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Original Article

Influence of Geography and Socio-Economic Factors on the Oral Microbiome of Indonesian Women

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ABSTRACT

Research exploring the effects of geographic and socioeconomic factors on the oral microbiome is limited. According to the 2018 Indonesian Basic Health Research (RISKESDAS), non-communicable diseases exhibited an upward trend compared to the 2013 survey. Conditions such as diabetes, heart disease, hypertension, and obesity were more prevalent in urban settings than in rural ones. Notably, these diseases affected women more frequently than men. This pilot study sought to assess oral health status and the oral microbiome from tongue dorsum samples in healthy Indonesian women residing in urban and rural regions. A total of 20 women (aged 21–47 years) were enrolled, including 10 from West Jakarta (DKI Jakarta province) representing an urban environment and 10 from Ende, Nangapanda (East Nusa Tenggara province) representing a rural environment. Oral hygiene was assessed using the Simplified Oral Hygiene Index (OHI-S) based on Greene and Vermillion criteria, with participants categorized into three groups. High-throughput sequencing of bacterial DNA was conducted using the Illumina iSeq 100 platform. Principal coordinate analysis revealed clear distinctions in bacterial community composition between urban and rural groups. Urban women showed greater microbial diversity and modified community structures linked to manifest oral conditions. Two genera, Prevotella and Leptotrichia, were significantly enriched in urban samples (adjusted $p < 0.01$), driving these differences independently of personal oral hygiene. Linear discriminant analysis effect size (LEfSe) identified multiple urban-specific biomarkers. At the species level, Leptotrichia wadei, Prevotella melaninogenica, Prevotella jejuni, and Prevotella histicola demonstrated strong potential to differentiate urban from rural oral microbiomes. Additionally, SparCC network analysis indicated that co-occurrence patterns within the core oral microbiome were niche-specific across the two populations. This represents the inaugural pilot investigation into the oral microbiome composition among Indonesian women from urban and rural settings. The findings indicate geographic-specific patterns in the female oral microbiome. These distinctive microbial profiles in Indonesian women may be associated with region-specific dietary practices, cultural behaviors, and socioeconomic factors in the studied cohorts.

Keywords: Oral microbiome, Women, Urban-rural differences, Indonesia, Pilot study

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Introduction

The human oral cavity has traditionally been estimated to harbor approximately 700 bacterial species [1]. Advances in high-throughput DNA sequencing, however, have revealed the presence of around 19,000 phylotypes, including many previously unculturable taxa [2]. Emerging evidence suggests that alterations in the oral microbiome during health and disease states—such as periodontitis and dental caries [3-5]—may contribute to various non-communicable diseases, including cardiovascular disease, stroke, cancer, chronic respiratory conditions, and diabetes [6-10]. These associations are influenced by multiple factors, including host genetics, oral hygiene practices [1], geographic location, dietary patterns, socioeconomic status, and lifestyle choices [1].

Indonesia, the most populous country in Southeast Asia and the fourth most populous nation globally, had a

population of 273,523,615 in 2020, comprising diverse ethnic groups [11]. As of 2007, the country was home to an estimated 633 distinct ethnic groups [12, 13], each with unique cultural practices and lifestyles, distributed across 34 provinces [12, 13]. Imbalances in oral microbiome composition have been linked to oral conditions like periodontitis and caries, as well as systemic diseases such as diabetes mellitus [14], cardiovascular disorders [15], and rheumatoid arthritis [16]. In Indonesia, the prevalence of diabetes, heart disease, and rheumatoid arthritis is higher in urban areas (1.9%, 1.6%, and 0.3%, respectively) compared to rural areas (1.0%, 1.3%, and 0.2%, respectively), with these preventable non-communicable diseases affecting women more than men [13]. Oral diseases also remain highly prevalent, with caries being widespread [17] and periodontitis affecting 73.1% of the population [18]. Such disease susceptibility may exhibit ethnic variations in highly diverse populations, prompting investigation into geographic influences on the oral microbiome and their implications for oral health. Prior research has identified ethnicity-associated clustering of microbial communities in saliva and subgingival plaque among genetically distinct populations across countries [19, 20]. Notably, marked differences in oral microbiome diversity have been observed among African populations, potentially driven by variations in traditional subsistence strategies, lifestyles, diets, and caries prevalence [19, 21]. Collectively, these findings highlight geography as a complex interplay of genetic, environmental, and cultural elements that shape the human oral microbiome.

Intriguingly, gender differences in periodontal health have been noted in Indonesian urban populations, where women exhibit reduced calculus accumulation but increased deep pocket formation according to the Community Periodontal Index of Treatment Needs (CPITN) [22]. On average, women tend to engage in less physical activity than men [23]. Rural women, however, participate in more household-related activities (mild to moderate intensity) compared to their urban counterparts [24, 25]. Women's caloric intake is also prone to fluctuations across the lifespan [26]. Reduced physical activity and variable energy intake are directly associated with higher risks of non-communicable diseases [27]. Furthermore, contemporary lifestyles—characterized by ready access to high-calorie foods, sedentary habits, and limited exercise—exacerbate the onset of these conditions [28].

Assessing technical variations across studies while accounting for gender-, geography-, ethnicity-, and lifestyle-related differences in healthy microbiome compositions poses significant challenges [29]. Nonetheless, profiling microbial communities is essential for identifying disease-associated dysbioses, restoring balanced configurations, and elucidating the features of healthy microbiomes across diverse ecological niches.

Although microbial variations across cultural contexts have been explored in other countries [29], no studies, to our knowledge, have investigated the impact of geographic and socioeconomic factors on oral health and the oral microbiome in Indonesia. This pilot study, therefore, aimed to evaluate oral health status and characterize the oral microbiome from tongue dorsum samples of healthy Indonesian women living in urban and rural settings.

Materials and Methods

Ethical statement

This study received approval from the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (protocol number: 1060/UN2.F1/ETIK/PPM.00.02/2019). All participants provided voluntary involvement and signed written informed consent forms covering all aspects of the study. Procedures were performed in full compliance with the Declaration of Helsinki for research involving human subjects.

Participants

Twenty women aged 20–45 years were recruited for this pilot study. Ten resided in Jakarta (the capital city), representing an urban population, while the other ten were from Ende, Nangapanda in East Nusa Tenggara, representing a rural population. Inclusion criteria comprised Indonesian female citizens who had not taken antibiotics or antihistamines and had not undergone periodontal treatment in the preceding three months. Exclusion criteria included history of gastrointestinal surgery, antibiotic use within the past three months, smoking, consumption of prebiotics or probiotics, vegetarian or vegan diets, use of nutritional or ergogenic supplements, and pregnancy or breastfeeding. Oral hygiene was assessed using the Simplified Oral Hygiene Index (OHI-S) according to Greene and Vermillion criteria [30], and participants were classified into three categories: good, fair, or poor. Tongue dorsum samples were collected immediately following the oral hygiene evaluation.

Tongue swab collection

Tongue samples were obtained by swabbing the dorsal surface of the tongue with a sterile cotton swab. The procedure was performed in duplicate for each

participant. Swabs were immediately placed into individual 1.5-mL microcentrifuge tubes containing 1 mL of sterile phosphate-buffered saline (PBS).

DNA extraction and preparation

Genomic DNA was isolated from the tongue swabs using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA concentration and purity were quantified using a Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Subsequent steps, including PCR amplification, purification of amplicons, library construction, and sequencing, are detailed below. DNA samples from all participants were labeled, pooled equitably, and thoroughly mixed in a single tube.

Library preparation and sequencing

PCR amplification targeting the V3–V4 hypervariable region of the bacterial 16S rRNA gene was conducted for library preparation. Primers incorporating Illumina overhang adapter sequences were used: forward primer (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′) and reverse primer (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′). Each 25-µL reaction contained 2.5 µL of template DNA (5 ng/µL), 5 µL each of forward and reverse primers (1 µM final concentration), and 12.5 µL of 2× KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Boston, MA, USA). Thermal cycling conditions consisted of initial denaturation at 95°C for 3 min, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. Amplicons were verified by electrophoresis on 1% (w/v) agarose gels in 1× Tris-Acetate-EDTA (TAE) buffer at 110 V for 15 min, yielding bands at approximately 550 bp.

Following amplification, a initial cleanup was performed using AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) to remove primers and primer dimers, in accordance with Illumina's 16S amplicon preparation guidelines. Dual-index adapters were then attached via index PCR using the Nextera DNA UD Indexes Set A (Illumina, San Diego, CA, USA). Each 50-µL indexing reaction included 5 µL of cleaned amplicon DNA, 10 µL of Nextera indexes, 25 µL of 2× KAPA HiFi HotStart ReadyMix, and 10 µL of PCR-grade water. A second cleanup with AMPure XP beads was performed on the indexed libraries. Final libraries were visualized by gel electrophoresis under the same conditions, showing bands at approximately 630 bp. Library preparation and sequencing were carried out on the Illumina iSeq 100 platform at the MiCORE Laboratory, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia.

Sequence processing and taxonomic annotation

Sequencing quality was initially assessed using Illumina Local Run Manager v2.0 and Sequencing Analysis Viewer v2.4.7. FASTQ files passing quality control were processed on the Galaxy public server (usegalaxy.org) [31]. Per-sample quality metrics were evaluated with FASTQC v0.72. High-quality reads were aligned to reference sequences using Bowtie2 v2.3.4.3 [32] with default settings. Sequencing depth was calculated from BAM files using the Genome Analysis Toolkit [33]. Taxonomic classification was performed via the 16S Metagenomics workflow in Local Run Manager v2.0, employing an Illumina-curated Greengenes database (version 13_5). A total of 612,962 reads were clustered into 1,317 operational taxonomic units (OTUs) at 97% similarity threshold. Rarefaction curves indicated adequate sequencing depth. Raw sequencing data are publicly available under BioProject accession PRJNA745286.

Microbiota data analysis

Alpha diversity metrics (Chao1 estimator and observed richness) along with rarefaction curves were computed and visualized using the MicrobiomeAnalystR platform [34, 35], leveraging the "phyloseq," "ggplot2," and "microbiomeseq" R packages (code available at https://github.com/xia-lab/MicrobiomeAnalystR).

Beta diversity and community structure differences were evaluated using algorithms implemented in MicrobiomeAnalystR. Non-metric multidimensional scaling (NMDS) ordination based on Bray–Curtis dissimilarity was employed, with stress values ≤0.20 deemed acceptable. Group differences were statistically tested using permutational multivariate analysis of variance (PERMANOVA) [36]. Similarity and dispersion homogeneity between urban and rural groups were further assessed with analysis of similarities (ANOSIM) [37, 38].

Microbiota data analysis (continued)

Network inference was performed using the SparCC algorithm [39], which is specifically tailored for compositional microbiome data. SparCC employs log-ratio transformations and iterative approximations to infer correlations, identifying genus-level pairs that deviate from background associations in urban versus rural populations, as well as in relation to oral hygiene status (OHI-S). Correlations were considered robust if the absolute Spearman correlation coefficient exceeded 0.6 and the p-value was <0.05. Pseudo p-values were

derived from SparCC correlation estimates within the MicrobiomeAnalystR platform. Only associations meeting these criteria (absolute R > 0.6, p < 0.05) were visualized in network plots.

Differential abundance at the species level was evaluated using STAMP software [40] with Fisher's exact test for multi-group comparisons. Significance was determined at a corrected p-value <0.01 (Storey FDR correction), and results were further filtered by q-value <0.05 and effect size >0.05.

Potential biomarker taxa distinguishing the two geographic groups were identified through linear discriminant analysis effect size (LEfSe) [41], implemented via the Huttenhower Lab tool on the Galaxy web platform [42]. The analysis began with non-parametric Kruskal–Wallis rank-sum tests (α = 0.01) to detect differentially abundant features across urban and rural groups. LDA was then applied to estimate effect sizes, using an all-against-all comparison strategy and an LDA score threshold of >2.0 for discriminative taxa. Differential abundance analysis at phylum, genus, and species levels was also conducted using the DESeq2 package [43] in MicrobiomeAnalystR. Features with relative abundance >0.1% and present in >50% of samples in at least one group were included unless otherwise stated.

Statistical analysis

Comparisons of continuous and categorical variables were performed using two-tailed Mann–Whitney U tests and Kruskal–Wallis tests, respectively. Differential abundance was expressed as log2 fold change, with multiple testing correction via the Benjamini–Hochberg procedure (FDR <10%). Features were deemed statistically significant if the adjusted p-value was <0.05.

Results and Discussion

Participant characteristics and oral microbiome differences between urban and rural groups

Urban participants were recruited from Jakarta (population ~10 million), with a mean age of 34 years (SD ±10.5). Rural participants were from villages in Ende and Nangapanda, East Nusa Tenggara (combined population ~87,269), with a mean age of 34 years (SD ±4.2). The rural sites are located approximately 1,500–4,360 km from Jakarta. Overall mean age across all participants was 34 years (SD ±7.8). A higher proportion of urban women exhibited good OHI-S scores compared to rural women.

To evaluate geographic differences in oral bacterial communities, microbial richness and diversity were examined. Chao1 richness estimates were significantly higher in urban samples than in rural samples (Mann–Whitney U test, p = 0.035; **Figure 1a**). When stratified by OHI-S category across locations, species richness showed no significant differences (Kruskal–Wallis χ^2 = 4.268, p = 0.118), though richness tended to be greater in samples with good or fair oral hygiene than in those with poor hygiene **(Figure 1c)**.

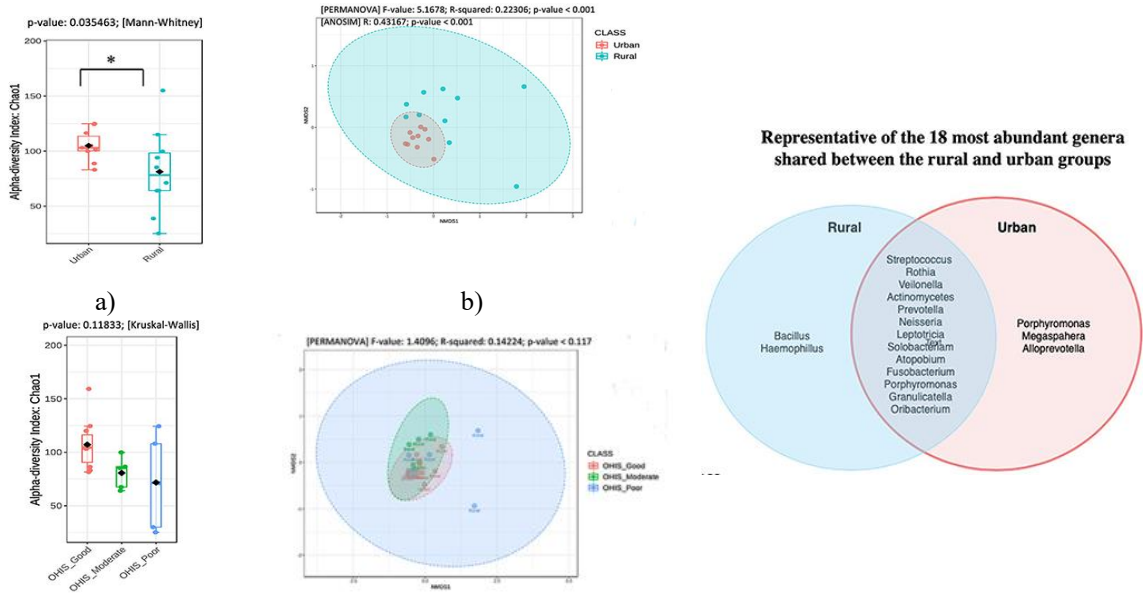


Figure 1. Overview of Oral Microbiota Diversity and Community Structure in Urban and Rural Populations.

a and c) Alpha diversity metrics for urban versus rural participants and stratified by oral hygiene status (OHI-S). Boxplots illustrate the distribution of diversity values across groups. Statistical comparisons were

performed using two-tailed Mann–Whitney U tests and Kruskal–Wallis tests. Shown are richness indices (observed OTUs and Chao1) for the oral microbiomes of women from urban and rural areas. Letters denote statistically significant differences ($p < 0.05$). (b and d) Beta diversity visualized by non-metric multidimensional scaling (NMDS) ordination based on Bray–Curtis dissimilarity, depicting microbial community structures for the two geographic groups and across OHI-S categories, respectively. Inset boxplots display inter-sample distances between locations and within OHI-S strata. Corresponding R^2 and p-values from PERMANOVA are indicated. Letters mark significant differences (*$p < 0.05$; ***$p < 0.001$). (e) Venn diagram illustrating the 18 most abundant genera shared between rural and urban groups.

Geographic differences in oral microbiome composition

Bacterial community structures differed markedly between urban and rural participants, as shown by NMDS ordination (**Figure 1b**). Samples from the two locations formed separate clusters (PERMANOVA: $R^2 = 0.223$, $p < 0.001$; NMDS stress = 0.084). ANOSIM confirmed significant compositional differences ($R = 0.43$, $p < 0.001$). In contrast, no significant variation was observed across OHI-S categories within each location (PERMANOVA: $R^2 = 0.141$, $p = 0.119$; NMDS stress = 0.097; **Figure 1d**). The overlap in the 18 most dominant genera between urban and rural groups is presented in **Figure 1e**.

Taxonomic differences in the oral microbiome between urban and rural areas

Differentially abundant phyla and genera were identified using DESeq2 with age adjustment (adjusted $p < 0.05$). Five major bacterial phyla accounted for relative abundances >2% across all samples **(Figure 2a)**. Firmicutes dominated, comprising 38% of sequences in urban samples and 52% in rural samples. Bacteroidetes (23% urban vs. 11% rural) and Fusobacteria (20% urban vs. 3% rural) were more abundant in urban participants, while Actinobacteria (9% urban vs. 19% rural) and Proteobacteria (8% urban vs. 13% rural) were enriched in rural participants. Significant enrichments included Bacteroidetes and Fusobacteria in urban samples (DESeq2 adjusted $p < 0.001$; $\log_2$ fold change −3.4 to −2.7) and Firmicutes, Actinobacteria, and Spirochaetes in rural samples (DESeq2 adjusted $p < 0.001$; $\log_2$ fold change 1.4 to 3.5).

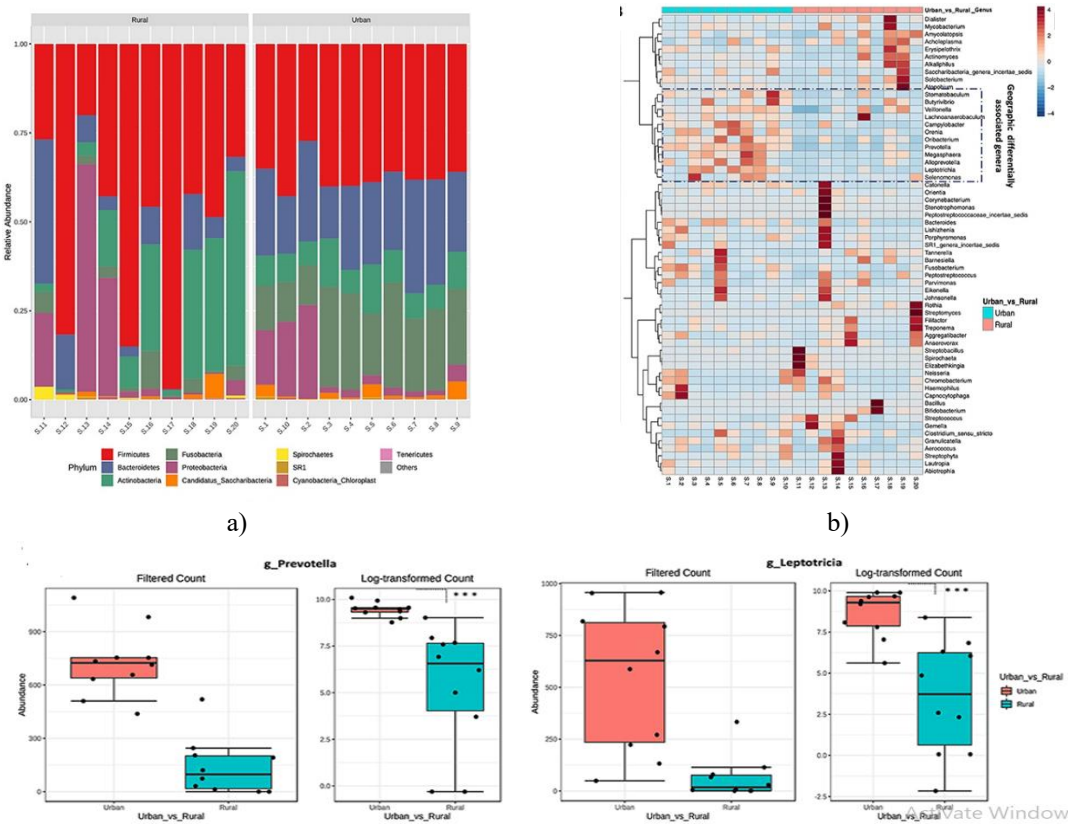


Figure 2. Taxonomic Composition of the Oral Microbiome in Urban and Rural Women.

(a) RelativeROID Relative abundance of the top 10 bacterial phyla in urban versus rural participants. (b) Heatmap displaying the most abundant genera across individual samples. Color intensity reflects z-score normalized relative abundances (row-scaled; mean = 0, SD = 1), with blue indicating low abundance and red indicating high abundance. The heatmap was generated using the R heatmap package with default settings. (c) Genera with the most significant differential abundance among the top 10 predominant taxa (average relative abundance >3%). Shown are Prevotella and Leptotrichia. DESeq2 analysis was performed, with $\log_2$ fold change values reported and multiple comparisons corrected using Benjamini–Hochberg (FDR <10%). ***Adjusted $p < 1.0 \times 10^{-4}$.

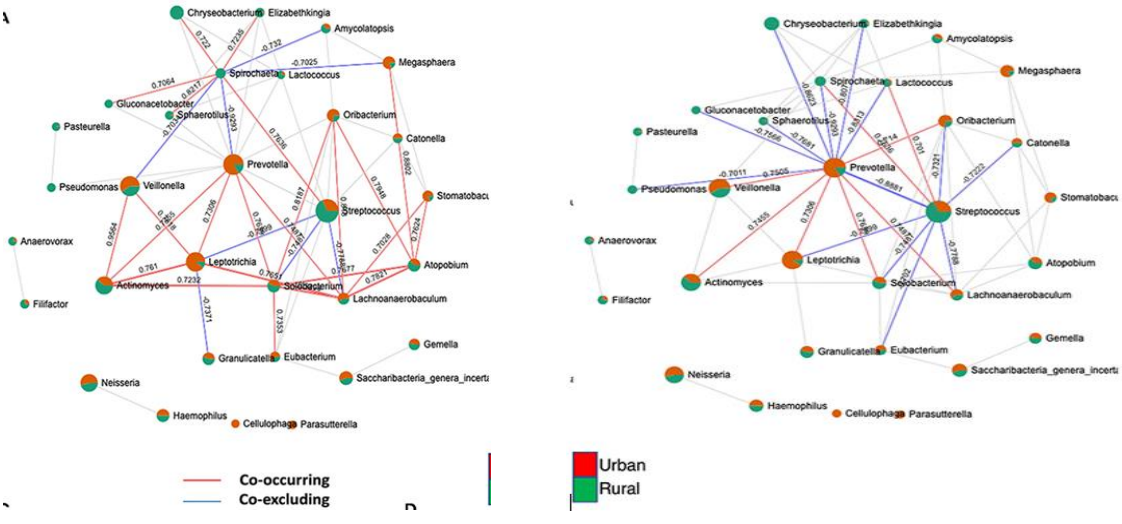
The top 10 genera from each sample (35 genera total across all samples) were visualized in a heatmap to highlight abundance patterns **(Figure 2b)**. Eight genera exhibited average relative abundances >3% in both groups. In rural samples, Streptococcus was dominant (30%), followed by Bacillus (10%), Rothia (9%), Veillonella (8%), Actinomyces and Actinobacteria (both ~7%), Neisseria (4%), and Prevotella (4%). In urban samples, Prevotella was the most abundant (21%), followed by Leptotrichia (16%), Streptococcus (15%), Veillonella (12%), Neisseria (6%), Actinomyces (5%), Fusobacterium (4%), and Rothia (3%). DESeq2 identified significant enrichment of Prevotella and Leptotrichia in urban participants (adjusted $p < 0.001$; $\log_2$ fold change −3.4 to −2.7; **Figure 2c**). No significant phylum- or genus-level differences were associated with OHI-S scores within either location.

Ecological interactions in the oral microbiome across urban and rural settings

To explore whether urbanization influences microbial interactions, co-occurrence networks were constructed separately for urban and rural groups using the SparCC algorithm [39, 44], which accurately infers linear relationships in compositional data and captures both direct and indirect ecological associations.

Across the full dataset, 165 significant robust correlations involving 70 genera were detected, comprising 165 positive and 95 negative associations. After FDR correction and filtering for OTUs with >10 counts in each group, distinct patterns emerged **(Figure 3a)**. Seven OTUs displayed particularly strong correlations conserved across both populations (absolute correlation coefficient ≥0.7, $p < 0.01$ for positive; absolute correlation coefficient >0.7, $p \leq 0.01$ for negative; **Figure 3c**). These OTUs, representing core microbiome members (up to 16% relative abundance in urban and <8% in rural samples), included Lachnoanaerobaculum, Atopobium, Leptotrichia, and Oribacterium (higher in urban) as well as Solobacterium, Actinomyces, and Spirochaeta (higher in rural). Positive correlations predominated among these taxa **(Figures 3c and 3d)**, suggesting shared ecological preferences and cooperative interactions within each niche.

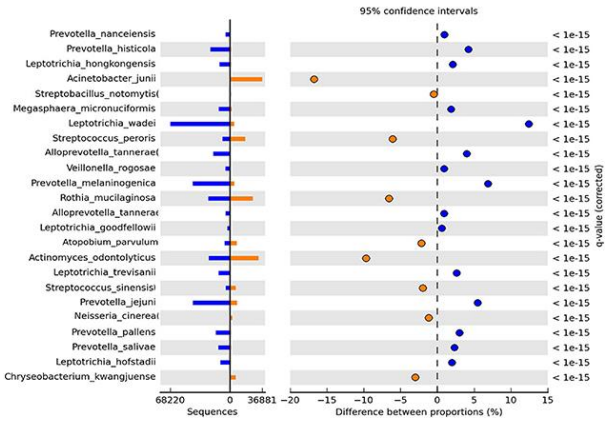
In contrast, dominant core genera exhibited more frequent negative associations: Streptococcus in rural samples and Prevotella in urban samples (absolute correlation >0.7, $p < 0.05$; **Figure 3b**). These patterns indicate that the predominant members of the core oral microbiome (>20% relative abundance) display location-specific competitive or exclusionary interactions, potentially contributing to the maintenance of ecological balance within each geographic setting.



a) b)

Phylum	Most Resolved Taxonomy	Higher Abundance group	Rank[a] [(=corr)|≥0.7 and p-value < 0.01]
Firmicutes	Lachnoanaerobaculum	Urban group	1
Firmicutes	Solobacterium	Rural group	2
Actinobacteria	Atopobium	Urban group	3
Actinobacteria	Actinomyces	Rural group	4
Fusobacteria	Leptotrichia	Urban group	5
Firmicutes	Oribacterium	Urban group	6
Spirochaetes	Spirochaeta	Rural group	7

c)



d)

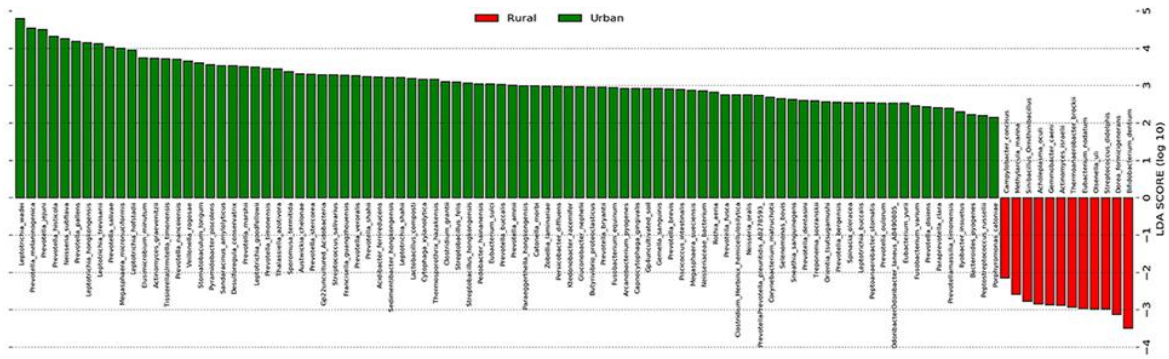
Figure 3. Co-occurrence Networks in the Oral Microbiome Across Populations

SparCC correlations are shown for |r| > 0.6 and p < 0.05. Nodes represent genera, with node size proportional to median relative abundance. Edges indicate correlations: solid red lines for positive (co-occurrence) and solid blue lines for negative (co-exclusion) associations (n = 165). Green nodes denote genera more associated with urban samples, while red nodes indicate those more linked to rural samples among shared genera. (A) Overall SparCC correlation network for urban versus rural oral microbiomes (|r| > 0.6, p < 0.05). (B) Subnetwork highlighting interactions involving dominant core genera (Streptococcus and Prevotella) across locations (|r| > 0.7, p < 0.05). (C) Top-ranking OTUs contributing to geographic co-occurrence patterns (|r| > 0.7, p < 0.01). (D) Differential bacterial taxa at the species level between locations, identified using STAMP (p < 0.01, q-value < 0.05, effect size > 0.05), illustrating key variations among dominant core microbiome members.

Differentially abundant biomarker taxa between urban and rural groups

To identify taxa driving geographic distinctions, linear discriminant analysis effect size (LEfSe) was applied at the OTU level. A total of 100 species exhibited LDA scores >2.0 **(Figure 4a)**. Among these, 10 urban-enriched species (LDA score >4.0) from three genera and 12 rural-enriched species (LDA score >3.0) from five genera were detected. Ten clades emerged as the most statistically and biologically consistent discriminators, exclusively enriched in urban participants **(Figure 4b)**. Key urban-associated biomarkers at the species level included Leptotrichia wadei, Prevotella melaninogenica, Prevotella jejuni, Prevotella histicola, Neisseria subflava, Prevotella pallens, Leptotrichia hongkongensis, Leptotrichia trevisanii, Prevotella salivae, and Megasphaera micronuciformis, primarily from the genera Prevotella, Leptotrichia, and Neisseria (**Figures 4b, 4c**). These biomarkers were enriched in urban women independently of oral hygiene status.

Rural-associated biomarkers were predominantly from the phyla Actinobacteria and Firmicutes (Clostridia class), including genera such as Bifidobacterium, Streptococcus, and Actinomyces. The most prominent species (LDA score >2.8) were Bifidobacterium dentium, Dorea formicigenerans, Actinomyces israelii, and Eubacterium nodatum, which were exclusive to rural participants.



a)

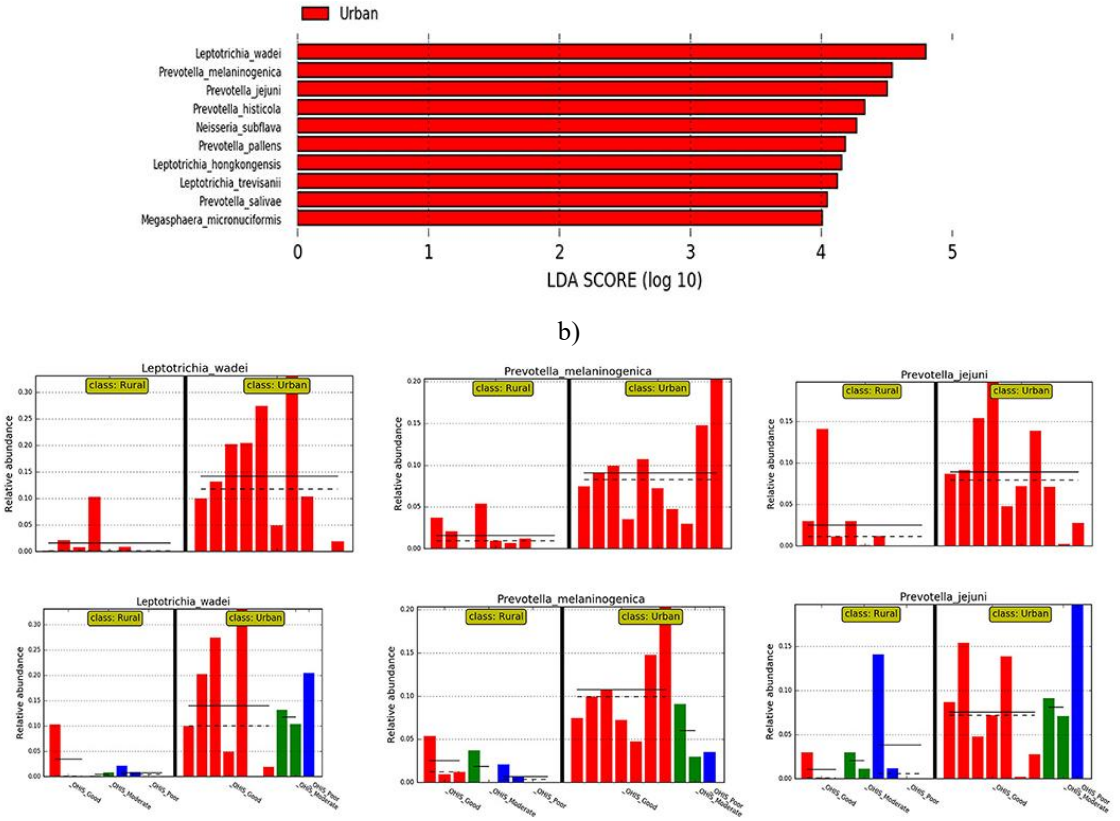


Figure 4. Linear Discriminant Analysis Effect Size (LEfSe) Results for Urban and Rural Oral Microbiomes

LEfSe identifies taxa that differ significantly between biological conditions while quantifying the magnitude of these differences. Histograms display LDA scores for differentially abundant taxa between urban and rural groups. Bar length reflects the effect size of specific taxa within each group at the species level. LDA scores indicate the degree of consistent abundance differences between microbial communities in the two classes. These histograms highlight clades responsible for the most pronounced community distinctions. (a) Discriminative features shown at LDA score threshold >2.0. (b) Discriminative features at a higher stringency threshold of LDA score >3.0. (c) Histogram of the most significantly enriched species (within the 0.01 abundance interval): Leptotrichia wadei and Prevotella melaninogenica in urban versus rural samples, further stratified by OHI-S categories (red: good; blue: moderate; green: poor).

DESeq2 analysis, using variance-stabilized data and a negative binomial model (default parameters), confirmed and ranked differentially abundant taxa. With greater statistical stringency, DESeq2 corroborated LEfSe findings, identifying marked enrichments in urban samples for species from Leptotrichia (L. wadei, L. hongkongensis, L. trevisanii) and Prevotella (P. melaninogenica, P. histicola, P. pallens, P. salivae).

Many 16S rRNA-based studies of the oral microbiome have focused solely on differential taxon abundance in relation to oral diseases [45, 46], often overlooking geography as a structuring factor. This pilot study presents the first evidence highlighting the role of geographic location in shaping oral microbiome composition among Indonesian women. Our findings indicate that geography and associated lifestyle factors significantly influence the oral microbiome in these urban and rural populations.

Urban participants exhibited significantly higher bacterial richness and diversity compared to their rural counterparts. Although improved OHI-S scores trended toward greater alpha diversity, these associations did not reach statistical significance in inter-category comparisons.

Beta diversity analyses (NMDS and PERMANOVA) reinforced these observations, revealing distinct clustering of microbial communities by location, with limited taxon overlap. No clear separation was evident based on oral hygiene status within each region. The Venn diagram illustrated that while a substantial portion of OTUs were shared between groups—potentially reflecting common influences such as age, core human physiology, or broad dietary elements—a smaller subset formed location-specific clusters, likely

driven by environmental, cultural, or socioeconomic factors.

All Actinomyces species implicated in human actinomycotic infections are part of the indigenous facultative pathogenic microbiota on mucosal surfaces, particularly in the oral cavity, with A. israelii being the primary etiologic agent [47, 48]. However, these taxa were detected only sporadically in the oral microbiomes of the rural women in this study. This observation aligns with prior evidence suggesting that the oral cavity may not serve as a primary reservoir for these putative caries- and periodontitis-associated pathogens.

Conversely, the majority of urban-enriched biomarkers are recognized opportunistic pathogens linked to oral diseases, halitosis, and compromised oral health, including Leptotrichia wadei, Prevotella melaninogenica, P. histicola, P. pallens, and Megasphaera micronuciformis [49]. These emerged as exclusive urban taxonomic biomarkers (LDA score >4, DESeq2 adjusted $p < 0.001$; $\log_2$ fold change −3.9 to −3.3). Previous research has associated the Prevotella pallens group [50] and L. wadei with halitosis, the latter showing elevated abundance in tongue coatings from halitosis patients compared to healthy controls [51]. High levels of P. histicola, P. melaninogenica, certain Veillonella spp., and Streptococcus spp. have also been correlated with poorer oral health outcomes [49]. Furthermore, Megasphaera spp., including M. micronuciformis, are increased in periodontitis [52]. Notably, studies on salivary microbiomes have linked Prevotella spp. (including P. histicola) to caries-active states in both adults and children with early childhood caries [53, 54]. Collectively, evidence indicates that Prevotella may exacerbate periodontitis by promoting Th17-mediated neutrophil recruitment. This is supported by findings in systemic inflammatory conditions, such as rheumatoid arthritis (RA), where patients without periodontitis exhibit enrichment of periodontitis-associated Prevotella species (e.g., P. melaninogenica, P. denticola, P. histicola, P. nigrescens, P. oulorum, and P. maculosa) [55]. Elevated inflammatory mediators in periodontal tissues of individuals with RA and related diseases likely influence disease progression [56]. Thus, disruptions in local or systemic inflammation can impair microbial homeostasis, enhancing bacterial pathogenicity and susceptibility to periodontal disease [55].

The marked enrichment of Prevotella in urban women—despite the absence of clinical periodontitis—highlights its potential role in microbiome perturbation. The presence of these taxa, combined with unassessed underlying systemic conditions, may predispose individuals to future oral diseases.

Intriguingly, these dysbiotic taxa showed no significant association with individual OHI-S scores. This suggests that geographic differences in oral microbiome composition are largely independent of personal oral hygiene practices, even though urban women displayed better OHI-S scores overall. Such disparities may stem from uneven distribution and access to oral health services, with rural residents often facing limited education on oral care and fewer facilities [57-59]. Another contributing factor in rural populations could be the traditional practice of betel leaf chewing, rooted in beliefs that it strengthens teeth, freshens breath, relieves stress, and provides pleasure akin to smoking [59, 60]. However, no scientific evidence supports these benefits; instead, betel chewing is associated with increased risks of periodontal disease, premalignant lesions, tooth abrasion, and fractures [57, 61]. This habit persists in rural communities due to cultural norms, social influences, and inadequate dental health education and counseling.

Future investigations should explore correlations between these microbial genera, better chewing practices, and halitosis severity [62]. Overall, these findings support the hypothesis that distinct cultural practices, socioeconomic factors, and dietary habits drive the observed geographic variations in oral microbiome profiles.

Furthermore, interpreting taxon abundance in oral microbiomes from distinct geographic locations requires consideration of community-wide ecological interactions. Co-occurrence networks reveal tendencies for species to associate positively or negatively within specific niches. A key advantage of such analyses is the identification of hub or keystone OTUs—highly connected taxa that disproportionately influence microbiome structure [63]. In our study, keystone OTUs from the core microbiome (≤16% relative abundance) exhibited stronger positive co-occurrence patterns, particularly involving differentially abundant genera like Leptotrichia. This suggests that these keystone taxa may critically affect overall microbiome function independent of their abundance [64], reflecting shared environmental preferences across rural and urban settings. In contrast, dominant core genera displayed more negative correlations, indicating niche-specific competitive interactions unique to each location.

Comparisons of co-occurrence networks across studies should be approached cautiously due to limited existing research on geographic influences on the oral microbiome and variability in methodologies, such as

differing correlation thresholds [65] or definitions of keystone taxa [64].

The tongue-derived microbiomes in both groups were dominated by eight genera, with Streptococcus and Prevotella emerging as the most prominent. The salivary microbiome is known to largely reflect tongue biofilm communities. The papillate dorsal tongue surface typically harbors anaerobe-enriched populations (e.g., Prevotella and Veillonella), while the ventral surface favors aerotolerant taxa like Streptococcus and Gemella [66]. Consistent with this, tongue biofilms are frequently dominated by Prevotella and Streptococcus [2], and three genera—Prevotella, Streptococcus, and Veillonella—ranked among the top four most abundant in both our urban and rural cohorts.

This study has several limitations. First, only healthy individuals were included. Second, convenience sampling was employed in this pilot investigation, resulting in a small sample size insufficient for definitive conclusions. Third, larger future studies should incorporate both genders, detailed dietary assessments, and broader socioeconomic variables.

Conclusion

Despite these limitations, this pilot study offers the first characterization of geographic influences on the oral microbiome among Indonesian women in urban and rural settings. We observed distinct, location-specific microbial patterns in female participants. Co-occurrence analyses revealed unique interaction networks within dominant core microbiomes across the two populations. Biomarker identification highlighted several taxa associated with poorer oral health in both groups, with certain species potentially contributing to oral dysbiosis independently of individual hygiene status. Urban-enriched biomarkers, in particular, may have clinical implications. These differences are likely driven by region-specific dietary practices, cultural traditions, and socioeconomic factors. Larger-scale studies are warranted to validate and expand upon these preliminary findings.

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