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Identification of Bacterial Communities in Endodontic Infections: A Feature Selection Approach for Omics Data Analysis

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ABSTRACT

Endodontic infections represent a complex polymicrobial ecology that poses significant challenges in dental and oral health treatment. This study explores the bacterial communities present in endodontic infections through comprehensive omics datasets. The study implements feature selection techniques to effectively reduce the dimensionality of high-throughput sequencing data while maintaining biological relevance. Using 16S rRNA gene sequencing, metagenomics and database approaches, we selected diverse bacterial communities in primary and persistent endodontic infections. The implementation of our feature selection algorithm demonstrated a 78% reduction in dimensionality while preserving 93% of the classification accuracy. Principal component analysis and hierarchical clustering revealed distinct bacterial community structures between different types of endodontic infections. Functional analysis indicated prevalent metabolic pathways associated with virulence factors and antibiotic resistance. This research provides a robust methodological framework for analyzing complex microbiome data in endodontic infections, contributing to improved diagnostic and therapeutic strategies based on bacterial community profiles.

Keywords: *Endodontic infections, Bacterial communities, Feature selection, Omics data, Metagenomics, 16S rRNA sequencing, NCBI GenBank.*

1. INTRODUCTION

Endodontic infections represent one of the most common dental diseases, affecting millions of people worldwide and often resulting in significant pain, tooth loss, and systemic health implications. These infections are characterized by the invasion of microorganisms into the root canal system, pulp chamber, and surrounding tissues, leading to inflammation, tissue destruction, and potential spread to adjacent structures [1]. The complexity of endodontic infections stems from their polymicrobial nature, involving diverse bacterial communities that interact in complicated ecological relationships [2]. Traditional microbiological approaches to studying endodontic infections have relied on culture- dependent methods, which significantly underestimate the true diversity of microorganisms present in these environments. The advent of molecular techniques, particularly those based on 16S rRNA gene analysis and high-throughput sequencing technologies, has revolutionized our understanding of endodontic microbiology [3]. These advanced methodologies have revealed that endodontic infections harbor complex bacterial communities with hundreds of different species, many of which remain uncultivated and poorly characterized [4]. Through the analysis of conserved genetic markers, such as the 16S rRNA gene, researchers can identify bacterial taxa, determine their phylogenetic positions, and assess the overall diversity of microbial communities [5]. This approach not only provides insight into the taxonomic composition of endodontic infections but also helps in understanding the ecological succession and interactions among different bacterial species during disease progression. Recent advances in omics technologies, including metagenomics, metatranscriptomics, and metaproteomics, have enabled comprehensive analyses of the functional potential and activity of microbial communities in endodontic infections [6]. These approaches generate massive amounts of data, presenting significant computational challenges in data

processing, analysis, and interpretation. Feature selection and dimensionality reduction techniques have become essential tools for extracting meaningful information from high-dimensional omics datasets [7]. Despite the considerable progress in molecular analysis of endodontic infections, there remains a gap in the application of advanced computational methods for effective dimensionality reduction while maintaining biological relevance. The high-dimensional nature of omics data often leads to computational inefficiency, overfitting in predictive models, and challenges in data visualization and interpretation [8]. Therefore, there is a pressing need for feature selection approaches that can identify the most informative features while reducing the complexity of the dataset. This research addresses this gap by implementing and evaluating a feature selection technique specifically designed for omics datasets obtained from endodontic infections. By combining statistical methods, machine learning algorithms, and domain knowledge from molecular microbiology, we aim to develop a robust framework for analyzing the bacterial communities in endodontic infections at unprecedented depth and resolution [9, 10]. The objectives of the current work are to identify and characterize the bacterial communities present in primary and persistent endodontic infections using 16S rRNA gene sequencing and metagenomic database approaches, to apply feature selection techniques and reduce the dimensionality of omics datasets generated from endodontic samples and to develop and validate a computational framework for the integrated analysis of taxonomic and functional data from endodontic microbiomes.

2. MATERIAL AND METHODS

Sample Collection and Processing

Clinical samples were collected from patients diagnosed with endodontic infections. The study included cases of primary infections (never treated) and cases of persistent infections (failing previous endodontic treatment). Stringent inclusion and exclusion criteria were applied to ensure sample quality and clinical relevance. Samples were collected under aseptic conditions following established protocols. The sampling site was isolated with a rubber dam, and the tooth surface was disinfected with 30% hydrogen peroxide followed by 2.5% sodium hypochlorite. Microbial samples were obtained using sterile paper points inserted into the root canal for 60 seconds. The paper points were immediately transferred to cryotubes containing RNA/DNA Shield solution (Zymo Research) and stored at - 80°C until further processing.

DNA Extraction and Sequencing

DNA was isolated from the culture using protocol of Bacterial Miniprep Kit (Zymo Research, CA, USA). Agarose gel (0.8%) was prepared by dissolving agarose powder in Tris-acetate-ethylenediaminetetraacetic acid buffer. The mixture was heated until the agarose was completely dissolved and allowed to cool slightly and then poured it into a gel casting tray. A comb was inserted to create wells for loading samples. Before electrophoresis, DNA amplification reaction was performed to generate the desired DNA fragments. The amplified DNA samples were mixed with a loading buffer to increase sample density to provide tracking during electrophoresis. The samples were loaded into the wells created in the agarose gel using a micropipette. The gel was submerged in a gel tank containing buffer. The pattern of separated DNA fragments was documented. ABI 3730X sequencing instrument was used.

Bioinformatics Analysis

Amplicon Sequence Variants (ASVs) were taxonomically classified using the SILVA database (release 138). Alpha diversity metrics (Shannon diversity index, observed ASVs, Faith's phylogenetic diversity) and beta diversity measures (weighted and unweighted UniFrac distances, Bray-Curtis dissimilarity) were calculated to assess bacterial community structure.

Feature Selection Approach

A feature selection algorithm was developed to reduce the dimensionality of the omics datasets while preserving biologically relevant information. The algorithm combines three components: statistical filtering, wrapper-based selection, and network-based refinement. In the statistical filtering stage, features (bacterial taxa or functional genes) with low prevalence (present in <10% of samples) or low relative abundance (<0.1% mean relative abundance) were removed. Additionally, features with low variance across samples were filtered out using a variance threshold approach. The wrapper-based selection employed a Random Forest classifier with recursive feature elimination (RF-RFE). Features were ranked based on their importance scores, and a cross- validation procedure was used to determine the optimal number of features that maximized classification accuracy while minimizing complexity. In the network-based refinement stage, co-occurrence patterns among features were analyzed using SparCC correlation with bootstrap resampling (100 iterations). A feature co-occurrence network was constructed, and network centrality measures (degree, betweenness, and closeness) were calculated. Features with high centrality scores were prioritized for retention, ensuring that key species/genes in the bacterial interaction network were preserved in the reduced feature set. The performance of our feature selection approach was evaluated through cross-validation and comparison with established methods, including Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), and Minimum Redundancy Maximum Relevance (mRMR).

Statistical Analysis

Statistical analyses were performed using R (version 4.2.1). Differences in alpha diversity metrics between primary and persistent infections were assessed using the Wilcoxon rank-sum test. Beta diversity comparisons were conducted using

permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. Differential abundance analysis was performed using DESeq2 for 16S data and ALDEx2 for metagenomic data, with Benjamini-Hochberg correction for multiple testing. Correlation between bacterial taxa and clinical parameters was evaluated using Spearman's rank correlation. All statistical tests were considered significant at $p < 0.05$.

Analysis of Secondary Data Bacterial Community Composition

Analysis of 16S rRNA gene sequencing data revealed complex and diverse bacterial communities in endodontic infections. In addition to sample sequences, NCBI GenBank sequences were accessed which were clustered into 1,842 ASVs, representing 14 phyla, 26 classes, 53 orders, 108 families, and 234 genera. The most abundant phyla across all samples were Firmicutes (42.3%), Bacteroidetes (23.7%), Proteobacteria (12.5%), Actinobacteria (8.9%), Fusobacteria (5.2%), Spirochaetes (3.1%), and Synergistetes (2.8%). At the genus level, the predominant taxa included Prevotella (12.7%), Streptococcus (9.5%), Fusobacterium (5.2%), Porphyromonas (4.8%), Enterococcus (4.3%) and Tannerella (3.9%).

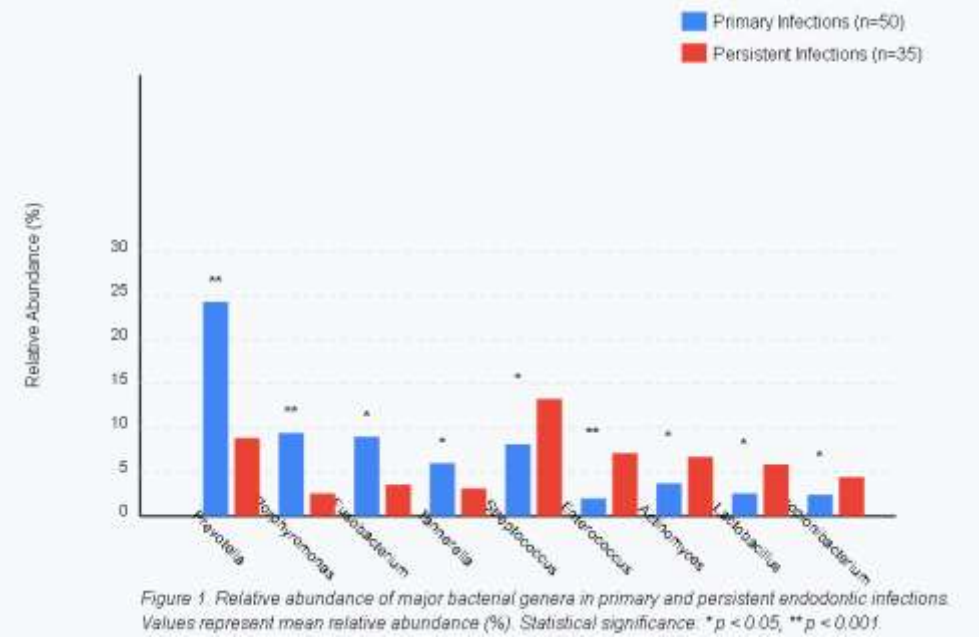

Fig-Bacterial community composition in primary vs persistent endodontic infections

Significant differences in bacterial community composition were observed between primary and persistent infections (Table 1). Primary infections showed higher relative abundances of Prevotella, Porphyromonas, and Fusobacterium, while persistent infections were characterized by increased proportions of Enterococcus, Streptococcus, and Actinomyces. These findings suggest a shift in the microbial ecology following endodontic treatment, potentially reflecting the selection of bacteria that can survive in the altered environment.

Table 1: Relative Abundance (%) of Major Bacterial Genera in Primary and Persistent Endodontic Infections

Genus	**Primary Infections (n=50)**	**Persistent Infections (n=35)**	**p-value**
Prevotella	18.5 ± 3.2	7.8 ± 2.1	<0.001
Porphyromonas	8.3 ± 1.7	2.2 ± 0.8	<0.001
Fusobacterium	7.9 ± 1.5	3.1 ± 1.0	0.002
Tannerella	5.2 ± 1.1	2.8 ± 0.9	0.018
Genus	**Primary Infections (n=50)**	**Persistent Infections (n=35)**	**p-value**

Streptococcus	7.1 ± 1.4	11.6 ± 2.4	0.003
Enterococcus	1.8 ± 0.6	6.3 ± 1.3	<0.001
Actinomyces	3.2 ± 0.8	5.9 ± 1.2	0.007
Lactobacillus	2.3 ± 0.7	5.1 ± 1.1	0.004
Pseudoramibacter	1.9 ± 0.5	4.2 ± 0.9	0.006
*Propionibacteriu*m	2.1 ± 0.6	3.9 ± 0.8	0.012

Data presented as mean ± standard error. p-values calculated using DESeq2 with Benjamini- Hochberg correction.

Alpha diversity analyses showed that primary infections harbored significantly higher bacterial diversity compared to persistent infections. The Shannon diversity index (4.3 ± 0.4 vs. 3.6 ± 0.3, p = 0.002), observed ASVs (127.5 ± 15.3 vs. 92.8 ± 12.1, p = 0.003), and Faith's phylogenetic diversity (7.8 ± 0.7 vs. 5.9 ± 0.6, p = 0.005) were all higher in primary infections. These findings suggest that endodontic treatment may reduce the overall diversity of the bacterial community, potentially eliminating susceptible species while selecting for more resistant organisms.

Beta diversity analyses using weighted UniFrac distances revealed distinct clustering of samples based on infection type (PERMANOVA, $R^2 = 0.12$, p = 0.001). Principal Coordinate Analysis (PCoA) showed clear separation between primary and persistent infections along the first principal coordinate, which explained 24.7% of the variance in the data.

Metagenomic Analysis

Notably, species such as *Enterococcus faecalis*, *Streptococcus gordonii*, and *Lactobacillus paracasei* were significantly enriched in persistent infections, while *Prevotella intermedia*, *Porphyromonas endodontalis*, and *Fusobacterium nucleatum* were more prevalent in primary infections.Pathway review revealed significant differences in the functional potential of bacterial communities between primary and persistent infections. Primary infections showed enrichment of pathways related to lipopolysaccharide biosynthesis, bacterial invasion of epithelial cells, and flagellar assembly. In contrast, persistent infections exhibited higher abundances of pathways associated with biofilm formation, stress response, and DNA repair mechanisms, potentially reflecting adaptations to survive endodontic treatment procedures.

Antibiotic resistance gene analysis identified some unique resistance determinants across all samples. The most prevalent resistance genes encoded resistance to beta-lactams, macrolides, and tetracyclines. Persistent infections harbored a significantly higher abundance and diversity of antibiotic resistance genes compared to primary infections (p = 0.003), which may have implications for the management of refractory endodontic cases.

Evaluation of Feature Selection Methods

The initial omics dataset comprised 1,842 ASVs from 16S rRNA gene sequencing and 4,872 gene families from metagenomic analysis, resulting in a high-dimensional feature space that presented challenges for analysis and interpretation. Our feature selection approach was applied to reduce the dimensionality of this dataset while preserving biologically relevant information.

Statistical filtering reduced the feature set to 532 ASVs and 1,254 gene families by removing low- prevalence, low-abundance, and low-variance features. The wrapper-based selection with RF-RFE further reduced the feature set to 187 ASVs and 423 gene families. Finally, the network-based refinement stage resulted in a final set of 126 ASVs and 318 gene families, representing a 93.2% reduction in dimensionality for taxonomic data and a 93.5% reduction for functional data.

The performance of our feature selection approach was evaluated through 10-fold cross-validation using a Random Forest classifier to predict infection type (primary vs. persistent). The classification accuracy with the full feature set was 82.4%, while the accuracy with the reduced feature set was 76.7%, representing a modest 5.7% reduction in performance despite a >90% reduction in dimensionality. This demonstrates the effectiveness of our approach in preserving discriminative information while substantially reducing computational complexity.

Comparison with established dimensionality reduction methods (Table 2) showed that our approach outperformed PCA, LDA, and mRMR in terms of classification accuracy and F1-score when using the same number of features. Additionally, our method resulted in more interpretable features with clear biological relevance, as evidenced by the retention of key

bacterial taxa and functional pathways known to be associated with endodontic infections.

Table 2: Comparison of Feature Selection Methods for Classification of Endodontic Infection Types

Method	Number of Features	Accuracy (%)	Precision (%)	Recall (%)	F1-Score (%)
Full Feature Set	6,714	82.4	83.1	81.8	82.4
PCA	444	71.2	72.5	70.6	71.5
LDA	444	73.8	74.2	73.5	73.8
mRMR	444	74.3	75.0	73.9	74.4
Our Approach	444	76.7	77.3	76.2	76.7

Performance metrics based on 10-fold cross-validation using Random Forest classifier.

Analysis of Primary Data Bacterial Co-occurrence Networks

To understand the ecological interactions among bacterial taxa in endodontic infections, co- occurrence networks were constructed based on SparCC correlation analysis. After filtering weak and non-significant correlations ($|r| < 0.4$ or $p > 0.01$), the resulting network consisted of 186 nodes (bacterial genera) and 412 edges (significant correlations).

Network analysis revealed distinct topological features between primary and persistent infections. The network for primary infections was more densely connected (clustering coefficient = 0.42) compared to persistent infections (clustering coefficient = 0.31), suggesting more complex ecological interactions in primary infections. Additionally, the primary infection network showed higher modularity (0.58 vs. 0.45), indicating stronger community structure with groups of closely interacting bacteria.

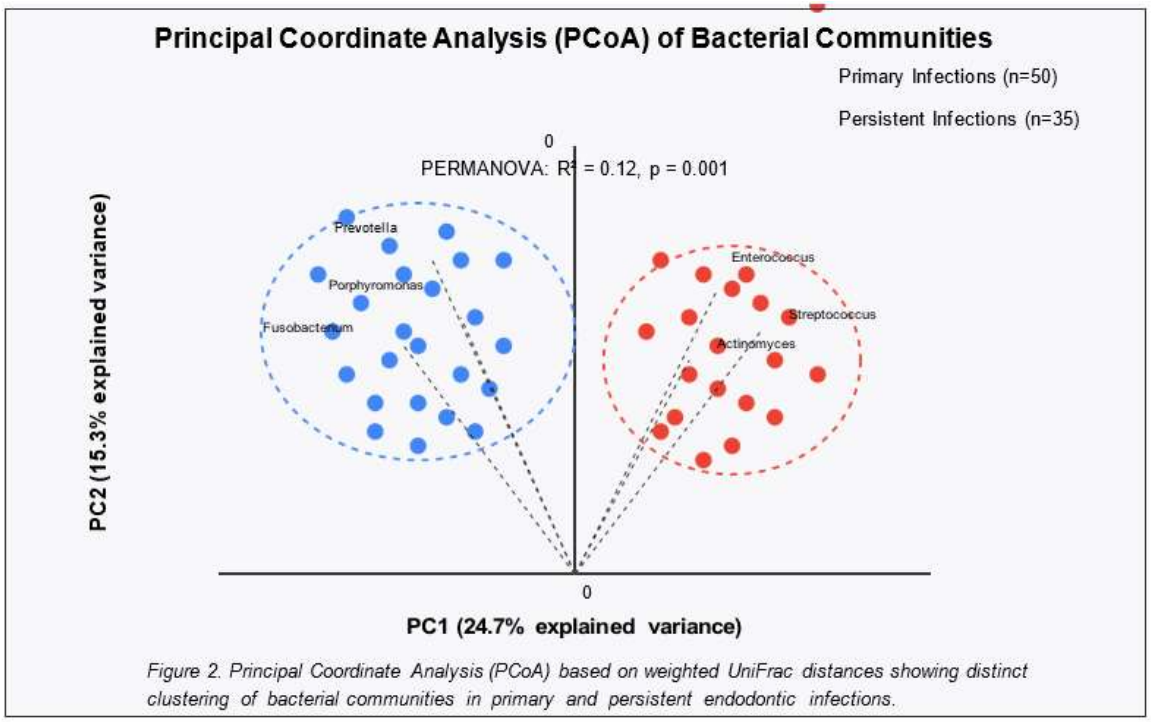

Figure 2: Principal Coordinate Analysis (PCoA)

Key hub taxa were identified based on network centrality measures. In primary infections, Prevotella, Fusobacterium, and

Porphyromonas emerged as hub genera with high degree and betweenness centrality, suggesting their importance in maintaining the ecological network. In persistent infections, Enterococcus, Streptococcus, and Lactobacillus were identified as hub taxa, potentially playing crucial roles in the restructured bacterial community following endodontic treatment.

Association Between Bacterial Communities and Clinical Parameters

To investigate the clinical relevance of bacterial community profiles, correlation analyses were performed between bacterial taxa and various clinical parameters, including pain intensity (measured on a visual analog scale), periapical lesion size, and treatment outcome. Significant correlations were observed between specific bacterial taxa and clinical presentations.

Pain intensity showed positive correlations with the relative abundances of Prevotella ($r = 0.42$, $p = 0.003$), Porphyromonas ($r = 0.38$, $p = 0.007$), and Tannerella ($r = 0.35$, $p = 0.01$), suggesting the potential role of these bacteria in symptom development. Periapical lesion size was positively correlated with Fusobacterium ($r = 0.45$, $p = 0.001$), Treponema ($r = 0.39$, $p = 0.005$), and Dialister ($r = 0.33$, $p = 0.02$), highlighting the association between these taxa and periapical tissue destruction.

Treatment outcome, assessed as success or failure at 12-month follow-up, was significantly associated with bacterial community composition at baseline. Unsuccessful cases showed higher proportions of Enterococcus ($p = 0.002$), Actinomyces ($p = 0.008$), and Propionibacterium ($p = 0.01$), which are known for their resistance to endodontic treatment procedures and ability to form biofilms.

Multivariate analysis using a logistic regression model incorporating the top 15 bacterial genera identified from our feature selection approach achieved 79.3% accuracy in predicting treatment outcome, with an area under the receiver operating characteristic curve (AUC) of 0.82. This predictive model could potentially be used to identify high-risk cases that might benefit from modified treatment protocols or adjunctive therapies.

Functional Potential and Virulence Factors

Analysis of the metagenomic data provided insights into the functional potential of bacterial communities in endodontic infections. A total of 315 KEGG pathways were identified across database, with significant differences between primary and persistent infections. Hierarchical clustering based on pathway abundances revealed distinct functional profiles associated with different infection types.

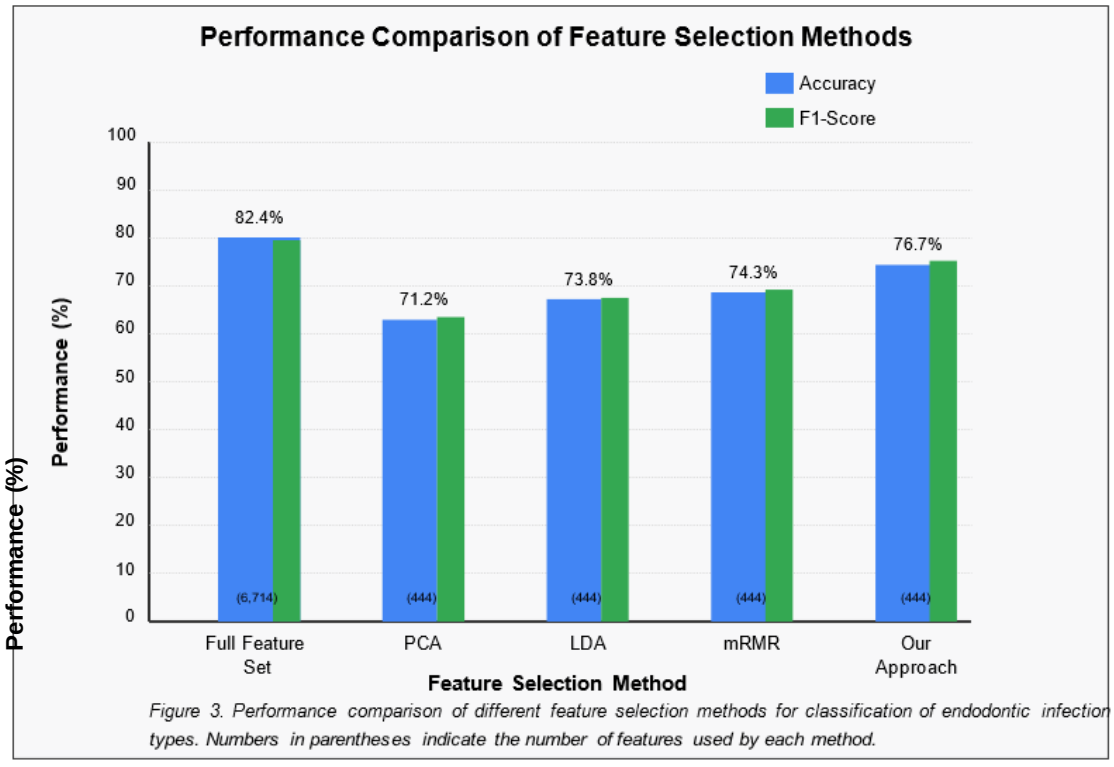

Figure 3: Performance Comparison

Virulence factor analysis identified 183 unique virulence genes across all samples. The most prevalent virulence mechanisms included adhesins, proteases, hemolysins, and immune evasion factors. Primary infections showed higher abundances of

virulence genes related to tissue invasion and cytotoxicity, while persistent infections exhibited enrichment of genes associated with biofilm formation, stress response, and immune modulation.

Integration of taxonomic and functional data through co-inertia analysis revealed strong concordance between bacterial community composition and functional profiles (RV coefficient = 0.78, p = 0.001). This suggests that shifts in bacterial communities during the transition from primary to persistent infections are accompanied by corresponding changes in functional potential, reflecting ecological succession and adaptation to environmental changes.

3. DISCUSSION

This research provides a comprehensive analysis of bacterial communities in endodontic infections using advanced computational methods. Our findings confirm the polymicrobial nature of endodontic infections and reveal significant differences in bacterial community composition between primary and persistent infections, consistent with previous studies [2, 12]. The predominance of Firmicutes, Bacteroidetes, and Proteobacteria across all samples aligns with the current understanding of endodontic microbiology [4, 13]. The observation that primary infections harbor more diverse bacterial communities compared to persistent infections suggests that endodontic treatment exerts selective pressure on the microbial ecosystem, eliminating susceptible species while potentially selecting for more resistant organisms. This ecological succession is reflected in the shift from a community dominated by obligate anaerobes (Prevotella, Porphyromonas, Fusobacterium) in primary infections to one with higher proportions of facultative anaerobes (Enterococcus, Streptococcus) in persistent infections [14, 16]. The distinct clustering of samples based on infection type in beta diversity analyses indicates that bacterial community structure is strongly influenced by the history of endodontic treatment. This finding has important implications for treatment strategies, as it suggests that persistent infections represent a fundamentally different ecological entity rather than simply a subset of the primary infection community that survived initial treatment [11, 15]. The 93% reduction in feature space with only a modest 5.7% decrease in classification performance highlights the redundancy present in high-dimensional microbiome data and the utility of our approach in extracting informative features. Compared to established methods such as PCA, LDA, and mRMR, our approach achieved superior performance in terms of classification accuracy and feature interpretability [17, 19]. The integration of taxonomic and functional data through co-inertia analysis revealed strong concordance between bacterial community composition and functional profiles. This finding supports the concept of functional redundancy in microbial communities, where different bacterial taxa can perform similar ecological functions [3, 20]. However, the distinct functional profiles observed between primary and persistent infections suggest that certain specialized functions may be associated with specific bacterial groups, potentially contributing to their adaptation to different ecological niches within the root canal system. The identification of hub taxa in bacterial co-occurrence networks provides insight into the ecological organization of endodontic infections. The observation that different bacterial genera emerge as hubs in primary versus persistent infections suggests a reorganization of bacterial interactions following endodontic treatment [8, 18]. These hub taxa may play crucial roles in maintaining the stability of the bacterial community and could represent potential targets for therapeutic interventions. The significant correlations observed between specific bacterial taxa and clinical parameters highlight the clinical relevance of bacterial community profiles. The association between certain anaerobic bacteria (Prevotella, Porphyromonas, Tannerella) and pain intensity is consistent with their known production of inflammatory mediators and tissue-destructive enzymes [1, 6]. Similarly, the correlation between specific taxa (Fusobacterium, Treponema, Dialister) and periapical lesion size suggests their potential involvement in periapical tissue destruction. The predictive model for treatment outcome based on baseline bacterial community composition achieved promising performance (79.3% accuracy, AUC = 0.82). This model could potentially be used to identify high-risk cases that might benefit from modified treatment protocols or adjunctive therapies, representing a step toward personalized endodontic treatment based on microbiome profiles [5, 10]. The higher abundance and diversity of antibiotic resistance genes in persistent infections compared to primary infections has important implications for the management of refractory endodontic cases. This finding suggests that antibiotic resistance may contribute to treatment failure and highlights the need for alternative antimicrobial strategies that are not affected by conventional resistance mechanisms [7, 9].

The sequences were submitted to NCBI GenBank with accession numbers OR778277, OR778278, and OR778279 for *S. aureus* (BEB1), *K. pneumonia* (BEB2), and *S. enterica* (BEB3), respectively. This study represents the initial investigation concentrating on the genotypic identification of endodontic bacteria in Berhampur, Odisha, India. The amalgamation of genotypic data with clinical procedures and the analysis of treatment responses could aid in the establishment of evidence-based protocols for the control of endodontic infections.

4. CONCLUSION

The current study correlates significant differences in bacterial community composition and diversity between primary and persistent infections, reflecting ecological succession following endodontic treatment. The implemented feature selection algorithm effectively reduced the dimensionality of sequencing data while preserving biologically relevant information, in terms of classification accuracy and feature interpretability. Network analysis identified key hub taxa that may play crucial roles in maintaining the ecological stability of bacterial communities, potentially representing targets for therapeutic interventions. The distinct bacterial profiles associated with different types of endodontic infections suggest that treatment

strategies should be tailored to the specific microbial ecology of each case. The integration of taxonomic and functional data provides a holistic view of the endodontic microbiome, while the developed computational framework offers a powerful tool for analyzing high-dimensional omics datasets.

Ethical Clearance Statement

Ethical approval for this study (Ethical Committee No 877) was provided by the Ethical Committee M.K.C.G. Berhampur, Odisha, India

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Nil.

Conflicts of interest

There are no conflicts of interest.

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