

# PEDIATRIC ORAL MICROBIOME AND ITS EFFECT ON GASTRO-INTESTINAL SYMPTOMS

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**Abstract – Objective:** This pilot study assessed whether there was any relationship between the oral microbiome and its effect on gastrointestinal symptoms in children.

**Materials and Methods:** Thirty-six children aged 8 to 12 years were assigned to the following groups: 1) control group with no gastrointestinal symptoms, and 2) experimental group with gastrointestinal symptoms. Gastrointestinal symptoms were assessed using a questionnaire completed by the parents. Dental plaque was collected from the maxillary right first molar region. DNA was extracted, and the samples were sent for 16S sequencing. Data were evaluated by the Mann-Whitney U test.

**Results:** The comparison between both groups in terms of composition of microbes revealed no significant differences. However, the number of *Fusobacterium* was significant in the experimental group ( $p=0.03$ ).

**Conclusions:** Our preliminary findings indicated no strong differential abundance between the groups, except for *Fusobacterium*, which showed a significant increase in the experimental group with reported gastrointestinal symptoms.

**Keywords:** Gastrointestinal symptoms, Plaque, Oral microbiome, Children.

## INTRODUCTION

The human gastrointestinal tract is one of the major host-environment interactions and hosts a highly varied microbial population collectively known as the gut microbiome<sup>1</sup>. This complex ecosystem includes bacteria, archaea, viruses, and eukaryotic microorganisms that contribute to host metabolism, immunological regulation, and maintenance of intestinal homeostasis<sup>2</sup>. Several variables impact the gut microbiome's composition and function, including host genetics, diet, environmental exposures, and microbial interactions<sup>3</sup>.

The oral microbiome is one of the most diverse microbial communities in the human body and consists of more than 700 bacterial species colonizing oral niches such as saliva, mucosal surfaces, and dental plaque biofilms<sup>4</sup>. As the first entry point to the digestive system, the oral cavity serves as a significant source of microorganisms introduced into the gastrointestinal tract through chewing



and swallowing<sup>5</sup>. It has been estimated that approximately  $10^{11}$  bacterial cells are swallowed daily with saliva, suggesting a continuous microbial flow from the oral cavity to the gastrointestinal tract<sup>4</sup>.

Increasing evidence highlights the concept of the oral-gut axis, which refers to the dynamic ecological and functional interactions between microbial communities in the oral cavity and the intestine<sup>6</sup>. Oral bacteria may translocate to the gastrointestinal tract through saliva and, under conditions of microbial imbalance or impaired host defenses, may contribute to intestinal microbial dysbiosis<sup>6</sup>. Several oral taxa, including *Streptococcus*, *Fusobacterium*, and *Prevotella*, have been identified in the intestinal microbiome and have been associated with inflammatory and metabolic diseases<sup>7</sup>.

These interactions may be particularly relevant during childhood, when microbial communities are still developing and stabilizing<sup>8</sup>. Early-life microbiome establishment plays a critical role in shaping immune system maturation, metabolic pathways, and gastrointestinal physiology<sup>8</sup>. Disruptions in microbial composition during early life have been associated with gastrointestinal disorders such as functional abdominal pain, diarrhea, and other pediatric gastrointestinal symptoms<sup>9</sup>. Despite increasing evidence of interactions between the oral and intestinal microbiota, the relationship between the pediatric oral microbiome and gastrointestinal symptoms remains poorly characterized. Understanding these interactions may provide insights into microbial mechanisms underlying gastrointestinal symptom development and may contribute to the identification of potential microbial biomarkers and therapeutic targets.

## Aims and Objectives of the Study

The objective of this study is to correlate the oral bacterial strains with self-reported gastrointestinal symptoms among children at Rutgers School of Dental Medicine. The use of this prospective pilot study model would help direct future care and collaboration in the pediatric population. This data would promote multidisciplinary collaboration across Rutgers Health when assessing patient health and elucidate the vital role of the oral microbiome in the maintenance of health and homeostasis in the pediatric population. This study would lead to innovative pathways for not only the collaboration of clinicians and scientists but also studying ways to help direct better health practices in the community and innovation in the treatment of global health issues by assessing the oral microbiome.

The aims of this study were:

1. to study bacteria in the pediatric oral microbiome of patients who receive treatment at Rutgers School of Dental Medicine,
2. to assess whether the bacterial strains have an influence on the reported gastrointestinal symptoms in pediatric patients,
3. to have substantial baseline data in a healthy pediatric population.

## MATERIALS AND METHODS

The present study was a prospective pilot study conducted in the Department of Pediatric Dentistry at Rutgers School of Dental Medicine, Newark, New Jersey. Children aged 8 to 12 years were selected from among patients visiting the Department of Pediatric Dentistry from December 2024 to February 2025 based on the selection criteria of this study. The study was approved by the Rutgers School of Dental Medicine, Rutgers University, Newark, New Jersey, USA, Ethical Committee with protocol number Pro2024001210 and date November 6<sup>th</sup>, 2024. Written informed consent was obtained from parents or legal guardians, and assent was obtained from all participating children prior to enrollment in the study.

The study team worked daily in this department and provided dental care for these patients. The study was explained to the parent and the child in a semi-private operatory. A thorough medical history of the patients was obtained, and the parents and patients were asked about their willingness to participate.

The inclusion criteria were: 1) healthy children, 2) age between 8 and 12 years, and 3) routine dental appointments.

The exclusion criteria were: 1) medically compromised children, 2) presence of subgingival plaque, 3) presence of calculus or radiographic evidence of bone loss, 4) emergency dental visits, 5) history of feeding disorders or acid reflux, 6) use of long-term medications or antibiotic therapy within the past 3 months.

## Study Design

The selected patients were divided into 2 groups:

- Group 1: control group without gastrointestinal symptoms – 18 patients.
- Group 2: experimental group with gastrointestinal symptoms – 18 patients.

## Procedure

The protocol was explained verbally, and time was given for parents and subjects to ask questions. The process was only proceeded if the parents and patient were comfortable. If they refused, all treatment was continued as normal. But if they agreed, the parents were asked to sign the informed consent form, and the child was asked to sign the assent form in their preferred language (English or Spanish). The patient's parents also completed a survey regarding gastrointestinal symptoms validated by Walker, Caplan-Dover, and Rasquin-Weber in 2000 in its original validated form<sup>10</sup>. The child was assigned to the experimental group despite one self-reported GI symptom.

After consent and assent, the participant underwent a dental examination, and plaque samples were collected from the maxillary right first molar area using a swab only. The samples were then transported on ice and stored in the Oral Biology Department at Rutgers School of Dental Medicine in a freezer at -80°C. The samples were labeled with a de-identified number. The samples were then processed for DNA extraction and sent to the SeqCoast Genomics lab (Portsmouth, New Hampshire, USA) for 16S sequencing to evaluate potential bacterial genera.

There were no risks beyond those associated with a routine dental examination. Breach of confidentiality was managed by de-identification and data security measures. Unique identification numbers were assigned to each patient, and a tracking form was used to ensure that all data were complete and properly recorded. All documents were stored in a locked cabinet. There were no expenses to the parent/patient other than those incurred for routine dental treatment.

## Statistical Analysis

All collected data were entered into an Excel sheet and subjected to statistical analysis. The parameters between the two groups were compared using the Mann-Whitney U test. Statistical significance was set at a  $p$ -value of  $< 0.05$ .

## RESULTS

The microbial profiles across all samples consisted exclusively of bacterial taxa, with no detectable presence of fungi, archaea, or other eukaryotic microorganisms (Figures 1-3). The overall composition of microbial communities was comparable between the control and experimental groups. The analysis demonstrated that *Fusobacteriota* species showed a statistically significantly higher abundance in the experimental group compared to the control group ( $p = 0.03$ ) (Table 1). The difference in abundance (experimental – control) for *Fusobacteriota* was 3837.43. Actinobacteria, Firmicutes, Bacteroidota, and Proteobacteria showed differences in abundance between the groups; however, these differences were not statistically significant ( $p > 0.05$ ). Microbial diversity, assessed using the Shannon index, was similar between the two groups. The mean Shannon index was  $5.99 \pm 0.85$  in the control group and  $6.02 \pm 0.97$  in the experimental group (Table 2). This difference was not statistically significant ( $p > 0.05$ ), indicating comparable levels of microbial richness and evenness between groups.

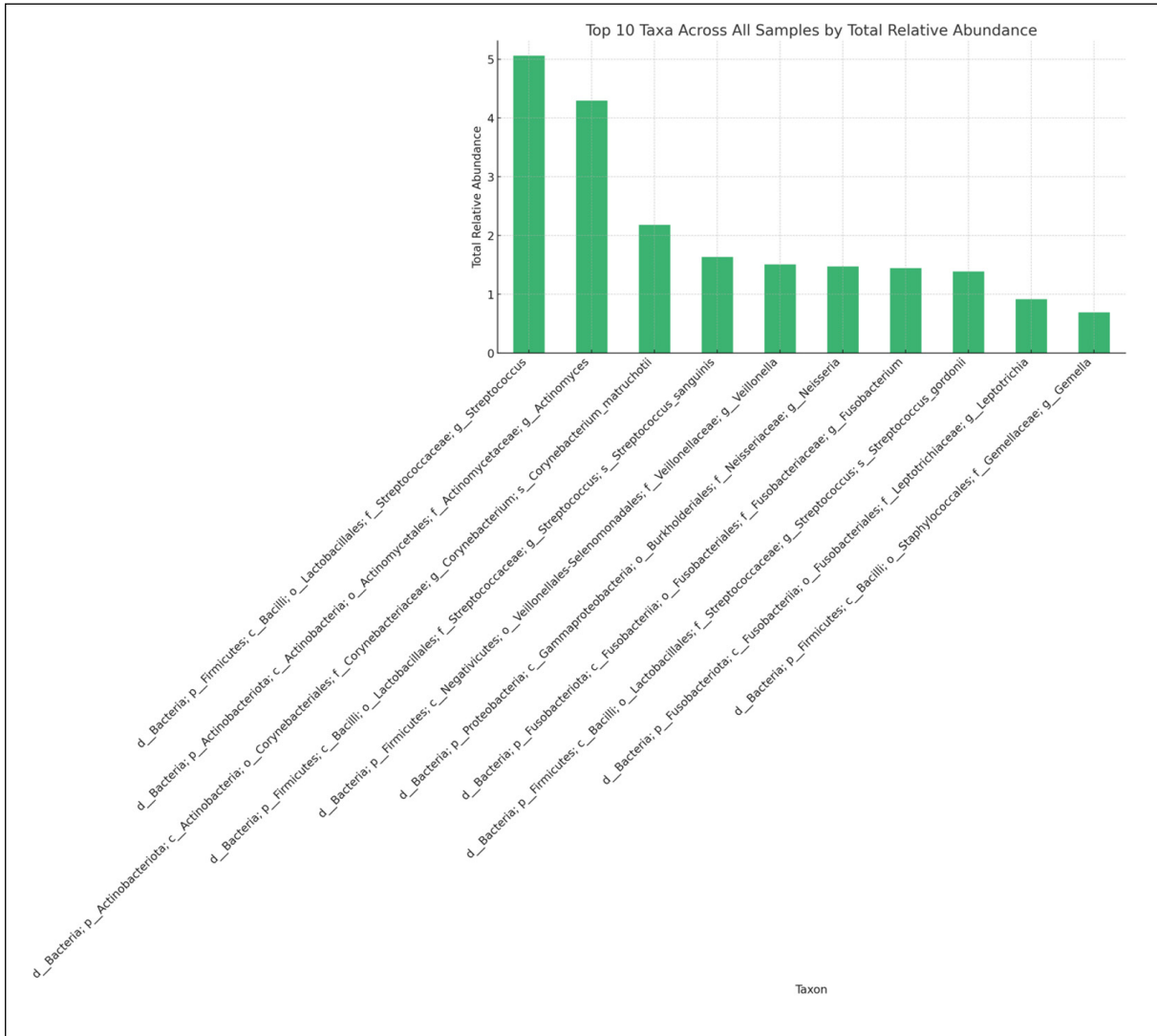


Figure 1. Top 10 taxa by relative abundance across all samples.

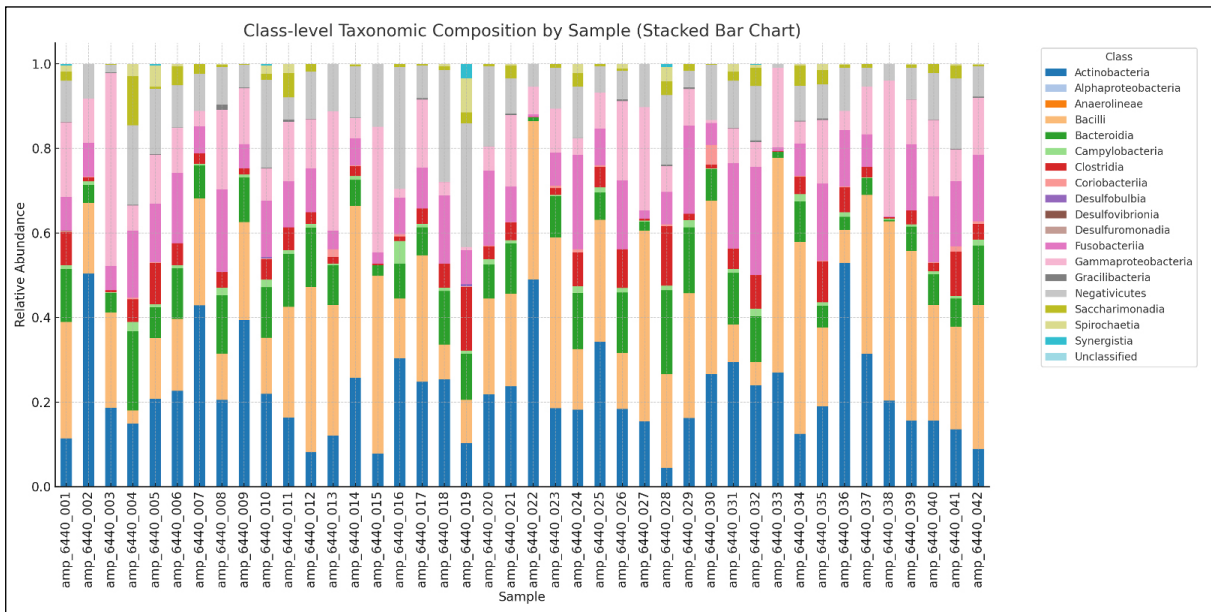


Figure 2. Class level composition as per samples.

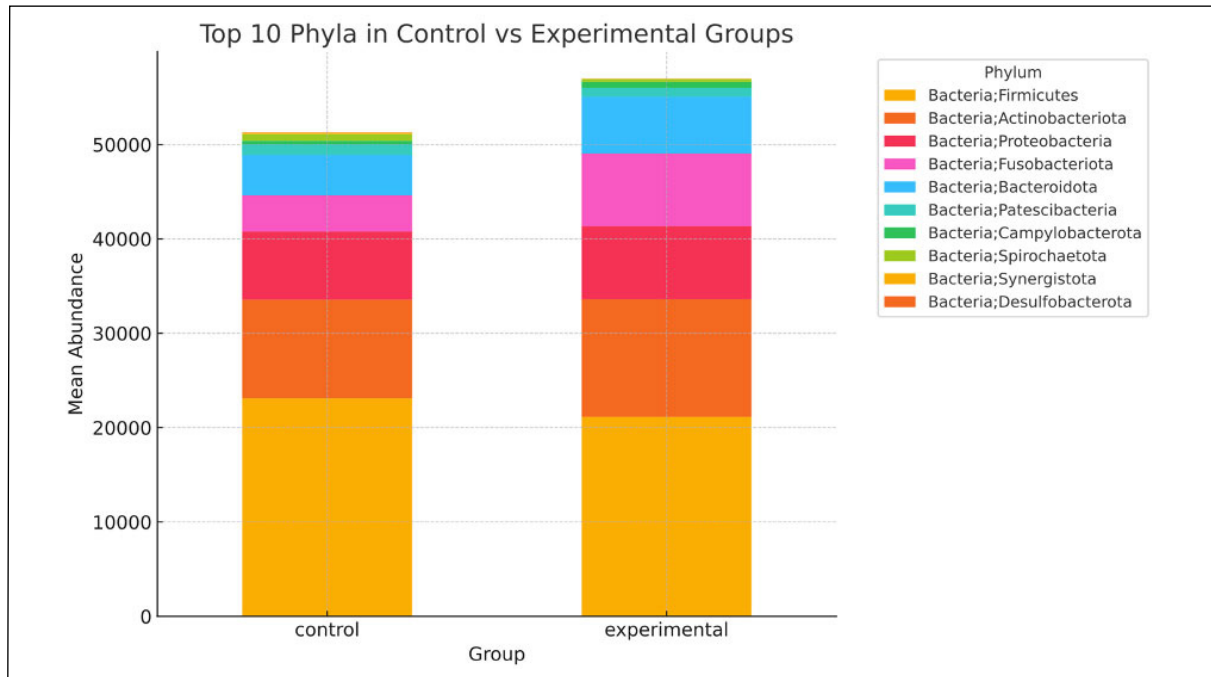


Figure 3. Comparison between both groups at the phylum level.

TABLE 1. THE TABLE SHOWS THE DIFFERENCE IN THE NUMBER OF BACTERIA BETWEEN THE TWO GROUPS. FUSOBACTERIOTA SHOWED A STATISTICALLY SIGNIFICANT DIFFERENCE BETWEEN CONTROL AND EXPERIMENTAL GROUPS ( $p \approx 0.03$ ).

Phylum	Control (Mean ± SD)	Experimental (Mean ± SD)
Fusobacteriota	0.149 ± 0.073	0.193 ± 0.045
Actinobacteriota	0.226 ± 0.111	0.229 ± 0.120
Firmicutes	0.395 ± 0.102	0.427 ± 0.107
Bacteroidota	0.098 ± 0.039	0.081 ± 0.054
Proteobacteria	0.235 ± 0.121	0.238 ± 0.128

TABLE 2. TABLE 2 SHOWS THE MEAN SHANNON VALUES OF BOTH GROUPS. SHANNON'S VALUES DESCRIBE BOTH THE RICHNESS AND EVENNESS OF SPECIES OF BACTERIA IN A GIVEN SAMPLE. THE HIGHER THE VALUE, THE HIGHER THE DIVERSITY OF THE SPECIES.

Group	Mean Shannon	Standard Deviation	Min	Max
Control	5.99	0.85	4.01	7.48
Experimental	6.02	0.97	3.33	7.33

$p > 0.05$ , not significant

## DISCUSSION

The present study demonstrated a significant difference in the abundance of *Fusobacterium* species between the control and experimental groups. Members of this genus are anaerobic gram-negative bacteria commonly found in the oral cavity, with *Fusobacterium nucleatum* and *Fusobacterium necrophorum* representing the most frequently isolated species<sup>11</sup>. These bacteria have been implicated in a variety of clinical conditions, including abscess formation, bacteremia, puerperal infections, and septic shock<sup>11</sup>.

Scholars<sup>12</sup> have demonstrated an association between *F. nucleatum* and colorectal cancer. Indeed, Komiya et al<sup>12</sup> identified genetically identical strains of *F. nucleatum* in both saliva and colorectal tumor tissues, suggesting that the oral cavity may serve as a potential reservoir for intestinal colonization by this bacterium. Similar findings were reported in metagenomic analyses demonstrating enrichment of *F. nucleatum* in colorectal tumor microbiota compared with healthy intestinal tissues<sup>7</sup>.

At a biological level, *F. nucleatum* is capable of adhering to intestinal epithelial cells through the Fap2 adhesin, which binds to the tumor-associated carbohydrate Gal-GalNAc expressed on colorectal tumor cells<sup>13</sup>. In addition, the FadA adhesin has been shown to activate the Wnt/ $\beta$ -catenin signaling pathway, thereby promoting tumor cell proliferation and inflammatory responses within the intestinal microenvironment<sup>14</sup>.

Consistent with our findings, previous microbiome studies have reported that *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* are dominant bacterial phyla in both oral and intestinal microbial communities<sup>15</sup>. Analyses of salivary microbiota have shown that *Streptococcus* is typically the most abundant genus, followed by *Actinomyces odontolyticus* and *Prevotella melaninogenica*, which have been associated with host metabolic status and microbial ecological balance<sup>16</sup>. These findings support the similarity between oral and intestinal microbial ecosystems and are consistent with the microbial patterns observed in the present study.

## CONCLUSIONS

In this study, the microbial species were similar in both groups. There was no strong differential abundance between the groups, except for *Fusobacterium*, which showed significant abundance in the experimental group. The results of this study look promising.

## Limitations

This study has several limitations that should be considered when interpreting the findings. The sample size was relatively small, which may have limited our ability to detect subtle differences in microbial composition between the groups. In addition, dietary habits and environmental factors—both known to influence the oral and gut microbiome—were not fully controlled and may have affected the results. The study was also conducted at a single academic center, which may limit the applicability of these findings to other pediatric populations. Finally, it was not possible to determine cause-and-effect relationships or assess how changes in the oral microbiome may relate to gastrointestinal symptoms over time.

Future studies with larger and more diverse populations are needed to validate the observed associations between oral microbial composition and gastrointestinal symptoms. Longitudinal investigations may help clarify the temporal relationship between oral microbial changes and the development of gastrointestinal disease.

## Conflicts of Interest

The authors declare no conflicts of interest.

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## Authors' Contribution

Jaai Rane: Methodology, Writing – Original draft. Madhu Mohan: Conceptualization, Supervision, Writing – review and editing. Carla Cugini: Conceptualization, Supervision, Writing – review and editing, Data analysis.

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**Data Availability Statement**

The datasets used in this study are not publicly available, but can be obtained from the corresponding author upon reasonable request.

**Use of Artificial Intelligence (AI)**

No generative AI or AI-assisted technologies were used during the writing or development of this manuscript.

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