

# Effect of taper and metallurgy on cleaning efficacy of endodontic files in primary teeth: An *in-vitro* study

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## ABSTRACT

To determine the effect of metallurgy and taper on primary teeth root canals infected with *Enterococcus faecalis*, by stainless steel (SS) and nickel-titanium (Ni-Ti) endodontic files. Ninety primary teeth root canals were used, in which the canals were sterilized after being enlarged with #15 K-file and then contaminated with an inoculation of a broth of *E. faecalis*. The teeth were divided into two groups, A and B. A is served as the control group and B as an experimental group. Experimental group was further divided into five subgroups (subgroup B1 – SS K-files: 2% taper, B2 – SS H-files: 2% taper, B3 – SS Flare files: 5% taper, B4 – Ni-Ti: 2% taper, and subgroup B5 – Ni-Ti: 6% taper) based on the metallurgy and taper of different endodontic files used for instrumentation. The post bacterial samples were collected, and were seeded on plates for analysis. After the incubation period, analysis of colony forming units/200 µL was done. The data thus obtained were subjected to statistical analysis. Ni-Ti H and ProTaper group: The 6% taper showed a significant bacterial reduction ( $P < 0.05$ ) and produced cleaner canals than SS K-files: 2% taper, SS H-files: 2% taper, and Ni-Ti K-files: 2% taper. However, there was not much of difference between Ni-Ti H and ProTaper: The 6% taper and SS Flare files: The 5% taper ( $P > 0.05$ ). It was concluded that metallurgy does not play a significant role in cleaning efficiency of root canals, whereas taper does influence the bacterial reduction and hence produce cleaner canals in primary teeth.

**Key words:** *Enterococcus faecalis*, Instrumentation, Primary teeth, Root canal preparation

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## INTRODUCTION

The main goal of microbiological research and chemomechanical preparation is to completely eradicate intracanal bacterial population or at least reduce them to levels that are compatible with periradicular tissue healing. Bacteria persisting after chemomechanical preparation techniques have been shown to negatively influence the treatment outcome.<sup>[1]</sup> Thus, the success of endodontic treatment depends upon, how effectively the clinician is in eradicating the bacteria from the canal.

The endodontic research assumes special importance in finding methods and materials to predictably eradicate

the root canal infection. Of the procedures for root canal treatment, two steps assume special relevance with regard to bacterial elimination, that is, biomechanical preparation and interappointment intracanal medicaments.<sup>[2]</sup> Biomechanical preparation is of paramount importance for root canal disinfection.

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Mechanical preparation is often the first means of bacterial reduction during endodontic treatment of infected root canals.<sup>[2]</sup> Ingle and Zeldow<sup>[3]</sup> reported 20% reduction of intracanal bacteria after instrumentation only using sterile water.

For long, root canal instruments were manufactured using stainless steel (SS) only. Hatton *et al.*,<sup>[4]</sup> reported that canals prepared with SS instruments were only superficially cleaned, and much of the pulp tissue was not removed. SS files have also been shown to create aberrations, probably as a result of the inherent stiffness of SS, which is compounded by instrument design and canal shape. Similarly, Weine *et al.*,<sup>[5]</sup> concluded in his study that most instrumentation techniques with SS instruments in curved canals result in apical transportation. This makes obtaining a successful apical seal more difficult.

Newer instruments have been prepared for root canal preparation and debridement. Rotary nickel-titanium (Ni-Ti) instruments with increased taper and different design have also been recently developed. Many studies<sup>[6,7]</sup> have shown Ni-Ti superior to SS with the advantages of maintaining original shape and curvature of canal, less likely chance of procedural errors, short treatment time, better tapered canal for irrigation, and obturation. Barr *et al.*,<sup>[8]</sup> used Ni-Ti profile 0.04 taper rotary instruments for primary root canal preparation and concluded that the use of Ni-Ti rotary files for root canal preparation in primary teeth was lucrative and faster and resulted in consistently uniform and predictable fillings. However, the breakage of instruments has been a concern by some and lack of tactile feedback from others.<sup>[9]</sup> This has led to the introduction of hand operated a version of Ni-Ti rotary.

Nevertheless, there are some cases in which the treatment has followed the highest technical standards and yet failure results. Studies have revealed that the chemomechanical preparation *per se*, predictably does not render root canals bacteria free, with about 40-60% of the prepared canals still containing cultivable bacteria.<sup>[10]</sup> Among them, facultative anaerobic and Gram-positive bacteria such as enterococcus and actinomyces are more commonly found and are more resistant to instrumentation.<sup>[11]</sup>

*Enterococcus faecalis* is a microorganism commonly detected in asymptomatic, persistent endodontic infections, and its prevalence in such infections ranges from 24% to 77%.<sup>[12]</sup> Hence, it is the most commonly found species in root-filled teeth evincing recalcitrant periradicular lesions as a consequence, a role in the causation of treatment failure.<sup>[13]</sup>

Although it has been demonstrated that the newer instruments and techniques improve shaping of canal, few studies have evaluated their capability in eliminating root canal infection, such studies have reported that rotary and hand instrumentation techniques were equally effective for reducing intracanal bacteria.<sup>[2,14]</sup> However, few studies have evaluated instrumentation techniques using greater taper (GT) files for its efficacy in eliminating intracanal bacteria.<sup>[9]</sup>

Therefore, the purpose of this study was to evaluate the effect of taper and metallurgy on intracanal bacterial reduction.

## MATERIALS AND METHODS

The study was conducted on 90 primary teeth root canals. Teeth that were indicated only for extraction were collected from Department of Pediatric Dentistry of Himachal Dental College and other dental colleges located in India. After cleaning, all the teeth were stored in normal saline (Alkem Lab., India) until used.

### Exclusion criteria

Teeth with:

1. Any signs of internal resorption,
2. Any signs of lateral resorption,
3. Root length lesser than 6 mm, and
4. Root canal foramina wider than #20 K-file were not included in the study.

Materials and product details of various endodontic files used for the study are shown in Table 1.

### Methodology

#### Preparation of samples

To standardize, all extracted teeth were decoronated from the cemento-enamel junction. Pulp extirpation was

**Table 1: Metallurgy and product details of various endodontic files**

Metallurgy	Type of file used	Manufacturer	Length used (mm)	Sizes used	Taper (%)
SS	K-file	Mani Inc., Japan	21	15, 20, 25, 30	2
SS	H-file	Mani Inc., Japan	21	15, 20, 25, 30	2
SS	Flare files	Mani Inc., Japan	21	15, 20, 25, 30	5
Ni-Ti	K-file	Dentarc, India	21	15, 20, 25, 30	2
Ni-Ti	Hand ProTaper	Dentsply Maillefer	21	S <sub>x</sub> , S <sub>1</sub> , S <sub>2</sub> , F <sub>1</sub> , F <sub>2</sub> , F <sub>3</sub>	6

Ni-Ti: Nickel-titanium, SS: Stainless steel

done using #15 K-file. Root apices were then sealed with cyanoacrylate. Later root canals were mounted in plaster and autoclaved in 45 mL Falcon tubes (Polylab Pvt. Ltd., New Delhi, India).

#### Isolation of microbial strain

*E. faecalis* pure culture was procured on selective media of *E. faecalis* confirmatory agar. Colonies were transferred to Tryptic Soya Broth, after incubation of 48 h at 37°C the turbid broth was used for inoculating *E. faecalis* in root canals.

#### Inoculation of Bacteria in root canals

Falcon tubes containing root canal samples were opened under laminar flow hood [Figure 1]. Following which 5 µL of turbid Tryptic Soya Broth was transferred to root canals and incubated at 37°C for 24 h. After a short incubation, re-inoculation was done to maintain the bacterial survival in root canals and incubated at 37°C for 48 h.

#### Collection of bacterial samples from root canals

For a collection of bacterial samples from root canals, root canals were opened in inoculation chamber under laminar flow hood, and 5-10 µL of sterile peptonated water was transferred to root canals. #20 paper points were inserted into each root canal for 10 s. Paper points were then transferred to Eppendorf tubes (Eppendorf tubes Pvt. Ltd., New Delhi, India) containing 1 mL of sterile Tryptic Soya Broth.

#### Cleaning and shaping of root canals

Samples were divided into two groups, A and B. "A" served as the control group and "B" as an experimental group. The experimental group was further divided into five subgroups based on the metallurgy and taper of different endodontic files used for instrumentation as shown in Table 2. Canals were irrigated with sterile distilled water. After every root canal instrumentation files were sterilized in Glass Bead Sterilizer (Denfort International, India) at 250°C for 15 s.



**Figure 1:** Turbid Tryptic Soya Broth being transferred to root canals

#### Estimation of colony forming units

The processes of a collection of bacterial samples from root canals were repeated, which were then subjected to serial dilution of  $10^{-3}$  and 200 µL of the serially diluted solution was dispensed on *E. faecalis* confirmatory agar medium and incubated. After incubation, colony forming units were counted [Figure 2] and subjected to formula:

$$\text{Number of bacteria/ml} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution factor}}$$

## RESULTS AND OBSERVATION

The data analysis was done using Statistical Package for the Social Sciences (SPSS) Version 11.5 (Chicago, IL, USA). The analysis of variance was used to test the significance of the difference between the means of given variables in two groups and five subgroups groups. Tukey's *post-hoc* test was used for multiple comparisons between the groups. The level of significance was fixed at 0.05.

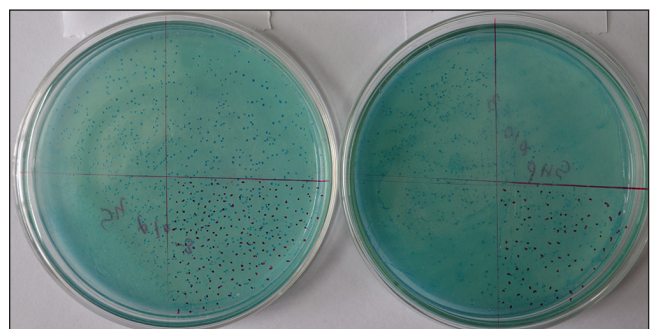
Descriptive statistics showed the mean difference in the bacterial count and mean bacterial percentage reduction as shown in Table 3 and Graph 1.

The analysis of variance demonstrated that there were significant differences between different groups both

**Table 2: Grouping of samples into control group and experimental groups**

Groups			
	Instrumentation	Taper	Number of samples
Group A	Control group (no instrumentation was done)	—	15
Group B			
Subgroups	Instrumentation	Taper (%)	Number of samples
B1	SS K-files	2	15
B2	SS H-files	2	15
B3	SS Flare files	5	15
B4	Ni-Ti K-files	2	15
B5	Ni-Ti Hand ProTaper	6	15

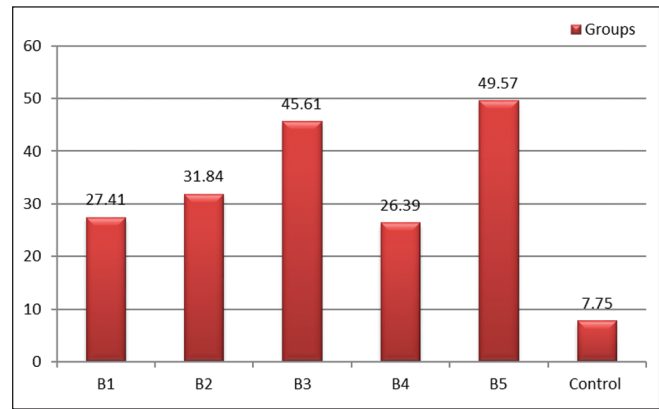
Ni-Ti: Nickel-titanium, SS: Stainless steel



**Figure 2:** Counting of colony forming units in both pre- and post-samples

in terms of bacterial count, as well as the percentage bacterial reduction ( $P < 0.05$ ).

Post-hoc test for multiple comparisons showed that, there was statistically significant difference ( $P < 0.05$ ) in the mean percentage bacterial reduction between B1 (SS K-files: 2% taper), B2 (SS H-files: 2% taper), B4 (Ni-Ti K-files: 2% taper), and B5 (Ni-Ti Hand ProTaper: 6% taper). However, group B3 (SS Flare files: 5% taper) and group B5 (Ni-Ti Hand ProTaper: 6% taper) showed no statistical significant difference ( $P > 0.05$ ). Same were observed with the group B1 (SS K-files: 2% taper) and group B4 (Ni-Ti K-files: 2% taper) [Table 4].



Graph 1: Demonstrates percentage of bacterial reduction

**Table 3: Mean difference in bacterial count exhibited by the experimental and the control group**

Groups	n	Mean	SD	95% CI for mean		Minimum	Maximum
				Lower bound	Upper bound		
Difference in bacterial count							
B1	15	116.67	27.126	101.64	131.69	84	180
B2	15	106.80	34.040	87.95	125.65	64	188
B3	15	166.67	53.000	137.32	196.02	116	292
B4	15	87.60	14.327	79.67	95.53	64	112
B5	15	170.36	74.886	127.12	213.60	76	316
Control	15	27.00	4.243	3.000	−11.12	65.12	24

SD: Standard deviation, CI: Confidence interval

**Table 4: Comparison of different groups for percentage of bacterial reduction using “post-hoc” test**

Multiple comparisons						
Dependent variable	Subgroup	Mean difference	SE	Significant	95% CI	
					Lower bound	Upper bound
Bacterial reduction (%)	B1					
	B2	-4.42667	4.20396	1	-16.6186	7.7653
	B3	-18.19667*	4.20396	<0.001**	-30.3886	-6.0047
	B4	1.02667	4.20396	1	-11.1653	13.2186
	B5	-22.15810*	4.27838	<0.001**	-34.5658	-9.7504
	B2					
	B1	4.42667	4.20396	1	-7.7653	16.6186
	B3	-13.77000*	4.20396	0.017*	-25.9619	-1.5781
	B4	5.45333	4.20396	1	-6.7386	17.6453
	B5	-17.73143*	4.27838	0.001**	-30.1392	-5.3237
	B3					
	B1	18.19667*	4.20396	<0.001**	6.0047	30.3886
	B2	13.77000*	4.20396	0.017*	1.5781	25.9619
	B4	19.22333*	4.20396	<0.001**	7.0314	31.4153
	B5	-3.96143	4.27838	1	-16.3692	8.4463
	B4					
	B1	-1.02667	4.20396	1	-13.2186	11.1653
	B2	-5.45333	4.20396	1	-17.6453	6.7386
	B3	-19.22333*	4.20396	<0.001**	-31.4153	-7.0314
	B5	-23.18476*	4.27838	<0.001**	-35.5925	-10.7770
	B5					
	B1	22.15810*	4.27838	<0.001**	9.7504	34.5658
	B2	17.73143*	4.27838	0.001**	5.3237	30.1392
	B3	3.96143	4.27838	1	-8.4463	16.3692
	B4	23.18476*	4.27838	<0.001**	10.7770	35.5925

\*The mean difference is significant at the 0.05 level. CI: Confidence interval, SE: Standard error, \*\*Significant <0.001



## DISCUSSION

The pursuit of an ideal endodontic instrument, to clean infected root canal has long presented a challenge. To effectively treat endodontic infections, clinicians must recognize the cause and effect of the microbial invasion of the dental pulp space and the surrounding periradicular tissues.<sup>[15]</sup> Accurate knowledge of the occurrence of the major putative endodontic pathogens and their implication in pathogenesis of periradicular diseases has the potential to afford subsidies for the development of antimicrobial strategies effective in treating both primary and persistent or secondary endodontic infections.

The root canal system is a selective habitat that allows the growth of certain species of bacteria in preference to others. Unlike most putative endodontic pathogens frequently found in primary infections, *E. faecalis* can colonize root canals in single infections.<sup>[1]</sup> *E. faecalis* is significantly associated with treatment failures, whereas this species was detected in 18% of the cases of primary endodontic infections, its prevalence in root-filled teeth was much higher: 67% of the cases.<sup>[13]</sup> These findings suggest that *E. faecalis* can be inhibited by the mixed bacterial consortium commonly present in primary infections and that the bleak environmental conditions within filled root canals may not prevent its survival.

*E. faecalis* is a facultative anaerobic and Gram-positive bacterium. As described by Sherman,<sup>[16]</sup> enterococci can grow at 10°C and 45°C, at pH 9.6, in 6.5% NaCl broth, and also can survive at 60°C for 30 min. This bacterium significantly penetrates the interior of dentinal tubules as a consequence of the collagen's attraction for this microorganism.<sup>[17]</sup> Besides a greater dentin penetration, this bacterium can develop better conditions for survival in the root canal system, where it has been observed to persist for periods of 4 weeks to as long as 12 months inside the tubules, even in obturated canals. The virulence of *E. faecalis* may also be related to its resistance to intracanal medicaments.<sup>[17]</sup> Hence, *E. faecalis* was chosen for the present study.

In literature, several methods have been reported to evaluate the efficiency of endodontic instruments, which includes morphometric analysis, microscopic observation, the amount of extruded debris, and a bacterial assessment.<sup>[2]</sup> Although, the value of canal sampling in clinical practice has been questioned, the elimination or reduction of intracanal bacteria remains a prime objective. Hence, the assessment of bacteriological means was selected for the present study.

A practical and quantitative method for recovering microbes from infected root canals is essential for a

quantitative study such as this. The sampling technique as used by Machado et al.<sup>[18]</sup> was chosen for the current study because of its practical use in the clinical setting, high recovery rate, and stability of samples. The extraction of *E. faecalis* with paper points followed the method used by Siqueira et al.<sup>[19,20]</sup> and the sampling and transport medium used in the present study was Tryptic Soya Broth. These are considered suitable for sampling, in which existing bacteria remain viable and the chance for reproduction, which could alter the results are reduced.

There are several factors that contribute to the clinical success of the endodontic procedure, such as biomechanical preparation of root canals, intracanal medicament used, root canal filling material, and the type of restoration done. The effect of mechanical instrumentation on bacterial reduction without using any antimicrobial irrigation is reported in the literature.<sup>[2]</sup> The mechanical preparation alone itself can reduce the intracanal bacterial count sufficient enough to be quantitatively evaluated using appropriate sampling technique. Byström and Sundqvist<sup>[21]</sup> reported samples were taken after hand filing with saline irrigation usually contained 2-3-fold fewer bacterial count than initial specimens. In some cases, the reduction was even higher. Therefore, the bacterial reduction with different endodontic instruments was the prime objective in the present study.

SS K-files are one of the conventional and widely used endodontic instruments for canal cleaning and shaping. Many studies have reported their advantages over other instruments. Yin et al.<sup>[14]</sup> reported that K-files removed a greater amount of volumetric dentin and left lesser uninstrumented canal area than rotary ProTaper files. This fact leads us to check their cleaning efficiency. Considering them the gold standard in endodontics, they were used for comparison against Ni-Ti Hand ProTaper, Ni-Ti K-files (2% taper), SS H-files, and Flare files.

The effective cleaning of root canals may also be influenced by the taper or by the apical size of the root canal preparation.<sup>[22]</sup> Smith et al.<sup>[22]</sup> did a 5 years retrospective study, and concluded that a flared canal with complete obturation had a success rate of 7.75% higher than the older techniques. Therefore in the present study, files with GT such as Ni-Ti ProTaper: 6% taper and SS Flare files: 5% taper, were used and compared to 2% tapered SS K-file, H-file, and Ni-Ti K-file.

There are many advantages of Ni-Ti rotary instruments quoted in literature. Barr et al.<sup>[8]</sup> used Ni-Ti Profile 0.04 taper rotary instruments for primary root canal preparation, and concluded that the use of Ni-Ti files for root canal preparation in primary teeth was cost

effective and rapidly resulting in consistently uniform and predictable obturation. However, other studies found clinical success in primary molars with a modified protocol using ProTaper files. Kuo *et al.* (2006)<sup>[23]</sup> concluded that ProTaper Ni-Ti rotary files can be safely and efficiently applied for root canal preparation in primary molars.

In the present study, Ni-Ti H and ProTaper (6% taper) showed the largest amount of mean bacterial percentage reduction followed by SS Flare files (5% taper), H-file, SS K-file, and Ni-Ti K-file (2% taper), in that order. Statistically, there was significant difference between Ni-Ti H and ProTaper: 6% taper (subgroup B5), Ni-Ti K-file: 2% taper (Subgroup B4), SS Flare file: 5% taper (subgroup B3), and SS K-file: 2% taper (subgroup B1). These findings strongly confirm the findings of Cheung and Liu<sup>[9]</sup> and Huang *et al.*<sup>[24]</sup> that the taper observed more is the mean bacterial percentage reduction seen in samples. This could be explained by the fact that, the larger preparations can incorporate more anatomical irregularities and allow the removal of a substantial number of bacterial cells from the root canal. In addition, the instrument to larger file sizes can also result in better irrigant exchange in the apical third of the canal.

The clinical utility of an endodontic instrument is defined by its ability to endure the stress and strain, ease of its applicability, time, and iatrogenic errors. Much attention has been directed toward root canal preparation techniques with Ni-Ti rotary instruments. Numerous studies<sup>[6-9]</sup> have reported that they could efficiently create smooth, predetermined funnel-form shapes with minimal risk of ledging and transportation. Marending *et al.*<sup>[25]</sup> concluded in his prospective study using SS K-files and Ni-Ti rotary, that, the type of instrument had no significant effect on treatment outcome.

Statistically, no significant difference was observed between group B5 (Ni-Ti ProTaper) and B3 (SS Flare file), and group B1 (SS K-file) and B4 (Ni-Ti K-file). The results of groups with Ni-Ti alloy and SS alloy were complementary to the findings of Marending *et al.*,<sup>[25]</sup> Iqbal *et al.*,<sup>[26]</sup> and Fleming *et al.*,<sup>[27]</sup> but in contrast, to the study done by Pettiette *et al.*<sup>[28,29]</sup> who demonstrated better success rates for teeth instrumented with Ni-Ti instruments. The reason for this could be, as the study was conducted in two parts and by undergraduate students, in the first part of their two-part clinical trial, 60 molar teeth were treated by inexperienced dental students, and root canal transportation and procedural errors were evaluated. In the second part of this investigation, 1-year success rates of same teeth used in the aforementioned study were compared, and the comparison of success rates was based primarily on the interpretation of the periapical status of the teeth. Furthermore, the follow-up period of this trial

was only 1-year, and the number of teeth subsequently evaluated was small.

A significant reduction in bacteria such as between Ni-Ti H and ProTaper, and Ni-Ti K-file was observed after GT files were used for cleaning and shaping. All the experimental groups reduced a significant amount of bacterial population than the control group. However, none of the samples was completely sterile. It should be noted that a further reduction of viable bacteria could be expected with the use of antimicrobial irrigants such as sodium hypochlorite and ethylenediaminetetraacetic acid.

Only primary teeth root canals (curved or straight) were taken in the study, as they possess very different morphological characteristics<sup>[30]</sup> allowing a more diverse evaluation of the instrument techniques. Since the results of present study strongly favor the association of taper of instrument and bacterial count, hence, it seems logical that the larger file sizes, with different design and metallurgy, can bring the bacterial count close to zero. If so, it may be easier to reach these larger sizes with minimal procedural errors and maintaining remaining radicular dentin in physiologic limits of the tooth to prevent any complications such as compromised restorability, fracture susceptibility, and canal path alterations.

The canal types sampled in this study are not purely representative of all anatomical canal configurations one may encounter when treating patients for pulpectomy. Consequently, other canals may pose more challenge to conventional or Ni-Ti instruments. This emphasizes the need for additional antimicrobial measures such as antimicrobial irrigants and bactericidal intracanal medicaments.

## CONCLUSION

All the endodontic file systems used in the present study significantly reduced the amount of bacteria in the mechanical disinfection of the root canal system, demonstrating that they are suitable for this purpose. Based on the present methodology and obtained results, in the present study following conclusions were made:

1. None of the endodontic file system investigated, produced completely sterile canals.
2. Metallurgy does not play an important role other than reduction in procedural errors and less the apically extruded debris with Ni-Ti instruments.
3. More the taper, more is the removal of intracanal dentin and significant is the bacterial reduction.

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### Conflicts of interest

There are no conflicts of interest.

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