

A cross-sectional metagenomic analysis of the microbial ecology in symptomatic apical periodontitis – An *in vivo* study

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Abstract

Background: Symptomatic apical periodontitis (SAP) is a painful inflammatory disease driven by root canal infection. A detailed understanding of its microbial ecology, compared to a noninfectious baseline, is needed.

Aims: This study aimed to characterize the microbial ecology of SAP using 16S ribosomal (RNA) 16S rRNA metagenomic sequencing and compare it to control teeth undergoing root canal treatment after trauma.

Materials and Methods: This cross-sectional study included 10 patients with SAP and 10 control patients. Pulpal samples were collected aseptically. Microbial DNA was extracted, and the full-length 16S rRNA gene was sequenced through Oxford Nanopore Technology. Analysis was performed using QIIME2.

Statistical Analysis Used: Microbial abundances and diversity indices were compared using an independent samples *t*-test or Mann–Whitney *U*-test ($P < 0.05$ significant).

Results: The SAP microbiome was dysbiotic and enriched in anaerobes. *Veillonella parvula* was highly abundant in SAP (mean 13.1%) but absent in controls. Species like *Dialister pneumosintes* and *Prevotella melaninogenica* were found almost exclusively in SAP. Commensals including *Faecalibacterium prausnitzii* were significantly reduced.

Conclusion: SAP is associated with a distinct microbial signature defined by the enrichment of anaerobic pathobionts and a loss of commensals, revealing a polymicrobial, dysbiotic community.

Keywords: 16S ribosomal RNA; metagenomics; microbiota; periapical periodontitis

INTRODUCTION

Apical periodontitis (AP) is a prevalent inflammatory disease of the periapical tissues, primarily caused by microbial infection of the root canal system.^[1,2] It is a frequent

sequela of dental caries, pulp necrosis, or trauma and can be classified as symptomatic AP (SAP) or asymptomatic AP based on clinical presentation.^[3] SAP is characterized by spontaneous pain, tenderness to percussion, and radiographic evidence of a periapical radiolucency, often reflecting an acute inflammatory response.^[4]

The microbiota associated with AP is complex and polymicrobial, dominated by anaerobic bacteria.^[5] Traditional culture-based techniques have provided a foundational

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understanding but fail to capture the full diversity of these communities, particularly fastidious or unculturable species.^[6] While molecular methods such as polymerase chain reaction improved detection, they are limited by primer specificity.^[7] The advent of next-generation sequencing (NGS) has revolutionized this field, enabling comprehensive, culture-independent analysis and revealing unprecedented microbial diversity in endodontic infections.^[8,9]

A key concept in understanding these infections is microbial ecology, which emphasizes the complex interactions within the community and with the host, influencing both pathogenesis and treatment outcomes.^[10] The root canal, as a necrotic, low-oxygen environment, selects for a specialized anaerobic microbiome where certain “keystone” species can disproportionately drive dysbiosis and inflammation.^[11]

While NGS studies have advanced our knowledge, the application of long-read sequencing (e.g., Oxford Nanopore Technology) for high-resolution, species-level profiling of the SAP microbiome remains limited. A detailed characterization of the SAP microbiota is needed to identify the specific microbial drivers of acute symptoms. Therefore, this study aimed to investigate the microbial ecology of SAP in adult permanent teeth using Oxford Nanopore-based full-length 16S ribosomal RNA (16S rRNA) sequencing and compare it to a noninfectious control baseline. The scope of this work was to establish baseline data on the microbial diversity and dominant taxa involved in SAP.

MATERIALS AND METHODS

Study design

This was a cross-sectional *in vivo* clinical study.

Ethical considerations

The study protocol was reviewed and approved by the institutional ethics committee. Written informed consent was obtained from all participants prior to their enrollment.

Setting

The study was conducted in a private dental college and hospital. Patient recruitment and sample collection took place between June 2, 2025, and September 30, 2025.

Participants

A total of 20 adult patients were enrolled and divided into two groups:

- Group I (control, n = 10): Patients with permanent teeth indicated for intentional root canal treatment following acute trauma, representing a noninfectious, baseline state

- Group II (SAP, n = 10): Patients with permanent teeth clinically and radiographically diagnosed with SAP. Diagnosis was based on spontaneous pain, tenderness to percussion, and radiographic evidence of periapical radiolucency.

Inclusion criteria

Inclusion criteria were adult patients (aged ≥18 years), teeth meeting the above group criteria, and no antibiotic use within the past 3 months.

Exclusion criteria

Exclusion criteria included pediatric patients, systemic diseases (e.g., diabetes and immunocompromised conditions), previous endodontic treatment, endo-perio lesions, and pregnant or lactating women. All consecutive patients who met the inclusion criteria and provided informed consent were enrolled.

Variables

The primary outcome variables were the relative abundance and diversity of the root canal microbiome, measured through 16S rRNA gene sequencing. The primary exposure variables were the clinical disease state, categorized as either “control” (trauma) or “SAP.”

Data sources/measurement

Sample collection

Under strict asepsis, teeth were isolated with a rubber dam. The operative field was disinfected with 2.5% sodium hypochlorite. After access opening with a sterile bur (Dentsply Maillefer, Ballaigues, Switzerland), pulpal tissue remnants were collected using sterile broaches (Dentsply Maillefer, Ballaigues, Switzerland). The samples were transferred into sterile tubes containing lysis and binding and elution buffers provided in the UltraNucleo Genomic DNA Isolation Kit (Canvax Biotech S.L., Córdoba, Spain) and immediately stored at –80°C.

DNA sequencing and analysis

Genomic DNA was extracted from the clinical samples using the UltraNucleo Genomic DNA Isolation Kit (Canvax Biotech S.L., Córdoba, Spain) according to the manufacturer’s instructions. The full-length 16S rRNA gene (~1500 bp) was amplified using universal bacterial primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-ACG GCT ACC TTG TTA CGA CTT-3'). Library preparation was performed using the SQK-LSK114 ligation sequencing kit (Oxford Nanopore Technologies, Oxford, UK), and sequencing was carried out on a MinION Mk1C device (Oxford Nanopore Technologies). The resulting sequences were processed and analyzed using the QIIME2 pipeline for taxonomic classification.

Bias

To minimize bias and contamination, stringent aseptic protocols were followed during sample collection, including

rubber dam isolation and surface disinfection. To prevent selection bias, consecutive eligible patients were enrolled. To account for confounding, patients with recent antibiotic use or systemic conditions known to alter the microbiome were excluded. The use of a control group (trauma teeth) allowed for a direct comparison to a noninfectious baseline.

Study size

The sample size was calculated *a priori* using G*Power software (version 3.1.9.7 - developed by Heinrich-Heine University Düsseldorf, 2020). Based on an expected medium effect size ($d = 0.8$), with an alpha error of 0.05 and a power of 80%, a total sample size of 20 (10 per group) was determined to be sufficient for statistical comparisons.

Quantitative variables

The primary quantitative variables were the relative abundances of microbial taxa (from phylum to species level), expressed as percentages. Alpha diversity indices (e.g., Shannon and Chao1) were treated as continuous variables. These variables were derived directly from the QIIME2 output and were used in subsequent statistical analyses without further transformation or grouping.

Statistical methods

Statistical analysis was performed using IBM SPSS Statistics (Version 26.0, IBM Corp., Armonk, NY, USA). The normality of data distribution for continuous variables was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. For comparing microbial abundances and diversity indices between the two groups, the independent samples *t*-test was used for normally distributed data, and the Mann–Whitney *U*-test was used for nonnormally distributed data. $P < 0.05$ was considered statistically significant. No data were missing for the primary microbial analysis of the 20 included samples.

RESULTS

Participants

During the recruitment period, a total of 28 patients were

assessed for eligibility. Of these, eight were excluded (five did not meet the inclusion criteria, and three traumatized, declined to participate). A final cohort of 20 participants was enrolled and allocated equally into the two groups. All 20 participants completed the study, and all collected samples yielded sufficient DNA for sequencing and were included in the final analysis, resulting in no missing data.

Descriptive data

The study included 20 patients (13 males and 7 females) with a mean age of 40.1 years. Age-wise, two participants were aged 18–27 years (10%), seven were aged 28–37 years (35%), eight were aged 38–47 years (40%), and three were aged 48–57 years (15%).

Microbial composition and diversity

The most abundant classification in the control group was unclassified bacteria (mean –46.47%), followed by commensals such as *Faecalibacterium prausnitzii* (6.43%) and *Lautropia mirabilis* (8.18%) [Table 1]. In stark contrast, the SAP microbiome was characterized by a significant enrichment of the anaerobe *Veillonella parvula* (mean 13.1%), which was entirely absent in controls [Table 2]. Other taxa, such as *Dialister pneumosintes*, *Leptotrichia wadei*, and *Prevotella melaninogenica*, were exclusively or predominantly found in SAP samples. Several commensal species, including *Escherichia coli*, *Neisseria gonorrhoeae*, and *Streptococcus agalactiae*, were significantly reduced in the SAP group.

Statistical comparisons of key taxa

Multiple species showed statistically significant differences in abundance between the groups ($P < 0.05$). Species that were significantly enriched in SAP included *V. parvula*, *L. wadei*, *D. pneumosintes*, *Haemophilus parainfluenzae*, and several *Streptococcus* species. Species that were significantly depleted in SAP included *F. prausnitzii*, *N. gonorrhoeae*, and *Agathobacter rectalis*.

Community-level shifts

The dysbiotic shift was evident at higher taxonomic levels. SAP samples demonstrated a marked increase in the phylum Bacillota (Firmicutes), which surged to 43.46% compared to

Table 1: The abundance of bacterial species at species level in Group I (control)

Bacterial species	Control, mean±SD	SAP, mean±SD	P	Note
<i>Veillonella parvula</i>	0.0±0.0	13.1±2.5	<0.001	Exclusive to SAP
<i>Segatella copri</i>	5.4±2.1	10.9±1.8	0.003	Enriched in SAP
<i>Leptotrichia wadei</i>	0.0±0.0	3.5±0.9	<0.001	Exclusive to SAP
<i>Dialister pneumosintes</i>	0.0±0.0	0.5±0.3	0.012	Exclusive to SAP
<i>Prevotella melaninogenica</i>	0.0±0.0	0.8±0.4	0.008	Exclusive to SAP
<i>Haemophilus parainfluenzae</i>	0.4±0.6	3.3±2.1	0.005	Enriched in SAP
<i>Streptococcus thermophilus</i>	0.1±0.1	2.0±1.1	0.001	Enriched in SAP
<i>Faecalibacterium prausnitzii</i>	6.4±1.8	2.3±1.0	0.005	Depleted in SAP
<i>Lautropia mirabilis</i>	8.2±3.5	1.1±0.2	0.002	Depleted in SAP
<i>Neisseria gonorrhoeae</i>	4.4±1.9	0.5±0.3	0.001	Depleted in SAP
<i>Streptococcus agalactiae</i>	4.3±2.7	1.1±0.3	0.010	Depleted in SAP

SAP: Symptomatic apical periodontitis, SD: Standard deviation

Table 2: The abundance of bacterial species at species level in Group II (symptomatic apical periodontitis)

Taxonomic level/Group	Control, mean±SD	SAP, mean±SD	P
Phylum: Bacillota	8.8±3.2	43.5±5.1	<0.001
Phylum: Pseudomonadota	25.8±6.1	5.9±2.3	<0.001
Phylum: Fusobacteria	0.0±0.0	6.9±1.8	<0.001
Class: Negativicutes	3.0±1.5	37.9±4.7	<0.001

SAP: Symptomatic apical periodontitis, SD: Standard deviation

8.82% in controls. Concurrently, the phylum Pseudomonadota declined sharply from 25.81% in controls to 5.94% in SAP. The class Negativicutes was significantly increased in SAP (37.9%) compared to controls (2.98%). Fusobacteriota, absent in controls, emerged in SAP with a mean abundance of 6.89%.

DISCUSSION

This study used full-length 16S rRNA sequencing to provide a high-resolution taxonomic profile of the microbial ecology in SAP. Our findings strongly support the concept that SAP represents a polymicrobial dysbiosis, characterized by a significant shift from a diverse community in controls to one dominated by specific anaerobic pathobionts.

The control group's microbiota, though not sterile, included species like *Faecalibacterium prausnitzii*, a commensal with known anti-inflammatory properties,^[12] alongside other oral residents. This baseline diversity is consistent with other NGS studies of root canal microbiota.^[13,14] In stark contrast, the SAP microbiome was markedly enriched with opportunistic anaerobes. A dominant finding was the profound abundance of *V. parvula*, which was entirely absent in controls. *V. parvula* is a lactate-fermenter known for its role in early biofilm formation and metabolic synergy with other bacteria like streptococci, which can exacerbate virulence and sustain periapical inflammation.^[15]

Other taxa were exclusively or predominantly detected in SAP, underscoring a community-level shift. *D. pneumosintes*, detected only in SAP, is a recognized endodontic pathogen noted for its association with symptoms and resistance to intracanal medicaments.^[16] Similarly, the significant enrichment of *L. wadei* and *P. melaninogenica*, both linked to refractory infections and abscesses, highlights a consortium tailored for persistence in an inflamed, hypoxic environment.^[5,9] The detection of such a wide range of taxa, including *Actinomyces* and *Streptococcus* species, aligns with the complex communities reported in both primary and persistent endodontic infections.^[17,18] Concurrently, commensal species such as *L. mirabilis* were significantly depleted, completing the picture of a dysbiotic state defined by both pathogen enrichment and commensal loss.

This taxonomic shift implies significant functional consequences. The enriched anaerobic bacteria are

metabolically specialized for low-oxygen environments and often produce virulence factors such as proteases and short-chain fatty acids (e.g., butyrate and succinate) that can directly exacerbate tissue destruction and immune evasion.^[11,19] The co-detection of typically gut-associated species such as *Megasphaera elsdenii* and *Segatella hominis*, albeit in low abundance, hints at possible microbial translocation into the inflammatory root canal niche, a phenomenon warranting further investigation.^[20]

Our findings align with a modern ecological understanding of endodontic disease, moving beyond a focus on single pathogens like *Enterococcus faecalis*.^[21,22] While *E. faecalis* remains a significant pathogen in refractory cases, its role in primary symptomatic infections like those studied here may be less prominent, as demonstrated by its low abundance and the dominance of other taxa such as *Veillonella* and *Dialister*. Our results, powered by high-resolution sequencing, contribute to a more nuanced view of the pathogenic consortia in SAP.^[23] Instead, SAP appears to be driven by a dynamic, interactive microbial network. The consistent presence of organisms such as *D. pneumosintes* and *V. parvula* could serve as potential biomarkers for disease severity. It is important to note that our methodological choices define the scope of our findings. The 16S rRNA sequencing used here excels at taxonomic identification, especially with full-length reads for species-level accuracy, but it provides a functional profile of the community.^[24] For a true functional understanding, techniques like Metatranscriptomics, which sequence RNA to reveal actively expressed genes, are required.^[25]

This study has limitations. Its cross-sectional design provides only a single snapshot in time. Future research should adopt longitudinal designs and incorporate functional metagenomic approaches. This will help connect the microbial signatures we identified to their active role in disease progression and the host response.

CONCLUSION

This *in vivo* metagenomic analysis defines a distinct dysbiotic microbiome associated with SAP. The SAP community was characterized by a marked enrichment of anaerobic pathobionts and a significant loss of commensal species. *V. parvula* emerged as a dominant, potential keystone organism, entirely absent in controls. The exclusive presence of *D. pneumosintes* and significant enrichment of other inflammation-driving taxa such as *L. wadei* and *P. melaninogenica* highlight a pathogenic consortium optimized for persistence in the root canal environment. These findings confirm that SAP is a manifestation of a polymicrobial dysbiotic ecosystem, reinforcing the importance of microbial ecology in understanding endodontic disease pathogenesis.

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Conflicts of interest

There are no conflicts of interest.

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