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# Advances in Human Microbiome as an Emerging Tool in Forensics

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**ABSTRACT: Microbiome refers to the total microbial genes or the sum of all microscopic life forms including bacteria, fungi, archaea, protozoa and viruses, inhabiting the human body. Forensic microbiology is an evolving science based on the identification of individual microbiomes related to a crime scene. The core human microbiome from birth to death has peculiar set of organisms which can aid in identification of time and cause of death. In fact, microbial signatures are unique to individuals and considered as fingerprints. In addition, this emerging science can be useful in determining the location-based information of a person by linking the environment microbiota to the victim and to individuals' core microbiome. Moreover, Post-Mortem Microbiome PMM including group of bacteria-thanatomicrobiome associated with the host after death are helpful tool for investigation. Furthermore, forensic microbiology can help in the interpretation of infectious diseases cases that result in sudden unexpected death. Microbial forensics can also be used to estimate the Post-Mortem Interval PMI. In addition, the gut microbiome inhabiting the digestive tract play a significant role in behavioral response to addictive drugs as well as anxiety and depression-like behavior. The microbiome and metabolites produced can provide evidence of individual's alcohol, drug addiction or psychosis suggesting significant gut-brain interactions in drug addiction. Despite the numerous mentioned application of microbiology in forensic science, forensic examiners sometimes ignore these evidences and prefer to deal with other marks found in the crime scene. The objective of the present review was to highlight the significance of forensic microbiology field by discussing several applications of human microbiome which can be considered as an emerging tool during forensic investigation.**

**Keywords:** Forensic Microbiology, Microbiome, Post-mortem Microbiology, Thanatomicrobiome, Cause of death, Gut microbiome.

## I. INTRODUCTION

In accordance with Human Microbiome Project (HMP), the human body is composed of healthy cells and microbes with ten times more microbes than human cells. Human microbiota are characterized by different anatomical positions of healthy individuals [1, 2]. The normal flora or microbiota inhabiting different tissues and organs play vital functions in the human health and diseases [3].

With the advent of sequencing and metagenomics, a new emerging science came to light 'Forensic microbiology or Microbial Forensics'. Forensic microbiology is a developing science defined as the application of scientific approach to the examination and analysis of microbiological evidence associated with bioterrorism, biocrimes, hoaxes, or the accidental release of microbiological agents [4, 5]. Forensic microbiology was first recognized through the *Bacillus anthracis* or Anthrax attacks at the USA postal service in 2001 where the United States was not prepared to illustrate the biological data related to the bioterrorist attack [6, 7]. As a result, microbial forensics was developed to provide a vigorous forensic ability to support the investigators in biocrime and bioterrorist attacks. In 2009, forensic microbiology outlined the source of *Bacillus anthracis* from injectional anthrax circumstances between heroin consumers in Scotland [8]. Later, it traced the source of the Haitian cholera epidemic following the 2010 earthquake and the 2011 *Escherichia coli* O104:H4 outbreak in Germany [9, 10]. Today, microbial forensics has been expanded from biocrime and bioterrorism to include the human microbiome identification, especially microbiome associated with the host after death defined as thanatomicrobiome [11] as well as Post-Mortem Interval PMI estimation [12, 13]. In addition, microbial forensics is used in tracing transmission of some microbes and viruses such as human immunodeficiency virus HIV and hepatitis C virus HCV in sexual-assaults or other related crimes [14, 15]. The forensic prospective of microorganisms becomes obvious due to developments in molecular biology and genetics in addition to numerous areas including microbiology, forensic science, epidemiology as well as the law enforcement and public health societies.

The aim of the present review was to provide better understanding of forensic microbiology as an emerging field. This review is exceptional and different from previous studies since numerous emerging applications of microbiology during forensic investigations were discussed. Among these, the review focused on the microbial fingerprints for individual identification and geolocation. Also, post-mortem microbiome PMM together with the thanatomicrobiome as a tool for forensic investigation were considered. Moreover, the

use of microbial forensic for Post-Mortem Interval PMI estimation and the importance of microbes in investigation of Sudden Unexpected Death in Infancy SUDI involving infectious diseases were discussed. In addition, Substance Use Disorders SUD and Gut Microbiota–Brain Axis were highlighted.

## II. MICROBIOMES FROM SAMPLING TO FORENSIC APPLICATIONS

In forensic microbiology, the microbiome analysis pathway include proper sample collection (stool, saliva, skin) followed by Polymerase Chain Reaction PCR, sequencing and analysis using sophisticated software as the main applications of microbiomes in forensic science. Fig. 1 illustrates all steps involved in the microbiome analysis pathway. The sample collection or sampling can be realized from different microbial resources including human, environment or any object from crime scene. This step is very critical since sample contamination, time of collection as well as the storage of these samples could be challenging. After sampling, various methodologies can be applied for the sequencing for microbiomes quantification. Over the last 15 years, sequencing and bioinformatics advances allowed the characterization of microorganisms for a diversity of human forensic applications. Among the common sequencing technologies, 16S-ribosomal sequencing 16S rRNA, Whole-genome sequencing WGS, Next-generation sequencing NGS are widely used in microbial forensics. The widely common methodology used for examination of the microbial taxonomic repartition in the sample is the targeted sequencing of the variable regions of 16S ribosomal RNA gene or 16S rRNA. The comparison of whole 16S rRNA gene sequences has been broadly used to create a taxonomic connections among prokaryotic strains. 16S rRNA gene sequencing has wide applications in forensic science including the biological samples identification, individual microbiome identification (personalized microbiome), postmortem interval estimation as well as geographical locations. Recently, researchers have examined the soil bacterial profile through 16S rRNA gene and linked the soil with their location of origin [16, 17].

Due to the improvement of sequencing technologies and reduction of sequencing costs, Whole Genome Sequencing WGS methods have been developed to analyze the human microbiome rather than 16S rRNA gene sequencing. In addition to 16S rRNA and WGS, Next-generation sequencing NGS has been used to sequence total DNA extracts from any sample allowing the identification of different bacterial taxa and strains. NGS does not require any additional fragment analysis and directly generates sequence information in comparison to traditional techniques. NGS used to analyze a microbial community have several applications in forensic science including the sample's origin source, personal identification as well as site body [18].

Following the microbial DNA sequencing, the microbiome analysis addresses several forensic applications including personal identification, geolocation as well as thanatomicrobiome, post-mortem interval PMI estimation and cause of death including the sudden unexpected death (Fig. 1).

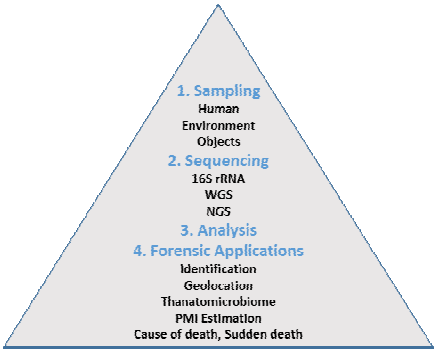


**Fig. 1.** Microbiome analysis pathway.

The analysis of microbial evidences include several steps: sampling, sequencing, analysis and forensic applications. All forensic applications were discussed in next sections (A; B; C; D; E).

### *A. Microbial Fingerprints for Identification and Geolocation*

When comparing microbial DNA to human DNA, microbial DNA is more resistible than human DNA due to the fact that microbial DNA is protected by the cell envelope considered as a biological barrier protector from the degradation. Hence, the importance of microbial evidences for forensic examination.

In forensics, the identification of a single individual from a population through personal microbiomes has been recognized by numerous researchers who have demonstrated the potential use of bacterial profiles to identify an individual and link an individual to an object [19, 20].

In addition, in a sexual assault crime, an individual's microbiome left on the victim's body can link a suspect to a crime and a crime to a specific site in the suspect's body. In fact, the human microbial groups are categorized depending on their taxonomic, metagenomics, and metabolic diversity which are variable depending on human body sites. Dobay *et al.*, (2019) has investigated the microbiome of six different body sites through sequencing studies of the 16S rRNA gene and proved that bacterial markers can identify body fluid and tissues in forensic cases [21]. Among the microbiomes human body site, the human oral cavity contains more than 700 bacterial species which can be analyzed through 16S rRNA gene sequence comparison with a predominance of *Streptococcus* [22, 23]. Kennedy *et al.*, (2012) showed that microbial DNA amplified by PCR from bite marks and teeth can offer supporting data in the identification of suspects by collecting bite-mark and teeth swabs from 16 participants and analyzing their microbiomes by sequence comparison of streptococcal DNA [24]. Moreover, if bite-marks are found on the victim's body, the microbial fingerprints in saliva, especially Streptococcus salivarius can help in identifying the suspect [25]. The microbial DNA of bacteria, fungi, viruses in human saliva combined with other saliva biomarkers such as proteomic and genomic biomarker can not only identify a person but also provide data about the lifestyle of an individual and the consumption of any drug thus helping the forensic analysis [26].

In addition to applications of microbial forensics in individual identification, researchers have proposed the forensic prospective of the microbial evidences left by individuals on physical surfaces such as the mobile phones that carry the individual microbial signature as well as any keyboard [27]. In fact, forensic microbiology can not only link microbial evidences to an individual but also to a specific geographical area since microbial communities differ between different geographical areas [28]. The microbial evidences vary upon locations depending on environmental conditions such as soil, climate as well as microbe's host. Knowing the specific microbiomes component of the host, forensic examiners can attribute microbial evidences to special geographic area [29]. Some researchers have established a relationship between microbiome samples gathered from body sites and hosts' country of origin [30]. Other researchers have highlighted the importance of gut microbiome in personal geolocation [31].

*B. Post-Mortem Microbiome PMM including Thanatomicrobiome as a tool for forensic investigation*

Each individual has numerous microbial flora associated to skin and mucous membrane. This flora appears after birth and remains until death. A human body contains about 1013 cells and 1014 bacteria, 10 times more microbes than cells [3]. In living person, the immune system inhibits the presence of microbes in internal organs and body fluids. However, after death, due to cessation of blood movement and immunological barriers, microbes move from mucosal membrane into blood and tissues before necropsy [2, 3]. The speed of microbes' proliferation throughout the dead body is altered by environmental conditions including temperature, humidity and absence or presence of wounds which can enhance microbes' entry [32]. After death, the microbiome is known as 'Post-Mortem Microbiome' or PMM. PMM can be divided into thanatomicrobiome defined as microbial succession in decomposing remains involving microbes present in internal organs and biological fluids after death, and epinecrotic microbes present at the surface of decomposing residues [33, 34]. PMM is considered as a useful emerging biomarker to help the medico-legal and forensic examiner during investigations [3]. In forensic cases, when deaths are not witnessed or when inconsistent crime has been reported, Thanatomicrobiome can provide a crucial data regarding interactions between microorganisms and their mammalian hosts [35]. Recently, researchers highlighted the concept that the nature and quantity of microbes in human thanatomicrobiome are variable depending on organs, time and temperature [36] and that the conditions of death including time and location can be estimated through analysis of thanatomicrobiome [37].

Javan *et al.,* (2017) examined the PMM of a cohort of 45 corpses in fresh and bloat stages and showed that 95% of post-mortem liver and spleen involved Clostridium spp, a gram positive anaerobes and symbiotic bacteria located in healthy intestines. The internal locations were studied by two hypervariable regions of 16S rRNA gene. They revealed that V4 and V3-4 hypervariable testing support individual representative assessments of thanatomicrobiome [35]. In other thanatomicrobiome studies of post-mortem human samples, researchers have discovered a number of *Clostridium* spp., including *C. sordellii, C. difficile, C. bartlettii, C. bifermentans, C. limosum, C. haemolyticum, C. botulinum*, and *C. novyi* by using next-generation sequencing of 16S rRNA gene amplicons [38].

*C. Post-Mortem Interval PMI estimation*

Post-mortem interval PMI or Time Since Death TSD, time elapsed between death and discovery of the corpse, is one of the most difficult tasks in forensic investigation. For many years, PMI has been estimated through examination of physical changes occurring after death, named post-mortem changes. These changes including livor mortis, rigor mortis, algor mortis, saponification, putrefaction and skeletonization are considered as early post-mortem changes and cannot continue to late post-mortem, thus providing an approximate PMI estimation [39]. Recently, numerous approaches have been proposed for estimating PMI. Post-mortem change of microbial communities has been suggested as a forensic tool for PMI estimation [40]. Several researches have showed post-mortem changes in microbes during mammalian decay related to skin, gastrointestinal and rectal areas, oral, nasal and ear cavities as well as cadaver-associated soils [41-43]. PMI estimation was realized through different model-based statistical approaches and machine learning approaches [41]. Machine learning is considered as an effective tool to estimate PMI through models construction using post-mortem changes of entire microorganisms' community [44]. The quantification of each microbial taxa can be predicted through marker gene including 16S rRNA, 18S rRNA. Different regression models were used to show the relationship between microbiome composition and decomposition time thus helping in PMI estimation. The Random Forest regression, a common used approach for PMI estimation, constitutes a group of machine learning technique suitable for a set of decision trees on subsamples of the data set and combines all results to develop regression accuracy [45, 46]. Belk *et al.,* (2018) predicted PMI through Random Forest regression models on diverse corpse and environment samples including grave soil, torso's skin, head's skin, gene markers including the 16S rRNA genetic marker and taxonomic levels [47].

*D. Relationship between isolated microbes and infectious cause of death in Forensic Microbiology*

In Forensic microbiology, post-mortem blood and internal organs culture techniques can be helpful for recognizing a number of microbial indicators of particular types of death including the sudden death. Depending on infectious syndrome's evidence, a microbe isolated after death can be measured as a contributing cause of death. Christoffersen (2015) conducted microbiological tests for 42 autopsies and found that fifty one microorganisms, mostly composed of bacteria, where present in 37 cases and that in 19 cases, these microorganisms were considered as cause of death by comparing to cases microbiome ante-mortem [48].

Sudden death is a non-anticipated, non-violent incidence in which death happens promptly or after six hours of symptoms' appearance, in non-hospitalized individuals in regular life activities. Investigating sudden

death cases is very challenging and has been mainly linked to primary cardiac death or abrupt respiratory decline. Recently, the benefits of microbiological investigations in all cases of Sudden Unexpected Deaths in Infancy SUDI were discussed by several researchers and a number of SUDI cases were linked to infectious disease [49]. Fernández-Rodríguez *et al.,* (2019) proposed a general PMM collection techniques in various situations including sudden deaths by reducing the use of invasive autopsy and suggested the use of PMM in PMI estimation [50].

Rambaud *et al.*, (1999) examined PMM on 57 cases of SUDI aged from 1 to 57 months and found that cultural analysis on tissue and blood samples showed both bacteria mainly *Escherichia coli*, and viruses, especially Enterovirus C [51]. To investigate 121 SUDI infants less than 2 years, Sehgal *et al.*, (2019) analyzed the microbiological and virological evidence in organs and biological fluids. They showed that cultures were positive for pathogens in 49% cases, for postmortem flora/non-potentially pathogenic microbes in 73% cases, for both postmortem flora and pathogenic microbes in 32% cases, and negative in 10% cases [52]. Limelette *et al.*, (2013) studied the SUDI of four and a half month-old baby during his sleep and showed that microbiological analysis revealed an infection of respiratory syncytial virus complicated by a bacterial infection due to *Haemophilus influenza* [53]. Recently, a retrospective study in Ontario Canada considered 7506 unexpected deaths between Jan 2016 – Dec 2017 and showed that 6% of unexpected deaths were due to infectious diseases including bacterial pneumonia (most common infectious syndrome), peritonitis, myocarditis, pyelonephritis, *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella* spp, and *Escherichia coli* [52]. Sehgal *et al.*, (2019) concluded that infectious diseases can lead to unexpected death mainly due to bacterial pneumonia with high proportion of gram-positive bacteria.

In addition to bacteria, viruses have been proposed as a potential marker in SUDI. Fernàndez-Rodrìguez *et al.*, (2006) investigated 64 cases of SUDI. They analyzed different organs and body fluids using Polymerase Chain Reaction PCR, serology, viral culture and various microscopy techniques. A number of viruses were identified including adenovirus, HHV-6, CMV, RSV, EBV and Influenza A. In accordance with the pathological examination, the cause of death was due to a virological infection only in two cases [54].

Eleven cases of SUDI in comparison to control samples were analyzed by using quantitative real-time PCR for virus detection including HHV-6, EBV, and CMV. The findings proposed that herpesvirus infections mainly caused by EBV and HHV-6 could be associated with some SUDI cases [55]. The role of post-mortem virology in establishing the cause of death was highlighted in 546 SUDI cases performed between 1996 and 2005 (Weber *et al.,* 2010). Viruses were identified as cause of death in 4% of cases including enterovirus, RSV, HSV, CMV, adenovirus and influenza virus [56].

Moreover, some researchers consider that SUDI kids have a stimulated immune system by the presence of several viruses including rhinovirus, cytomegalovirus, respiratory syncytial virus, *Bordetella pertussis*, enterovirus, and parvovirus as well as the presence of bacteria including *Staphylococcus aureus*, *Clostridium difficile*, and *Escherichia coli* which can lead to death [57, 58].

*E. Substance Use Disorders SUD and Gut Microbiota–Brain Axis*

Forensic Psychology is defined as the application of psychology to the court of law. In forensic psychology, understanding the psychological mechanisms linked to Substance Use Disorders SUDs including alcoholism and drug addiction is crucial for forensic examiners.

Researchers have showed that the gut microbiota-brain axis, bidirectional communication between gut microbiome and brain, influences SUDs [59, 60]. In fact, the microbiome and metabolites produced by gut microbes can provide evidence of individual's drug addiction. Concerning the alcohol use disorders, Gorky and Schwaber (2016) proposed a mechanism explaining the gut microbial perturbation by alcoholism showing the way this perturbation contributes to neuroinflammatory effects on emotion due to increase in the pathogenic colonies [61].

Moreover, the gut microbiota–brain axis have been associated to neurodevelopment and neuropsychiatric disorders including anxiety, depression [62, 63]. Researchers have shown in animal models that gut bacteria affect the Central Nervous System CNS homeostasis by altering the gene expression and neurochemical metabolites thus affecting behaviors and performance. A spectrum of behavioral changes have been recognized in Germ-Free mice in comparison to conventionally raised mice [64]. Recently, researchers have started to examine in human the relationship between the gut microbiota and human neurodevelopment.

Despite all researches, the role of gut microbiota-brain axis in SUD should be further explored. The National Institute on Drug Abuse NIDA has funded 4 millions $ to Jackson Laboratory JAX researchers to explore this field.

## III. DISCUSSION

During the last decades, human microbiome was proposed as an emerging tool in forensics along with conventional evidences that can be found in a crime scene. In the present review, we explored the microbiome analysis pathway from sampling to forensic applications insisting on the importance of an appropriate sample collection followed by different approaches used for the sequencing for microbiomes quantification. Although, the development of sequencing technologies, the costs remain the major drawback to consider microbes' analysis in forensics. We discussed diverse applications of microbiology in forensic science. Among these, microbial fingerprints for identification and geolocation were documented by several researchers who showed that a single individual can be identified from a population through human microbiomes [19, 20, 28]. Moreover, although the microbiome proliferation after death is affected by several environmental factors, PMM using thanatomicrobiome can be an important tool for forensic investigation [33, 34]. In addition, microbial forensics can be helpful in PMI estimation where several model-based statistical and machine learning approaches were considered [43]. Researchers have

also considered that isolated microbes and/or viruses after death can be helpful in estimating the cause of death focusing on the Sudden Unexpected Deaths in Infancy SUDI [49]. Finally, the role of gut microbiome-brain axis in investigating the mechanism implicated in cases involving drug addiction was discussed [62].

Recently, the novel virus Severe Acute Respiratory Syndrome SARS2 causing the coronavirus disease COVID-19 has reached 185 countries with an increasing number of deaths [65]. Some researchers have suggested a summary of recommendations for forensic pathologists and odontologists who perform an identification of dental autopsies of unidentified human remains with unknown medical history. These unidentified human remains could be suspected Sars-Cov2 positive deaths. Hence, forensic practitioners should increase protection awareness in the medicolegal death investigation of infectious diseases.

## IV. CONCLUSION

Altogether, this review allow a better understanding of the role of microbiology in medico-legal practice and forensic examination.

## V. FUTURE SCOPE

Although, all mentioned applications, microbial forensics is still not well explored and forensic examiners will prefer to use biological evidences including hair, blood, saliva, etc. where DNA fingerprinting is obvious. Hence, researches should highlight the importance of microbiology in forensic investigation. Also, the significance of SARS 2 novel virus in identification of individuals in addition to several applications should be explored in the future in the field of forensic microbiology.


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