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ORIGINAL ARTICLE

Evaluation of different disinfection protocols for gutta-percha cones contaminated by microorganisms associated with improper handling by the professional

Avaliação de diferentes protocolos de desinfecção para cones de guta-percha contaminados por microrganismos associados ao manuseio inadequado pelo profissional

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KEYWORDS
Gutta-percha
Decontamination
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ABSTRACT

Objective: to evaluate the effectiveness of decontamination of different protocols for disinfection of gutta-percha cones contaminated by *Staphylococcus aureus*. **Method:** previously contaminated gutta-percha cones were submitted for contact with other disinfecting agents at different times. Subsequently, the protocols were evaluated for the growth of microorganisms by observing the color change of the medium contained in the tubes. A comparative qualitative analysis was performed to assess contamination differences between *Staphylococcus aureus* ATCC 25923 and Enterococcus faecalis ATCC 29212. **Result:** In the qualitative study, no difference was observed regarding the contamination of gutta-percha cones by the microorganisms tested. All agents tested had their effectiveness over the decontamination action of gutta-percha cones contaminated in both exposure times. **Conclusion:** the *Staphylococcus* genus is the most frequently found after contamination due to the breakdown of the aseptic chain suggesting a bacterial model for experiments associated with contamination due to inadequate professional manipulation of dental materials. All the agents showed an effective decontamination action of contaminated gutta-percha.

PALAVRAS-CHAVE
Guta-percha
Descontaminação
Staphylococcus

RESUMO

Objetivo: avaliar a eficácia da descontaminação de diferentes protocolos de desinfecção de cones de guta-percha contaminados por *Staphylococcus aureus*. **Método:** cones de guta-percha previamente contaminados foram submetidos ao contato com diferentes agentes desinfetantes por períodos distintos. Posteriormente, os protocolos foram avaliados quanto ao crescimento de micro-organismos por meio da observação da alteração da cor do meio contido nos tubos. Uma análise qualitativa comparativa foi realizada entre o material contaminado com *Staphylococcus aureus* ATCC 25923 e Enterococcus faecalis ATCC 29212. **Resultado:** Na análise qualitativa, nenhuma diferença foi observada quanto

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à contaminação dos cones de guta-percha pelos micro-organismos testados. Todos os agentes testados mostraram eficácia na ação de descontaminação dos cones de guta-percha contaminados, em ambos os tempos de exposição. **Conclusão:** O gênero *Staphylococcus* é o mais frequentemente encontrado após a contaminação devido à quebra da cadeia asséptica, sugerindo um modelo bacteriano para experimentos associados à contaminação por manipulação inadequada dos materiais dentários. Todos os agentes mostraram uma ação eficaz de descontaminação dos cones de guta-percha contaminados.

INTRODUCTION

Endodontic treatment aims to prevent infections that may affect the root canal system (RCS) or to eradicate them when already present, through chemical and mechanical disinfection techniques[1]. If performed correctly, this treatment has high success rates, with failures often attributed to the persistence and resistance of microorganisms, dental anatomical changes, and technical errors during endodontic therapy[2]. Root sealing is a crucial step for the success of endodontic treatment. The combination of materials and sealing cements promotes the formation of an airtight and three-dimensional seal that prevents microbial contamination in the RCS and the appearance or persistence of periapical lesions[1,3].

Gutta-percha cones are the most widely used filling materials in endodontics, presenting biocompatibility, dimensional stability, radiopacity, thermoplasticity, easy removal, and antimicrobial activity (attributed to the zinc oxide in its composition)[3,4]. However, because it is manufactured in a non-sterile environment and cannot be sterilized in an autoclave, it is a material that can carry microorganisms into the RCS if not properly disinfected before being used in the filling step[3]. Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are the most commonly used compounds for the chemical decontamination of gutta-percha cones. However, despite the literature presenting satisfactory results with these antimicrobials, there is no consensus on the best protocol or disinfecting agent for this material[3,4].

In vitro studies reported endodontic issues are often conducted using *Enterococcus faecalis* as a microbiological model[5-7]. However, because this microorganism is part of the oral microbiota and is classically associated with persistent endodontic lesions and infections, it could not be considered an exogenous microorganism introduced into the RCS, as it may already be colonizing the region and not introduced by the contaminated filling material[6,7].

Staphylococcus is a bacterial genus associated with the breakdown of the aseptic chain and is one of the most common microorganisms isolated from procedure gloves[8,9]. Therefore, it could be a relevant microorganism, linking the contamination of the gutta-percha cone to improper handling by the professional and the breakdown of the aseptic chain during the filling phase[10]. This study aims to use different disinfection protocols to evaluate the effectiveness of chemical decontamination of gutta-percha cones that were previously contaminated, contributing to the identification of the best disinfection method for the material used in sealing the RCS.

METHODS

Selection of disinfectant solutions and gutta-percha cones

Three disinfectant agents were tested using different contact times in the disinfection protocols. The most relevant agents in research and the most commonly used in dental offices were selected, making them easily accessible to professionals. These agents were: sodium hypochlorite (NaOCl) at concentrations of 0.5%, 1%, 2.5%, and 5.25%; chlorhexidine (CHX) at 2%; and ethanol (ethyl alcohol) at 70%. At the end of the disinfection protocol, the gutta-percha cones were rinsed to remove any residual disinfectants, to not interfere in the evaluations.

All these agents were tested at contact times of 1 and 5 minutes. For the negative control, sterile saline solution was used for 1 and 5-minute periods. To compare the effectiveness of the disinfecting actions, the 5.25% concentration was used as a reference, as reported by Amaral et al.[4].

Microorganisms conditions

Bacterial strains of *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 were used. The strains were reactivated from the stock of the microbiological collection at the Department of Microbiology and Immunology of the Faculty of Medical Sciences at UERJ (Rio de Janeiro, RJ, Brazil).

Mannitol salt liquid culture medium

The Mannitol Salt Agar medium (HiMedia Laboratories Pvt.Ltd) was selected because it provides ideal conditions for the growth of the microorganism chosen as the contaminant (*ATCC 25923*) and for the color change in the culture medium from red to yellow, in case of growth.

The culture medium was filtered to remove agar, converting it into a liquid state. The liquid medium promotes greater nutrient availability and microbial proliferation.

Microbiological analysis and group division

In a container, 42 #80 gutta-percha cones (DiaDent, Diadema, SP, Brazil, batch 56535) were immersed in a saline suspension with the *S. aureus* strain (ATCC 25923) at a turbidity corresponding to 0.5 on the McFarland nephelometric scale (approximately 1.5×10^8 CFU/mL) for 10 minutes to ensure the effective contamination of the tested material.

Next, the cones were divided into 14 groups, and each group was exposed to one of the previously selected disinfecting agents for contact times of 1 minute and

5 minutes. After the stipulated contact time, each sample was submerged in a sterile test tube containing the Mannitol Salt liquid culture medium, as previously described, and kept in an incubator at 37°C for 48 hours. The tubes were evaluated at 24 and 48 hours. The visual results regarding microbial growth were recorded based on the color change of the medium: positive (yellow) and negative (red). The negative control group consisted of samples previously contaminated with *S. aureus* and immersed in saline.

The experiment was performed in triplicate, with the triplicate applied to each group formed and subjected to the established protocols.

Qualitative analysis of gutta-percha cone contamination

For the qualitative analysis of the contamination on the surface of the gutta-percha cones, the rolling plate method described by Pereira-Ribeiro et al.[11] and adapted for the material in question was used.

The #80 gutta-percha cone (DiaDent, D) was immersed in a test tube containing a saline suspension with the *S. aureus* ATCC 25923 strain at a turbidity corresponding to 0.5 on the McFarland nephelometric scale (approximately 1.5×10^8 CFU/mL) for 10 minutes. The same methodology was performed using the *Enterococcus faecalis* ATCC 29212 strain, and non-contaminated saline solution, for comparative analysis

After the contamination period, the rolling technique was applied to the materials on the blood agar medium, which was then placed in an incubator at 37°C for 24 hours to observe bacterial growth. The experiment was conducted in triplicate, and the qualitative analysis was performed through direct visualization.

RESULTS

After the incubation period, changes in the color of the medium contained in the tubes were observed. A positive result (yellow color change) was observed only in the control group, which did not undergo decontamination (Figure 1).

It was verified that all the tested agents, both at the 1-minute and 5-minute periods, inhibited the viability of the contaminating microorganism on the tested material. Except for the negative control (saline), no positive result was shown in any of the tubes. The results are outlined in (Table 1).

The qualitative analysis using the rolling technique showed no difference in the contamination profile between the *S. aureus* ATCC 25923 strain and the *E. faecalis* ATCC 29212 strain. However, a difference was observed compared to the material immersed only in saline (Figure 2).

DISCUSSION

Staphylococcus aureus are microorganisms belonging to the microbiota of human skin and mucous membranes and rarely cause diseases in healthy hosts. As part of this microbiota, they can colonize materials or environments that have come into contact with the individual or the professional who handled them without proper aseptic precautions. Thus, the literature reports the involvement of species from this genus in opportunistic infections associated with the breakdown of the aseptic chain[8-10,12].

Table 1 – Results of bacterial growth evaluation.

Substance	Concentration	Time (min)	Result
Sodium Hypochlorite (NaOCl)	5.25%	1	Negative
	5.25%	5	Negative
	2.5%	1	Negative
	2.5%	5	Negative
	1%	1	Negative
	1%	5	Negative
	0.5%	1	Negative
	0.5%	5	Negative
Chlorhexidine (CHX)	2%	1	Negative
	2%	5	Negative
Ethanol (C_2H_6O)	70%	1	Negative
	70%	5	Negative
Distilled Water (H_2O)	-	1	Positive
	-	5	Positive

Negative - no bacterial growth; Positive - bacterial growth occurred.

Staphylococcus aureus is reported as a species with a high expression of virulence factors associated with their high capacity to adapt to different community and hospital environments, their ability to colonize and form biofilms on various surfaces, and their genetic plasticity, which allows them to acquire and express genes from other bacterial genera and species[8-10,12].

Gutta-percha is a material used in the obturation phase of the RCS. Different studies report if this material is not properly disinfected, it can carry microorganisms into the interior of the RCS[3,4]. The rolling method on agar is used for qualitative evaluation of the bacterial surface contamination profile[11]. According to the results obtained, the growth profile of the *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 strains did not show any observable difference regarding the adhesion profile on the surface of Gutta-percha.

Despite containing zinc oxide in its composition, which could confer antimicrobial action, some studies discuss this activity, showing that cones taken directly from their original packaging were contaminated for the operator, thus reaffirming the importance and necessity of prior chemical decontamination before handling them during the obturation phase of the RCS[13].

The most commonly used disinfectant agent in endodontics is sodium hypochlorite (NaOCl) at various concentrations. This substance has broad-spectrum antimicrobial activity and also exhibits proteolytic activity, which aids in cleaning and degrading residual organic material present in the RCS. The NaOCl is considered a very effective method for decontaminating gutta-percha cones[3,14-16].

This study demonstrated that NaOCl at concentrations of 0.5%, 1%, 2.5%, and 5.25% were effective against the infecting agent *Staphylococcus aureus* at both 1-minute and 5-minute

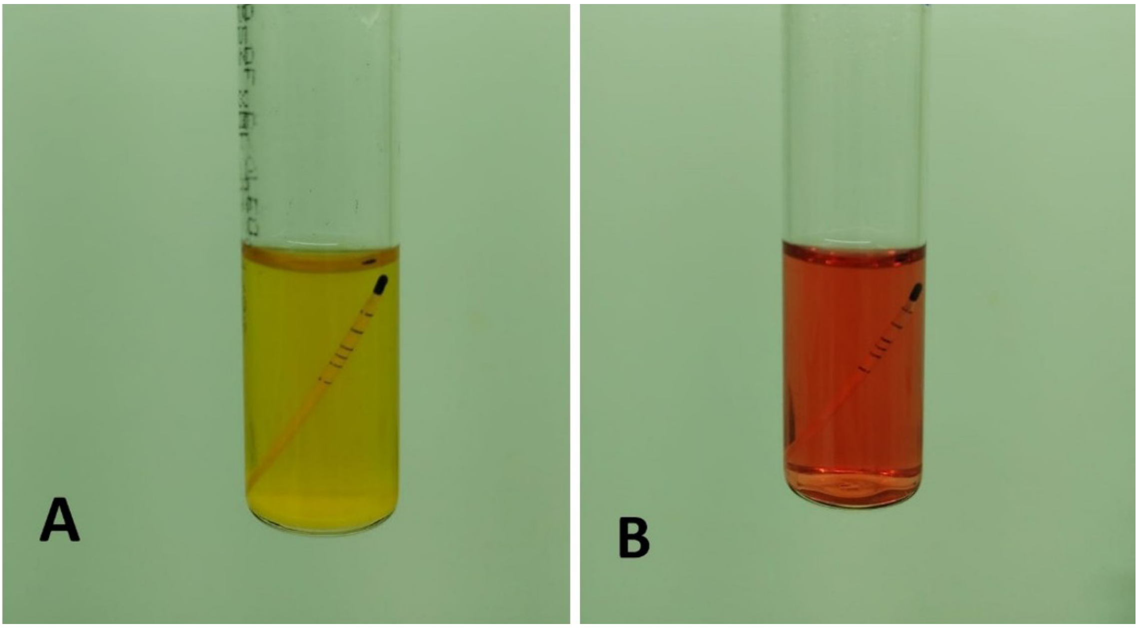

Figure 1 – (A) Positive result, indicating bacterial growth; (B) Negative result, indicating no bacterial growth.

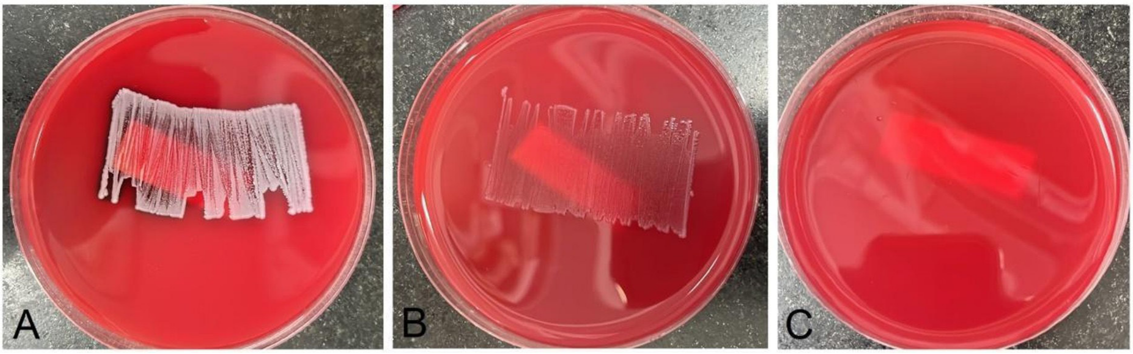

Figure 2 – (A) Rolling of the gutta-percha cone contaminated by *S. aureus* ATCC 25923; (B) Rolling of the gutta-percha cone contaminated by *E. faecalis* ATCC 29212; (C) Rolling of the gutta-percha cone immersed in sterile saline solution.

immersion times. Previous studies have shown that 5.25% sodium hypochlorite produces effective bactericidal activity against *S. aureus*[10,17]. The results showed antimicrobial action even at the lowest concentration (0.5%) and with the shortest exposure time (1 minute).

Chlorhexidine (CHX) has been used in endodontics as an irrigating substance or an intracanal medicament due to its broad-spectrum antimicrobial activity, substantivity, and lower cytotoxicity than NaOCl, among other properties. It is recommended as an alternative to sodium hypochlorite, especially in incomplete apical development and root resorption[18]. CHX at a concentration of 2% was also one of the agents selected for chemical disinfection in this study. An adequate disinfection profile was observed in the gutta-percha cones exposed to this agent, with no bacterial growth after exposure for 1 and 5 minutes.

The CHX and sodium hypochlorite are the chemical substances most used in endodontics both for clinical use as a disinfectant for clinical materials[19]. However, due to its presence in dental offices, primarily as a surface disinfectant, 70% ethanol was presented in this study as an alternative chemical agent for decontaminating gutta-percha cones. The results demonstrated its efficacy in decontaminating the gutta-percha cones against *S. aureus* at both 1-minute and 5-minute exposure times.

CONCLUSION

Staphylococcus genus is the most frequently found after contamination due to the breakdown of the aseptic chain. The qualitative analysis showed no significant difference in contamination between *Staphylococcus aureus* and *Enterococcus faecalis*. Thus, *S. aureus* can be suggested as a bacterial model for experiments associated with contamination due to inadequate professional manipulation of dental materials.

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Furthermore, according to the methodology used and the results obtained, it can be concluded that all the tested agents (NaOCl at 0.5% to 5.25%, CHX at 2%, and 70% ethanol) were proven effective for the decontamination of gutta-percha cones against *S. aureus* in both the 1-minute and 5-minute exposure times.

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