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Original Article

Assessing the Contamination of the External Surfaces of Reused Calen® System Tubes

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Abstract

Objective: To determine the presence of contamination on the external surface of reused Calen® System tubes before (TA) and after (TB) the action of four disinfectants: 0.12% chlorhexidine, 2% glutaraldehyde, 70 % alcohol and 1% sodium hypochlorite. **Material and Methods:** Microbiological collection of 20 tubes from dental clinics was performed with the aid of sterile swabs moistened in saline and rubbed for one minute on their surfaces and inserted in tubes containing 4 ml BHI and incubated under microaerophilic conditions (37°C for 24h) for further Mc Farland (F0.5 to F10) and Gram staining analyses. For disinfection (TB), cotton wicks with 1 ml of disinfectant or distilled water (control) was rubbed for 30 seconds, and after drying by evaporation, new samples were collected from the surfaces, which were submitted to the same incubation and Gram staining process for the analyses of slides. **Results:** Prevalence of F4 and F5 in the same proportion in TA and F1 in TB. Gram + cocci were present in 100% of TA and TB, and prevalence of Gram + cocci, Gram + and – bacilli, spores, Gram - cocci and vibrios in descending order. **Conclusion:** All external surfaces of reused Calen® System tubes were contaminated before and after disinfection. In descending order, the disinfectant that provided a greater reduction in the Mc Farland scale and thus a greater reduction in the number of microorganisms was 1% sodium hypochlorite, followed by 2% glutaraldehyde, 0.12% chlorhexidine, 70% alcohol and distilled water (control).

Keywords: Endodontics; Bacteria; Decontamination.

Introduction

Calcium hydroxide has been extensively used in dentistry as intracanal dressing in cases of impossibility of endodontic treatment performed in a single session and also between sessions in primary teeth with pulp necrosis [1]. It has been shown that this substance has pronounced antibacterial effect against most microorganisms isolated from root canals. However, its antibacterial activity applies only to microorganisms in direct contact with the substance, in situations where pH is extremely alkaline [2]. There are several ways for placing medications, including injection techniques [3]. Ca (OH) 2 may be presented in the form of paste conditioned in tubes for immediate use and marketed as Calen® paste (S.S. White, Rio de Janeiro, Brazil).

Cross contamination is a serious problem that involves health professionals, especially in dentistry [4,5]. It is the transmission of infectious agents between patients and staff in a clinical environment and can occur by direct contact with tissues, secretions or blood droplets containing contaminants or through the cutting edges of contaminated instruments that have not been properly sterilized [6].

One of the main sources of contamination in dental offices is the use of equipment coupled with water / air jet systems, producing aerosols that serve as vehicle for the spread of microorganisms around the operative field [7]. The hands of the professional contaminated by saliva and blood are also of fundamental importance in the cross-infection process, when the professional fails to perform a thorough anti-sepsis at the end of the treatment to a patient and beginning of treatment to others [8]. This step is often overlooked by dentists [9].

Disinfection is defined as the elimination of microorganisms in the vegetative form from fixed surfaces using various chemical agents [10]. Considering that all patients should be treated as potentially infected, procedures for infection control must be the same for all without distinction [11]. The adequate selection of the disinfectant agent determines the success of the disinfection process [10]. Chlorinated compounds, such as sodium hypochlorite, are recommended for disinfection by soaking of nonmetallic materials, since they have corrosive action on metals. Glutaraldehyde, in turn, is used for sterilizing heat sensitive items, metal, plastic, rubber and instruments that can be disinfected by soaking [12,13].

In a previous study, it was found that disinfection with 1% sodium hypochlorite and 70% alcohol for 30 seconds was effective in decontaminating periapical radiographic films [10]. With respect to the disinfecting action of chlorhexidine, it was concluded that 5% chlorhexidine alcohol solution is quite effective in reducing the action of microorganisms, mainly gram-positive bacteria [9,14].

Healthcare professionals need to be aware of what is an ideal disinfectant, for what purpose it will be used, what is the target microbial population, what are the advantages and disadvantages of the product, taking into account risks, cost and benefits [9].

The indiscriminate manipulation of Calen® system tubes in dental offices is a fact to be studied in order to establish the degree of surface contamination and the type of bacterial strains

found in them. If not sterilized after use, this tube can become a vehicle for pathogens to come into contact with living tissues.

The aim of this study was to verify the presence of microorganisms on the external surface of reused Calen® system tubes and analyze by microbiological methods the action of four disinfectants (70% alcohol, 0.12% chlorhexidine, 2% glutaraldehyde and 1% sodium hypochlorite) and distilled water as control used in dental offices in the disinfection process of these tubes.

Material and Methods

This is a quantitative, descriptive and cross-sectional study. The collection of tubes occurred in two dental offices belonging to a private university and in eight private dental offices in the city of Fortaleza, Ceará, totaling 10 locations.

Microbiological collection was performed from the external surface of 20 Calen® System tubes, 11 tubes of Ca (OH) 2 paste and 9 tubes of glycerin, after their use in the clinical practice.

The collection procedure occurred as follows: the laboratory material was stored in a cooler under refrigeration and taken to the offices where the collection was carried out with procedure gloves, always close to the flame and with the aid of sterile swabs moistened in saline. Samples were collected throughout the extent of the external surface of tubes, including their ends sealed by rubber structures (piston and diaphragm) for 1 minute. After collection, swabs were inserted into sterile test tubes containing 4 ml BHI (Brain Heart Infusion), kept under refrigeration until arrival at the laboratory of microbiology of the University of Fortaleza. The enumerated and identified test tubes were then incubated in bacteriological incubator under microaerophilic conditions at 37 ° C for 24 hours, waiting for microbiological growth.

After the incubation period, microbiological growth was confirmed in the test tubes by the turbidity degree found in them. Microbiological growth was estimated using the Mc Farland scale. Then, the types of bacteria colonies, and their morphology and arrangement were identified through Gram staining technique and slides were analyzed under optical microscope with 100x magnification.

To evaluate the efficiency of methods for the disinfection of the external surface of Calen® system tubes, a new sample collection was carried out after the use of 1% sodium hypochlorite, 0.12% chlorhexidine, 70% ethanol, 2% glutaraldehyde and distilled water. A randomly selected disinfectant was used for each tube. For technical reasons, 5 tubes were eliminated in this phase, remaining 15 tubes to be analyzed before and after disinfection, 9 tubes of Ca (OH)2 paste, 6 tubes of glycerin, and of these, 5 tubes were disinfected with 1% sodium hypochlorite, 3 tubes with 0.12% chlorhexidine, 2 tubes with 70% ethanol, 3 tubes with 2% glutaraldehyde and 2 tubes with distilled water.

The disinfection of tubes was performed with the aid of a cotton swab moistened in 1 ml of each disinfectant solution, rubbed on the external surface of tubes for 30 seconds. After drying by evaporation, new samples were collected from the surfaces that have been submitted to the same incubation process and Gram staining for analysis of slides.

Results

According to the results obtained, there was contamination on the external surface of all 15 tubes analyzed before and after disinfection with 70% alcohol, 1% sodium hypochlorite, 2% glutaraldehyde, 0.12% chlorhexidine and distilled water, regardless of the material contained in the tubes, Calen® paste or glycerin.

According to the McFarland scale, which ranges from F0.5 to F10, before disinfection, predominant turbidity F4 and F5 in the same proportion was observed. After use of selected disinfectants, the predominant turbidity was F1. In one of the tubes decontaminated with 70% alcohol, turbidity increased from F4 to F7. In descending order, the disinfectant that provided a greater reduction in the Mc Farland scale and thus in the number of microorganisms was 1% sodium hypochlorite, followed by 2% glutaraldehyde, 0.12% chlorhexidine, 70% alcohol and distilled water, as shown in Table 1.

Table 1. Contamination of the external surface of tubes before and after the use of decontaminating agents according to the Mc Farland scale.

Disinfection stage	1% Hypochlorite	2%Glutaraldehyde	0.12%Chlorhexidine	70% Alcohol	Distilled water
Before	F5	F4	F2/F4/F5	F4/F3	F4/F5
After	F1	F1	F1/F2/F5	F7/F2	F3/F4

Slides stained by the Gram's Method allowed identifying the types of microorganisms after analysis under optical microscope with 100x magnification. It was observed that the microorganisms that prevailed before and after decontamination were Gram + cocci, which were present in 100% of samples. In descending order, predominant microorganisms were Gram + cocci, Gram + and – bacilli, spores, Gram - cocci and vibrios, the latter being present in only one slide, as shown in Table 2.

Table 2. Prevalence of microorganism in the contamination of the external surface of tubes before and after the use of decontaminating agents by the Gram staining technique.

Disinfection stage	1% Hypochlorite	2% Glutaraldehyde	0.12% Chlorhexidine	70% Alcohol	Distilled water
Before	G + Cocci G +/- bacilli	G + Cocci G +/- bacilli Spores	G + Cocci G +/- bacilli Spores	G + Cocci G +/- bacilli Víbrios	G + Cocci G - bacilli
After	G + Cocci G + bacilli Spores	G + Cocci G - bacilli	G + Cocci	G + Cocci G - bacilli	G + Cocci G +/- bacilli Spores

Discussion

The risk of occurrence of cross-contamination during dental practice is a real and proven fact, and the dentist is responsible for developing ways for preventing contamination between patients and between patients and dental staff.

This study aimed to verify the contamination of the external surface of reused Calen® System tubes in ten dental offices, since contamination of surfaces present in dental offices was found, becoming a cross-infection [9,7,10]. Efficient protocols for the disinfection of contaminated surfaces should be developed, considering that all patients are potentially infected [11,15].

In this research, samples were incubated in a bacteriological incubator under microaerophilic conditions at 37°C for 24 hours [12]. In studies assessing the contamination of toothbrushes by enterobacteria, aerobic jars for incubation were used, which is a method similar to that proposed in the present study, differing by its higher oxygen concentration [16]. Studies aimed at checking the contamination intensity of triple-pointed disposable syringes used anaerobic jars during the incubation period to provide for the growth of anaerobic bacteria. The use of anaerobic jars during the incubation period to assess the contamination of triple-pointed disposable syringes has been proposed in order to provide the growth of anaerobic bacteria [17].

The limit used of 24 hours is required for bacteria to grow and to confirm the results, avoiding false-negative results. After the incubation period, microbiological growth was verified through the Mc Farland scale, correlating turbidity with the presence of positive microorganisms [18].

The Gram staining method was used for making the slides, making it possible to identify morphotinctorial features and arrangements of microorganisms found [19].

The previous cleaning the dental office surfaces is an item that should not be overlooked, as it can determine the success of the disinfection [20]. In this study, four disinfecting agents were used: 1% sodium hypochlorite 0.12% chlorhexidine, 70% alcohol, 2% glutaraldehyde, and distilled water as control.

Although none of the disinfecting agents has been effective in decontaminating the tubes, it was found that 1% sodium hypochlorite and 2% glutaraldehyde solutions showed greater effectiveness in reducing the count of microorganisms, and the results of this study are consistent with a research in which these solutions showed great efficacy against the microorganisms tested [12]. The action of sodium hypochlorite was evaluated in different concentrations for the disinfection of gutta-percha cones and this chemical agent was effective even at the smallest concentration tested (2.25%) [21]. In studies on the effectiveness of denture disinfection methods in the control of in vitro colonization by Candida albicans, it was found that 1% sodium hypochlorite solution proved to be very effective [22].

Usually, 70% alcohol is chosen as the first-choice disinfectant agent. However, the results of this research showed that the action of this product was not effective on the bacteria found, corroborating studies that have demonstrated its failure in the disinfection of surfaces when compared to sodium hypochlorite at different concentrations, revealing better antimicrobial activity, with bactericidal, fungicidal and sporicidal properties against the microorganisms tested [8]. The fact that turbidity increased from F4 to F7 in one of the tubes decontaminated with 70% alcohol can be explained by the reduced sample size.

A study found that the use of 70% alcohol by the rubbing method was effective in surface decontamination 10, which justifies the choice of the rubbing method in this study. Another study also found effective in the disinfection protocol with 70% alcohol, but also with 2% glutaraldehyde [15].

In this study, the chlorhexidine concentration used was 0.12% and its result as a disinfectant agent was not considered satisfactory, corroborating findings that demonstrated the effective action of this disinfectant solution only at concentrations from 1% [9,7,23].

No other study was not found in literature about the contamination of the external surface of Calen® system tubes, highlighting the originality of this work. This fact also reveals the need for further studies on the subject with a larger sample with the use of the substances used in this study at higher concentrations and even with other disinfectant solutions, enabling a better discussion and confirmation of results.

Due to the reuse of Calen® ®system tubes, the microorganisms present on their external surface may, through one of its ends (diaphragm), infiltrate into it, thus contaminating their inner contents. Therefore, assessing the contamination of the inner surface of tubes and the medium required for the incubation of existing bacteria under aerobic and anaerobic conditions become an interesting topic to be discussed in a further work.

Conclusion

According to the methodology used, it could be conclude that:

1. All external surfaces of reused Calen® system tubes were contaminated before and after disinfection with all substances tested.

2. Cocci, bacilli, vibrios and spores were found, especially gram-positive cocci.

3. In descending order, the disinfectant that provided a greater reduction in the Mc Farland scale and thus in the number of microorganisms was 1% sodium hypochlorite, followed by 2% glutaraldehyde, 0.12% chlorhexidine, 70% alcohol.

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