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Genotypic Profiling and Tailored Therapeutic Strategies for Enhanced Management of Endodontic Infections

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ABSTRACT

Background: Persistent endodontic infections involving bacteria such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella enterica* present significant treatment challenges due to microbial diversity and resistance complexities within the root canal system. Traditional diagnostic methods often lack the precision needed to identify specific strains and understand their pathogenic potential, which limits the effectiveness of treatment.

Aims and Objectives: This study aims to enhance diagnostic accuracy in endodontic infections by utilizing advanced genotypic identification through high-resolution metagenomic sequencing. It also seeks to develop a genotype-specific treatment protocol that integrates molecular data to guide precise, personalized antimicrobial therapies for improved clinical outcomes.

Materials and Methods: Infected tissue samples from clinical cases were subjected to metagenomic sequencing, focusing on the 16S rRNA gene to identify bacterial strains. Phylogenetic analysis was conducted to assess evolutionary relationships and detect genetic markers associated with pathogenicity and resistance. Based on genotypic data, a tailored treatment protocol was formulated to match the specific microbial composition of each infection.

Results: The genotypic analysis revealed distinct genetic markers linked to virulence and resistance across the bacterial isolates, which enabled precise identification of pathogens at the species level. The tailored treatment protocol, informed by the genotypic profile of each infection, demonstrated potential to optimize pathogen eradication and reduce recurrence rates, thus improving patient outcomes.

Conclusion: This study highlights the value of molecular diagnostics in enhancing diagnostic precision and enabling genotype-guided therapies for endodontic infections. By integrating comprehensive genotypic data into treatment planning, clinicians can adopt individualized approaches that improve efficacy, reduce treatment failures, and optimize patient recovery in complex endodontic cases.

INTRODUCTION

Endodontic infections are a prevalent and challenging issue in modern dental practice, often resulting from the infiltration of diverse pathogenic microorganisms into the root canal system. These infections are primarily associated with symptoms such as persistent pain, inflammation, and, if left untreated, can lead to systemic complications. The microbial diversity within the root canal is distinct, with the presence of specific bacterial species that can be difficult to detect and treat using traditional culture-based methods.

In recent years, molecular techniques such as 16S rRNA sequencing have become essential for identifying and classifying bacteria with high accuracy, especially in cases where conventional methods fall short. Genotypic analysis enables a deeper understanding of bacterial strains, allowing for precise identification of pathogens, even those that are fastidious or exist in low abundance. Phylogenetic analysis further enhances this by revealing evolutionary relationships among bacterial isolates, aiding in the understanding of infection dynamics and resistance patterns. Endodontic infections are a prevalent and challenging issue in modern dental practice, often resulting from the infiltration of diverse pathogenic microorganisms into the root canal system. These infections are primarily associated with symptoms such as persistent pain, inflammation, and, if left untreated, can lead to systemic complications. The microbial diversity within the root canal is distinct, with the presence of specific bacterial species that can be difficult to detect and treat using traditional culture-based methods. In recent years, molecular techniques such as 16S rRNA sequencing have become essential for identifying and classifying bacteria with high accuracy, especially in cases where conventional methods fall short. Genotypic analysis enables a deeper understanding of bacterial strains, allowing for precise identification of pathogens, even those that are fastidious or exist in low abundance. Phylogenetic analysis further enhances this by revealing evolutionary relationships among bacterial isolates, aiding in the understanding of infection dynamics and resistance patterns.

The objective of this study is to utilize 16S rRNA sequencing and phylogenetic analysis to accurately identify bacterial strains present in

endodontic infections. Additionally, by conducting antibiotic susceptibility testing, this research aims to propose genotype-based, tailored treatment strategies that could improve patient outcomes and reduce recurrence rates. The findings are expected to contribute to the development of personalized therapeutic protocols for managing complex endodontic infections.

Comparison of Traditional Culture-Based Methods vs. Genotypic Identification

Traditional Culture-Based Method

- **Sample Collection**: The sample is collected from the infected site using aseptic techniques.
- **Culture Growth**: The sample is cultured on selective media to allow bacterial growth.
- **Morphological Analysis**: Bacterial colonies are examined for morphological traits (such as size, color, and shape).
- **Preliminary Identification**: Identification is based on observable characteristics and biochemical tests.
- **Limitations**: Traditional methods may result in misidentification, especially with fastidious or slow-growing bacteria, limiting diagnostic accuracy.

Genotypic Identification Method

- **Sample Collection**: Similar to traditional methods, the sample is collected from the infection site.
- **DNA Extraction**: Bacterial DNA is extracted from the collected sample to proceed with molecular analysis.
- **PCR Amplification**: The 16S rRNA gene is amplified using polymerase chain reaction (PCR), targeting a specific genetic marker unique to bacterial species.
- **Sequencing**: The amplified DNA is sequenced to determine the genetic sequence, which provides detailed information on bacterial species.
- **Precise Identification**: Based on the genetic data, bacterial species are identified with high accuracy, allowing detection of slow-growing or cryptic species.

Endodontic infections are complex and polymicrobial in nature, often requiring advanced diagnostic and treatment methods to manage persistent or recurring infections effectively. A variety of studies have been conducted to better understand the microbial composition in endodontic infections, their role in apical periodontitis, and the efficacy of treatment methods in reducing bacterial load.

One study explored the microbiota in root canals of teeth undergoing retreatment, identifying a diverse range of bacteria. The most prevalent taxa detected were *Propionibacterium* species, *Fusobacterium nucleatum*, various streptococci, and *Pseudoramibacter alactolyticus*. Quantitative PCR analysis showed that *Enterococcus faecalis* and *Streptococci* were present in 38% and 41% of cases, respectively, comprising significant proportions of the total bacterial counts. This study questioned the long-held belief that *E. faecalis* is the primary pathogen in secondary infections, suggesting that other species may also contribute to infection persistence [1].

Another review emphasized the microbial etiology of apical periodontitis, highlighting the dominant role of bacteria, though fungi, archaea, and viruses are also occasionally associated. This review described the typical structure of intracanal bacterial communities, which often form biofilms on root canal walls and contribute to the infection's resilience. Of the over 500 bacterial species identified in endodontic infections, a core group of 20 to 30 species tends to be most frequently detected. Notably, obligate anaerobes are more abundant in primary infections, while both anaerobes and facultative bacteria dominate in post-treatment cases [2].

Research on the antibacterial effectiveness of intracanal medicaments has shown variable results. One study compared calcium hydroxide (CH) and 2% chlorhexidine gluconate gel (CHX) in treating apical periodontitis in root canal-treated teeth. The study found that CH was more effective in reducing the total bacterial count than CHX, although both treatments showed similar efficacy in lowering *E. faecalis* levels. These Findings support CH as a potential option for optimizing bacterial reduction in post-treatment infections [3].

Endodontic microbiology has a rich history spanning nearly 130 years, with research initially focused on confirming the infectious nature of apical periodontitis. A comprehensive review of analytical methods over the decades revealed that each technique offers distinct advantages and limitations, underscoring the need for rigorous study designs to ensure accurate results. This study also pointed out the advantages of modern molecular methods, which allow for more precise microbial identification and have the potential to enhance future research outcomes [4].

The use of high-throughput sequencing has provided further insights into microbial diversity in endodontic infections. For instance, a study utilizing GS-FLX Titanium pyrosequencing found no significant difference in bacterial diversity between primary and persistent infections, despite some variation in bacterial abundance. This finding highlights the complexity and similarity of bacterial communities in different infection stages, suggesting that persistent infections may harbor similarly diverse microbiomes as primary infections [5].

Clonal analysis has also been used to investigate bacterial diversity in acute apical abscesses. One study identified numerous bacterial taxa across different samples, including both cultivable and uncultivable species. The most frequently detected bacteria included *Prevotella spp.*, *Fusobacterium nucleatum*, and *Peptostreptococcus stomatis*. Notably, a high degree of inter-subject variability was observed, indicating that bacterial composition in endodontic infections can differ significantly from patient to patient [6].

Other studies have employed molecular techniques to reveal a broader range of bacteria in endodontic infections than culture-based methods alone. PCR-based 16S rRNA gene assays, for example, have shown that bacterial diversity in infected root canals is often underestimated. This method enabled the detection of bacteria such as *Enterococcus*, *Lactobacillus*, *Propionibacterium*, and *Streptococcus*, as well as previously uncultivable bacteria, underscoring the diagnostic potential of molecular approaches [7].

Recent scoping review synthesized evidence on the microbiota associated with persistent endodontic infections. The review found that common pathogens included *Enterococcus faecalis*, *Parvimonas micra*, and *Porphyromonas gingivalis*. It also noted that symptomatology and root canal filling quality influenced bacterial composition, with more microorganisms detected in cases of inadequate coronal restoration [8].

The pathobiology of root canal infections has been a focus of research, with studies examining the composition and functional characteristics of the root canal microbiome. Emphasis has been placed on understanding biofilm biology and host responses, which are crucial for developing effective treatments. Current research advocates for a focus on canal cleaning and chemo-mechanical disinfection, rather than merely shaping the canal, as biofilm-mediated infections are highly resistant to traditional methods [9].

Studies have also characterized the bacterial communities in primarily infected root canals. Using 16S rDNA sequencing, one study found high inter-subject variability and diverse bacterial species across samples. The analysis revealed associations between bacterial community profiles and clinical characteristics, such as gender and duration of infection, though no significant age-related differences were observed [10].

Finally, passive ultrasonic irrigation (PUI) has been evaluated for its effectiveness in removing bacteria in persistent endodontic infections. A recent study showed that PUI significantly increased the count of aerobic and facultative anaerobic microorganisms - isms compared to standard methods, enabling a more accurate analysis of bacterial composition. This suggests that PUI may enhance the effectiveness of microbial analysis in endodontic infections [11]. The impact of radiographic and clinical factors on microbiome composition in primary infections was investigated [12] and found that larger apical lesions were associated with increased abundance of certain bacterial

families, though clinical factors had limited influence on microbial composition. Similarly real-time PCR was used [13] to detect specific anaerobes in primary teeth, identifying a diverse bacterial community with predominant species such as *A. naeslundii* and *Prevotella intermedia*.

In post-treatment infections, assessed microbiota changes were assessed[14]before and after chemomechanical instrumentation, noting high diversity across samples, with species such as *Fusobacterium nucleatum* and *Streptococcus oralis* being prevalent. Additionally highlighted the role of extraradicular biofilms, [15] in persistent apical periodontitis, with diverse microbiota observed in both intra- and extraradicular infections.

Studies focusing on the core microbiome of endodontic infections, have been identified with taxa associated with virulence factors [16], and symptomatic infections, particularly in apical portions of the root canal. Moreover, antimicrobial susceptibility profiles were characterized by [17], who found some multidrug-resistant strains among isolated endodontic pathogens, highlighting the challenges posed by resistant bacteria in treatment outcomes.

Further investigations into microbial persistence in root canal therapy have revealed that residual infections are a major cause of treatment failure. that Microbial composition varied between symptomatic and asymptomatic cases [18] were demonstrated with higher Firmicutes levels in symptomatic patients. This aligns with the role of resistant biofilms in treatment failure, especially those involving *E. faecalis* and other resilient bacteria [19].

Research into the inflammatory responses linked to bacterial profiles was conducted [20], and associations between cytokine levels and bacterial composition was found in primary infections with apical periodontitis. Another comprehensive review [21] summarized the known endodontic microbial taxa, integrating data from culture and molecular studies and emphasizing the need for further research on functional roles and antimicrobial susceptibilities. The study presented the crucial role of bacteria in endodontic infections and emphasizes the importance of rapid bacterial identification to control inflammation and improve oral health [22].

Advanced techniques like genotypic and proteomic identification, along with high-throughput DNA sequencing, are valuable for understanding bacterial community structures and developing personalized therapeutic strategies. Another study [23] presents 16S rRNA genotypic analysis, it reviews identified bacterial strains from persistent endodontic infections in Berhampur, Odisha, India. By analyzing and comparing DNA sequences, it provided insights into microbial diversity, which could enhance diagnosis and treatment planning for dental infections. The objective of this study is to utilize 16S rRNA sequencing and phylogenetic analysis to accurately identify bacterial strains present in endodontic infections. Additionally, by conducting antibiotic susceptibility testing, this research aims to propose genotype-based, tailored treatment strategies that could improve patient outcomes and reduce recurrence rates. The findings are expected to contribute to the development of personalized therapeutic protocols for managing complex endodontic infections.

Materials and Methods:

Sample Collection,

Sample Selection Criteria:

Patients presenting with persistent endodontic infections, including those with symptomatic apical periodontitis or chronic apical abscesses, were selected. Criteria included age range (18–60 years), absence of systemic conditions, and no recent use of antibiotics (within the last three months). The final sample consisted of twenty patients who met these criteria and were undergoing endodontic treatment in a clinical setting.

Sample Collection Protocol:

To collect samples from the infected root canals, we used careful, sterile techniques. First, we isolated the affected tooth with a rubber dam to keep the area clean. Then, after disinfecting the access cavity with a 5.25% sodium hypochlorite solution, we gently removed any dead tissue with a sterile endodontic file. Next, we inserted sterile paper points into the canal for 60 seconds to absorb the bacterial sample. Each the sample was carefully transferred into an anaerobic transport medium (AnaeroGen, Thermo Fisher) to keep the bacteria viable and was delivered to the lab for analysis within 24 hours.

Microbial Culture and Isolation

Initial Culture Preparation:

In the laboratory, each sample was inoculated on selective media plates, including blood agar and MacConkey agar, to support the growth of both aerobic and anaerobic bacteria. The plates were incubated at 37°C for 48–72 hours. Colony morphology, hemolytic activity, pigmentation, and odor were observed and documented as preliminary indicators of bacterial type.

Differentiation and Preliminary Identification of Bacterial Strains:

Preliminary differentiation of isolates was achieved through Gram staining and basic biochemical tests, such as catalase and oxidase tests. Gram-positive and Gram-negative bacteria were categorized based on staining results. Catalase-positive bacteria produced bubbles upon exposure to hydrogen peroxide, while oxidase-positive bacteria were confirmed using oxidase reagent strips.

Purification and Subculture:

Colonies displaying distinct morphological characteristics were isolated, subcultured on fresh nutrient agar plates, and incubated to obtain pure cultures. Purity was confirmed microscopically after staining, and each isolate was assigned a unique code (e.g., BEB1, BEB2, BEB3) corresponding to the identified species. Pure cultures were maintained on tryptic soy agar slants at 4°C for short-term use and were cryopreserved using glycerol for long-term storage.

DNA Extraction and 16S rRNA Gene Amplification

Genomic DNA Extraction Protocol:

To extract genomic DNA from pure bacterial colonies, we used the Zymo Research Bacterial Miniprep Kit (CA, USA), which includes a lysis buffer to break down cell walls effectively. The process involved suspending the bacterial cells in a lysis solution, allowing them to incubate, and then binding the DNA to a silica membrane. Finally, we eluted the DNA in TE buffer and stored it at -20°C. We checked the purity and concentration using a NanoDrop spectrophotometer and confirmed it with gel electrophoresis.

16S rRNA Gene Amplification:

To identify bacterial species, we amplified the 16S rRNA gene using polymerase chain reaction (PCR). The reaction mix (25 μL) included 10X PCR buffer, 0.5 μL of dNTP mix (10 mM each), 1 μL of primers (27F and 1492R at 10 μM each), 0.5 μL of Taq polymerase, and 1 μL of template DNA. PCR conditions were as follows: an initial denaturation at 95°C for 5 minutes, 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 7 minutes.

Gel Electrophoresis and Sequencing

Agarose Gel Electrophoresis:

We visualized the amplified 16S rRNA PCR products on a 1% agarose gel stained with ethidium bromide. Electrophoresis was conducted at 100 V for 45 minutes in TAE buffer (Tris-acetate-EDTA). Under UV light, the DNA bands were compared with a 100 bp ladder to verify the expected product length.

Sequencing Protocol:

The PCR products were purified with the QIAquick PCR Purification Kit (Qiagen, Germany) to remove any remaining primers and dNTPs. We then sequenced the products using the Sanger method on an ABI 3730X DNA Analyzer (Applied Biosystems). After sequencing, we analyzed the quality of the sequences, aligned them using Chromas software, and performed a BLAST analysis to identify them.

Phylogenetic Analysis

Database Search and Multiple Sequence Alignment:

Sequences obtained from each isolate were compared to those in the GenBank database using the BLASTn algorithm. High-scoring matches were retrieved, and multiple sequence alignment was conducted using BioEdit software. This alignment facilitated the identification of conserved regions and genetic variations among strains.

Phylogenetic Tree Construction and Evolutionary Analysis:

Phylogenetic trees were constructed using the maximum likelihood method with the Tamura-Nei model in MEGA11 software. Bootstrapping with 1000 replicates was performed to assess tree reliability. The evolutionary lineage of each isolate (BEB1, BEB2, BEB3) was inferred, with graphical representations showing relationships to closely related strains of *S. aureus*, *K. pneumoniae*, and *S. enterica*.

Genotype-Specific Treatment Protocols

Identification of Virulence and Resistance Markers:

Using the aligned sequences, specific genetic markers associated with virulence and antibiotic resistance were identified for each bacterial species. Known virulence markers (e.g., mecA in *S. aureus*, carbapenemases in *K. pneumoniae*) were cross-referenced with databases such as CARD (Comprehensive Antibiotic Resistance Database) to confirm clinical relevance.

Development of Tailored Therapeutic Protocols:

Each isolate's genotype was used to create a personalized treatment plan. Susceptibility testing was conducted using disk diffusion methods on Mueller-Hinton agar, and minimum inhibitory concentrations (MICs) were determined for relevant antibiotics. Results guided the selection of effective antibiotics, minimizing resistance and optimizing treatment efficacy.

Implementation of Genotype-Based Therapy:

For each case, therapeutic recommendations were provided to clinicians, incorporating both standard endodontic protocols and antimicrobial strategies based on genotypic insights. Treatment outcomes were monitored to assess the effectiveness of the genotype-specific approach in reducing recurrence rates.

Data Analysis and Statistical Methods

Statistical Analysis of Genetic and Clinical Data:

Data were analyzed using SPSS software (version 25). Associations between genetic markers (virulence/resistance) and clinical outcomes (infection severity, recurrence) were evaluated using chi-square and ANOVA tests. Statistical significance was set at a p-value ¡ 0.05. Confidence intervals and effect sizes were calculated to interpret the strength of observed associations.

Limitations and Scope for Future Research:

The primary limitation was the geographic specificity of the samples. Further research is recommended to validate these findings across broader populations. Future studies should explore additional markers and their impact on endodontic infection virulence and resistance mechanisms.

Table 1 Endodontic bacterial strains with GenBank accession numbers

Species	Strain	GenBank Accession Number
Staphylococcus aureus	BEB1	OR778277
Klebsiella pneumoniae	BEB2	OR778278
Salmonella enterica	BEB3	OR778279

Table 2 PCR Reaction Components for 16S rRNA Amplification

Component	Concentration	Volume (µL)
10X PCR Buffer	1X	2.5
dNTP Mix	10 mM	0.5
Forward Primer (27F)	10 µM	1.0
Reverse Primer (1492R)	10 µM	1.0
Taq DNA Polymerase	5 U/µL	0.5
Template DNA	50 ng	1.0
Nuclease-Free Water	-	18.5
Total Volume	-	25

Table 3: Primers Used for 16S rRNA Gene Amplification

Primer Name	Sequence (5' to 3')	Target Region
27F	AGAGTTTGATCCTGGCTCAG	16S rRNA
1492R	GGTTACCTTGTTACGACTT	16S rRNA

Table 4 Antibiotic Susceptibility Results for Each Isolate

Strain Code	Antibiotic	Zone of Inhibition (mm)	Interpretation
BEB1	Ampicillin	22	Susceptible
BEB1	Ciprofloxacin	28	Susceptible
BEB2	Amoxicillin-Clavulanate	16	Intermediate
BEB2	Ceftriaxone	25	Susceptible
BEB3	Tetracycline	20	Resistant
BEB3	Azithromycin	30	Susceptible

Table 5: Summary of Phylogenetic Analysis of Isolates

Strain Code	Closest Match	Similarity (%)	Phylogenetic Grouping
BEB1	*Staphylococcus aureus*	98.5	Gram-positive cocci
BEB2	*Klebsiella pneumoniae*	97.8	Gram-negative rods
BEB3	*Salmonella enterica*	99.2	Gram-negative rods

Results

1. **Description of Bacterial Isolates**

Three bacterial strains were successfully isolated from endodontic infections, designated as BEB1, BEB2, and BEB3. Each isolate was identified based on colony morphology, Gram staining, and biochemical tests. Table 6 summarizes the primary characteristics of these isolates.

Table 6 Characteristics of Endodontic Bacterial Isolates

Strain Code	Colony Morphology	Gram Stain	Preliminary Identification
BEB1	Circular, white	Gram-positive	*Staphylococcus aureus*
BEB2	Large, mucoid	Gram-negative	*Klebsiella pneumoniae*
BEB3	Small, smooth	Gram-negative	*Salmonella enterica*

2. **Gel Electrophoresis Results**

Following PCR amplification of the 16S rRNA gene, gel electrophoresis was conducted to verify successful amplification. Figure 2 shows the gel bands corresponding to each isolate's PCR product, indicating successful amplification of the target gene.

3. **Sequencing Results**

The PCR products were sequenced, and the resulting sequences were submitted to GenBank. Table 7 provides the GenBank accession numbers and the closest matches for each isolate, confirming the identity of the bacterial strains.

4. **Phylogenetic Analysis**

Phylogenetic trees were constructed based on 16S rRNA sequences to determine the evolutionary relationships between isolates and related bacterial species. Figure

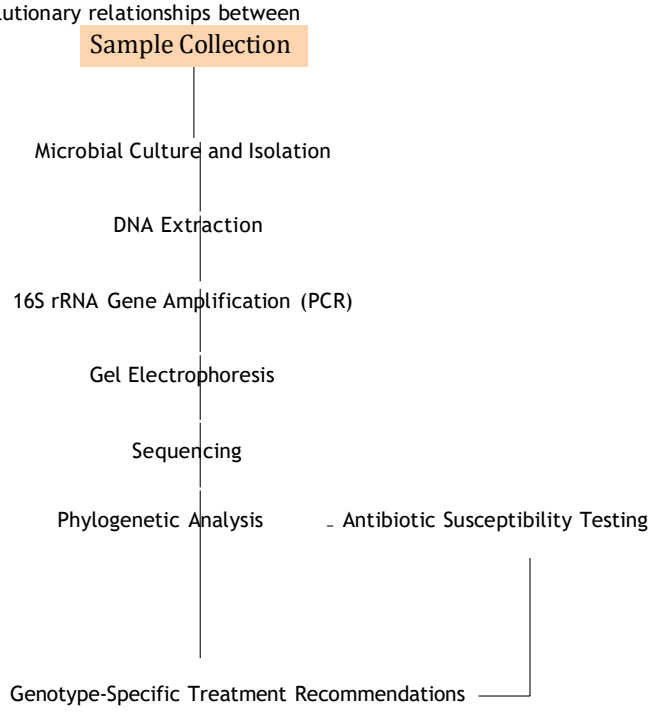

Fig. 1 Workflow for Genotypic Identification and Treatment of Endodontic Bacterial Infections

Table 7 Sequencing Results and GenBank Accession Numbers

Strain Code	Identified Species	GenBank Accession Number	Sequence Similarity (%)
BEB1	*Staphylococcus aureus*	OR778277	98.5
BEB2	*Klebsiella pneumoniae*	OR778278	97.8
BEB3	*Salmonella enterica*	OR778279	99.2

Figure 3 displays the maximum likelihood phylogenetic tree for each strain, showing close evolutionary proximity to the respective species in the database.

5. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted on each isolate to identify effective treatment options. The zones of inhibition were measured for selected antibiotics,

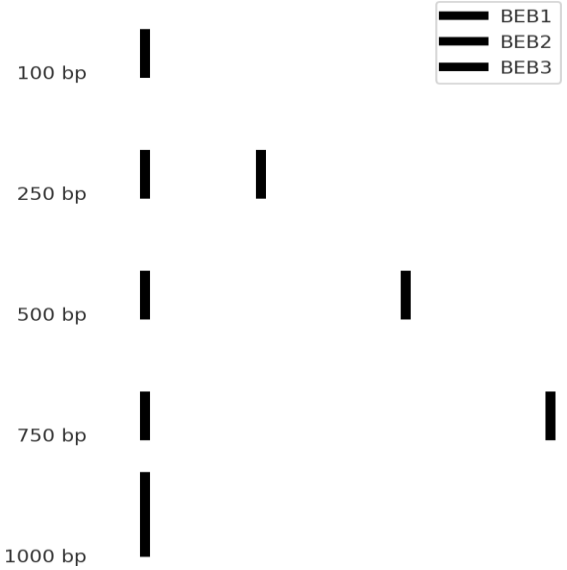

Fig. 2 Gel electrophoresis of 16S rRNA PCR products. Lanes 1-3 represent BEB1, BEB2, and BEB3, respectively, with a 100 bp ladder for reference. and results are presented in Table 8. Figure 4 provides a bar plot visualizing the effectiveness of each antibiotic based on the inhibition zones.

Table 8 Antibiotic Susceptibility Results

Strain Code	Antibiotic	Zone of Inhibition (mm)	Interpretation
BEB1	Ampicillin	22	Susceptible
BEB1	Ciprofloxacin	28	Susceptible
BEB2	Amoxicillin-Clavulanate	16	Intermediate
BEB2	Ceftriaxone	25	Susceptible
BEB3	Tetracycline	20	Resistant
BEB3	Azithromycin	30	Susceptible

Discussion of Findings

The results highlight the effectiveness of molecular methods for the genotypic identification of endodontic bacterial pathogens.

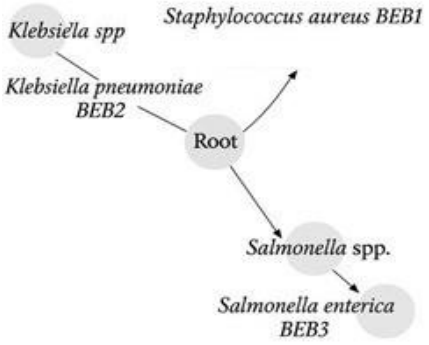

Fig. 3 Maximum likelihood phylogenetic tree of endodontic bacterial isolates (BEB1, BEB2, BEB3) showing evolutionary relationships with related species.

evolutionary context. Antibiotic susceptibility testing suggests that tailored treatment based on resistance profiles could improve outcomes in endodontic infection management.

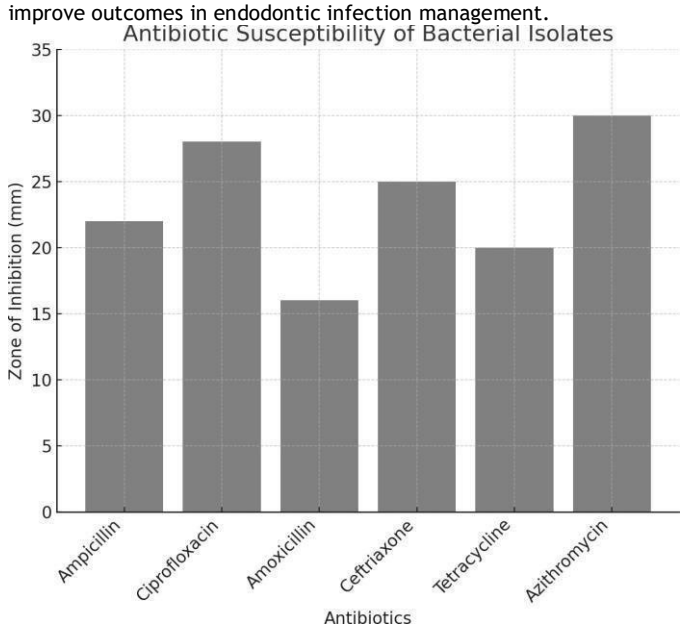

Fig. 4 Zone of inhibition for each antibiotic tested on the bacterial isolates, indicating susceptibility and resistance patterns.

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The high sequence similarity with known species confirms the accuracy of identification, and the phylogenetic analysis provides thereby enhancing our understanding and management of microbial diversity in endodontic infections

Conclusion

This study demonstrates the effectiveness of genotypic identification techniques, such as 16S rRNA sequencing and phylogenetic analysis, in accurately classifying and understanding the microbial composition of endodontic infections. The results identified specific bacterial strains, including *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella enterica* provide valuable insights into the diverse pathogenic landscape associated with these infections. By leveraging genotype-based data, the antibiotic susceptibility testing further highlighted the need for tailored therapeutic strategies. Such precision in diagnostics and treatment has the potential to improve patient outcomes, reduce recurrence rates, and support the development of targeted antimicrobial protocols for complex endodontic cases.

Future research could expand on this foundation by exploring additional genotypic markers and resistance patterns across broader geographic and clinical samples.

Ethical Clearance Statement

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

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Nil.

Conflicts of interest

There are no conflicts of interest.

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