IN VITRO STUDY OF ANTIBACTERIAL ORAL CAPACITY THROUGH FIVE MOUTHWASHES

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Abstract: The mouthwashes effectiveness containing essential oils or antiplaque substances has been demonstrated in numerous studies. By our study we aimed at highlighting in vitro ability of new mouthwashes. Methods. Microbial agents deployed in dental plaque were seeded on specific culture media for Streptococcus mutans, Lactobacillus and Candida albicans. From the colonies developed we implemented in a liquid culture medium, a standard number of pathogens that were maintained for 1-3 and 5 minutes contact with the studied mouthwashes. After the contact period the samples were again seeded in appropriate culture media and incubated for 48 hours at 27 °C. Results. Cultures obtained were interpreted according to the development of microbial / fungal thus, establishing antiseptic rinses in control of microflora. Conclusions. Recommendations of mouthwashes are desirable to take into account the disease we want to fight with, for their antimicrobial effect and some possible adverse effects.

INTRODUCTION

The first mention of mouthwashes in therapeutic purposes dates back 2 700 years ago BC, recorded by Chinese medicine as rinsing with urine.

The same treatments by rinsing were later practiced in ancient Greece and in addition, there are also used mixtures of oil, aniseed, mint and white wine to combat halitosis.

In the Middle Ages, in the sixteenth century, there are mentioned mouthwashes after brushing, recommended to save the dental illness as a heated mixture made of vinegar, and wine myrrh.(1,2,3)

An important step in the evolution of the rinse treatment was marked by the introduction of chlorhexidine in the composition of mouthwash by Professor Löe in Denmark (1960).

There followed a great impetus for achieving various types of mouthwashes with instructions to combat halitosis, tooth decay or gum disease.(4,5)

On the other hand recent studies conducted in the United Kingdom and Australia claim that using mouth waters intensively and on long term without a dentist recommendation, can have some serious side effects.

One of adverse effect is the disturbance of oral bacteria ecology that convert nitrate to nitrite.

The reduced plasma level of nitrites is associated with physiological growth of blood pressure with 2 or 3 units.(6,7)

Other studies refer to the increased alcohol content of the mouthwashes, allowing the carcinogenic substances such as nicotine to penetrate more easily into the oral tissues increasing the risk of oral cancer.(8)

Currently, there are available a wide variety of mouthwashes that can be generally classified as: mouthwashes containing fluoride, mouthwashes containing natural plant extracts and mouthwashes that contain Chlorhexidine or Listerine antiseptic type.

PURPOSE

Through our study we aimed at testing the in vitro effect of 5 antiseptic mouthwashes, to the most common problems caused by Streptococcus mutans, Lactobacillus and Candida albicans.

MATERIALS AND METHODS

Mouthwashes in the study were selected according to their contents in active substances as follows:

- Aslamed (Farmec Cluj-Napoca) contains chamomile extract, chlorhexidine and a special argil. According to the prospectus, the clay in the composition contains more than 20 trace elements (iron, calcium, magnesium, potassium, copper, zinc, cobalt, fluor etc.)
- Pell Amar (Pell Amar Cosmetics LLC) contains mud extracts from Balta Albă (Buzău) lake. In the composition of this mouthwash, there is also an organometallic and enzymatic complex with biotrophic, regenerative, antiinflammatory and analgesic properties, with Chamomila recutita and Echinacea palida extract.
- Colgate Plax Sensitive without alcohol (Colgate Palmolive) contains cetyl pyridinium chloride and sodium fluoride as active substances. According to the prospectus, it is indicated for a fresh breath and for a strong tooth enamel. The prospectus also mentions a 99.9% destruction of oral bacteria thus providing protection for 12 hours.
- Extra Parodontax chlorhexidine 0.2%, alcohol free (Glaxo Smith Kline) contains 0.2% chlorhexidine digluconate which adheres to tooth surfaces and soft tissue so the effect is maintained for 12 hours and also it may depart from 3.5 times more plaque than by simply brushing, according to the prospectus.
- Listerine Cool Mint (Johnson & Johnson) is considered the first mouthwash sold since 1914. It has in its composition as active ingredient Listerine (hydroalcoholic products based on essential oils extracted from

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plants: 0.042% menthol, 0.064% thymol, 0.092% eucalyptol and 0.06% methyl salicylate dissolved in alcohol with a content of 21.6%. Listerine contains phenolic compounds which has bactericidal effect by preventing the bacterial aggregation, destroying cell membranes or inhibiting the enzymes or toxins activity.

For testing the ability of these antibacterial mouthwashes we deployed bacteria from the plaque composition by chewing a paraffin block from CRT bacteria IntroKit (Ivoclar Vivadent).

From stimulated saliva we seeded on selective culture media kits per se, and we kept incubated at 37 $^{\circ}$ C for 72 hours, according to the prospectus.

The colonies formed were compared with the kit scale confirming the increased clinical risk for both Lactobacillus and Streptococcus mutans.

After primary seeding, the bacterial subcultures were transferred on agar medium 5% sheep blood and incubated at $37 \degree C$ for 48 hours.

For Candida albicans, the seeding was done from stimulated saliva on Saburaud culture medium, at 37 $^{\circ}$ C for 48 hours and then maintained at 22 $^{\circ}$ C for 48 hours.

After the development of colony forming units (CFU) and verification of cultural smear, they were imersed in simple liquid broth medium.

Standardization of cultures density was performed by densitometry McFarland (Biosan) at the concentration work of 0.3 UmcFarland.

The working dilution of the five mouthwashes was obtained by placing 1 ml mouthwash and 500 μl bacterial / fungi suspension, in sterile tubes.

RESULTS

To test the antimicrobial capacity in time, we proceeded to sowing after 1, 3 and 5 minutes, on specific solid culture media. As a control we used the physiological saline solution.

To highlight the cultures according to the time of contact with mouthwash, culture media were separated into 3 sections, using sterile microbiological loop flame. Candida albicans cultural issues developed after 48 h incubation at 37 $^{\circ}$ C is shown in figure no. 2.

Figure no. 2. Development cultures after different contact time with mouthwash



Assessment of crop development in mouth rinses suspension study was conducted according to the following protocol:

For lack of colonies in 100%

+ For this rare colony in 75%

++ For many colonies mostly confluent line sowing in 50%

+++ For very abundant culture developed

Centralization of results is represented in table no. 1.

Table no. 1. Representation of mouthwash antimicrobial capacity

		CONTACT TIME								
PRODUCT		1 minute			3 minutes			5 minutes		
		S.M.	L	CA.	S.M.	L.	C.A.	SM.	L	CA
1.	ASLAMED with chamomile special argil and chlorhesidine	++	-	#	+	-	+	-	-	+
2	PELLAMAR	+++	++++	+++	+++	+++	+++	+++	+++	+++
3	Colgate Plax Sensitive	+++	-	++	+++	-	+	++	-	-
4	PARODONTAX extra 0,2%	++	-	+	+	-	-	-	-	-
5.	LISTERINE Coolmint Antibacterial Mouthwash	+++	-	+	+++	-	+	++	-	+
	MARTORS.F.	++++	++++	++++	++++	++++	++++	++++	++++	++++

DISCUSSIONS

The results of in vitro tests indicate very different values for studied mouthwashes.

The antibacterial effect of lactobacilli was obtained from the first minute of contact with Parodontax, Aslamed, Colgate Plax Sensitive and Listerine mouthwash.

The antibacterial effects on streptococcus and rinses Candida albicans vary depending on the type of contact.

The best results after 1 minute of contact were obtained for Aslamed and Parodontax mouthwash.

Results slightly delayed for the effect on Streptococcus mutans Colgate Plax occur in sensitive waters and Listerine.

The strong effect of rinses containing chlorhexidine is confirmed by numerous studies.(9,10,11,12,13)

Not to negate, the effects of biotrophic regenerative, anti-inflammatory and analgesic effects in our study we obtained the lowest regardless of the time of action for Pell Amar mouthwash.

This leads us to think to indicate Pell Amar mouthwash without contraindications for patients presenting oral halitosis associated with blood hypertension.(7,14)

Not to minimize or increase, the effect of antiseptic rinses in vitro, in vivo study on their association with individual tooth brushing and the use of dental floss certainly helps removing the plaque with beneficial effects on oral health.

CONCLUSIONS

Bactericidal activity of mouthwashes varies depending on their chemical composition.

Rinsing with mouthwash should not be indicated for all patients, but only those who have chronic troubles in the plaque control, causing caries, gingivitis and bad breath mouth.

Recommendation of mouthwashes as individual methods helper of oral hygiene should be done by the dentist depending on individual diseases.

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