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Efectividad de diferentes protocolos de desinfección / esterilización para instrumental de ortodoncia sensible al calor

Artículo Original/Original Article

Efectividad de diferentes protocolos de desinfección / esterilización para instrumental de ortodoncia sensible al calor

Effectiveness of different disinfections/sterilization protocols for heat sensitive orthodontics instruments

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Resumen

Objetivo: El objetivo de este trabajo fue evaluar la efectividad de diferentes protocolos desinfección/esterilización para instrumental termosensible de ortodoncia. **Métodos:** Se aplicaron ocho protocolos a instrumentos ortodónticos (n = 120). El instrumental se limpió con detergentes bi o trienzimáticos y se desinfectó con glutaraldehído 2,5%, o hipoclorito al 1% u ortoftalaldehído 0,55%. Después de aplicar cada protocolo, se tomaron muestras de los instrumentos y se cultivaron en agar MacConkey y Aged CLED. Para *Staphylococcus spp*, se utilizó una prueba de coagulasa. Los instrumentos se consideraron contaminados cuando el recuento de UFC de los cultivos fue superior a 10⁵ UFC / ml. **Resultados:** Se encontraron las mismas proporciones de contaminación al usar glutaraldehído e hipoclorito de sodio, y no se encontraron diferencias significativas con el uso de detergentes bi o trienzimáticos. De los tres desinfectantes utilizados, ortoftalaldehído fue significativamente más eficiente, ya que nunca se encontró contaminación cuando se el mismo fue utilizado. Los microorganismos aislados de los diferentes instrumentos fueron *Micrococcus spp*, *Corynebacterium spp*, *Bacillus spp* y *Staphylococcus* coagulasa-negativo. **Conclusiones:** El ortoftalaldehído fue significativamente más eficiente como desinfectante. Los microorganismos aislados fueron *Micrococcus spp*, *Corynebacterium spp*, *Bacillus spp* y *Staphylococcus* coagulasa-negativo. Aunque el porcentaje de instrumentos contaminados fue bajo en relación con el total monitorizado, cualquier instrumento contaminado implica riesgos, para un paciente o profesional, de una enfermedad infecciosa o contagiosa.

PALABRAS CLAVES: ortodoncia, instrumental dental, desinfección, esterilización.

Abstract

Objective: The aim of this work was to assess effectiveness of different disinfections/ high level disinfection protocols for heat-sensitive orthodontics instruments. **Methods:** Eight protocols were applied to orthodontic instruments (n=120); they were cleaned with dual- or triple-enzyme detergent and disinfected with glutaraldehyde 2.5% or hypochlorite 1% or orthophthalaldehyde 0.55%. After applying each protocol, samples were taken from the instruments and cultured in MacConkey Agar and CLED Agar media. For *Staphylococcus* spp, a coagulase test was used. Instruments were considered contaminated when the CFU count from the cultures was greater than 10⁵ CFU/ml. **Results:** The same proportion of contamination was found when using glutaraldehyde and sodium hypochlorite, and no significant differences were found with the use of dual-enzyme and triple-enzyme detergents. Of the three disinfectants used, orthophthalaldehyde was significantly more efficient, since contamination was never found when it was used. The microorganisms isolated from the different instruments were *Micrococcus*spp, *Corynebacterium* spp, *Bacillus* spp and coagulase-negative *Staphylococcus*. **Conclusions:** Orthophthalaldehyde was significantly more efficient as a disinfectant. The microorganisms isolated were *Micrococcus* spp, *Corynebacterium* spp, *Bacillus* spp and coagulase-negative *Staphylococcus*. Although the percentage of contaminated instruments was low in relation to the total monitored, any contaminated instrument implies risks to a patient or a professional of an infectious or contagious disease.

KEY WORDS: orthodontic, dental instruments, disinfection, sterilization

Introduction

Infection control is given great importance in today's practice of dentistry¹. Many oral and systemic disease-causing organisms are easily transmitted from the oral cavity as they have long latent incubation periods². Dental health personnel are constantly exposed to the threat of infection by occupational exposures to a variety of microbial pathogens, and therefore the prevention of cross-contamination among dentists, dental staff, and patients is a major concern in dental practice³. Awareness of efficient sterilization techniques occupies centre stage in the prevention of the spread of infectious diseases².

Articles related to the transmission of infectious agents in dentistry have focused on the instruments as possible vehicles of disease transmission⁴⁻⁵, increasing the risk of cross-infection. Orthodontics has always been characterized by a high rotation of patients and by the use of a variety of metal, acrylic and elastomeric instruments, and so orthodontists is at high risk of exposure to serious pathogens and must take adequate precautions to guard themselves against their transfer².

As several instruments used in orthodontics are elastomeric and/or acrylic, to which heat sterilization techniques cannot be applied, the aim of this work was to assess effectiveness of different disinfections/ high level disinfection protocols for heat-sensitive orthodontics instruments routinely used in clinical orthodontic care.

Methods

Acrylic and elastomeric instruments commonly used in the clinical practice of orthodontics were monitored (n=120). The elastomeric elements were: ligature sticks (SANI-TIE, Dentsply GAC International, USA) (**LI**), spools of elastomeric chains (Maximum Power Chain Orthotechnology, USA) (**CH**) and the acrylic elements were: intraoral cheek retractors (OdontoMatriz 2000, Buenos Aires, Argentina) (**RE**) (Figure 1).

We mainly analyzed the action of glutaraldehyde, sodium hypochlorite and orthophthalaldehyde compounds.

Eight protocols effectiveness were measured:

Protocol A was applied to LI, CH, and RE (Table 1).

Protocols B and C were applied to LI, CH (Table 1).

Protocols D, E, F, G, H were applied to RE (Table 2).

Table 1: Description of protocols applied to spools of elastomeric chains and to ligature sticks. Ref. CH: spools of elastomeric chains, LI: ligature stick.

	DISINFECTION	RINSING	DRIED
PROTOCOL A (CH n=10) (LI n=10)	Spraying with aqueous solutions of 2.5% glutaraldehyde (<i>Surgibac G, Tracker Medical, Buenos Aires, Argentina</i>) and leaving it to act for 5 minutes	Under running water for 5 minutes.	With disposable medical grade paper towels (<i>Axon, Córdoba, Argentina</i>).
PROTOCOL B (CH n=10) (LI n=10)	CH: spraying the reel of chain (unwound) with aqueous solution of glutaraldehyde 2.5%, leaving it to act for 10 minutes and immersing the end of the chain (15 cm) in the same solution for 10 minutes. LI: immersion in aqueous solution of glutaraldehyde 2.5 %, leaving it to act for 30 minutes	Under running water for 5 minutes.	With disposable medical grade paper towels
PROTOCOL C (CH n=10) (LI n=10)	CH: spraying the reel of chain (unwound) with aqueous solution of hypochlorite 1% (<i>Active chlorine 55g/liter, Ayudin, CLOROX, Argentina</i>), leaving it to act for 10 minutes and immersing the end of the chain (15 cm) in the same solution for 10 minutes. LI: immersion in aqueous solution of hypochlorite 1% leaving it to act for 10 minutes.	Under running water for 5 minutes.	With disposable medical grade paper towels

The protocols applied were based on those of Irazuzta et al.⁶ and Zarate et al.⁷.

Bacteriological testing

After applying each protocol, samples were taken from the instruments for bacteriological analysis, using plastic

tubes with a sterile cotton swab (Eurotubo, Barcelona, Spain) to which later thioglycollate broth (Britania, Buenos Aires, Argentina) was added with indicator and they were then incubated for 24 hs at 37°C.

The following bacteriological testing was performed on tubes that displayed macroscopic turbidity:

1. Gram staining for the preliminary identification of the bacteria by morphotypes.
2. Culture in following media: MacConkey Agar (Britania, Buenos Aires, Argentina), and CLED Agar (Britania, Buenos Aires, Argentina). In each case, three lines with a 5 ml calibrated loop, for quantification of the bacterial load. Incubation at 37°C for 24 h.
3. Gram staining of the bacteria that developed.
4. Biochemical tests for typification. For *Staphylococcus*, a coagulase test was used.
5. Instruments were considered contaminated when the CFU count taken from the cultures of the samples was greater than 10⁵ CFU/ml.

Statistical analysis:

The variables considered in this study were: a) Infected (<10⁵ CFU/ml) / uninfected (10⁵ CFU/ml) – binary variable -and b) protocols applied – polytomous nominal variable -.

The following analysis was made for the type of variables:

1. The statistical description of the data was made by relative frequencies expressed in percentages, calculated as the number of contaminated instruments over the total of instruments used in each technique.
2. The evaluation of a significant association between the different protocols and bacterial infection/non-infection was performed by Chi Squared test and p-value <0.05 was set for statistical significance.

Results

Eight protocols were applied and a total of 120 instruments were analyzed. The contamination presented for each protocol is shown in Table 3.

When using glutaraldehyde and sodium hypochlorite for disinfection for LI and CH, the same proportion of contamination was found (Table 3).

Of the three disinfectants used, orthophthalaldehyde was significantly more efficient, as contamination was never found when this was used, while the same proportions of contamination were found with hypochlorite and glutaraldehyde (Table 3).

With regard to the RE no significant differences were found between the use of dual-enzyme and triple-enzyme detergents, and the same proportions of contamination

were found when using glutaraldehyde and sodium hypochlorite.

The microorganisms isolated from the different instruments were *Micrococcus* spp, *Corynebacterium* spp, *Bacillus* spp and coagulase-negative *Staphylococcus*.

DISCUSSION

In this work, we set out to check the effectiveness of different disinfection and high level disinfection (HLD) protocols, applied to heat-sensitive instruments that are used routinely in orthodontics. The daily use of plastic and elastomeric instruments at every clinical step prevents them being sterilized in ethylene oxide (which is the gold standard for sterilizing these instruments), because they would have to be sent to specialized processing stations with adequate safety levels, requiring time and, what is more important, a high cost. Given the risk of cross-infection generated through these instruments, it is very important to know the effectiveness of the different disinfectants and high-level disinfectants and protocol their use for clinical orthodontic practice.

We analyzed the action of glutaraldehyde and sodium hypochlorite compounds, because these are routinely used for the disinfection of heat-sensitive instruments by the general dental community in our country. In addition, we tested the effectiveness of orthophthalaldehyde, although this disinfectant is not known in our dental community because of its high cost.

Sodium hypochlorite is an inexpensive and effective disinfectant; it has bacterial, fungicidal, virucidal and sporicidal activity, but it is readily inactivated by organic matter⁸. Also, the effectiveness of hypochlorites as disinfecting agents is influenced by factors such as concentration, pH value, and temperature⁹. We always found a percentage of contamination when sodium hypochlorite was used. The disinfection was always performed by immersing the instruments in an aqueous solution of hypochlorite 1% or by spraying the reel of chain and immersing the end of the chain as described above. Bustos et al.¹⁰ showed the disinfecting power of sodium hypochlorite for printing materials used at concentrations of 0.5% for 5 minutes but we left it to act for 10 minutes and still contamination was found; if the immersion time in sodium hypochlorite solution is increased, it permeates the material with its intense odor and the taste is stronger, causing discomfort to the patient and, over time, it also alters the plastic and elastomeric material. The worldwide use of sodium hypochlorite in dentistry is as a root canal irrigating solution due to its efficacy for pulpal dissolution and antimicrobial activity¹¹. As a disinfectant, it is considered a good option for surfaces such as those in the cuspidor, as it has been shown to reduce the microbial load of Gram

positive bacteria and completely eliminate Gram negative bacteria¹².

Glutaraldehyde is a chemical agent used for high level disinfection that is capable of reducing the population of sporulated microorganisms, is unaltered in the presence of inorganic material and does not react with synthetic materials or detergents. When we used the 2.5% solution of glutaraldehyde in different ways, we obtained different results. When glutaraldehyde was *sprayed* on the instruments, contamination was found. This process was ineffective because it was seen to produce a heterogeneous disinfection and only a reduction of microorganisms and is therefore contraindicated⁶. It is important to note that, when the glutaraldehyde solution was sprayed (in protocol A), the first step of decontamination and cleaning was not performed; this is not recommended even though this is normal practice among orthodontists when they work with many patients in a clinical day.

For validating high-level disinfection (HLD) for instruments with glutaraldehyde solution, authors like Frãpilãet al.¹³ recommend that they must be immersed in 2% glutaraldehyde for 30 min. We found that, when instruments were *immersed* in glutaraldehyde there was no contamination. The RE and LI were immersed completely in the solution and the spools of CH were opened and sprayed and the ends immersed in the product for 30 minutes. We did this because normally the elastomeric chain is cut off the roll with scissors and inserted in the oral cavity and so it can be contaminated during processing, packaging and manipulation by the dental assistant or orthodontist, prior to reaching its final destination in the oral cavity¹⁴. Pithon et al.¹⁵ also recommend immersing the ends of the chains in disinfectant, because these are always in contact with the patient and the orthodontist indirectly contaminates the reel that contains the material, which may trigger a cross-infection. We agree with this author and with others such as Supronoet al.¹⁶, Reddy et al.¹⁷ and Gutierrez et al.¹² and we recommend the use of glutaraldehyde as a disinfectant based on the research results.

With respect to orthophthalaldehyde, after decontamination and cleaning, we immersed the instruments in this solution and none of them presented contamination. Orthophthalaldehyde has excellent mycobactericidal activity and is used for HLD at a 0.55% concentration for 12 minutes¹³. It can be recommended instead of glutaraldehyde, because of its low toxicity. It is a compound that emits little vapor and has not yet been shown to be carcinogenic. A study by Rutala¹⁸ demonstrated that orthophthalaldehyde acts well against glutaraldehyde-resistant microorganisms such as mycobacteria and *Bacillus subtilis*. Orthophthalaldehyde is a high-level disinfectant however, and skin damage that seems to have been caused by its improper use has

been observed in isolated instances¹⁹. Various studies have validated the use of OPA as a high level disinfectant for endoscopes^{20, 21}. No studies have been found related to the disinfection/sterilization of dentistry instruments using orthophthalaldehyde.

For RE decontamination and cleaning, plastic containers with double- or triple-enzyme detergents were used but we observed no significant differences between these. When the retractors were washed with double-enzyme detergent and submerged in glutaraldehyde for 30 minutes, contamination was found. This could be related to incorrect cleaning of these instruments, because it is common in clinical rooms where there is a high rotation of patients to take just a short time to treat them, especially for cleaning and decontamination. Retractors are widely used for retracting cheeks and lips in order to obtain an adequate clinical photograph for monitoring treatment progress and, consequently, they come into contact with saliva and possibly blood²². The correct cleaning of instruments is fundamental for increasing the effectiveness of decontamination as, when well-performed, it physically removes all the remains of organic matter⁸. For a correct and effective decontamination, enzyme detergents for medical use are recommended as cleansing solutions²³. Whitworth et al.²⁴ showed that this step increases the effectiveness of the cleaning processes, because the waste that remains in instruments is difficult to remove and the detergents remove the organic matter, even in the least accessible places. They also stimulate a process that diminishes risks in the work environment, by a significant reduction of microorganisms when contaminated instruments are handled. Although in our study no significant differences were found in the use of double- and triple-enzyme detergents, when the instruments present blood or tissue debris the use of triple-enzyme detergents is recommended.

The types of bacteria isolated and identified in the cultures of samples taken from instruments were: coagulase-negative *Staphylococcus*, *Corynebacterium* spp, *Bacillus* spp and *Micrococcus* spp. In a study made by Rabello et al.²⁵ in forceps, clamps and drills, contamination was found with *Corynebacterium*, *Micrococcus*, coagulase-negative *Staphylococcus* and *Bacillus* spp. All these microorganisms are part of the normal flora of humans and of the environment, which suggests a possible source of contamination in the overpopulation of the clinical rooms in which the study was made. It should be remembered that the dentist's office has been classified by the CDC at a Biosafety Level of 2, which implies "restricted access"²⁶. In addition, many human diseases are caused by microorganisms that are part of the normal flora of skin, mucous membranes and other body cavities of healthy

individuals; they develop when those microorganisms are taken to locations where they are not usually found²⁷.

Coagulase-negative *Staphylococcus* (CNS) are found in 63% of human buccal cavities; although these microorganisms were for many years considered clinically insignificant, they have now been associated with numerous human diseases such as urinary infections, endocarditis, cardiovascular infections, encephalitis and hospital-acquired infections²⁸. Among the CNS, *Staphylococcus epidermidis* most likely to cause infections, being introduced into the body from the skin by means of medical instruments²⁹.

Orthodontic professionals should know that all clinical instruments in orthodontics must be considered critical or semi-critical. This means applying sterilization protocols for heat-resistant or HDL for heat-sensitive instruments.

In this study, the percentage of contaminated instruments was low (10.83%) in relation to the total monitored, but we consider that any contaminated instrument implies risks to a patient or the professional of acquiring an infectious or contagious disease.

The presence of coagulase-negative *Staphylococcus* should be considered as a contamination marker.

This work enables orthodontic professionals, whether in private offices or in dental training institutions, to become aware of the possibility of generating cross-infections through the instruments used in their normal clinical practice.

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Figure 1:Acrylic and elastomeric orthodontic instruments monitored.A and E: acrylic intraoral cheek retractors; B and C: ligature sticks; D: spools of elastomeric chains

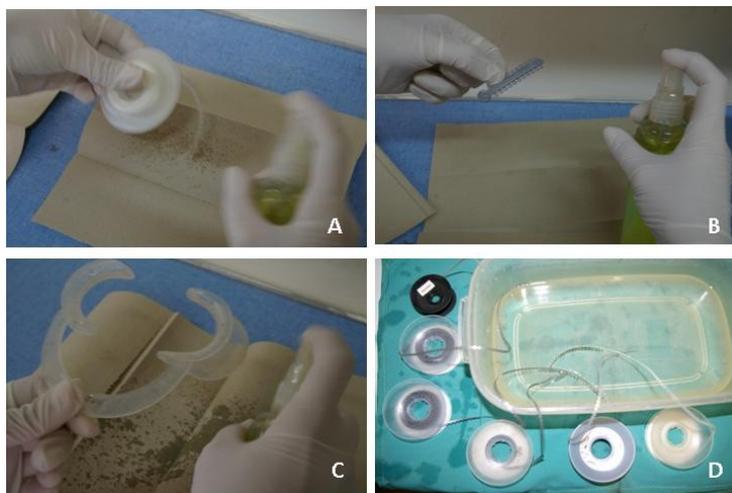


Figure 2: Disinfection process. A, B and C: Spraying with aqueous solutions of 2.5% glutaraldehyde; D: immersion of the end of the chain in aqueous solutions of 2.5% glutaraldehyde

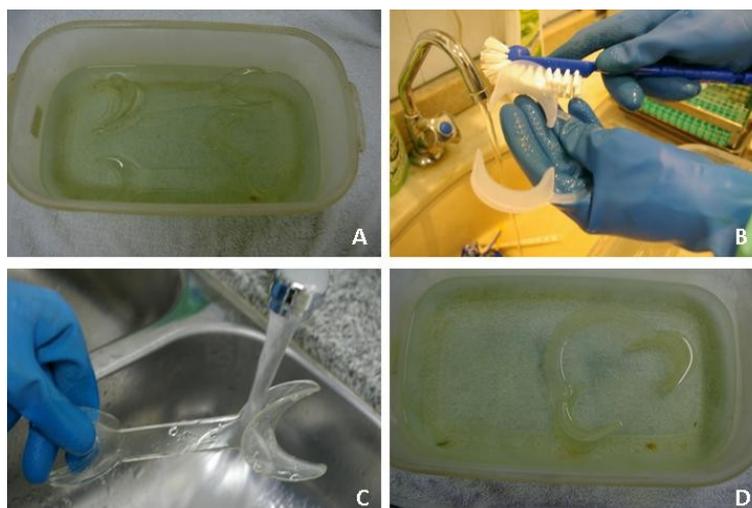


Figure 3: Retractors: A: decontamination by immersion in aqueous solution of enzyme, nonionic detergent; B: cleaning by brushing with hard-bristle non-metallic brush; C: rinsing under running water for 5 minutes; D: Disinfection by immersion in aqueous solution of glutaraldehyde 2.5% for 30 minute

Table 2: Description of protocols applied to intraoral cheek retractors. Ref. *RE: intraoral cheek retractors*

	DECONTAMINATION	RINSING/ DRIED	DISINFECTION	RINSING/ DRIED
PROTOCOL A (RE n= 10)			Spraying with aqueous solutions of 2.5% glutaraldehyde(<i>Surgibac G, Tracker Medical, Buenos Aires, Argentina</i>) and leaving it to act for 5 minutes	Under running water for 5 minutes. With disposable medical grade paper towels (<i>Axon, Córdoba, Argentina</i>).
PROTOCOL D (RE n= 10)	Immersion in aqueous solution of dual-enzyme, nonionic detergent (Bacter Z, Densell; Buenos Aires, Argentina) (Figure 3A)	Under running water for 5 minutes/ With disposable medical grade paper towels	By immersion in aqueous solution of glutaraldehyde 2.5% for 30 minutes.	Under running water for 5 minutes/ With disposable medical grade paper towels
PROTOCOL E (RE n= 10)	Immersion in aqueous solution of dual-enzyme, nonionic detergent, for 10 minutes	Under running water for 5 minutes/ With disposable medical grade paper towels	Immersion in aqueous solution of hypochlorite 1% leaving it to act for 10 minutes (Figure 3D).	Under running water for 5 minutes/ With disposable medical grade paper towels
PROTOCOL F (RE n= 10)	Immersion in aqueous solution of dual-enzyme, nonionic detergent, for 10 minutes	Under running water for 5 minutes/ With disposable medical grade paper towels	Immersion in orthophthalaldehyde solution at 0.55% (<i>CIDEX OPA Solution, Johnson & Johnson Medical Ltd, Coronation Road, Ascot, Berkshire SL5 9EY, UK</i>) for 12 minutes.	Under running water for 5 minutes/ With disposable medical grade paper towels
PROTOCOL G (RE n= 10)	Immersion in aqueous solution of triple-enzyme, nonionic detergent (Surgizime, Tracker medical SRL, Buenos Aires, Argentina)	With disposable medical grade paper towels	Immersion in aqueous solution of glutaraldehyde 2.5% for 30 minutes.	Under running water for 5 minutes/ With disposable medical grade paper towels
PROTOCOL H (RE n= 10)	Immersion in aqueous solution of triple-enzyme, nonionic detergent	With disposable medical grade paper towels	Immersion in orthophthalaldehyde for 60 minutes.	Under distilled water /With disposable medical grade paper towels

Table 3: Percentage of contaminated instruments and percentage of contaminating microorganisms found in each protocol. Ref. *CH*: spools of elastomeric chains; *LI* ligature sticks; *RE* intraoral cheek retractors; *AF*: absolute frequency

INSTRUMENT	PROTOCOL	DECONTAMINATION	DESINFECTION	CONTAMINATION % (AF)	MICROORGANISM
LI (n= 10)	A	Non done	Glutaraldehyde (spraying)	10 (1)	<i>Coagulase-negative Staphylococcus</i>
LI (n= 10)	B	Non done	Glutaraldehyde (immersion)	without contamination	
LI (n= 10)	C	Non done	Hypochlorite (immersion)	10 (1)	<i>Micococcuspp</i>
CH (n= 10)	A	Non done	Glutaraldehyde (spraying)	10 (1)	<i>Coagulase-negative Staphylococcus</i>
CH (n= 10)	B	Non done	Glutaraldehyde (immersion)	without contamination	
CH (n= 10)	C	Non done	Hypochlorite (immersion)	20 (2)	<i>Bacillus spp</i>
RE (n= 10)	A	Non done	Glutaraldehyde (spraying)	30 (3)	<i>Coagulase-negative Staphylococcus</i>
RE (n= 10)	D	Dual-enzyme, nonionic detergent (immersion)	Glutaraldehyde (immersion)	20 (2)	<i>Bacillus spp</i> ; <i>Micococcuspp</i>
RE (n= 10)	E	Dual-enzyme, nonionic detergent (immersion)	Hypochlorite (immersion)	30 (3)	<i>Bacillus spp</i> ; <i>Coagulase-negative Staphylococcus</i> ; <i>Micococcuspp</i>
RE (n= 10)	F	Dual-enzyme, nonionic detergent (immersion)	Orthophthalaldehyde (immersion)	without contamination	
RE (n= 10)	G	Triple-enzyme, nonionic detergent (immersion)	Glutaraldehyde (immersion)	without contamination	
RE (n= 10)	H	Triple-enzyme, nonionic detergent (immersion)	Orthophthalaldehyde (immersion)	without contamination	

