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Influence of endodontic instrument-holder on sterilization efficacy. A pilot in-vitro study.

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Abstract: Infection control is crucial in dentistry and various methods have been designed to assure its efficacy. However, little information exists regarding the influence it could have the instrument-holder of endodontic files. The aim of this research was to determine the influence of three instruments-holders on sterilization efficacy of endodontic files. **Methods:** A pilot in-vitro study. 60 endodontic files were contaminated by biomechanical preparation of extracted molars with periapical abscess, then processed according to the standard washing method. The endodontic files were divided into 3 groups (n = 20) and assigned to 3 instrument-holders: Metallic box (MB), surgical gauze (SG) and synthetic sponge (SS). Then, the files were packaged and sterilized by autoclaving (134°C/45min). Microbiological culture was performed in thioglycolate solution for each endodontic file (37°C/5days). **Results:** The overall sterilization efficacy was 91.7%, 80% for MB, 100% for SS, and 95% for SG, with no statistically significant differences (p = 0.06) between the groups. **Conclusions:** The lack of differences in the efficacy of sterilization may be due to the reduced sample; therefore, a full-size study is necessary to confirm this outcomes. The results of this study discourage the use of the MB as instrument-holder until a full-size study can confirm this data.

Keywords: endodontics, sterilization, efficacy, instrument.

Influencia del porta-instrumento endodóntico en la eficacia de esterilización. Estudio piloto in-vitro.

Resumen: El control de infecciones es crucial en odontología y variados métodos se han diseñado para asegurar su eficacia. Sin embargo, existe poca información respecto a la influencia que podría tener el porta-instrumento de limas endodónticas. El objetivo de esta investigación es determinar la influencia de 3 porta-instrumentos sobre la eficacia de esterilización de limas endodónticas. **Método:** Estudio in-vitro piloto. 60 limas endodónticas fueron contaminadas mediante preparación biomecánica de molares extraídos con diagnóstico de absceso periapical, procesadas según el método de lavado estándar, divididas en 3 grupos (n=20) y asignadas a 3 porta-instrumentos: caja metálica (CM), gasa quirúrgica (GQ) y esponja sintética (ES); luego empacadas y esterilizadas en autoclave (134°C/45min). Se realizó un cultivo microbiológico en solución de Tioglicolato de cada una de las limas (37°C/5días). **Resultados:** La eficacia de esterilización general fue 91,7%, para CM de 80%, ES de 100% y GQ de 95%; no se hallaron diferencias estadísticamente significativas (p=0,06). **Conclusiones:** La falta de diferencias en la eficacia de esterilización puede deberse a la muestra reducida, por lo que se necesita realizar un estudio con tamaño completo. Los resultados de este estudio desaconsejan el uso de la CM como porta-instrumento hasta que un estudio de tamaño completo confirme estos datos.

Palabras clave: endodoncia, esterilización, eficacia, instrumento.

Introduction.

Infection control is crucial in dental practice due to the presence of several contagious agents who cause varied diseases¹, some of which are serious like the Creutzfeld-Jacob disease². For this reason, the use of an aseptic technique in root canal therapy is imperative to achieve a successful treatment. Also, all of the steps should be taken to avoid the introduction of contaminated agents into the root canal and the periapical region³⁻⁵.

In daily clinical practice, instruments of many sizes and shape are used; several of which make debris elimination difficult. It is known that the endodontic reamers challenge the washing process, because of their complex, small structure⁶. Furthermore, the number of times that the instrument has been sterilized

influence directly on the efficacy⁷.

Some studies demonstrated a high degree of contamination of some instrument in their own original package⁸, meanwhile other studies demonstrated no presence of infectious agents, having made cultives of instrument directly taken from the original package⁹.

Studies of the sterilization method demonstrate a better efficacy of the autoclave steam method above others^{1, 3, 10, 11}. In the washing stage, one of the most effective combinations is the manual debris elimination and pre-soaking with enzymatic detergents⁹. This can be improved with the use of the ultrasonic cleaner and the automatic washer machine for surgical instruments¹². Also, there are other studies of the effectiveness of the pre-sterilization processes and the combined use of them^{7, 13}.

There is a lack of research focused on the impact of the instrument-holders which are used for endodontic instrument sterilization and this are centered basically in the usage of synthetic sponge, with almost 100% of efficacy^{10, 14}, in other words the sponge does not affect the sterilization process. Because this, does not exist enough information about the interference that might have the instrument-holder in the efficacy of the sterilization process.

The aim of this research is to determine the influence of three instrument-holders – metallic box, surgical gauze and synthetic sponge – on sterilization efficacy.

Materials and methods.

Design: In-vitro study. An exploratory experimental study was performed.

Analysis unit: Endodontic hand files (Ø15-80/25mm) (Maillefer, Switzerland) were used: H files, K files and Reamers.

Selection criteria: Inclusion criteria was endodontic hand files used in the preparation of teeth with apical abscess diagnostic and an acceptable conservation state (no broken parts and no stretched or bended, all files had 10 or fewer uses); any files with oxide was excluded.

Sample Size: Was determined according to the general estimation for a proportion with: Significance level of 5%, a priori sterilization efficacy of 96.7% and a standard error of 5%; resulting in a sample of 59 instruments per intervention. Due to the exploratory nature of this study, a third of the required sample was used: 20 files for each one of three interventions tested.

Intervention: In a first step, three teeth with the endodontic diagnosis of apical abscess (acute or chronic) were obtained in the Clinic of Dental Urgency at Universidad de Concepción Dental School. Later, the teeth were treated biomechanically with the endodontic instruments with the aim of contamination, as in a root canal treatment (Figure 1).

Then, the instruments were submitted to the sterilization process (Figure 2), as described:

1. Set under the waterjet.
2. Pre-soaked in enzymatic detergent for 15 minutes.
3. Washed rigorously with water and neutral soap.
4. Dried with gauze.
5. All of the instruments were randomly put in different instrument-holders in groups of four:
 - a. Surgical Gauze
 - b. Synthetic Sponge
 - c. Metallic box

6. Packed in Steam Heat Bag for Autoclave (PMG, Mexico).
7. Sent to the Dental School Sterilization Center, where the bags were sealed and sterilized in Autoclave (Matachana, Model S100. Mexico) with the standard program (45 minutes / 135°C).

After the sterilization, the bags were carried to the Department of Microbiology, Faculty of Biological Sciences, Universidad de Concepción, where they conducted a culture procedure. It consisted of an aseptic transfer of the endodontic instruments to sterile tubes with Thioglycolate solution then incubated at 37°C for five days. Thioglycolate solution allows the growth of aerobic and anaerobic species.

Outcome: The efficacy of sterilizations was

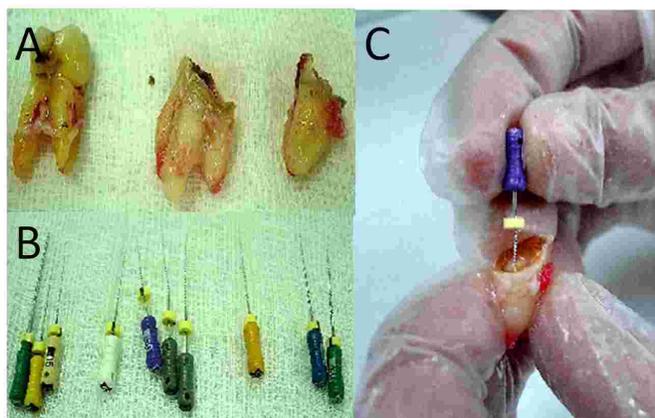


Figure 1: Use and contamination of endodontic files. A. Extracted molars with infected root canal; B. Endodontic files; C. Canal root preparation.

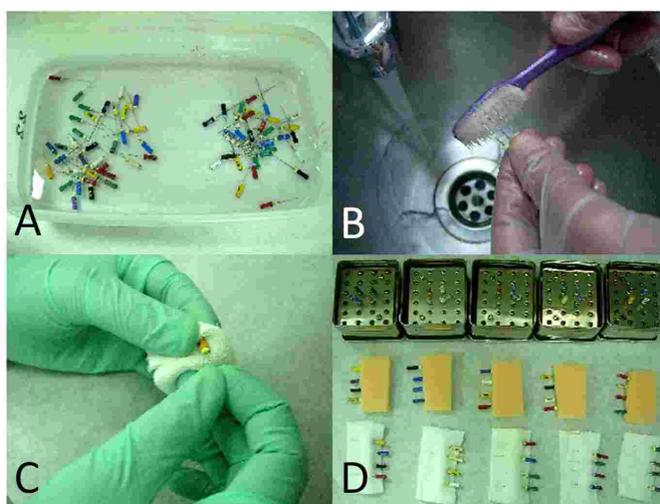


Figure 2: Sterilization process and assignment to each instrument holder. A. Enzymatic detergent (15 min.); B. Manual removal of residues with a toothbrush; C. Drying with surgical and sterile gauze; D. Assignment and package.

determined by the absence of microbiological growth in the test tube, according to the evaluation of the microbiologist.

Statistical analysis: Efficacy rate was calculated for each intervention. The statistical differences between them was estimated by the Halton-Freeman extension of the Fisher exact probability test for a two-rows by three-columns contingency table, a $p < 0.05$ was considered.

Results.

Results of sterilization efficacy for each instrument holder are presented in Table 1.

There was no significant statistical differences between the interventions groups ($p = 0.0617$).

Instrument holder	Sterilized	Not sterilized
Surgical gauze	95% (19)	5% (1)
Metallic Box	80% (16)	20% (4)
Synthetic sponge	100% (20)	0% (0)
Total	91.7% (55)	8.3% (5)

Tabla 1.
Sterilization efficacy by instrument holder.

Discussion.

There are very few studies focused on determining the influence of the instrument-holder in the sterilization of endodontic files. Meanwhile, Kuritani *et al*⁴ give a 96.67% of effectiveness for autoclave sterilization using contaminated sponges, Boyd *et al*⁰ confirm a 100% effective sterilization of endodontic hand instruments by using synthetic sponges as the instrument-holder, placed in sealed bags and sterilized by autoclaving. The results of this study confirm those in the literature,

with values very close to 100% for synthetic sponge as holder of endodontic files.

However, there are no other studies that account for other instrument-holders, which make comparison of results difficult. Despite the above, the influence of the metallic box brought the worst consequences to the sterilization process, which is also partially in line with what is described in the literature for instruments having shapes and/or sizes that are difficult in the sterilization process.^{6,7}

For example, the metallic box may have facilitated the accumulation of debris, which prevented proper sterilization by re-contaminating the instrument after the washing process. This hypothesis should be tested in future research.

It is imperative to mention some limitations of this study. First, this is a pilot study, and the sample size is not adequate to avoid Type II error in the statistical test applied. This is evident in the case of the low rate of sterilization to the metallic box, which was not statistically significant. A simulation of the Freeman extension of the Fisher exact test probability maintaining sterilization rates and tripling the sample size does determine differences ($p < .05$).

Another limitation relates to the in vitro methodology used, so these results are not directly transferable to clinical practice.

Despite this, the results of this study recommend avoidance of the metallic box as an instrument-holder for sterilization of endodontic files until the realization of a new study with the required sample size and usual clinical conditions can confirm or refute the differences found.

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