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Microbiological analysis in forensic identification and machine learning: a review *

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Abstract

Microorganisms that share human body space and live in the surrounding environment carry specific microbial signatures in each individual and location. They interact continuously with the surrounding environment and this interaction may provide useful information. In the body of a deceased, microorganisms are called as thanatomicrobiome. This fact presents a promising alternative tool for human identification from the traditional identification methods (fingerprint and DNA analysis) in cases where the samples for those methods are unattainable. Microbiological analysis introduces the possibility of using microbiomes as identifiers in forensic investigation and their values can be directed into four aspects; PMI inference, individual identification, tissue or fluid identification and geolocation inference. Rapid technological advancement in artificial intelligence has been widely applied to a lot of disciplines including forensic science. The use of artificial intelligence enhances the accuracy of microbial identification. Numerous machine learning frameworks have been created such as RF, SVM, and ANN alongside the expansion of metagenomic databases. Along with this, concerns regarding limitation and ethical implication of the AI application in forensic microbiology arise. As more research are conducted, people may become aware of the importance of microbiome data and their privacy. There are also concerns regarding AI-generated analysis reports being used in judicial processes because of how inexplicable the way AI works. This paper summarizes the application of microbiological analysis in forensic identification and development of forensic microbiology with the aid of artificial intelligence, specifically the machine learning technique with few examples leaning towards forensic odontology (samples from oral cavity).

Keywords: machine learning, forensic identification, microbiological, artificial intelligence

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Introduction

Microbiological analysis is becoming a more valuable part of forensic science. It is often viewed with such a minor role and only known for human DNA analysis in human identification (1). Though it is very small and cannot be seen with naked eyes, microorganisms are widely spread not only on humans but also the environment we are living in. They are great identification tools as they are proven to have individual specificity as microbial communities comprising fungi, bacteria, protozoa and viruses (2). Thus, forensic microbiology offers a new dimension of human identification other than DNA analysis. When a body of the deceased is found in a degraded condition where primary identification methods such as DNA or fingerprints are not attainable, forensic microbiology is useful to help solve the problem by making use of the microbial signatures of the human microbiome (2,3). Forensic microbiology was first recognized globally in 2001 when a bioterrorism attack occurred by spreading Bacillus anthrax through mailing service in the US (3).

From then on, there is an increased need for forensic microbiology applications because cases of infectious disease of iatrogenic origin, deaths caused by microbial origin and cases of violent death are emerging. The increasing use of implant and transplant nowadays is related to infections of iatrogenic disease (4). In cases where the cause of death is microbial origin, forensic microbiology will make it possible to trace the origin of the microorganisms involved and to reconstruct the chain of contagion. Additionally, characterization of the microorganisms in microbiological analysis will help in reconstruction of violent death cases (4,5).

When combining traditional methods of forensic microbiology with the cutting-edge technology nowadays for example artificial intelligence (machine learning in specific), further improvement in forensic microbiology will be achieved. Traditional method in forensic microbiology, microbial culture, is said to be low efficient and has a low degree of quantitative analysis. Technological advancement in microbiome and metagenomics may make it possible for shifting forensic microbiology from traditional method to genome sequencing technology with a combination of advances in bioinformatics. In other words, utilizing AI to manage the big data of metagenomics in forensic identification can make microbiological analysis more time effective with better quality. Some examples have been taken into action like using machine learning programs to run analysis, interpret and store microbiological samples data in identifying a deceased. There are still some challenges in conducting microbiological analysis in forensic identification regardless of the technological advancement as an aid. For instance, standardized post-mortem sampling procedures and analyses. There is also the instability of the samples overtime which is affected by various external and internal factors (5). Last, challenges in distinguishing individual differences when they are cohabitating or having close interaction that leads to similar microbial profiles (4). In terms of applying AI into forensic microbiology, there are also concerns such as the validity of AI-generated reports as evidence and ethical implications. This paper serves for the purpose of reviewing the application of microbiological analysis in forensic identification and how far AI technologies have been applied as an aid in forensic microbiology.

Microbiological analysis in forensic identification

Microbiological analysis is the biological, biochemical or chemical methods to identify and even enumerate microorganisms in samples. In forensic microbiology, it simply uses traditional, culture-based microbiology and molecular analysis to find out more information about the sample that is obtained from a body of the deceased or the surrounding environment where the body of the deceased is found (4,5). The information will then become useful for legal proceedings in a case of sudden death or crime investigations. Forensic microbiology is now understood to be useful in analysing evidence from criminal and civil cases ranging from accidental release of biological agents, biocrimes, bioterrorism, etc (3). It is recognised first during a bioterror attack relating to Bacillus anthracis that are spread by US mailing service in 2001 and with the help of genome sequencing and comparative genomics the case was solved.3,5 Recent studies found out the application value of microorganism in forensic identification can be divided into four aspects which are postmortem interval inference, individual identification, tissue/fluid identification and geolocation inference (5,6). Although microorganism comprises of fungi, bacteria, protozoa and viruses, bacteria usually become the main focus for its diversity and primary involvement in decomposition (4).

Postmortem Interval (PMI) Inference

When a body of an unidentified deceased is found, its time of death is usually unknown. However, there is one way to reveal the time of death, postmortem interval (PMI) in other terms, by using microbiological analysis (6). Just like a living human, the body of the deceased is inhabited by a diverse number of microorganisms distributed through different anatomical sites (7). This microbial community which is also called as thanatomicrobiome (postmortem microbiota) plays a significant role in the decomposition process (4,8). They in turn will determine the microbial succession of the dead body mass in each anatomical site (4). That is why this method of PMI inference is generally associated with the decomposition process which can be classified into 6 stages described by Payne. A cadaver will decompose through these stages: fresh, bloated, active decay, advanced decay, dry and remains stage (9).

Oral cavity can become one of the key sources for microbiological analysis for PMI inference purposes as it is known as one of the most diverse microbial community and second most complex after the gastrointestinal tract microbial community. A study by Adserias-Garriga et al found out different oral microbial populations in different stages of body decomposition. Firmicutes and Actinobacteria are the most prominent population in the fresh stage of decomposition, Tennericutes in bloat stage, and a different population of Firmicutes in advanced decay (10). This method of estimating PMI shows a new perspective of forensic identification but there is limitation in estimating PMI of a dead body found in an uncontrolled environment (6).

Individual identification

The distributed microbiota in different anatomical sites have obvious individual differences that can be used for individual identification. Based on the studies that have been done, skin, oral cavity and intestine are the unique sites for identification purposes in forensic (6). Each person in this world carries their own unique sets of microorganisms. Identifying a person using their unique sets of microorganisms can be done in two ways; culture-dependent and culture-independent methods (11,12). The culture-dependent method is traditionally used and described as bacterial cultures and even fungal cultures. These microbial cultures will go through microbial profiling to determine their genus, species and strain. Historically, the determination is via bacterial morphology under the microscope (13). The fact that culture-dependent method is considered to be slow and has a lot of limitations resulted in the advancement of the culture-independent method.

Microbial profiling using the culture-independent method means analysing the genetic material of the microbiotas without the need for culturing them (14). Recent advancement in this method used in forensic identification is the next-generation sequencing (NGS). It consists of amplicon-based sequencing and metagenomic shotgun sequencing (15). Forensic DNA analysis initially relies on the genotyping of the short tandem repeats from the DNA of the individual himself either by PCR or capillary electrophoresis (14). Some cases cannot go through this DNA analysis because of the degraded or inadequate DNA that will result in highly degraded samples for analysis. Therefore, microorganisms from the body of the deceased can provide the DNA needed for further microbial analysis in individual identification. Bacterial DNA is proven to be more protected than human DNA from environmental factors (16). Thus, the use of the two next generation sequencing techniques which are mainly used for characterizing the microbiome (14).

Amplicon-based sequencing is the most popular NGS technology in forensic microbiological analysis. It targets 16S ribosomal RNA marker gene for bacteria and archaea but for fungi, the target is the internal transcribed spacer (ITS) (14,16). It will provide genus level resolution. Meanwhile the shotgun metagenomics sequencing makes it possible for the simultaneous detection of all genetic material in a sample which then provide identification of the entire microbiome including bacteria, fungi, and viruses within a sample in a strain level resolution (14).

A study by Phan et al investigated bacterial profiling as an indicator of human characteristics mainly of sex and ethnicity (17). Bacterial community obtained from touched objects that were originally DNA-free was analysed using 16S rRNA sequencing and Qiime pipeline. The result of their study is two potential bacterial biomarkers identified which are Lactococcus for diet type and Alloiococcus for sex and ethnicity. The absence of Lactococcus could be indicative of Chinese diet while the presence of Alloiococcus could be indicative of Caucasian ethnicity and male gender (17).

Tissue/ fluid identification

Other than the body itself, stain that is related to biological traces and body fluids collected in a crime scene can be identified for their bodily origin (6). It can also become important evidence in a crime scene event reconstruction and clarification of the activities that took place such as sexual assault. A study of 6 bodily fluids (saliva, semen, vaginal fluid, menstrual blood, skin and peripheral blood) that have been exposed indoor for 30 days shows that they still exhibit microbial signature that are characteristic of their body site of origin for forensic identification purposes (18). The dominant microbiome found in skin, saliva, semen is Propionibacterium, Prevotella and Bacteroides respectively. While the dominant microbiome found in vaginal fluid and menstrual blood is Lactobacillus (18).

There are various methods in forensic science for tissue or body fluid identification such as catalytic, enzymatic and immunologic tests. Recent advancements lead to bacterial DNA-based identification of tissue or body fluid (19). Once again, oral cavity has become one of the main focuses in fluid identification for forensic casework. Body fluid found in crime scenes may be saliva from an individual. Hence, there are methods in detecting the presence of saliva in body fluid collected from crime scenes. The conventional method is testing the presence of α-amylase but this enzyme can also be found in other body fluid like semen and urine. That is why a more reliable marker is needed to discriminate saliva from other body fluids. Studies found that streptococci as the most abundant bacteria in saliva can be used as a marker for detecting the presence of saliva. Specifically, Streptococcus salivarius and streptococcus mutans were detected using PCR based on study of Hiroaki et al. This detection via PCR is said to be sufficient enough to determine the presence of saliva from a body fluid sample. Between the two, Streptococcus salivarius is more reliable as a marker because of the fact that it can be found mainly on the dorsum of tongue (20). Another study found another saliva-specific bacterium that is possible as a marker which is Veillonella atypica (19).

Geolocation inference

In forensic science, there is a link between crime scene investigations, the suspect and an object or location (6). That is why collecting samples from a crime scene must be done meticulously and even environmental samples like soil, water and plant may be a source of valuable information (14). This leads to the concept of geolocation in which a certain place can contain characteristic microbiota that is different from other places. There is also an ancient axiom “every contact leaves trace” that can be applied in analysis of an individual microbiome and possibly establish where they have been (21). This is because individuals carry microbial signatures that are reflective of their environment. When a person touches an object in a certain place, he/she can pick up microorganisms on that object or transfer his/her microorganism to the object.

Human microbiomes are easily spread everywhere in the human environment by three primary mechanisms. The first mechanism is by direct contact of a human with any surfaces or objects. Second, breathing can cause the emission of aerosol particles. These aerosol particles can also come from hair, skin and clothing because of airflow. Third, dust containing previously shed human skin cells, hair, and other human microbiome-loaded particles may be resuspended in the atmosphere (22). That is why environment samples in forensic investigations can contain not only human DNA signature but also the human microbial signature. Thus, analysing this sample can provide information regarding the people interacting with that specific environment previously.

For example, excellent microbial samples can be obtained from the soil in a crime scene. Soil can easily adhere under shoe sole, fingernails, tire treads, weapons and clothing so it is easily misplaced during a crime. It is proven that soil exhibit specific physical and chemical characteristics of the microbial communities that in turn can be related to the specificity of a crime scene (23). A study by Habtom et al found that soil microbial samples may differ in communities within different distances of the location they are found (24). In other words, the farther apart two microbial communities of soil were located, the more they differed.

Machine learning as an aid in forensic microbiology

Forensic microbiology is considered still in its infancy stage but the advancement of artificial intelligence (AI) in medical disciplines may help in paving a brighter future of the field. AI in medical disciplines is made to help the process of diagnosis and choosing best therapeutic options for the patients. Meanwhile, for forensic microbiology, AI can get a role in managing the accumulation of vast amounts of complex data (big data), analyzing and interpreting them with a better quality and shorter turnaround time (3).

History of AI

Artificial intelligence can be traced back as first introduced in the 1940s and 1950s by a British mathematician named Alan Turing who believed that machines can be developed into smart devices and can be tested for their intelligence. He explained that belief in the Turing Test concept which became the benchmark of identifying the intelligence of a machine or artificial system. This concept states that a machine is said to be intelligent if it cannot be distinguished from human when a human is interacting with the machine and another human at the same time.3 At this point, the term AI was not introduced yet but later in 1956 it was introduced by John McCarthy and Marvin Minsky. After that, AI went through a lot of successful development for nearly two decades and reached its revolutionary phase in the 1990s and 2000s (3,21).

AI now consists of three main techniques; machine learning, deep learning and natural language processing (NLP) (3). This came from a theory of learning which is Hebbian Learning by Donald Hebb in the 1940s which replicate the way neurons process information in the human brain (21). This theory leads to the research of Artificial Neural Networks. In 2015, artificial neural network research managed to reach the state of deep learning when AlphaGo was created. A system was created to have the ability of interpreting external data correctly, learning from those data and finally using those learnings to achieve specific goals and tasks through flexible adaptation (21). Nowadays, artificial neural networks and deep learning construct the basis of most AI applications.

Application of machine learning in forensic microbiology

In medical disciplines, machine learning is the predominant technique of AI used, primarily for structured data generated from hospital laboratories (3). Machine learning is a subfield of AI that gives computers or systems the capability to self-learn and improve from experience and exposure to more data (25,26). It includes the artificial neural network and support vector machine that basically let computers and systems mimic the way human learn. This proves that AI can also be applied in managing the big data in forensic microbiology. AI is already being used in image analysis, culture interpretation and antimicrobial susceptibility testing in diagnostic microbiology laboratories. It is also utilised in the analysis, interpretation and storage of large volumes of complex data in these laboratories (3).

There is already a big database that may be used in forensic microbiology known as the Human Microbiome Project (HMP) launched in 2007 through its applicability is more for clinical filed. The project was originally aiming to explore the composition and distribution of microbial communities in different parts of the human body. Now it is slowly building a microbial genome sequences database from numerous large-scale programs that produce large amounts of data over the past years (14). Thus, the most challenging application of AI in forensic microbiology is to develop and analyze various computationally generated algorithms for microorganism identification by comparing the samples with the existing databases. A lot of researchers have tried this. For example, an algorithm called computer vision can be used to analyze various large and complex images and compare them with the databases for culture identification purposes in diagnostic microbiology. There is already one called CellaVision that was created in Lund, Sweden (3,27). This example may be applied in forensic microbiology in the near future.

There are also some machine learning models namely random forest (RF), support vector machine (SVM) and AdaBoost used widely in forensic microbiology and slowly becoming a promising tool for forensic casework (5). An RF model was used for investigating pubic hair bacteria for individual identification with an accuracy of 2.7 ± 5.8% (28). A study using the brain, heart and cecum of mice as the site of origin for microbiological sample applied machine learning models RF, SVM and ANN to estimate PMI with an accuracy with a mean absolute error (MAE) of 1.5 h with a variability of 0.8 h over a period of 24 h (29). The study concludes the combination of microbiological analysis and machine learning models can serve as a reliable and accurate technology in PMI inference. Huang et al constructed a machine learning framework to establish geolocations from microbiome samples and it reflected accuracy of 86% (30).

Machine learning algorithms are rapidly being used in the healthcare field including for diagnosing disease and classifying bacterial species. These algorithms are expected to easily handle large amounts of dataset and compare the dataset to predict a specific disease or bacterial species. To make the algorithms that are valid for use, training process is done using a certain amount of data as a way to learn to accomplish a task from any data given later. Support vector machine is a classical ML algorithm that have been widely used in microbial analysis. It can be used in high-dimensional data but only effective on small datasets and may face difficulties in solving multiclassification task (31). Meanwhile, random forest has a higher accuracy and is suitable when running on large datasets (31,32).

Limitation and considerations in machine learning application

Considering forensic microbiology itself still has many limitations that need to be addressed, the application of AI into the field also has its own limitations and concerns. A lot of researchers have developed machine learning programs that can be used as an aid in microbiological analysis in the effort of human identification in forensic investigations and mostly proved the usefulness of the program. There is such a thing called black box of AI which means AI whose internal workings and decision-making processes are not known to its users. This could be designed intentionally by the developers or as a result of the training process. When used for microbiological analysis in forensic identification, AI may contain inexplicability in regards to the method of data entry and obtaining conclusions in predicting specific microbiota. Hence, AI may not completely and theoretically generate real results of microbiological analysis (5,33). This fact may possess problems when making AI-generated reports as evidence in judicial process because the inexplicability may be difficult to understand in courtroom and there is lack of accountability because of the way AI works as it may evolve in unforeseen ways resulting in their programmers hold accountable for any wrong happenings (3).

In addition, previous studies in microbiome, especially using rapid sequencing technologies have created large databases storing human microbial samples from those studies' collections. These databases may raise questions regarding ethical implications of sampling human microbiomes. Continuing forward, more research will be conducted to establish forensic microbiology as an important tool either in forensic science or other fields. We may need more microbiome samples from individuals for the purposes of research. Privacy invasion of biological data may become the concern of the targeted individuals for obtaining samples (34). Therefore, privacy and safety measures must be applied when collecting and storing microbiome data to prevent this concern. Although in reality there is still a lack of specific regulation on microbiome data (34). Nevertheless, the idea of using those databases to create a forensic microbiome biobank could contribute to the improvement of many aspects in microbiological analysis of forensic science. It will then lead to forensic microbiology gaining recognition as more than “side activity” in forensic investigation.

Conclusion

Forensic microbiology is a subdiscipline that utilizes microbiological expertise to assist forensic investigations. It opens up the possibility of detecting minimal amounts of microorganisms either from humans or the environment in forensic investigation. Advancement in this field allows for PMI and geolocation inference, individual identification and tissue or fluid identification using culture-independent microbial profiling which is microbial gene sequencing. Up to date, the main focus of AI application in forensic microbiology is utilisation of the big data provided by gene sequencing using machine learning. A lot of machine learning algorithms have been created to help in analysing microbiological samples. This will help in increasing effectiveness in microbiological analysis in terms of time and cost. Slowly, this will pave the way for more AI applications that are proven to be helpful in forensic microbiology. However, there are concerns regarding guideline the selection of machine learning algorithm that is deemed applicable and yield valid interpretation in microbiological analysis as well as in forensic microbiology. With a lot of limitations and knowledge gaps still present, future research may be conducted with the objective of making advancement in the field and recognising the full potential of microbiology for forensic investigations alongside the advancement of AI application.

Declaration of Interest

None

Author Contributions

AZ, EIA and ANST contributed to the conceptualization of this study and writing the original draft of the manuscript. EIA is the study supervisor. All authors have contributed and approved the final draft of the manuscript.

Statement on the use of artificial intelligence in manuscript preparation

Artificial intelligence was not used in the preparation of this manuscript.

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