Forensic Science International: Reports 9 (2024) 100355

Contents lists available at ScienceDirect

Forensic Science International: Reports

journal homepage: www.sciencedirect.com/journal/forensic-science-international-reports

Review

Emerging methods of human microbiome analysis and its forensic applications: Review

Amy Arabella Singh, Moses Okpeku *

University of KwaZulu-Natal, Westville, University Road, Westville, Durban, KwaZulu-Natal, South Africa

ARTICLE INFO

Keywords:
Geographical identification
Personal identification
Postmortem analysis
Sexual assault cases

ABSTRACT

The human microbiome comprises trillions of microbial cells, primarily bacteria, found in places such as the gut, oral cavity, and skin. The human microbiome holds significant potential in forensic investigations, with important applications in post-mortem analysis, geographical identifications, sexual assault cases, and personal identification. The human microbiome displays significant variations, which can be utilized to distinguish individuals based on their distinctive microbial signatures, thus facilitating identification. Microbiome composition varies geographically and can be explored for identification of individuals' origin or location. In addition, changes observed in microbial composition during different stages of decomposition can provide valuable insight into the time and location of death which can assist in forensic investigations. However, the use of microbiomes in forensic investigation is still developing and under-utilized, especially in the developing world. This article reviewed emerging microbiome analyses, their strengths, limitations, and potential for future research in forensic investigations. In particular five microbiomic forensic applications were looked at; postmortem analysis, geographical identification, sexual assault cases, bite marks and personal identification. Despite challenges and limitations associated with storage sensitivity, limited samples, and lack of standardization, the use of microbiomes in forensic investigation is quite promising. However, future research should focus on more extensive and standardized studies to overcome these challenges and fully harness the potential of the human microbiome in forensic investigations, enabling its practical application in various forensic scenarios.

Introduction

The human microbiome consists of the genes of trillions of microbial cells, usually bacteria, often found in places such as the gut, oral cavity and skin [1]. The actual definition of the human microbiome has been difficult due to confusion in terminology. This is because microbiota and microbiome are often used interchangeably but there is a distinction; while microbiota represent the microbial organisms found in the human body, microbiome is the gene catalogue for the microbiota [1]. There are also different microbe species and quantities found in different locations of the body, which was first discovered by Antonie van Leewenhoek in the 1680s [2]. The human microbiome helps in the development and maintenance of various functions and operations [3]. Specifically, it helps in maintaining homeostasis in the host, host nutrition, development of the immune system and resistance to colonization of pathogenic microbes [4].

Through culture-independent methods and phylogenetics, scientists have been able to catalogue and compare microbe communities. This has evolved into a helpful tool in forensic science, especially for forensic investigations where less DNA evidence is present [5]. DNA fingerprinting has been very important for forensic science since the 1980s [6]. Variable number tandem repeats (VNTRs) and minisatellites were the most common markers used in the 1980s and are still used, to a lesser degree, today [7]. As technology continues to advance, new DNA fingerprinting techniques have been developed such as short tandem repeats (STRs) typing and mitochondrial DNA (mtDNA) typing [8]. These techniques have reduced the cost of DNA fingerprinting and as such has expanded this practice [9].

An emerging field of the human microbiome holds the potential to enhance forensic investigations such as by providing information that can be used to identify the origin of unknown samples by identifying unique strains of microbes that characterise every individual [10,11]. Understanding microbial signature analysis techniques is crucial for forensic use in this emerging field, where further research is needed on aspects like deposition time, standardization, privacy, and technology development. This article comprehensively aims to review current and

* Correspondence to: University Road, Westville, Private Bag X 54001, Durban 4000, South Africa.
E-mail address: OkpekuM@ukzn.ac.za (M. Okpeku).

https://doi.org/10.1016/j.fsir.2024.100355
Received 12 October 2023; Received in revised form 8 January 2024; Accepted 17 January 2024
Available online 22 January 2024

emerging microbiome analysis methods, their strengths, limitations and future research needs.

Bacteria make up the bulk of the human microbial communities but archaea, viruses and eukaryotes are also present in smaller quantities [12]. There is between 500–1000 bacterial species alone at any one time in the human body [13]. Bacteroidetes and Firmicutes make up the bulk of the microbial community in adult humans [14]. The Methanogenic archaea specifically *Methanobrevibacter smithii,* eukaryotes, such as yeast types, and phage viruses are present [15]. An accumulation of the genotypes of all these species make-up a human microbiome that is exceptionally larger than the native human genome [16]. The microbiome of humans is highly variable. Not only is there variability between different individuals but there is also variability withing the same individual over time and across different body sites. Even in cases where the genetic makeup of individuals are identical such as in monozygotic twins, environmental factors play a significant role in shaping the microbiome which will emphasize the individuality of the microbiome [14].

An emerging field of the human microbiome holds the potential to enhance forensic investigations such as by providing information that can be used to identify the origin of unknown samples by identifying unique strains of microbes that characterise every individual [10,11]. Understanding microbial signature analysis techniques is crucial for forensic use in this emerging field, where further research is needed on aspects like deposition time, standardization, privacy, and technology development. This article comprehensively aims to review current and emerging microbiome analysis methods, their strengths, limitations and future research needs.

Methodology

Search condition: A systematic review of the human microbiome in relation to forensic science was conducted following PRISMA guidelines. articles were selected from the Google Scholar, Web of Science and Scopus databases between June and July 2023, using following search words (“forensic” or “forensics”), (“microbiome”, “microbes”, or “microbial”), (“analysis”, “identification”, “cases” or “techniques”) and (“geographical”, “personal”, “postmortem”, “marks”, “assault”) as keywords. A total of 371 articles were found.

Exclusion criteria: Articles with one or more of the key words used but not related to the human microbiome were excluded. 261 articles that failed the exclusion criteria were excluded from the review.

Inclusion criteria: The selection process involved a meticulous assessment of each study’s abstract and title, followed by a readthrough of the article in its entirety. If the paper contained information relating to the review title with focus on human microbiome, it was selected for inclusion. Once selected, the article title, abstract and citation were added to a table in Microsoft Word 2019 while the articles themselves were downloaded and stored.

Human microbiome variation

The human microbiome is highly variable between individuals due to factors such as lifestyle, geography, body region, and diet [11]. The microbiome is unique to each individual, with between 80–90 % difference between individuals [17]. There are substantial differences between free-living microbial communities and those within the human body which shows a co-evolution of the human microbes with the human ecosystem [18]. The human genome has genomic variation but overall, individuals share about 99.9 % of the same genetic material [19]. The variation present in the human microbiome is due to how humans develop, with the first microbes usually colonizing a human shortly after birth. A vaginal delivery will result in the infant having a microbiome similar to that of its mother’s vagina whereas a Caesarean section delivery will result in the infant’s microbiome resembling that of human skin [20]. After birth, the human gastrointestinal tract will continue to be colonized over an individual’s life, most frequently during the first few years of life [21]. Research has found that a person’s lifestyle influences their microbiome community including their diet, location, health status, if they have pets, smoke and the people the form intimate relationships with [22]. Despite the variability, the emerging methods of human microbiome analysis have shown potential in forensic applications, such as shedding light on processes such as decomposition, cause of death, location of death, and connecting a suspect with an item or place. There are, however, still concerns regarding the application of this new field, such as microbiomic variation, classification challenges, limited biomass and lack of standardisation that still need to be addressed before microbiome analysis becomes a frequently used forensic analysis technique [23].

The human body is inhabited by a vast number of microorganisms, including bacteria, archaea, fungi, protists, and viruses. Fig. 1 shows some of the microbial species found in the human body. This does differ, based on many factors such as lifestyle, geography, body region and diet. For example, *Streptococcus* is found in higher amounts in the oral cavity of some humans [24]. A higher consumption of carbohydrates leads to an increase in certain species of fungi in humans such as *Candida albicans* whilst a diet high in protein has an almost negative abundance of the fungal species [25,26]. Some of the most prevalent fungal species are *Saccharomyces cerevisiae*, *Malassezia restricta* and *Candida albicans* [27]. Viruses also inhabit the human body and will differ depending on the abovementioned factors. Cutaneous β and γ human papillomaviruses are examples of viruses that can be found on human skin [28]. Certain protozoan phyla namely Blastocystis, Entamoeba and Enteromonas have been found on most populations around the world with sample evidence from North and South America, Europe and Africa [29].

The human microbiome interacts with the external environment, and when contact occurs with external surfaces, microbes from the skin can be transferred onto these surfaces [30]. The microbe composition on the touched surface will resemble the microbe composition specific to the individual that touched the surface. It has been shown that it is possible to indicate who touched the surface and with what finger or body part they touched the surface with, with 95 % accuracy [31].

The variation in the human microbiome between individuals allow for its analysis in the forensic science field, which can shed light on processes such as decomposition, cause of death, location of death, and connecting a suspect with an item or place [32]. There are, however, still concerns regarding the application of this new field such as microbiomic variation, classification challenges, limited biomass and lack of standardization that still need to be addressed before microbiome analysis becomes a frequently used forensic analysis technique. The emerging methods of human microbiome analysis have shown potential in forensic science applications, such as supporting crime scene investigations, human identification, detection of body fluids and geographic location of individuals.

Forensic application of the human microbiome

Advancements in next-generation sequencing methods and cost reductions have allowed for phylogenetic mapping and showcasing of the variation that exists within the human microbiome [33]. Research into this started with a focus on small subunit ribosomal sequences, and utilized traditional typing techniques [34]. The abundance of specific microbial genera can help predict features about an individual, most notably their sex, with 79 % accuracy [35]. This works best in enclosed spaces such as a bedroom because there is less influence from environmental microbial communities. The microbiome signature is strongest immediately after the contact but can still be sampled months after [36]. The potential use of microbial DNA in a forensic application utilises samples from diverse locations such as hair and skin. Analysis looks at the distinct signatures of the microbiome, forensic indicators and microbial-derived data. Forensic indicators include time or death and country of origin [22].

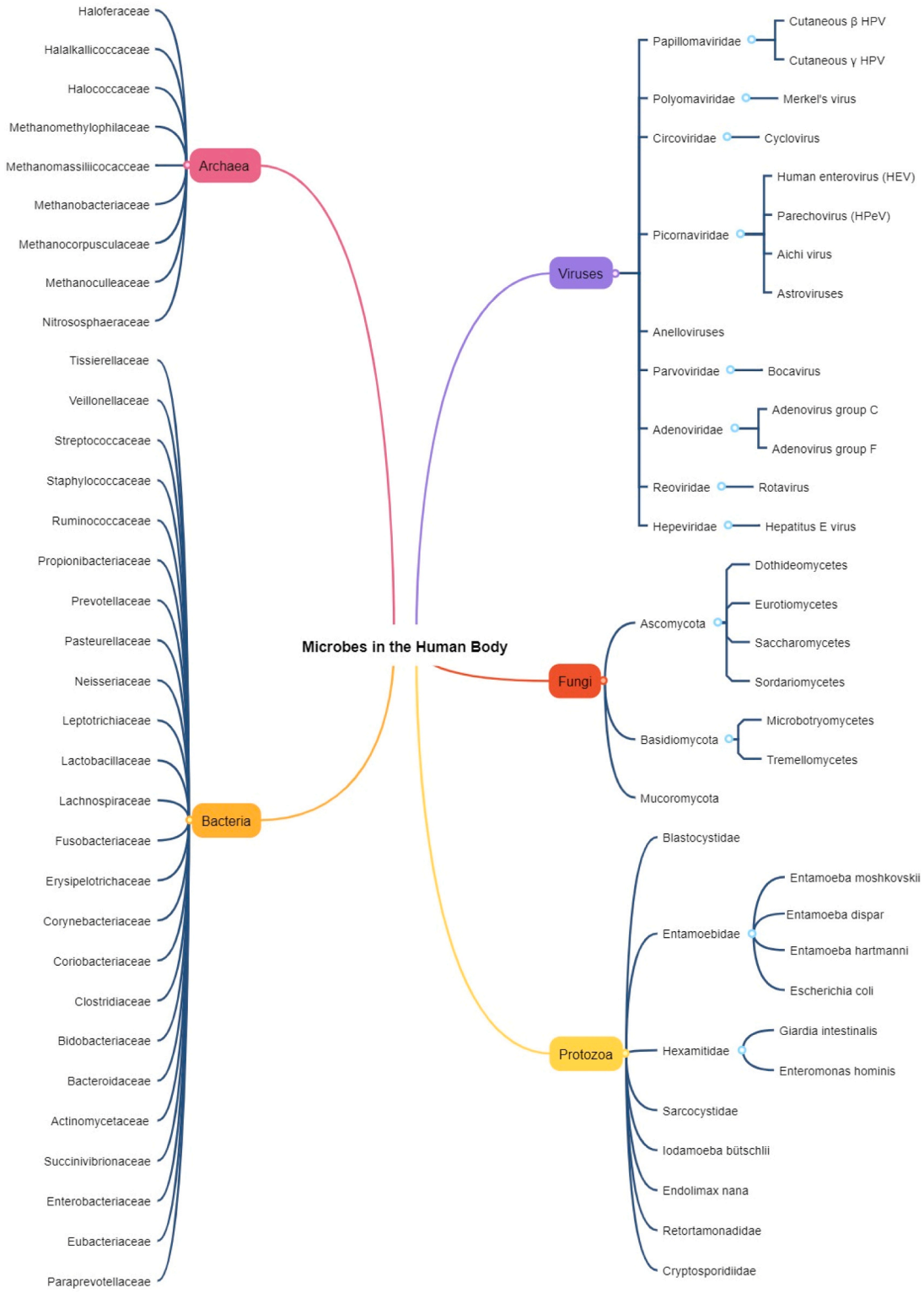

Fig. 1. Generalized overview of microbial diversity in the human body [24]; [25]; [26]; [27]; [28]; [29].

Microbiomes that have been collected from surfaces such as cell phones show a partial microbial signature of their user and partially of the environment [37]. Scientists can differentiate between the human and environmental microbiome and this creates possibilities of linking a suspect to a crime scene [38], especially when no DNA evidence is found. Something that helps scientists discriminate between both microbiomes is an understanding that most microbial species that are found in humans have been sequenced whereas about 50 % of the environmental microbiome has not [39].

The human microbiome can be used to study ethnicity, as the microbiome fingerprint of an individual can be traced to the genomic background of the individual's ancestors [40]. Each geographical location also has its own unique microbial composition, even specific to cities and towns due to climate variation, energy sources and human metabolism. Therefore, the microbiome can be used to link a person with a location, which has the potential to help in events such as human

trafficking [5,41]. Even buildings have unique signatures that can connect a suspect to a city and even the specific building [42]. The gut microbiome is usually analysed for geographic analysis, and this sample can be collected from toilets [43]. Saliva and skin can also be used for geographic analysis [22]. The human microbiome can also be used in the case of a decomposing body to identify the time of death or postmortem interval (PMI) of the person. There are certain microbes that appear at different times post-death and also in different locations of the body [44]. Models have been developed to estimate the time of death by looking at the distribution and abundance of these microbes [45].

Human microbiome analysis

Microbiome applications for forensic analysis can be grouped into five categories, postmortem analysis, geographical identification, person identification, sexual assault cases and bite marks.

Postmortem analysis

After death, the immune system weakens, allowing microbes to invade the body. Changes to the body that occur after death influence microbial community composition [32]. As decomposition progresses, aerobic organisms are replaced by anaerobic organisms due to oxygen depletion and gas accumulation [44]. There are five stages of decomposition; fresh, bloat, active decay, advanced decay and dry. The *Clostridium* species is the dominant species during decomposition of a body [46]. The advanced decomposition stage is composed of soil microorganisms, due to the increase in carbon and other nutrient levels. The dry remains stage is mainly associated with spore-forming microorganisms, allowing colonization of new ecological conditions [32]. The changes that occur to the body after death are predictable [47]. These predictable changes in microbial composition after death can help determine the PMI, location and cause of death [48]. The Random Forest method is an optimal approach for the analysis of PMI [49]. Many studies that look at postmortem microbial community composition use animals such as pigs and rabbits [50,51]. Different studies have analysed various anatomical sites to estimate PMI [52–57].

Hu et al. [52] used the Random Forest algorithm to look at human intestine microbiome data. The study looked specifically at samples from the appendix and colon to see if there is a difference in microbiome composition between these two locations. It also used the Random Forest algorithm to generate a PMI estimation model. The study found that there were differences between the colon and appendix microbiomes, and they were sometimes more significant than differences between individuals. The appendix was shown to have more diversity and more biomarkers than the colon after death, onetheless, there were not significant differences in diversity between the appendix and colon at the same PMI. The appendix looks like a better region to sample when determining PMI. To validate the use of the appendix as a sampling site for PMI estimation, the researchers analysed the succession patterns of the appendix microbiome after death. They observed predictable changes in the abundance of certain microbial taxa. Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria were found to be predominant in the appendix during decomposition, with Firmicutes becoming the most abundant species over time. The abundance of Proteobacteria decreased slightly with increasing PMI. The study also employed the Random Forest algorithm to identify significant biomarkers associated with corpse decomposition. Several potential characteristic biomarkers were detected at different intervals, suggesting their usefulness in estimating PMI. The accuracy of PMI prediction, however, decreased for longer PMIs, possibly due to a smaller sample size in the late PMI period.

The oral microbial composition changes during the different stages of human decomposition, and distinct bacterial communities are observed in each stage. Adserias-Garriga et al. [53] conducted a study that looked at the oral microbial composition of the palate, tongue, cheek, dental surfaces and internal mucosa at each stage of decomposition. The results showed that the microbial composition changed at each stage of decomposition. For example, the fresh stage had indigenous oral microbiome, whilst the bloat stage had Peptostreptococcaceae, Enterococcaceae, and Bacteroidaceae. This shows that in postmortem analysis, by looking at the microbes present, investigators can determine which stage of decomposition the body is at and also determine an estimated time of death.

A study by Pechal et al. [54] looked at whether the microbiome shortly after death can provide indication of an individual's health status. Samples were collected in postmortem intervals between 24 to 73 h. The results showed that antemortem microbes can be used to indicate health status however the longer the postmortem interval, the less accurate the results. The authors also stated that the interval can decrease in conditions such as extreme temperatures which affect microbe composition.

Microbiome analysis of frozen bodies undergoing thawing reveals that during this process, microbial diversity increases, with specific changes observed in different anatomical sites. Pechal et al. [55] investigated two bodies from a freeze and found that there was an increase in the microbe diversity when the bodies were thawed, specifically in the regions of the nostrils, eyes and rectum. This has important implications for forensic science, as it suggests that the microbiome can be used to determine the time of death and other factors even in frozen bodies. Reports, however, on how freezing human-related samples affect the microbiome have been varied, and it is essential to examine freezing effects immediately after the freeze-thaw process and prior to decomposition. This is especially important after the publication of a case study that showed a shift in post-mortem microbial community abundance and structure during the thawing of two paediatric cases. Overall storing bodies frozen preserves the composition and abundance of their microbiome, and bacterial community composition in the rectum post-mortem is significantly different from other anatomical areas, regardless of freezing [58].

Studies have shown that sex differences in postmortem microbiomes can be identified by looking at variations in microbial abundance and composition between male and female cadavers [54,56]. An investigation encompassed diverse anatomical regions including the blood and heart, and revealed that alterations in microbial communities were contingent upon both the corpse's sex and the PMI [57]. Bell et al. [56] conducted research that specifically explored cardiac tissue in 10 cadavers with PMIs ranging from six to 58 h, and it substantiated the observed distinctions in microbiomes between the male and females corpses. Notably both studies identified an elevated presence of *Streptococcus* in males and an increased prevalence of *Pseudomonas* and *Clostridium* in females. These investigations underscore the potential of microbiome analysis for estimating PMI and gaining insight into the shifts occurring within microbial communities during the process of decomposition.

Geographical identification

The human microbiome varies depending on the location of individuals [59], which is due to factors such as diet, lifestyle, and level of industrialisation. This variation in microbiome composition can be used in forensic analyses to catalogue every region around the world. Analysis of microbiomes from different geographical locations has shown detectable geolocation signals between populations, even for populations living within the same city [60].

Research conducted by Walker et al. [60] revealed notable distinctions among populations when examining individuals from 12 cities across seven countries. Principal Component Analysis (PCA) revealed that samples from the same city clustered closely together. Machine learning analysis, particularly using Random Forest (RF) and Support Vector Machine (SVM) classifiers, proved effective in predicting the city of origin for unknown samples. This finding has promising implications

for forensic applications and shows that the microbiome can be used to cluster individuals based on geographic location.

Brinkac et al. [61] explored the realm of human hair shaft microbiota. Hair samples were collected from various body areas of adult participants residing in Maryland, California and Virginia. This study included both male and female participants from diverse ethnic backgrounds. Each individual contributed multiple hair samples, resulting in a total of 42 samples from the scalp and 32 from the pubic area. The profiling of the microbiota involved targeting the V4 region of the 16 S rRNA gene.

The research revealed that hair shaft communities differ from those found in hair follicles and are more akin to the surrounding skin. This has implications for forensic applications involving scalp hair, as hair characteristics such as length influence the diversity of microbial communities. Longer scalp hair shows higher alpha diversity than shorter hair, a pattern not observed in pubic hair. The research revealed that *Staphylococcus*, commonly found on the skin, is prevalent in hair, while *Propionibacterium*, a typical skin and hair follicle dweller, is conspicuously absent. This absence may be due to the hair shaft's unfavourable environment, characterized by higher oxygen levels and lower sebum content than the hair follicle. This variation complicates the assessment of hair microbiota variability and poses challenges for forensic applications involving scalp hair.

Additionally, research has uncovered geographic variations in hair microbial communities, with scalp hair exhibiting more potential for geolocation prediction compared to pubic hair. The interaction, however, of scalp hair with the environment suggests that any geographic signature might be transient or linked to environmental and lifestyle factors. This distinction underscores the value of hair microbiota in forensic contexts, with scalp and pubic hair serving specific roles in scenarios such as geolocation, gender/individual identification, or biomarker detection. More research is needed with expanded sample sizes and conducting longitudinal studies which will enhance our understanding of the utility of both hair types in forensic investigations.

Nagasawa et al. [62] conducted a study to determine the origin of corpses using microbial geolocation. This study focused on the presence of *Helicobacter pylori* (*H. pylori*) DNA in gastric mucosal samples obtained from cadavers during forensic autopsies. Gastric mucosa tissues were collected from 177 cadavers, 123 male and 54 female. The primary nationality was Japanese. *Helicobacter pylori* is found in about 50 % of the global population. *H. pylori* DNA was successfully detected in more than 50 % of forensic autopsy samples, even in cases of severe cadaver decomposition. Samples were collected from cadavers with external causes of death such as burns or drowning, and *H. pylori* DNA was still detectable.

The study employed phylogenetic analysis of the *vacA* gene to estimate the geographic origin of cadavers. It successfully classified cadavers into three major clusters: East Asian, Western, and Southeast Asian, based on *H. pylori* strains detected.

The detection rate of *H. pylori* DNA was compared to other viral markers used for determining the geographic origin of unidentified cadavers. *H. pylori* DNA detection had a lower prevalence than varicella-zoster virus but higher than John Cunningham virus and Epstein-Barr virus. The study acknowledges limitations in the certainty of geographic origin determination based solely on *H. pylori* strains. It recommends combining *H. pylori* typing with other microbial marker-based methods for more accurate results. This method could be useful for investigative teams seeking comprehensive information on unidentified cadavers.

A review by Moitas et al. [63] included several recent studies regarding forensic microbiology and geographical location. Clarke et al. [64] aimed to distinguish between geographically diverse populations, even those living in the same city, using oral and stool microbiome samples from 206 female participants in four regions: Barbados, Santiago (Chile), Pretoria (South Africa), and Bangkok (Thailand). The V4 region of the 16 S rRNA gene was targeted for microbiota profiling. Participants from different regions exhibited mostly similar dietary and lifestyle habits, with over half having a normal BMI, consuming starch-heavy diets, and differing in tobacco exposure and pet ownership.

In stool microbiomes, the top five most dominant taxa were *Bacteroides*, *Prevotella_9*, *Faecalibacterium*, *Alistipes*, and unclassified *Eubacterium*. *Faecalibacterium*, an anti-inflammatory commensal, was more abundant in South African individuals and less in Thai individuals. Lifestyle factors like smoking minimally influenced stool microbiota. This information can be used in forensic analyses to catalogue every region around the world and to identify the geographic origin of an individual.

Oral microbiomes exhibited significant differences in Bacteroidetes and Proteobacteria phyla between countries. The top dominant oral microbiota taxa included two *Prevotellaceae* genera, *Pasteurellaceae* unclassified, *Haemophilus*, *Streptococcus*, *Gemelia*, *Veillonella*, and *Neisseria*. The oral microbiome contained a higher percentage of bacteria with geographic specificity compared to stool samples. Lifestyle and behaviour factors had a stronger influence on the oral microbiota compared to stool microbial composition in this study.

Geographic variation in the human microbiome has been observed in both oral and stool microbiota. Oral microbiota demonstrated higher discriminatory power (16 %) based on geographic origin than stool microbiota (less than 8 %). Lifestyle behaviours and diet also contributed to the microbiome differences within sub-regions.

Even after accounting for lifestyle and metadata, a significant geographic signal remained for both oral and stool microbiota. Neighbourhood sub-regions within Chile and Barbados showed differences in microbiomes, particularly in oral microbiota. Lifestyle behaviours and diet also contributed to microbiome differences within sub-regions. The geolocation effect remained strong even after considering metadata differences. Differential abundance taxa amplified the geolocation signal in both oral and stool microbiota. Understanding how external factors, such as diet and environment, affect microbiota structure is crucial. Establishing reference databases for geographic inference using microbiomes is essential.

Habtom et al. [65] conducted a study on soil microbial communities to understand the differences at both local (meters) and regional (kilometres) scales. Soil sampling was conducted at five research sites across Israel, covering a geographic distance of 260 km. the microbial profiling was carried out using Terminal Restriction Fragment Length Polymorphism (TRFLP) analysis and high-throughput sequencing (HTS) targeting the V4 region of the 16 S rRNA gene. It was found that the location of the site had a more significant impact on microbial community composition than the specific soil type. The sites formed tight clusters in the ordination plot, regardless of the soil types they comprised. Within each site, however, bacterial communities from different soil types remained significantly different. A strong correlation between average annual precipitation and soil community composition across all sites was observed. Soil community composition was most correlated with soil sodium and soil ammonium levels in certain regions.

A distance-decay analysis was conducted to examine the relationship between geographical distances and microbial community differences. A significant correlation was found, indicating that as the geographical distance between soil samples increased, the dissimilarity between their microbial communities also increased. This relationship was observed across different soil types and ranged from distances of tens of meters to tens of kilometres.

A likelihood ratio (LR) framework was used to quantitatively assess the probability that two soil samples originate from the same source or different sources based on their microbial profiles. This approach can provide valuable evidence in criminal investigations, offering the capability to pinpoint the source of a soil sample with high accuracy, potentially down to within 25 m. There are, however, challenges in implementing this method in forensic casework, including issues related to sample storage, standardization of soil DNA processing, and the analysis of mixed soil samples. Nevertheless, the study contributes to the

growing body of research in forensic soil genetics, demonstrating the potential of soil microbial analysis as a forensic tool for determining the origin of soil samples in criminal investigations.

Importantly, the study demonstrated the statistical and repetitive distinguishability between different geographic locations within the same soil type and different soil types within the same geographic location. This underscored the potential utility of soil microbiomes as tools in forensic sciences. Challenges such as temporal limitations, sample storage, lack of standardized protocols for soil DNA analysis, and the inability to analyze samples with soil mixtures need to be addressed in future research.

Single-sample studies have limitations in terms of scope and sample size, while the lack of uniformity in metadata reporting hinders meta-analysis efforts. To address these limitations, Singh et al. [66] created the Forensic Microbiome Database (FMD). The FMD includes 20,820 16 S rRNA NGS samples procured from various anatomical regions across individuals hailing from diverse geographic locations, spanning 35 different countries and representing 138 cities. Predominantly the samples (roughly 50 %) were derived from stool samples, with saliva and other oral locations following suit. The predictive accuracy stood at 80.5 % for cities, and 92.1 % for countries. There was evidence of cross-contamination as the vaginal samples were classified as stool samples and were dominated by the same genus.

The articles highlight the importance of combining microbiome analysis with other methods to enhance accuracy. Emerging methods of human microbiome analysis and its forensic applications include the use of different samples depending on the study focus; such as hair, and soil. Geographical location and environmental factors significantly influence microbial communities in both human and soil samples.

Person identification

The human microbiome is unique to every individual and exhibits relative stability over time [17,67]. This dual characteristic renders the microbiome a promising instrument for forensic analysis. The human hand, which is an extension of the skin, leaves microbial evidence on touched surfaces, making it a potential source of forensic identification [68].

Park et al. [69] aimed to assess the potential of human microbiota for individual discrimination in the context of forensic science. The study involved 15 volunteers, and 686 bacterial strains were successfully isolated and identified using 16 S rRNA gene sequencing. The genus *Staphylococcus* was detected in all participants, with *Staphylococcus epidermidis* being the most prevalent species (found in 14 subjects). The genus *Micrococcus*, primarily *Micrococcus yunnanensis*, was also commonly found among the participants (11 subjects). *Enhydrobacter aerosaccus* was detected in most participants (12 subjects) within the Gammaproteobacteria phylum. The analysis was done analysing only aerobic bacteria, further studies involving anaerobic bacterial isolation would provide a more comprehensive understanding of the total bacterial community on palms. Among the isolates, certain minor species were unique to specific individuals, potentially serving as markers for personal identification. Additionally, major species, particularly within the *Staphylococcus* genus, exhibited potential as molecular markers at the subspecies level. Future research should focus on expanding the sample size and conducting statistical analyses to identify individual-specific bacteria more comprehensively.

Richardson et al. [70] investigated microbial communities in individuals in private and public areas using machine learning models. Personal samples were collected from 37 participants residing in 28 separate dorm rooms. Initially, only individuals' hands were sampled at the first time point. Subsequently, at three additional time points, samples were taken from the desk, floor, fitted bed sheet, interior doorknobs of each participant's room, as well as from the dominant hand and shoe of the participant. Common surfaces were also sampled, including tables in the dormitory lounge and the handle of the entry door to the lounge, floors, door handles of bathrooms, hallway floors, and elevator buttons. To predict which individual's hands a surface had interacted with, bed sheets, desks, and door handles of the participant rooms were the most informative surfaces. Random forest models were used for classification and calculated error ratios to evaluate their performance. It's worth noting that classification errors were observed for specific individuals, and the primary source of error was the presence of roommates. In fact, there was a linear relationship between classification error and the number of roommates, with error increasing by 18 % points for each additional roommate.

Microbial signatures associated with skin remain stable over time. The presence of roommates posed a challenge to classification, as interactions between individuals and the exchange of microbes affected the accuracy of forensic inference. Notably, the interaction networks in the dormitory were shaped by floor-associated and hand-associated samples, with hands serving as key points of microbial transmission between individuals.

The findings underscore the potential of microbial signature analysis as a forensic tool but also highlight the need for further optimization and consideration of factors such as clustering methodology, the presence of roommates, and the stability of microbial signatures over time. Metagenomic methods that rely on metagenomic markers represent a promising avenue for future research, as they offer greater accuracy and flexibility in microbial signature analysis.

Schmedes et al. [67] presented a novel approach for accurately attributing skin microbiome samples to individual donors, even over an extended time span of more than 2.5 years. Skin microbiome shotgun metagenomic data sets was obtained from 12 healthy individuals, covering 17 different body sites and sampled at three distinct time points. Two methods were used for distinguishing skin microbiomes from different individuals, 1-nearest-neighbor (1NN) and regularized multinomial logistic regression (RMLR). The classifier achieved high accuracy across various body sites, with the Mb (shirt) and Hp (palm) sites demonstrating remarkably accurate classification rates of 97 % and 96 %, respectively, using 1NN classification based on nucleotide diversity. These results are especially noteworthy for the hand, which is subject to frequent microbial recolonization due to daily activities and typically exhibits limited shared phylotypes (approximately 17 %) between different individuals.

Previous studies have reported similar classification accuracy when attributing microbiome samples from various surfaces, such as phone surfaces, to their respective owners. Classification accuracy typically dropped when assessing samples collected over longer intervals, as observed by Franzosa et al. [71]. Notably, the sample size was limited, with three intraindividual samples available for each body site. Larger sample sizes are essential for further validation and the development of statistical models to account for microbiome classification likelihood. Additionally, the research does not address the applicability of the data to real or mock forensic scenarios, where informative targets may need enrichment. Targeted enrichment and sequencing using a panel of selected informative markers may be the ideal approach for microbiome profiling in forensic identification, especially for low-biomass and potentially degraded samples.

Wilkins et al. [72] aimed to link a place of residence to the skin microbiome of individuals. Researchers collected skin, surface, and air samples from nine residences in Hong Kong across four seasons. The V4 region of the 16 S rRNA gene was amplified using the 515 F/806 R primer pair. The analysis of surface samples confirmed that the majority of household surface microbiota originated from the skin of the occupants. The most prevalent family across all surface samples was Moraxellaceae, primarily composed of the skin-colonizing genus Acinetobacter. Among the top ten most abundant families were those closely associated with human skin, such as Staphylococcaceae, Micrococcaceae, Corynebacteriaceae, and Streptococcaceae.

The study attempted microbial matching of individuals to their places of residence based on comparison of skin microbiota to household

surface microbiota traces, to determine whether accurate microbial matching could be achieved even with large time delays between skin and surface sampling. It was found that skin and surface samples from the same residence and season were more similar than those from different residences in the same season, confirming that household occupants are the primary source of household surface microbiota. The research highlights the potential of using skin microbiome analysis for forensic human identification and also notes that the primer set (515 F/806 R) used in this study may have underrepresented the phylum Actinobacteria, including significant human skin genus, Propionibacterium. Matching accuracy decreased when surface samples were collected a season or more after skin samples, with no accurate matches made after a delay of three seasons. Similarly, when used skin samples were collected after surface samples, the accuracy fell to 33 % for delays of two or three seasons. This suggests that the accuracy of microbiota matching is influenced by the time delay between sample collections. Skin microbiota changes over time, and traces deposited on surfaces begin to degrade within hours, even without cleaning or mechanical removal. This points out a limitation for using skin microbiota in forensic applications. Future research in microbiota matching might benefit from utilizing additional or alternative primer sets that are more suitable for detecting human taxa. Targeted enrichment and sequencing using a panel of selected informative markers may be the ideal approach for microbiome profiling, especially for low-biomass and potentially degraded samples.

The salivary microbiome can also be used for human identification. A study by Leake et al. [73] demonstrated that the salivary microbiome exhibits significant biodiversity, and through a PCR-based metagenomic approach, it was possible to differentiate between two unrelated individuals. Saliva samples were collected from two consenting healthy adult individuals. To achieve comprehensive coverage of the salivary microbiome, three distinct targets were selected: the 16 S rRNA gene and *rpoB*. For *rpoB*, two pairs of primers were employed, targeting *Streptococci* (*rpoB1*) and other bacterial species (*rpoB2*). The 16 S rRNA primers were designed to amplify the V5 region, while two sets of *rpoB* primers covered the V1 region. For both *rpoB1* and 16 S rRNA, Firmicutes dominated, constituting over 90 % and 70 % of the population, respectively. For *rpoB2*, Actinobacteria accounted for over 90 % of the population. The varying taxonomic compositions observed with each *rpoB* primer pair are expected, as they were designed to amplify different taxa, demonstrating the advantage of targeting multiple regions of the same target gene.

Given the use of the HiSeq2000 for analysis, a machine capable of producing over one billion reads, the minimum number of sequences needed to distinguish between two individuals was initially unknown. The analysis revealed that, for *rpoB2*, which provided the least separation, a minimum of 50,000 sequences was necessary to adequately differentiate the two individuals. When considering individual targets, 16 S rRNA offered the best separation. However, combining 16 S rRNA and *rpoB1* improved separation further. Combining all three targets yielded the best results, with significantly improved separation achieved with 50,000 sequences. Combining three targets allowed the detection of certain genera down to the species and even strain level. Specifically, while 16 S rRNA detects *Streptococcus* at the genus level and occasionally the species level, *rpoB* enables detection at the species/strain level, providing a deeper characterization of this segment of the saliva microbiome. This is significant because *Streptococcus* constitutes approximately 80 % of Firmicutes, the most abundant phylum. The study also addressed the impact of antibiotics on the salivary microbiome, highlighting the need to collect a reference sample post-treatment when the microbiome has returned to its pre-antibiotic state. Moreover, the article considered the resilience of bacterial DNA to external factors, highlighting the potential advantages of using microbiota-based forensic investigation. Bacterial DNA is more resistant to degradation from UV light, heat, and humidity compared to human DNA, as bacterial DNA is circular, condensed, and protected by a cell wall.

Further research is needed to explore the full potential and limitations of this technique, including its applicability to twin differentiation and the relationship between specific species and lifestyle habits. The small sample size used limits the generalizability of the results, and further research with larger sample sizes is needed.

The “touch microbiome” is the trace microbial DNA found on surfaces that can be considered as forensic markers similar to “touch DNA”. A pilot by Procopio et al. [74] aimed to investigate the utility of "touch microbiome" analyses for personal identification, comparing different sample types obtained from skin swabs and fingerprint swabs on glass slides. The study also aimed to assess the transferability and survival of the "touch microbiome" on a surface, as well as identify donor-specific taxa (DCTs) transferred to the glass surface that may have forensic relevance for identification purposes. There were 11 participants, five were male, six were female, spanning different age groups from 20 to 70 years located in Italy for at least three generations. All individuals were in good health and had not undergone courses of antibiotics or antifungals. Samples were collected from the skin and fingerprint. Skin samples were taken by swabbing the palm of their dominant hand and fingerprint samples were taken by having participants touch glass slides. The highly variable V4 region of the 16 S rRNA gene was targeted.

The results showed that the "Chargeswitch® Forensic DNA" kit (specifically designed for extracting human DNA) was more effective than the "QIAamp PowerFecal Pro" kit (used for microbial DNA) on both skin and glass swabs, likely due to its specificity for extracting small amounts of DNA from various forensic samples. The "QIAamp PowerFecal Pro" kit, designed for extracting microbial DNA from richer sources like feces, performed less efficiently on fingerprint swabs. The analysis of the skin microbiome could complement human DNA typing, especially when classical STR polymorphism analysis fails, as in this study. This suggests that skin microbiome analysis could provide valuable information for forensic applications, particularly in cases involving degraded or low.

The human microbiome, particularly skin and salivary microbiota, has the potential to be a valuable tool in forensic analysis and individual identification. Studies have shown that microbial profiles among individuals are unique, and certain bacterial species or strains are specific to particular individuals, offering a basis for personal identification.

The temporal stability of skin and salivary microbiota is crucial for forensic applications that may involve samples collected over extended periods. microbial analysis can link individuals to specific locations or surfaces, and the impact of factors like time delays on accuracy should be considered. Using multiple targets or regions within microbial DNA can improve accuracy in distinguishing between individuals. The resilience of bacterial DNA to external factors, such as UV light, heat, and humidity, compared to human DNA can be advantageous for forensic investigations. Overall, these studies collectively highlight the potential of microbial analysis as a valuable tool in forensic science.

Sexual assault

During sexual contact, microbes can be transferred between individuals. Since the human microbiome is quite individualized, it could potentially be possible to use it in sexual assault cases [75]. The genital microbiome, in particular, had been studied for its potential in detecting sexual assault. Preliminary data on both the penile and vaginal microbiome demonstrate potential value in cases of sexual assault.

Williams and Gibson [76] looked at the stability of the pubic hair microbiome in a six-month period. They looked at pubic mound hair samples from 43 people who self-identified as free of sexually transmitted diseases (STDs) and yeast infections, this included 12 partner pairs. Across all samples, the predominant bacterial phyla were Actinobacteria (49.1 %), Firmicutes (46.6 %), and Proteobacteria (2.9 %). The most common genera, representing over 77 % of the microbiome, included Corynebacteria (29.2 %), Staphylococci (21.5 %),

Propionibacteria (15.4 %), and Lactobacilli (11.5 %). Notably, there was differential abundance of genus-level OTUs between males and females. Out of the 109 OTUs enriched in males, 34 were associated with Corynebacterium, while 23 OTUs associated with Lactobacillus were more abundant in females. This observation aligns with previous findings that Lactobacillus predominates in vaginal fluids, suggesting its utility as a biomarker for identifying vaginal secretions [77-79]. Unfortunately, demographic classifications such as race and age were not sufficiently populated to facilitate meaningful comparisons of differentially abundant OTUs.

The results presented in this study suggest that the pubic hair and pubic mound microbiomes have the potential to serve as a tool for distinguishing between individuals, with implications for forensic investigations. The microbiome in this area could differentiate one individual from another, consistent with findings in other body regions. Couples also tended to exhibit more microbiome similarity than unrelated individuals, although this similarity does not necessarily arise solely from sexual contact. It may be due to shared environments or indirect contact between couples, as microbiome transfer from the skin to other surfaces is known to occur readily. The study's observational nature and the participants' unregulated behaviour did not allow the researchers to directly quantify transfer probabilities associated with unwanted sexual assaults. Controlled experiments involving sample collection immediately before and after sexual contact could help determine the extent and duration of mixing. However, such experiments would be challenging to conduct due to logistical and ethical considerations. Despite these challenges, the pubic microbiome holds promise for forensic applications. While it may not provide positive identification of a suspect in most cases, it could confirm sexual contact, exculpate a suspect, or provide other forms of assistance to victims. Moreover, combining microbiome analysis with mitochondrial DNA analysis of a hair found at a crime scene could potentially lead to more definitive individual identification.

The article by Williams and Gibson [75] found that the pubic hair microbiome has the potential to serve as a tool for distinguishing between individuals, with individuality and gender as the primary sources of variation. The study involved six healthy adult participants. The ability to correctly identify the individual from whom a sample originated was influenced by both intra-individual and inter-individual variation. It study also found differential abundance between genders. Nonetheless, heterogeneity within women in the variance of gender-biased taxa suggests limited use as gender-specific markers. The article's observational nature and the participants' unregulated behaviour did not allow the researchers to directly quantify transfer probabilities associated with unwanted sexual assaults. Even so the pubic microbiome holds promise for forensic applications, as it could confirm sexual contact, exculpate a suspect, or provide other forms of assistance to victims.

The pubic hair and pubic mound microbiomes have the potential to serve as a tool for distinguishing between individuals, with implications for forensic investigations. Further research is needed to address issues such as mixed samples, person-dependent hair-to-hair variation, and transfer probabilities associated with unwanted sexual assaults. Additionally, the variability induced by sample collection and handling has not been systematically assessed, and future research is needed to validate optimal collection, storage, and analysis methods. Despite these challenges, the human microbiome holds promise for forensic applications, and combining microbiome analysis with other methods could potentially lead to more definitive individual identification.

The use of pubic hair microbiome analysis in forensic science faces several challenges that need to be addressed. These challenges include microbiome transfer, sample collection, DNA extraction, sequencing and batch effects. Technical replicates, high sequencing quality, and batch processing can help reduce technical sources of variation. Guidelines for sample collections and analysis will evolve as datasets grow, potentially exploring metagenomics sequencing for higher resolution.

The identification of microbial signatures in bodily fluids can aid in forensic investigations, particularly in sexual assault cases. Zou et al. [80] introduced a PCR-based approach for the identification of microbial signatures to determine body fluid origin in a Han Chinese population. DNA samples extracted from the forensic specimens in this study followed conventional procedures for genomic DNA purification, with the inclusion of lysozyme and mutanolysin during a pre-incubation step. Consequently, mixed DNA samples were obtained comprising both human and bacterial DNA. These samples were then utilized for PCR-based microbial signature detection and individual STR typing. Successful profiling of vaginal fluid, saliva, and faeces samples was achieved using an STR typing kit. The pre-incubation step incorporating lysozyme and mutanolysin allowed for the preparation of DNA samples for both microbial signature identification and individual STR typing, streamlining detection processes and minimizing sample requirements—crucial for situations where DNA quantities are limited, such as in forensic investigations. The study identified microbial markers including the 16 S rRNA genes of *L. crispatus, L. gasseri, L. jensenii, L. iners,* and *A. vaginae,* the 16 S rRNA genes and GTF enzyme genes of *S. salivarius* and *S. mutans,* the 16 S rRNA genes of E. spp., the *rpoB* genes of *B. uniformis* and *B. vulgatus*, and the α-1–6 *mannanase* gene of *B. thetaiotaomicron*, were successfully detected in DNA samples at their expected sizes. The results showed that *L. crispatus* and *L. jensenii* serve as specific markers for the identification of vaginal fluid, while *L. gasseri, L. iners,* and *A. vaginae* exhibit lower specificity. However, it's worth noting that *L. crispatus* was detected in some female urine samples, likely due to contamination with vaginal fluid, consistent with previous studies by Akutsu et al. [81].

Contrary to a previous study conducted in Japan by Nakanishi et al. [82], *S. salivarius* and *S. mutans* were not specific to saliva. This discrepancy may be attributed to differences in dietary habits between the Han Chinese and Japanese populations, which can influence oral and gut microflora. Diet-induced variations can lead to differences in the composition of human oral and gut microbiota. In feces, *B. uniformis* and *B. thetaiotaomicron* exhibited higher specificity compared to E. spp. and *B. vulgatus.* These results are consistent with prior findings indicating that *B. uniformis* and *B. thetaiotaomicron* are not detected in body fluid samples other than feces. Notably, similar to *L. crispatus* in female urine, *B. uniformis* was also detected in some male urine samples, which we considered as potential sample contamination.

Karadayi et al. [83] evaluated the microbiota taxonomic profiles of male saliva, female breast skin, and mixed samples to investigate whether saliva can be detected in the microbiota profiles of mixed samples. In this study, a total of 44 samples were collected from four unrelated healthy couples, including female breast skin samples without saliva, male saliva samples, and mixed samples (breast skin with saliva).

The study found that bacterial DNA from saliva can be detected in mixed samples up to 48 h after exposure. Notably, mixed samples displayed a bacterial composition closer to saliva samples than to breast skin samples.

The researchers employed statistical and bioinformatic analyses to determine the microbial distance between saliva samples and mixed samples, with results showing that mixed samples collected up to 48 h after saliva transfer predominantly matched the saliva sample from the contributing individual. Future research is needed to validate and expand upon these results, including exploring different case scenarios, body regions, longer time intervals, and larger participant groups. Additionally, diverse sample types, such as cotton swabs containing vaginal fluid from sexual assault cases and cigarette butts with saliva should also be considered. Finally, the development of rapid detection methods based on multiplex PCR or real-time PCR for various body fluids and stains obtained from crime scenes should be pursued.

Dixon et al. [84] investigates the potential use of microbiome analysis, employing Massively Parallel Sequencing (MPS), to identify unique bacterial signatures on genitals before and after intercourse, aiming to develop a method for perpetrator identification in sexual assault cases

with mixed DNA profiles. Vaginal and penile skin samples were obtained from six healthy, consensual male-female couples with no history of sexually transmitted infections or reproductive medical conditions. Genital microflora samples were collected by each participant within male-female pairs. The study found that both male and female genital microbiomes displayed an increase in total genera post-coitus, with male samples showing the most significant changes in abundance, particularly in the increased relative abundance of *Lactobacillus*. Additionally, taxonomic analysis identified the presence of certain genera, such as *Haemophilus* and *Streptococcus*, post-coitus, which are not typically found in the genital microbiome and may have been transferred during sexual contact or sampling. The findings suggest that microbial transfer during sexual intercourse can have a significant impact on the genital microbiome, highlighting the potential forensic applications of microbiome analysis in cases involving sexual contact and the need for further research to explore microbiota restoration timelines and implications for determining time since intercourse (TSI).

Further research should expand the scope to encompass additional bacteria found in various body fluids and utilise larger specimen sets to confirm whether the taxa are genuinely unique to individuals and to obtain more detailed genetic information about the bacteria. The survey responses collected from participants did not clearly indicate the number of days since menses at baseline sample collection. Cyclic variations could have influenced the results, and future research should collect more specific information about menses. It is also important to include questions about oral intercourse in future surveys to investigate potential microbial transfer from oral contact. Due to the nature of this study, the risk of hand cross-contamination is likely. Future research in this field should consider including hand swabs alongside genital swabs to assess similarities and potential cross-contamination during intercourse.

The studies discussed share a common theme of exploring the forensic potential of microbial analysis in cases involving sexual contact and individual identification. Williams and Gibson [75], Karadayi et al. [83], and Dixon et al. [84] all highlight the individuality of microbial profiles in various body regions, including the pubic hair, genitalia, and breast skin. These distinct microbial signatures offer the potential for individual identification. Williams and Gibson [75] identified gender-specific markers within the pubic hair microbiome. Similarly, Zou et al. [80] identified microbial markers specific to body fluids like saliva, vaginal fluid, and feces. These markers can assist in determining the origin of bodily fluids in forensic investigations. Dixon et al. [84] and Karadayi et al. [83] investigate microbial transfer during sexual contact. Dixon et al. [84] demonstrated changes in microbial diversity and composition post-coitus, while Karadayi et al. [83] detected microbial transfer between sexual partners. These findings have implications for establishing recent sexual contact in forensic cases.

Bite marks

A bite mark is defined as a physical impression on the skin or other surfaces resulting from the application of pressure by teeth [85]. Human bite marks are predominantly encountered in cases of homicide, sexual assault, and child abuse [86]. In a systematic review conducted by Moitas et al. [87], several papers were examined with regard to the utilization of microbial signatures transferred during biting incidents for forensic purposes, which will be discussed.

Elliot et al. [88] aimed to utilize pyrolysis mass spectrometry (Py-MS) and statistical mass spectra analysis to create distinctive profiles for various strains of *Streptococcus salivarius*, with the goal of potentially assisting in identifying suspects in assault or rape cases involving bite-marks by demonstrating the strain-level differentiation of *S. salivarius* based on the source of the isolate. Using this method, the researchers were able to distinguish between two individuals.

In the investigation conducted by Kennedy et al. [89], a total of 16 volunteers who intentionally self-inflicted bites on their upper arms were subjected to a sampling procedure, wherein bite marks and samples from both upper and lower anterior teeth were meticulously swabbed. Subsequently, DNA was directly extracted from these collected samples, followed by purification, amplification, and pyro-sequencing targeting specific genetic loci, including the 16 S rRNA gene, the 16S–23 S rRNA intergenic spacer region (ITS), the endoribonuclease P (*rnpB*) locus, and the RNA polymerase beta subunit (*rpoβ*) locus.

The findings of this investigation illuminated a critical distinction: the analysis of the *rpoβ* locus exhibited a notably superior capacity to accurately differentiate between samples when contrasted with pyro-sequencing approaches targeting streptococcal 16 S ribosomal RNA (16 S rRNA) or the ^{16}S–^{23}S intergenic spacer (ITS). The assessment of streptococcal DNA across these three genetic regions, specifically aimed at distinguishing between the study participants, revealed compelling results. Notably, the probability of successfully matching bite mark samples with their respective teeth samples stood at 92 % for ITS, an impressive 99 % for 16 S rRNA, and an unequivocal 100 % for *rpoβ*, with a calculated confidence interval of 95 %. The species identified within the bite mark and teeth samples, across all three targeted loci, consistently comprised *Streptococcus mitis*, *Streptococcus oralis*, and *Streptococcus cristatus*. Notably, *S. mitis* predominated on the tooth surfaces, imparting a distinctive influence on the observed outcomes. This distinction arises from the selectivity of *rpoβ* primers for *S. mitis*, whereas the 16 S rRNA and ITS primers encompass a broader range of streptococcal species. Consequently, the robust discriminatory power of *rpoβ* emanates from its exclusive scrutiny of a species marked by profound genotypic diversity, thereby affording it a remarkable specificity rating of 100 %.

From a forensic perspective, it is crucial to ensure the temporal stability of oral streptococcal populations. Genetic analyses have shown that oral streptococcal populations are dynamic, with species numbers and proportions fluctuating over time. Nevertheless, dominant strains of streptococci tend to be retained over longer periods. Approximately 20 % of all *S. mitis* genotypes recovered from the buccal mucosae of participants were detected in repeated samplings over a 10-month period, and almost 50 % of *S. mitis* and *S. oralis* genotypes from two individuals were detected two years after initial sampling. Rahimi et al. [90] found that between 20–78 % of bacterial genotypes were recovered from the same teeth 12 months later. Therefore, prompt sampling increases the likelihood of matching bite mark sequence data to that of a suspected assailant.

Rahimi et al. [90] aimed to assess the effectiveness of AP-PCR (Arbitrarily primed polymerase chain reaction) in identifying the biter, investigating the natural distribution of oral *Streptococcus* genotypes, and examining their recoverability after a 12-month period. Bacterial samples were collected from the biting edges of the lower incisors of eight volunteers who had not used mouthwashes or received antibiotics in the previous three months. All collected samples underwent culture, and the Streptococcal genotypes were derived from 50 randomly selected bacterial colonies from each sample. The analysis of the streptococcal DNA revealed the ability to differentiate 106 genotypes, with each individual harbouring between eight to 23 distinct strains. By comparing the amplicon profiles with those of the 8 participants, the bacteria were unequivocally matched to the respective biter. Conversely, the bacteria from an additional bite mark (from an unrelated individual) could not be associated with any of the 8 participants. The study also evaluated the temporal stability of *Streptococcus* genotypes, collecting additional samples of the incisors from each participant for analysis after a 12-month period. The results indicated that between 20 % to 78 % of the catalogued bacterial genotypes were recoverable after this time frame. Furthermore, throughout the duration of the study, none of the bacterial genotypes were shared among the participants. Top of Form.

The AP-PCR method used in the study enabled efficient analysis of a large number of bacteria, showing potential for forensic applications, particularly when human DNA recovery is challenging. Further research

is needed to determine the frequency of specific streptococcal genotypes among populations. Overall, the study suggests that this method could provide valuable supportive evidence in forensic bite mark analysis when human DNA is not recoverable, potentially linking a suspect to a crime.

Hsu et al. [91] conducted a study to investigate the feasibility of directly amplifying bacterial DNA recovered from bite marks for comparison with oral samples. Experimental bite marks and streptococcal DNA analysis procedures were conducted involving 24 healthy individuals. Participants created bite marks on their upper arms, which were sampled after three hours. Swabs from bite marks, adjacent unbitten control sites, lingual surfaces, and biting edges of lower incisors were processed to extract DNA. Denaturing gradient gel electrophoresis (DGGE) was used to compare amplicons from these samples based on a specific 16 S ribosomal DNA region.

When comparing the amplicon patterns generated from bite marks with at least 6 DNA bands, 8 out of 15 cases matched with the corresponding incisors, exhibiting a correlation coefficient greater than 0.70. This study lends support to the potential use of a microbiological approach in analysing bite marks by directly amplifying streptococcal DNA obtained from the bite mark itself. Top of FormThe research lends support to a microbiologically-based approach for analysing bite marks. While the recovery and analysis of human DNA from the biter should always take precedence, when this proves unsuccessful, the same sample (swabbed from the bite mark) presents an opportunity for microbial analysis by amplifying streptococcal DNA for comparison with amplicons derived from a suspect's dentition.

Brown et al. [92] investigated the potential for recovering *Streptococcus salivarius* from ten-microliter samples of whole saliva applied to human skin and aimed to determine the time frame within which *S. salivarius* could be retrieved. The saliva samples were plated on a selective medium known as Mitis-Salivarius agar, specifically designed for the identification of these bacteria, allowing for the assessment of the duration of *S. salivarius* recoverability on human skin.

The findings indicated a reduction of 44.8 % per hour in the overall count of *Streptococcus* bacteria and a 43.9 % per hour decline in *S. salivarius* specifically. While the rate of decline in recoverable bacteria seemed high, it is important to note that even after several hours, substantial numbers of recoverable streptococci remain, especially considering the small initial inocula used. *S. salivarius* typically colonizes the tongue and is found there in higher proportions than in any other part of the oral cavity. Hence, it is reasonable to expect a larger inoculum when the tongue is involved. Licking rather than biting, resulted in the bacterial counts recovered after 8–9 h being considerably higher. Establishing a suitable "fingerprint" typing scheme for oral bacteria could potentially provide valuable evidence pertaining to the identity of a suspect in such cases.

In conclusion, these studies collectively emphasize the potential of utilizing microbial analysis, particularly focusing on *Streptococcus* species, as a valuable tool in forensic investigations involving bite marks. Several key findings are highlighted; strain-level differentiation was shown by Elliot et al. [88] which demonstrated the ability to distinguish between individuals based on strain-level differences in *Streptococcus salivarius*. The research conducted by Kennedy et al. [89] and Rahimi et al. [90] underscored the importance of temporal stability in oral streptococcal populations. Dominant strains tend to persist over time, increasing the likelihood of matching bite mark sequences to suspects.

Overall, these articles collectively support the notion that microbial signatures, especially those related to *Streptococcus* species, can play a crucial role in forensic bite mark analysis. While human DNA analysis remains a priority, microbial analysis offers a valuable complementary approach, providing additional evidence for identifying suspects and aiding in criminal investigations.

Novelty of this work

The use of the microbiome in forensic investigation is still developing and under-utilized, especially in the developing world. Microbiome applications for forensic analysis can be grouped into five categories, postmortem analysis, geographical identification, person identification, sexual assault cases and bite marks. By the exploration of emerging microbiome analyses, their strengths, limitations, and potential for future research in forensic investigations, the review highlights challenges and limitations associated with these applications, and suggestions for future research focus to overcome these challenges and fully harness the potential of the human microbiome in forensic investigations, enabling its practical application in various forensic scenarios.

Limitations

The emerging microbiome techniques provides a lot of potential for the expansion of forensic investigations. There is still a long way to go before these techniques become used as frequently as traditional DNA typing techniques. The following are some limitations of using the human microbiome in forensic analysis.

Undoubtedly, the primary challenge in (forensic) microbiome research resides in the absence of consensus regarding experimental and analytical methodologies across various scientific communities. This dearth of agreement significantly hinders the ability to compare findings between studies, making it challenging, if not unfeasible, to conduct meta-analyses encompassing data from multiple studies and to provide an accurate assessment of the current state and future potential of forensic microbiome research [93]. Microbiomic data is new and is still being developed, therefore there is lack of standardization laws that govern how samples should be collected and used and particularly concerning its application in forensic contexts. This is needed as using these tools to identify individuals make the current laws and standards too complex. Additionally, the microbiome can reveal information about the lifestyle of an individual as well as possibly indicate certain diseases which could be a possible privacy issue [94].

A critical issue that plagues the field is the lack of consensus both in the experimental and analytical methods adopted by various scientific communities. This absence of a standardized framework hampers the ability to effectively compare results across different studies, thereby impeding the meta-analysis of data from multiple sources. Consequently, this fragmentation within the field inhibits the establishment of a comprehensive overview of the current state and future prospects of forensic microbiome research. The necessity for rigorous validation and reliability assessment of the methods and protocols employed in microbial profiling is also lacking. In the context of forensic microbiome research, the stakes are particularly high, as microbial profiles may be considered as crucial evidence in legal cases. Therefore, it becomes imperative to ensure that the methods utilized meet specific standards set forth by accreditation bodies. Accreditation bodies may need to develop new, specialized standards tailored to the unique requirements of microbial profiling in forensic applications. Moreover, there is the potential role of agencies that set standards and accredit laboratories. In the future, such agencies could play a pivotal role in upholding high standards of admissibility for microbial profiles as forensic evidence. These standards would need to encompass various aspects, including data generation, analysis, and reporting. Ensuring that forensic practitioners adhere to these standards is essential, and proficiency testing could be a means of assessment [93].

A notable constraint associated with employing geolocation data in microbiome investigations lies in the susceptibility to result disparities arising from shifts in an individual's lifestyle. This limitation is primarily attributed to the substantial influence of lifestyle on the composition of the microbiome, thus introducing a potential confounding factor into the outcomes of such investigations [22]. Even minor perturbations,

such as the onset of an illness or travel-related changes, possess the capacity to induce noticeable alterations in an individual's microbiome composition, and these modifications can manifest within a relatively short timeframe, often spanning only a few days [95]. A plausible remedy to mitigate the aforementioned limitation has been proposed in extant literature. A study has posited that the gut microbiome exhibits a degree of stability contingent upon an individual's body mass and age [96]. This observation suggests that, despite the microbiome's susceptibility to lifestyle-related changes, factors such as body mass and age may serve as stabilizing influences, potentially attenuating the impact of lifestyle variations on the microbiome's composition.

As observed in the preceding context concerning postmortem interval (PMI) estimations, the precision of such estimations is contingent upon several influential factors, including but not limited to seasonal variations, ambient temperature fluctuations, and soil composition [97]; [98]. These factors exert their influence by modulating the microbial community inhabiting the cadaveric ecosystem. Consequently, the predictive models and methodologies employed for PMI determination may necessitate distinct considerations and adjustments when applied in dissimilar geographic locations or environmental settings [22].

Subsequently, the challenge of microbiomic data availability comes into focus. Typically, forensic investigations hinge upon genetic datasets and comprehensive genomic sequences. Nevertheless, when considering microbiomic data, its availability remains constrained. While the Human Microbiome Project embarked on the endeavour of characterizing the human microbiome, the intrinsic diversity of microbial communities has rendered this undertaking an ongoing and evolving effort [22]. Moreover, a substantial proportion of sequences derived from microbiomic studies cannot be classification [99].

Another issue is the matter of storage. The human microbiome exhibits remarkable sensitivity, and thus, the methodology employed for storage, the temperature conditions maintained during storage, and the duration of storage can exert substantial influence over the microbiome's compositional integrity [100,101]. Freezing represents a viable preservation strategy, provided that suitable stabilizers are incorporated into the sample. However, it is essential to acknowledge that the repeated cycles of freezing and thawing, coupled with the length of storage, could potentially introduce perturbations to the microbial composition. These are the conditions typically encountered by evidence during forensic investigations, thus underscoring the imperative to identify alternative strategies to mitigate this limitation [102].

Time constitutes another constraint of significance. Whether situated within the external environment, associated with an object, or subjected to postmortem analysis, the human microbiome exhibits limited stability [103]. Temporal considerations are prone to diminishing the precision of results, as elucidated by Hu et al. [52], primarily due to microbial degradation or assimilation into the surrounding external microbiome. Furthermore, it is worth noting that the extent of variation attributable to temporal factors can diverge contingent upon the anatomical source of the sample microbiome; for instance, the gut microbiome tends to exhibit greater stability compared to other anatomical regions [22].

Lastly, a limitation pertaining to the biomass available for sampling, which is typically quite constrained and can potentially exert adverse effects on research outcomes. Given the disparities in available biomass across distinct anatomical regions of the human body, conducting correlative studies becomes notably challenging [104]. When dealing with trace quantities of biomass, the risk of contamination is substantially heightened [105].

Future perspectives

Future research and development are important to harness the full potential of human microbiome analysis. Research should also explore the use of the microbiome to determine the geographic origin of samples and associate individuals with objects or spaces they have interacted with. Additionally, studies should evaluate the transfer probabilities and the maintenance of microbiome mixtures in cases of sexual assault. There is a need to evaluate the sensitivity of current technologies in obtaining microbiome profiles from limited biomass samples commonly encountered in forensic investigations. Standardization protocols for microbiological sample collection are necessary to optimize results and ensure consistency across different investigations. The development of reliable and comprehensive microbiome databases, including metadata associated with humans, would support broader applications in forensic science. Standardized processes for sample collection, storage, and analysis should be implemented to minimize contamination and maintain the integrity of microbiomes.

Conclusion

The human microbiome holds considerable potential in the field of forensic sciences, encompassing various applications including persona identification, geographical identification, sexual assault cases and postmortem analysis. The microbiome displays significant variation, which can be utilized to distinguish individuals based on their distinctive microbial signatures, thus facilitating individual identification. Moreover, the microbiome composition varies geographically, enabling identification of an individual's origin or location by looking at their microbial profile. In postmortem analysis, the changes observed in microbial composition during each stage of decomposition can provide valuable insights into the time and location of death which can assist in forensic investigations. Microbiome analysis also shows promise as a valuable tool in sexual assault cases, as it allows for the confirmation of sexual contact or the exoneration of suspects by looking at the microbiomic composition. Notably, the stability of the pubic hair microbiome and the transfer of saliva microbes offer potential forensic evidence in sexual assault cases. Despite certain challenges and limitations, such as sample size and standardization issues, the analysis of the human microbiome holds tremendous promise as a complementary tool to traditional forensic techniques, expanding our capabilities in forensic analysis.

Financial disclosure

None reported.

CRediT authorship contribution statement

Okpeku Moses: Conceptualization, Methodology, Supervision, Writing – review & editing. **Singh Amy Arabella:** Conceptualization, Data curation, Investigation, Validation, Visualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] L.K. Ursell, J.L. Metcalf, L.W. Parfrey, R. Knight, Defining the human microbiome, Nutr. Rev. 70 (2012) S38–S44.
[2] C. Dobell, The discovery of the intestinal protozoa of man, J. R. Soc. Med. 13 (1920) 1–15.
[3] S. Thomas, J. Izard, E. Walsh, K. Batich, P. Chongsathidkiet, G. Clarke, D.A. Sela, A.J. Muller, J.M. Mullin, K. Albert, J.P. Gilligan, K. DiGuilio, R. Dilbarova, W. Alexander, G.C. Prendergast, The host microbiome regulates and maintains human health: a primer and perspective for non-microbiologists, Cancer Res. 77 (2017) 1783–1812.
[4] G.A. Ogunrinola, J.O. Oyewale, O.O. Oshamika, G.I. Olasehinde, The human microbiome and its impacts on health, Int. J. Microbiol. 2020 (2020) 8045646.
[5] N.R. Pace, A molecular view of microbial diversity and the biosphere, Science 276 (1997) 734–740.

[6] A.J. Jeffreys, J.F. Brookfield, R. Semeonoff, Positive identification of an immigration test-case using human DNA fingerprints, Nature 317 (1985) 818–819.
[7] Y. Nakamura, M. Leppert, P. O'Connell, R. Wolff, T. Holm, M. Culver, C. Martin, E. Fujimoto, M. Hoff, E. Kumlin, Variable number of tandem repeat (VNTR) markers for human gene mapping, Science 235 (1987) 1616–1622.
[8] M.A. Jobling, P. Gill, Encoded evidence: DNA in forensic analysis, Nat. Rev. Genet. 5 (2004) 739–751.
[9] C. Borsting, N. Morling, Next generation sequencing and its applications in forensic genetics, Forensic Sci. Int.: Genet. 18 (2015) 78–89.
[10] T.H. Clarke, A. Gomez, H. Singh, K.E. Nelson, L.M. Brinkac, Integrating the microbiome as a resource in the forensics toolkit, Forensic Sci. Int.: Genet. 30 (2017) 141–147.
[11] H.W. Cho, Y.B. Eom, Forensic analysis of human microbiome in skin and body fluids based on geographic location. Frontiers in Cellular and Infection, Front. Cell. Infect. Microbiol. 11 (2021) 695191.
[12] J.R. Marchesi, Prokaryotic and eukaryotic diversity of the human gut, Adv. Appl. Microbiol. 72 (2010) 43–62.
[13] J.A. Gilbert, M.J. Blaser, J.G. Caporaso, J.K. Jansson, S.V. Lynch, R. Knight, Current understanding of the human microbiome, Nat. Med. 24 (2018) 392–400.
[14] L.K. Ursell, J.C. Clemente, J.R. Rideout, D. Gevers, J.G. Caporaso, R. Knight, The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites, J. Allergy Clin. Immunol. 129 (2012) 1204–1208.
[15] A. Reyes, M. Haynes, N. Hanson, F.E. Angly, A.C. Heath, F. Rohwer, J.I. Gordon, Viruses in the faecal microbiota of monozygotic twins and their mothers, Nature 466 (2010) 334–338.
[16] K.J. Locey, J.T. Lennon, Scaling laws predict global microbial diversity, Proc. Natl. Acad. Sci. 113 (2016) 5970–5975.
[17] P.J. Turnbaugh, M. Hamady, T. Yatsunenko, B.L. Cantarel, A. Duncan, R.E. Ley, M.L. Sogin, W.J. Jones, B.A. Roe, J.P. Affourtit, M. Egholm, B. Henrissat, A. C. Heath, R. Knight, J.I. Gordon, A core gut microbiome in obese and lean twins, Nature 457 (2009) 480–484.
[18] R.E. Ley, C.A. Lozupone, M. Hamady, R. Knight, J.I. Gordon, Worlds within worlds: evolution of the vertebrate gut microbiota, Nat. Rev. Microbiol. 6 (2008) 776–788.
[19] Z. Ahmed, S. Zeeshan, D. Mendhe, X. Dong, Human gene and disease associations for clinical-genomics and precision medicine research, Clin. Translat. Med. 10 (2020) 297–318.
[20] M.G. Dominguez-Bello, E.K. Costello, M. Contreras, M. Magris, G. Hidalgo, N. Fierer, R. Knight, Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 11971–11975.
[21] C. Palmer, E.M. Bik, D.B. DiGiulio, D.A. Relman, P.O. Brown, Development of the human infant intestinal microbiota, PLoS Biol. 5 (2007) e177.
[22] T.H. Clarke, A. Gomez, H. Singh, K.E. Nelson, L.M. Brinkac, Integrating the microbiome as a resource in the forensics toolkit, Forensic Sci. Int: Genet. 30 (2017) 141–147.
[23] D. Roy, S. Tomo, P. Purohit, P. Setia, Microbiome in death and beyond: current vistas and future trends, Front. Ecol. Evol. 9 (2021) 630397.
[24] A. Gonzalez, Y. Vazquez-Baeza, R. Knight, Snapshot: The human microbiome, Cell 158 (2014).
[25] C. Hoffmann, S. Dollive, S. Grunberg, J. Chen, H. Li, G.D. Wu, J.D. Lewis, F. D. Bushman, Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents, PLoS One 8 (2013) e66019.
[26] R. Mohammadzadeh, A. Mahnert, S. Duller, C. Moissl-Eichinger, Archaeal key-residents within the human microbiome: characteristics, interactions and involvement in health and disease, Curr. Opin. Microbiol. 67 (2022) 102146.
[27] A.K. Nash, T.A. Auchtung, M.C. Wong, D.P. Smith, J.R. Gesell, M.C. Ross, C. J. Stewart, G.A. Metcalf, D.M. Muzny, R.A. Gibbs, N.J. Ajami, J.F. Petrosino, The gut mycobiome of the human microbiome project healthy cohort, Microbiome 5 (2017).
[28] M. Lecuit, M. Eloit, The human virome: new tools and concepts, Trends Microbiol. 21 (2013) 510–515.
[29] A. Lokmer, A. Cian, A. Froment, N. Gantois, E. Viscogliosi, M. Chabé, L. Ségurel, Use of shotgun metagenomics for the identification of protozoa in the gut microbiota of healthy individuals from worldwide populations with various industrialization levels, PLoS One 14 (2019) e0211139.
[30] J.F. Meadow, A.E. Altrichter, S.W. Kembel, M. Moriyama, T.K. O'Connor, A. M. Womack, G.Z. Brown, J.L. Green, B.J.M. Bohannan, Bacterial communities on classroom surfaces vary with human contact, Microbiome 2 (2014).
[31] N. Fierer, C.L. Lauber, N. Zhou, D. McDonald, E.K. Costello, R. Knight, Forensic identification using skin bacterial communities, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 6477–6481.
[32] M.G. Garcia, M.D. Perez-Carceles, E. Osuna, I. Legaz, Impact of the human microbiome in forensic sciences: a systematic review, Appl. Environ. Microbiol. 86 (2020).
[33] The Human Microbiome Project Consortium, Structure, function and diversity of the healthy human microbiome, Nature 486 (2012) 207.
[34] I.C. Anderson, J.W.G. Cairney, Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques, Environ. Microbiol. 6 (2004) 769–779.
[35] J.C. Luongo, A. Barberan, R. Hacker-Cary, E.E. Morgan, S.L. Miller, N. Fierer, Microbial analyses of airborne dust collected from dormitory rooms predict the sex of occupants, Indoor Air 27 (2017) 338–344.
[36] S.W. Kembel, E. Jones, J. Kline, D. Northcutt, J. Stenson, A.M. Womack, B. J. Bohannan, G.Z. Brown, J.L. Green, Architectural design influences the diversity and structure of the built environment microbiome, ISME J. 6 (2012) 1469–1479.
[37] S.J. Song, C. Lauber, E.K. Costello, C.A. Lozupone, G. Humphrey, D. Berg-Lyons, J.G. Caporaso, D. Knights, J.C. Clemente, S. Nakielny, J.I. Gordon, N. Fierer, R. Knight, Cohabiting family members share microbiota with one another and with their dogs, Elife 2 (2013) e00458.
[38] I. Kuiper, Microbial forensics: next-generation sequencing as catalyst: The use of new sequencing technologies to analyze whole microbial communities could become a powerful tool for forensic and criminal investigations, EMBO Rep. 17 (2016) 1085–1087.
[39] V. Torsvik, L. Ovreas, Microbial diversity and function in soil: from genes to ecosystems, Curr. Opin. Microbiol. 5 (2002) 240–245.
[40] J. Ma, C. Coarfa, X. Qin, P.E. Bonnen, A. Milosavljevic, J. Versalovic, K. Aagaard, mtDNA haplogroup and single nucleotide polymorphisms structure human microbiome communities, BMC Genom. 15 (2014) 257.
[41] S.H. Katsanis, J. Kim, M.A. Minear, S. Chandrasekharan, J.K. Wagner, Preliminary perspectives on DNA collection in anti-human trafficking efforts, Recent Adv. DNA Gene Seq. 8 (2014) 78–90.
[42] J. Chase, J. Fouquier, M. Zare, D.L. Sonderegger, R. Knight, S.T. Kelley, J. Siegel, J.G. Caporaso, Geography and location are the primary drivers of office microbiome composition, Am. Soc. Microbiol. J. 1 (2016).
[43] G.E. Flores, S.T. Bates, D. Knights, C.L. Lauber, J. Stombaugh, R. Knight, N. Fierer, Microbial biogeography of public restroom surfaces, PLoS One 6 (2011) e28132.
[44] I. Can, G.T. Javan, A.E. Pozhitkov, P.A. Noble, Distinctive thanatomicrobiome signatures found in the blood and internal organs of humans, J. Microbiol. Methods 106 (2014) 1–7.
[45] H.R. Johnson, D.D. Trinidad, S. Guzman, Z. Khan, J.V. Parziale, J.M. DeBruyn, N. H. Lents, A machine learning approach for using the postmortem skin microbiome to estimate the postmortem interval, PLoS One 11 (2016) e0167370.
[46] G.T. Javan, S.J. Finley, T. Smith, J. Miller, J.E. Wilkinson, Cadaver thanatomicrobiome signatures: The ubiquitous nature of Clostridium species in human decomposition, Front. Microbiol. 8 (2017) 2096.
[47] J.L. Metcalf, L. Wegener Parfrey, A. Gonzalez, C.L. Lauber, D. Knights, G. Ackermann, G.C. Humphrey, M.J. Gebert, W. Van Treuren, D. Berg-Lyons, K. Keepers, Y. Guo, J. Bullard, N. Fierer, D.O. Carter, R. Knight, A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system, Elife 2 (2013) e01104.
[48] J.L. Metcalf, Estimating the postmortem interval using microbes: knowledge gaps and a path to technology adoption, Forensic Sci. Int.: Genet. 38 (2019) 211–218.
[49] J.L. Metcalf, Z.Z. Xu, S. Weiss, S. Lax, W. Van Treuren, E.R. Hyde, S.J. Song, A. Amir, P. Larsen, N. Sangwan, D. Haarmann, G.C. Humphrey, G. Ackermann, L. R. Thompson, C. Lauber, A. Bibat, C. Nicholas, M.J. Gebert, J.F. Petrosino, S. C. Reed, J.A. Gilbert, A.M. Lynne, S.R. Bucheli, D.O. Carter, R. Knight, Microbial community assembly and metabolic function during mammalian corpse decomposition, Science 351 (2016) 158–162.
[50] J.L. Pechal, T.L. Crippen, M.E. Benbow, A.M. Tarone, S. Sowd, J.K. Tomberlin, The potential use of bacterial community succession in forensics as described by high throughput metagenomic sequencing, Int. J. Leg. Med. 128 (2014) 193–205.
[51] K.E. Lawrence, K.C. Lam, A. Morgun, N. Shulzhenko, C.V. Löhr, Effect of temperature and time on the thanatomicrobiome of the cecum, ileum, kidney, and lung of domestic rabbits, J. Vet. Diagn. Investig. 31 (2019) 155–163.
[52] L. Hu, Y. Xing, P. Jiang, L. Gan, F. Zhao, W. Peng, W. Li, Y. Tong, S. Deng, Predicting the postmortem interval using human intestinal microbiome data and random forest algorithm, Sci. Justice 61 (2021) 516–527.
[53] J. Adserias-Garriga, N.M. Quijada, M. Hernandez, D. Rodríguez Lázaro, D. Steadman, L.J. Garcia-Gil, Dynamics of the oral microbiota as a tool to estimate time since death, Mol. Oral. Microbiol. 32 (2017).
[54] J.L. Pechal, C.J. Schmidt, H.R. Jordan, M.E. Benbow, A large-scale survey of the postmortem human microbiome, and its potential to provide insight into the living health condition, Sci. Rep. 8 (2018).
[55] J.L. Pechal, C.J. Schmidt, H.R. Jordan, M.E. Benbow, Frozen: Thawing and its effect on the postmortem microbiome in two pediatric cases, J. Forensic Sci. 62 (2017) 1399–1405.
[56] C.R. Bell, J.E. Wilkinson, B.K. Robertson, G.T. Javan, Sex-related differences in the thanatomicrobiome in postmortem heart samples using bacterial gene regions V1-2 and V4, Lett. Appl. Microbiol. 67 (2018) 144–153.
[57] G.T. Javan, S.J. Finley, I. Can, J.E. Wilkinson, J.D. Hanson, A.M. Tarone, Human thanatomicrobiome succession and time since death, Sci. Rep. 6 (2016) 1–9.
[58] N. Ogbanga, A. Nelson, S. Gino, D.J. Wescott, H.L. Mickleburgh, T.P. Gocha, N. Procopio, The impact of freezing on the post-mortem human microbiome, Front. Ecol. Evol. 11 (2023) 1151001.
[59] V.K. Gupta, S. Paul, C. Dutta, Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity, Front. Microbiol. 8 (2017) 1162.
[60] A.R. Walker, S. Datta, Identification of city specific important bacterial signature for the MetaSUB CAMDA challenge microbiome data, Biol. Direct 14 (2019) 11.
[61] L. Brinkac, T.H. Clarke, H. Singh, C. Greco, A. Gomez, M.G. Torralba, B. Frank, K. E. Nelson, Spatial and environmental variation of the human hair microbiota, Sci. Rep. 8 (2018) 9017.
[62] S. Nagasawa, H. Motani-Saitoh, H. Inoue, H. Iwase, Geographic diversity of Helicobacter pylori in cadavers: forensic estimation of geographical origin, Forensic Sci. Int. 229 (2013) 7–12.
[63] B. Moitas, I.M. Caldas, B. Sampaio-Maia, Forensic microbiology and geographical location: a systematic review, Aust. J. Forensic Sci. (2023) 1–16.

[64] T. Clarke, L. Brinkac, C. Greco, A.T. Alleyne, P. Carrasco, C. Inostroza, T. Tau, W. Wisitrasameewong, M.G. Torralba, K. Nelson, H. Singh, Sampling from four geographically divergent young female populations demonstrates forensic geolocation potential in microbiomes, Sci. Rep. 12 (2022) 18547.
[65] H. Habtom, Z. Pasternak, O. Matan, C. Azulay, R. Gafny, E. Jurkevitch, Applying microbial biogeography in soil forensics, Forensic Sci. Int.: Genet. 38 (2019) 195–203.
[66] H. Singh, T. Clarke, L. Brinkac, C. Greco, K.E. Nelson, Forensic microbiome database: a tool for forensic geolocation meta-analysis using publicly available 16S rRNA microbiome sequencing, Front. Microbiol. 12 (2021) 644861.
[67] S.E. Schmedes, A.E. Woerner, B. Budowle, Forensic human identification using skin microbiomes, Appl. Environ. Microbiol. 83 (2017).
[68] M.E. Cantrell, E. Sylvestre, H.C. Wharton, R. Scheidegger, L. Curchod, D.M. Gute, J. Griffiths, T.R. Julian, A.J. Pickering, Hands are frequently contaminated with fecal bacteria and Enteric pathogens globally: a systematic review and meta-analysis, ACS Environ. Au 3 (2023) 123–134.
[69] J. Park, S.J. Kim, J.A. Lee, J.W. Kim, S.B. Kim, Microbial forensic analysis of human-associated bacteria inhabiting hand surface, Forensic Sci. Int.: Genet. Suppl. Ser. 6 (2017) e510–e512.
[70] M. Richardson, N. Gottel, J.A. Gilbert, S. Lax, Microbial similarity between students in a common dormitory environment reveals the forensic potential of individual microbial signatures, mBio 10 (2019).
[71] E.A. Franzosa, K. Huang, J.F. Meadow, D. Gevers, K.P. Lemon, B.J.M. Bohannan, C. Huttenhower, Identifying personal microbiomes using metagenomic codes, Proc. Natl. Acad. Sci. U. S. A. 112 (2015).
[72] D. Wilkins, M.H.Y. Leung, P.K.H. Lee, Microbiota fingerprints lose individually identifying features over time, Microbiome 5 (2017).
[73] S.L. Leake, M. Pagni, L. Falquet, F. Taroni, G. Greub, The salivary microbiome for differentiating individuals: proof of principle, Microbes Infect. 18 (2016) 399–405.
[74] N. Procopio, F. Lovisolo, G. Sguazzi, S. Ghignone, S. Voyron, M. Migliario, F. Renò, F. Sellitto, G. D'Angiolella, P. Tozzo, L. Caenazzo, S. Gino, Touch microbiome as a potential tool for forensic investigation: a pilot study, J. Forensic Leg. Med. 82 (2021) 102223.
[75] D.W. Williams, G. Gibson, Individualization of pubic hair bacterial communities and the effects of storage time and temperature, Forensic Sci. Int.: Genet. 26 (2017) 12–20.
[76] D.W. Williams, G. Gibson, Classification of individuals and the potential to detect sexual contact using the microbiome of the pubic region, Forensic Sci. Int.: Genet. 41 (2019) 177–187.
[77] M. Doi, S. Gamo, T. Okiura, H. Nishimukai, M. Asano, A simple identification method for vaginal secretions using relative quantification of Lactobacillus DNA, Forensic Sci. Int.: Genet. 12 (2014) 93–99.
[78] J. Ravel, P. Gajer, Z. Abdo, G.M. Schneider, S.S.K. Koenig, S.L. McCulle, S. Karlebach, R. Gorle, J. Russell, C.O. Tacket, R.M. Brotman, C.C. Davis, K. Ault, L. Peralta, L.J. Forney, Vaginal microbiome of reproductive-age women, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 4680–4687.
[79] M. Vaneechoutte, The human vaginal microbial community, Res. Microbiol. 168 (2017) 811–825.
[80] K.N. Zou, L.J. Ren, Y. Ping, K. Ma, H. Li, Y. Cao, H.G. Zhou, Y.L. Wei, Identification of vaginal fluid, saliva, and feces using microbial signatures in a Han Chinese population, J. Forensic Leg. Med. 43 (2016) 126–131.
[81] T. Akutsu, H. Motani, K. Watanabe, H. Iwase, K. Sakurada, Detection of bacterial 16S ribosomal RNA genes for forensic identification of vaginal fluid, Leg. Med. 14 (2012) 160–162.
[82] H. Nakanishi, A. Kido, T. Ohmori, A. Takada, M. Hara, N. Adachi, K. Saito, A novel method for the identification of saliva by detecting oral streptococci using PCR, Forensic Sci. Int. 183 (2009) 20–23.
[83] S. Karadayi, T. Arasoglu, I. Akmayan, B. Karadayı, Assessment of the exclusion potential of suspects by using microbial signature in sexual assault cases: a scenario-based experimental study, Forensic Sci. Int. 325 (2021) 110886.
[84] R. Dixon, S. Egan, S. Hughes, B. Chapman, The sexome - a proof of concept study into microbial transfer between heterosexual couples after sexual intercourse, Forensic Sci. Int. 348 (2023) 111711.
[85] D.M. Kennedy, J.-A.L. Stanton, J.A. Garcia, C. Mason, C.J. Rand, J.A. Kieser, G. R. Tompkins, Microbial analysis of bite marks by sequence comparison of streptococcal DNA, PLoS One 7 (2012) e51757.
[86] A.J. Freeman, D.R. Senn, D.M. Arendt, Seven hundred seventy eight bite marks: analysis by anatomic location, victim and biter demographics, type of crime, and legal disposition, J. Forensic Sci. 50 (2005) 1436–1443.
[87] B. Moitas, I.M. Caldas, B. Sampaio-Maia, Forensic microbiology and bite marks: a systematic review, J. Odonto-Stomatol. 40 (2022) 44–51.
[88] T.R. Elliot, A.H. Rogers, J.R. Haverkamp, D. Groothuis, Analytical pyrolysis of Streptococcus saliivarius as and aid to identification in bite-mark investigation, Forensic Sci. Int. 26 (1984) 131–137.
[89] D.M. Kennedy, J.-A.L. Stanton, J.A. García, C. Mason, C.J. Rand, J.A. Kieser, G. R. Tompkins, Microbial analysis of bite marks by sequence comparison of streptococcal DNA, PLoS One 7 (2012) e51757.
[90] M. Rahimi, N.C.K. Heng, J.A. Kieser, G.R. Tompkins, Genotypic comparison of bacteria recovered from human bite marks and teeth using arbitrarily primed PCR, J. Appl. Microbiol. 99 (2005) 1265–1270.
[91] L. Hsu, D. Power, J. Upritchard, J. Burton, R. Friedlander, J. Horswell, C. MacDonald, J. Kieser, G. Tompkins, Amplification of oral streptococcal DNA from human incisors and bite marks, Curr. Microbiol. 65 (2012) 207–211.
[92] K.A. Brown, T.R. Elliot, A.H. Rogers, J.C. Thonard, The survival of oral streptococci on human skin and its implication in bite-mark investigation, Forensic Sci. Int. 26 (1984) 193–197.
[93] C. Diez Lopez, A. Vidaki, M. Kayser, Integrating the human microbiome in the forensic toolkit: Current bottlenecks and future solutions, Forensic Sci. Int.: Genet. 56 (2022) 102627.
[94] B.J. Koops, M. Schellekens, Forensic DNA phenotyping, STLR 9 (2008).
[95] L.A. David, A.C. Materna, J. Friedman, M.I. Campos-Baptista, M.C. Blackburn, A. Perrotta, S.E. Erdman, E.J. Alm, Host lifestyle affects human microbiota on daily timescales, Genome Biol. 15 (2014) R89.
[96] J.S. Escobar, B. Klotz, B.E. Valdes, G.M. Agudelo, The gut microbiota of Colombians differs from that of Americans, Europeans and Asians, BMC Microbiol. 14 (2014) 311.
[97] D.O. Carter, J.L. Metcalf, A. Bibat, R. Knight, Seasonal variation of postmortem microbial communities, Forensic Sci. Med. Pathol. 11 (2015) 202–207.
[98] K.L. Cobaugh, S.M. Schaeffer, J.M. DeBruyn, Functional and structural succession of soil microbial communities below decomposing human cadavers, PLoS One 10 (2015) e0130201.
[99] S. Yooseph, C. Andrews-Pfannkoch, A. Tenney, J. McQuaid, S. Williamson, M. Thiagarajan, D. Brami, L. Zeigler-Allen, J. Hoffman, J.B. Goll, D. Fadrosh, J. Glass, M.D. Adams, R. Friedman, J.C. Venter, A metagenomic framework for the study of airborne microbial communities, PLoS One 8 (2013) e81862.
[100] M.B. Jones, W.C. Nierman, Y. Shan, B.C. Frank, A. Spoering, L. Ling, A. Peoples, A. Zullo, K. Lewis, K.E. Nelson, Reducing the bottleneck in discovery of novel antibiotics, Microb. Ecol. 73 (2017) 658–667.
[101] J.M. Choo, L.E.X. Leong, G.B. Rogers, Sample storage conditions significantly influence faecal microbiome profiles, Sci. Rep. 5 (2015) 16350.
[102] E.A. Franzosa, K. Huang, J.F. Meadow, D. Gevers, K.P. Lemon, B.J.M. Bohannan, C. Huttenhower, Identifying personal microbiomes using metagenomic codes, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) E2930–E2938.
[103] F. Plaza-Onate, J.M. Batto, C. Juste, J. Fadlallah, C. Fougeroux, D. Gouas, N. Pons, S. Kennedy, F. Levenez, J. Dore, S.D. Ehrlich, G. Gorochov, M. Larsen, Quality control of microbiota metagenomics by k-mer analysis, BMC Genom. 16 (2015) 183.
[104] S.J. Salter, M.J. Cox, E.M. Turek, S.T. Calus, W.O. Cookson, M.F. Moffatt, P. Turner, J. Parkhill, N.J. Loman, A.W. Walker, Reagent and laboratory contamination can critically impact sequence-based microbiome analyses, BMC Biol. 12 (2014) 87.
[105] K.M. Hewitt, C.P. Gerba, S.L. Maxwell, S.T. Kelley, Office space bacterial abundance and diversity in three metropolitan areas, PLoS One 7 (2012) e37849.

Further reading

[1] M. Ghemrawi, A.R. Torres, G. Duncan, R. Colwell, M. Dadlani, B. McCord, The genital microbiome and its potential for detecting sexual assault, Forensic Sci. Int.: Genet. 51 (2021) 102432.
[2] J. De Alcaraz-Fossoul, Y. Wang, R. Liu, M. Mancenido, P.A. Marshall, C. Núñez, J. Broatch, L. Ferry, Microbes in fingerprints: A source for dating crime evidence? Forensic Sci. Int: Genet. 65 (2023) 102883.