



## Root Caries Diagnosis By Metagenomic Approach - An Untouched Page

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### Abstract

Root caries is a distinct type of dental decay that damages the roots of teeth. Unlike traditional dental caries, which typically involve the enamel and dentin, root caries occurs when the roots of teeth become exposed due to receding gingival, periodontal disease or other predisposing factors. This can leave the softer, more vulnerable root tissue exposed to bacteria and acid, leading to decay and eventual tooth loss if left untreated. Sumney et al. defined root caries as “a cavitation below the cemento-enamel junction, not usually involving the adjacent enamel, typically discoloured, softened, ill-defined, and involving both cementum and underlying dentine.”<sup>1</sup> The oral microbiota differ among various geographic regions and ethnicities. However few studies in literature have evaluated the risk factors and microbial host factors associated with root caries in the Indian population. According to a study by Maklennan et al, 2025, prevalence of root caries in India is 66.7%.<sup>2</sup> Root caries is qualitatively and microbiologically different from coronal caries.

**Keywords:** caries, prevalence, metagenomics, molecular analysis

### Introduction

In 2013, a study conducted by Ashok Aparna et al reported that *Streptococcus mutans*, *Lactobacillus* and *Actinomyces* sp. were higher in the older group (55-75 years) as compared to middle-aged group (35-44 years) in South Indian population with *Streptococcus mutans* being the highest in number followed by *Lactobacillus* and *Actinomyces* spp. respectively. Also, there is a significant association between the age group and the presence of *Lactobacillus*.<sup>3</sup>

In 2017, a study conducted by Mithra Hegde et al concluded that with the increase in age of population, more the teeth retained and higher the prevalence of root surface caries due to various factors such as untreated caries, diet, oral hygiene maintenance and systemic diseases.<sup>4</sup> A total of 660 patients with

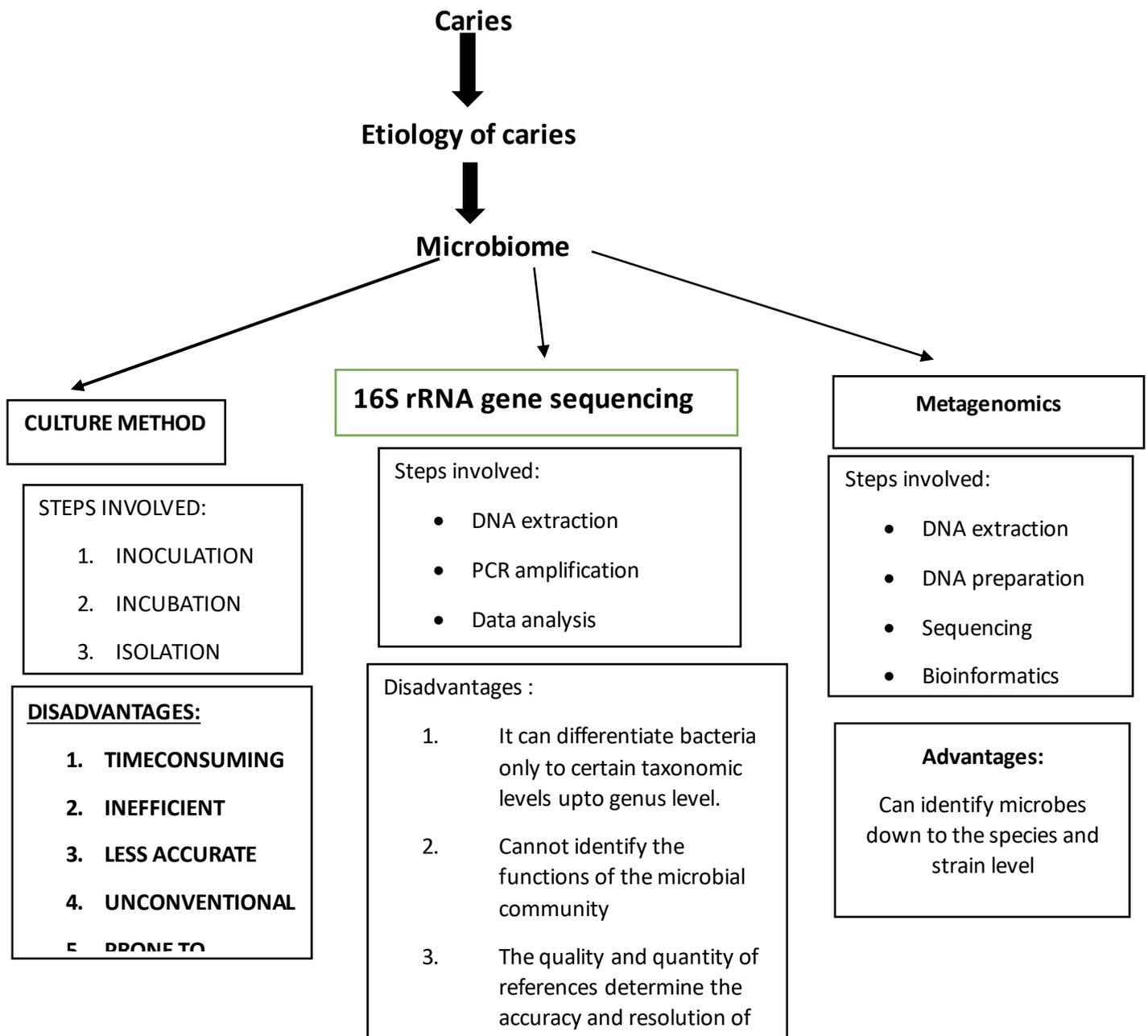
gingival recession which were included revealed root surface caries. Males exhibited higher root surface caries 11% than females in the age group of 56 to 65 years of age (36.36%). Intraoral distribution assessment of root surface caries revealed that the molars were most frequently infected with the buccal surface showing highest presence 32.92%. A correlation between root surface caries and diet, brushing habit and method was also observed.

In 2018, a study conducted by Jyothi Shetty et al reported that three out of ten persons in India may be affected by root caries, which is a serious health issue. To enhance the quality of life for the aged population, oral health policies and preventive actions should concentrate on this issue.<sup>5</sup>

In addition to the obvious topographical difference the composition and structure of root caries are different from those of enamel. The mineral content of the root surface is lower and the crystal size is substantially smaller than that of enamel, thus root surfaces have greater solubility than coronal surfaces leading to pulpal involvement and pain at shorter time duration.

Bacteria being the most predominant species, is significant in etiology of the diseases. Studies based on molecular ecology where 16S RNA was amplified from plaque samples revealed more than 600 bacterial species of which less than 50% are culturable. Members of this microflora are also participants in common oral cavity diseases like caries and periodontitis. Proper identification of the microflora is the utmost requirement to treat the disease properly.

**Different methods used to study the profile of oral microbiome:**



**Root Caries Can Be Assessed By:**

1. Screening of root caries by clinical examination
2. Salivary analysis of root caries active subjects
3. Molecular analysis of microbial species associated with root caries

### What Is Metagenomics?

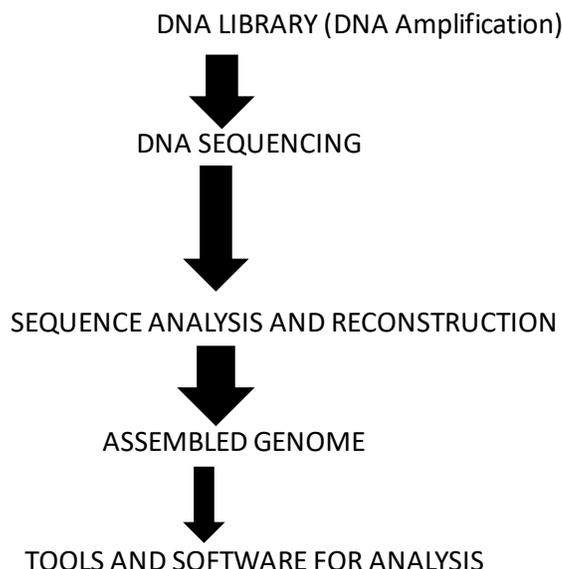
Metagenomics analysis is a technique to isolate, observe and study microbial DNA present in any environmental or biological sample and assess microbial diversity and functional variability within the existing microbial community. The term metagenomic analysis was coined by Handelsman and Goodman et al in 1988 marking the beginning of a new era in genetics.<sup>6</sup>

### Steps Involved In Metagenomics Of The Oral Flora:

1. Sample collection: Samples are collected from saliva, dental plaque or caries.
2. DNA extraction: In this, the microbes break open to release their DNA.
3. DNA sequencing: Here the sequencing machines (illumina) is used to read the DNA code of all microbes in the sample.
4. Quality check and filtering: Here the poor quality data or human DNA is removed.
5. Bio-informatic analysis: The software tools are used to group DNA sequences and identify which microbes are present. Then the sequences are compared to the databases.
6. Functional analysis: Here what microbes can do is evaluated. Functional potential of microbial community is assured.
7. Interpretation and visualisation: Charts or graphs are created to show which microbes are common or unique. Conclusions are drawn regarding the oral health or disease.

### Metagenomics Of Oral Flora Working Principle:

Sample is collected. DNA is obtained from the sample. The nucleic acid is wrapped in nuclear envelope in the cell. The DNA is extracted from the cell after lysis of the cell wall or cell membrane.



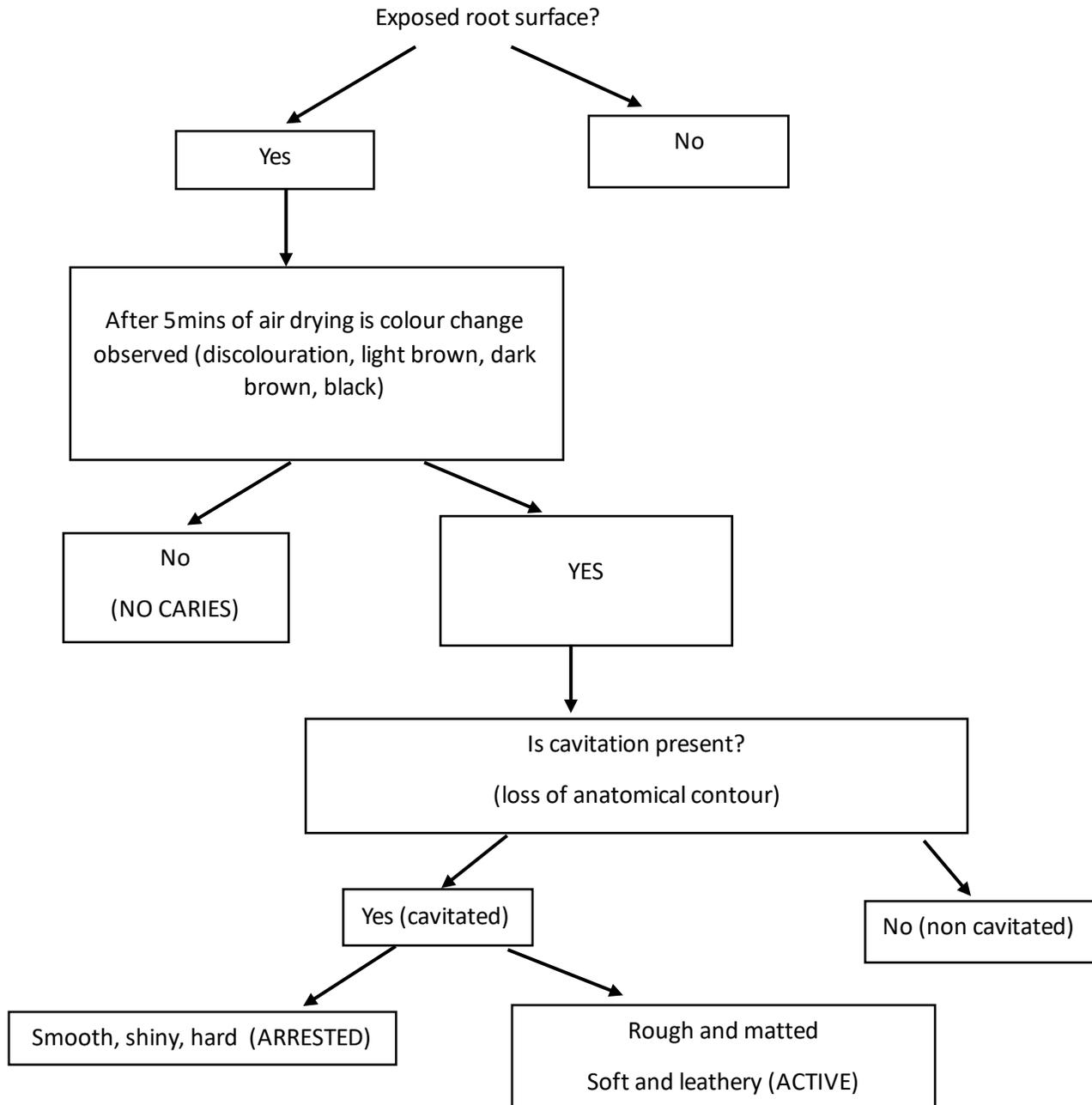
Advantages of the metagenomics:

1. Speed
2. Accuracy
3. Precise
4. Many microorganisms can be studied in a single experiment. Vast microbial community can be included.

By metagenomics we can assess:

1. The bacterial diversity in root caries.
2. The association of specific bacterial species or bacterial species or bacterial communities with healthy and carious roots through this approach.

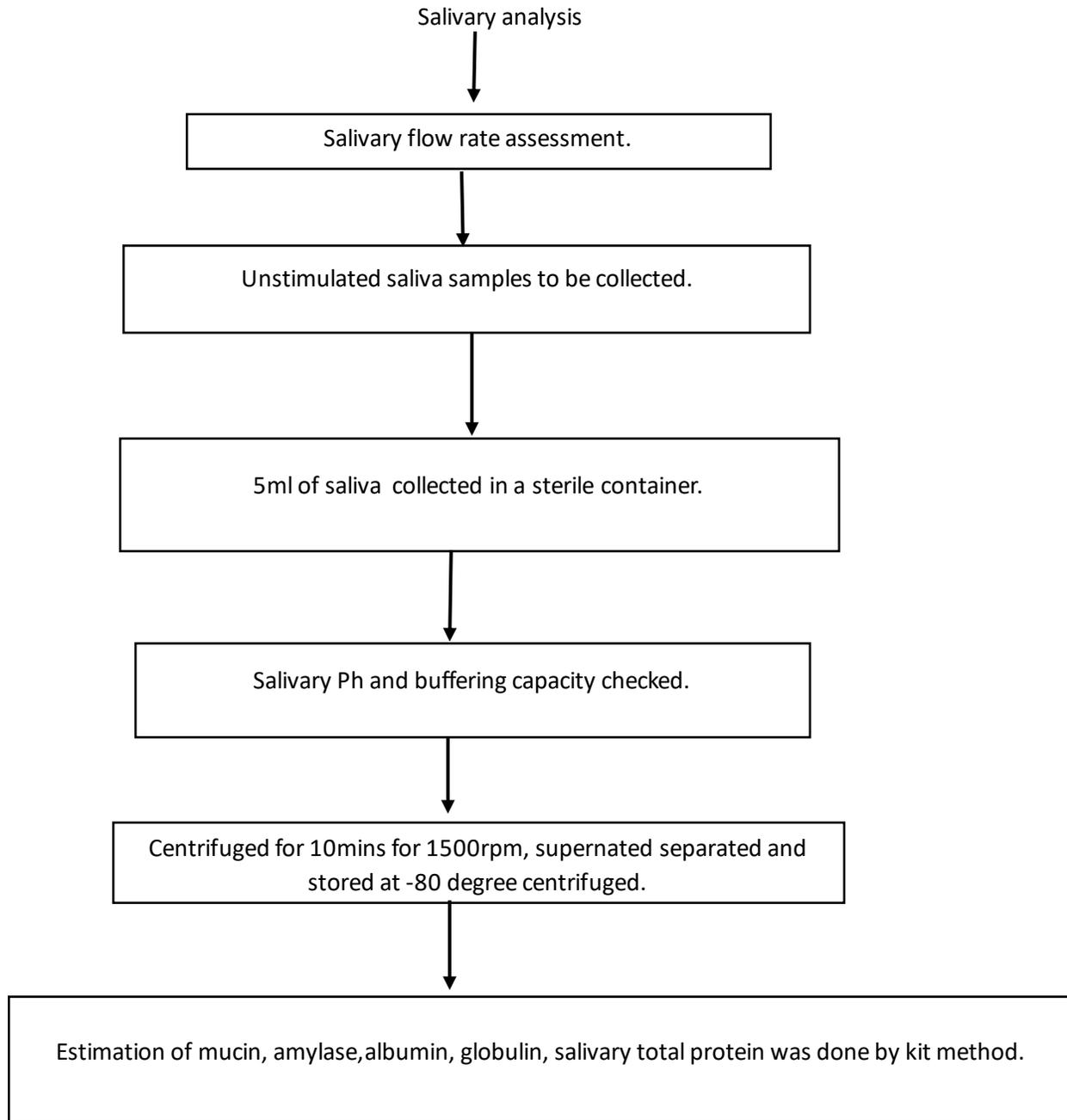
**Clinical diagnosis of root caries using international caries classification and management system(ICCMS)7:**



Based on the results of the clinical examination:

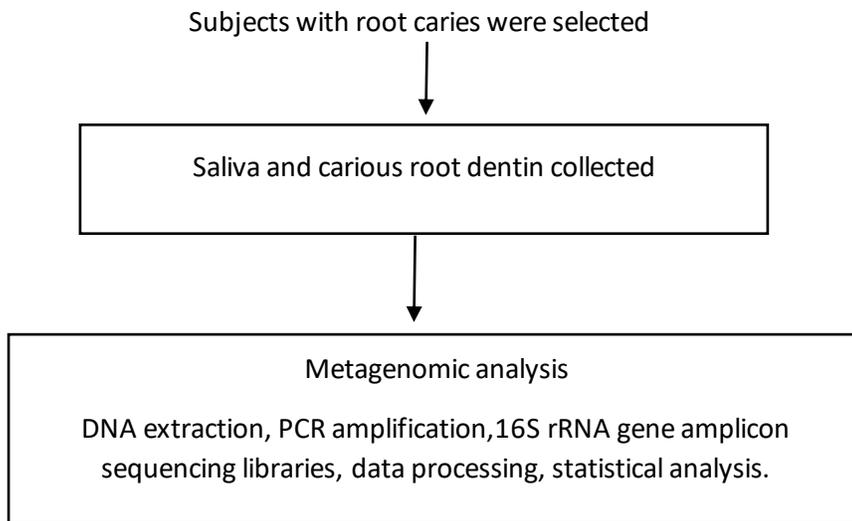
The prevalence of root caries was found to be 7.5% and the root caries index is 17.66% according to Jyothi Shetty et al. multiple logistic regression analysis showed an association between the variables age, exposed root surfaces and systemic conditions.

**Salivary analysis for detection of root caries:**



The study by Jyothi Shetty et al demonstrated that reduced salivary flow and buffer capacity along with lower albumin levels and high mucin-1 concentrations are present in individuals with root caries.<sup>8</sup>

**Molecular analysis of microbial species associated with root caries**



Predominant microflora in root caries:

If the sample is extracted from caries or from saliva the microbial load also varies.

ROOT CARIES SAMPLE	SALIVA SAMPLE
Prevotella	Streptococcus
Streptococcus	Lactobacillus
Lactobacillus	Prevotella
Actinomyces	Bifidobacterium
Fusobacterium	Fusobacterium
Oisenella	Neisseria
Bifidibacterium	Rothia
Pseudomonus	Enterococcus

Predominant microflora in coronal sample:

If the sample is extracted from caries or from saliva the microbial load also varies

Coronal caries sample	Saliva sample
Streptococcus	Prevotella
Oisenella	Fusobacterium
Actinomyces	Leptotrichia
Lactobacillus	Staphylococcus
Parascardovia	Veillonella
Cornebacterium	Lactobacillus
Neisseria	Neisseria

Sulphuro spirillum

There is a significant variation between certain samples, reinforcing the concept that oral microbiota composition changes with caries progression. These observations highlight microbial diversity differences between oral health conditions which could be useful in identifying biomarkers for early caries detection and targeted interventions. These charts show the differences between the microflora associated with root caries and coronal caries which may be relevant for understanding disease specific microbial signatures.

Metagenomic analysis identified Prevotella as the main predominant bacterial genus in root caries along with other bacterial species. Variation in the microbial profile of the clinical presentation of root caries, its prevalence and risk indicators could be associated with the change in lifestyle or oral hygiene practices and host defence mechanism.

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