Original article

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Microbial contamination of brand new nickel-titanium endodontic instruments

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Abstract

*Aims.*This study aims to estimate the microbial presence on the surface of different brand new NiTi endodontic instruments for clinical use.

Materials and Methods. Eleven different types of NiTi rotary endodontic instruments, obtained from their fresh opened original packages, were assigned to three different groups, according to packaging type and sterilization and tested for bacterial contamination. Isolated bacteria were identified by using standard microbiological methods and then counted.

Differences observed in groups were analyzed statistically by using the one-way analysis of variance (ANOVA) for dependent samples and the Tukey HSD post hoc test.

Results. Statistical differences were found between instruments delivered in plastic boxes which bacterial count resulted higher than those obtained from instruments delivered in blisters ($p<0.01$).

Conclusions. Some brand new endodontic instruments showed degrees of bacterial contamination that both quantitatively and qualitatively deserve to be considered in clinical procedures. ***Clin Ter 2019; 170(4):e258-261. doi: 10.7417/CT.2019.2144***

Key words: *Endodontic Instruments, Bacteria, Root canal treatment, Contamination*

Introduction

The endodontic space of a healthy tooth is sterile; root canal treatment aims to resolve infections and prevent future bacterial contaminations. Bacterial pathogens can diffuse through the apical foramen from the endodontic space to periapical bone, where they can cause even severe infections. (1). Degenerative processes and the need for rehabilitative procedures justify the execution of root canal treatment also in the absence of an infection: also in this cases complete instrumentation of canals, removal of debris and sealing of the endodontic space is essential for long term success to prevent a new contamination (2,3).

Although the most frequent cause of failure in endodontics is an inadequate procedure (4-6), it is well known that in some cases failure occurs even if the highest technical standards have been followed (7). Multiple factors have been associated with the failure of a root canal treatment that conducts to an incomplete bacterial removal (8,9). *Enterococcus faecalis*, is known to be resistant to the sudden and massive ecological changes determined by root canal treatment and its presence has been reported in literature as a leading agent of secondary failures (10).

Many producers deliver endodontic instruments without sterilizing them and frequently such instruments are used as they are deluivered. Autoclaving is the standard sterilization procedure for instruments adopted in every dental practice and data from the literature show that autoclaving does not alter mechanical properties of NiTi rotary instruments (11,12).

This study aims to evaluate quantitatively and qualitatively microorganisms contaminating the surface of various brand new endodontic instruments.

Materials and methods

Eleven different types of NiTi rotary endodontic instruments (24 instruments for each type), taken from their original packages, were tested for bacterial contamination (Table 1). All tested instruments were of the same size (#25) and length (25 mm) (with the exception of Path Files, Dentsply, York, Pennsylvania, USA). Overall 144 instruments delivered in sterile packages, 24 instruments delivered as non-sterilized in sealed blisters and 96 instruments delivered as non-sterilized in plastic boxes were tested.

For microbiological analyses, instruments were aseptically removed from their original packages, under a class 2 vertical flow safety cabin, and individually transferred into sterile 5 ml conical tubes containing 2 ml of sterile phosphate buffered saline pH 7.2 (PBS). Tubes were then vortexed for 5 minutes to detach eventual adherent bacteria.

Instruments were removed aseptically from the tubes that were then centrifuged 15 minutes at 10000xg. Following centrifugation PBS was carefully removed by inverting tubes and 0.2 ml of sterile PBS were added to each tube to re-suspend bacteria. The resulting suspensions were then plated

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on BD Columbia Agar plates supplemented with 5% Sheep Blood (BD Italia, Milan, Italy) and incubated at 37°C for up to 5 days. Each plate was inspected daily to mark visible bacterial colonies by a sharp-ended permanent marker. At the end of incubation bacterial colonies were counted and data were recorded as colony forming units (CFU/instrument). Standard morphological methods (colony morphology, cell morphology and Gram stain reaction) were used to analyse bacterial colonies grown on agar plates.

If judged of interest (i.e. when standard methods suggested the presence of potentially pathogenic bacteria), single colonies were further streaked for isolation in pure culture on appropriate solid media, and identified at the species level by standard microbiological methods (13).

Significance of differences between bacterial counts obtained from the different tested instruments were evaluated by performing the one-way analysis of variance (ANOVA) for dependent samples. The Tukey HSD test was performed as post hoc test to detect differences between groups of instruments.

Results

Bacterial counts obtained from the tested instruments are reported in Table 2 (individual counts obtained from each instrument) and Figure 1 (mean bacterial counts from each type of instrument ± standard deviation).

As expected, no bacterial colonies were detected from instruments delivered in sterile packages. On the contrary, all instruments delivered as non-sterilized gave rise to the development of bacterial colonies (Table 2).

Table 1. List of endodontic instruments used in this study.

Q.ty	Instrument	Manufacturer	Packaging	Sterile
24	HyFlex EDM	Coltene	Blister	Yes
24	Wave one Gold	Dentsply	Blister	Yes
24	Mtwo	Sweden&Martina	Blister	Yes
24	Protaper	Dentsply	Blister	Yes
24	One Shape	Micro Mega	Blister	Yes
24	F6 Skytaper	Komet	Blister	Yes
24	M3 Rotary Files	United Dental	Blister	No
24	Path File*	Dentsply	Plastic box	No
24	TF Adaptive	Sybron	Plastic box	No
24	Profile Vortex	Dentsply	Plastic box	No
24	K3	Sybron	Plastic box	No

Table 2. Bacterial counts (reported as colony forming units/instruments) obtained from each tested instrument: #instruments delivered in blister; ##instruments delivered in plastic boxes.

M3 Rotary Files United dental #	Path File Dentsply ##	TF Adaptive Sybron ##	Profile Vortex Dentsply ##	K3 Sybron ##
19	124	86	178	156
26	166	129	115	112
21	258	118	147	168
28	189	167	226	137
32	97	134	132	186
24	233	142	184	142
34	149	97	207	117
18	174	114	249	173
29	217	151	181	202
27	132	108	154	164
23	201	125	172	119
31	158	153	232	143
22	122	94	183	161
31	227	136	129	174
26	141	143	142	132
25	139	137	180	126
32	167	116	203	154
28	118	161	213	142
20	193	129	167	167
27	231	119	188	138
33	153	132	145	195
19	177	92	138	127
25	181	101	157	188
22	224	109	121	171

Bacterial counts obtained from instruments commercialized as non-sterilized in plastic boxes (Table 1)(overall mean count 156.1 ± 37.9 cfu/instrument) resulted significantly higher ($P<0.01$) than those obtained from M3 rotary files (United Dental, Changzhou, China), commercialized as non-sterilized in sealed single instrument blisters (mean count 25.9 ± 4.8 cfu/instrument) (Figure 1). Significant differences were detected between bacterial counts obtained from TF Adaptive (Kerr, Orange, Usa) instruments (mean bacterial count 123.9 ± 21.1 cfu/instrument) on one side and and Profile Vortex (Dentsply Sirona, York, Pennsylvania, USA) (mean bacterial count 172.6 ± 36.3 cfu/instrument), or Path files (Dentsply Sirona, York, Pennsylvania, USA) (mean bacterial count 173.8 ± 42.8 cfu/instrument), ($P<0.05$ and >0.01).

No potentially pathogenic species were detected among bacterial contaminants grown from M3 rotary files (delivered single packaged, non-sterilized, in blisters). On the contrary, some potentially pathogenic species (*Pseudomonas aeruginosa*, *Enterococcus* spp., coagulase positive and negative *Staphylococcus* spp.) were detected among bacterial contaminants grown from instruments delivered non-sterilized in plastic boxes (Table 2 and Figure 1).

Discussion

The main goal of root canal treatment is to remove dental pulp and eventual microbial contaminants and shape the endodontic so as to enable to seal it in the absence of potentially dangerous bacterial species. In most cases this implies active removal of pathogenic bacteria that have caused an endodontic infection. In certain instances root canal treatment is performed in the absence of any bacterial contamination. In any case the introduction of exogenous bacteria during endodontic treatment should be reduced as much as possible.

According to data presented in this study, instruments delivered as non-sterilized, show various degrees of bacterial contamination and packaging modalities significantly influence contamination level both quantitatively and qualitatively.

In fact, the packaging modality resulted to be a relevant variable for the bacterial contamination of endodontic instruments. Instruments delivered in sealed packages were shown to be poorly contaminated by few environmental and non-pathogenic bacterial species. Instruments delivered in non-sterilized plastic boxes resulted to be contaminated by a significant higher number of bacteria. Moreover, bacteria isolated from instruments delivered in open plastic boxes included species that could potentially affect the success of an endodontic treatment.

Microbiological analysis revealed that all tested instruments packaged in plastic boxes (i.e. Path Files, TF Adaptive, Profile Vortex and K3) were contaminated by bacterial species that are known agents of human infections (*Pseudomonas aeruginosa*, *Enterococcus* spp., coagulase positive and negative *Staphylococcus* spp.). Among these bacterial species, *P. aeruginosa* and *Staphylococcus aureus* deserve particular consideration as they are well known agents of therapy resistant osteomyelitis (14,15) and *E. faecalis* is also a known agent of persistent endodontic infections (16,17).

Conclusions

Data suggest that instruments packaged in non-sterile plastic boxes could vehicle pathogens and should not consequently be used without preliminary autoclave sterilization; instruments delivered in sealed blisters resulted microbiologically safe although data on this aspect could be affected by the limited number of instruments that were tested.

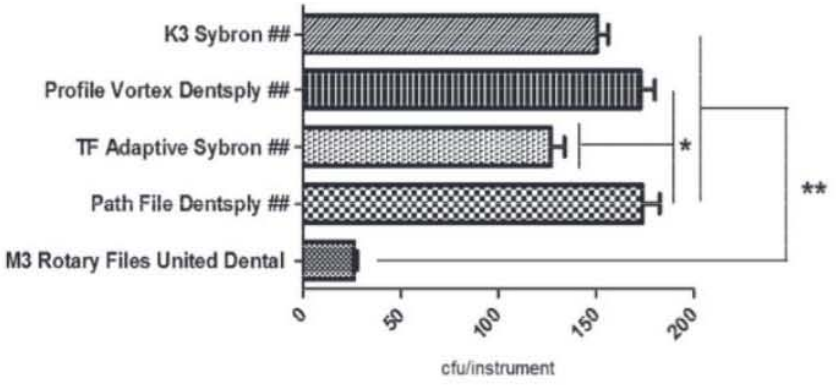

Fig. 1. Mean bacterial counts (reported as colony forming units / instrument) (±SD) obtained with the different groups of instruments. ## instruments delivered in plastic boxes. * values of P in the range >0.01-≤0.05 indicating the existence of significant differences between groups, ** values of P≤0.01 indicating the existence of very significant differences between groups.

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