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Antimicrobial Efficiency of Some Antiseptic Products on Endodontic Micoflora Isolated from Gangrenous Pulp Tissue

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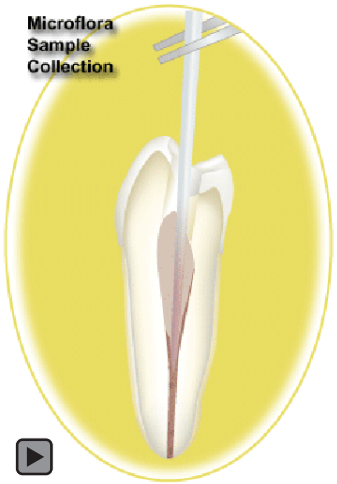

Abstract

Aim: The aims of the study are to investigate the bactericidal effect of three antiseptics (chlorhexidine solution, povidone-iodine solution, and Walkhoff solution) and to determine the minimum inhibitory concentration and their effect on different microbial species.

Methods and Materials: The study was performed on microflora derived from root canals with simple and complicated pulp gangrene and on pure strains of *Enterococcus* and *Candida albicans*.

Results: Chlorhexidine and povidone-iodine proved to have antibacterial and antifungal effects if used in the treatment of pulp gangrene and in cases not responding to conventional therapy.

Conclusion: According to the obtained results, the spectrum of antibacterial agents used in infected canal irrigation can be enlarged to include the agents tested.

Keywords: Antiseptics, chlorhexidine, povidone iodine, Walkhoff solution, pulp gangrene, culture medium

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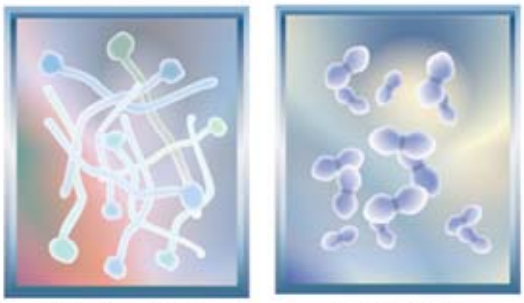
Introduction

The ecological medium in a root canal is complex. Many factors may influence bacterial selection to form the microflora in the canal. Adequate nutrition is essential for bacterial growth, and diverse bacteria species have different nutritional requirements and the availability of nutrients varies in a root canal. The main sources of nutrients are: necrotic pulp tissue, inflammatory exudates, and tissue fluids from apical foramen and lateral canals. Inflammatory exudate contains serum and blood components. If there is a direct communication with the oral cavity, then saliva can be considered another nutritional source. Pulp debris, tissue fluids, and saliva that provide proteins are promoting factors for bacterial growth. On the other hand, microflora relying on energy production from carbohydrate fermentation have little chance to grow in a root canal environment.[1,2,3]

The living conditions of the infective microbial flora in the root canal system are presumably dependent on the redox potential (amount of oxygen) which is quite low. Even if there is direct contact, the oxygen level will remain low especially in the apical portion of the canal. Facultative aerobic bacteria can initiate the colonization of the pulp chamber, but the lack of oxygen will favor the growth of anaerobic bacteria.



Candida albicans Enterococcus

The aims of the study are to investigate the bactericidal effect of three antiseptics and to determine the minimum inhibitory concentration and their effect on different microbial species. The three antiseptics tested at various concentrations were: chlorhexidine gluconate solution, povidone-iodine solution, and Walkhoff solution. The study was performed on microflora isolated from root canals with simple and complicated pulp gangrene and on pure strains of *Enterococcus* and *Candida albicans* which present high resistance to irrigant solutions, i.e., NaOCl, oxygenated water, and calcium hydroxide. These microorganisms are often responsible for endodontic treatment failures.

Methods and Materials

Sample Collection

The following procedure was utilized for testing the three irrigating solutions:

- Use of gloves and rubber dam during sample collection to reduce the incidence of re-infection. The pulp chamber was opened with a sterile bur at low speed and irrigated with sterile water. The exposed pulp content was removed with a sterile spoon excavator.
- A pathway to the apex was created by inserting a #15 K-file, then a sterile absorbent point was inserted in the canal to reach the apical portion and allowed to remain in place to absorb as much exudate as possible. If the paper point was dry upon withdrawal from the canal, then another paper point was moistened with transport medium and inserted into to the canal to obtain a sample. Paper points were removed using sterilized cotton forceps and immediately placed in a transport tube. For multi-radicular teeth, all the paper points were placed in the same tube.
- Sample tubes were identified with the patient's name, date, involved tooth, and then sent to laboratory to culture and perform microbial tests.

Test Antiseptic Solutions

The following are the antiseptic solutions and their concentrations used in the study:

- Chlorhexidine gluconate (2%, 1%, 0.5%, 0.1%, 0.05%)
- Povidone-iodine (3%, 2%, 1%, 0.5%, 0.1%)
- Walkhoff solution (3% camphorated paramonochlorophenol)

Bacterial Strains

The following bacterial strains were used in the study:

- Polymorphic microfora isolated from gangrenous pulp tissue
- *Enterococcus faecalis ATCC 29212*
- *Candida albicans ATCC 10231*

Antibacterial activity was evaluated using dark field microscopy.

Sample Collection and Culturing

The same method of sample collection and culturing was used for all three bacterial strains as described below. The culture medium varied for each strain as indicated.

Polymorphic Microflora

Three samples of polymorphic microflora were collected from different canals under aseptic conditions and strict isolation of the canals using a rubber dam. The samples were introduced in Broth R.C. (HiMedia Laboratoires Pvt. Limited, India) and transported to the laboratory where 200µl of microbial sample was spread onto Wilgrins-Chalgren agar (Oxoid, United Kingdom) with 5% human blood (25ml/plaque ø90mm). The agar plates were dried in thermostat for 15 minutes then 5µl of the test agents with the above mentioned concentrations were spotted onto the plates. The plates were incubated 48 hours at 37°C in a Gas-Pak anaerobic jar. Three tests were done for each examined antiseptic being evaluated. Sterile distilled water was used for dilution, and the 3% Walkhoff solution served as a control.

Enterococcus fecalis ATCC 29212

Nutritive agar (BioTech, United Kingdom) was used to transport the samples and Mueller-Hinton agar (BioTech, United Kingdom) was used for culturing. A culture was developed on the surface of nutritive agar inoculated with the tested strain for 24 hours. Using this culture a microbial suspension of 0.5 Mc Farland was prepared using a BioMerieux Kit (SA, Marcy-1" Etoile, France). The Mc Farland turbidity standard is used for standardization of numbers of bacteria when required by procedures or for susceptibility testing. The basic 0.5 Mc Farland Standard contains approximately $1x10^7$ to $1x10^8$ CFU/ml ($1x10^{10}$ to $1x10^{11}$ CFU/L). Then 200µl of the microbial suspension was then spread onto a Mueller-Hinton agar (25ml/plaque ø90mm). The agar plate was dried, then 5µl of the test agents with the above mentioned concentrations were spotted onto the plate.

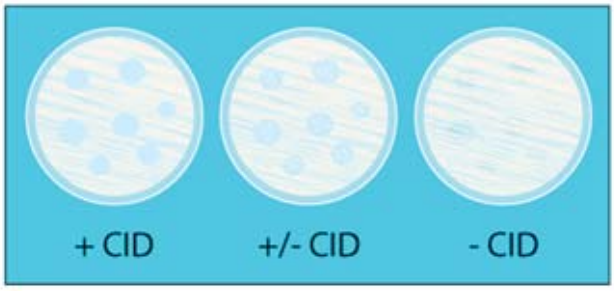
Candida albicans ATCC 10231

Sabouraud agar (Biotech, Marea Britanie) was used for transporting and culturing. A culture was developed on the surface of Sabouraud agar inoculated with the tested strain for 24 hours. Using this culture, we prepared a microbial suspension of 1 Mc Farland (turbidity standard) by using a BioMerieux Kit. Then 200µl of the microbial suspension was spread onto Sabouraud agar (25ml/plaque ø90mm). The agar plate was dried then 5µl of the test agents with the above mentioned concentrations were spotted onto the plate.

Culture Analysis

A CID test (bacterial growth inhibition against the tested microorganisms) was evaluated by examining the spot area (lysis area without colonies) following incubation at 30°C for 24 hours.

+ CID = Substantial zone of inhibition (visible lysis area without resistant colonies in the spot area).
+/- CID = Weak zone of inhibition(lysis area with unclear contour, with resistant colonies in spot the area).
- CID = No zone of inhibition (absence of lyisis area, developed colonies in the spot area)



Results

Polymorphic Microflora

Endodontic microflora extracted from teeth with pulp gangrene were tested to determine the minimum inhibitory concentration for two antiseptics: chlorhexidine gluconate and povidone-iodine as shown in Table 1. The inhibition patterns on the agar plate are shown in Figure 1.

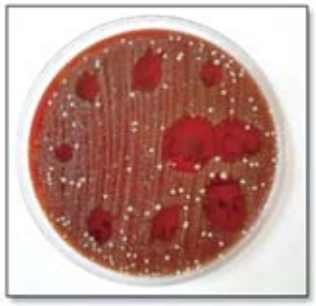


Figure 1. The culture plate reveals a similar positive CID effect of chlorhexidine and povidone-iodine on polymorphic microflora with well defined bacterial inhibition areas (minimum active concentration).

The chlorhexidine gluconate concentrations tested were: 2%, 1%, 0.5%, 0.1%, and 0.05%. The active minimum concentration was found to be 0.05%. Povidone-iodine concentrations tested were: 3%, 2%, 1%, 0.5%, and 0.1%. The active minimum concentration was found to be 1.0%.

Enterococcus fecalis ATCC 29212

Tests for the minimum inhibitory concentration for chlorhexidine gluconate and povidone-iodine on *Enterococcus fecalis* were conducted. The chlorhexidine gluconate concentrations tested were: 2%, 1%, 0.5%, 0.1%, and 0.05% with the active minimum concentration found to be 0.05%.

Figure 2. The culture plate reveals a similar positive CID effect of chlorhexidine and povidone iodine on *Enterococcus fecalis* with well defined bacterial inhibition areas (minimum active concentration).

Povidone-iodine concentrations tested were: 3%, 2%, 1%, 0.5%, and 0.1% with the active minimum concentration found to be 1% (Figure 2).

Candida albicans ATCC 10231

Tests for the minimum inhibitory concentration for chlorhexidine gluconate and povidone-iodine on *Candida albicans ATCC 10231* were conducted. The chlorhexidine gluconate concentrations tested were: 2%, 1%, 0.5%, 0.1%, and 0.05% with the active minimum concentration found to be 0.5%. Povidone-iodine concentrations tested were: 3%, 2%, 1%, 0.5%, and 0.1% with the active minimum concentration found to be 1% (Figure 3).

Figure 3. The culture plate reveals a similar positive CID effect of chlorhexidine and povidone-iodine on *Candida albicans* with well defined bacterial inhibition areas (minimum active concentration).

The antimicrobial effect of Walkhoff solution, which served as the control, was positive for all tested microorganisms as shown in Table 2.

Discussion

Many researchers and clinicians have demonstrated microorganisms play an important role in pulpal and periapical infection. Kakehashi showed the presence of microorganisms can provoke irreversible pulp reactions.8 Bystrõm and Sundqvist demonstrated the presence of bacteria in non vital pulp tissue is associated with bone destruction.9

Not all microorganisms can induce periapical reactions, but the type and the number of the microorganisms play a major role in every case. Some bacteria produce more aggressive and more active agents than others. As a result, some bacteria from root canals have a greater pathogenicity than others.

Because of the improvement in bacteriological techniques, it is possible to cultivate most

Table 1. Comparison between the antimicrobial effect of minimum inhibitory concentrations of chlorhexidine gluconate and povidone-iodine on different microbial strains.

Tested strain	Minimum Active Concentration	
	Chlorhexidine	Povidone-Iodine
Endodontic polymicroflora sample 1	0.05%	1%
Endodontic polymicroflora sample 2	0.05%	1%
Endodontic polymicroflora sample 3	0.05%	1%
Enterococcus faecalis ATCC 29212	0.05%	1%
Candida albicans ATCC 10231	***0.5%***	1%

Table 2. The antimicrobial effect of Walkhoff solution (control) on the tested microorganisms.

Microorganisms Tested	Antimicrobial Effect
Endodontic polymicroflora sample 1	+
Endodontic polymicroflora sample 2	+
Endodontic polymicroflora sample 3	+
Enterococcus faecalis ATCC 2921	+
Candida albicans ATCC 10231	+

microorganisms associated with endodontic infections. Consequently, there are numerous scientific data suggesting most endodontic diseases correlated primarily or secondary with the presence of bacteria. Therefore, it is essential for clinicians to have an extensive knowledge of the relationship between the oral microflora and the pulp and periapical tissues.

Recent studies on endodontic microbiology have showed the role of Gram negative anaerobic bacteria and proved an intimate relation between the symptomatic cases and some specific bacteria.

C. albicans is a commensal yeast that lives in the oral cavity.[10] This opportunist microorganism is also present in healthy individuals. A characteristic feature of *candida* is its manner of development when the host offers conditions and nutrients for its growth and reproduction as listed below.[11]

- Natural factors—normal and pathologic modifications in physiological status of the host
- Diet factors—diet rich in carbohydrates and poor in vitamins
- Mechanical factors—poor fitted prosthesis
- Latrogenic factors—administration of large spectrum of antibiotics or cortico-therapy[10]

Candida can penetrate the pulp through the dentinal tubules, dentinal caries, trauma, or through root canal during canal treatment.[12] Natural factors like the ecological modifications in an infected canal and iatrogenic factors such as canal medications allow *candida* to thrive through the elimination of other competing microorganisms.

Clinically *candida* species present a high resistance to calcium hydroxide.[13] Its resistance to calcium hydroxide and its ability to penetrate into the lateral canals and dentinal tubules are responsible for its presence in persistent apical periodontitis.[13]

Enterococcus fecalis is a Gram positive facultative anaerobic bacteria and it is implicated in persistent root canal infections.[14,15,16,17] It has been used in different studies[18,19,20] because of

its high level of resistance to a large spectrum of antibacterial agents. *Enterococcus* can be isolated even after calcium hydroxide treatment. These microorganisms can be absent initially in the root canal, but they can gain access to the canal environment during endodontic therapy and their presence requiring a special therapeutic strategy.

Ferraz[21] showed chlorhexidine can be used as an intracanal medication or canal irrigant. The comparison *in vitro* between chlorhexidine and NaOCl showed both compounds have an equal effectiveness as antibacterial agents.[22]

It is crucial to note topical treatment with chlorhexidine can produce various symptoms of immediate hypersensitivity (anaphylactic reaction).[23,24] *In vitro* the toxicity of chlorhexidine on human gingival cells depends on time exposure.[25] Boyce found chlorhexidine at 0.05% concentration is toxic for both human cells and for microorganisms.[26]

Chlorhexidine has a very large antibacterial spectrum.[27] The results of the present study confirmed the findings obtained by Ohara[28] demonstrating the antibacterial activity of chlorhexidine (solution, gel) was superior to that of NaOCl. However, the gel form of 0.2% chlorhexidine required two hours to produce negative cultures.

The present study tested the antibacterial activity of chlorhexidine and povidone-iodine in direct contact with a *Enterococcus fecalis* culture. Povidone-iodine (polyvinylpyrolidone-iodine) can represent a valid alternative agent because of its substantial antibacterial activity; low potential to develop adverse reactions; wide availability; ease of handling; and low price.

Severyns AM, et al.[22] evaluated the toxicity of a few irrigating solutions through microscopic observations.[29] Povidone-iodine (10%) proved to be a very irritating solution provoking secondary thrombosis. Chlorhexidine at concentrations of 0.05%, 0.02%, and 0.001% had a very low toxicity compared with physiological serum.

Povidone-iodine solutions are widely used because of their high antiseptic activity. However, maximum concentration seems to be toxic for the involved cells in wound healing. The effect of povidone-iodine in cutaneous injuries especially after chemical burning was studied by Wormser U, et al. They found the activity of matrix metalloproteinase-9 (MMP-9) is influenced by povidone-iodine.[30] The authors noted in addition to its reduced collagenolitic activity povidone-iodine protects the skin against chemical aggression.

A 3% concentration of Walkhoff solution has an antibacterial effect on all the tested microorganisms. Moreover, Walkhoff solution is active on candida confirming its bactericidal and antifungal effect.

Phenol and its derivative compounds like paramonochlorphenol and cresol mixed with camphor (camphorated solutions) or with phormaldehide (phormocresol) were used for a long time as intracanal medications. These compounds produce contact necrosis when used in high concentrations. Spangberg and Bystrõm had demonstrated their irritating and toxic effects on vital tissues.[31,32]

Conclusions

The present study evaluated the antimicrobial effect of different irrigating solutions, including chlorhexidine, camphorated paramonochlorphenol (Walkhoff solution), and povidone-iodine. The minimum active concentration of chlorhexidine for all the tested strains is the same 0.05%, excepting *candida* which requires a minimum active concentration of 0.5%. The minimum active concentration of povidone-iodine is 1% making it a potential irrigating agent in infected root canals.

The effect of these solutions on microflora collected from infected canals as well as on bacteria from pure culture proved these products are highly effective, the minimum inhibitory concentration being under the concentrations used in other applications.

According to the obtained results, the spectrum of antibacterial agents used in infected canal irrigation can be widened. Chlorhexidine and povidone-iodine proved to have antibacterial and antifungal effects when used in the treatment of pulp gangrene and in cases failing to respond to conventional therapy.

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