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Oral microbiomes as forensic markers of origin and migration: Insights from an underrepresented population, Nigeria

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ABSTRACT

The oral microbiome is shaped by environmental and host-associated factors, suggesting its utility for human profiling in forensics, particularly as an indicator of geographic origin and human migration. Using high-throughput sequencing and machine learning models, the predictive ability of the oral microbiome was assessed to determine the country of origin of the donors, using samples of individuals across 6 countries. The impact of migration on the predictive ability of the oral microbiome was also assessed through a longitudinal study of Nigerian migrants over a six-month period following migration. By analysing the oral microbiome at various time points during this timeframe, this study explores the influence of migration on the oral microbiome to provide insights into its applicability in forensic investigations. Our findings demonstrate that distinct microbial profiles correlate with the six geographic regions assessed in this study. Furthermore, the longitudinal sampling of Nigerian migrants revealed initial shifts in their microbiome profile, followed by a recovery to the original microbiome profile of Nigerian locals, observed after six months. These results highlight the forensic potential of the oral microbiome for geographic origin attribution, in migration tracking, and for providing intelligence information useful for forensic purposes.

1. Introduction

The human oral microbiome holds untapped potential for forensic human profiling due to its significant variability across individuals and populations [1-3]. As a diverse microbial ecosystem comprising over 700 species [4], the oral microbiome is shaped by environmental, genetic, and lifestyle factors [5-7], providing unique insights into an individual's identity and background. Hence, unlike other forensic markers such as DNA or fingerprints, the oral microbiome can potentially reflect dynamic factors such as diet [8], health status [9], lifestyle [10-13], and environmental exposures [14], making it a novel and complementary tool in forensic science.

One of the key advantages of using the oral microbiome for forensic profiling lies in its ability to vary geographically and culturally [15]. Studies have shown that populations from different regions exhibit distinct microbial signatures, driven in part by dietary habits and cultural practices [16,14,17,18]. A recent study [19] also showed significant differences in saliary microbiome between two similar populations, Poles and Serbians The assessment of oral microbiota as a potential forensic profiling tool to determine the geographic origin of an individual is primarily based on the principle that the oral microbiome is influenced by genetic and dietary factors – the combination of which can potentially be uniquely personified.

Beyond diet, other environmental and behavioural factors play a significant role in shaping oral microbial communities. Oral hygiene practices, smoking, and exposure to pollutants have all been shown to influence microbial composition [10,20]. For instance, smokers typically exhibit a reduced diversity in their oral microbiome, with a higher prevalence of pathogenic species, potentially offering forensic clues regarding lifestyle habits [21]. Additionally, access to clean water and exposure to industrial pollutants could leave microbial imprints that reflect an individual's environmental surroundings [22,23]. These environmental markers provide additional layers of information in forensic contexts, where understanding an individual's environmental

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history or habits can be pivotal.

Moreover, underlying health conditions, such as celiac disease [24,25], diabetes [26,27], and periodontitis [28,29], may alter the oral microbiome. These alterations may be characterized by shifts in microbial diversity or an overrepresentation of specific taxa associated with disease states. In forensic investigations, such microbiome signatures could provide valuable insights into an individual's health status at the time of death, potentially aiding in personal identification or contributing to the reconstruction of their medical history.

The application of microbial forensics is still in its infancy, but early studies suggest that microbial profiling, including the use of oral microbiota, could potentially be used to trace geographic origins [30-33] or to establish connections between suspects and specific environments [34]. Thus, oral microbial profiles could complement traditional forensic tools such as DNA and fingerprinting. A recent global study showed regional variation, with microbiome patterns clustering in ways that are detectable to machine learning models [35]. While the study focused on the gut microbiome, the oral microbiome has also been shown to exhibit individual and population-level variability [1,36,37]. Given that oral swab samples are non-invasive and easily collected in forensic investigations [38], exploring geographic variation in the oral microbiome could provide valuable investigative information about a person's geographic origin. If similar regional patterns exist in the oral microbiome, this approach could further enhance forensic human profiling.

This study, therefore, explores human oral microbial taxa across countries for the purpose of forensic biogeographical identification. It also aims to explore the influence of migration on the oral microbiome, specifically Nigerian migration to the UK, while documenting oral microbial communities within understudied populations (Nigeria), as publicly available microbiome data are populated primarily by samples from high income economies [39]. Finally, to understand the effects of migration, this study also assesses the ability of using machine learning models to accurately predict an individual's country-of-origin across a 6-month period after migration.

2. Methodology

2.1. Study design

To explore the aims of this study, both published oral microbiome data from other research and data generated from samples collected specifically for the purpose of this study were analysed. Hence, oral microbiome data of individuals from six countries – Nigeria, Italy (ITA), China (CHN), South Africa (SA), Thailand (THAI) and the United States of America (USA) – was assessed.

Fastq files for the ITA cohort was generated from previous research [37] conducted by the authors and added to this study. For the CHN, SA, THAI and USA cohorts, raw sequencing data (fastq files) were retrieved from The National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA). The search inclusion criteria on NCBI for samples yielding fastq files for these cohorts are listed as follows.

- only samples obtained from the buccal mucosa to reflect typical forensic sampling
- run on Ilumina MiSeq
- amplification of the 16S rRNA gene V4 region; and
- sample donors aged 25 – 40 years old.

For the Nigerian group, buccal swab samples were collected from 100 unrelated individuals, consisting of 50 locals (NG) and 50 migrants (Migrant). Participants in the NG cohort were required to: be local Nigerian residents who had not travelled abroad for at least one year prior to sampling; be aged between 25 and 40 years had not used antibiotics within six months before sample collection; refrain from eating for at least 30 min prior to sampling.

In the second Nigerian cohort, **Migrant**, the criteria were modified to enrol Nigerians who had migrated to the United Kingdom (UK) within six months of the initial sampling. All other criteria, excluding local residency and abroad travel, remained the same as with the NG cohort. Furthermore, to explore any effects on the oral microbiome due to continued residence in the UK after migration, the volunteers in the Migrant cohort were sampled twice, in addition to the initial sampling. While some donors dropped out of the study at different timepoints due to various personal reasons, a total of 41 (of 50) individuals were consistently sampled across all timepoints. Hence, sample collection from Migrant cohort occurred at:

- T0 (initial sampling),
- T1 (3 months after T0), and
- T2 (6 months after T2).

Sample collection for all Nigerian participants was carried out using the SMART-eNAT® kit (COPAN Group, Copan Italia, Brescia, Italy). Because the other cohorts (CHN, SA, THAI, USA, and ITA) were originally sampled using cotton swabs, we conducted a small-scale validation to ensure that differences in collection method did not significantly affect the microbiome data. Buccal samples were collected from four individuals in Migrant group using both cotton swabs and the SMART-eNAT® kit. Results from these paired samples, shown in Supplementary Figure 1, indicate that microbiome profiles obtained from either collection method are comparable. The purpose of including two cohorts originally from the same country was to assess the effects of migration on the oral microbiome

A description of each cohort, including the cohort size and the BioProject numbers, is given in Table 1. However, donor-specific data are available online through each study's associated NCBI projects.

2.2. Sample collection and storage

Regarding the sample collection method for the NG and Migrant cohorts, participants were required to self-collect by rubbing sterile swabs on the inside of their buccal cavity, specifically around the left and right cheeks. This was done for a duration of 15 s in each area – as in standard forensic sample collection practice. This method replicated the sample collection procedure for Italian cohort, as described in another study [37]. Samples collected with the SMART eNAT® kit were shipped to Northumbria University, UK, at room temperature, following manufacturer's instructions. The kit, containing a DNA stabilizing medium, allowed sample transport without the need for accompanying dry ice. All swab samples (on arrival to Northumbria University, UK) were stored at −20°C until subsequent analysis. Oral samples collected in this study from participants were obtained only after receiving documented informed consent from all donors. This study was approved by Northumbria University's Ethics Committee, UK, under the project reference number 1792.

Table 1
Table illustrating countries included in study and the number of donors associated with each group. Demographic data of specific participants are listed under the relevant BioProject on the NCBI database.

S/N	Country	Cohort	No of Donors	NCBI BioProject
1.	Nigeria	NG (locals)	50	PRJNA1150994
2.	Nigeria	Migrant T0	50	
3.	Nigeria	Migrant T1	44	
4.	Nigeria	Migrant T2	41	
5.	Italy	ITA	50	PRJNA1150994
6.	China	CHN	53	PRJNA765405
7.	South Africa	SA	44	PRJNA545251
8.	Thailand	THAI	32	
9.	United States of America	USA	21	PRJNA801882

2.3. DNA isolation and sequencing

Microbial DNA was isolated from 195 collected swab samples (189 samples from 185 Nigerian participants (this includes 4 replicate SMART eNAT® swabs) and 6 extraction and sequencing controls) using the DNeasy 96 PowerSoil Pro Kit from Qiagen (Qiagen, Hilden, Germany) with a slightly modified protocol. To ensure efficient release of microbial cells from the swabs into the solution, the collection tubes containing SMART-eNAT® swab samples were vortexed briefly, and 250 µl of the preservation medium was added to the PowerBead Pro Plate before proceeding with the manufacturer's protocol.

All sequencing was conducted at the NU-OMICS DNA sequencing facility (Northumbria University in Newcastle, UK) on an Illumina MiSeq platform. Amplicon sequencing efforts were focused on the 16S rRNA gene, V4 region, and the protocol for sequencing was carried out following the MiSeq Wet Lab SOP guidelines documented in detail by Kozich et al. [40].

2.4. Integrated bioinformatic processing of all cohorts

To ensure technical comparability and minimise batch effects between datasets originating from different studies, all available raw FASTQ files, including those from Nigeria, Italy, China, South Africa, Thailand and the USA, were processed jointly through the same QIIME 2 (Version 2025.7) [41] pipeline to ensure consistency across cohorts. The complete workflow is provided in Supplementary Methods S1. In summary, paired-end reads were imported via the QIIME 2 manifest format and demultiplexed using qiime tools import with Phred33V2 encoding. Sequence quality profiles were visualised (*qiime demux summarize*) and informed trimming parameters of 242 bp (forward) and 229 bp (reverse).

Amplicon sequence variants (ASVs) were obtained with the DADA2 plugin (*qiime dada2 denoise-paired*) and the consensus method for chimera removal (*–p-chimera-method consensus*). The resulting feature table, representative sequences, and denoising statistics were retained as.qza artefacts. Taxonomy was assigned using a naïve Bayes classifier trained on the Human Oral Microbiome Database (HOMD RefSeq V16.02). Additional taxonomic assignments were also attempted using the SILVA 138.1 database; however, the results were similar to those obtained with the HOMD database. Therefore, we opted to retain the HOMD assignments. Unassigned ASVs at different taxonomic levels may represent previously unclassified taxa or reflect the intrinsic limitations of 16S rRNA sequencing. These ASVs were retained for subsequent analyses, as they may still constitute important features for the machine learning algorithms. Reference sequences and taxonomy were imported into QIIME 2 and the V4 region (515 F/806 R) extracted (*qiime feature-classifier extract-reads*) before classifier training (*fit-classifier-naive-bayes*). Classification was performed with *classify-sklearn* and phylogenetic trees for downstream diversity analyses were built with *qiime phylogeny align-to-tree-mafft-fasttree*.

All read processing was executed using identical parameters, ensuring that ASV calling, chimera removal, and taxonomic assignment were consistent across all countries. The resulting feature table and taxonomy were then imported into R (v4.3.1) via phyloseq (v1.46.0) for downstream analyses where reads classed as Mitochondria and Chloroplasts were excluded. Additionally, ASVs identified in the negative controls (both extraction and sequencing negatives) were excluded from further analyses using the decontam package (v. 1.30).

Differential abundance testing to explore significantly different taxa across cohorts was performed using DESeq2 (v1.42.1) in R on the raw ASV count table, as DESeq2 analysis accounts for differences in sequencing depth between samples. The design formula used *Cohort* as the primary variable of interest, while controlling for *Sex* as a factor. Log_2 fold changes were estimated using the Wald test and adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR < 0.05).

All analysis were conducted at ASV level, however for the purpose of clarity in reporting, taxonomy is displayed at phylum, family or genus level (or NA where taxonomic identification at reporting level is not available).

2.5. Random forest classification

A random forest classifier was implemented using the caret package in R to evaluate the predictive capacity of oral microbiome profiles for determining country of origin. The dataset was split into training (70 %) and test (30 %) sets using stratified sampling with createDataPartition() to preserve class distributions. Model training was performed on the training set using 10-fold cross-validation (trainControl(method = "cv", number = 10)), with performance evaluated via multiclass metrics (summaryFunction = multiClassSummary) and class probabilities enabled (classProbs = TRUE).

The model was trained using the train() function with method = "rf", and the optimal number of variables randomly sampled at each split (mtry) was selected based on minimal log-loss. The final model was then applied to the test set, and predictive performance was evaluated using a confusion matrix comparing predicted versus true country labels. The full taxonomic assignment and first 8 characters of each ASV sequence identified as important for classification prediction can be found in Supplementary Table 1.

Finally, to understand the impact of migration on predicting an individual's country-of -origin for forensic profiling purposes, microbiome data of the Migrant group at all timepoints were passed to the machine learning model built to determine geographic origin with the six countries.

3. Results

In total, 395 samples representing six countries were processed together through a single QIIME 2 pipeline as described previously (Table 2). Across all datasets, 15,541,700 high-quality paired-end reads were obtained, yielding 707 amplicon sequence variants (ASVs). The average sequencing depth per sample was 39,346 reads (median = 26,615). Overall table sparsity was 0.897, indicating that approximately 10 % of the feature matrix contained non-zero abundances.

3.1. Geographic variation in the oral microbiome

Analysis was conducted to understand measures of diversity across samples of individuals from the six countries; China (CHN), Nigerian locals (NG), United States of America (USA), South Africa (SA), Thailand (THAI), and Italy (ITA). Alpha diversity analysis using the Shannon index, and statistical analysis with Kruskal-Wallis' test revealed significant differences in microbial diversity among the countries ($p = 7.8e\text{-}16$). Post hoc Dunn test comparisons (indicated with blue letters in Fig. 1A) indicated significant differences across the countries, with higher diversities in NG and CHN cohorts compared to others. Other alpha diversity indicators (Observed, Chao1, ACE, and Simpson) are reported in Supplementary Figure 2.

Fig. 1B illustrates beta diversity using Principal Coordinates Analysis (PCoA) based on Bray-Curtis distances. The clustering of microbial communities by cohort highlights the distinct composition of oral microbiomes across countries. PERMANOVA tests confirmed significant differences between countries, explaining approximately 29.6 % of the variation in microbial composition ($R^2 = 0.296$, $p = 0.001$). This suggests that geographic location is an important factor influencing oral microbial composition, with the remaining variation likely explained by individual factors such as genetics, lifestyle, and environmental exposures.

3.1.1. Taxa differences across countries

The relative abundance of oral microbial communities across six

Table 2
Summary of sequencing coverage and taxonomic richness for each cohort. All taxa labelled "unassigned", "uncultured" or NA at any taxonomic level are counted as a single taxon per label. Consequently, richness at lower taxonomic levels may be smaller than at higher levels when ASVs could not be fully classified. This reflects the limitations of taxonomic assignment rather than true biological richness across Phylum, Class, Order, Family, Genus or Species level.

Cohort	n_samples	total_reads	mean_reads	median_reads	min_reads	max_reads	n_ASVs	Phylum	Class	Order	Family	Genus	Species
CHN	53	319,584	6030	4566	1066	22,059	268	15	22	47	75	102	82
ITA	50	5875,563	117,511	102,174	12,647	337,025	312	13	20	48	76	123	108
NG	50	1632,822	32,656	33,042	11,919	50,150	287	13	23	51	80	118	98
SA	44	776,811	17,655	18,556	652	25,238	235	12	18	37	62	94	90
THAI	32	1035,599	32,362	33,454	17,081	42,361	221	10	15	42	68	97	77
USA	21	1504,439	71,640	73,997	24,507	118,483	236	10	15	36	63	96	89
Migrant T0	50	2131,039	42,621	45,232	1477	102,200	625	23	43	97	162	283	155
Migrant T1	44	1198,276	27,234	22,292	1458	291,204	270	14	22	48	75	115	95
Migrant T2	45	1013,563	22,524	24,979	4778	35,515	291	12	22	49	78	119	105

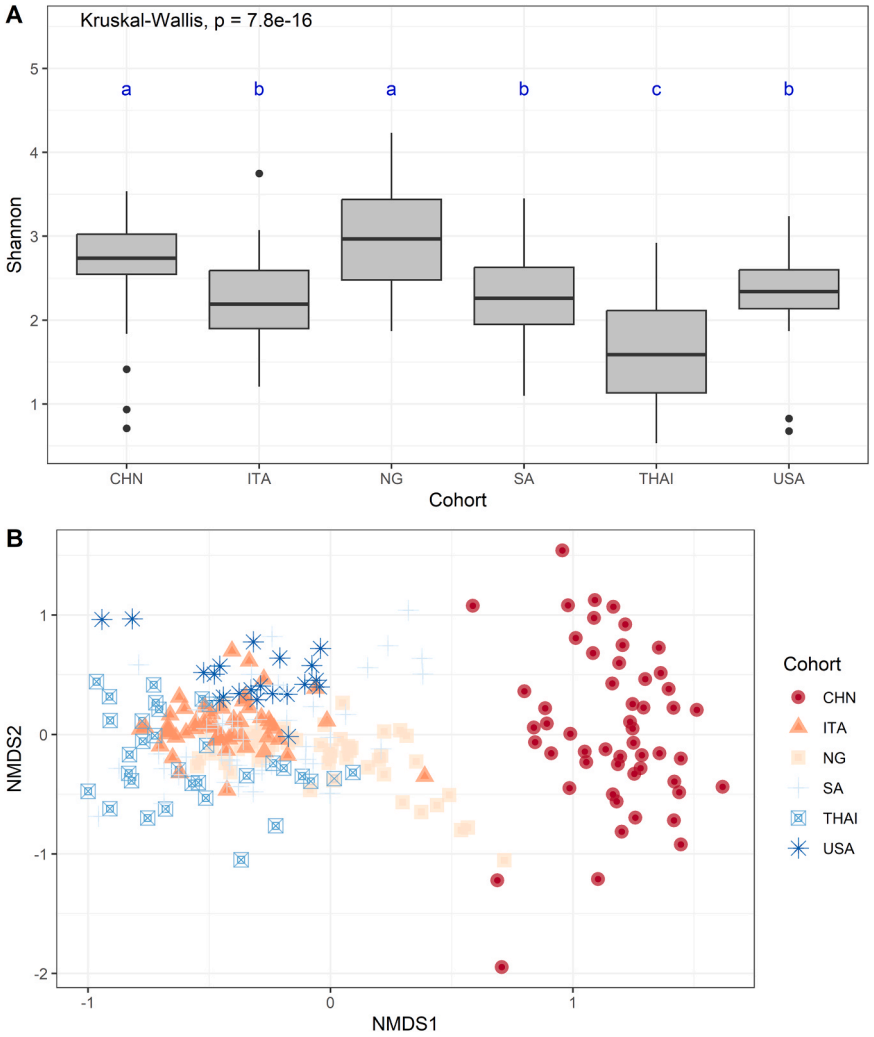

Fig. 1. **(A)** Oral microbiome diversity across six international cohorts: China (CHN), Italy (ITA), Nigeria (NG), South Africa (SA), Thailand (THAI), and the United States of America (USA). Significant differences are indicated by Kruskal-Wallis p-value (p = 7.8e-16) and Dunn test results are displayed in blue letters to show which groups are similar. Cohorts with the same letter are similar and significantly different to cohorts with a different letter. **(B)** Principal Coordinates Analysis (PCoA) plot illustrating beta diversity of oral microbial communities based on Bray-Curtis distances.

countries, as depicted in Fig. 2, reveals considerable variation in phylum-level composition, likely influenced by cultural, dietary, and environmental factors. The most dominant phyla across all countries were Firmicutes, Bacteroidota, Proteobacteria, Fusobacteriota and Actinobacteriota, with notable differences in their proportions between countries. These findings are consistent with previous studies on global oral microbiome compositions [5,42].

The oral microbiome of individuals from the USA was predominantly composed of *Firmicutes* (70.0 %), followed by *Bacteroidota* (14.9 %), with smaller proportions of *Actinobacteriota* (6.48 %) and *Proteobacteria* (4.17 %). For the South African group, *Firmicutes* (56.2 %) and *Bacteroidota* (26.8 %) dominated the oral microbiome. In the Thai cohort, *Firmicutes* dominated the microbiome (52.1 %), but *Proteobacteria* represented a substantial portion (23.7 %). This contrasts with lower *Proteobacteria* levels in the USA and Italian cohorts, highlighting the potential influence of regional dietary habits. The Nigerian cohort also

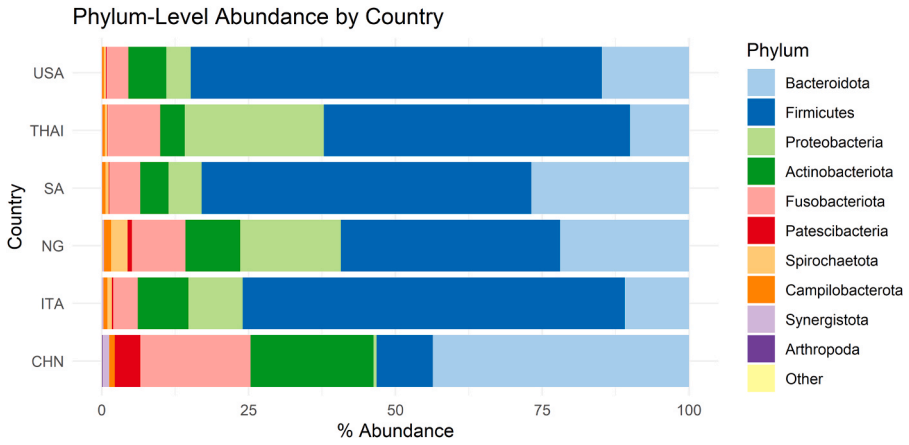

Fig. 2. Stacked bar chart illustrating the relative abundance of microbial phyla across represented countries.

presented a complex microbial community, with significant proportions of *Firmicutes* (37.4 %), *Bacteroidota* (21.9 %), and a notably high level of *Proteobacteria* (17.1 %). Furthermore, *Spirochaetota* (2.78 %) was found in greater abundance within this cohort compared to others.

The Italian cohort was also dominated by *Firmicutes* (65.1 %) and had a lower abundance of *Bacteroidota* (10.9 %), *Proteobacteria* (9.25 %) and *Actinobacteriota* (8.64 %) were also present in moderate proportions, with smaller amounts of *Fusobacteriota* (4.16 %) and

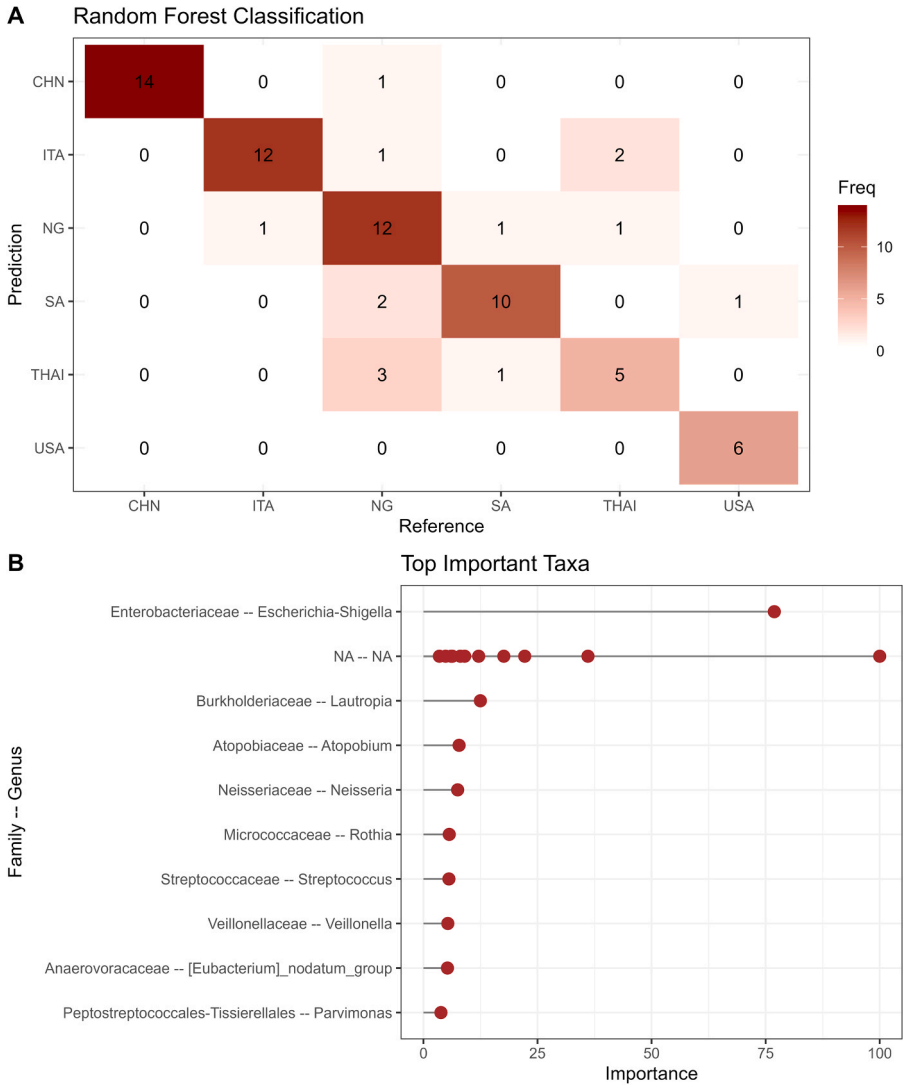

Fig. 3. (A) Random Forest classification model showing predicted vs actual country assignments of individuals. **(B)** Top 10 bacterial biomarkers ranked in descending order according to their importance to the accuracy of the model.

Patescibacteria (0.227 %). The Chinese cohort presented a unique profile with high proportions of *Bacteroidota* (43.6 %), *Actinobacteriota* (20.9 %) and Fusobacteriota (18.8 %), and low levels of *Firmicutes* (9.59 %) compared to other countries. The near absence of *Proteobacteria* (0.531 %) in the Chinese group contrasts sharply with other countries, particularly Thailand and Nigeria, where *Proteobacteria* is much more abundant. Additionally, the Chinese cohort shows a higher presence of *Patescibacteria* (4.35 %) and *Synergistota* (1.10 %), both of which have been associated with oral and gut health [43].

To identify taxa that were significantly represented across the countries, differential abundance analysis was conducted with DESeq2 at ASV level. 30 distinct taxa were identified following the analysis. The plot in Fig. 3 shows clustering of the differentially abundant genera across the six countries, further suggesting that these taxa may be associated with the specific geographical populations. The relative abundance plots (Supplementary Figure 3) also strengthen this result by showing abundance variations of the genera across cohorts.

3.1.2. Random Forest Classification and Prediction

To explore the oral microbiome's ability to predict country-of-origin, a random forest model was built as described earlier. Results from this analysis achieved an overall accuracy of 86.4 %, with the best performance in terms of AUC (Area Under the ROC Curve; AUC = 0.981). Overall, the model demonstrated strong performance for most countries, particularly for CHN, supporting beta diversity results which showed distinct separation of Chinese microbial profiles from other countries.

In addition, the top 20 important taxa identified by the model to make these classification predictions are shown in Fig. 3B. Taxa from various genera such as *Escherichia-Shigella*, *Lautropia*, *Atopobium*, *Neisseria*, and *Rothia* were highly ranked, with the *Escherichia-Shigella* genus (ASV: d46e2205) being the most influential known genus in the model. A significant proportion of important taxa remained unidentified at the genus level (denoted as NA) and this highlights the limitations of amplicon sequencing in taxonomic resolution of microbial sequences and potentially highlights the importance of research on understudied populations (like Nigeria) and environments containing poorly characterised bacteria.

3.2. Impact assessment of migration

Migration presents a unique opportunity to explore how changes in environment, diet, and lifestyle affect the oral microbiome and subsequently its utility in forensic profiling. Thus, oral microbial communities of immigrants (Nigerian-born UK residents) were explored over a 6-month period and compared to Nigerian locals to explore how migration may influence the prediction accuracy of the random forest model and the implications for forensic profiling.

3.2.1. Nigerian migrants vs. Nigerian locals

The assessment of alpha diversity (in Supplementary Figure 4) using the Kruskal-Wallis test indicated no significant differences in alpha diversity between the migrant (Migrant) and local (NG) cohorts ($p = 0.27$). On the other hand, beta diversity analysis, as shown in the PCoA plot in Fig. 4, reveals a separation between the NG cohort and migrants at the point of initial sampling (T0). PERMANOVA analysis also shows a significant difference in microbial composition between the two groups ($R^2 = 0.1028$, $p = 0.001$).

However, the separation of Migrants from the NG cohort observed at T0 is not sustained at later time points. At T1 and T2, samples show less separation, indicating potential recovery of the local microbiome composition over time. Beta diversity analysis, excluding T0 samples, reveals a weaker, yet significant, differentiation between T1, T2, and the NG cohort ($R^2 = 0.03344$, $p = 0.003$).

3.2.2. Evaluation of random forest model on Nigerian migrants

The random forest model encountered significant challenges in predicting the correct country-of-origin for Nigerian migrants, especially at T0, where 39 of 50 samples (78 %) were incorrectly classified as Italian, and only 6 % (3 of 50 samples) were accurate. However, with progression of time, the model improved in identifying Nigerian samples correctly – with 26 of 44 samples (59 %) at T1 and 26 of 41 samples (63 %) at T2, shown in Fig. 5.

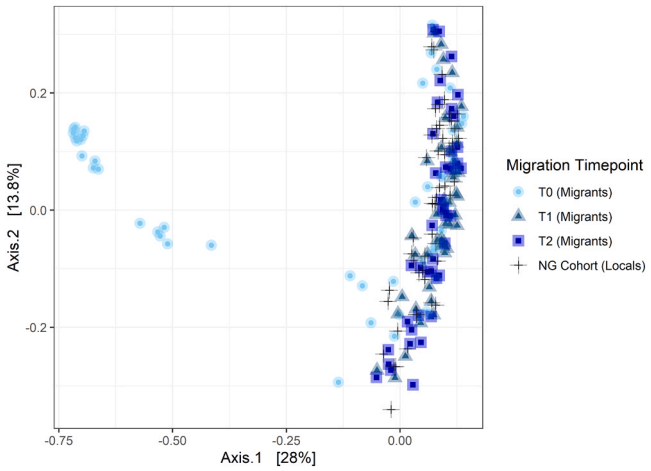

Fig. 4. PCoA plot showing distribution of Nigerian locals - NG - in comparison to Migrant group at all timepoints.

4. Discussion

4.1. Diversity assessments

The association of microbial community composition with geographic origin – across ethnicities and continents [30,31,44] – has been a subject in forensics for human profiling. Therefore, this study aimed to assess any inter-country variability in microbial composition and subsequently determine the potential of the oral microbiome to predict country-of-origin. High alpha diversity seen in NG and CHN cohorts may be driven by traditional diets rich in fibrous, plant-based foods, which are known to support a diverse microbiome [45]. This aligns with previous studies that have shown links to increased microbial diversity in African and East Asian diets [46], which often contain a high proportion of unprocessed foods [47-50]. Conversely, the lower diversity observed in ITA and USA cohorts could be linked to processed or industrialized diets, associated with reduced microbial diversity [51, 52]. Interestingly, despite the similarities between Chinese and Thai diets, the THAI cohort showed the lowest alpha diversity. This adds support to other research [15,53], demonstrating that geographical, environmental or lifestyle factors may also significantly influence oral microbiota composition.

These findings offer promising insights into the potential for using the oral microbiome as a tool for forensic biogeographic profiling. Both alpha and beta diversity analyses indicate significant differences between cohorts, suggesting that the composition of the oral microbiome could serve as a biomarker for identifying an individual's country of origin. However, challenges remain in the practical application of this approach. Factors such as migration [54], health host genetics [55], antibiotic use [56], and diet variability [57] may affect the stability and reliability of these microbial signatures over time.

4.2. Microbial composition

The variation in the relative abundance of various taxa across the countries at phylum level also potentially indicate geographical influence on the oral microbiome. Urbanization and westernized dietary patterns, characterized by high consumption of processed foods and

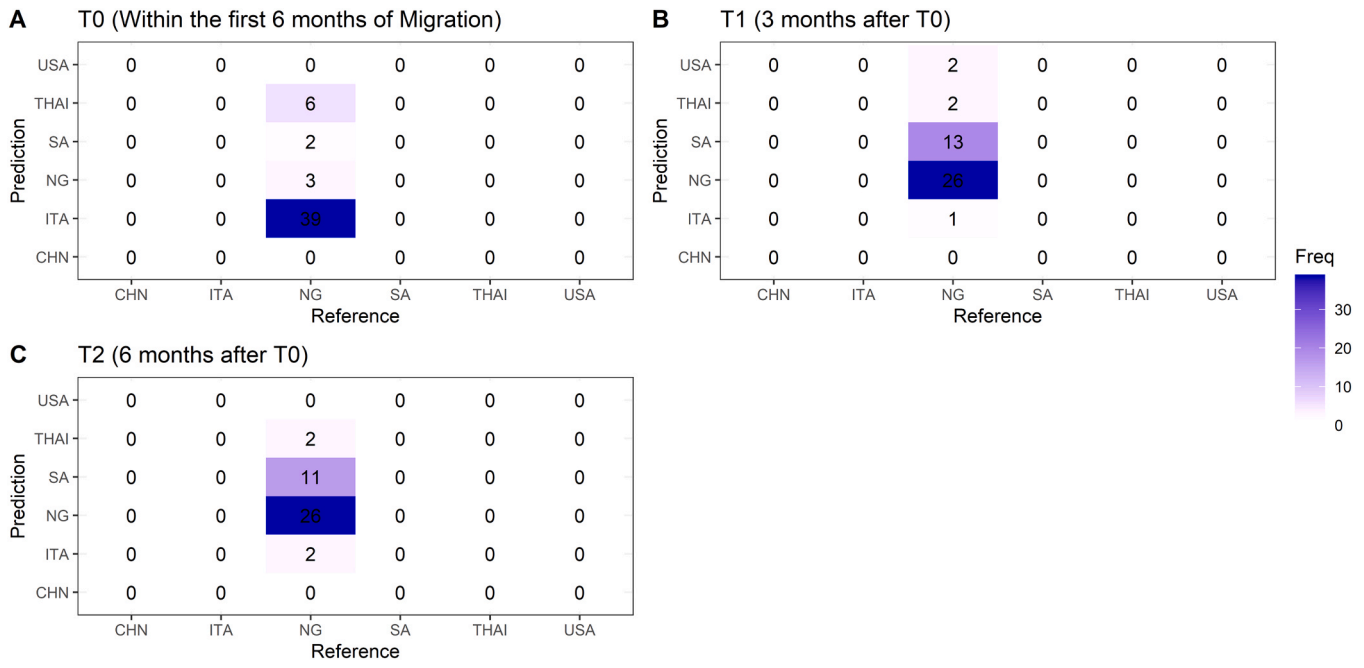

Fig. 5. Random Forest classification. Country of origin prediction of Migrant group at all timepoints – (A) T0, (B) T1 (C) T2 – using RF model built with Nigerian locals.

refined sugars, have been associated with increased proportions of *Firmicutes*-associated bacteria [58,59], and is seen in the USA and South African cohorts. Additionally, the relatively low proportions of *Proteobacteria* seen in both countries compared to others may further give insights to health status as reduced abundance of the phylum has been reported as a possible indicator of diabetes [60,61]. This is however controversial as another study [62] associated increased levels of *Proteobacteria* with diabetes. The similarity in microbial profiles of both countries, despite their geographic and cultural differences, potentially reflects the impact of increasing urbanization and adoption of Westernized diets in parts of the South African population [63]. Mixed diet patterns are more common in South African urban populations and may include both traditional and westernized dietary elements [64].

On the other hand, a diet rich in vegetables, fermented foods, and lower intake of processed sugars could explain the high abundance of *Proteobacteria* [65] in the Thailand cohort. A study reporting high consumption of spicy foods and fermented products such as fish sauce and pickled vegetables, have indicated that this may contribute to the proliferation of Firmicutes in the Thai oral microbiome [66].

Furthermore, higher levels of *Patescibacteria* were observed in the Chinese cohort. Recent genome-resolved analyses have identified *Patescibacteria* as one of the most prevalent and phylogenetically diverse phyla in the human oral microbiome [67]. The prominence of this phylum, known for its broad phylogenetic diversity, may partly explain the greater microbial richness and distinct community structure observed in the CHN cohort.

The Nigerian cohort displayed a more diverse microbiome, with higher levels of *Proteobacteria*, suggesting a diet rich in fibre and plant-based nutrients [68]. This contrasts with the lower levels of *Proteobacteria* in Western cohorts, reinforcing the idea that dietary factors play a crucial role in shaping microbial communities [69]. The microbiome profile of this cohort also showed higher abundance of *Spirochaetota*, which has been reported to be potentially linked to certain oral disease states [70,71]. However, traditional diets in sub-Saharan Africa, characterized by low levels of processed foods and high consumption of plant fibres, have been linked to increased microbial diversity and a more balanced phylum-level distribution [72].

In the Italian population assessed for this study, the increased abundance of *Firmicutes* may also reflect a westernised diet, however it also reflects the Mediterranean diet's high carbohydrate content (e.g., pasta, bread) and processed foods, which promote the growth of *Firmicutes* [73]. The Mediterranean diet has been associated with favourable microbiome outcomes, including a higher abundance of *Bacteroidota*, which are involved in the fermentation of complex carbohydrates and the production of beneficial metabolites [73]. The significant reduction of *Proteobacteria* in the Chinese group compared to others, may be linked to regional dietary habits emphasizing fermented foods, which can suppress the growth of *Proteobacteria* [74]. Hence the variation in abundance of these phyla across cohorts could serve as distinguishing markers for forensic profiling.

4.3. Differential abundance of Taxa

One of the most striking differences is the high association of *Catonella* and *Saccharimonadales* in the Chinese cohort (CHN), taxa that are nearly absent in other groups. Previous research has shown that *Catonella* is linked to periodontal diseases and oral inflammation, conditions that may be influenced by dietary patterns and/or oral hygiene practices in specific populations [75-77]. The presence of *Saccharimonadales* has been linked to coffee consumption [78] and could also point toward unique environmental exposures, such as differences in air quality and water composition. *Fretibacterium* and *Rothia* were also highly correlated with the CHN cohort. *Rothia* is a genus typically associated with oral dysbiosis and diseases like cancer [79,80]. Similarly, the genus *Fretibacterium*, which has been linked to subgingival biofilm in cases of periodontitis [81-83], might reflect differences in oral health status between China and other regions.

In the USA cohort, *Streptococcus* is highly abundant, consistent with other studies showing that this genus dominates the oral microbiome in Western populations [84]. *Streptococcus* is a key player in the oral biofilm and its dominance may be linked to the consumption of high-sugar diets common in the United States [85]. Interestingly, *Neisseria*, another genus commonly associated with oral health, shows a higher relative abundance in Nigerian (NG) and Thai (THAI) samples. *Neisseria* is known for its ability to metabolize nitrate, and its increased abundance could be reflective of environmental exposures such as nitrate levels in

drinking water [86].

These findings underscore the complexity of the oral microbiome and highlight how various factors, including diet, environmental exposures, and health status, shape the oral microbial landscape in different populations. In addition, social factors such as healthcare access, hygiene practices, and exposure to antibiotics or antimicrobials can influence oral microbiome composition. The unique combination of these factors and how they collectively influence the oral microbiome further adds a microbial signature that can be identifiable to a geographic population, which in turn underlines the untapped potential of the oral microbiome in forensic profiling.

4.4. Predicting country of origin

The results of the random forest model suggest that the human oral microbiome can serve as a useful tool for forensic profiling of individuals' geographic origins. The high overall accuracy and strong AUC values demonstrate that the microbiome holds promise as a predictive tool in forensic contexts, echoing findings from previous studies that have linked microbiome composition to various demographic factors, including diet, lifestyle, and environmental exposure [87,88,66,89,90,78].

Misclassifications in the model's predictions may be due to shared microbial signatures between misclassified countries, possibly due to similar dietary patterns or environmental factors that influence oral microbiome composition. For example, the NG cohort which showed the highest levels of misclassification, yet was never assigned to the USA cohort. This could be due to the traditional and plant-based diets that may be seen across the other countries but not prominently in westernised diets.

The taxa importance analysis revealed that members of the *Escherichia-Shigella* genus were the most predictive in distinguishing between populations. This taxon has been implicated in various health-related conditions including inflammatory bowel disease [91,92], and as such may provide further insights to health status as well as geographic origin in forensic profiling. Other prominent genera include *Neisseria* and *Veillonella*, both of which are well-known residents of the oral cavity and have been previously found to exhibit geographic variability in other studies [93,32,15,94].

Furthermore, two ASVs – c845eda9 (*Lautropia*) and d83f6018 (*Rothia*) – were identified as significant taxa in both differential abundance analysis and random forest importance rankings, inferring their utility in distinguishing between individuals from different countries based on their oral microbiome profiles. Both *Lautropia* and *Rothia* have also been shown to vary significantly across different populations and geographic regions [93,95,15,94]. The finding of these ASVs as significantly important in both differential abundance analysis and random forest importance assessments suggests their potential as biomarkers in biogeographical forensic profiling.

4.5. Predicting country of origin following migration

This study suggests that the overall richness and evenness of the oral microbiome do not undergo significant changes over the 6-month assessment period and/or between the migrant group and the local Nigerian cohort. Thus, while migration may introduce external environmental changes, the alpha diversity of the oral microbiome remains stable. This result aligns with previous reports stating that the oral microbiome may be more resilient to change than other microbial communities in the body, such as the gut microbiome [96,97]. The lack of significant shifts in alpha diversity over the six-month post-migration period also suggests that oral microbiome richness and evenness are less sensitive to short-term environmental, or lifestyle changes associated with migration.

Beta diversity assessments however indicate that some changes to oral microbial communities occur initially after migration. This could reflect adaptation to new dietary patterns, hygiene habits, and other environmental factors in the UK. Similar findings have been reported in studies of immigrants, where migration was shown to impact the composition of the microbiome [98,99,54]. Additionally, further analysis of the Migrant group over the 6-month period indicates that while the oral microbiome composition differs between Nigerian migrants and the local NG cohort at T0, this difference diminishes over time. The separation seen at T0 may reflect immediate changes in the microbiome due to the new environment, including shifts in diet and lifestyle. However, the similarity at T1 and T2 in comparison to Nigerian locals (NG Cohort) suggests that the microbiome may revert to former microbial profiles – particularly in the event that individuals intentionally seek out their local diets after migration – before gradually adapting to the new environment or showing signs of stabilization. A similar study involving the assessment of stool samples from USA migrants also showed that changes to the microbiome become pronounced approximately 9 months following migration [54]. The observations suggest that environmental and dietary factors related to migration may initially alter the microbiome, but with extended residence in the new country, microbial communities may adapt or stabilize as individuals settle into their new environment.

This is supported by the results of the analysis when attempting to predict the country of origin of the Nigerian migrants at the different sample collection timepoints (initial collection – T0; 3 months later – T1; 6 months later – T2) as prediction accuracy was lowest in the first instance but improved over time. This early misclassification further supports the hypothesis that the oral microbiomes of migrants rapidly shift upon relocation, becoming less reflective of their country of origin, and the increasing accuracy may indicate a possible reversion in microbial communities of migrants to local microbial profiles as migrants settle into their new communities.

Notably, the highest frequency of incorrect predictions across T1 and T2 was for South Africa, which, like Nigeria, is located on the African continent. This regional proximity could suggest shared microbial traits across populations due to similar environmental or dietary factors. While this might present challenges for forensic profiling at country-level, it shows potential for ethnic or regional identification.

The findings of this study underscore the potential of oral microbiome profiling as a powerful tool in forensic science. The distinct microbial compositions observed across the six countries highlight how the oral microbiome may serve as a marker for geographical origin, dietary and environmental exposures. For example, the low abundance of *Proteobacteria* in the Chinese cohort compared to other countries, as well as the higher presence of *Patescibacteria* and *Synergistota*, suggests region-specific microbial patterns that could assist in identifying individuals from similar environments. Similarly, the elevated levels of *Spirochaetota* in the Nigerian cohort could serve as a potential marker for the population.

Furthermore, the results also highlight limitations of using oral microbiome data alone for forensic profiling, especially in individuals who have migrated and experienced environmental changes. The strong misclassification at T0 suggests that microbiomes of migrants quickly adapt to their new surroundings, making it challenging to reliably determine their country of origin initially. However, the increasing convergence between the oral microbiomes of Nigerian migrants and Nigerian locals, as well as South Africans following further sampling at T1 and T2, underscore its potential use for forensic profiling with respect to biogeographic location (continental) and ethnicity. Additionally, further insights to microbiome changes during migration could help to not only predict geographic origin but also estimate the timing of migration.

4.6. Technical considerations and batch effects

One technical limitation in studies such as this one that includes analysis of microbiome data obtained from other studies is the potential

technical confounding factor arising from different collection kits, extraction chemistries, or sequencing runs. It is important to consider potential limitations, including the possibility of batch effects due to differences in sample processing across labs and countries. While all raw sequencing data were processed together in this study to minimize such effects, variations in sample handling or sequencing conditions between different labs could introduce biases.

In this study, all available raw FASTQ files were re-processed jointly through an identical QIIME 2–DADA2 pipeline, ensuring that read trimming, denoising, and taxonomic assignment were performed under the same parameters and classifier. Although this approach substantially reduces batch effects caused by upstream laboratory variation, it does not totally eliminate them. Remaining sources of variation, such as subtle differences in DNA extraction efficiency or run-specific biases, may still contribute to between-cohort differences. Future work using unified sampling kits and mock communities could further clarify the proportion of biological versus technical variation. Nonetheless, the consistent processing of raw sequencing data described here provides confidence that the observed geographic signatures primarily reflect biological rather than technical differences.

In forensic investigations, microbial profiling can potentially add an additional layer of context to traditional DNA analysis, offering clues about an individual's lifestyle, habits, and regional origins. The unique microbial patterns identified in this study could provide valuable information to support other conventional evidentiary types. As microbial forensics continues to evolve, the ability to analyse oral microbiomes may become an integral part of human identification efforts, offering a novel and dynamic tool for forensic profiling.

The findings of this study stress the importance of exploring diverse populations in understanding the robustness and utility of the microbiome for forensic profiling. It is essential to stress however, that this study is based on a very small group of individuals (approx. 50) from only six countries. Therefore, future validation of these findings in studies that focus on larger and more diverse populations, would be essential in understanding the oral microbiome, its applications in forensic science for biogeographic identification and the effects of migration on its ability to determine country-of-origin.

CRediT authorship contribution statement

Noemi Procopio: Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Richard Somiari:** Writing – review & editing, Conceptualization. **Darren Smith:** Writing – review & editing, Validation, Supervision, Resources, Project administration. **Andrew Nelson:** Writing – review & editing, Validation, Supervision, Investigation. **Nengi Ogbanga:** Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation, Conceptualization.

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.

The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigen.2025.103395.

References

[1] J.L. Baker, J.L. Mark Welch, K.M. Kauffman, J.S. McLean, X. He, The oral microbiome: diversity, biogeography and human health, 22, Nat. Rev. Microbiol. 2023 22 (2) (2023) 89–104, https://doi.org/10.1038/s41579-023-00963-6.

[2] M.W. Hall, N. Singh, K.F. Ng, D.K. Lam, M.B. Goldberg, H.C. Tenenbaum, et al., Inter-personal diversity and temporal dynamics of dental, tongue, and salivary microbiota in the healthy oral cavity, 3, 1–7, npj Biofilms Micro 2017 3 (1) (2017), https://doi.org/10.1038/s41522-016-0011-0.

[3] D.R. Utter, J.L. Mark Welch, G.G. Borisy, Individuality, stability, and variability of the plaque microbiome, Front Microbiol 7 (2016) 185459, https://doi.org/10.3389/FMICB.2016.00564/BIBTEX.

[4] M. Kilian, I.L.C. Chapple, M. Hannig, P.D. Marsh, V. Meuric, A.M.L. Pedersen, et al., The oral microbiome - an update for oral healthcare professionals, Br. Dent. J. 221 (2016) 657–666, https://doi.org/10.1038/SJ.BDJ.2016.865.

[5] F.E. Dewhirst, T. Chen, J. Izard, B.J. Paster, A.C.R. Tanner, W.H. Yu, et al., The Human Oral Microbiome, J. Bacteriol. 192 (2010) 5002, https://doi.org/10.1128/JB.00542-10.

[6] K.M. Yu, H.S. Cho, A.M. Lee, J.W. Lee, S.K. Lim, Analysis of the influence of host lifestyle (coffee consumption, drinking, and smoking) on Korean oral microbiome, Forensic Sci. Int Genet 68 (2024) 102942, https://doi.org/10.1016/J.FSIGEN.2023.102942.

[7] E. Zaura, B.J.F. Keijser, S.M. Huse, W. Crielaard, Defining the healthy "core microbiome" of oral microbial communities, BMC Microbiol 9 (2009) 259, https://doi.org/10.1186/1471-2180-9-259.

[8] A.A. Ribeiro, M.A. Azcarate-Peril, M.B. Cadenas, N. Butz, B.J. Paster, T. Chen, et al., The oral bacterial microbiome of occlusal surfaces in children and its association with diet and caries (Available at:), PLoS One 12 (2017) e0180621, https://doi.org/10.1371/journal.pone.0180621.

[9] D. Bourgeois, L.S. Gonçalves, J. Lima-Junior, C. da, F. Carrouel, Editorial: The Oral Microbiome Is a Key Factor in Oral and Systemic Health, Front Microbiol 13 (2022), https://doi.org/10.3389/fmicb.2022.855668.

[10] A.M.L. Benn, N.C.K. Heng, W.M. Thomson, C.H. Sissons, L.S. Gellen, A.R. Gray, et al., Associations of sex, oral hygiene and smoking with oral species in distinct habitats at age 32 years, Eur. J. Oral. Sci. 130 (2022) e12829, https://doi.org/10.1111/eos.12829.

[11] C. Huang, G. Shi, Smoking and microbiome in oral, airway, gut and some systemic diseases, 17, 1–15, J. Transl. Med. 2019 17 (1) (2019), https://doi.org/10.1186/S12967-019-1971-7.

[12] H. Shi, H. Niina, C. Anna-Paola, H. Rebecca, J. Lingjing, P. Laxmi, et al., Human Skin, Oral, and Gut Microbiomes Predict Chronological Age, e00630-19, mSystems 5 (2022), https://doi.org/10.1128/mSystems.00630-19.

[13] A.S. Widyarman, C.F. Theodorea, N.S. Udawatte, A.M. Drestia, E.W. Bachtiar, T. E. Astoeti, et al., Diversity of Oral Microbiome of Women From Urban and Rural Areas of Indonesia: A Pilot Study, Front. Oral. Health 2 (2021) 738306, https://doi.org/10.3389/FROH.2021.738306/BIBTEX.

[14] G. Ma, Y. Qiao, H. Shi, J. Zhou, Y. Li, Comparison of the oral microbiota structure among people from the same ethnic group living in different environments, Biomed. Res Int 2022 (2022) 6544497, https://doi.org/10.1155/2022/6544497.

[15] I. Nasidze, J. Li, D. Quinque, K. Tang, M. Stoneking, Global diversity in the human salivary microbiome, Genome Res 19 (2009) 636–643, https://doi.org/10.1101/GR.084616.108.

[16] M. Handsley-Davis, K. Kapellas, L.M. Jamieson, J. Hedges, E. Skelly, J. Kaidonis, et al., Heritage-specific oral microbiota in Indigenous Australian dental calculus, Evol. Med Public Health 10 (2022) 352–362, https://doi.org/10.1093/emph/eoac024.

[17] A.T. Ozga, K. Sankaranarayanan, R.Y. Tito, A.J. Obregon-Tito, M.W. Foster, G. Tallbull, et al., Oral microbiome diversity among Cheyenne and Arapaho individuals from Oklahoma, Am. J. Phys. Anthr. 161 (2016) 321–327, https://doi.org/10.1002/ajpa.23033.

[18] J. Wang, J. Feng, Y. Zhu, D. Li, J. Wang, W. Chi, Diversity and Biogeography of Human Oral Saliva Microbial Communities Revealed by the Earth Microbiome Project, Front Microbiol 13 (2022). Available at: ⟨https://www.frontiersin.org/articles/10.3389/fmicb.2022.931065⟩.

[19] K. Skonieczna, N. Kovacevic-Grujicic, A. Srivastava, M. Gawrych, M. Ciesielka, N. Rana, D. Drakulic, M. Mojsin, M. Milivojevic, M. Stevanovic, G. Teresiński, T. Grzybowski, Salivary microbiome signatures of Poles and Serbians and its potential for prediction of biogeographic ancestry, –103173. doi:https://doi.org/, Forensic Sci. Int. Genet. 74 (2024) 103173, https://doi.org/10.1016/j.fsigen.2024.103173.

[20] J. Wu, B.A. Peters, C. Dominianni, Y. Zhang, Z. Pei, L. Yang, et al., Cigarette smoking and the oral microbiome in a large study of American adults, ISME J. 10 (2016) 2435–2446, https://doi.org/10.1038/ismej.2016.37.

[21] A.Y. Shchipkova, H.N. Nagaraja, P.S. Kumar, Subgingival microbial profiles of smokers with periodontitis, J. Dent. Res 89 (2010) 1247–1253, https://doi.org/10.1177/0022034510377203.

[22] I. Kalabiska, D. Annar, Z. Keki, Z. Borbas, H.P. Bhattoa, A. Zsakai, The oral microbiome profile of water polo players aged 16–20, 11, 216, Sports 2023 11 (2023) *216*, https://doi.org/10.3390/SPORTS11110216.

[23] I.B. Stanaway, J.C. Wallace, A. Shojaie, W.C. Griffith, S. Hong, C.S. Wilder, et al., Human oral buccal microbiomes are associated with farmworker status and azinphos-methyl agricultural pesticide exposure, Appl. Environ. Microbiol 83 (2017), https://doi.org/10.1128/AEM.02149-16/SUPPL_FILE/ZAM999117596SD1.XLSX.

[24] D. Poddighe, A. Kushugulova, Salivary Microbiome in Pediatric and Adult Celiac Disease, Front Cell Infect. Microbiol 11 (2021) 625162, https://doi.org/10.3389/FCIMB.2021.625162/BIBTEX.
[25] N. Tian, L. Faller, D.A. Leffler, C.P. Kelly, J. Hansen, J.A. Bosch, et al., Salivary gluten degradation and oral microbial profiles in healthy individuals and celiac disease patients, Appl. Environ. Microbiol 83 (2017), https://doi.org/10.1128/AEM.03330-16/SUPPL_FILE/ZAM999117723SD2.XLS.
[26] J. Long, Q. Cai, M. Steinwandel, M.K. Hargreaves, S.R. Bordenstein, W.J. Blot, et al., Association of oral microbiome with type 2 diabetes risk, J. Periodontal Res 52 (2017) 636–643, https://doi.org/10.1111/JRE.12432.
[27] A. Shaalan, S. Lee, C. Feart, E. Garcia-Esquinas, D. Gomez-Cabrero, E. Lopez-Garcia, et al., Alterations in the Oral Microbiome Associated With Diabetes, Overweight, and Dietary Components, Front Nutr. 9 (2022) 914715, https://doi.org/10.3389/FNUT.2022.914715/BIBTEX.
[28] M. Di Stefano, A. Polizzi, S. Santonocito, A. Romano, T. Lombardi, G. Isola, Impact of oral microbiome in periodontal health and periodontitis: a critical review on prevention and treatment, 23, 5142, Int. J. Mol. Sci. 2022 23 (2022) *5142*, https://doi.org/10.3390/IJMS23095142.
[29] H. Qin, G. Li, X. Xu, C. Zhang, W. Zhong, S. Xu, et al., The role of oral microbiome in periodontitis under diabetes mellitus, J. Oral. Microbiol 14 (2022), https://doi.org/10.1080/20002297.2022.2078031.
[30] H.W. Cho, Y.Bin Eom, Forensic analysis of human microbiome in skin and body fluids based on geographic location, Front Cell Infect. Microbiol 11 (2021) 695191, https://doi.org/10.3389/FCIMB.2021.695191/BIBTEX.
[31] T. Clarke, L. Brinkac, C. Greco, A.T. Alleyne, P. Carrasco, C. Inotroza, et al., Sampling from four geographically divergent young female populations demonstrates forensic geolocation potential in microbiomes, 12, 1–10, Sci. Rep. 2022 12 (1) (2022), https://doi.org/10.1038/s41598-022-21779-z.
[32] 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, https://doi.org/10.1016/J.FSIGEN.2017.06.008.
[33] A.A. Singh, M. Okpeku, Emerging methods of human microbiome analysis and its forensic applications: Review, Forensic Science International Reports 9 (2024) 100355, https://doi.org/10.1016/J.FSIR.2024.100355.
[34] Cocking, J.H. Turley, R. Sgt, V.Y. Fofanov, K. Samuels-Crow, B. Hungate, R.L. Mau, et al., Forensic analysis of Soil Microbiomes: Linking Evidence to a Geographic Location, 07.10.198044. bioRxiv 2020 (2020) https://doi.org/10.1101/2020.07.10.198044.
[35] R.J. Abdill, S.P. Graham, V. Rubinetti, M. Ahmadian, P. Hicks, A. Chetty, et al., Integration of 168,000 samples reveals global patterns of the human gut microbiome, Cell 0 (2025), https://doi.org/10.1016/J.CELL.2024.12.017.
[36] T. Clarke, L. Brinkac, C. Greco, A.T. Alleyne, P. Carrasco, C. Inotroza, et al., Sampling from four geographically divergent young female populations demonstrates forensic geolocation potential in microbiomes, 12, 1–10, Sci. Rep. 2022 12 (1) (2022), https://doi.org/10.1038/s41598-022-21779-z.
[37] N. Ogbanga, A. Nelson, S. Ghignone, S. Voyron, F. Lovisolo, G. Sguazzi, et al., The Oral Microbiome for Geographic Origin: An Italian Study, Forensic Sci. Int Genet 64 (2023) 102841, https://doi.org/10.1016/j.fsigen.2023.102841.
[38] M. Upadhyay, P. Shrivastava, K. Verma, B. Joshi, Recent advancements in identification and detection of saliva as forensic evidence: a review, Egypt J. Forensic Sci. 13 (2023) 1–13, https://doi.org/10.1186/S41935-023-00336-3/TABLES/3.
[39] R.J. Abdill, E.M. Adamowicz, R. Blekhman, Public human microbiome data are dominated by highly developed countries, PLoS Biol. 20 (2022) e3001536, https://doi.org/10.1371/JOURNAL.PBIO.3001536.
[40] J.J. Kozich, S.L. Westcott, N.T. Baxter, S.K. Highlander, P.D. Schloss, Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform, Appl. Environ. Microbiol 79 (2013) 5112–5120, https://doi.org/10.1128/AEM.01043-13.
[41] E. Bolyen, J.R. Rideout, M.R. Dillon, N.A. Bokulich, C.C. Abnet, G.A. Al-Ghalith, et al., Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2, 37, Nat. Biotechnol. 2019 37 (8) (2019) 852–857, https://doi.org/10.1038/s41587-019-0209-9.
[42] D. Verma, P.K. Garg, A.K. Dubey, Insights into the human oral microbiome, Arch. Microbiol 200 (2018) 525–540, https://doi.org/10.1007/S00203-018-1505-3.
[43] L. Gao, T. Xu, G. Huang, S. Jiang, Y. Gu, F. Chen, Oral microbiomes: more and more importance in oral cavity and whole body, Protein Cell 9 (2018) 488, https://doi.org/10.1007/S13238-018-0548-1.
[44] V.K. Gupta, S. Paul, C. Dutta, Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity, Front Microbiol 8 (2017) 237451, https://doi.org/10.3389/FMICB.2017.01162/BIBTEX.
[45] K. Makki, E.C. Deehan, J. Walter, F. Bäckhed, The impact of dietary fiber on gut microbiota in host health and disease, Cell Host Microbe 23 (2018) 705–715, https://doi.org/10.1016/J.CHOM.2018.05.012.
[46] B. Senghor, C. Sokhna, R. Ruimy, J.C. Lagier, Gut microbiota diversity according to dietary habits and geographical provenance, Hum. Micro J. 78 (2018) 1–9, https://doi.org/10.1016/J.HUMIC.2018.01.001.
[47] M.G. Khonje, M. Qaim, Modernization of African food retailing and (un)healthy food consumption, 11, 4306, Sustainability 2019 11 (2019) *4306*, https://doi.org/10.3390/SU11164306.
[48] F. Kroll, E.C. Swart, R.A. Annan, A.M. Thow, D. Neves, C. Apprey, et al., Mapping Obesogenic Food Environments in South Africa and Ghana: Correlations and Contradictions, 11, 3924. Sustainability 2019 11 (2019) *3924*, https://doi.org/10.3390/SU11143924.
[49] F.Y. Zhai, S.F. Du, Z.H. Wang, J.G. Zhang, W.W. Du, B.M. Popkin, Dynamics of the Chinese diet and the role of urbanicity, 1991-2011, Obes. Rev. 15 (2014) 16–26, https://doi.org/10.1111/OBR.12124/SUPPINFO.
[50] T. Zhong, Z. Si, J. Crush, Z. Xu, X. Huang, S. Scott, et al., The impact of proximity to wet markets and supermarkets on household dietary diversity in Nanjing City, China, 10, 1465, Sustainability 2018 10 (2018) *1465*, https://doi.org/10.3390/SU10051465.
[51] J.L. Broussard, S. Devkota, The changing microbial landscape of Western society: Diet, dwellings and discordance, Mol. Metab. 5 (2016) 737–742, https://doi.org/10.1016/J.MOLMET.2016.07.007.
[52] M.G. Dominguez Bello, R. Knight, J.A. Gilbert, M.J. Blaser, Preserving microbial diversity Microbiota humans all cultures are needed ensure health future generations. Science 362 (1979) 33–34, https://doi.org/10.1126/SCIENCE.AAU8816/ASSET/DBFBB2AC-DFF5-45BE-B0E7-42192AA99E49/ASSETS/GRAPHIC/362_33_F1.JPEG.
[53] M.R. Mason, H.N. Nagaraja, T. Camerlengo, V. Joshi, P.S. Kumar, Deep Sequencing Identifies Ethnicity-Specific Bacterial Signatures in the Oral Microbiome, PLoS One 8 (2013) e77287, https://doi.org/10.1371/JOURNAL.PONE.0077287.
[54] P. Vangay, A.J. Johnson, T.L. Ward, G.A. Al-Ghalith, R.R. Shields-Cutler, B. M. Hillmann, et al., U.S. immigration westernizes the human gut microbiome, Cell 175 (2018) 962, https://doi.org/10.1016/J.CELL.2018.10.029.
[55] R. Blekhman, J.K. Goodrich, K. Huang, Q. Sun, R. Bukowski, J.T. Bell, et al., Host genetic variation impacts microbiome composition across human body sites, Genome Biol. 16 (2015) 1–12, https://doi.org/10.1186/S13059-015-0759-1/FIGURES/4.
[56] L.F. Gomez-Arango, H.L. Barrett, H.D. McIntyre, L.K. Callaway, M. Morrison, M. D. Nitert, Antibiotic treatment at delivery shapes the initial oral microbiome in neonates, 7, 1–10, Sci. Rep. 2017 7 (1) (2017), https://doi.org/10.1038/srep43481.
[57] L.S. Weyrich, S. Duchene, J. Soubrier, L. Arriola, B. Llamas, J. Breen, et al., Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus, Nature 544 (2017) 357–361, https://doi.org/10.1038/NATURE21674.
[58] S. Ramne, L. Brunkwall, U. Ericson, N. Gray, G.G.C. Kuhnle, P.M. Nilsson, et al., Gut microbiota composition in relation to intake of added sugar, sugar-sweetened beverages and artificially sweetened beverages in the Malmö Offspring Study, Eur. J. Nutr. 60 (2021) 2087–2097, https://doi.org/10.1007/S00394-020-02392-0/TABLES/3.
[59] A. Severino, E. Tohumcu, L. Tamai, P. Dargenio, S. Porcari, D. Rondinella, et al., The microbiome-driven impact of western diet in the development of noncommunicable chronic disorders, Best. Pr. Res Clin. Gastroenterol. (2024) 101923, https://doi.org/10.1016/J.BPG.2024.101923.
[60] T.E. Matsha, Y. Prince, S. Davids, U. Chikte, R.T. Erasmus, A.P. Kengne, et al., Oral Microbiome Signatures in Diabetes Mellitus and Periodontal Disease, J. Dent. Res 99 (2020) 658–665, https://doi.org/10.1177/0022034520913818/ASSET/IMAGES/LARGE/10.1177_0022034520913818-FIG4.JPEG.
[61] X. Sun, M. Li, L. Xia, Z. Fang, S. Yu, J. Gao, et al., Alteration of salivary microbiome in periodontitis with or without type-2 diabetes mellitus and metformin treatment, 10, 1–14, Sci. Rep. 2020 10 (1) (2020), https://doi.org/10.1038/s41598-020-72035-1.
[62] Y. Yang, S. Liu, Y. Wang, Z. Wang, W. Ding, X. Sun, et al., Changes of saliva microbiota in the onset and after the treatment of diabetes in patients with periodontitis, Aging 12 (2020) 13090–13114, https://doi.org/10.18632/AGING.103399.
[63] Vorster, H.H., K. A, M. B. M, V. C. S, M. U. E, W. M. P, & W.-V. E (2011). The nutrition transition in South Africa: a complex set of determinants for obesity. 12 (5), 342–349 - Google Search. Obesity Reviews. Available at: https://www.google.com/search?q=Vorster%2C+H.+H.%2C+Kruger%2C+A.%2C+Margetts%2C+B.+M.%2C+Venter%2C+C.+S.%2C+Macintyre%2C+U.+E.%2C+Wissing%2C+M.+P.%2C+…+%26+Wentzel-Viljoen%2C+E.+(2011).+The+nutrition+transition+in+South+Africa%3A+a+complex+set+of+determinants+for+obesity.+In+Obesity+Reviews%2C+12(5)%2C+342%E2%80%93349&sourceid=chrome&ie=UTF-8 (Accessed September 20, 2024).
[64] O.H. Oduaran, F.B. Tamburini, V. Sahibdeen, R. Brewster, F.X. Gómez-Olivé, K. Kahn, et al., Gut microbiome profiling of a rural and urban South African cohort reveals biomarkers of a population in lifestyle transition, BMC Microbiol 20 (2020) 1–17, https://doi.org/10.1186/S12866-020-02017-W/METRICS.
[65] E.O. Méndez-Salazar, M.G. Ortiz-López, M.D.L.Á. Granados-Silvestre, B. Palacios-González, M. Menjivar, Altered gut microbiota and compositional changes in firmicutes and proteobacteria in mexican undernourished and obese children, Front Microbiol 9 (2018) 308672, https://doi.org/10.3389/FMICB.2018.02494/BIBTEX.
[66] B. Senghor, C. Sokhna, R. Ruimy, J.C. Lagier, Gut microbiota diversity according to dietary habits and geographical provenance, Hum. Micro J. 78 (2018) 1–9, https://doi.org/10.1016/J.HUMIC.2018.01.001.
[67] Cha, Jun Hyung, Nayeon Kim, Junyeong Ma, Sungho Lee, Geon Koh, Sunmo Yang, Samuel Beck, Iksu Byeon, Byungwook Lee, and Insuk Lee. "A high-quality genomic catalog of the human oral microbiome broadens its phylogeny and clinical insights." *Cell Host & Microbe* (2025).
[68] S.J.D. O'Keefe, J.V. Li, L. Lahti, J. Ou, F. Carbonero, K. Mohammed, et al., Fat, fibre and cancer risk in African Americans and rural Africans, 6, 1–14, Nat. Commun. 2015 6 (1) (2015), https://doi.org/10.1038/ncomms7342.
[69] C. De Filippo, D. Cavalieri, M. Di Paola, M. Ramazzotti, J.B. Poullet, S. Massart, et al., Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa, Proc. Natl. Acad. Sci. USA 107 (2010) 14691–14696, https://doi.org/10.1073/PNAS.1005963107/SUPPL_FILE/PNAS.201005963SI.PDF.

[70] D. Kawamoto, R. Borges, R.A. Ribeiro, R.F. de Souza, P.P.P. Amado, L. Saraiva, et al., Oral dysbiosis in severe forms of periodontitis is associated with gut dysbiosis and correlated with salivary inflammatory mediators: a preliminary study, Front. Oral. Health 2 (2021), https://doi.org/10.3389/froh.2021.722495.
[71] Y. Wang, F. Yang, Y. Wang, S. Deng, R. Zhu, Alterations and correlations in dental plaque microbial communities and metabolome characteristics in patients with caries, periodontitis, and comorbid diseases, BMC Oral. Health 24 (2024) 1–10, https://doi.org/10.1186/S12903-023-03785-3/FIGURES/6.
[72] A.J. Obregon-Tito, R.Y. Tito, J. Metcalf, K. Sankaranarayanan, J.C. Clemente, L. K. Ursell, et al., Subsistence strategies in traditional societies distinguish gut microbiomes, Nat. Commun. 6 (2015), https://doi.org/10.1038/NCOMMS7505.
[73] F. De Filippis, N. Pellegrini, L. Vannini, I.B. Jeffery, A. La Storia, L. Laghi, et al., High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome, Gut 65 (2016) 1812–1821, https://doi.org/10.1136/GUTJNL-2015-309957.
[74] E. Dimidi, S.R. Cox, M. Rossi, K. Whelan, Fermented foods: definitions and characteristics, impact on the gut microbiota and effects on gastrointestinal health and disease, Nutrients 11 (2019), https://doi.org/10.3390/NU11081806.
[75] A. Antezack, M. Boxberger, B.La Scola, V. Monnet-Corti, Isolation and Description of Catonella massiliensis sp. nov., a Novel Catonella Species, Isolated from a Stable Periodontitis Subject, 10, 367, Pathogens 2021 10 (2021) *367*, https://doi.org/10.3390/PATHOGENS10030367.
[76] A.L. Griffen, C.J. Beall, J.H. Campbell, N.D. Firestone, P.S. Kumar, Z.K. Yang, et al., Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing, ISME J. 6 (2012) 1176–1185, https://doi.org/10.1038/ISMEJ.2011.191.
[77] G. Isola, S. Santonocito, S.M. Lupi, A. Polizzi, R. Sclafani, R. Patini, et al., Periodontal Health and Disease in the Context of Systemic Diseases, Mediat. Inflamm. 2023 (2023) 9720947, https://doi.org/10.1155/2023/9720947.
[78] K.M. Yu, H.S. Cho, A.M. Lee, J.W. Lee, S.K. Lim, Analysis of the influence of host lifestyle (coffee consumption, drinking, and smoking) on Korean oral microbiome, Forensic Sci. Int Genet 68 (2024) 102942, https://doi.org/10.1016/J.FSIGEN.2023.102942.
[79] A. Amer, S. Galvin, C.M. Healy, G.P. Moran, The microbiome of potentially malignant oral leukoplakia exhibits enrichment for Fusobacterium, Leptotrichia, Campylobacter, and Rothia species, Front Microbiol 8 (2017) 302760, https://doi.org/10.3389/FMICB.2017.02391/BIBTEX.
[80] J.L.C. Mougeot, M.F. Beckman, H.C. Langdon, M.T. Brennan, F.B. Mougeot, Oral Microbiome Signatures in Hematological Cancers Reveal Predominance of Actinomyces and Rothia Species, 9, 4068, J. Clin. Med. 2020 9 (2020) *4068*, https://doi.org/10.3390/JCM9124068.
[81] M. Iniesta, C. Chamorro, N. Ambrosio, M.J. Marín, M. Sanz, D. Herrera, Subgingival microbiome in periodontal health, gingivitis and different stages of periodontitis, J. Clin. Periodo 50 (2023) 905–920, https://doi.org/10.1111/JCPE.13793.
[82] T. Khemwong, H. Kobayashi, Y. Ikeda, T. Matsuura, T. Sudo, C. Kano, et al., Fretibacterium sp. human oral taxon 360 is a novel biomarker for periodontitis screening in the Japanese population, PLoS One 14 (2019) e0218266, https://doi.org/10.1371/JOURNAL.PONE.0218266.
[83] Y.-H. Lee, H.J. Park, S.-J. Jeong, J. Jung, G.-J. Lee, J.-Y. Hong, et al., Oral. Micro Profile Gingivitis Periodo Gener. Seq. (2023), https://doi.org/10.21203/RS.3.RS-3530768/V1.
[84] J.R. Willis, T. Gabaldón, The Human Oral Microbiome in Health and Disease: From Sequences to Ecosystems, 8, 308, Microorganisms 2020 8 (2020) *308*, https://doi.org/10.3390/MICROORGANISMS8020308.
[85] M. del P. Angarita-Díaz, C. Fong, C.M. Bedoya-Correa, C.L. Cabrera-Arango, Does high sugar intake really alter the oral microbiota?: A systematic review, Clin. Exp. Dent. Res 8 (2022) 1376–1390, https://doi.org/10.1002/CRE2.640.
[86] T. Willmott, H.J. Serrage, E.C. Cottrell, G.J. Humphreys, J. Myers, P.M. Campbell, et al., Investigating the association between nitrate dosing and nitrite generation by the human oral microbiota in continuous culture, Appl. Environ. Microbiol 90 (2024), https://doi.org/10.1128/AEM.02035-23.
[87] L. Franceschetti, G. Lodetti, A. Blandino, A. Amadasi, V. Bugelli, Exploring the role of the human microbiome in forensic identification: opportunities and challenges, Int J. Leg. Med 138 (2024) 1891–1905, https://doi.org/10.1007/S00414-024-03217-Z/TABLES/1.
[88] K. Phan, M. Barash, X. Spindler, P. Gunn, C. Roux, Retrieving forensic information about the donor through bacterial profiling, Int J. Leg. Med 134 (2020) 21–29, https://doi.org/10.1007/S00414-019-02069-2/FIGURES/4.
[89] A.A. Singh, M. Okpeku, Emerging methods of human microbiome analysis and its forensic applications: Review, Forensic Science International Reports 9 (2024) 100355, https://doi.org/10.1016/J.FSIR.2024.100355.
[90] S. Sun, H. Zhang, L. Ye, L. Huang, J. Du, X. Liang, et al., Combined analysis of the microbiome and metabolome to reveal the characteristics of saliva from different diets: a comparison among vegans, seafood-based omnivores, and red meat (beef and lamb) omnivores, Front Microbiol 15 (2024) 1419686, https://doi.org/10.3389/FMICB.2024.1419686/BIBTEX.
[91] M.M.H. Abdelbary, M. Hatting, A. Bott, A. Dahlhausen, D. Keller, C. Trautwein, et al., The oral-gut axis: Salivary and fecal microbiome dysbiosis in patients with inflammatory bowel disease, Front Cell Infect. Microbiol 12 (2022) 1010853, https://doi.org/10.3389/FCIMB.2022.1010853/BIBTEX.
[92] B. Xiang, C. Runzhi, Z. Min, Z. Yuanqi, P. Xiang, W. Wei, et al., P1214 The crosstalk between oral and intestinal microbiota in inflammatory bowel disease patients, i2158–i2158, J. Crohns Colitis 18 (2024), https://doi.org/10.1093/ECCO-JCC/JJAD212.1344.
[93] H.-W. Cho, Y.-B. Eom, Forensic analysis of human microbiome in skin and body fluids based on geographic location, Front Cell Infect. Microbiol 11 (2021). Available at: ⟨https://www.frontiersin.org/articles/10.3389/fcimb.2021.695191⟩.
[94] Y. Wu, X. Chi, Q. Zhang, F. Chen, X. Deng, Characterization of the salivary microbiome in people with obesity, PeerJ 2018 e4458 (2018), https://doi.org/10.7717/PEERJ.4458/SUPP-14.
[95] I. Mashima, C.F. Theodorea, B. Thaweboon, S. Thaweboon, T. Vichayanrat, F. A. Scannapieco, et al., Characterization of the salivary microbiome in healthy Thai children, Asian Pac. J. Trop. Med 12 (2019) 163–169, https://doi.org/10.4103/1995-7645.257116.
[96] B.T. Rosier, P.D. Marsh, A. Mira, Resilience of the Oral Microbiota in Health: Mechanisms That Prevent Dysbiosis, J. Dent. Res 97 (2018) 371–380, https://doi.org/10.1177/0022034517742139/ASSET/IMAGES/LARGE/10.1177_0022034517742139-FIG6.JPEG.
[97] W.G. Wade, Resilience of the oral microbiome, Periodontol 2000 86 (2021) 113–122, https://doi.org/10.1111/PRD.12365.
[98] K.L. Hoffman, D.S. Hutchinson, J. Fowler, D.P. Smith, N.J. Ajami, H. Zhao, et al., Oral microbiota reveals signs of acculturation in Mexican American women, PLoS One 13 (2018) e0194100, https://doi.org/10.1371/JOURNAL.PONE.0194100.
[99] N.S. Shad, N.I. Shaikh, S.A. Cunningham, Migration Spurs Changes in the Human Microbiome: a Review, J. Racial Ethn. Health Disparities (2023) 1–15, https://doi.org/10.1007/S40615-023-01813-0/TABLES/2.