
EVALUATION OF THE EFFICACY OF SEVERAL ORAL ANTISEPTICS ON *STREPTOCOCCUS MUTANS*

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RESUMO: Com o objectivo de demonstrar o efeito "in vitro" de vários antissépticos orais sobre os *Streptococcus mutans*, utilizaram-se os seguintes produtos comerciais: Hibitane Dental, Cariax, Betadine Solução Oral, Oraldene, Mentamida e Resorborina. Para cada um foi calculado a Concentração Inibitória Mínima (MIC), tendo sido o Cariax (Clorhexidina e Flúor) aquele que apresentou a MIC menor. O Betadine (Polivinilpirrolidona iodada), pelo contrário, apresentou a MIC maior. A Mentadima (Hexetidina e Benzidamida) mostrou uma MIC menor que o Oraldene (Hexetidina). A relação entre a concentração do antisséptico e o diâmetro da zona inibidora nas culturas de *Streptococcus mutans* foi feita utilizando o teste de difusão em disco de Kirby-Bauer, o qual utiliza equações exponenciais. Esta relação permite-nos calcular a sensibilidade ou resistência do *Streptococcus mutans* relativamente aos agentes antimicrobianos. Acreditamos que a Hexetidina e/ou a Benzidamida são superiores à Clorhexidina em utilização prolongada devido aos efeitos secundários desta última. A utilização de antissépticos orais e outras medidas de higiene reduzem significativamente os *Streptococcus mutans*, no entanto o seu efeito é transitório. Por isso é fundamental instruir o paciente sobre técnicas de higiene oral motivando-o periodicamente para essa tarefa. Sugerimos que sejam feitas colheitas bacterianas para estudo da flora oral e para determinar a sua susceptibilidade aos agentes antimicrobianos, permitindo-nos a prescrição de um antisséptico oral específico.

ABSTRACTS: In order to demonstrate the effect "in vitro" of several commercialized oral antiseptics on *Streptococcus mutans*, we have used the following: Hibitane Dental, Cariax, Betadine Mouthwash, Oraldene, Mentamide and Resorborine. Minimum Inhibitory Concentrations (MICs), were determined for every one, being Cariax (Chlorhexidine and Fluoride) the one which exhibited the lowest MIC. Meanwhile, Betadine (Povidone Iodine) presented the highest one. Mentamide (Hexetidine and Benzidamine) showed a less important MIC than Oraldene (Hexetidine). The relationship between antiseptic concentration and inhibitory zone diameters from *Streptococcus mutans* culture, was achieved by Kirby-Bauer disk diffusion test, which was represented by exponential equations. This relationship lets us calculate the sensibility or resistance of the *Streptococcus mutans* against antimicrobials. We believe that Hexetidine and/or Benzidamine are better than chlorhexidine in prolonged usage because its side-effects. The antiseptics oral administration and other hygienic measures cause an important reduction of *Streptococcus mutans*, but it is a transitory effect. For this reason, it is necessary to give some hygienic measures continuously, having in mind the individuals need. We suggest the possibility of performing bacterial sampling in order to study oral flora, and so, to determine its susceptibility against antimicrobials, allowing us the prescription in each case of a specific oral antiseptic.

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INTRODUCTION

Relationship between dental decay and *Streptococcus mutans* (*S. mutans*) was well established several years ago (1,2,3). A large number of studies have showed that *S. mutans* is the organism most frequently isolated from human dental plaque (4,5,6). The cariogenicity of *S. mutans* depends on some factors, which include the ability of the organism to colonize the tooth surfaces and to synthesize adherent glucans from sucrose, the length of time the pH remains below the critical for demineralization of the enamel and the quantity of the acid produced (3,7,8).

Several local antimicrobials can be considered to be used against bacterial plaque development. The initial demonstration of Chlorhexidine gluconate as an inhibitory dental plaque formation agent, was made in 1970 (9). Chlorhexidine in caries prevention has been evaluated in some different investigations (10,11,12) and has showed up an additive benefit through the combination of this antiseptic and fluoride (13).

Quaternary ammonium compounds (Benzalconium chloride), have shown also an antiplaque effect (14).

Phenolic compounds have been used in mouthrinses at high concentration, having made visible reductions in plaque (15). The aim of the present investigation was to determine the effect "in vitro" on *S. mutans* isolated the several commercialized oral antiseptics and to compare their efficacy.

MATERIALS AND METHODS

S. mutans type strain (from Spanish Collection of Type Cultures under the number CECT 479), has been used to determine its susceptibility to the following oral antiseptics, which are commercialized and whose main components are indicated in brackets:

- Hibitane Dental (Chlorhexidine digluconate 0.2 g/100 ml)
 - Cariax (Chlorhexidine digluconate 0.2 g + Fluoride 0.022 g/100 ml)
 - Betadine mouthwash (Povidone Iodine 7.5 g/100 ml)
 - Oraldene (Hexetidine 100 mg/100 ml)
 - Mentamide (Hexetidine 100 mg + Benzidamine ClH 100 mg/100 ml)

— Resorborine (Benzalconium chloride 100 mg + Resorcionol 2 g/100 ml)

Minimum Inhibitory Concentrations (MICs) from each antiseptic were performed by a macrodilution methods as it is described by the NCCLS dilutions standard M 7-T (16) and others authors (17). The inoculum was prepared by suspending enough growth from a 24 h. agar plate in Mueller-Hinton broth to yield turbidity equivalent to a 0.5 McFarland standard.

The antiseptic disk were made in our laboratory by adding 0.025 ml of increasing solutions of the product, which contained enough drug (40 times) to give the final desired concentration, to each blank 6 mm disk.

S. mutans broth suspension was streaked evenly in 3 planes onto the surface of the Mueller-Hinton Agar in Petri dishes with a sterile cotton swab. After the inoculum was dried, the antiseptic disks were placed on the medium with flamed forceps (18). After a 24 h. incubation, the inhibition zone was measured with a ruler (including the 6 mm disk). Each antiseptic was tested 6 times with several dilutions. The number of dilutions for each one was variable until the inhibitory zone diameter was evident (from 4 to 12 dilutions). Exponential regression equation ($y = a \cdot e^{bx}$) represents the relationship between each concentration of the antiseptic in mcg/disk (y) and the mean of the size inhibition zone in mm (x). Exponential curves were plotted in scattergrams and the corresponding determination coefficients were calculated.

In accordance with these data and evaluating the standard deviation of the size inhibition zone by each concentration, we have calculated the minimum size diameter which is necessary to determine the susceptibility of *S. mutans* to the antiseptics tested.

RESULTS

TABLE I. — Shows the MICs of the oral antiseptics on *S. mutans* type strain. There were remarkable differences in the susceptibility obtained from each product. Cariax exhibits the lowest MIC because of the combined effects of fluoride and chlorhexidine. Meanwhile Betadine (Povidone Iodine) represent the highest one. Mentamide has a less important MIC than Oraldene, perhaps by synergic action of both, Hexetidine and Benzidamine.

EVALUATION OF THE EFFICACY OF SEVERAL ORAL ANTISEPTICS ON *STREPTOCOCCUS MUTANS*

TABLE I

MICs of the oral antiseptics on *Streptococcus mutans* type strain

<u>Antiseptics</u>	<u>Dilution</u>	<u>MICs (mcg/ml)</u>
Hibitane Dental	1/256	7,81
Cariax	1/4000	0,50
Betadine Mouthwash	1/32	2353,75
Oraldene	1/400	3,90
Mentamide	1/1500	1,33
Resorborine	1/512	41,01

The following TABLES (II, III and IV), compare the inhibition zone size diameter in mm, produced around disk with their corresponding potency (the equivalent dilution from commercial antiseptics is indicated). In all cases, we can observe an exponential decreasing of the diameter size in accordance with the power of the disk (disk potency).

TABLE V, summarizes the Minimum Inhibitory zone diameter produced in a culture of the *S. mutans* grown on Mueller-Hinton Agar medium in Petri dishes for every antiseptic tested, with regard some determined potency disk, in order

to consider if it is sensitive or resistant. These sizes were calculated in accordance with the mean (m) and standard deviation (s) obtained from 6 measures (m + 2s) for each concentration.

FIGURE 1, includes the curves representing the relationship between size of inhibition zone means in mm (x) and antiseptic concentration in mcg/disk (y), with the expression of the exponential equation and the determination coefficients (r^2), for every antiseptic. These coefficients revealed, in all cases, an excellent correlation between concentration (potency disk) and their inhibition zone diameters.

TABLE II

Inhibition zone size diameter (mm), produced by each antiseptic in relationship with disks potency (mcg/d) on *Streptococcus mutans* type strain

<u>Dilution</u>	<u>HIBITANE DENTAL</u>		<u>CARIAX</u>	
	<u>Disk potency</u>	<u>Size diameter</u>	<u>Disk potency</u>	<u>Size diameter</u>
1/1	50	19.0	50	27.0
1/2	25	17.0	25	24.0
1/4	12.50	15.0	12.50	21.3
1/5	10	14.0	10	19.6
1/8	6.25	13.5	6.25	18.0
1/10	5	13.0	5	16.0
1/16	3.12	9.5	3.12	14.5
1/20	2.50	8.5	2.50	12.6
1/32			1.56	11.1
1/40			1.25	10.2
1/64			0.78	9.3
1/80			0.62	8.3

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TABLE III

Inhibition zone size diameter (mm), produced by each antiseptic in relationship with disks potency (mcg/d) on *Streptococcus mutans* type strain

Dilution	BETADINE MOUTHWASH		ORALDENE	
	Disk potency	Size diameter	Disk potency	Size diameter
1/1	1875	11.3	25	30.6
1/2	937.50	8.8	12.50	24.6
1/4	468.75	7.6	6.25	19.3
1/5	375	7.0	5	16.6
1/8			3.12	15.6
1/10			2.50	14.0
1/16			1.56	11.3
1/20			1.25	9.0

TABLE IV

Inhibition zone size diameter (mm), produced by each antiseptic in relationship with disks potency (mcg/d) on *Streptococcus mutans* type strain

Dilution	MENTAMIDE		RESORBORINE	
	Disk potency	Size diameter	Disk potency	Size diameter
1/1	50	14.9	531.12	14.8
1/2	25	12.6	265.56	12.8
1/4	12.5	11.2	132.78	11.3
1/5	10	10.3	106.22	9.5
1/8	6.25	8.7	66.39	7.8
1/10	5	7.7	53.11	7.0
1/16	3.12	7.0		

TABLE V

Minimum inhibitory zone diameter (mm) in accordance with disk potency (mcg/d) on *Streptococcus mutans* type strain

Antiseptics	Disk potency	Resis.	Sensi.
Hibitane Dental	5	12.5	13.8
Cariax	5	15.5	16.6
Betadine Mouthwash	1000	8.5	10.0
Oraldene	5	16.0	17.2
Mentamide	10	10.0	11.5
Resorborine	10	9.0	10.4

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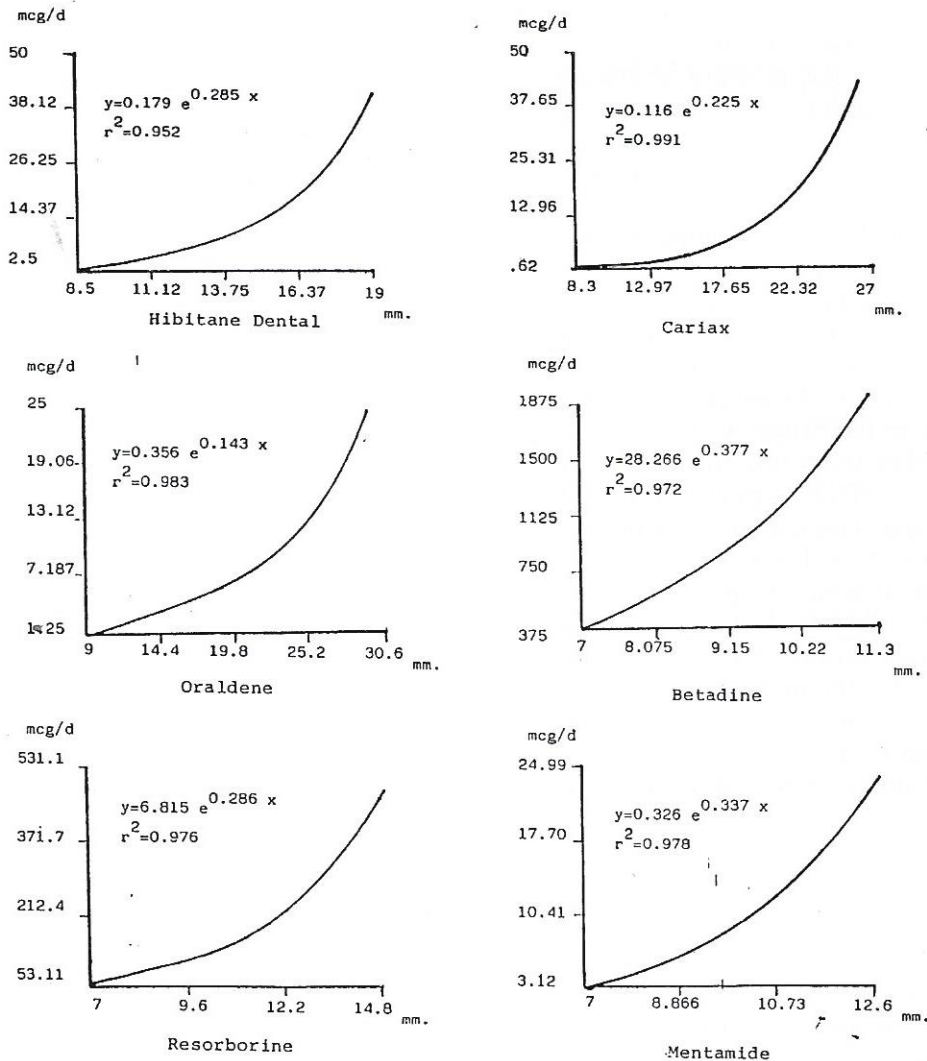


Fig. 1 — Relationship between size of inhibition zone (x) and antiseptic concentration (y).

DISCUSSION

Our studies have revealed that chlorhexidine combined with fluoride (Cariax) "in vitro" has greater effect than chlorhexidine alone (Hibitane Dental), this fact is in line with other authors (13). The products with Hexetidine (Oraldene and Mentamide), show a MIC on *S. mutans* smaller than chlorhexidine. In contrast, the antiplaque effect of Hexetidine is smaller if compared with that of chlorhexidine (19).

We believe that Hexetidine alone (Oraldene) or combined with Benzidamine (Mentamide) is a suitable oral antiseptic, better than chlorhexidine. Although it may be used too (20), it has an

unpleasant taste and produces a brown colouration of the teeth, mucous membranes and tongue (21). Because of these reasons its long-term use is limited (22). However, Hexetidine at high concentration may cause mucosal erosions and that is why we suggest its use in mouthwash at 0.1%, in order to prevent oral infection in high caries risk patients pre and post oral surgery and other purposes, and also to prevent nosocomial pneumonia in patients receiving continuous mechanical ventilation, because this might be caused, in many cases, by oropharyngeal flora (23).

Axelsson et al. (24) in a longitudinal study of the effects of some oral hygiene measures on *S. mutans*, carried out in one hundred and eighty-

seven schoolchildren distributed into three groups, who had received different sanitary actions as mechanical tooth-cleaning, chlorhexine treatment, oral hygiene instructions....., showed that there was a significant reduction of *S. mutans* in saliva as well as on approximal tooth surfaces in all groups, but no significant differences among them.

In agreement with these authors, we believe that all hygienic measures cause a reduction of *S. mutans* and decrease dental caries risk, although with a transitory effect. It is necessary to apply several of those measures continuously and with individualized intervals. We suggest that bacterial samplings might be performed in every visit to the dentist's in order to study oral microflora and to determine its susceptibility against antimicrobials (mainly *S. mutans*). This can make us prescribe a specific oral antiseptic and other hygienical actions based on individual need. We propose the use of the Kirby-Bauer (18) disk diffusion technique to study the antimicrobial susceptibility, because it is a wellknown test for the microbiologists and also results to be easy. However, in case of the antiseptics, every laboratory must achieve its own standards about sensibility on *S. mutans* or other isolated organisms.

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EVALUATION OF THE EFFICACY OF SEVERAL ORAL
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