ORIGINAL ARTICLE

Effect of Traditional and Truss Access Cavity Designs on Residual Bacterial Levels in Mandibular First Molars: An In Vitro Confocal Microscopy Study

Aim *To quantitatively compare residual bacterial levels in the pulp chambers of mandibular first molars prepared using traditional endodontic access cavities (TEC) and truss access cavities (TAC) following standardized cleaning and shaping procedures.*
Methodology *This in vitro experimental study included thirty extracted human mandibular first molars, randomly assigned to two groups (n = 15 each): TEC and TAC. After access cavity preparation, all specimens were inoculated with Actinomyces naeslundii and incubated for 10 days to allow biofilm formation. The distal canals were instrumented using Vortex Blue rotary files with continuous irrigation using 5.25% sodium hypochlorite at a flow rate of 1 mL/min. A final irrigation protocol consisting of 5 mL of 5.25% sodium hypochlorite, 17% ethylenediaminetetraacetic acid, and 5 mL of sterile saline was applied. Specimens were sectioned and analyzed using confocal laser scanning microscopy to quantify residual bacterial presence. Percentage bacterial reduction was calculated, and intergroup comparisons were performed using an independent two-sample t test (α = 0.05).*
Results *The mean (± SD) percentage of bacterial reduction was 32.7% ± 8.0 in the TEC group and 28.4% ± 4.8 in the TAC group. No statistically significant difference was observed between the groups (P = 0.08).*
Conclusion *Under the conditions of this in vitro study, truss access cavities demonstrated bacterial reduction comparable to traditional endodontic access cavities following cleaning and shaping. These findings suggest that truss access designs may be considered a minimally invasive alternative without compromising intracanal disinfection.*

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Introduction

The primary objective of root canal treatment is to eliminate harmful pathogens from the complex root canal system to preserve teeth and restore their function in the oral cavity (1). However, a combination of structural and microbiological factors may lead to failure of endodontically treated teeth (ETT) (2, 3). Structural factors can include loss of tooth structure from extensive caries or size of access cavity preparation, excess removal of radicular dentin during instrumentation, improper selection of post, or proteolytic and demineralizing actions of root canal irrigants (4-6). Microbiological factors can include incomplete debridement of pulpal tissues and remaining dentinal debris or viable microorganisms (7).

Historically, successful endodontic treatment has emphasized the complete removal of caries and the importance of root canal disinfection. Traditional endodontic access cavities (TEC) are commonly used in endodontic treatment and often result in premature, structural failure-derived loss of ETT, but highly flexible heat-treated rotary instruments and enhanced illumination and imaging technology have decreased the overall failure rate (8,9). Recently, the concept of minimally invasive endodontics has been suggested to increase the success of ETT, promote the preservation of hard dental tissues during root canal treatment, and improve longevity and functionality (10). Despite ongoing debate about the size of the access cavity and root canal preparation, no consensus exists regarding the best approach to ETT (11). Endodontic therapy involves cleaning and shaping, disinfection, and 3-dimensional obturation of the root canal system. During root canal treatment, the goal of an access cavity design is to obtain straight-line access for complete debridement of pulp and to enhance the cleaning and shaping of the root canal system (8). When TEC design is used, the roof of the pulp chamber is completely removed to improve access to the coronal part of the root canal system (12). This process frequently results in removal of tooth structure, which weakens the tooth in the pericervical area, and predisposes it to fractures, which may cause the tooth to be nonrestorable (9-11).

Minimally invasive access cavity preparations, also known as conservative endodontic access cavity designs (CEC), are intended to preserve the pericervical dentin by retaining as much of the pulp chamber roof as possible with the aim of improving the fracture resistance of ETT (13). Truss access cavity (TAC) is a type of CEC that involves creating separate access cavities to treat each canal individually. For example, with mandibular molars, one access cavity is made for the mesial canals and another for the distal canals (14). The primary goal of TEC is to maximize dentin preservation by leaving a truss of dentin between access cavities, thus improving the fracture resistance of ETT (13-15). Improper post selection is a well-documented reason for the failure of ETT (16,17), so a benefit of using TAC is a decreased need for post placement for restoring ETT. In addition, TAC has been reported as less time consuming than TEC (18). However, if the access cavity is too small, it can be challenging to locate the orifices of canals. As such, it can be difficult to carry out the necessary cleaning, shaping, and obturation of the root canal system and may lead to endodontic mishaps like transportation and deviation of root canals (6,10,11,18-21). The importance of preserving dentin cannot be overemphasized and must be balanced with disinfecting abilities to achieve successful endodontic treatment. To date, studies have focused on the fracture resistance of ETT with various types of CEC, but there is limited data about the debridement of the root canals using CEC (14,21-24).

Endodontic infections are typically

polymicrobial in nature and often involve several microbial species that work synergistically to cause infection and progression of pulpal disease (25). Actinomycetes bacteria are anaerobic, Gram-positive rods that are frequently seen in primary and secondary endodontic infections. Actinomycetes work synergistically with Pseudopropionibacterium species, leading to persistent extraradicular infections (25,26). Both bacteria are members of the order of Actinomycetota and have the ability to colonize on external root surfaces. This colonization is the primary cause of persistent endodontic lesions, even in the absence of endodontic infection. In particular, the presence of Actinomyces naeslundii in a root canal system has been correlated with failure of endodontic treatment since this bacterium is difficult to eradicate by chemomechanical debridement (27,28). However, a study by Radclifffe et al. (25) reported several concentrations of sodium hypochlorite lowered the number of colony forming units of Actinomyces naeslundii below the detection limit after 10 seconds of irrigation.

Confocal laser scanning microscopy (CLSM) is a reliable method for quantifying bacteria viability and identifying associations between bacterial presence and endodontic infections (29). To quantify bacteria, fluorescent dyes are applied to a biofilm, and they then penetrate the DNA and RNA of bacterial cells. This procedure allows differentiation between living and dead bacterial cells in the root canal system and is useful for studying the antimicrobial efficacy of endodontic treatments before and after instrumentation (30). Given the complexity and bacterial concerns of successful root canal treatment, Neelakantan et al (14) investigated outcomes when using a TEC or TAC design for treatment. They reported debridement of the pulp chambers of mandibular molars was significantly compromised when TAC was performed (14). Because the TAC design retains a truss of dentin between mesial and distal access cavities in mandibular molars, the authors were able to evaluate the pulp chambers for reduction in microbial load (14). They concluded that the presence of inaccessible bacteria beneath the truss may affect overall success of endodontic treatment (14). Although many studies have investigated the association between fracture resistance of ETT and different cavity designs (19,20,22,24,31,32), few have investigated microbial reduction of ETT when comparing TEC and TAC (21,24,33). Further, to our knowledge, no studies have investigated TEC and TAC for root canals inoculated with Actinomyces naeslundii.

Therefore, the purpose of the current study was to evaluate the impact of access cavity types on the quantity of residual bacteria in the pulp chamber of extracted mandibular first molars after cleaning and shaping during root canal treatment. Specifically, we compared TEC and TAC designs for root canals inoculated with A. naeslundii and hypothesized there would be significant differences in the quantity of residual bacteria between designs.

Materials And Methods

Sample Selection and Preparation

Based on a previous study comparing instrumentation efficacy between TEC and CEC [19], we performed a sample size calculation using G*Power 3.1 software for Windows (Heinrich Heine-Universität Dusseldorf, Germany). Using the effect size of 1.04, α of 0.05, 80% statistical power, and allocation ratio of 1:1 from that study (19), we calculated our sample size required at least 26 teeth to observe a significant difference. Based on this outcome, we included 15 teeth for our 2 experimental groups (TEC and TAC).

Sample Size Analysis

To evaluate the impact of TEC and TAC on residual bacteria after cleaning and shaping during root canal treatment,

Figure 1
TEC and TAC designs used on mandibular first molars and identification of the section used for bacterial quantification.

30 non-carious mandibular first molars were used. Teeth had been extracted and stored in 10% formalin until use (14). To be included in the study sample, teeth were examined under a high magnification microscope (Global microscope, St. Louis, MO, USA) for cracks or vertical root fractures in mesial or distal roots (5,31). If a tooth had a history of previously initiated root canals or root canal treatment, the presence of internal or external resorptive defects, or severe calcifications, it was excluded from the sample. Once teeth were selected for inclusion, they were evaluated for anatomical similarity. To verify all teeth were as similar as possible, we measured from the occlusal surface to the root apex and measured the mesiodistal and buccolingual dimensions of crowns (32). Teeth with 2 mesial and 1 distal canal were selected based on periapical radiographs. For further standardization of the study sample, additional periapical radiographs were obtained from mesiodistal and buccolingual angles (31,33). The debridement of hard and soft tissues remnants on teeth was manually performed using an ultrasonic scaler. In addition to the 30 teeth for the TEC and TAC groups (15 per each experimental group), we also included 4 controls in the study. Control teeth were prepared using TEC with normal saline as an irrigant, and 2 autoclaved teeth served as negative controls (20,24).

The 30 teeth were randomly divided into the 2 experimental groups. Next, TEC or TAC was performed on the mandibular molars under a high magnification microscope using a size #4 round carbide bur and an Endo Z bur (Dentsply Sirona Endodontics, Ballaigues, Switzerland) with a high-speed handpiece and copious amounts of water (Figure 1). The TEC was performed according to current guidelines to achieve complete deroofing of the pulp chamber and provide straight-line access to the root canals with no undercuts (12). For TAC, mesial and distal slots of oval cavities were made to gain access over the individual canals on the mesial and distal sides. The roof of the pulp chamber was left intact between the mesial and distal cavities beneath the truss of the tooth (14). Be-

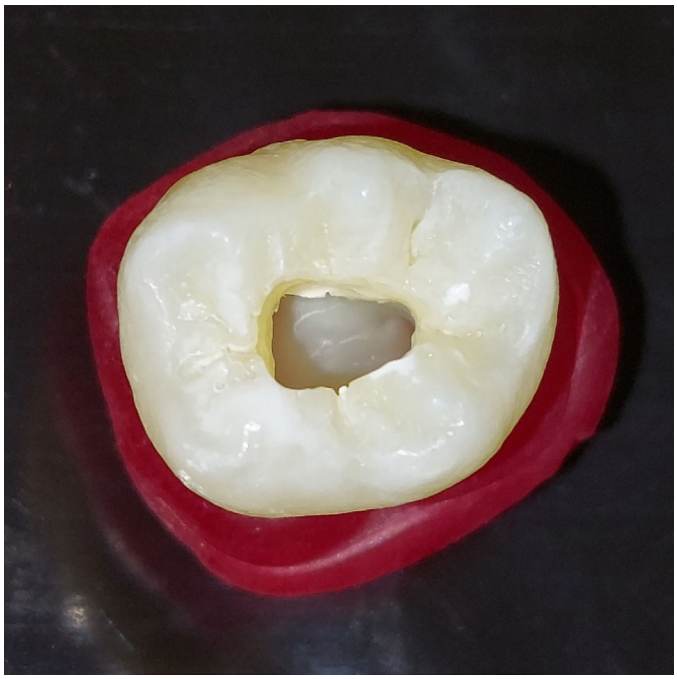

Fig. 1A

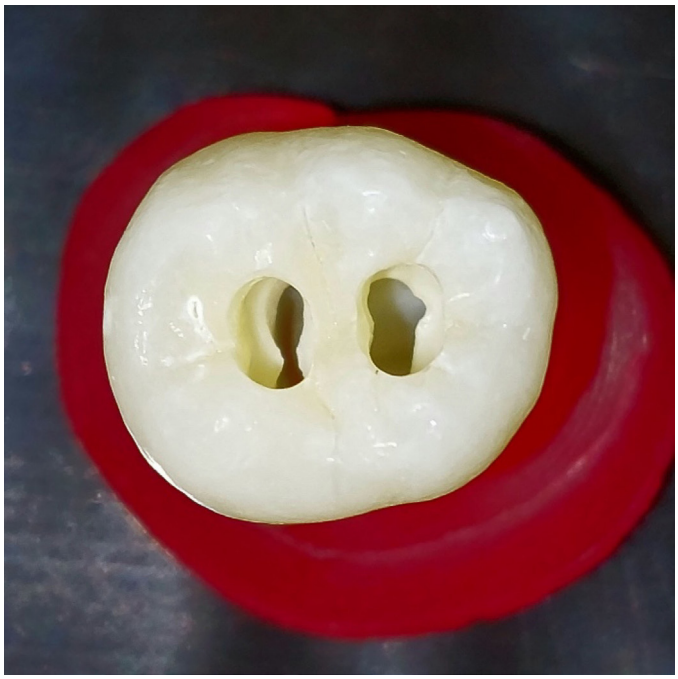

Fig. 1B

cause all teeth in our sample had similar dimensions, we were able to attain some degree of standardization in the size of the access cavities. However, no attempt made to modify the size of the access cavities to gain better access to the canals once cleaning and shaping was initiated. All TEC and TAC procedure were completed by the same endodontics specialist.
The distal canal of the tooth was selected for instrumentation and inoculation of the bacterial sample (Figure 1). The crowns of the teeth were adjusted to have a uniform working length of 18 mm in the distal canals. The apical patency of the distal canal was confirmed by using a #10 K-file (Dentsply Sirona Endodontics, Ballaigues, Switzerland) to advance it out of the apical foramen. The canal was gauged up to a #15 K-file, and the working length was determined to be 1 mm short of the apical foramen. All canals were irrigated using 2 mL of 5.25% NaOCl solution performed at 2 mm short of the working length. To neutralize the effect of the sodium hypochlorite, the teeth were immersed in 10% sodium thiosulfate solution for 4 hours followed by a sterile solution for 20 hours. The external surface of the tooth was painted with 2 layers of nail polish to prevent contamination and to seal the apical foramen (23).

Inoculation of Teeth with Actinomyces naeslundii

Root canals were inoculated with 100 µL of an Actinomyces naeslundii culture (ATCC 12104 LGC, UK). After inoculation, the bacterial suspension was transferred to the working length of the distal root using a manual #15 K-file. The teeth were then incubated by submersion in Eppendorf tubes containing blood heart infusion broth. The incubation time was 10 days under anaerobic conditions. The culture medium was refreshed every 3 days. The growth of Actinomyces naeslundii was confirmed through observation of the turbidity of the solution in the Eppendorf tubes during the incubation period (24).

Root Canal Instrumentation

Teeth were mounted on a metal mounting block, and root canal treatment was performed under a laminar flow hood following strict disinfection protocols. Every treatment was completed by the same individual. All Vortex Blue rotary files (Dentsply Sirona Endodontics, Ballaigues, Switzerland) were attached to a 16:1 gear reduction handpiece (Dentsply, Sirona). Files were used passively to advance into the canal and progress apically. Minimal apical pressure was applied on activation, and 3 easy amplitudes were used in each pass. As suggested by the manufacturer of the Vortex Blue rotary file system, the distal canals in all TEC and TAC teeth were enlarged in a sequence of 15/06, 20/06, 25/06, and 30/06. A standardized volume and duration of irrigant were used for both groups. The canal was irrigated with 1 mL/min of 5.25% NaOCl after every rotary instrument and was recapitulated to working length using a #10 K-file. A hypodermic irrigation needle (30-gauge, side vented, Max-i-Probe, Patterson Dental, USA) was placed passively in the canal and 1 mm short of the apical foramen. The #10 K-file was cleaned with sterile gauze after each recapitulation. A final rinse of 5 mL of 5.25% NaOCl was completed at a rate of 1 mL/min followed by 1 mL/min of rinsing with 17% ethylenediaminetetraacetic acid. An additional rinse was performed using 5 mL/min of sterile saline (34).
The time required to perform the instrumentation and irrigation was recorded and standardized for all (TEC, TAC, and control) groups. Quantification of bacterial load evaluation was performed using CSLM. All teeth were cross-sectioned mesiodistally with a 0.3-mm IsoMet saw at 200 rpm and continuous water. Teeth were stained with BacLight LIVE/DEAD stain (Invitrogen, Carlsbad, CA, USA) for 15 minutes. The CLSM was set at excitation and emission wavelengths of 480 nm

Figure 2
CLSM images of live (green) and dead (red) bacteria in the pulp chambers of teeth using TEC (2A-2B) or TAC (2C-2D) designs for root canal treatment.

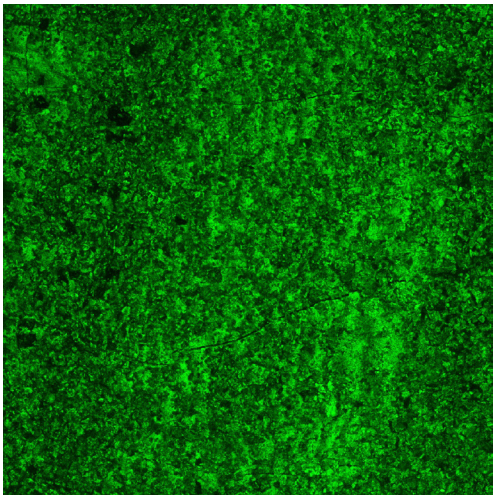

Fig. 2A

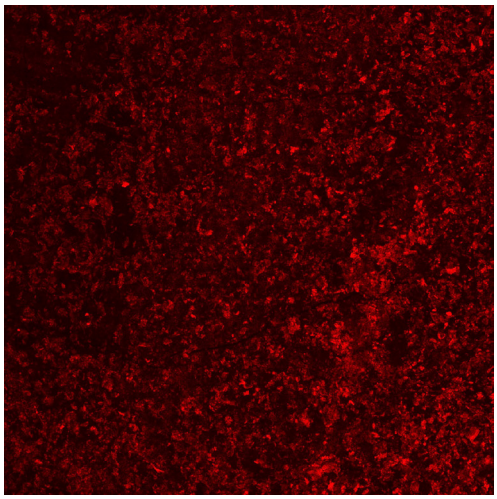

Fig. 2B

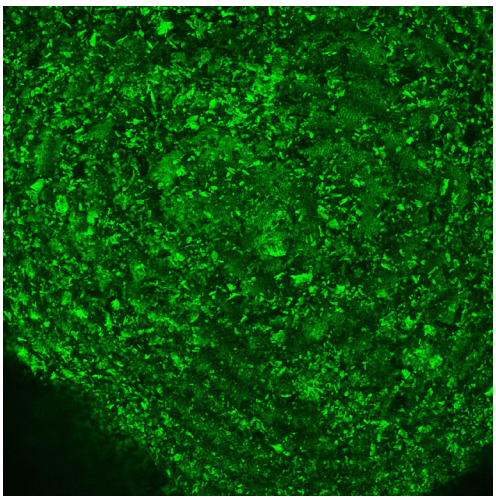

Fig. 2C

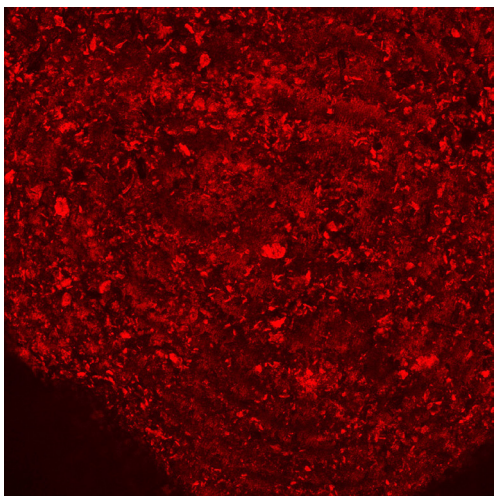

Fig. 2D

and 500 nm. Next, fluorescein diacetate dye was applied, and a 40× magnification oil lens was used to evaluate the teeth. The resulting images were analyzed with ImageJ (NIH, LOCI, University of Wisconsin) to quantify live (stained green) and dead (stained red) bacteria (Figure 2). The measured red and green fluorescence intensities were used to calculate the percentage of dead bacteria for both live and dead bacteria (21). Blinding of the operator was not feasible due to the nature of the access cavity designs. However, the examiner responsible for confocal laser scanning microscopy image analysis was blinded to group allocation.

Statistical Analysis

Bacterial load for all teeth in the TEC and TAC groups was summarized as percentage of bacterial reduction mean and standard deviation (SD). To verify normal distribution of data for percentage of bacterial reduction, a Shapiro-Wilk test was used. For non-normally distributed data, a Wilcoxon-Mann Whitney test was used. For normally distributed data, a 2-sample independent t test was used to evaluate differences in the percentage of bacterial reduction between the TEC and TAC

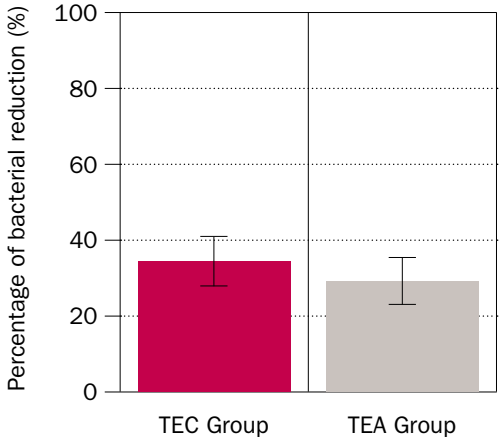

Figure 3
Percentage of bacterial reduction for the TEC and TAC groups.

groups. IBM SPSS Statistics for Windows, Version 27.0 (Armonk, NY; IBM Corp.) statistical software was used for all analyses, and a $P < .05$ was considered significant.

Results

The mean (SD) percentage of bacterial reduction for Actinomyces naeslundii was 32.7 (8.0) with a range of 23.3-48.6 for the TEC group and 28.4 (4.8) with a range of 21.6-39.2 for the TAC group. No difference was found between groups ($P = .08$) (Figure 3).

Discussion

The present in vitro study evaluated the effect of access cavity design on residual bacterial levels in the pulp chamber of mandibular first molars following standardized cleaning and shaping procedures. The results demonstrated no statistically significant difference in the percentage reduction of Actinomyces naeslundii between TEC and TAC. Accordingly, the null hypothesis was rejected. These findings are in agreement with previous laboratory investigations reporting comparable bacterial reduction between conventional and conservative access cavity designs when standardized instrumentation and irrigation protocols are employed (21,23,34).

Effective elimination of intracanal microorganisms is essential for periapical healing and long-term success of endodontic treatment (1). Access cavity design plays a pivotal role in facilitating straight-line access, adequate canal instrumentation, and effective irrigant delivery (18). Traditional access cavity preparation, which involves complete deroofing of the pulp chamber, provides unobstructed access to canal orifices and may enhance irrigant penetration (12). However, this approach is associated with substantial loss of coronal and pericervical dentin, which has been shown to compromise fracture resistance and increase susceptibility to catastrophic failure of endodontically treated teeth (9,13,35).

Fracture remains one of the leading causes of failure in endodontically treated teeth (35). Several studies have demonstrated that conservative access cavity designs, including TAC, improve fracture resistance and result in more favourable fracture patterns compared with TEC (19,32,35). Kishen (35) reported enhanced fracture resistance in teeth prepared with TAC, emphasizing the importance of preserving pericervical dentin. These findings underscore the need to balance structural preservation with effective microbial control to optimize clinical outcomes.

The absence of a significant difference in bacterial reduction between TEC and TAC in the present study suggests that access cavity design alone may have a limited influence on microbial reduction when effective chemomechanical preparation is achieved. Instead, disinfection outcomes appear to be more strongly influenced by instrumentation protocols, irrigant concentration, volume, and contact time. Similar observations were reported by Tüfenkçi and Yılmaz (23), who found no significant difference in Enterococcus faecalis reduction between traditional and conservative access cavities. El-Tayeb and Nabeel (21) also demonstrated comparable bacterial reduction between TEC and TAC, while noting inferior disinfection with ultraconserva-

tive access cavities.

Studies reporting reduced bacterial reduction with ultraconservative access designs suggest that excessive restriction of coronal access may create coronal interferences, limiting effective instrumentation and irrigant delivery (21,33). In contrast, TAC provides independent access pathways to mesial and distal canals while preserving coronal dentin, potentially minimizing such interferences. Neelakantan et al. (14) reported increased residual pulp tissue beneath the truss in TAC preparations but found no significant differences in canal debridement, supporting the concept that adequate canal access can still be achieved with TAC designs.

Instrumentation system selection and canal morphology also influence bacterial reduction. Vieira et al. (20) reported increased residual bacteria with conservative access cavities when using XP-Endo Shaper instruments, possibly due to the interaction between access design and instrument kinematics. Conversely, Krishan et al. (19) reported a higher percentage of untouched canal walls in mandibular molars prepared with conservative access cavities, particularly in oval distal canals, highlighting the importance of instrument selection when employing minimally invasive access designs. The choice of Actinomyces naeslundii as the test microorganism in the present study was deliberate and clinically relevant. Actinomyces naeslundii is a gram-positive, facultative anaerobe frequently associated with persistent primary and secondary endodontic infections and has been implicated in cases of post-treatment apical periodontitis (26–28). Its ability to penetrate dentinal tubules, resist chemomechanical debridement, and survive in harsh intracanal environments makes it a suitable and challenging organism for evaluating disinfection efficacy (26,27). Furthermore, Actinomyces naeslundii has been reported to colonize external root surfaces, contributing to persistent periapical pathology even after technically adequate root canal treatment (28). Therefore, its use in this study enhances the clinical relevance of the findings.

Irrigant-related factors also play a critical role in bacterial elimination. Although NaOCl remains the gold standard irrigant, its antimicrobial efficacy is influenced by concentration, volume, and contact time (28,36,37). Lower concentrations may require prolonged exposure to achieve comparable antimicrobial effects. Given the complex anatomy of the root canal system, including isthmuses and accessory canals, adjunctive irrigation activation techniques such as ultrasonic or laser-assisted irrigation may further enhance disinfection, particularly when conservative access cavity designs are used. Several limitations of this study should be acknowledged. Standardization of canal anatomy was based on periapical radiographs; the use of micro–computed tomography could have provided more precise assessment of canal morphology and reduced anatomical variability. Additionally, only conventional syringe irrigation was evaluated, and adjunctive irrigation activation techniques were not assessed. The in vitro nature of the study also limits direct extrapolation of the findings to clinical conditions. There is a need for future studies focussing on employing novel adjunctives for disinfection (38-40) and also incorporating antimicrobial components into endodontic biomaterials (41).

Within the limitations of this in vitro investigation, bacterial reduction of Actinomyces naeslundii was comparable between traditional endodontic and truss access cavity designs following standardized cleaning and shaping procedures. These findings, in conjunction with existing literature (9,21,23), suggest that TAC may be considered a viable minimally invasive alternative to TEC without compromising intracanal disinfection. Further studies incorporating advanced irrigation strategies, guided access techniques, and three-

dimensional imaging are warranted to optimize disinfection (42), while preserving tooth structure (43).

Conclusion

Within the limitations of this in vitro study, no statistically significant difference was observed in the reduction of Actinomyces naeslundii in the pulp chamber region between TEC and TAC designs following standardized cleaning and shaping procedures. These findings indicate that both access cavity designs provide comparable disinfection of the pulp chamber when effective chemomechanical preparation is performed. Therefore, TAC may be considered a viable minimally invasive alternative to TEC without compromising bacterial reduction.

Clinical Relevance
Preservation of coronal and pericervical dentin is an important consideration in contemporary endodontic practice. The findings of this study suggest that TAC designs can achieve bacterial reduction comparable to TEC when standard instrumentation and irrigation protocols are used. Accordingly, TAC may be clinically adopted in selected cases to support a minimally invasive endodontic approach while maintaining effective root canal disinfection.

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Conflicts of Interest: None

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