Submitted: 21 September, 2022 Accepted: 07 December, 2022 Published: 03 March, 2023 DOI:10.22514/jocpd.2023.009

ORIGINAL RESAERCH

Journal of Clinical Pediatric Dentistry

Effectiveness of pediatric rotary, rotary and reciprocating instrumentations on bacterial load reduction in primary molars: an *ex vivo* comparative study

Esra Oz[1,*], Berna Gulseren Timur[2], Emel Sesli Cetin[3], Goksel Bilir[3]

[1]Department of Pedodontics, Faculty of Dentistry, Suleyman Demirel University, 32040, Isparta, Turkey
[2]Kartal Oral and Health Center, 34880, Istanbul, Turkey
[3]Department of Microbiology, Faculty of Medicine, Suleyman Demirel University, 32040, Isparta, Turkey

***Correspondence**
esrakaraagac@sdu.edu.tr
(Esra Oz)

Abstract

This study aimed to evaluate the efficacy of intracanal *Enterococcus faecalis* reduction using pediatric rotary (EndoArt Pedo Kit Blue, EasyInSmile X-Baby and Denco Kids), rotary (ProTaper Next) and reciprocating (WaveOne Gold) file systems through microbiological analyses in primary molars. Seventy-five mandibular primary second molars were selected and divided into five instrumentation groups and a negative control group. After incubation, five roots were used to confirm biofilm formation on the root canals. Before and after instrumentation, bacterial samples were collected. The bacterial load reduction was statistically analyzed by using Kruskall-Wallis and Dunn post hoc tests at a significance level of 0.05. Denco Kids and EndoArt Pedo Kit Blue promoted higher bacterial reduction than EasyInSmile X-Baby systems. There was no difference in bacterial reduction between ProTaper Next rotary file systems and other groups. Among the single-file techniques, instrumentation with the Denco Kids rotary system showed a more significant bacterial load reduction than WaveOne Gold ($p < 0.05$). All systems used in the study reduced bacterial counts from root canals in primary teeth. Further studies are required to generate more information about the use of pediatric rotary file systems in clinics.

Keywords

Endodontics; *Enterococcus faecalis*; Primary molars; Rotary files

1. Introduction

Anaerobic microorganisms are the main etiological factor of primary endodontic infections in primary teeth [1]. The preferred treatment for irreversible pulp inflammation or necrosis due to caries and trauma might be pulpectomy [2]. The success of pulpectomy depends on effectively eliminating of microorganisms from root canals. Nevertheless, endodontic treatment in primary teeth is complicated due to the physiological root resorption, morphology of root canals, less thickness of radicular dentin, and children's limited cooperation time [3].

In pediatric dentistry, Nickel-Titanium (NiTi) rotary instruments which are designed primarily for permanent teeth have been started to be used routinely to investigate treatment success in primary teeth. The observed advantages were a decrease in mechanical preparation time and effective cleaning and shaping of canals, which resulted in uniform and conical-shaped canals, promoted optimal obturation quality, and increased patient cooperation [3–7]. On the contrary, lateral perforations, microcracks, and fracture risk of instruments were the major concerns of using rotary file systems in primary teeth [8].

Considering the needs in pediatric endodontics, pediatric rotary files have been developed to overcome the problems and improve treatment success [9]. These files working in rotational motions are shorter than rotary file systems manufactured for permanent teeth and have a triangular cross-section. The limited research on pediatric rotary file systems published thus far has reported its use in the shaping and dentin removal of dentin, the taper of preparation, and apical debris extrusion [9–11].

Enterococcus faecalis (*E. faecalis*) is a gram-positive facultative anaerobic bacteria involved in the persistence of the infection in root canal systems of primary teeth [12]. Since *E. faecalis* can colonize and form biofilm structures on root canal walls, difficulties are encountered in its removal during endodontic treatment procedures [13]. Complex root canal systems of primary teeth can cause ineffective instrumentation which can lead to incomplete cleaning of the root canal systems [14]. Many NiTi rotary file systems manufactured primarily for permanent teeth have been used to overcome the limitations and improve cleaning and disinfection procedures in primary teeth. However, few studies have investigated the cleaning effectiveness of NiTi rotary files manufactured primarily for

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permanent teeth in primary teeth using *E. faecalis* [3, 14, 15]. To our knowledge, there is no study evaluating the ability of newly developed pediatric rotary files to reduce *E. faecalis* from root canals in the literature. There is a need to understand the effectiveness of pediatric rotary file systems in reducing microbial load according to the rotary and reciprocating file systems manufactured for permanent teeth. Consequently, this present study aimed to evaluate the efficacy of intracanal *E. faecalis* reduction using pediatric rotary, rotary and reciprocating file systems through microbiological analyses.

The null hypothesis (H0) was that there would be no difference in the effectiveness of the rotary file systems designed for permanent teeth and the pediatric rotary file systems on bacterial load reduction from root canals.

2. Materials and method

The manuscript of this laboratory study has been written according to the Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 Guidelines [16]. The PRILE 2021 flowchart summarizes the key steps (Supplementary material).

2.1 Sample selection

The sample size was calculated using G Power® (v 3.1.9.2., Kiel University, Kiel, Germany). Based on the results of a previous study [17], for an alpha (α) type error of 0.05, a beta (β) power of 0.80, and statistical power of 95%, a minimum of 5 samples per instrumentation group were required to highlight the impact of the intracanal bacterial reduction. The sample size was increased due to the problems that may arise during the study. The final sample was 60 (12 for each group) for the instrumentation group.

For the analysis, primary mandibular second molars (n = 75) were selected from healthy children aged between 4–6 years with a diagnosis of extraction due to pulp necrosis and radiographically visible chronic apical infections. Straight and round mesial canals of primary mandibular second molars were checked by digital radiographs (Visualix Gendex Dental Systems, Monza, Italy) from the mesio-distal and bucco-lingual directions with 65 kVp, 8 mA, and 0.1 sec. The reasons for selecting the mesial roots of primary mandibular second molars were less ramification, and narrower and rounder shape compared to distal roots [18]. Teeth with no canal calcification, no root defects, moderate root angulations according to Schneider classification [19], and at least 2/3 of the root length remaining were included. The teeth with oval root canals, external and internal resorption, root caries, treated root canal, and resorption of >2/3rd of the root were excluded.

2.2 Tooth preparation

Specimens were collected and cleaned of the soft tissue residues with a brush. Teeth were stored in a 0.5% thymol solution at room temperature for 48 h and then kept in distilled water until use in experiments.

In determining the mesial root canal length, an evaluation was made according to the standard root lengths before root resorption [20], and the root length was standardized to be 9 ± 1 mm. Due to extensive caries in the majority of the teeth, the crowns were removed from the enamel-cementum section using a diamond fissure bur (G&Z Instrumente, Lustenau, Austria) under water cooling in order to ensure standardization. Each tooth was sectioned at the furcation. The distal root was discarded, and the mesial was kept for investigation.

The access opening of each root canal was checked, and the working length was established as 1 mm behind the apical foramen with a size 10 K-file (Mani, Tokyo, Japan). Initial instrumentation was performed with a size 15 K-file up to working length to aid contamination of the root canal system with *E. faecalis.* The canal opening of the other mesial root, which was not going to be processed, was covered with compomer filling (Dyract XP; Dentsply De Trey, Konstanz, Germany).

The smear layer was removed with 3 mL of 17% Ethylenediaminetetraacetic Acid (EDTA) and then 3 mL of 2.5% Sodium Hypochlorite (NaOCl) solutions. The teeth were stored in 10% Sodium Thiosulphate ($Na_2S_2O_3$) for 4 h and sterile saline for 20 h. The apical foramina were sealed with nail varnish.

Each of the roots was separately placed in a 1.5-mL Eppendorf tube with a sterilized brain heart infusion broth (BHI) (Difco Laboratories, Detroit, MI, United States of America), and sterilization was performed in an autoclave for 15 min at 121 ℃. After sterilization, the roots were kept in an incubator at 35 ± 2 ℃ for 48 h.

Using a block randomization method, 70 roots were split into 5 instrumentation groups (n = 60, 12 roots for each group) and a negative control group (n = 10, 2 roots for each group). Five roots as a positive control group were used to confirm biofilm formation on the root canals after incubation (Fig. 1).

2.3 Contamination of roots with Enterococcus faecalis

Roots were infected using a 24-h pure culture suspension of an *E. faecalis* strain (ATCC 29212) cultivated in BHI broth. The turbidity of *E. faecalis* strain was adjusted to 0.5 McFarland (1×10^8 CFU/mL) by using the photospectrometric method. Each of the root canals was filled with 5 μL of this *E. faecalis* suspension (5×10^5 CFU/root canal) using sterilized micropipettes; sterilized size 15 K-files were used to transfer the suspension to the root canal length with up-and-down and circumferential movements. Roots were incubated at 35 ± 2 ℃ for 7 days, and during this time, the BHI was removed and refilled every 48 h. The medium's turbidity during the period of incubation confirmed the growth of *E. faecalis*, and the purity of the cultures was tested through the use of Gram staining and colony morphology on Columbia Agar with 5% Sheep Blood (CA-SB) (Becton Dickinson GmbH, Heidelberg, Germany) (Fig. 2).

2.4 Root canal preparation

All root canal instrumentations and bacteria sampling before (S1) and after (S2) root canal instrumentations were performed by a single trained operator (B.G.T.).

Before taking samples from the root canals, the root canal wall was scraped with a sterile size 15 K-file with up-and-down and circumferential movements along the working length to

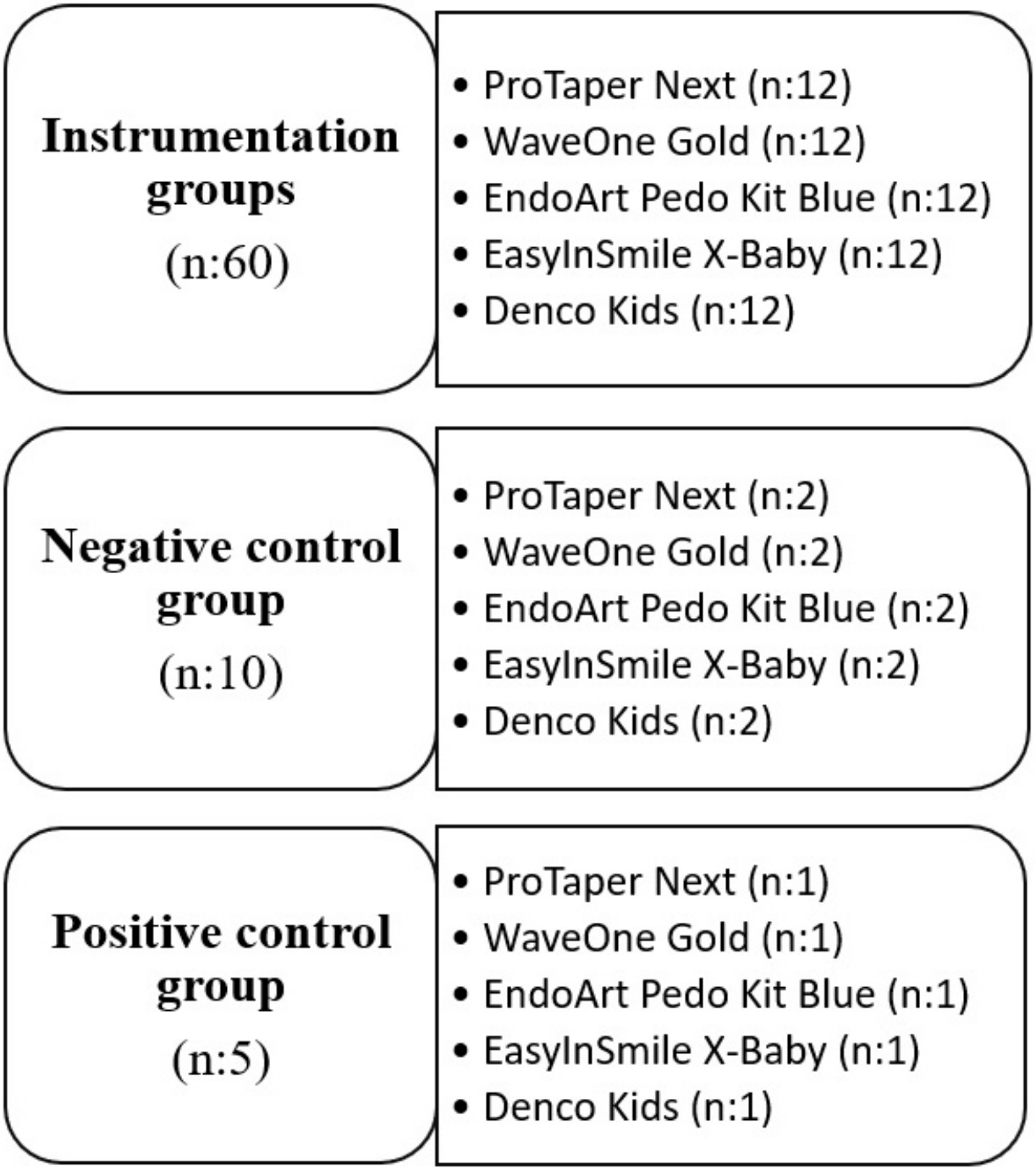


FIGURE 1. Study groups.

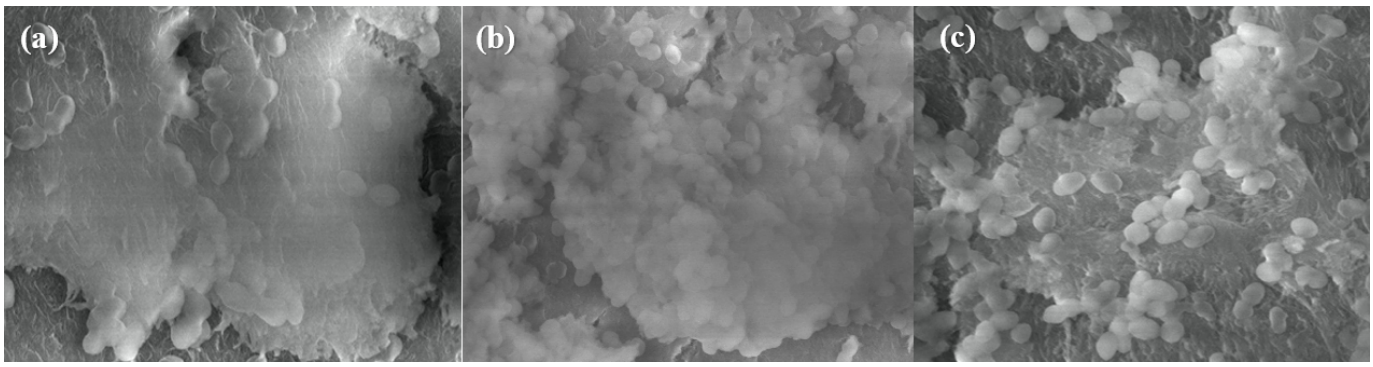
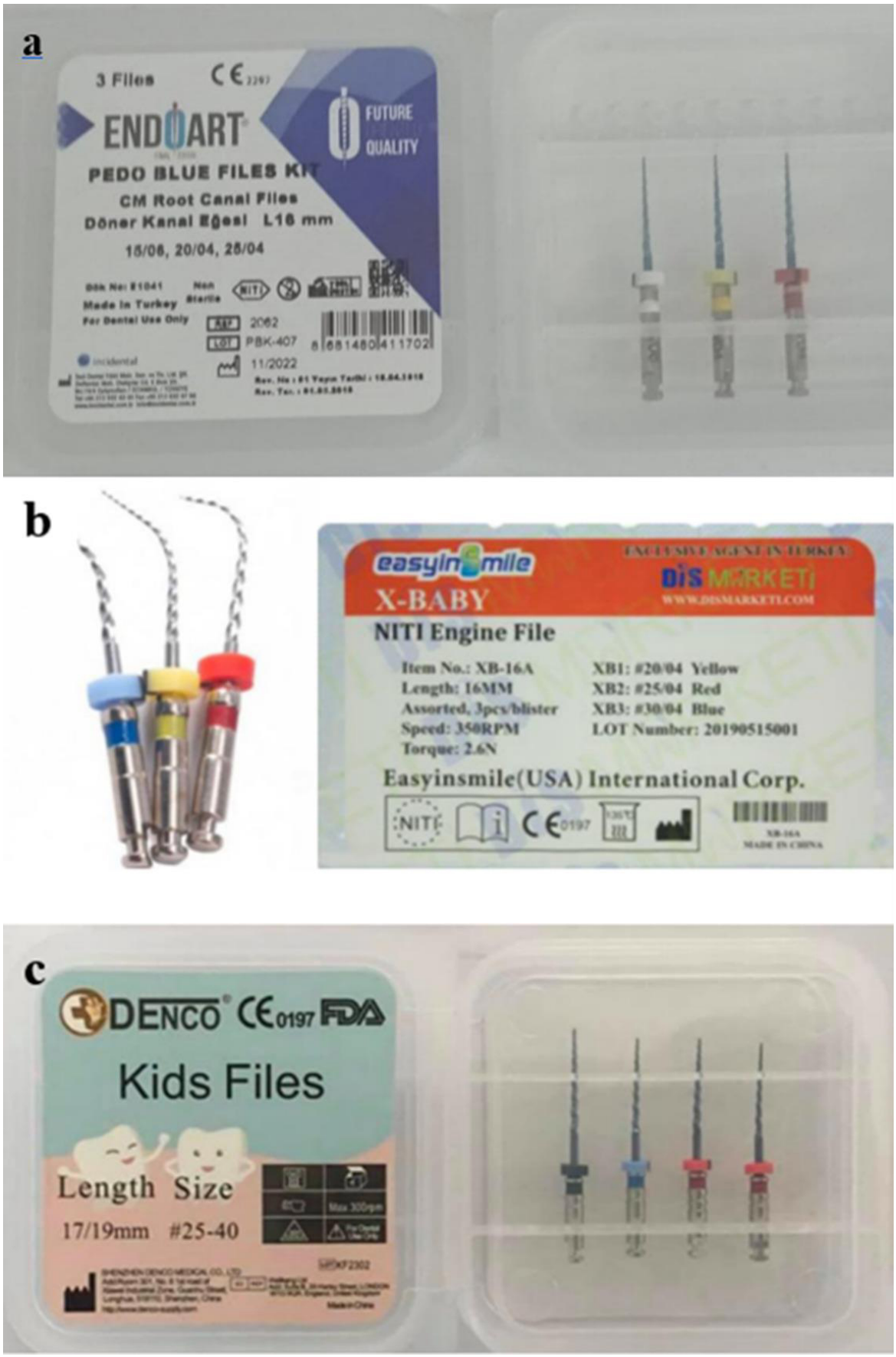


FIGURE 2. SEM images of the biofilm-like structure formed in the coronal (a), middle (b), and apical (c) regions of the root canal (20,000× magnification).



F I G U R E 3. Pediatric rotary file systems used in this study (a. EndoArt Pedo Kit Blue, b. EasyInSmile X-Baby, c. Denco Kids).

release the bacteria attached to the canal walls. First samples (S1) were collected using a sterile size 15 paper point which was placed at the working length for 1 min.

All instrumentations were performed by a torque-controlled endomotor (Woodpecker Motopex; Guilin Woodpecker Medical Instrument Co., Ltd, Guangxi, China). For each group, the speed and torque of the systems were set according to the manufacturer's instructions. The final apical diameter was set to #25 in all rotary systems to establish standardization as stated in pediatric pulpectomy guidelines [21]. WaveOne Gold and Protaper Next have a taper of .07-mm in reciprocal motion and .06-mm in rotational motion, respectively, with a tip size of #25. The ProTaper Next rotary instrument system uses two files to prepare the apical dimension of the root canal to #25, while the WaveOne Gold system uses one file. All pediatric rotary systems were prepared up to #25/.04. To prepare the apical dimension of the canal at #25, the EndoArt Pedo Kit Blue rotary file system consists of three files, the EasyInSmile X-Baby system consists of two files, and Denco Kids is a single-file system (Table 1) (Fig. 3). In all rotary instrument systems, the mechanical preparation of root canals was performed using the crown-down method. Root canals were irrigated with 3 mL of saline for each canal during and after instrumentation, using the syringe which was inserted 1 mm back from the working length. Two non-contaminated root canals for each group were instrumented for each of the preparation techniques and were used as negative controls. To overcome the risk of instrument fracture, the number of uses for each instrument was limited to three times. After the rotary instrumentation process was completed, sterile size 20 paper points were placed and were left for 1 min in the root canals, then second bacterial samples (S2) were performed.

2.5 Bacterial load counts

The paper points used to collect the bacteria samples were inserted into the tubes containing 1 mL of 0.85% saline solution, and vortexed for 1 min, and then diluted 10-fold in sterile saline. Each diluted sample of 0.1 mL was placed onto CA-SB agar plates and incubated at 35 ± 2 °C for 24 h. The amount of countable bacterial growth was counted as colony-forming units (CFUs), and the number of CFU per mL was determined by multiplying the dilution factor of the counted plate.

2.6 Scanning electron microscopy analysis

Using a block randomization method, five selected roots after contamination with *E. faecalis* (positive control group) and two roots from each experimental group after bacteria sample collection were fixed in 10% formalin for 1 week, fractured longitudinally, and dehydrated in ethanol solutions (70%, 90% and 100%). Coronal, middle, and apical third regions of the root canal walls were evaluated topographically for biofilm formation by a Scanning Electron Microscope (SEM) under 10,000× and 20,000× magnifications for the prepared samples.

2.7 Statistical analysis

Data were analyzed with the SPSS Statistics™ program (version 23.0, SPSS Chicago, IL, abbreviation of state, USA). For the microbiological analysis, the counts of *E. faecalis* bacteria in CFU/mL were transformed to a logarithmic base of 10. The effectiveness of rotary file systems on microbial load reduction was analyzed statistically by using Kruskall-Wallis and Dunn post hoc tests. p value < 0.05 was considered statistically significant.

3. Results

After one-week incubation period, biofilm-like structures were observed in the SEM images of the samples taken from the root canal walls of the teeth infected with *E. faecalis* (Fig. 4). In the negative control groups, no bacterial growth was observed. There were statistically significant differences in bacterial reduction after instrumentation of the root canals ($p = 0.003$). The comparison between pediatric rotary file groups showed that Denco Kids and EndoArt Pedo Kit Blue promoted higher bacterial reduction compared to the EasyInSmile X-Baby systems, but no significant difference was found between Denco Kids and EndoArt Pedo Kit Blue ($p > 0.05$). There was no difference in bacterial reduction between ProTaper Next rotary file systems and other groups. Among the single-file techniques, instrumentation with the Denco Kids pediatric rotary file system showed a more significant bacterial load reduction compared to WaveOne Gold ($p < 0.05$) (Table 2).

In the present study, in the SEM images of root canals in which canal shaping was performed by rotary file systems, it was observed that *E. faecalis* was not completely removed from the coronal, middle, and apical third regions of the root canals; the dentinal tubules were plugged with debris in the middle and apical regions, and the dentinal tubules were partially open in the coronal region (Figs. 5,6).

4. Discussion

Mechanical preparation is an important step for successful endodontic treatment in the removal of microorganisms from the infected root canals. In pediatric endodontics, many NiTi rotary file systems manufactured for permanent teeth have been used in order to perform root canal shaping easily in a short time. Some of them have multi-file concepts in rotation motion, while others work with reciprocating single-file system [22]. In this study WaveOne Gold, a NiTi single-file instrumentation system working with a reciprocating kinematics and ProTaper Next NiTi multiple file system working with rotational motion were preferred.

The WaveOne Gold single-file reciprocating motion system, which undergoes heat treatment during its production and is produced by Gold Wire® alloy, is similar in length to manual files and is simpler to use with younger children due to its short file handle [23]. It has been stated that its use in primary teeth promotes less canal transportation and faster canal preparation compared to rotary instrumentation systems with rotational motion, which may be due to the fact that the system grasps less dentin tissue and has a better canal-centering ability [24].

The ProTaper Next multiple-file rotary system, which oper-

TABLE 1. The torque, speed and used files of rotary systems.

Groups	N	Files	Working principles
ProTaper Next (PTN; Dentsply Maillefer, Ballaigues, Switzerland)	12	X1 (17/.04), X2 (25/.06)	300 rpm, 2.0 Ncm torque, Rotational motion
WaveOne Gold (WOG; Dentsply Maillefer, Ballaigues, Switzerland)	12	Primary 25/.07	300 rpm, 2.0 Ncm torque, Reciprocal motion
EndoArt Pedo Kit Blue (Inci Dental, Istanbul, Turkey)	12	15/.06, 20/.04, 25/.04	300 rpm, 1.5 Ncm, 1.0 Ncm, 1.0 Ncm torque, respectively, Rotational motion
EasyInSmile X-Baby (EasyInsmile International Corp., Çangşa, China)	12	20/.04, 25/.04	350 rpm, 2.5 Ncm torque, Rotational motion
Denco Kids (Yesil Dental, Istanbul, Turkey)	12	25/.04	300 rpm, 2.0 Ncm torque, Rotational motion

TABLE 2. Bacterial reduction in samples after being prepared with rotary file systems (median, standard deviation, minimum-maximum log10 CFU/mL).

Rotary File System	n	Before (S1)	After (S2)	Reduction (S1–S2)	Reduction Min-Max	Percentage of reduction (%)
Protaper Next	12	6.00 (0.89)	4.17 (0.94)	5.98^{ab} (0.89)	4.30–7.60	0.945
WaveOne Gold	12	5.60 (0.46)	4.65 (1.07)	5.45^{b} (0.47)	4.59–6.00	0.857
EndoArt Pedo Kit Blue	12	6.10 (0.69)	4.50 (1.05)	6.00^{a} (0.68)	4.95–7.23	0.936
EasyInSmile X-Baby	12	5.40 (0.47)	3.75 (0.67)	5.15^{b} (0.48)	4.95–6.00	0.967
Denco Kids	12	6.70 (1.04)	4.00 (0.81)	6.66^{a} (1.06)	4.95–8.30	0.969

SD: Standard Deviation, Min: minimum, Max: maximum, Different letters denote statistically significant differences.

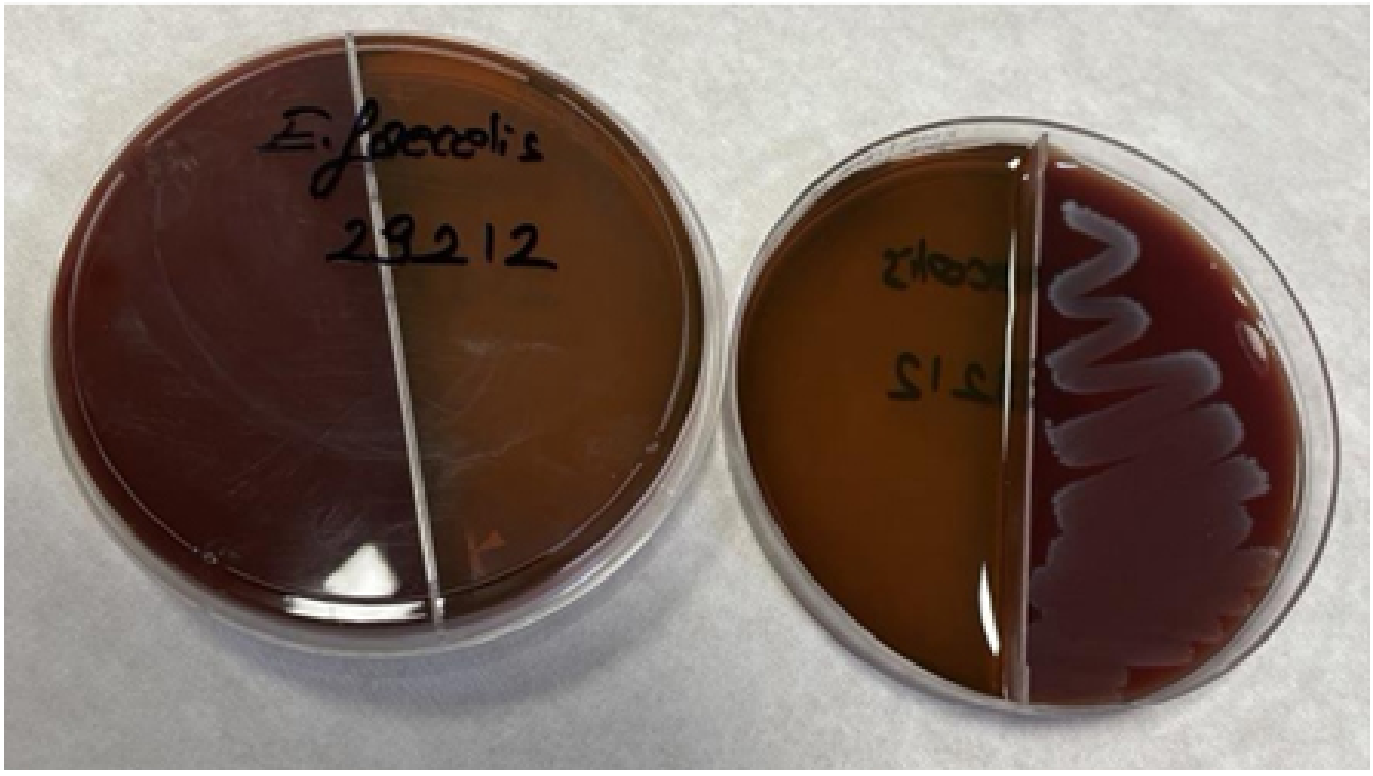


FIGURE 4. *E. faecalis* strain cultured on blood agar medium.

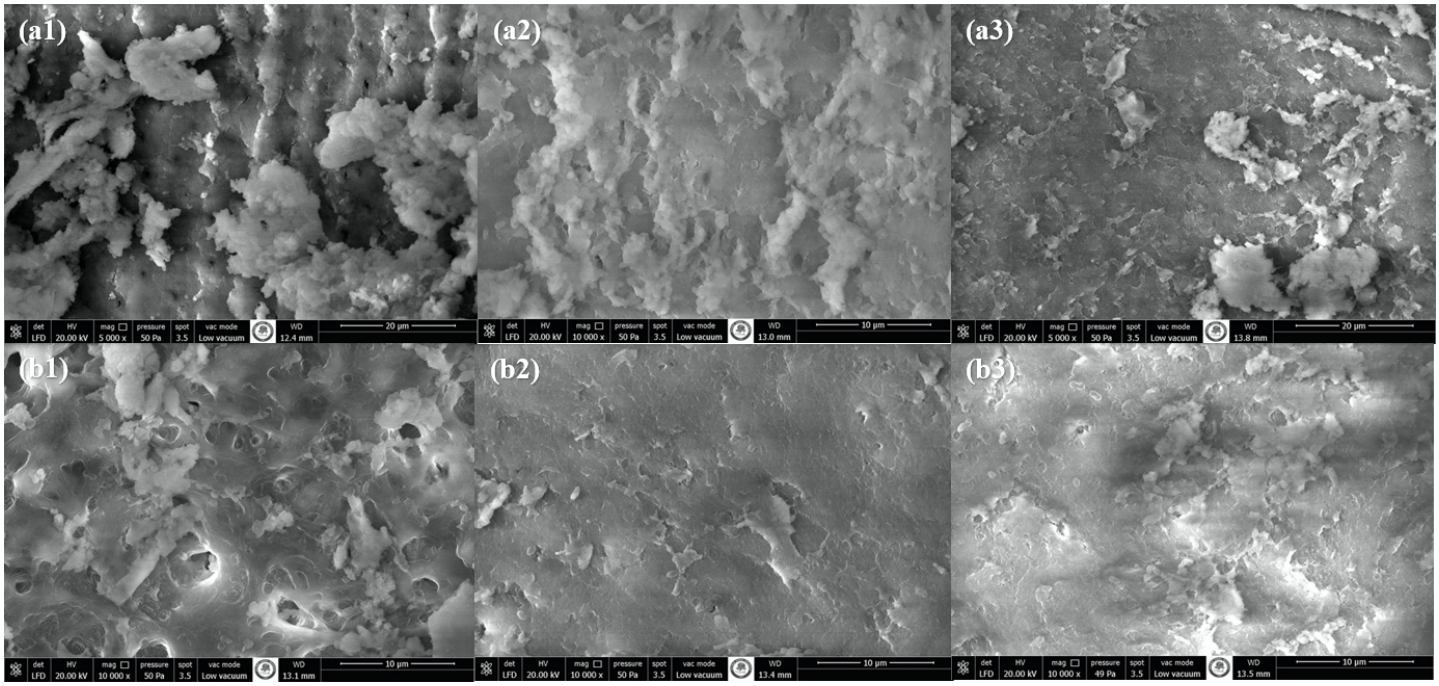


FIGURE 5. Instrumentation with Protaper Next (a1; coronal, a2; middle, a3; apical) and Waveone Gold (b1; coronal, b2; middle, b3; apical) (10,000× magnification).

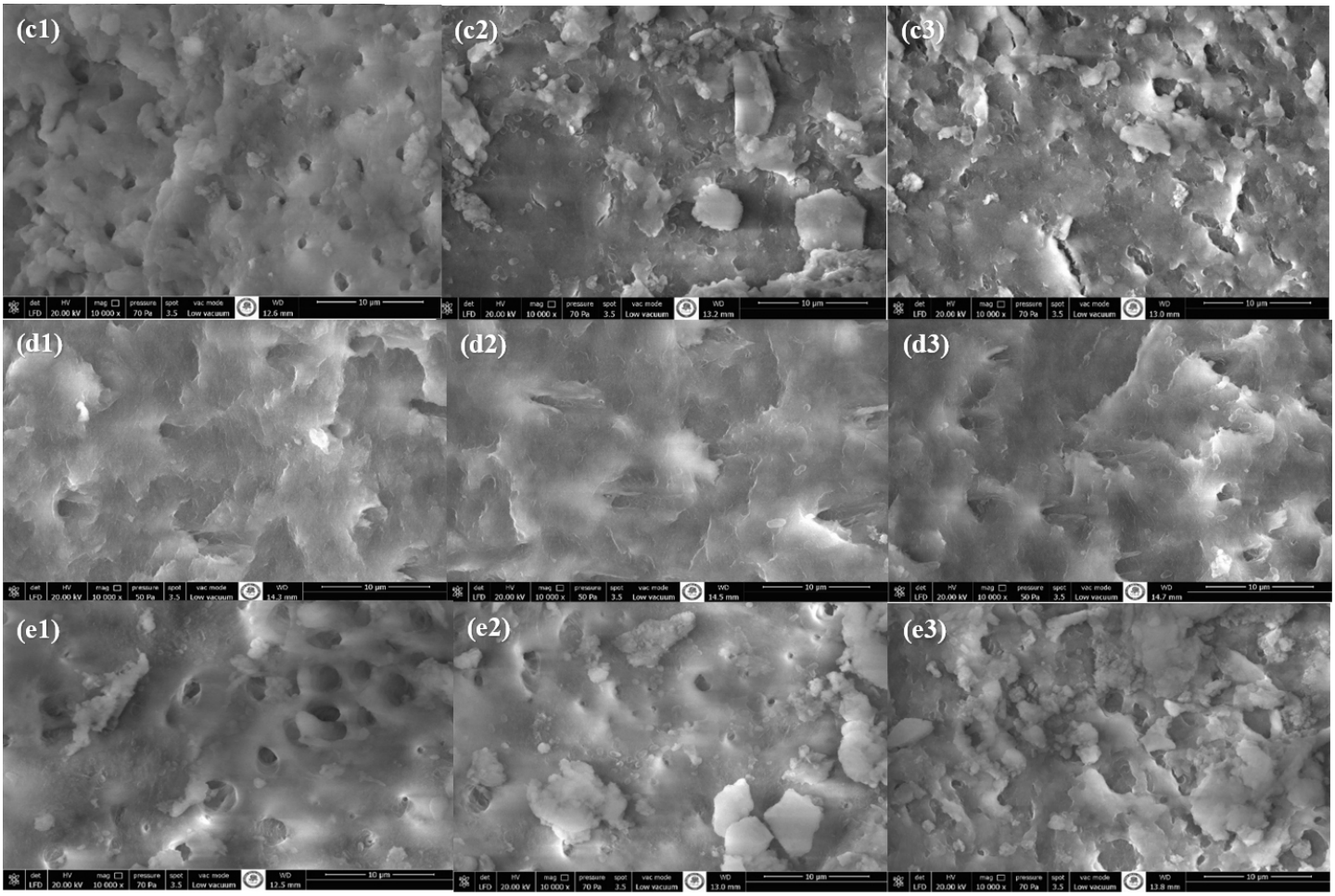


FIGURE 6. Instrumentation with EndoArt Pedo Kit Blue (c1; coronal, c2; middle, c3; apical), EasyIn Smile X-Baby (d1; coronal, d2; middle, d3; apical), Denco Kids (e1; coronal, e2; middle, e3; apical) (10,000× magnification).

ates with rotation motion, has an off-centered, rectangular, and cross-section design with M-Wire® alloy, which contributes to its increased flexibility. This design reduces the contact between the file and the canal wall, reducing the screw-in and taper-lock effect and allowing it to adhere to the root canal anatomy during shaping [25]. In addition, ProTaper Next has been shown to be the least time-consuming rotary file system in terms of shaping and cleaning the root canals of primary molar teeth [26].

Pinheiro *et al.*[3] (2012) evaluated the effectiveness of manual, hybrid, and rotary instrumentation techniques for removing *E. faecalis* from the root canals of primary molar teeth using the ProTaper rotary and K-type files. It was reported that the hybrid system was the most effective technique. The researchers stated that bacterial load reduction was affected by the increased exposure of microorganisms to irrigation solutions, which was supported by the simultaneous movements of manual and rotary instruments [3].In addition, another study reported that both the ProTaper and WaveOne rotary file systems had lower rates of bacterial load reduction in the root canals of primary teeth (ProTaper 89.36%, WaveOne 78.10%), with no statistically significant difference between them [14]. In our study, there was no difference in bacterial reduction between ProTaper Next and WaveOne Gold rotary file systems. Although there is no study evaluating the effectiveness of removing *E. faecalis* from the root canals of primary teeth using ProTaper Next and WaveOne Gold rotary file systems, in accordance with the data obtained from these studies [3, 14], it is thought that several factors, such as the primary teeth and the anatomy of root canals used in the study, the selected incubation time, bacterial strains, irrigation solutions, methods of bacterial collection, preparation technique, and the design and kinematics of the instrumentations, may be responsible for the results.

Üreyen *et al.* [27] (2019) evaluated the efficiency of using chemomechanical preparation with single-file systems (WaveOne Gold, Hyflex EDM and XP-endo Shaper) in reducing *E. faecalis* within the straight and round root canals of mandibular premolar teeth. They found that Hyflex EDM and XP-endo Shaper resulted in a significantly higher bacterial reduction than WaveOne Gold [27]. de Brito *et al.* [28] (2016) evaluated the bacterial reduction achieved by ProTaper Next, ProTaper Universal and WaveOne systems in root canals of mandibular premolars contaminated with *E. faecalis*. The WaveOne/saline solution group showed a lower level of bacterial reduction than the other groups. Groups in which irrigation was performed with NaOCl showed a higher level of bacterial reduction when compared with saline solution irrigation. This study showed that effective irrigation solutions are more important in terms of antibacterial activity during mechanical instrumentation, regardless of which method is used [28]. Considering the anatomical, chemical and histological differences between permanent and primary teeth, the results of these studies on permanent teeth cannot be transferred to primary teeth.

Considering the tapers of the rotary file systems used, it is considered an acceptable result that the WaveOne Gold system had the highest bacterial load reduction since it has the highest taper (.07). However, there was no statistically significant difference in bacterial reduction efficacy between the ProTaper Next and WaveOne Gold rotary file systems in this study. This result supports the idea that taper does not affect the shaping and cleaning efficiency of root canals [29]. The WaveOne Gold file system creates higher amounts of debris, constricting the debris laterally in the isthmus and protrusions of the canal walls, resulting in an inability to remove intracanal bacteria [30]. Considering that root canals in primary teeth have a thin dentin structure, creating areas susceptible to perforation using files with a higher taper causes higher stress on root canal walls (effect of on formation of dentinal cracks). As such our study shows that the use of files with larger tapers is not more effective in removing intracanal *E. faecalis.*

When rotary file systems produced for permanent teeth were used in canal preparation of primary teeth, 76.5% of the root canal surface remained unprepared [31]. It was found that to design pediatric rotary file systems that were more suitable for the structural features and root anatomy of primary teeth and that could reach even the narrowest root canals of primary teeth, the features of files, such as appropriate length, taper, and flexibility were important [32]. The pediatric rotary file systems used in this study have similar designs (triquetrous) and kinematic properties, and the tapers and apical sizes (25/.04) of the master apical file used in the preparation are the same. The differences between the groups in terms of the amount of bacterial load reduction support the view that the number of files used in the systems is a factor that affects bacterial reduction efficacy in root canals [30]. The highest intracanal bacterial reduction occurred after preparation with the Denco Kids pediatric rotary system using a single-file. Considering that single-file rotary systems have the advantages of shortening the treatment time and improving the safety of the shaping procedures [7], it can be said that the Denco Kids rotary file systems can be used more effectively than the other systems tested in this study in endodontic treatment of primary teeth. It was also found that this file system is superior to WaveOne Gold, the single-file system for permanent teeth, in terms of bacterial load reduction. In our study, although there were no differences in the amount of intracanal *E. faecalis* removed between the ProTaper Next and other rotary file groups, it is thought that the use of the former may be limited in root canal treatments of primary teeth, as it uses two files and time is an important factor during endodontic treatment in children. Because no study has examined the effectiveness of pediatric rotary file systems on bacterial load reduction in primary molars, the results of this study cannot be directly compared with other studies.

In the present study, the crown-down technique was used for canal preparation. This method depends on the early flaring of the coronal area of the canal, which may increase the amount of debris directed toward the orifice area during preparation. While the rotary file systems working with the principle of the rotational movement mostly perform shaping in the coronal and middle third regions of the root canals, the final files of these systems can generally perform shaping in the apical third region of the root canals [33]. Because the ProTaper Next rotary file system shapes the dentin during the rotatory motion by gripping it with a larger circumference than its own, there is less compression on the canal walls, and

hence does not result in constriction in the dentin tubules [34]. This finding partially overlaps with what was observed in the SEM images taken from the sample that used the ProTaper Next file system in our study. In the SEM images taken after shaping with the WaveOne Gold rotary file system, it was observed that the dentinal tubules were covered with a smear layer in the middle and apical third regions of the root canal. This can be attributed to the fact that WaveOne Gold file systems, which work with reciprocating motion, create more debris and smear layer than rotary motion systems and pushes the debris along the dentinal walls toward the lateral canals and apex with each reciprocating motion [27]. The SEM images of root canals instrumented with pediatric rotary files were found to be similar, but the dentinal tubules were plugged with more debris, especially in the coronal region of the EasyInSmile X-Baby group, in which less bacterial reduction was observed compared to the groups instrumented with other pediatric rotary file systems. Bacteria was still apparent in the dentine layers of all samples.

In this study, there were statistically significant differences in bacterial reduction after instrumentation of the root canals. Thus, the null hypothesis was rejected.

Limitations of this study include that only the mesial canals of mandibular second primary molars were taken into account, small amounts of live bacteria cannot be detected due to the culture method used and bacteria located areas such as isthmus may not be detected by paper point sampling, it is likely that the results would change under clinical conditions due to *E. faecalis* interacting with other microorganisms. Considering the mixed bacterial flora in the clinical settings, the obtained data cannot be directly extrapolated to the clinical conditions. In addition, the obtained results depend on the experience of the observer. Not applying to blind of the evaluator increased the risk of bias. Despite these limitations, as the first study to our knowledge to evaluate the rates of removal of *E. faecalis* from root canals by the rotary file systems developed specifically for primary teeth, this study provides significant insights to physicians for adapting the findings obtained from the study to clinical conditions and serves as a stepping stone for other studies to be conducted in the future.

The lack of studies regarding bacterial load reduction using rotary and pediatric rotary file systems in primary teeth and the obtained results in this study emphasize the importance of more research in this field. Further investigations should be performed to identify the reduction of *E. faecalis* in root canals of primary teeth by using different file systems and irrigation solutions.

5. Conclusions

All systems used in the study reduced bacterial counts from root canals in primary teeth. Although there was no difference in terms of the amount of bacterial load reduction between the rotary file systems produced for permanent teeth, we concluded that using a single-file in pediatric rotary systems can be more effective than using multiple files due to the characteristics of the root canals and the shortening of the treatment period when single files are used.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author.

AUTHOR CONTRIBUTIONS

EO and ESC—designed the research study. BGT and GB—performed the research. EO and BGT—wrote the manuscript. All authors analyzed the data. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Research Ethics Committee of the University of Suleyman Demirel, Isparta, Turkey (2021/313) and conducted by the Declaration of Helsinki. All parents/caregivers of the children provided written informed consent before collecting extracted teeth.

ACKNOWLEDGMENT

The authors are grateful to Ozgur Koskan, Assoc. Prof. at Isparta University of Applied Science, Faculty of Agriculture, Department of Animal Science, who assisted with the statistical analyses of this study.

FUNDING

The present study was supported by Department of Scientific Research Projects, Suleyman Demirel University (Grant/Award Number: TDH-2021-8394).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.jocpd.com/files/article/1631487266636349440/attachment/Supplementary%20material.docx.

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How to cite this article: Esra Oz, Berna Gulseren Timur, Emel Sesli Cetin, Goksel Bilir. Effectiveness of pediatric rotary, rotary and reciprocating instrumentations on bacterial load reduction in primary molars: an ex vivo comparative study. Journal of Clinical Pediatric Dentistry. 2023; 47(2): 30-39. doi: 10.22514/jocpd.2023.009.