Assessment of various endodontic instrumentation systems on the amount of apically extruded bacteria - An *in vitro* study

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**Abstract**

**Aim:** The objective of this *in vitro* study was to assess the effect of different endodontic instrumentation systems on the amount of apically extruded bacteria.

**Materials and Methods:** One hundred and twenty freshly extracted human mandibular premolars with single canal were collected. Endodontic access cavities were prepared and then contaminated with an *Enterococcus faecalis* suspension (ATCC 29212). After incubation at 37°C for 24 h, the root canals were instrumented with K flare files, F360 Single file system, K3XF files, Heroshaper files, Protaper Next files, and Hyflex EDM Single file system. During instrumentation, apically extruded bacteria were collected in the vials containing 0.9% NaCl. Samples were taken from the vials and incubated in brain–heart infusion agar medium for 24 h.

**Statistical Analysis:** The number of colony-forming units was determined, and data were statistically analyzed using one-way analysis of variance and *post hoc* Tukey test.

**Results:** Significant difference was found between the rotary and hand instrumentation systems.

**Conclusions:** Both rotary and hand instrumentation systems extruded intracanal bacteria through the apical foramen, Group 1 (Hand K Flare files) showed maximum, whereas, Group 5 (Protaper Next) and Group 6 (Hyflex EDM) showed the least extrusion.

**Keywords:** Apical extrusion; *Enterococcus faecalis*; hand and rotary instrumentation system

**INTRODUCTION**

Successful endodontic treatment depends on fundamentals of debridement, disinfection, and obturation, which are contributive to periradicular healing. All irritants must be eliminated from the root canal by cleaning and shaping without causing injury to the periradicular tissues. However, at the time of preparation, dentin chips, pulp tissue-containing microorganisms, and/or irrigants may be forced into the periradicular tissues and result in inflammation, postoperative pain, and delay in periapical healing.[1] The extruded material often referred to as “worm of necrotic debris” has been associated with flare-ups.[2] The factors responsible for pain during endodontic treatment encompass mechanical (overinstrumentation), chemical (irrigants, overextended filling materials), and/or microbial injury to periradicular tissues. Despite this, the prevailing cause of inter-appointment flare-ups remains the bacterial infection caused by apical extrusion of microorganisms and their by-products from the root.

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It is well reported that all preparation techniques extrude some amount of debris even with preparations kept short of the apical foramen; however, the amount of debris extrusion into the periapical tissues may vary in pursuance of preparation technique and file system used. There has been a rapid advancement in instrumentation systems through the last decade, and many have been investigated for their debris extrusion potential. Moreover, studies have shown that though qualitative factors are not under the control of the practitioner, quantitative factors may be controlled by selecting techniques to provide gradual access to the canal terminus, which grants the containment of the amount of irritants propelled periapically. The aim of this in vitro study was to assess the amount of apical extrusion of bacteria from extracted teeth after canal instrumentation using one manual file system (K Flare files), two rotary single-file systems (F360 and Hyflex EDM), and three rotary multifile systems (Protaper Next, K3F and Heroshaper). Null hypothesis considered was that all the systems had the same amount of apical debris extrusion.

MATERIALS AND METHODS

One hundred and twenty freshly extracted single-rooted mandibular premolars (for orthodontic/periodontal reasons) with mature apices and curvature between 0° and 10° were collected. Ethical clearance was taken. Soft tissue and calculus were removed with periodontal scaler (Guilin Woodpecker). Radiographic assessment was made to check each tooth for the presence of single canal without calcification. Teeth were stored in physiological saline (Alkem Laboratories Pvt Ltd) until used. Tooth length was standardized to 18 mm by decoronation using a disk. The access cavity was prepared using Endo Z bur (Dentsply, Maillefer, Switzerland). Working length was established 1 mm short of the apical foramen with #10 K file (Mani, Utsunomiya, Japan) and pulp was extirpated using a fine barbed broach (Dentsply, Maillefer, Switzerland).

For each tooth, a glass vial model was used for the assessment of bacterial extrusion [Figure 1]. The teeth were inserted in center of the rubber stopper and fixed to it at the cementoenamel junction by using the cyanoacrylate glue (Pidilite, India). The external surfaces of the roots were dried and coated twice with nail varnish to prevent microleakage through the lateral canals. Teeth were stored in physiological saline (Alkem Laboratories Pvt Ltd) until used. Tooth length was standardized to 18 mm by decoronation using a disk. The access cavity was prepared using Endo Z bur (Dentsply, Maillefer, Switzerland). Working length was established 1 mm short of the apical foramen with #10 K file (Mani, Utsunomiya, Japan) and pulp was extirpated using a fine barbed broach (Dentsply, Maillefer, Switzerland).

A standard suspension of Enterococcus faecalis was cultured in brain–heart infusion broth, and the turbidity was adjusted to 0.5 Mcfarland standard so as to ensure that bacterial concentration was 1.5 × 10⁸ colony-forming units (CFU)/ml. E. faecalis was chosen because of its implication as the possible microbial factor in therapy-resistant apical periodontitis. Each root canal was filled with 20 μl E. faecalis suspension (ATCC 29212) with sterile micropipettes, and a sterile 10 K file was used to carry bacteria down the length of the canals. The infected root canals were then incubated at 37°C for 24 h.

The contaminated samples were then randomly divided into six experimental groups (n = 20) and prepared in a crown down fashion using K flare files (Mani, Utsunomiya, Japan), K3XF (SybronEndo, Orange, California), Heroshaper (MicroMega, Besancon, France), Protaper Next (Dentsply Maillefer, Switzerland) and F360 (Komet, Germany).

Group I Hand K Flare files

The preparation was done in crown-down manner while gradually decreasing the instrument size from #40, with an increment of 1 mm for each file until size 15 till the working length and then preparation of apex till #25.

Group II F360 file

A #25 file with taper of 0.04 was used in rotational speed of 250–350 rpm, with torque value of 1.8 Ncm. The file was used in a “pecking motion” to prepare the canals.

Group III K3XF files

K3 instruments were used in a sequence of #25, 0.06 taper until half of the working length followed by #25, 0.06 taper...
between half and 2/3rd of working length, and finally, #20 and #25 of 0.04 taper respectively to the working length.

**Group IV Hero Shaper files**

In Hero Shaper files, endoflare was used to enlarge the orifices and then a #30 file with a 0.04 taper was initially introduced in two-thirds of the working length. Shaping was completed with #20 and #25 file with a 0.04 taper at the working length.

**Group V Protaper Next files**

ProTaper Next files were used in the sequence X1 and X2 at 300 rpm and 2 N-cm torque. The instrumentation sequence was #17, 0.04 taper followed by #25, 0.06 taper.

**Group VI Hyflex EDM files**

The instruments were used in a gentle in-and-out motion with a rotational speed of 400 rpm and 2.50 N-m torque. The HyFlex files were used with the sequence of #25, 0.12 taper as an orifice opener followed by #10, 0.05 taper to create the glide path and then #25+one file for enlargement of the root canals up to the working length.

In each experimental group, the canals were irrigated with 1 ml 0.9% saline solution by using 30G side vented needle (Dentsply, Maillefer, Switzerland) with 5 ml syringe in between instruments with a total volume of 10 ml irrigation in each group. The canal preparations were carried on each specimen under a Class I laminar airflow cabinet to prevent airborne bacterial contamination [Figure 1].

After the completion of the root canal preparation, 100 µl saline was taken from each vial and inoculated on the brain–heart infusion agar prepared in petridishes. The petridishes were then incubated at 37°C for 24 h and CFU's for each tooth were calculated and log-transformed (base 10). The determination of debris extrusion was done by another operator who was blinded with respect to assorted groups. The obtained values were statistically analyzed with one-way analysis of variance and *post hoc* Tukey test.

**RESULTS**

The mean and the standard deviation values of each experimental group are shown in Table 1. The result of one-way ANOVA showed that there was a significant difference in the amount of apically extruded bacteria using different file systems. The between-group variance was much higher than the within-group variance [Table 2]. Once the significance was established, *post hoc* Tukey's test was applied to study pairwise mean difference. It was found that Group 1 (Hand K Flare files) had the maximum CFUs/ml. Furthermore, Group 5 (Protaper Next) and Group 6 (Hyflex EDM) showed similar CFU's and least of all [Figure 2].

**DISCUSSION**

In the present study, maximum extrusion was seen with Hand K-Flare files as compared to other rotary groups (mean count = 231.75 CFUs/ml). This observation was in accordance with previously done studies. The reason for more extrusion could be the file acting as a piston in the radicular area that tends to push the debris. Flare files are nickel-titanium hand files with a unique 5% taper and a triangular cross-section. The flare files undergo thermal processing, which provides them flexibility, sharpness, and efficiency compared to hand K stainless steel files.

F360 showed second highest debris extrusion (mean count = 59.1 CFUs/ml) in the current study. These files have a thin instrument core to deliver a high level of cutting efficiency while respecting natural root canal morphology. Although the preparation time greatly reduced with this single file rotary system but the debris extrusion potential was still more than many multifile rotary systems, as mentioned in the study done by Bürklein et al.

K3XF also showed debris extrusion (mean count = 19.7 CFUs/ml) but significantly less than K Flare and F360 file system. This could be due to its variable pitch, which helps to prevent the screwing-in effect of the instrument. The increasing flute helical angle from tip to handle helps to dislodge the dentin chips from the working area and carry them coronally to the orifice. This result was in agreement with previously done studies.

HERO Shaper instruments showed minimal debris extrusion (mean count = 13.1 CFUs/ml) but statistically more than ProTaper Next and Hyflex EDM file systems. The file has an adapted pitch, i.e., more tapered an instrument longer is its pitch. Lengthening the pitch increases flexibility and cutting efficiency of the files. The files have a positive rake angle, which is responsible for better debris evacuation. The blade shows a triple helix cutting edge resulting in better force balance. Helix angle shows a gradual increase starting from the tip of the instrument, along the working length, which prevents the instrument from binding in the root canal by the screw-like action and thus reducing the debris extrusion potential.
In the current study, the debris extrusion potential of ProTaper Next was slightly higher (mean count = 3.95 CFUs/ml) than Hyflex EDM but not statistically significant. ProTaper Next is a multifile system having an off-centered rectangular cross-section and progressive and regressive percentage tapers on a single file. This design helps to reduce the contact between file and canal surface, followed by a reduction in taper lock and screwing effect, which leads to efficiency enhancement.\[18\] It produces asymmetrical rotary motion because of 2 point contact of file. Clinically, the advantages of this file are the swaggering effect, which reduces the taper lock, more cross-sectional area for better cutting, loading, and removal of debris.\[19,20\] This may account for the reduced amount of debris extrusion as compared to other groups. The finding was similar to an earlier study done by Topçuoğlu et al.\[21\]

Hyflex EDM is a single-file system used with a continuous rotary movement. It has a change in the taper of file from \(D_0\) to \(D_{16}\) (i.e., 25/0.08 and 25/0.04 at coronal portion) progressively. This file is manufactured by electrical discharge machining technology, which allows for superior canal tracking. Hyflex EDM has three different cross-sections, namely, quadratic in apical, trapezoidal in the middle, triangular in coronal part; which contributes to the least number of instruments and increases cutting efficiency.\[22\] This could be the reason for the least debris extrusion in this file system (mean count = 2.2 CFUs/ml).

Varying amount of apical extrusion, when compared to previous studies, were due to difference in selected teeth, file systems and irrigation protocols followed. Results suggest that practitioners should be aware about the debris extrusion potential of different instrumentation systems, which can probably be made the basis for the selection of a specific instrument system. Therefore, with the combined use of crown down technique, negative pressure irrigation, and the correct choice of rotary instrumentation system

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**Table 2: Summary of post hoc Tukey test showing pair wise mean difference along with P value**

| Group comparison                        | Mean difference | P    | Tukey test | Significance |
|-----------------------------------------|-----------------|------|------------|--------------|
| Hand K flare files - F 360              | 172.65          | <0.001 | 1>2        | Significant  |
| K3XF-Hand K flare files                 | -212.05         | <0.001| 1>3        | Significant  |
| Heroshaper-Hand K flare files           | -218.65         | <0.001| 1>4        | Significant  |
| Protaper Next-Hand K flare files        | -227.8          | <0.001| 1>5        | Significant  |
| Hyflex EDM-Hand K flare files           | -229.55         | <0.001| 1>6        | Significant  |
| K3XF-F 360                              | -39.4           | <0.001| 2>3        | Significant  |
| Heroshaper-F 360                        | -46             | <0.001| 2>4        | Significant  |
| Protaper Next-F 360                     | -55.15          | <0.001| 2>5        | Significant  |
| Hyflex EDM-F 360                        | -56.9           | <0.001| 2>6        | Significant  |
| K3XF-Heroshaper                         | 6.6             | <0.001| 3>4        | Significant  |
| Protaper Next-K3XF                      | -15.75          | <0.001| 3>5        | Significant  |
| K3XF-Hyflex EDM                         | 17.5            | <0.001| 3>6        | Significant  |
| Protaper Next-Heroshaper                | -9.15           | <0.001| 4>5        | Significant  |
| Hyflex EDM-Heroshaper                   | -10.9           | <0.001| 4>6        | Significant  |
| Protaper Next-Hyflex EDM                | 1.75            | 0.567 | 5=6        | Not significant |

**Figure 2:** Colony forming units/ml formed in each experimental group. (a) Group I: Hand K Flare files (b) Group II: F360 file (c) Group III: K3XF files (d) Group IV: Hero Shaper files (e) Group V: ProTaper Next files (f) Group VI: Hyflex EDM files
the operator can minimize the flare-ups by reducing the common error of periradicular extrusion of debris and irrigants.

Thus, further in vivo and in vitro research in this direction could provide more insight into the instrumentation systems responsible for the least apical extrusion of bacteria and debris.

CONCLUSIONS

Under the limitations of this study, the Hand K flare file system was associated with maximum extrusion of bacteria whereas ProTaper Next and Hyflex EDM file systems were found to be most efficient in terms of debris evacuation potential.

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Conflicts of interest
There are no conflicts of interest.

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