial succession with respect to climate, geography, and host-specific differences all creates a problem of standardization.¹³ Several discrepancies are brought along by differences in sampling, storage, sequencing platforms and bioinformatics pipelines. Moreover, there are concerns on the admissibility of evidence produced by NGS and ML-generated evidence reproducibility and explainability in courts where a court should find relevance in criminal cases. The trends of the future in this area are the requirements to create microbiome databases globally, the need to create explainable AI models to increase the validity of law, and the introduction of the portable sequencing technology into the field. It will be necessary to build interdisciplinary interaction between forensic scientists, microbiologists, data scientists, and legal experts to convert the microbiome-related PMI identification into a applicable, reliable, standardized and court admissible tool. Together with them, these developments demonstrate the potential of revolutions in NGS and ML application as an instrument of forensic microbiome studies, a huge leap to determining PMI correctly through technologically sound methods.

1. The Human Microbiome as a Postmortem Clock

Human body harbours trillions of microorganisms live and these communities remain in a dynamic condition even after death. This postmortem succession of microbes, known as thanatomicrobiome, has already become a hopeful approach to determining the postmortem interval (PMI). The microbial diversity and abundance has been proven to vary predictably overtime in different body habitats like oral cavity, gut, skin and the surrounding soil. This includes an instance where anaerobic outperforms

aerobic and facultative anaerobic bacteria such as Proteobacteria early in the decomposition phase, but anaerobic groups such as the Firmicutes and Bacteroidetes have the upper hand late in the decomposition phase.¹⁴ These series of time-varying-microbes can be adopted as biological biomarkers to determine a postmortem molecular clock. The microbiome are temporal or time literate such that they optimally utilize the conditions in the environment or the short time slots, and, can be applied to form array of stages of decomposition. However, the ambient temperature, humidity, genetic variation and geography of the host influence microbial succession causing variability and restricting the use of standard forensics.

2. Next-Generation Sequencing in Forensic Microbiome Profiling

The invention of Next-Generation sequencing (NGS) technologies has revolutionized the research on postmortem microbial communities significantly by offering an opportunity to profile it on a large scale and high resolution. Common techniques are 16S rRNA gene sequencing, which offers taxonomic classification of bacterial ports, shotgun metagenomics, which offers both functional and genomic information, and whole-genome sequencing (WGS) to offer a solution on a strain-by-strain basis. The techniques provide unparalleled width in separating culturable and unculturable microorganisms attached to decomposition. Forensic examination in recent times has revealed that NGS reveals never-ending microbial shifts according to the decomposition stage, enabling us to utilize it to estimate the PMI. The procedure typically involves sampling, DNA extraction, sequencing and bioinformatics analysis of the genetic material to map the microbial succession. The strength of NGS includes that it is sensitive, has the ability to detect low frequency taxa and is able to generate large datasets.¹⁵ However, there are some limitations like the cost of sequencing is expensive, possibility of contamination and specialized computational expertise are required. Despite these limitations, NGS has thereby firmly cemented its ownership as a standard of modern research into microbiomes in forensics as a powerful lens through which the changes that are experienced by the microbes following death may be observed.

3. Machine Learning Models in Microbiome-Based PMI Prediction

NGS provides high throughput data of microbial successions when compared to these multi-faceted datasets that require complex procedures to explain or interpret them. ML has turned out a groundbreaking tool to the investigation of forensic microbiome and can identify fine details and convert them into valuable PMI models. Random forests, Support Vector Machines and Neural Networks are learning algorithms that were used under the supervision of a supervisor to classify the microbial communities at various stages of decomposition. Thousands of microbial aspects may be processed concurrently and more accurate PMI approximations are generated through these models. The quality of the dataset and the environment that affects the prediction in some studies have recorded prediction accuracy as a few to more than a few days. Unsupervised learning (clustering) has also been applied to understand the dynamics in a microbial community without initially labeling it, thereby giving data about the natural succession pattern. In spite of their promise, the most formidable competition to ML models includes overfitting to small datasets, a lack of training data on varied population, and lacks model-interpretability. However, the synergy between NGS data with ML algorithms is a major step forward providing a calculation framework of converting microbial indicators into taking action based on forensic timelines.¹⁶

4. Toward an Integrated NGS - Machine Learning Framework

The actual power of microbiome-based PMI estimation is the compatibility that next-generation sequencing and machine learning techniques have. NGS on its own provides high management images of the succession and composition of microbial communities; nevertheless, the data itself is at times overwhelmingly voluminous and complex as regards quantity. Machine learning models act as an indispensable interface of interpretation, in the sense that they can infer patterns of meaning between these datasets, and interpret them within a predictive tool of estimating time since death. The integration of both these frameworks guarantees enhanced accuracy along with flexibility among environments, body locations, and stages of decomposition. Algorithm training can now be performed

on large and diverse datasets from NGS-based research and this provides forensic analysts with greater generalizability to a myriad of cases when more precise and hence superior reliable and admissible in court. This collaboration will eventually make raw sequence data a forensic clock a clock that leverages the ecological dynamics of microbes and computer intelligence to respond to perhaps the most challenging question in forensic death investigation: when did death occur.¹⁷

CHALLENGES AND FUTURE DIRECTIONS

Despite the fact that the microbiome-based postmortem interval (PMI) estimation reflects the potential ability to transform the world, a list of challenges deserves its traditional application. Host and climatic as well as environmental conditions can be enumerated as among the most remarkable challenges because the microbial succession are bound to vary under the different conditions of the environment. The existing temperature, or the humidity level and the soil composition and insect activity in this context can turn out to be humongous variables of confidence that put the microbial activities into uncertainty models. Another kind of limitation is the non-availability of big and heterogeneous data because most of the studies are limited to a controlled environment or to a small population. This makes one wonder whether the models can be useful in the forensic context. Additionally, the procedural form is resource exhausting since it requires high throughput sequencing and elaborate computational pipelines and therefore may prove impossible on inadequately equipped forensic labs. It is legally and ethically important as well because the legal and ethical requirements of the reproducibility and transparency are at times conflicting with certain of the machine learning algorithms that are considered black boxes. It is also worth noting that in the future thanatemiome profiles under different conditions reported by different geographies of decaying bodies should be established in huge standardized databases. Multicenter research studies would offer impressive training data so as to optimize the result of machine learning as multicentric and collaborative studies. It will also entail an equally obligatory development of explainable artificial intelligence (XAI) whereby, in addition to its ability to predict

correctly, the output of artificial intelligence should be explainable, to make the outputs in any legal activity open for scrutiny. Field-based microbiome profiling using newer portable sequencing technologies, like Oxford Nanopore, would also have the ability to speed up turnaround times. Moreover, microbiome, entomological, chemical, and histological data would provide the multimodal framework that would make results more valid and acceptable.

CONCLUSION

The time of death approximated has been an essential component of the forensic analysis from time immemorial, yet traditional methods prove useless regarding precision and environmental alterations. A revolution that can occur in regards to conquering this obstacle is the advent of next-generation sequencing and machine learning into microbiome research. The successional patterns of microbial populations are decipherable through this technology, provided that the individual trends are convertible into predictive signals that will be employed to estimate PMI on a scientifically sound and technologically sophisticated platform. Although gaps such as the lack of data, heterogeneity of the environment and admissibility in courts still need to be bridged, quick advances in sequencing platforms, bioinformatics pipelines, and transparent artificial intelligent are sure to negate such gaps. As more work is being done on the research employing standard procedures and common datasets, forensic science stands on the threshold of discovering the microbiome as a useful postmortem crop. This connection of microbial ecology to computational intelligence is ultimately resulting in the enhancement not just of the accuracy of the death-time estimation, but also of the science underpinning forensic assistance, the future steps are to publish forensic DNA as an integral component of regular forensic practice and procedure.

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