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In Vitro Testing of Susceptibility to Endodontic Irrigants and Disinfectants of Bacterial Strains Isolated from Chronic Apical Periodontitis

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Abstract

*The key factor in the production of pulp and periapical inflammation is represented by the presence of bacteria in the root canal system and the outcoming toxins. Bacterial biofilm is identifiable in the various areas of the endodontic space, but due to the complexity of the internal anatomy of the root canal, bacteria removing is yet difficult to obtain, a particularly important step for treatment success representing the medication choice. The purpose of this study was to determine the sensitivity spectrum of microbial strains isolated from chronic apical periodontitis teeth microbiota to different antimicrobials used in the chemomechanical disinfection stages: irrigants, local antiseptics, antibiotics.**Material and Methods.** Following tooth extractions, there were taken evidence of apical lesions chronic from twenty-nine patients; the identified microbial strains were identified based on the colony character, Gram stain, conventional biochemical tests and API kits. The isolated microbial strains were tested for susceptibility to different antibiotics and antiseptic substances, as well as irrigants of dental usage by using an adpated diffusion method. **Results:** Our study revealed that some of the analyzed strains were totally susceptible to conventional antiseptics. Analysis of irrigants inhibiton action and medicinal dressings currently used in endodontic practice (sodium hypochlorite 2.5% and 3%, 5.25%, 2% chlorhexidine gel and solution, EDTA) showed that strains isolated from chronic apical periodontitis were generally susceptible to their action. Disinfectants presented a lower level of antimicrobial effect in comparisson to that observed within antiseptics.**Conclusions:** Different types of microbial agents action is generally different depending on their type (antiseptics, chelating agents, irrigants, calcium hydroxide). Antiseptics presented the most efficient antimicrobial action. It was found that various antimicrobial agents had variable effects on different tested strains and generally, antiseptics exhibited a superior antimicrobial effect than the tested disinfectants.*

Keywords: *bacterial strains, chronic apical periodontitis, disinfectants*

1. Introduction

The key factor in the production of pulp and periapical inflammation is represented by the presence of bacteria in the root canal system and the outcoming toxins.Bacterial biofilm is

identifiable at the dentin endodontic space, apical ramifications, isthmus, intercommunications, lateral canals and external apical portion of the root surface. Due to the complexity of the endodontic system, infected dentinal tubules with microorganisms remain a difficult area to predictably disinfect [1]. The major objective of an endodontic treatment is bacterial elimination from ductal space and initiating healing consecutive periapical inflammation [2, 3]. The chemomechanical instrumentation, irrigants and medicinal dressings remove most of the bacteria and their metabolic products [4, 5]. However, periapical infection was observed after single-visit and multiple-visit treatment sessions [6, 7, 8], along with the persistence of microorganisms in the root apical portion of the endodontically tooth treated [9, 10, 11, 12]. The choice of endodontic medication is of special importance for the success of the treatment, the cells contained in biofilms developing a phenotypic resistance (behavioral) antimicrobial different from that determined by genetic resistance [13], explained by adherence to the substrate, interbacterial aggregation and adhesion, secretion of exopolymers and enzymes. Microbial resistance, natural or acquired, manifests itself through drug substance inefficiency on them.

The purpose of this study was to determine the sensitivity spectrum of microbial strains isolated from chronic apical periodontitis teeth microflora on different antimicrobials used in the stages of chemomechanical disinfection: irrigants, local antiseptics, antibiotics.

2. Materials and methods

2.1. Isolation and identification of the microbial strains

The apical lesion samples were taken from twenty- nine patients (15 men and 14 women) older than 20 years of age, in a dentistry clinic in Bucharest. From these, a number of 10 had inaccurate endodontic treatments, and 19 of them showed no endodontic treatment. All of the teeths presented fibrous chronic apical lesions that could be detected on individual radiographs, and the endodontic retreatment was not possible. There were 14 mandibular molars, 2 mandibular premolars, 3 upper premolars, 7 maxillary molars, 3 incisors. All the patients had radiographic evidence of apical periodontitis lesions. The extractions were made using aseptic and antiseptic techniques, under local anesthesia. After drawing out, by using aseptic techniques and sterile instruments, bacterial samples were taken and placed into a 2mL centrifuge tube containing 1.5 mL thioglycolat medium. The samples were immediately processed. The inoculated tubes were vortexed for a few seconds and then cultured on 10% sheep blood Brucella agar. The plates were incubated aerobically and anaerobically for 48 to 7 daysat37°C. The obtained microbial isolates were presumptively identified based on colony morphology, Gram staining and conventional biochemical tests (catalase and oxydase). The colonies were then identified by using the commercial biochemical kits API (bioMérieux, France): API Staph, APIStrep, API 20E, API NE, API 20A. The obtained isolates from samples collected in the dental office, under specific conditions of sterility and in accordance with legal provisions, identified, were tested for sensitivity to antibiotics, various antiseptic substances and irrigants commonly used in dental practice.

2.2. Testing the sensitivity of the various disinfectants was carried out by depositing substances on the previously seeded medium with a standard inoculum (0.5 McFarland) obtained from the strain of interest. For solutions it was used a volume of 10µl and for products presented as paste, a "fingerprinting" on the culture medium was accomplished. The seeded plates were incubated for 24 hours at 37^{0} C. The substances used in the study were presented in the table 1.

Table 1. Tested antimicrobial substances

Name	Action	Presentation	Composition
Rockle's	Antiseptic	Solution	Dexamethasone acetate 0.138g, 45,285g phenol, guaiacol 6,790g in 100ml solution.
Cresophene	Antiseptic	Solution	0,111g dexamethasone acetate, thymol and excipients 5g: parachlorphenol, racemic camphor in 100 g solution
Formacresol	Antiseptic	Solution	Tricrezol formaldehyde, glycerin and isopropyl alcohol,
Walckhoff	Antiseptic	Solution	Parachlorphenol 25%, 50% camphor, menthol 25%
Chlumsky	Antiseptic	Solution	Camphor 60g, 30g phenol, ethyl alcohol 10g
NaClO 2.5%	Irrigant	Solution	Aqueous solution of sodium hypochlorite containing 2.5%
Parcan	Irrigant	Solution	3% sodium hypochlorite, excipients for 100 g (sodium chloride, sodium carbonate, sodium hydroxide solution, sodium edetate, purified water)
Chloraxid	Irrigant	Solution	5.25% sodium hypochlorite
Chlor X	Irrigant	Solution	2% chlorhexidine solution
Soft prep	Chelation agent	Gel	17% EDTA (ethylenediaminetetraacetic acid)
Calasept	Medical dressing	Paste	Calcium hydroxide
Calplus	Medical dressing	Paste	Calcium hydroxide with iodoform
Gluco – chex 2%gel	Irrigant	Gel	Chlorhexidine digluconate, additional substances

3. Results and Discussions

In the present study there were isolated and investigated 17 microbial strains, belonging to the following genera and species: *Enterococcus faecium/faecalis* (6 strains), *Actinomyces naeslundii (3 strains), Streptococcus acidominimus* (2 strains), *Pantoea ssp (2 strains), Prevotella oralis, Prevotella intermedia, Eubacterium lentum, Streptococcus acidominimus, Veillonella parvula, Aerococcus viridans, Lactococcus lactis ssp cremoris, Staphylococcus epidermidis, Streptococcus oralis, Streptococcus intermedius.*

The strains susceptibility to the antimicrobial substances

Comparatively evaluating the effects of conventional antiseptics (solutions like Cresophene, Chlumsky, Walchkhoff, Rockles's, Formacresol) on isolated strains showed that Formacresol inhibitory effect is highly pronounced. A secondary efficacy was obtained from the Cresophene solution. Some of the tested strains were totally susceptible to the activity of conventional antiseptics *(Prevotella sp., Eubacterium sp., Veilonella sp.)* (fig. 1).

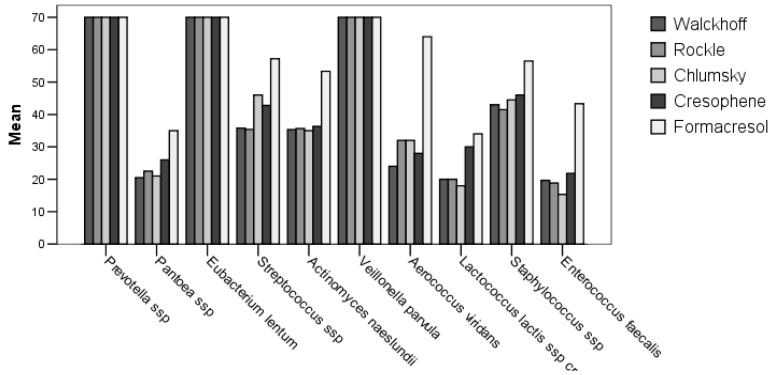

Figure 1. Antimicrobial activity of Walckhoff, Rockles's, Chlumsky, Cresophene, Formacresol antiseptics expressed through the average diameter of the growth inhibition area.

Analysis of the irrigants inhibitory action and of the medicinal dressings used in current endodontic practice (sodium hypochlorite 2.5% and 3%, 5.25%, 2% chlorhexidine gel and solution, EDTA) showed that strains isolated from chronic apical periodontitis are generally sensitive to their action. It was noticed that the chlorhexidine and 17% EDTA products revealed the highest antimicrobial activity. The comparative analysis of sodium hypochlorite activity at various concentrations revealed no great differences related to changes in concentration (fig. 2).

The tested strains were not sensitive to products with calcium hydroxide basis and calcium hydroxide iodoform. Therefore, we were unable to establish a match between the tested strains and their susceptibility to a particular antiseptic or irrigant within the same bacteria species exhibiting different resistance to antimicrobial substances. Some strains recorded low sensitivity, regardless of the microbial agent used *(Pantoea* sp*., Lactococcus sp., Enterococcus fecalis*), others recorded similar sensitivity to two agents, but different from the third one (*Aerococcus* sp. and *Prevotella* sp.), while some strains registered different sensitivity to the three tested agents *(Actinomyces* sp., *Sterptococcus* sp., *Staphylococcus* sp., *Eubacterium, Veillonella* sp.). Generally, the antiseptics recorded the highest sensitivity among the tested substances.

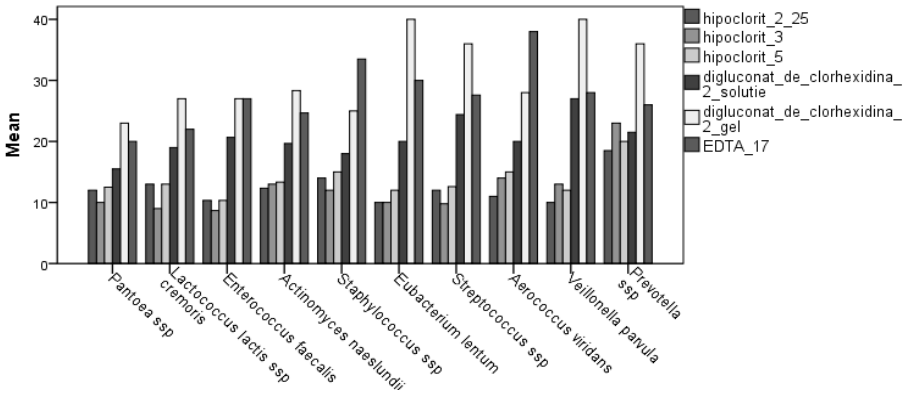

Figure 2. Antimicrobial activity of irrigants and chelation agents expressed through the average diameter of the growth inhibition area.

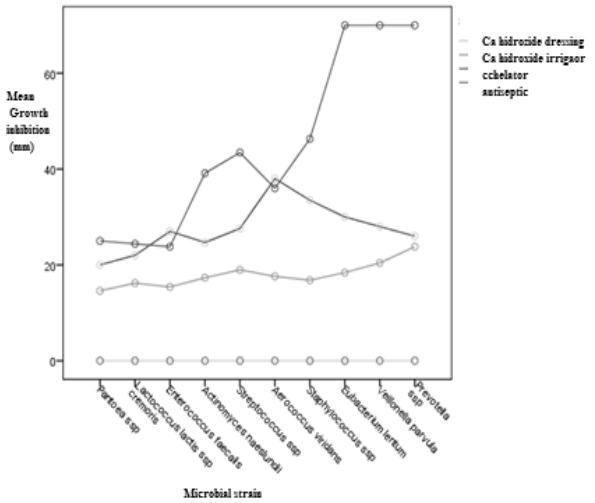

Figure 3. Comparative aspects regarding the sensitivity of strains to different categories of agents (antiseptics, irrigants and chelation agents).

Analyzing on what extent there is a different susceptibility of strains to antiseptics or irrigant substances, using a non-parametric Mann-Whitney test, it was observed that there was no statistically significant difference for all tested strains ($p < 0.05$). However, by analyzing the inhibition area obtained values, substantially increased values were observed for antiseptics as compared to disinfectants (**Table 3**), suggesting a higher efficiency of the first ones.

Table 3. Comparative susceptibility of strains to antiseptics and irrigants.

Agent	Mean	Median	
Antiseptic	40.02	32	p<0.001
Celation agent	27.13	25	
Irrigant	17.59	14	
CalciumHydroxide	0.00	0	
Kruskal-Wallis test			

No statistically significant differences between antiseptics and chelators were observed. Chlorhexidine has been used in endodontics both as irrigant and as intracanal medicine. It is active against a broad range of both Gram-positive organisms, including *Enterococcus faecalis*, Gram-negative bacteria, fungi [14]. As endodontic medication, it is more effective than calcium hydroxide against *Enterococccus faecalis* from the dentinal tubules [15, 16, 17]. Combined with other substances, including calcium hydroxide, calcium hydroxide with zinc oxide, the chlorhexidine antimicrobial activity is reduced [18, 19, 20]. A 2% chlorhexidine gel has a number of advantages over the 2% chlorhexidine solution, while its antimicrobial properties and biocompatibility are similar [21, 22]. Among the solutions with sodium hypochlorite, it was observed that the product containing 5.25% sodium hypochlorite best inhibits the development of microbial strains isolated from the apical periodontitis, which is consistent with several studies in the literature, but the effects of different concentrations are similar. As for the solutions of sodium hypochlorite, the more effective is the concentration of 5.25% at 40 mins contact [23], but also on a contact less than 30 mins [24]. Calcium hydroxide is used as dressing between treatment sessions. Its antimicrobial activity is related to the release of hydroxide ions (HO^{-}) in an aqueous medium, which is probably due to damage the bacterial cytoplasmic membrane, denaturation of proteins and DNA damage. To achieve effective disinfection, it is

required for 7 days [14]. In our study, products based on calcium hydroxide (Calasept and Calplus) have not influenced the development of the tested strains in the time that were in contact, their reading being achieved after 24 hours of incubation, while the literature specifying the effect of these compounds after 7 days. Sodium hypochlorite is considered as the first choice of irrigant, our study showing a higher antimicrobial efficiency for EDTA and chlorhexidine. These results can be explained by the different solubility and the ability of these substances to diffuse into environment and to generate a concentration gradient of microbial growth inhibition around the spot of substance deposited on the culture medium. Studies have shown the ability to develop *Enterococccus faecalis* biofilms in different environmental conditions and nutrients, even in the poor food [25, 26, 27]. In environments rich in nutrients, aerobic and anaerobic microorganism produces aggregates bacterial joined by channels of water, structure of biofilm classic, and in environments low in food producing adhesions irregular cell structure of the biofilm can be visualized by microscopy confocal laser (CLSM) [25, 26, 27]. Some *in vitro* studies have revealed that *Fusobacterium nucleatum* aggregates *Enterococcus faecalis* [25, 26, 27], acting at dentin and coexisting community represented by biofilm. Two of the microorganisms present in the endodontic space, isolated from us, namely *Enterococcus faecalis* and *Candida albicans,* have the ability to adjust to the new life and private environment of nutrients in space ductal following treatment, forming biofilm and might also invade dentin tubules [25, 28, 29, 30]. The quantitative evaluation and standardization of bacterial viability before and after endodontic disinfection protocol, represents a goal in this area in recent years various staining methods have been introduced in order to be able to view the dentin viable micro-organisms, as well as the dead ones. Zapata *et al* [31] have proven bacterial metabolic activity and viability in the dentinal canalicules for *Enterococcus faecalis* by two staining methods. Nagayoshi *et al* [32] used a model infected with bovine dentine and Parmar *et al* [33] used the dentinal tubules CLSM for highlighting the human mineralized viable and non-viable bacteria. All of these studies used methods of cultivation for viewing *Enterococcus faecalis* in the dentinal tubules. Considering the existence of factors such as variation in the size and diameter of dentinal tubular nutritional intake, expression of molecules key relationship [34], infected dentin is variable as density microbial or depth of penetration thereof, and it is difficult or almost impossible to quantify. Compared to SEM, CLSM for better dentinal tubules view of bacterial presence, it penetrates up to 10 mm beyond the surface of the sample, including the tubules that are not open to the surface. *Enterococcus faecalis* is considered extremely important as the pathogen in most cases of failure of endodontic treatment. Analysis of the inhibitory action of the 5 antiseptics tested (Rockles's, Cresophene, Formacresol, Walkhoff, Chlumsky) showed that Formacresol presented the most pronounced inhibitory effect on the tested strains, probably due to its chemical composition. Products based on calcium hydroxide (Calasept and Calplus) have influenced the development of tested strains, the time for action being only 24 hours. Results are influenced by the experimental model used, in which the diffusivity capacity of the substance has an important role.

4. Conclusions

Microbial strains isolated from chronic periodontitis lesions have generally different susceptibility to the tested substances, i.e. *Prevotella* sp. and *Veillonella* sp. being more susceptible than *Pantoea sp.*, *Lactococcus sp.* And *Enterococcocus faecalis*. The action of different types of microbial agents (antiseptic, chelating and irrigants, calcium hydroxide) is different, the most efficient antimicrobial action being exhibited by antiseptics, out of which

Formacresol was the strongest. CHX based irrigants agents seem to have an efficient / better or similar action as compared to hypochlorite. The analysis of the antimicrobial action of certain substances used in dentistry, such as irrigants (2.5% sodium hypochlorite or 3% and 5.25%, 2% chlorhexidine gel and solution, EDTA, calcium hydroxide, calcium hydroxide iodoform), showed that the microbial strains isolated from apical periodontitis are generally sensitive to them, particularly to 5.25% hypochlorite, chlorhexidine-based products and 17% EDTA. The used concentration influenced the antimicrobial activity of NaOCl, while the formulation (gel and solution) that of CHX.

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