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Review Article

Human oral microbiome as forensic biomarkers for individual identification: A systematic review

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ABSTRACT

Background: The oral microbiome is a promising and novel source of biomarkers for forensic identification. It offers distinct signatures that may differentiate individuals, sparking new avenues of research and discovery.

Aim and Objective: This systematic review is meticulously designed to comprehensively consolidate current research on oral microbiome signatures in forensic contexts, leaving no stone unturned in our quest for knowledge. It focuses explicitly on microbial diversity, community structure, and specific taxa as potential markers for individual identification.

Materials and Methods: To identify relevant studies published between 2014 and 2024, a comprehensive search of major scientific databases was conducted. Inclusion criteria encompassed peer-reviewed articles investigating oral microbiome diversity and specific microbial markers relevant to forensic individual identification. These studies were employed to evaluate the reliability and consistency of these biomarkers across diverse populations and environmental conditions.

Results: The review included 13 studies that met the criteria, revealing significant associations between specific oral microbial taxa and individual identification. This systematic review highlighted consistent discriminatory power and stability of microbial signatures across varied populations.

Conclusion: Oral microbiome analysis is promising to enhance forensic investigations by providing unique biomarkers for individual identification. However, challenges such as standardization of microbiome studies and influences of environmental factors on microbiome underscore the need for further research to validate and effectively implement oral microbiome data in forensic practice.

1. Introduction

Forensic identification mainly relies on physical characteristics, DNA profiling, and dental records. One of the most promising uses of the newly-emerging field of forensic microbiology is the estimation of the postmortem interval (PMI), or the amount of time elapsed since death. The human microbiome is crucial to the breakdown of postmortem tissues and is crucial to individual identification.[1] The human microbiota is made up of hundreds of different bacterial genera and species that are primarily grouped into four distinct phyla: the Pseudomonadota (former Proteobacteria), the Bacteroidota (former Bacteroidetes), the Bacillota (former Firmicutes), and the Actinomycetota (former Actinobacteria).[2]

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The oral cavity is home to a dynamic microflora that has a substantial impact on overall health. It is the second most diverse microbial location in the human body, next to the gastrointestinal tract.[3] Each person's oral microbiota maintains a somewhat distinct composition, despite varying degrees of disruption and it affects the many parts of the oral cavity (teeth, tongue, gingiva, saliva). More than 1000 bacterial species, representing more than 10 phyla, are counted there, with the most prevalent genera being Streptococcus, Fusobacterium, Lactobacillus, Actinomyces, Veilonella, and Neisseria. These phyla are primarily Bacillota, Bacteroidota, Pseudomonadota, Actinomycetota, and Fusobacteriota.[4]

Recent advancements in microbiome research have identified unique microbial signatures that remain stable and distinct to individuals over time.[5] The study of the oral microbiome has expanded beyond its role in oral diseases like dental caries, periodontal disease, and oral cancer, to encompass broader health conditions such as cardiovascular diseases, diabetes, and systemic infections.[6]

Microorganisms in the oral cavity play a crucial role in balancing health and disease, encompassing a vast diversity of species.[4] Studies show that individuals with periodontal disease have higher percentages of Spirochetes, Synergistetes, Firmicutes, and Chloroflexi, while healthy individuals have more Actinobacteria, especially Actinomyces.[7] High oral disease conditions also exhibit increased bacterial diversity, including Clostridiales cluster bacteria.[7]

The oral microbiome, a complex and diverse community of microorganisms residing in the oral cavity, has garnered significant attention with advancements in Next Generation Sequencing (NGS) and implementation of the Human Microbiome Project.[4] This growing field on microbiome research has illuminated the crucial roles that bacteria, fungi, viruses, and archaea play in maintaining oral health and contributing to disease processes.[8]

Recent research highlights the importance of understanding oral microbiome signatures for various applications, including forensic identification and personalized medicine.[5] Dysregulation of the oral microbiota is increasingly recognized as a key factor in various health conditions, driving the need for advanced detection and treatment methods.[7]

The oral microbiome holds great potential as a forensic biomarker for individual identification, as evidenced by various studies. Research has shown that the oral microbiome is highly individualized, with unique microbial markers that can vary between individuals, making it a promising tool for forensic applications.[9] Studies comparing oral microbiomes of individuals from different regions revealed shared core microbiota and region-specific taxa, indicating the potential for geographical provenience determination.[10] Additionally, the oral microbiome's role in postmortem events, known as the thanatomicrobiome, has been highlighted, showcasing the importance of microbial activity in the early stages of decomposition and the potential for forensic odontology applications.[2] The distinct signatures of oral microbiome's offer a valuable avenue for individual identification in forensic investigations, with further research needed to realize its full potential.

This review aims to amalgamate the current state of knowledge on the oral microbiome, covering recent advancements in microbiome characterization, gaps in existing research, and integrating microbiome data into personalized dentistry and forensic identification. By examining the roles of different microbial taxa and their interactions with host factors, this article provides a comprehensive overview of how oral microbiome research evolves and its implications for future studies and clinical applications.

2. Materials and Methods

2.1. Search strategy

A comprehensive search was conducted across major scientific databases like PubMed, Scopus, Web of Science, Elsevier, Google Scholar and Researchgate using relevant keywords such as 'Forensic individual identification', 'Forensic biomarkers' and 'Oral microbiome'. Studies published between 2014 and 2024 were included, focusing on human subjects and investigations of oral microbiome diversity and specific taxa associated with individual identification. Records that were not written in English, did not have full-text access or were categorised as commentary, conference abstracts, or posters were excluded based on certain criteria. We retrieved and carefully reviewed articles that fit our interest criteria, extracting relevant data for our study.

This protocol was registered with the PROSPERO International Prospective Register of Systematic Reviews (Registration number CRD42024581846).

2.2. Selection criteria

Selection process involved an initial screening of titles and abstracts, followed by a thorough review of full-text articles to confirm their relevance. The inclusion criteria mandated that studies focus on human subjects and investigate the use of oral microbiome diversity or specific microbial taxa as forensic biomarkers. Additionally, studies are needed to provide data on applying these microbiome signatures for individual identification. Studies not meeting these criterias were excluded, like those involving non-human subjects, non-English publications, or non-peer-reviewed content like case reports and general reviews.

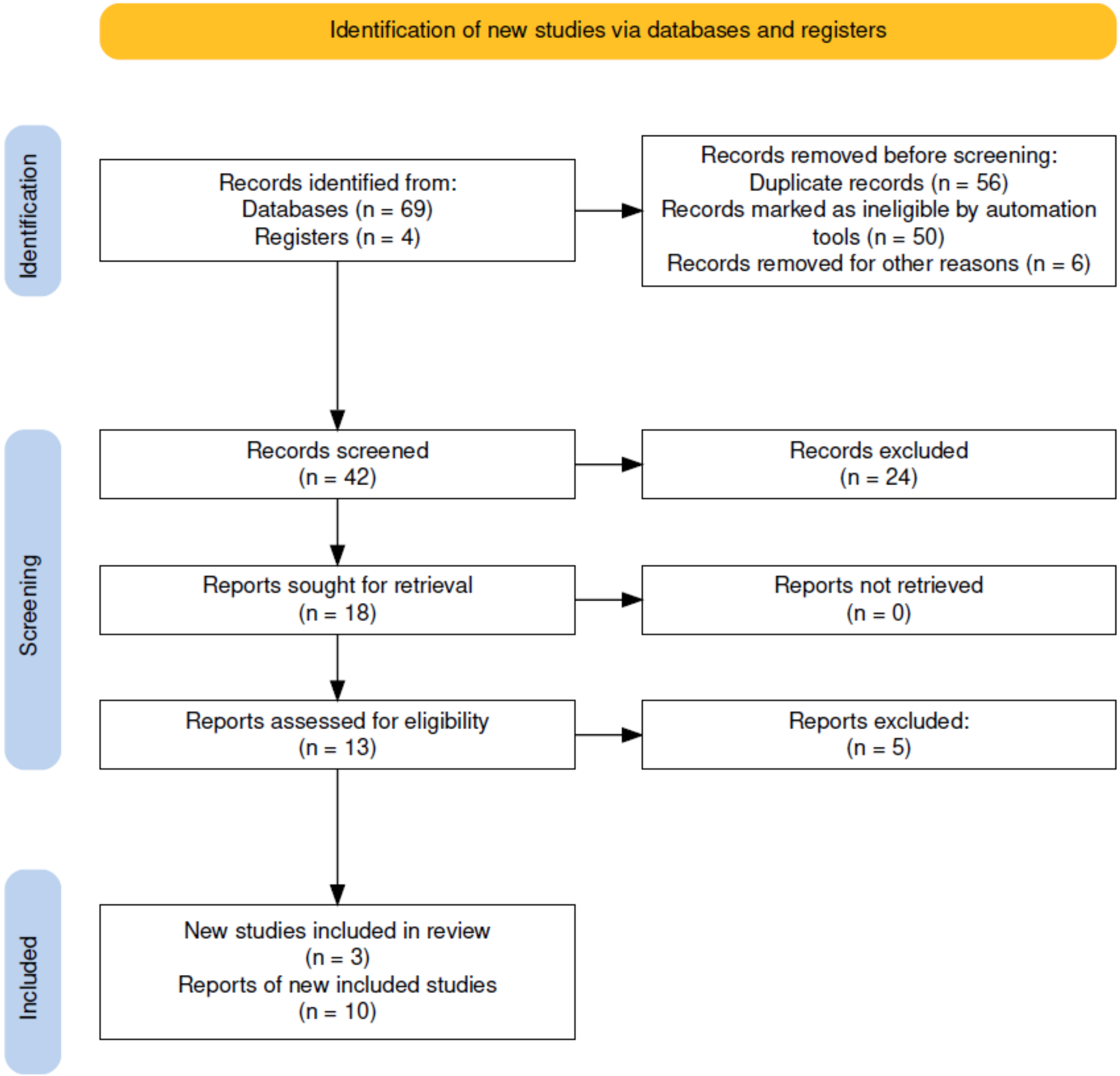


Diagram 1: PRISMA flow chart

2.3. *Quality assessment*

From the initial search results, 13 studies were selected based on their adherence to the inclusion criteria. Two independent reviewers used a standardized form to extract data. The data included study characteristics, sample sizes, methodologies for analyzing oral microbiome diversity, and findings on the effectiveness of microbiome signatures for individual identification. Reviewer disagreements during the data extraction were resolved through discussion to ensure accuracy and consistency.

Interobserver reliability was evaluated using Cohen's kappa statistic for the study selection and data extraction processes. Initial kappa scores were calculated to measure agreement, with discrepancies resolved through discussion to achieve consensus. The risk of bias across studies was visualized using a risk of bias graph (Figures 1 and 2) generated with Review Manager (RevMan) software, which displayed the proportion of studies categorized by risk levels in different domains.

3. Results

Thirteen studies met the inclusion criteria, encompassing diverse populations and methodologies. The studies primarily examined microbial diversity indices, specific taxa abundance, and microbial community structure as potential forensic biomarkers. The results are tabulated in Table 1.

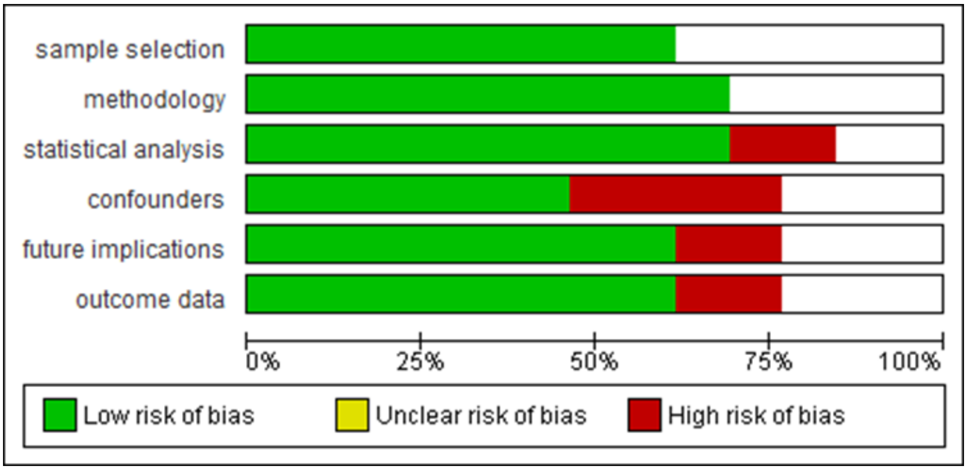
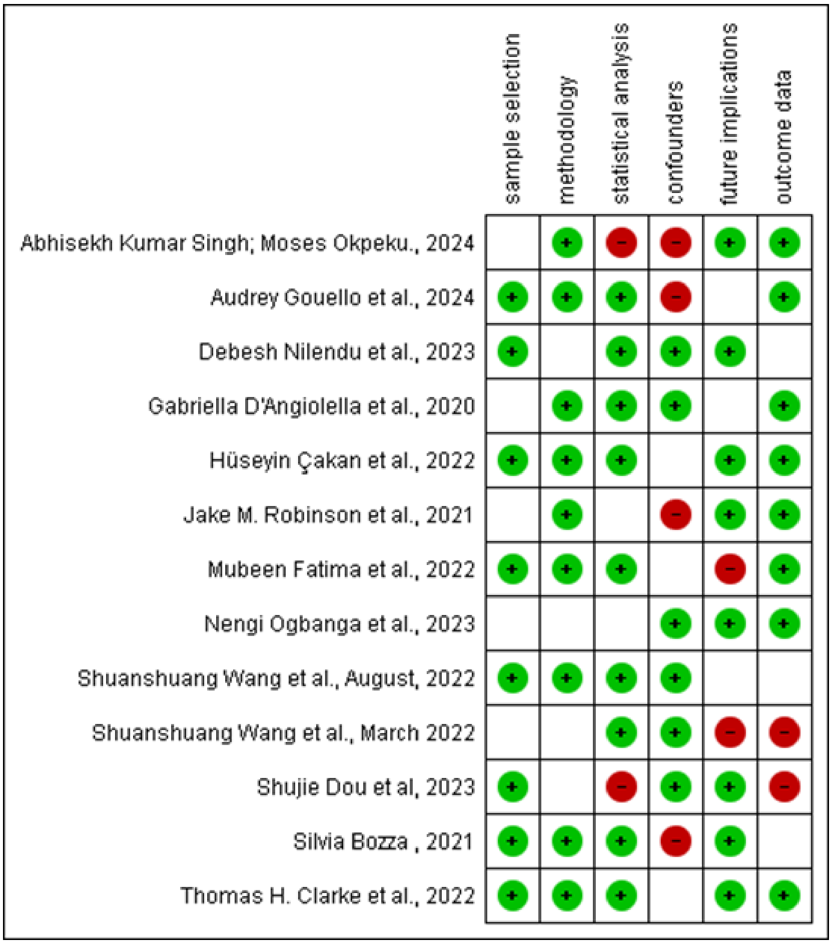


Figure 1: Graph displaying review authors' judgments on each risk of bias item, presented as percentages across all included studies



	sample selection	methodology	statistical analysis	confounders	future implications	outcome data
Abhisekh Kumar Singh; Moses Okpeku., 2024		+	−	−	+	+
Audrey Gouello et al., 2024	+	+	+	−		+
Debesh Nilendu et al., 2023	+		+	+	+	
Gabriella D'Angiolella et al., 2020		+	+	+		+
Hüseyin Çakan et al., 2022	+	+	+		+	+
Jake M. Robinson et al., 2021		+		−	+	+
Mubeen Fatima et al., 2022	+	+	+		−	+
Nengi Ogbanga et al., 2023				+	+	+
Shuanshuang Wang et al., August, 2022	+	+	+	+		
Shuanshuang Wang et al., March 2022			+	+	−	−
Shujie Dou et al, 2023	+		−	+	+	−
Silvia Bozza , 2021	+	+	+	−	+	
Thomas H. Clarke et al., 2022	+	+	+		+	+

Figure 2: Risk of bias summary: Review authors' assessments of the risk of bias for each included study

Table 1: Characteristics of included studies on oral microbiome signatures as forensic biomarkers for individual identification

S. No.	Author and year	Population Sample and Sampling Methods	Methods Used	Dataset	Results	Conclusions	Future implications	Limitations
1	Abhisekh Kumar Singh; Moses Okpeku., 2024[11]	371 articles	Next-generation sequencing	Review article	The human microbiome shows promise in forensic science for identifying individuals, determining geographic origins, aiding sexual assault cases, and analyzing post-mortem changes. Variations in microbial profiles can reveal location, and decomposition stages provide insights into time and place of death.	The microbiome displays significant variation, which can be utilized to distinguish individuals based on their distinctive microbial signatures, facilitating individual identification.	The microbiome can reveal information about an individual's lifestyle and potential diseases, raising privacy concerns. A major issue in the field is the lack of consensus among scientific communities on experimental and analytical methods.	Understanding microbial signature analysis is crucial for forensic use, requiring further research on deposition time, standardization, privacy, and technology development.
2	Shujie Dou et al, 2023[12]	Small sample size	Metagenomic sequencing on saliva and feces samples.	Saliva and feces samples from students and manual laborers	Results of ANOSIM showed that there were significant differences between male students and male laborers in saliva and fecal samples	Different oral and gut microbial communities between students and workers.	Explore larger sample sizes for more representative results.	Small sample size

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Table 1 continued

S. No.	Author and year	Population Sample and Sampling Methods	Methods Used	Dataset	Results	Conclusions	Future implications	Limitations
		Sampling methods not specified	Built random forest models with recursive feature elimination processes.	Data analyzed for taxonomic and functional annotations in five databases	RF models with 100% accuracy based on specific species/ pathways.	Microbiome information shows potential for occupational inference.	Investigate additional occupational sectors for comprehensive analysis.	Limited representativeness
3	Audrey Gouello et al., 2024[13]	Population sample size: Samples from healthy volunteers tested.	Next-generation sequencing technologies	Saliva, vaginal fluid, blood, semen, skin swabs	Bacterial communities in isolated fluids identified specific bacterial orders.	Bacterial communities in isolated fluids allow fluid identification.	Consolidate the identification protocol.	Limits of sensitivity and specificity in current identification methods.
		Sampling methods: Next-generation sequencing technologies used for bacterial community analysis.	Analysis of bacterial communities in biological fluids	Mixtures of biological fluids in a 1:1 ratio	Oral and vaginal microbiomes precluded interpretation in fluid mixtures.	Interpreting bacterial microbiomes in fluid mixtures is challenging.	Ensure reliability through further analyses.	The predominance of certain bacterial microbiomes inhibits interpretation in fluid mixtures.

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Table 1 continued

S. No.	**Author and year**	**Population Sample and Sampling Methods**	**Methods Used**	**Dataset**	**Results**	**Conclusions**	**Future implications**	**Limitations**
4	Nengi Ogbanga et al., 2023[14]	Population sample size: 50 individuals	V4 region of the 16S rRNA gene was sequenced from the extracted microbial DNA	Oral samples from individuals in Lombardy and Piedmont regions.	A core oral microbiome was present across all samples.	The oral microbiomes of individuals from the same country and similar ethnicity are mainly identical.	Investigate donor characterizing taxa for identification purposes.	Skin microbiome may be better for human identification.
		Sampling method: Oral samples obtained by swabbing oral mucosa	Comparison of oral and skin microbiomes for identification purposes.	Skin and oral microbiome samples from the same donors.	Skin microbiome may be a better discriminant for human identification.	The skin microbiome may be a better discriminant for human identification than the oral one.	Explore bacterial signatures linked to specific lifestyles for investigative purposes.	Additional studies are required for further investigation.
5	Debesh Nilendu et al., 2023[15]	-	DNA/RNA sequencing for estimating biological diversity during decomposition stages	DNA/RNA sequencing	Oral microorganisms respond early in postmortem decomposition.	Oral microflora plays a significant role in forensic odontology.	Study advanced technology applications in thanatomicrobiome research.	-
			Next-generation sequencing to study oral thanatomicrobiome and epinecrotic communities	Metagenomic sequencing	DNA/RNA sequencing estimates biological diversity during decomposition stages.	Forensic research emphasizes the importance of thanatomicrobiology in postmortem events.	Explore further the role of oral microflora in forensic odontology.	

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Table 1 continued

S. No.	Author and year	Population Sample and Sampling Methods	Methods Used	Dataset	Results	Conclusions	Future implications	Limitations
6	Shuanshuang Wang et al., August, 2022[16]	-	Analyzes potential application value of oral microorganisms in identification.	Human oral microbiome	Characteristics of human oral microorganisms and microbial molecular markers are described.	Human oral microbiome can be a new biomarker for identification.	Explore unique microbial communities for individual identification.	-
			Reviews research progress of human oral microorganisms in forensic identification.	Microbial molecular markers	Potential application value of microorganisms in forensic individual identification is analyzed.	Microbial molecular markers show potential in forensic individual identification.	Investigate potential of microbial molecular markers in forensic identification.	
7	Thomas H. Clarke et al., 2022[17]	Population sample size: 206 young and healthy females.	Sequencing of 16S rRNA from stool and oral microbiomes	Raw datasets available in the NCBI SRA database under Bioproject PRJNA545251	Geolocation signals detected in microbiomes of diverse populations.	Microbiomes can uniquely identify individuals and reveal lifestyle patterns.	Develop algorithms for detecting geographic signatures in human microbiota.	Incomplete understanding of confounding factors
		Sampling method: 16S rRNA sequences of stool and oral microbiomes.	Analysis of geolocation signals between diverse populations	Processed datasets can be analyzed through the Forensic Microbiome Database (FMD)	Lifestyle variables partially affect geolocation signals in microbiomes.	Geolocation signals detected in microbiomes from diverse populations.	Utilize raw datasets for testing robust algorithms in microbiome research.	The paucity of diverse sequences

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Table 1 continued

S. No.	Author and year	Population Sample and Sampling Methods	Methods Used	Dataset	Results	Conclusions	Future implications	Limitations
8	Shuanshuang Wang et al., March 2022[9]	70 data in training set, 30 in test set	16S rRNA gene amplicon sequencing	PRJNA 765405	The salivary and buccal mucosal microbiota showed significant differences in composition.	Saliva and buccal mucosa have different bacterial community compositions.	Study unique bacteria presence for personal discrimination.	Few studies on forensic personal identification need supplementation.
		Random forest model used for classification of sample type	Random forest model for classification of microbiota differences	Publicly available original contributions presented in the study.	The random forest model based on microbial differences can classify saliva and buccal mucosa.	Oral microbiome has potential for forensic investigations and personal discrimination.	Explore core oral microbiota composition differences for forensic applications.	Limited application of oral microbiota in forensic personal discrimination.
9	Hüseyin Çakan et al., 2022[18]	Population sample size: 30 subjects	Cultivated 60 swab samples in 4 different media.	60 swab samples from 30 subjects	Alpha-hemolytic streptococci were the most dominant species found.	Salivary microbiota can be unique for individual identification in forensics.	Study unique microbiota features for individual identification in forensic science.	DNA damage or insufficiency in crime scene evidence
		Sampling methods: Swab samples from mouth and bitten finger	Investigated reproducible microorganisms in saliva for individual identification.	Cultivated in 4 different media: Blood, Chocolate, Endo, Chrom	Neisseria sp bacteria related to smoking, Candida sp to oral hygiene.	Streptococci and Neisseria bacteria are dominant in salivary microbiota.	Investigate relationship between salivary microbiota and lifestyle habits like smoking.	Microbiota used when DNA is insufficient or degraded
10	Silvia Bozza, 2021[19]	-	Salivary microbiota profiles	Salivary microbiota profiles	Salivary microbiota profiles can be used in forensic investigation	Salivary microbiota can aid forensic investigations when DNA methods fail.	Explore further applications of salivary microbiome in forensic science.	Limitations of intuition in decision-making

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Table 1 continued

S. No.	Author and year	Population Sample and Sampling Methods	Methods Used	Dataset	Results	Conclusions	Future implications	Limitations
			Bayes' factor for quantifying evidence value	Questioned and control materials in saliva form	Bayes' factor quantifies the value of the evidence	Bayes' factor quantifies evidence for forensic discrimination purposes effectively.	Investigate the potential of Bayes' factor in other forensic fields.	Limitations of using cut-off values as a mean of decision
11	Gabriella D' Angiolella et al., 2020[20]	-	Followed PRISMA Guidelines for review.	-	Salivary microbiome can be used as a genetic signature in forensics.	Oral microbiome can be used as a genetic signature in forensics.	Relate oral microbiome variation to specific factors.	Caution needed in interpreting results.
			Reviewed studies on oral microbiome for forensic applications.		Oral microbial analysis could become a useful forensic tool.	Further studies needed to understand oral microbiome variations and influences.	Direct research towards filling current knowledge gaps.	Further studies required to relate oral microbiome variations to factors.
12	Mubeen Fatima et al., 2022[21]	Sample size limitations hinder complete exploration of microbiomics potential.	Sequencing techniques, 16SrRNA datasets comparison for forensic microbiome database.	16SrRNA datasets from different body sites	Microbiome and metagenomics used for forensic investigations.	Human microbiome crucial in forensics for relation and exclusion.	Explore human microbe effectiveness for research and forensic purposes.	Sample size limitation affects studies.
		Various sampling methods used for human microbiome analysis in forensics.	Leave one out cross validation analysis for prediction of sex.	Human oral microbiome database (HOMD)	Potential toolkit for futuristic forensic investigations.	Forensic microbiome database development needed for future forensic investigations.	Include diverse human metadata in reputable microbiome databases for forensics.	Model accuracies limit exploration of microbiomics potential.

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Table 1 continued

S. No.	Author and year	Population Sample and Sampling Methods	Methods Used	Dataset	Results	Conclusions	Future implications	Limitations
13	Jake M. Robinson et al., 2021[5]	Modest sample sizes in studies, e.g., male (n 3), female (n 4).	Analysis of over 100 papers on forensic microbiome applications	Over 100 papers on forensic microbiome applications analyzed	Microbiome analysis has the potential for forensic applications.	Microbiomics has potential applications in forensics.	Explore fungal and viral communities for forensic applications.	Soil samples are complex and heterogeneous.
		Pubic hair microbiomes analyzed with small sample sizes.	Emphasis on geolocation, personal identification, trace evidence, cause of death, and postmortem interval (PMI)	Autopsy results from 42 cases studied for cause of death	Further research is needed to validate results.	Challenges remain in terms of sample sizes and model accuracies.	Validate soil microbiome analysis sensitivity and reproducibility for forensics.	Microbiomes exhibit high diversity in space and time.

4. Discussion

The oral microbiome has become a focal point of research with the advent of next-generation sequencing technologies and the Human Microbiome Project, revolutionizing our understanding of microbial communities of oral cavity.[6] This review comprises the current literature on the oral microbiome, emphasizing recent advancements in microbiome characterization through cutting-edge technologies. These advances have facilitated a more nuanced understanding of the oral cavity's microbial diversity, including bacteria, fungi, archaea, and viruses.

The oral cavity is the second most diverse microbial site in the human body, following the gastrointestinal tract.[4] The complex interplay between these microorganisms is critical in maintaining tissue homeostasis and oral health.[22] Integrating oral microbiome analysis into forensic protocols holds promise for enhancing individual identification accuracy and expanding forensic investigative capabilities.[23] The stable nature of microbial signatures and their uniqueness to individuals underscore their potential as supplementary or alternative forensic markers.[11]

The main goal of current thanatomicrobiome genetic profiling techniques is to clarify the microbial communities that are present in a given sample (commonly referred to as thanatogenomics). Two methodological approaches are used to sequence microbial communities: (a) Amplicon (marker gene) sequencing, which amplifies variable regions of a highly conserved bacterial gene, like the 16S rRNA gene, allowing phylogenetic inference of taxonomic/genomic relationships; and (b) Whole genome shotgun sequencing, also known as metagenomics, which sequences all of the DNA present in a sample (i.e., all "meta"). These techniques, which offer quick, accurate, and highly informative approaches for bacterial population identification, are based on the extraction of genetic material from samples utilising DNA commercial extraction kits, which considerably facilitate the procedure.[24]

Recent studies have utilized advanced sequencing techniques to analyze microbial communities in various biological samples, including saliva, feces, blood, semen, and skin swabs, from diverse populations.[13] For instance, Shujie Dou et al. (2023) identified distinct oral and gut microbial communities between students and manual laborers, suggesting potential occupational markers.[12] Similarly, Audrey Gouello et al. (2024) demonstrated that specific bacterial communities in isolated fluids could accurately identify fluid sources, though challenges remain in interpreting fluid mixtures.[13]

Further research by Nengi Ogbanga et al. (2023) highlighted that while the oral microbiome is broadly similar among individuals from the same region, the skin microbiome may offer better discriminative power for human identification.[10] Studies such as those by Debesh Nilendu et al. (2023) and Shuanshuang Wang et al. (2022) have emphasized the role of oral microorganisms in forensic odontology and the distinct bacterial profiles in salivary and buccal mucosal microbiota, respectively, underlining their forensic potential.[15,16]

However, several common limitations across these studies include small sample sizes, limited sensitivity and specificity of current methods, and the need for more diverse and representative sequences. For example, Thomas H. Clarke et al. (2022) identified geolocation signals in microbiomes but noted the incomplete understanding of confounding factors. Hüseyin Çakan et al. (2022) and Silvia Bozza (2021) explored salivary microbiota for forensic identification, acknowledging the challenges posed by DNA degradation and the limitations of current decision-making tools.[17–19]

While well-established algorithms like k-nearest neighbours, random forest models, and neural networks have proven beneficial for both classification and regression tasks in microbiome forensic applications, machine learning techniques clearly offer advantages when handling complex and multidimensional microbiome data; however, this requires the quantitative computation of pertinent forensic parameters.[2] If microbes may be utilised in courtrooms successfully, it will depend on how well-suited the microbiome knowledge is for refining error rates associated with PMI estimates and other forensic applications. These methods are in complete accordance with the well-known Locard concept, "every contact leaves a trace," which recognised that tangible evidence would be left at almost all crime scenes.[25]

Future research should focus on more extensive and diverse populations, refine analytical methods, and develop comprehensive forensic microbiome databases to advance this field. Despite these challenges, the unique microbial profiles associated with individuals and specific conditions offer a promising new frontier in forensic science, with potential applications in identifying individuals, determining geographic origins, and understanding postmortem changes. Challenges such as environmental influences, sample collection variability, and standardization of analytical methods remain critical considerations. There should also be longitudinal studies to validate oral microbiome signatures across diverse populations and environmental conditions. Advancements in sequencing technologies and bioinformatics tools will facilitate more precise identification and interpretation of microbial data in forensic settings.

5. Conclusion

Since bacteria are easy to sample and have unique microbial communities for every individual, using them for forensic identification offers an appealing alternative to conventional approaches. To enhance uniformity and comparability and, eventually, fortify microbiology's standing in the forensic sciences, standardising the techniques used in microbiological forensic analysis and combining them into a single protocol is necessary. Oral microbiome signatures represent a promising frontier in forensic science, offering unique biomarkers for individual identification. This systematic review emphasizes current evidence, underscores the potential applicability of oral microbiome data in forensic contexts, and outlines key considerations for future research and implementation. These gaps in information can only be filled by thorough investigations, possibly assisted by machine learning models, which will pave the way for a dependable strategy regarding the employment of microbes in legal settings.

6. Source of Funding

None.

7. Conflict of Interest

Nil.

Acknowledgments

Nil.

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