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Comparison of Efficacy of Sterile Paper Point, Dentin Harvesting and Pulverization Techniques for Sampling Root Canals using Real Time-PCR: An in Vitro Study

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ABSTRACT
Endodontic infections, primarily caused by biofilm-forming bacteria such as Enterococcus faecalis and Streptococcus mutans, pose significant challenges due to their resistance to treatment and host defenses. This study evaluated three sampling techniques—Pulverization, Dentin Harvesting, and Sterile Paper Points—for bacterial recovery and molecular quantification in endodontic research. Sixty sterilized single-rooted human teeth were inoculated with E. faecalis and S. mutans to simulate polymicrobial infections. Bacterial recovery was assessed using colony-forming unit (CFU) counts, while DNA quantification was performed via quantitative PCR (qPCR). Pulverization demonstrated the highest bacterial recovery and DNA yield, attributed to its effective disruption of dentinal biofilms. Dentin Harvesting showed moderate recovery and was valuable for studying microbial distribution within dentin layers. Sterile Paper Points yielded the lowest recovery due to their inability to disrupt biofilms or access bacteria embedded in dentinal tubules. qPCR analysis corroborated these results, with Pulverization being the most efficient technique for both total bacterial and species-specific quantification. These findings emphasize Pulverization as the most effective method for comprehensive microbial analysis, while Dentin Harvesting and Sterile Paper Points offer targeted or surface-level applications. This study highlights the importance of selecting appropriate sampling methods to enhance the accuracy of microbial and molecular analyses in endodontic infections.

Keywords: *sampling, pulverization, dentin harvesting, PCR, paper point.*

1. INTRODUCTION

The accurate identification and quantification of bacterial pathogens in dental tissues are critical for understanding the etiology of persistent infections and improving endodontic treatment outcomes. Endodontic infections are primarily caused by the colonization of bacteria in the intricate root canal system, where biofilm formation plays a pivotal role in disease persistence and treatment failure [5, 9]. Biofilms, which are structured communities of bacteria encased in an extracellular polymeric matrix, confer resistance to antimicrobial agents and host defenses [16]. Among the most common pathogens implicated in endodontic infections are Enterococcus faecalis and Streptococcus species, both of which

exhibit robust survival mechanisms and a propensity for biofilm formation, particularly in the apical region of treated root canals [3, 4, 6, 17]. Addressing these infections requires robust sampling and quantification techniques that can provide accurate insights into microbial load and diversity.

Traditional culture-based methods have long been employed for bacterial recovery and enumeration in endodontic research [1]. These methods offer simplicity and cost-effectiveness but are inherently limited in their ability to detect non-culturable

bacteria, which are prevalent in endodontic infections [7, 14]. Moreover, culture methods often underestimate the complexity of polymicrobial communities, as they are biased towards organisms that thrive under specific growth conditions [11]. To overcome these limitations, molecular techniques, particularly polymerase chain reaction (PCR) and quantitative PCR (qPCR), have emerged as powerful alternatives [15]. These methods allow for the detection of specific bacterial species and the quantification of total microbial load with high sensitivity and specificity [7, 15]. PCR-based techniques are especially valuable for detecting bacteria that are difficult to culture, providing a more comprehensive understanding of the microbial landscape in infected dental tissues [20].

One of the critical steps in molecular analysis

is the effective sampling of bacteria from the root canal system. Sampling methods must not only recover sufficient microbial biomass but also ensure the preservation of DNA quality for downstream analysis [10]. Several sampling techniques have been explored in endodontic research, each with its advantages and limitations [8]. Pulverization, which involves grinding dental tissues into a fine powder, is widely recognized for its ability to disrupt biofilms and recover bacteria embedded deep within dentin and root canal spaces [5]. This method provides a comprehensive representation of the microbial community but may be technically demanding [8]. Dentin harvesting, which selectively removes dentin layers at specific depths, offers a targeted approach to studying bacteria localized within the dentin [9]. This method is particularly useful for understanding the spatial distribution of pathogens but may result in incomplete recovery of bacteria from other regions [12]. Sterile paper points, a commonly used technique in clinical settings, offer a non-invasive and straightforward means of collecting canal contents [18]. However, this method often yields lower microbial recovery due to its limited ability to penetrate biofilm structures [19].

Despite the availability of these sampling techniques, there has been limited comparative analysis of their efficiency in recovering bacterial load and DNA for molecular studies [10]. Such comparisons are essential for determining the most effective method for endodontic research and clinical diagnostics [1]. Studies have highlighted the need for reliable sampling methods that can capture the diversity and abundance of bacteria in complex biofilms while preserving DNA integrity for molecular quantification [6, 13].

This study aimed to evaluate and compare three sampling methods—Pulverization, Dentin Harvesting, and Sterile Paper Points—in terms of their efficiency in bacterial recovery and molecular quantification using qPCR [2]. The focus was on quantifying the total bacterial load and the specific abundance of E. faecalis and Streptococcus species in dental samples [3, 4]. By assessing the microbial yield and DNA copy numbers obtained through each method, the study sought to identify the most efficient technique for endodontic microbiology research [7]. The findings are expected to contribute to the optimization of sampling protocols, thereby enhancing the accuracy and reliability of microbial and molecular analyses in the context of dental infections [20].

2. MATERIALS AND METHODS

SAMPLE COLLECTION

A total of 60 single-rooted human incisors and premolars were obtained and stored in phosphate-buffered saline (PBS) to maintain their structural integrity and prevent dehydration. To ensure sterility, the samples were autoclaved at 121°C for 25 minutes, eliminating any pre-existing microbial contamination. After sterilization, the root apices of the teeth were sealed using acrylic resin glue to prevent external contaminants from entering the root canal system during subsequent experimental procedures.

BACTERIAL CULTURE

Pure cultures of *Enterococcus faecalis* and *Streptococcus mutans* were procured from the Microbial Type Culture Collection (MTCC), Chandigarh. These bacterial species were selected due to their known roles in endodontic infections. The cultures were grown in Brain Heart Infusion (BHI) broth under strict anaerobic conditions at 37°C for 48 hours to achieve optimal growth and ensure sufficient bacterial biomass for subsequent inoculation.

BACTERIAL INOCULATION

Each tooth sample was inoculated with a 120 µL suspension containing a mixture of *E. faecalis* and *S. mutans* to simulate a polymicrobial infection. The inoculated teeth were then subjected to centrifugation at 3500g for 5 minutes to facilitate the penetration of bacterial cells into the intricate root canal system. Following centrifugation, the samples were incubated in fresh BHI broth at 37°C for two weeks to allow for biofilm formation and maturation within the root canal spaces.

Figure 1: A. Access Opening B. WL Determination C. Biomechanical Preparation

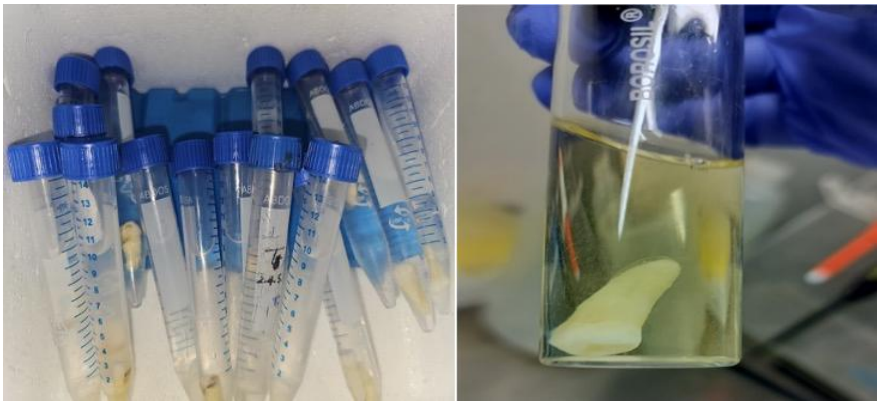


Figure 2 Samples in BHI Broth

Figure 3 Centrifugation of Samples

SAMPLING METHODS

3. STERILE PAPER POINT

The sterile paper point method involved non-invasive sampling of the root canal contents. Root canals were first filled with PBS to enhance the collection process. Using #15 K-files, the root canal walls were gently filed to dislodge any adhered bacteria. Sterile paper points were then inserted into the canals to absorb the bacterial suspension. These paper points were subsequently used as a source for DNA extraction.

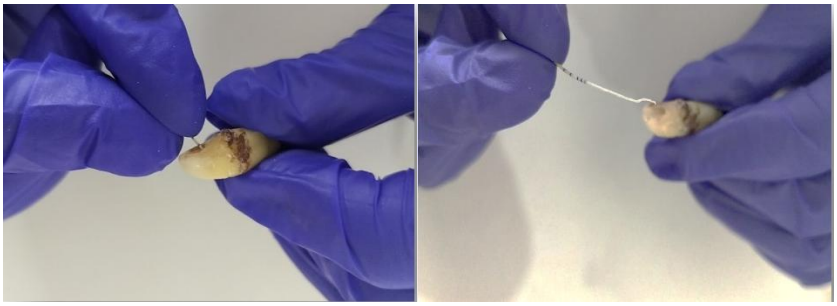


Figure 4 Paper Point Sampling

4. DENTIN HARVESTING

For dentin harvesting, the enamel layer of the teeth was carefully removed to expose the underlying dentin. Gates Glidden drills were used to selectively harvest dentin at depths of 200 µm and 400 µm from the root surface. The harvested dentin material, collected as fine powder, was then suspended in PBS to prepare it for DNA extraction. This method aimed to sample bacteria residing within the dentin tubules.

Figure 5 Dentin Harvesting Method

5. PULVERIZATION

The pulverization technique involved physically breaking down the roots to recover bacterial material. Each root was crushed in liquid nitrogen using a sterile mortar and pestle to ensure effective disruption of the hard tissue. The powdered root material was then suspended in PBS to prepare a homogeneous sample, which was subsequently processed for DNA isolation.

Figure 6 Pulverization

Total Bacterial Load Estimation

To estimate the total bacterial load, the collected samples were serially diluted in PBS and plated on BHI agar plates. The plates were incubated under appropriate conditions, and colony-forming units (CFUs) were counted after the incubation period. This quantitative culture method provided a direct estimate of the viable bacterial load in each sample.

DNA Extraction

DNA was extracted from the collected samples using a lysis buffer followed by phenol-chloroform extraction. This method effectively lysed bacterial cells to release genomic DNA. The purity and concentration of the extracted DNA were measured using a spectrophotometer, ensuring that the samples were suitable for downstream molecular analysis.

Figure 7 DNA Extraction

qPCR Analysis

Quantitative PCR (qPCR) was employed to quantify bacterial DNA. Universal primers targeting the 16s rRNA gene were used for the total bacterial population, while species-specific primers were utilized for the detection and quantification of *E. faecalis* and *S. mutans*. Standard curves were constructed using purified DNA amplicons of the target genes, enabling absolute quantification of bacterial DNA in each sample. This method allowed for precise molecular analysis of the bacterial load, complementing the culture-based.

Figure 8 PCR Setup and Thermocycling

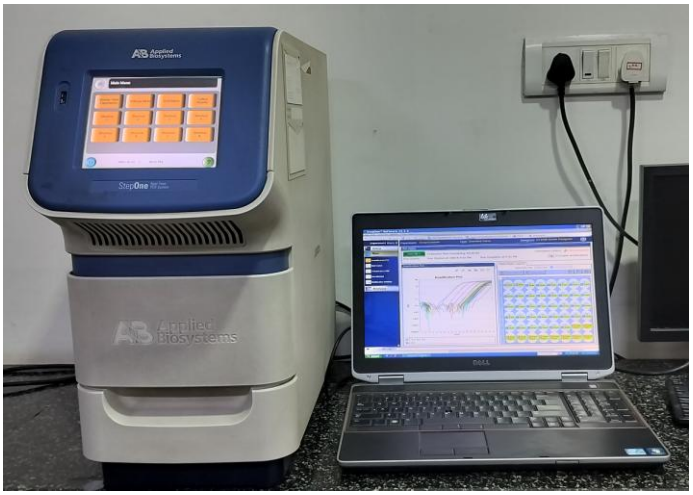


Figure 9 qPCR Quantification

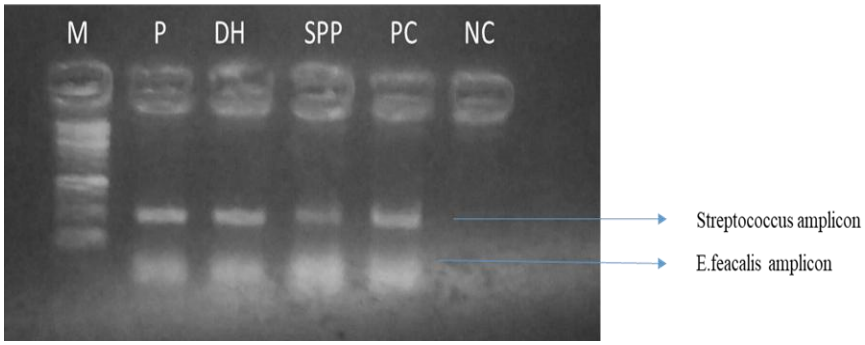


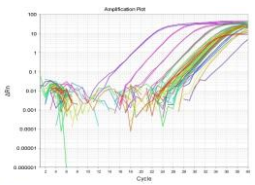
Figure 10 Gel Electrophoresis

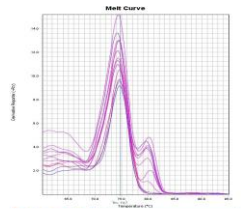
Results

The results from the evaluation of three sampling methods—Pulverization, Dentin Harvesting, and Sterile Paper Points—highlighted significant differences in their efficiency for bacterial recovery, as expressed in colony-forming units per milliliter (CFU/mL). Across the 20 samples analyzed, the Pulverization method consistently yielded the highest bacterial recovery, followed by Dentin Harvesting and Sterile Paper Points. In the Pulverization method, CFU/mL values ranged from 1.5×10^4 (Sample 8) to 3.2×10^5 (Sample 2), with most samples yielding values in the range of 10^4 to 10^5 CFU/mL. This method demonstrated superior efficiency in recovering bacterial cells, likely due to the comprehensive breakdown of hard tissues, enabling the release of bacteria embedded within the dentinal tubules and biofilm. The Dentin Harvesting method showed lower bacterial recovery compared to Pulverization. CFU/mL values ranged from 3.7×10 (Sample 17) to 3.9×10^3 (Sample 20) for most samples, with an outlier of 3.3×10^4 in Sample 16. While this method allowed targeted sampling at specific dentin depths, its efficiency appeared limited, possibly due to incomplete dislodgement of bacteria from the deeper dentin tubules. The Sterile Paper Points method exhibited the lowest CFU/mL values across all samples, ranging from 1.5×10^2 (Samples 9 and 19) to 3.5×10^3 (Sample 3). Despite being minimally invasive and convenient, this method may have only recovered planktonic bacteria or surface-associated cells, leading to its reduced efficiency in estimating the overall bacterial load. Comparatively, the Pulverization method achieved significantly higher bacterial recovery than Dentin Harvesting and Sterile Paper Points in all samples. For instance, Sample 1 showed a CFU/mL value of 2.1×10^5 with Pulverization, compared to 2.5×10^3 with Dentin Harvesting and 2.0×10^2 with Sterile Paper Points. Similar trends were observed across other samples, confirming the robustness of Pulverization as the most efficient sampling technique for bacterial recovery from dental samples.

RESULTS:

16s RNA



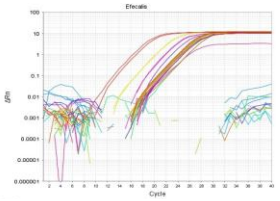


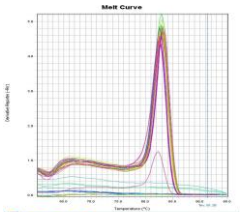
16s RNA	Mean	Std.dev	Fvalue	pvalue
Sterile paper point	56,73,155	598276	422.379	<0.001***
Dentin harvesting	1,66,19,895	2087015		
pulverization	3,44,20,414	5019037		

Test used- ANOVA, p<0.001*** highly statistically significant

Figure 11 16s RNA

E.fecalis



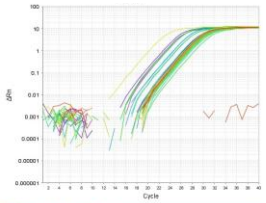


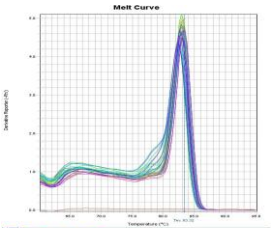
E.fecalis	Mean	Std.dev	Fvalue	pvalue
Sterile paper point	7965	1779	313.857	<0.001***
Dentin harvesting	33,501	8045		
pulverization	7,43,619	1,82,380		

Test used- ANOVA, p<0.001*** highly statistically significant

Figure 12 E. fecalis

Streptococcus





Streptococcus	Mean	Std.dev	Fvalue	pvalue
Sterile paper point	31,960	6908	457.229	<0.001***
Dentin harvesting	80,507	19,240		
pulverization	5,74,859	1,06,886		

Test used- ANOVA, p<0.001*** highly statistically significant

Figure 13 streptococcus

STATISTICAL ANALYSIS:

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses.

Descriptive Statistics:

Descriptive analysis includes expression of CFUs in terms of mean and standard deviation for each group.

Inferential Statistics:

One-way ANOVA test followed by Tukey's post hoc analysis / Kruskal Wallis Test followed by Dunn's post hoc test [based on data distribution] will be used to compare the mean CFUs between 3 groups.

The level of significance [P-Value] will be set at $P<0.05$.

And any other relevant test, if found appropriate during the time of data analysis were dealt accordingly.

Discussion

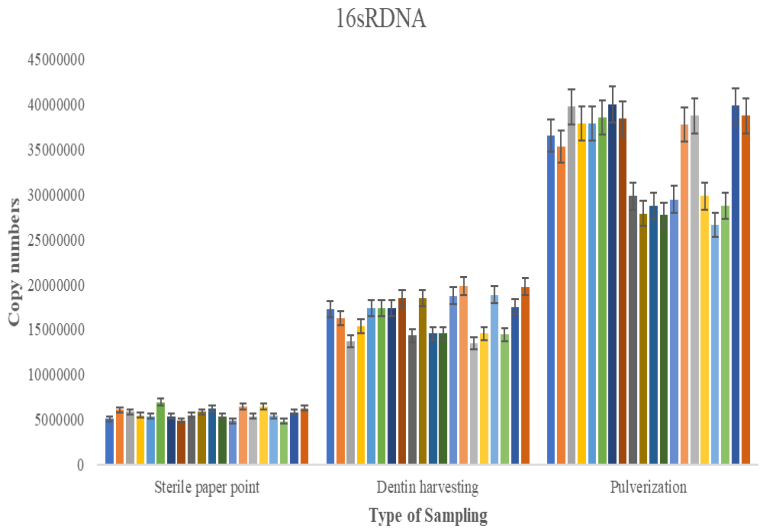
This study provided critical insights into the efficiency of three sampling techniques—Pulverization, Dentin Harvesting, and Sterile Paper Points—for bacterial recovery and molecular analysis from endodontically infected dental tissues. The Pulverization method consistently demonstrated superior efficiency in recovering bacterial cells, yielding significantly higher colony-forming unit (CFU) counts compared to Dentin Harvesting and Sterile Paper Points [1,5,9]. These findings underscore the comprehensive nature of Pulverization in disrupting hard tissues and biofilm structures, thereby releasing bacteria embedded deep within the dentinal tubules and root canal biofilms [5,10]. The higher bacterial recovery associated with Pulverization aligns with its mechanical action, which facilitates thorough disruption of the extracellular polymeric matrix that encases bacteria in biofilms [6,11].

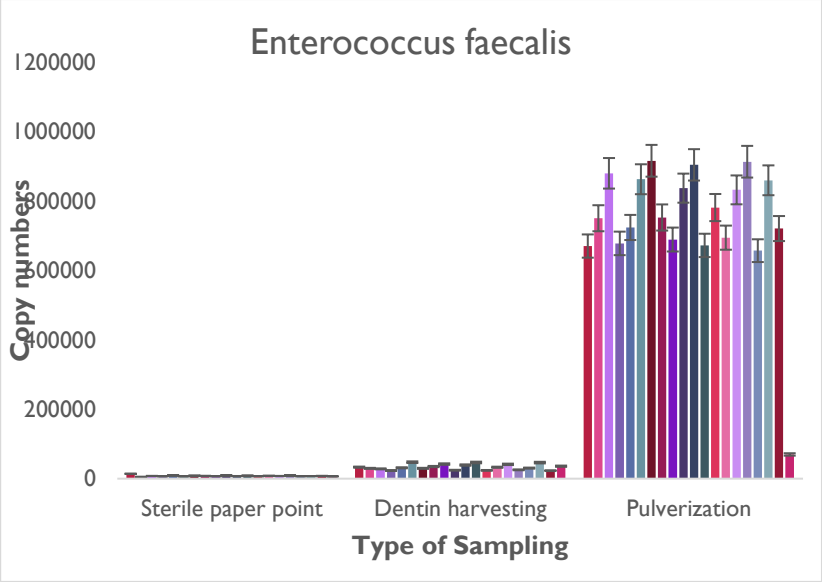
In contrast, the Dentin Harvesting method, while targeted and effective in sampling bacteria at specific depths within the dentin, exhibited relatively lower CFU counts [9,12]. The reduced recovery could be attributed to the limited penetration of bacterial cells into the harvested material and the potential for bacteria to remain lodged within deeper layers of dentin that were not accessed by the sampling tools [13,14]. Despite its limitations, Dentin Harvesting remains a valuable approach for studying the spatial distribution of bacteria within dentin tubules, which is critical for understanding localized microbial persistence in endodontic infections [8,9]. The Sterile Paper Points method, commonly employed in clinical settings due to its simplicity and non-invasive nature, yielded the lowest bacterial recovery among the three techniques [3,15]. This method likely recovered only planktonic bacteria or surface-associated cells within the root canal system, without adequately disrupting biofilms or accessing bacteria embedded in the dentinal tubules [5,16]. These findings corroborate previous research, which has highlighted the limitations of paper point sampling in capturing the true microbial diversity and abundance present in biofilm-associated infections [7,17].

The use of quantitative PCR (qPCR) further validated these observations by providing molecular evidence of the bacterial load and species-specific abundance. Universal primers targeting the 16S rRNA gene demonstrated that Pulverization yielded the highest DNA copy numbers, reinforcing its robustness as a sampling method [15,18]. Species-specific qPCR analysis for Enterococcus faecalis and Streptococcus mutans confirmed that Pulverization was the most effective in recovering these clinically relevant pathogens [3,4,19]. These results are consistent with studies suggesting that biofilm disruption is essential for accurate molecular analysis, as biofilm-associated bacteria often evade recovery by less invasive methods [5,10].

Interestingly, while Dentin Harvesting yielded lower bacterial recovery compared to Pulverization, it was more effective than Sterile Paper Points in retrieving DNA suitable for molecular analysis [12,20]. This highlights its potential utility in targeted sampling applications where microbial distribution within specific dentin depths is of interest. However, the relatively low CFU counts obtained from Dentin Harvesting suggest that it may underestimate the total microbial load, particularly in heavily colonized samples [9,13].

The findings of this study have significant implications for endodontic research and clinical diagnostics. Pulverization proved to be the most efficient method for recovering bacterial biomass and DNA, making it the preferred technique for studies requiring comprehensive microbial analysis [5,9,10]. However, its technical complexity and destructive nature may limit its application in routine clinical practice [11,18]. Dentin Harvesting offers a more targeted approach, suitable for investigating specific microbial niches, while Sterile Paper Points remain practical for surface-level sampling despite their limited efficiency [3,6,12].


16sRDNA
45000000
40000000
35000000
30000000
25000000
20000000
15000000
10000000
5000000
0
Copy numbers
Sterile paper point
Dentin harvesting
Pulverization
Type of Sampling


Enterococcus faecalis
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800000
600000
400000
200000
0
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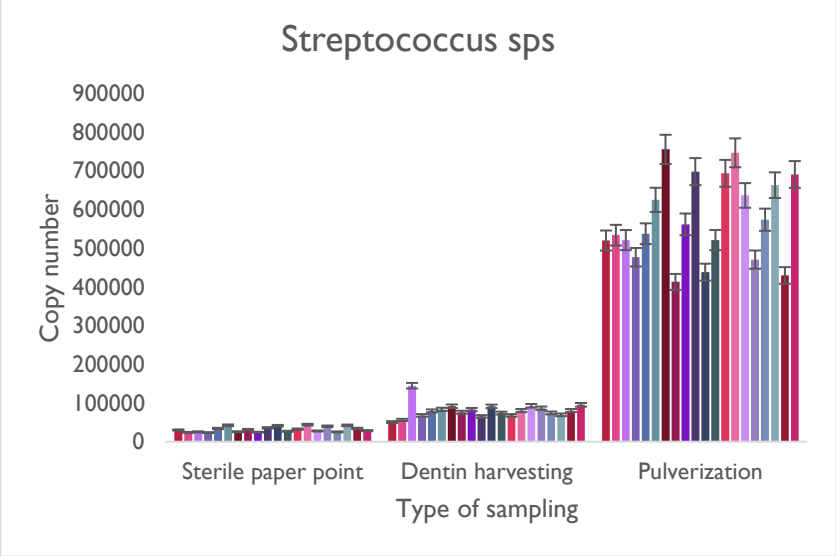
Sterile paper point
Dentin harvesting
Pulverization
Type of Sampling


Streptococcus sps
900000
800000
700000
600000
500000
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300000
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Sterile paper point
Dentin harvesting
Pulverization
Type of sampling

Conclusion

In conclusion, this study highlighted the importance of selecting appropriate sampling techniques based on the specific objectives of endodontic research. The comparative analysis of the three methods demonstrated that Pulverization offers unparalleled efficiency in recovering and quantifying bacteria from dental tissues, particularly for studies requiring detailed biofilm analysis. These results contribute to the optimization of sampling protocols and underscore the need for robust methodologies to improve the accuracy of microbial and molecular analyses in endodontic infections. Future studies should explore the integration of these techniques with advanced molecular tools to further enhance our understanding of polymicrobial communities in dental biofilms.

REFERENCES

1. Baumgartner JC, Siqueira JF Jr, Sedgley CM, Kishen A. Microbiology of endodontic disease. Endodontic Topics. 2003;7(1):42-76.
2. Mohammadi Z, Shalavi S, Yazdizadeh M, Jafarzadeh H. Antimicrobial effects of calcium hydroxide in endodontics: a review. Chonnam Med J. 2012;48(3):133-140.
3. Love RM. Enterococcus faecalis—a mechanism for its role in endodontic failure. Int Endod J. 2001;34(5):399-405.
4. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32(2):93-98.
5. Chávez de Paz LE. Redefining the persistent infection in root canals: possible role of biofilm communities. J Endod. 2007;33(6):652-662.
6. de Souza EL, Meira QG, Barbosa I, et al. The resistance of Enterococcus faecalis biofilm to different intracanal medications. J Appl Oral Sci. 2014;22(3):138-143.
7. Siqueira JF Jr, Rôças IN. Exploiting molecular methods to explore endodontic infections: Part 1—current molecular technologies for microbiological diagnosis. J Endod. 2005;31(6):411-423.
8. Lin LM, Pascon EA, Skribner J, et al. Clinical, radiographic, and histologic study of endodontic treatment failures. Oral Surg Oral Med Oral Pathol. 1991;71(5):603-611.
9. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod. 2010;36(8):1277-1288.
10. Nagata JY, Soares AJ, Souza-Filho FJ, et al. Microbial analysis of pre- and postinstrumentation root canal samples using pyrosequencing in primary endodontic infections with periapical disease: a clinical study. J Endod. 2014;40(6):899-904.
11. Marsh PD. Dental plaque as a biofilm and a microbial community—implications for health and disease. BMC Oral Health. 2006;6(Suppl 1):S14.
12. Khalifa W, Nokkaew N, Yang J, et al. Comparison of bacterial viability after exposure to endodontic irrigants. J Endod. 2019;45(7):858-862.
13. Kumar PS. Molecular and microbiologic insights into the oral microbiome and periodontal diseases. Periodontol 2000. 2013;64(1):226-237.
14. Sedgley CM, Lennan SL, Clewell DB. Prevalence, phenotype, and genotype of oral enterococci. Oral Microbiol Immunol. 2004;19(2):95-101.
15. Fouad AF, Barry J, Russo J, et al. PCR-based identification of bacteria associated with endodontic infections. J Clin Microbiol. 2002;40(9):3223-3231.
16. Neelakantan P, Romero M, Vera J, et al. Biofilms in endodontics: current status and future directions. Int J Mol Sci. 2017;18(8):1748.
17. Wang QQ, Zhang CF, Chu CH, et al. Prevalence of enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci. 2012;4(1):19-23.
18. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-398.
19. Ercan E, Dalli M, Dülgergil CT. In vitro assessment of the antibacterial efficiency of various endodontic irrigation solutions. J Appl Oral Sci. 2006;14(5):341-345.
20. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008;34(11):1291-1301.