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RESEARCH ARTICLE

THE EFFECT OF TWO ROTARY SYSTEMS VERSUS MANUAL INSTRUMENTATION ON THE REDUCTION OF ENTEROCOCCUS FAECALIS COUNT IN THE ROOT CANAL (AN IN VITRO STUDY)

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ABSTRACT

Introduction: The development of new endodontic technology is aimed at increasing ease and practicality. However, the basic principles of disinfection should also be considered because the presence of residual necrotic tissues and bacteria in the root canal has a direct effect on the outcome of endodontic therapy.

Aim of the study: To evaluate the effect of two rotary systems versus manual instrumentation on the reduction of *Enterococcus faecalis* count in the root canal.

Materials and Methods: Forty extracted mandibular premolars with single canals were inoculated with *Enterococcus faecalis* suspension (ATCC 29212). Specimens were divided according to the instrumentation technique into four parallel groups (n=10). Group I: ProTaper Next, Group II: One Shape Apical, Group III: K-Flexofiles and Group IV: No instrumentation (control group). Irrigation was performed using sterile saline solution. Bacterial samples were taken before, immediately after and one week after instrumentation. The samples were cultured on blood agar plates, incubated and the colony forming units were counted. Data was collected and then statistically analyzed.

Results: Significant bacterial reduction was observed in Groups I, II and III compared to the control group. Groups I, II and III also showed significant reduction in the bacterial count in immediate and final samples compared to the initial samples. No significant difference was found between the three groups immediately after instrumentation. However, Group I showed significantly higher bacterial reduction one week after instrumentation, compared to the initial sample and the other three groups.

Conclusions: All instrumentation techniques were equally significantly effective in reducing intracanal *E. faecalis* count immediately after instrumentation. After one week, bacterial growth was observed in all the groups; however, ProTaper Next significantly demonstrated the least amount of bacterial growth compared to One Shape Apical and K-Flexofiles.

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INTRODUCTION

Mechanical instrumentation, antimicrobial irrigation and intracanal medicaments are the commonly employed methods to eliminate intracanal microbial load (Byström and Sundqvist, 1981; Siqueira *et al*., 1999; Shuping *et al*., 2000).

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Microorganisms involved in primary endodontic infections are polymicrobial in nature and mainly include gram-negative anaerobic rods (Baumgartner and Falkler, 1991). However, persistent and secondary root canal infections mainly include a single species of predominantly gram-positive organisms. *Enterococcus faecalis* is usually the most commonly found bacterial species in such cases (Sundqvist *et al*., 1998). *Enterococcus faecalis* makes up a small proportion of the flora in untreated canals; however, it has a major role in the development of persistent peri-radicular lesions after root canal

treatment (Sundqvist *et al*., 1998) Enterococci have the ability to grow in the presence or absence of oxygen, penetrate deep into dentinal tubules and survive adverse environmental conditions such as extreme alkaline pH, high temperatures and scarce nutrition (Evans *et al*., 2002; Rôças *et al*., 2004; Pinheiro and Mayer, 2014; Chivatxaranukul *et al*., 2008). Since *E. faecalis* is the most resistant species among intracanal bacteria and in turn, has a direct effect on the outcome of root canal treatment, it was chosen for this study as a marker for bacterial disinfection. Several studies were conducted to compare the effect of rotary and manual instrumentation in mechanically reducing the bacterial count within root canals. Some have found no significant difference in bacterial reduction between manual and rotary techniques (Siqueira *et al*., 1999; Matos Neto *et al*., 2012), while others found rotary files to be more effective (Colak *et al*., 2005; Gorduysus *et al*., 2011). Two recently introduced rotary systems are ProTaper Next and One Shape Apical. ProTaper Next is a multiple file system while One Shape Apical is a single-file system. The file(s) of each system is unique in its design, degree of taper and type of motion in the canal, which in turn, may have a direct effect on the efficacy of each system in removing intracanal bacteria (Ruddle *et al*., 2013). Few studies were performed on the mechanical reduction of intracanal bacteria by ProTaper Next and to the best of our knowledge, no studies were performed on One Shape Apical regarding this means. Thus, our question is which of these rotary systems will be more efficient in mechanically reducing *E. faecalis* count in the root canal when compared to manual instrumentation? Our hypothesis is that there will be a difference in the amount of bacterial reduction by these instrumentation techniques due to the differences in their design and taper.

MATERIALS AND METHODS

This study was conducted in the High Institute of Public Health, Alexandria University, Egypt, from October 2015 to December 2015.

Preparation of teeth

Forty extracted human mandibular premolar teeth, with straight single roots, single canals and mature apices were selected for this study. The teeth were cleaned by a brush and rinsed with tap water to remove any tissue remnants, debris and blood on its surface. The teeth were then placed in a sterilization bag and sterilized in an autoclave at 121°C for 20 minutes and immediately stored afterwards in sterile saline to prevent its dehydration till the time of use. Conventional access opening was prepared in each tooth and the pulp tissue was totally removed. A size #10 K-Flexofile was inserted into the canal until its tip appeared just past the apical foramen to establish apical patency and this initial length was recorded. Working length was determined by subtracting 1 mm from this initial measurement. Teeth included in the study were only those in which the initial binding file in the canal was K-Flexofile size #15 or less. Canals were then instrumented under irrigation with sterile saline solution prior to insertion of each file size; from size #10 to size #20 K-Flexofiles, to provide enough space for injecting the bacterial suspension. The apical foramen and the external root surface of all teeth were coated with epoxy resin and left to dry to completely seal the root surface and prevent bacterial leakage during inoculation and mechanical instrumentation of the root canals. To facilitate handling, each tooth was mounted in a vertical position inside a sterile Eppendorf. This was done by preparing a hole in the center of each Eppendorf using a tapered fissure bur. The diameter of each hole was prepared to be slightly smaller than the cervical area of each tooth respectively. This allowed the insertion of the tooth through the hole by friction under pressure, up to the cementoenamel junction in order to be fixed in place, with the crown outside the Eppendorf and the root within it. Epoxy resin was again applied at the interface between the tooth and Eppendorf to avoid the leakage of bacteria. Each Eppendorf; with the tooth fixed within it, was placed in a sterilization bag and sterilized in an autoclave at 121°C for 20 minutes.

Preparation of the bacterial suspension

Enterococcus faecalis (ATCC 29212) was sub-cultured on a blood agar plate (Oxoid Ltd. Basingstoke, Hampshire, UK) and then incubated in microaerophilic conditions at 37°C for 24 hours by storing it in a firmly closed metal container with a candle inside it to consume oxygen and to maintain a 5% carbon dioxide concentration. After incubation, a suspension of *E. faecalis* in sterile saline was then prepared to a standard concentration of 0.5 McFarland ($1.5x10^8$ CFU/mL) and vortexed for 1 minute to obtain a homogenous suspension of the bacterial solution.

Inoculation of root canals with the bacterial suspension

Two layers of parafilm were wrapped around the coronal portion of each Eppendorf to maintain an appropriate seal, thus providing a reservoir for bacterial growth. Eppendorfs were placed standing vertically in a flynn base inside a sterile plastic container, with distilled water underneath to prevent dehydration of teeth during incubation. Each root canal was then injected with 250 µL of *E. faecalis* suspension using a 1 mL insulin syringe '30G' (BD, Franklin Lakes, USA), then carried by a sterile size #15 K-Flexofile to the full working length of the root canal. The plastic container, with the Eppendorfs inside, was firmly closed and incubated aerobically at 37°C for 24 hours to confirm complete contamination of the root canals.

Grouping

The samples were divided into four equal groups according to the instrumentation technique used and were labeled to facilitate identification.

Group I: Instrumentation using rotary **ProTaper Next files** (Dentsply Maillefer, Ballaigues, Switzerland)

Ten teeth were prepared by three ProTaper Next files; X1, X2 and X3 (#17/0.04, #25/0.06 and #30/0.07 respectively), with X-Smart motor in continuous rotation motion at a constant and stable speed of 300 rpm and a torque of 2 N.cm torque. Each file was used progressively down to the working length in a brushing motion and withdrawn once the working length was

reached. Apical patency was checked using size #10 K-Flexofile and the master apical file was X3 (#30/0.07).

Group II: Instrumentation using rotary **One Shape Apical file** (Micro-Mega, Besançon, France)

Ten teeth were prepared by single One Shape Apical 1 file (#30/0.06), with X-Smart motor in continuous rotation motion at 400 rpm and a torque of 2.5 N.cm. The cervical third of the root canal was first flared with EndoFlare, then using Apical 1 file, three in-and-out motions were gently performed in the apical direction. Apical patency was checked using size #10 K-Flexofile and the master apical file was Apical 1 (#30/0.06).

Group III: Instrumentation using manual **K-Flexofiles** (Dentsply Maillefer, Ballaigues, Switzerland)

Ten teeth were prepared by K-Flexofiles with step-back technique. Since these canals were instrumented before bacterial inoculation till size #20 K-Flexofile, instrumentation was performed at the working length starting from size #20 till #30 and then step-back technique was continued till size #45 K-Flexofile. Apical patency was checked using size #10 K-Flexofile and the master apical file was K-Flexofile (#30/0.02).

Group IV: No instrumentation **(control)**

Ten teeth were used as the control group. After bacterial inoculation, the root canals were not instrumented and only irrigation was performed.

Irrigation was performed in all groups with a total of 10 mL sterile saline solution by a disposable plastic syringe and NaviTip '30G' (Ultradent Products Inc. South Jordan, USA) to within 2 mm of the working length. In groups I and III, irrigation was repeated with the exchange of each file, while in group II irrigation was performed each time after file withdrawal.

Sampling technique and microbiological processing

Three samples were taken from each root canal:

In Groups I, II and III;
S1: Initial sample (before instrumentation)
S2: Immediate sample (immediately after instrumentation)
S3: Final sample (one week after instrumentation).

In Group IV;
The same samples were taken but before, immediately after and one week after *irrigation*.

For bacterial sample collection, a sterile paper point size #20 was placed inside each root canal till the working length for 1 minute using a sterile forceps. Each paper point was then dropped in a sterile screw cap pre-labeled tube size 2 mL containing 1 mL sterile saline solution. Each tube was vortexed for 20 second at high speed to obtain a homogenous suspension from the collected bacteria. From each tube, 10 µL of the bacterial suspension was obtained by a calibrated disposable loop and then streaked on corresponding blood agar plates. All plates were incubated microaerophilically at 37°C for 24 hours. After the incubation period, the colony-forming units (CFUs) that grew on the blood agar plate were counted using Stuart Digital Colony Counter (Stuart Scientific SC6, Keison, UK) and recorded.

Statistical analysis

To compare the results of the four groups, the data obtained from all the samples were recorded, tabulated, coded and fed to the statistical software IBM SPSS (Version 20). Statistical analysis was done by Friedman test and Paired *t*-test for intragroup analysis and by Kruskal-Wallis and One-way ANOVA tests for intergroup analysis. P value < 0.05 was considered to be statistically significant.

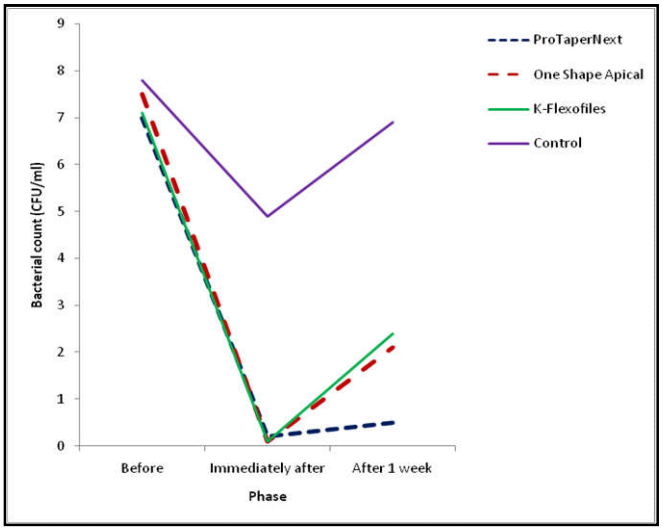
RESULTS

Analysis of the initial bacterial counts in the four groups revealed no significant differences between them and this indicates that the initial bacterial load was homogenous in all groups. Bacterial reduction was significantly superior in Groups I, II and III compared to Group IV (control group), which showed the least amount of bacterial reduction. Groups I, II and III showed significant reduction in the bacterial count immediately and one week after instrumentation compared to the initial samples and were equally statistically significant (P= 0.001). In contrast, bacterial reduction in Group IV was not statistically significant (P > 0.05). After one week, bacterial growth was observed in all groups (Table 1, Graph 1). However, Group I (ProTaper Next) was superior to the other groups showing the least amount of bacterial growth, thus demonstrating a significant reduction in the bacterial count after one week compared to One Shape Apical, K-Flexofiles and the control group respectively (Table 2, Graph 2).

Table 1. Comparison of the bacterial count before, immediately after and one week after instrumentation (x10^4 CFU/mL)

Group			Phase			P
			Before	Immediately After	After 1 Week	
I	ProTaper Next	Minimum	3.9	0.0	0.2	24.5 (0.001)*
		Maximum	16.8	0.3	1.6	
		Mean	8.6	0.1	0.6	
		SD	4.2	0.1	0.4	
		Median	7.0	0.2	0.5	
II	One Shape Apical	Minimum	3.9	0.0	0.2	23.4 (0.001)*
		Maximum	20.7	0.3	5.4	
		Mean	9.5	0.1	2.4	
		SD	5.6	0.1	2.0	
		Median	7.5	0.1	2.1	
III	K-Flexofiles	Minimum	3.7	0.0	1.4	23.9 (0.001)*
		Maximum	21.5	0.5	5.7	
		Mean	9.1	0.1	2.7	
		SD	6.1	0.2	1.5	
		Median	7.1	0.1	2.4	
IV	Control	Minimum	3.5	2.2	3.1	4.0 (0.130)
		Maximum	10.6	8.5	9.9	
		Mean	7.4	5.1	6.5	
		SD	2.6	2.0	2.2	
		Median	7.8	4.9	6.9	
P+			0.930	0.001*	0.001*	

P: adjusted P value of Friedman test * P < 0.05 (significant)
P+:adjusted P value of Kruskal-Wallis test

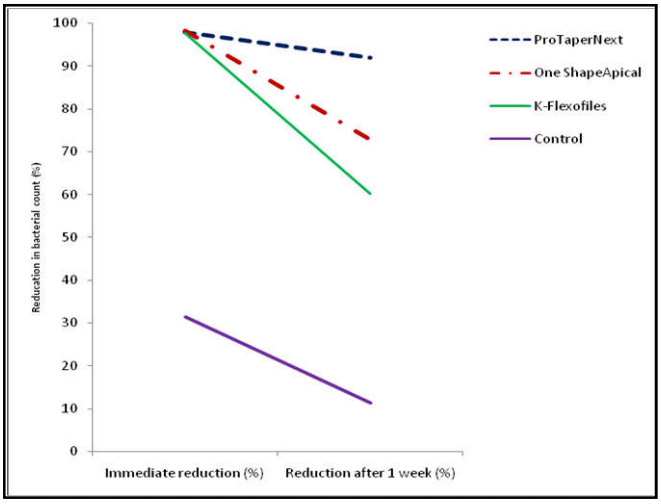


Graph 1. Comparison of the bacterial count before, immediately after and one week after instrumentation (x10^4 CFU/mL)

Table 2. Comparison of the mean percentage reduction in the bacterial count immediately after and one week after instrumentation (%)

Group		Immediate Reduction	Reduction After 1 Week		t (P)	
		Mean	SD	Mean	SD	
I	ProTaper Next	97.9	1.6	91.9 [II, III, IV]	4.8	0.001*
II	One Shape Apical	98.3	1.8	72.9	22.5	0.005*
III	K-Flexofiles	97.7	3.0	60.2	22.2	0.001*
IV	Control	31.4 [I, II, III]	9.4	11.4 [I, II, III]	3.6	0.001*
F (P)		0.001*	0.001*			

t: Paired-*t* test F: One-way ANOVA * P <0.05 (significant)



Graph 2. Comparison of the mean percentage reduction in the bacterial count immediately after and one week after instrumentation (%)

DISCUSSION

The control and elimination of microorganisms in the root canal system is highly important because they play an essential role in the development of pulpal and periapical disease and are the main cause of failure of endodontic therapy (Sundqvist, 1992; Gomes *et al*., 1996; Sakamoto *et al*., 2007). *Enterococcus faecalis* was specifically selected for this study because of its clinical significance, as it is the most commonly found species in persistent endodontic infections with a prevalence ranging from 30-90% and this makes it the primary organism associated with post-treatment failure (Molander *et al*., 1998; Pinheiro *et al*., 2003). Thus, a technique that is efficient for the reduction of *E. faecalis* from root canals may prove useful for the elimination of other less resistant microorganisms (Matos Neto *et al*., 2012). Moreover, *E. faecalis* was found to be the best microorganism to perform experimental penetration into dentinal tubules leading to gross infection (Peters *et al*., 2000; Dametto *et al*., 2005).

Mechanical instrumentation is considered the core method for bacterial reduction in infected root canals (Haapasalo *et al*., 2005). Thus, it was essential to evaluate the efficacy of the newly introduced ProTaper Next and One Shape Apical systems versus manual instrumentation on the reduction of *E. faecalis* count in the root canal, without the use of any antimicrobial irrigation or intracanal medication. The manual group was used as a reference for comparison as it is the most widely used instrumentation technique by many dentists (Machado *et al*., 2013).

The results of this study demonstrated that ProTaper Next, One Shape Apical and K-Flexofiles significantly reduced *E. faecalis* count in the root canals immediately after instrumentation, with no statistically significant difference between them. This could be attributed to the short duration of incubation (24 hours) of the infected teeth, which might have allowed bacteria to penetrate into the dentinal tubules to an extent that can be recovered even beyond the largest file size but failed to form dense populations in the more luminal parts of the dentinal tubules. Thus, no differences were found in the efficacy of these techniques to reduce intracanal bacteria immediately after instrumentation, in spite the differences in their taper and design (Aydin *et al*., 2007).

One week after instrumentation, bacterial growth was observed in all groups compared to the immediate sample after instrumentation. This supports the fact that re-growth of bacteria occurs in the root canals in between appointments (Machado *et al*., 2013; Dhingra *et al*., 2015). Our results was in agreement with Dhingra *et al*. (2015), who found bacterial growth in the root canal seven days after mechanical instrumentation with Wave One, ProTaper Next and ProTaper Universal. This was attributed to the presence of residual bacteria deep within dentinal tubules, lateral canals, fins and ramifications of the root canal system, which cannot be reached by mechanical instrumentation only, along with the insignificant antibacterial effect of the sterile saline solution. Thus, the bacteria could have multiplied and re-entered the main canal causing an increase in the bacterial count in the final sample. In contrast, Siqueira *et al*. (2007) found that using intracanal medicaments between appointments decreased the amount of bacterial growth. This supports the fact that mechanical instrumentation alone is never sufficient and the use of antibacterial irrigants and intracanal medicaments is fundamental in order to reach deep into dentinal tubules and other inaccessible areas of the root canal system. Thus, only then will optimal root canal disinfection be obtained, resulting in a long-term successful endodontic therapy.

The least amount of bacterial growth after one week was observed in the ProTaper Next group, followed by One Shape Apical, K-Flexofiles and finally the control group, which showed the maximum amount of bacterial growth. ProTaper Next was the only group that demonstrated a statistically significant reduction in *E. faecalis* count one week after instrumentation compared with the initial sample and compared to One Shape Apical, K-Flexofiles and the control group respectively. This could be due to the progressively tapered file design of ProTaper Next, having an off-centered rectangular cross-section, which generates a snake-like wave of motion along the active portion of the file termed as a "swaggering effect". This effect, compared to the action of a constant tapered file with a centered mass of rotation, serves to minimize the engagement between the file and dentin as there are only two contact points at any one time between the file and the canal wall. Thus, it provides more space for enhanced dentin cutting, as well as loading and removal of infected debris out of the canal with a decreased probability of lateral compaction of debris into the dentinal tubules, hence decreasing the intracanal bacterial count (Ruddle *et al*., 2013). Bergmans *et al.* (2001) stated that a progressive taper on a single file improves its cutting efficiency over a constant taper file i.e. One Shape Apical, by increasing the force per unit area of the file against the canal wall. They also stated that larger taper files remove more dentine. Moreover, the analysis of radicular thirds in various studies revealed that higher bacterial counts are found in the cervical third, followed by the middle, then the apical third (Nakamura *et al*., 2013; Nakamura *et al*., 2015). This is supported by the fact that the greatest number of dentinal tubules and the greatest extent of bacterial invasion into dentinal tubules are found in the cervical third, followed by the middle, then the apical third respectively (Love 1996; Matsuo *et al*., 2003; Souza *et al*., 2008).

Since ProTaper Next is the only system in our study having a progressively tapered file design, an off-centered rectangular cross-section and its final file taper is 0.07; which is larger than the rest of the file systems used, it could have resulted in more removal of dentin, especially in the cervical portion. In turn, it significantly eliminated bacteria which re-grew in this portion during the week more than One Shape Apical and K-Flexofiles respectively. Dhingra *et al* (2015) also found a significant reduction in bacterial count one week after instrumentation by ProTaper Next, Wave One and ProTaper Universal but no significant difference was found between them. This could be attributed to the large taper of Wave One file and the final file of ProTaper Universal (0.08 taper), as well as the progressive taper design of ProTaper Universal, which is similar to that of ProTaper Next. Thus, they might have eliminated bacteria in a similar manner.

In the control group, only irrigation with sterile saline solution was performed, the least amount of bacterial reduction was observed immediately and one week after irrigation compared to the three instrumentation groups. The bacterial reduction in the control group may be attributed to the basic ability of the irrigating solution to flush out debris from the root canals, in addition to the large volume (10 mL) used for irrigation in each sample, despite the absence of instrumentation. Vijaykumar *et al*. (2012) stated that the mechanical effects during irrigation are generated by the flow and back flow of the irrigant in the root canal. Thus, regardless of the type of irrigant used, the bacterial population inside the root canal was reduced by the mechanical effects of irrigation. However, when antimicrobial irrigants were used, a highly significant bacterial reduction was observed. Therefore, stressing the importance of using antimicrobial solutions for irrigation during mechanical preparation to provide maximum disinfection of the root canals.

Conclusion

Under the limitations of this study, it was concluded that ProTaper Next, One Shape Apical and manual instrumentation were equally significantly effective in reducing *E. faecalis* count in the root canals immediately after instrumentation. However, one week after instrumentation, ProTaper Next was significantly superior to One Shape Apical and manual instrumentation regarding this means. Bacterial growth was observed one week after instrumentation, which proves that mechanical instrumentation alone is never sufficient to obtain maximum disinfection of the root canals. Thus, we stress on the importance of using an antibacterial irrigant along with instrumentation to ensure a long-term successful endodontic treatment.

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