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Original papers

The *in vitro* activity of selected mouthrinses on standard strains of fungi

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ABSTRACT. An oral cavity hygiene plays a key role in prophylactic and therapeutic measures to prevent pathological changes caused by different viruses, bacteria, fungi and protozoa. It is important to maintain proper oral hygiene and assist the removal of potent pathogens; use of the mouthrinses can be one of method providing to these goal. The purpose of this study was to investigate the effects of selected mouthrinses on the standard strains of *Candida* presented in the oral cavity. Eight reference strains of fungi were investigated: *C. albicans* (CBS 2312), *C. albicans* (L 45), *C. albicans* (ATCC 24433), *C. dubliniensis* (CBS 7987), *C. glabrata* (CBS 862), *C. krusei* (CBS 573), *C. parapsilosis* (CBS 10947) and *C. tropicalis* (CBS 2424). Thirteen mouthrinses were used in the study, including pure chlorhexidine (CHX), and 12 commercially available varieties: Azulan, Colgate Plax Complete Care Sensitive, Corsodyl 0,2%, Curasept ADS 205, Dentosept, Dentosept A, Eludril Classic, Listerine Total care, Octenidol, Oral-B Pro-Expert Clinic Line, Sylveco and *Tinctura salviae*. The present study used a qualitative diffusion in agar gel-well plate method to evaluated the antifungal properties of mouthrinses. Among the 12 commercially available mouthrinses examined in the study, the following were not found to show antifungal activity: Azulan, Dentosept, Eludril Classic, Listerine Total care, *Tinctura salviae*. The largest inhibition zones were produced by Dentosept, Chlorhexidine and Colgate. The smallest inhibition zones were produced by Octenidol and Curasept. With regard to mouthwash type, statistically significant differences in growth inhibition zone diameter were found between the following pairs of fungi: *C. albicans* and *C. krusei* for Colgate without dilution and with 1:2 dilution; *C. albicans* and *C. glabrata* for Corsodryl without dilution and with 1:2 dilution; *C. albicans* and *C. dubliniensis* for Dentosept A without dilution; *C. glabrata* and *C. parapsilosis* with 1:2 dilution; *C. dubliniensis* and *C. tropicalis* for Sylveco without dilution, 1:2 dilution and 1:4 dilution; *C. dubliniensis* and *C. parapsilosis* for Sylveco without dilution, 1:2 dilution and 1:4 dilution. The lowest MIC values calculated from the Iinear regression equation, indicating the strongest potential activity, were obtained for Dentosept A, followed by chlorhexidine; the lowest activity, was calculated for Curasept and for Octenidol. Some of the tested mouthrinses have antimycotic properties at commercially available concentrations. In spite of the fact that chlorhexidine is thought to be the gold standard for mouthrinse use, Dentosept has stronger antimycotic activity and acts on a wider spectrum of fungi species. Chlorhexidine and Colgate do not appear to act against *C. tropicalis*, neither does Curaspet against *C.dubliniensis;* therefore, the determination of the fungus species is necessary.

Key words: *Candida* standard strains, mouthrinses, antifungal activity

Introduction

An oral cavity free of pathological changes is an integral part of health, and its hygiene plays a key role in prophylactic and therapeutic measures to prevent cavities, gingivitis and periodontitis; it also prevents inflammation of the oral mucosa by different viruses, bacteria, fungi and protozoa that create complex interactions with one another. A molecular biology study of the mycobiome of the oral cavity found it to be composed of 85 genera of fungi, most commonly *Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Cryptococcus*, *Fusarium*, *Geotrichum*, *Malassezia*, *Mucor*, *Rhizopus*, *Saccharomyces*, and *Scopulariopsis* belonging to 101 species [1].

It is therefore important to maintain proper oral hygiene and assist the removal of potent pathogens. Examples of such hygienisation treatments include dental brushing, soft plaque and scale removal, as

well as the use of dental floss and tapes, interdental brushes, brushes and scrapes for the tongue and various types of mouthrinse.

It is recommended that mouthrinses should be used before and after brushing, or immediately after the meal if there is no possibility to clean the teeth [2]. They are usually used for two reasons: 1. As a prophylactic measure in patients with good hygiene, who do not demonstrate any acute or chronic illnesses; 2. As treatment of disease states including local (gingivitis, periodontitis) and systemic (disorders of immunological system, chemotherapy) disorders [3]. Currently, several dozen mouthrinses are available, mainly containing natural substances, alcohol or chlorhexidine.

The purpose of this study was to investigate the effects of selected mouthrinses on the standard strains of *Candida* presented in the oral cavity.

Materials and Methods

Eight reference strains of fungi were investigated: *C. albicans* (CBS 2312), *C. albicans* (L 45), *C. albicans* (ATCC 24433), *C. dubliniensis* (CBS 7987), *C. glabrata* (CBS 862), *C. krusei* (CBS 573), *C. parapsilosis* (CBS 10947) and *C. tropicalis* (CBS 2424).

Thirteen mouthrinses were used in the study, including pure chlorhexidine (CHX), and 12 commercially available varieties: Azulan, Colgate Plax Complete Care Sensitive, Corsodyl 0,2%, Curasept ADS 205, Dentosept, Dentosept A, Eludril Classic, Listerine Total care, Octenidol, Oral-B Pro-Expert Clinic Line, Sylveco and *Tinctura salviae.*

Antifungal susceptibility tests are performed for various reasons, one being to compare the *in vitro* activity of new and existing agents. The present study used a qualitative diffusion in agar gel-well plate method. Briefly, 0.5 ml of liquid 24-hour culture on Sabouraud medium was transferred into 5 ml of liquid Sabouraud broth. From this dilution, 1 ml (10^6 fungal cells) of inoculum was transferred onto a 9 cm diameter Petri dish containing 30 ml of 3% Sabouraud agar at pH 5.6. The suspension was then evenly distributed on the surface of the plate using a crooked glass tube. After one hour of thermostatic incubation at 37°C, 10 mm diameter wells were formed in the medium using a sterile rotating drill. The wells were numbered and 100 µl amounts of subsequent dilutions of the studied mouthrinses were added to each well using an automatic pipette. The plates were then incubated for 24 hours at 37°C. The diameter of the growth inhibition zone (in mm) was measured for each well; each experience was repeated 3 times.

The activity curve of the compound for the particular fungal strain was plotted according to the Cartesian coordinate system: The *x*-axis showed logarithmic values of mouthrinses concentration while the y-axis showed the diameter of the zones of inhibited growth (given in mm) measured after 24 hours. The analyzed segment of the activity curve demonstrates a rectilinear course which reflects the relationship between the diameter of the zone of inhibited growth on agar and the concentration of the studied drug. The relationship is directly proportional and remains within the margin of error. The diameter of the zone of inhibited growth grew as the mouthrinse concentration increased. Activity curves were plotted for each strain to evaluate their susceptibility to a drug.

The Minimum Inhibitory Concentration (MIC) was calculated from the Iinear regression equation according to Kadlubowski [4]:

$$\log MIC - \overline{\log C_1} + \frac{\overline{\log C_2} - \overline{\log C_1}}{\overline{N}_2 - \overline{N}_1}(10 - \overline{N}_1)$$

where $\overline{N}_1$ and $\overline{N}_2$ are arithmetic means in two sets of zones diameters of inhibited growth and $\overline{\log C_1}$ and $\overline{\log C_2}$ are the means of the logarithms of mouthrinse concentrations in groups, N – diameter of inhibition zone in mm.

The results obtained in the Iinear dependence interval between the zone diameter N and the logarithm of the compound concentration were divided into two groups. For each group, mean values were calculated.

The degree of growth inhibition of the tested fungi by various mouth rinses was analysed. For the quantitative variables, the following characteristics were calculated: arithmetic mean ($\overline{x}$) and median (Me), mean and standard deviation (SD), and variation coefficient (v%) as a measure of variability. The minimum and maximum values were also given. As the distributions of the analyzed variable differed significantly from the normal distribution, nonparametric tests were used to compare the means. The Kruskal-Wallis test, a nonparametric equivalent of variance analysis for single classification, was used to compare several independent groups.

Table 1A – G.The diameter of growth inhibition zone (in mm) for different references species and particular mouthrinses ($\bar{x}$ ± SD/Min-Max)

Species	Dilution of chlorhexidine			
	1	0.5	0.25	0.125
C. albicans CBS 2312	16.0 ± 0.0 16 – 16	14 ± 0.0 14 – 14	12.0 ± 0.0 12 – 12	–
C. albicans L 45	29.0 ± 1.73 27 – 30	24.3 ± 1.15 23 – 25	20.0 ± 0.0 20 – 20	15 ± 0.0 15 – 15
C. albicans ATCC 24433	18 ± 0.0 18 – 18	16 ± 0.0 16 – 16	14 ± 0.0 14 – 14	12.0 ± 0.0 12 – 12
C. dubliniensis CBS 7987	20.0 ± 0.0 20 – 20	17.33 ± 0.58 17 – 18	15 ± 0.0 15 – 15	12.0 ± 0.0 12 – 12
C. glabrata CBS 862	18 ± 0.0 18 – 18	16 ± 0.0 16 – 16	14 ± 0.0 14 – 14	12.0 ± 0.0 12 – 12
C. krusei CBS 573	13 ± 0.0 13 – 13	–	–	–
C. parapsilosis CBS 10947	20.0 ± 0.0 20 – 20	17 ± 0.0 17 – 17	15 ± 0.0 15 – 15	13.0 ± 0.0 13 – 13
Statistics for dilution	H=9.832 p=0.0434	H=10.163 p=0.0378	–	–

Table 1B.

Species	Dilution of Colgate			
	1	0.5	0.25	0.125
C. albicans CBS 2312	17.67 ± 0.58 17 – 18	15.7 ± 0.58 15 – 16	13.0 ± 0.0 13 – 13	–
C. albicans L 45	16.0 ± 0.0 16 – 16	14.0 ± 0.0 14 – 14	12.0 ± 0.0 12 – 12	–
C. albicans ATCC 24433	12.7 ± 0.58 12 – 13	–	–	–
C. dubliniensis CBS 7987	20.0 ± 0.0 20 – 20	17.33 ± 0.58 17 – 18	15 ± 0.0 15 – 15	12.0 ± 0.0 12 – 12
C. glabrata CBS 862	18 ± 0.0 18 – 18	16 ± 0.0 16 – 16	14 ± 0.0 14 – 14	12.0 ± 0.0 12 – 12
C. krusei CBS 573	13 ± 0.0 13 – 13	–	–	–
C. parapsilosis CBS 10947	20.0 ± 0.0 20 – 20	17 ± 0.0 17 – 17	15 ± 0.0 15 – 15	13.0 ± 0.0 13 – 13
Statisticsfor dilution	H=9.832 p=0.0434	H=10.163 p=0.0378	–	–

Where statistically significant differences were found between the diameters of the growth inhibition zone in several compared groups, a Dunnett's pairwise comparison was used as a *post hoc* test. A comparison was regarded as statistically significant when $p < 0.05$. All calculations were performed using STATISTICA v.7.1.

Results

Among the 12 commercially available mouthrinses examined in the study, the following were not found to show antifungal activity: Azulan, Dentosept, Eludril Classic, Listerine Total care, *Tinctura salviae.*

Table 1C.

Species	Dilution of Corsodyl		
	1	0.5	0.25
C. albicans	18 ± 0.0	16 ± 0.0	14 ± 0.0
CBS 2312	18 – 18	16 – 16	14 – 14
C. albicans	20.0 ± 0.0	17 ± 0.0	13.0 ± 0.0
L 45	20 – 20	17 – 17	13 – 13
C. albicans	17 ± 0.0	15 ± 0.0	12.0 ± 0.0
ATCC 24433	17 – 17	15 – 15	12 – 12
C. dubliniensis	18 ± 0.0	16 ± 0.0	13.0 ± 0.0
CBS 7987	18 – 18	16 – 16	13 – 13
C. glabrata	12.0 ± 0.0	–	–
CBS 862	12 – 12		
C. krusei	16 ± 0.0	14 ± 0.0	12.0 ± 0.0
CBS 573	16 – 16	14 – 14	12 – 12
C. tropicalis	15.33 ± 0.58	13.0 ± 0.0	–
CBS 2424	15 – 16	13 – 13	
C. parapsilosis	15 ± 0.0	13.0 ± 0.0	–
CBS 10947	15 – 15	13 – 13	
Statistics for dilution	H=20.206 p=0.0011	H=20.476 p=0.0010	–

Table 1D.

Species	Dilution of Curasept	
	1	0.5
C. albicans	14 ± 0.0	12.0 ± 0.0
CBS 2312	14 – 14	12 – 12
C. albicans	–	–
L 45		
C. albicans	12.0 ± 0.0	–
ATCC 24433	12 – 12	
C. dubliniensis	–	–
CBS 7987		
C. glabrata	12.0 ± 0.0	–
CBS 862	12 – 12	
C. krusei	15 ± 0.0	13.0 ± 0.0
CBS 573	15 – 15	13 – 13
C. tropicalis	15 ± 0.0	13.0 ± 0.0
CBS 2424	15 – 15	13 – 13
C. parapsilosis	13.0 ± 0.0	–
CBS 10947	13 – 13	
Statistics for dilution	–	–

Table 1E.

Species	Dilution of Dentosept A			
	1	0.5	0.25	0.125
C. albicans	30.0 ± 0.0	25.0 ± 0.0	15 ± 0.0	13.0 ± 0.0
CBS 2312	30 – 30	25 – 25	15 – 15	13 – 13
C. albicans	30.0 ± 0.0	24.0 ± 1.73	18 ± 0.0	13.0 ± 0.0
L 45	30 – 30	22 – 25	18 – 18	13 – 13
C. albicans	26.33 ± 0.58	23.0 ± 0.0	20.0 ± 0.0	15 ± 0.0
ATCC 24433	26 – 27	23 – 23	20 – 20	15 – 15
C. dubliniensis	30.0 ± 0.0	25.0 ± 0.0	20.0 ± 0.0	15 ± 0.0
CBS 7987	30 – 30	25 – 25	20 – 20	15 – 15
C. glabrata	20.0 ± 0.0	17 ± 0.0	15 ± 0.0	–
CBS 862	20 – 20	17 – 17	15 – 15	

C. krusei	25.0 ± 0.0	20.0 ± 0.0	15 ± 0.0	–
CBS 573	25 – 25	20 – 20	15 – 15	
C. tropicalis	26.0 ± 0.0	20.67 ± 1.15	17 ± 0.0	14 ± 0.0
CBS 2424	26 – 26	20 – 22	17 – 17	14 – 14
C. parapsilosis	28.0 ± 0.0	25.33 ± 0.58	15 ± 0.0	–
CBS 10947	28 – 28	25 – 26	15 – 15	
Statistics for dilution	H=18.588 p=0.0023	H=19.815 p=0.0014	H=14.703 p=0.0117	H=19.462 p=0.0016

Table 1F.

Species	Dilution of Octenidol	
	1	0.5
C. albicans	15 ± 0.0	13.0 ± 0.0
CBS 2312	15 – 15	13 – 13
C. albicans	19.3 ± 1.15	16.3 ± 1.15
L 45	18 – 20	15 – 17
C. albicans	15 ± 0.0	12.0 ± 0.0
ATCC 24433	15 – 15	12 – 12
C. dubliniensis	15 ± 0.0	13.0 ± 0.0
CBS 7987	15 – 15	13 – 13
C. glabrata	15.67 ± 0.58	13.67 ± 0.58
CBS 862	15 – 16	13 – 14
C. krusei	13.0 ± 0.0	–
CBS 573	13 – 13	
C. tropicalis	15 ± 0.0	13.0 ± 0.0
CBS 2424	15 – 15	13 – 13
C. parapsilosis	15 ± 0.0	13.0 ± 0.0
CBS 10947	15 – 15	13 – 13
Statistics for dilution	H=13.880 p=0.0003	H=10.413 p=0.0643

Table 1G.

Species	Dilution of Sylveco		
	1	0.5	0.25
C. albicans	18 ± 0.0	15 ± 0.0	–
CBS 2312	18 – 18	15 – 15	
C. albicans	18 ± 0.0	15 ± 0.0	13.0 ± 0.0
L 45	18 – 18	15 – 15	13 – 13
C. albicans	20.0 ± 0.0	17 ± 0.0	13.0 ± 0.0
ATCC 24433	20 – 20	17 – 17	13 – 13
C. dubliniensis	22.67 ± 0.58	20.0 ± 0.0	18 ± 0.0
CBS 7987	22 – 23	20 – 20	18 – 18
C. glabrata	20.0 ± 0.0	15 ± 0.0	13.0 ± 0.0
CBS 862	20 – 20	15 – 15	13 – 13
C. krusei	17 ± 0.0	15 ± 0.0	13.0 ± 0.0
CBS 573	17 – 17	15 – 15	13 – 13
C. tropicalis	15 ± 0.0	13.0 ± 0.0	–
CBS 2424	15 – 15	13 – 13	
C. parapsilosis	15 ± 0.0	13.0 ± 0.0	–
CBS 10947	15 – 15	13 – 13	
Statistics for dilution	H=21.456 p=0.0007	H=20.374 p=0.0011	–

The largest inhibition zones were produced by Dentosept (mean scores without dilution – 26.92 mm; 1:2 – 22.5 mm; 1:4 – 16.88 and 1:8 – 12.5 mm), Chlorhexidine and Colgate (mean scores without dilution – 19.14 mm; 1:2 – 16.38 mm; 1:4 – 15.0 mm; 1:8 – 12.33 mm). The smallest inhibition zones were produced by Octenidol (mean scores without dilution – 15.38 mm; 1:2 – 13.0 mm; 1:4 – 10.81 mm) and Curasept (mean scores without dilution – 13.5 mm and 1:2 – 11.3 mm). The

Table 2. The values of MIC of different mouthrinses

Species	MIC							
	Chlorhe-xidine	Colgate	Corsodyl	Curasept	Dentosept A	Octenidol	Oral B	Sylveco
C. albicans CBS 2312	0.0625	0.1179	0.0625	0.25	0.0498	0.2265	0.2264	0.2204
C. albicans L45	0.0614	0.1250	0.1313	–	0.0318	0.1227	0.2264	0.1166
C. albicans ATCC24433	0.1250	0.1250	0.5	0.1340	0.5	0.0178	0.2726	0.2264
C. dubliniensis CBS 7987	0.0661	0.1250	0.1211	–	0.0625	0.2264	0.2264	0.0300
C. glabrata CBS 862	0.0625	0.0312	0.5	0.5	0.0992	0.1114	0.0603	0.1250
C. krusei CBS 573	0.50	0.2264	0.1250	0.3715	0.1250	0.5	0.2264	0.1114
C. parapsilosis CBS 10947	0.0566	0.2264	0.2264	0.3535	0.1384	0.2234	0.2264	0.2264
C. tropicalis CBS 2424	–	–	0.2311	0.2264	0.1933	0.1114	0.2264	0.2264

diameter of the growth inhibition zones for the various reference species and mouthrinses ($\bar{x}$± SD/Min-Max) are summarized in Tables 1A–G.

A statistically significant difference ($p<0.05$) in growth inhibition diameter was found for various species of fungus in the reference group: without dilution and 0.5 dilution of Chlorhexidine, Colgate, Corsodyl, Octenidol, Oral-B and Sylveco, and for all dilutions of Dentosept A. No statistically significant difference was found between any pairs (Chlorhexidine – 0–2.424; $p> 0.05$, 0.197–2.993; $p>0.05$; Oral-B – $p>0.05$).

With regard to mouthwash type, statistically significant differences in growth inhibition zone diameter were found between the following pairs of fungi: *C. albicans* and *C. krusei* for Colgate without dilution and with 1:2 dilution (respectively: $z=2.451$; $p=0.0142$ and $z=2.404$; $p=0.016$); *C. albicans* and *C. glabrata* for Corsodryl without dilution and with 1:2 dilution (respectively: $z=3.500$; $p=0.00697$ and $p<0.01$); *C. albicans* and *C. dubliniensis* for Dentosept A without dilution ($z=3.11$; $p=0.027$); *C. glabrata* and *C. parapsilosis* with 1:2 dilution ($z=3.175$; $p=0.02244$); *C. dubliniensis* and *C. tropicalis* for Sylveco without dilution, 1:2 dilution and 1:4 dilution (respectively: $z=3.377$; $p=0.01097$ and $z=3.118$; $p=0.02734$); *C. dubliniensis* and *C. parapsilosis* for Sylveco without dilution, 1:2 dilution and 1:4 dilution (respectively: $z=3.377$; $p=0.01097$ and $z=3.118$; $p=0.02734$).

The lowest MIC values calculated from the linear regression equation, indicating the strongest potential activity, were obtained for Dentosept A ($\bar{x}$= 0.0817), followed by chlorhexidine ($\bar{x}$ = 0.0827); however, it was not active against *C. tropicalis* (Table 2 and Fig. 1,2). In contrast, the highest MIC, and hence the lowest activity, was calculated for Curasept ($\bar{x}$ = 0.2897) and for Octenidol ($\bar{x}$ = 0.2121). Interestingly, *C. dubliniensis* ($\bar{x}$ = 0.1225) was found to be the most sensitive to the examined mouthrinses and *C. krusei* the most resistant ($\bar{x}$ = 0.2735).

Discussion

Correct oral hygiene is essential for preventing infection. One way to maintain proper oral hygiene is to use mouthrinses that have bactericidal, fungicidal, protozoonicidal/bacteriostatic, fungistatic or protozoonostatic abilities against the wide range of potentially invasive pathogens. The golden standard for this purpose is to use chlorhexidine (CHX) itself or the formulation that contains it [5].

As the agar diffusion method is the most widely used of the various techniques employed to determine the sensitivity of standard fungal strains to mouthrinses, it was used in the present study; however, the MIC of each mouthrinse was also calculated. It is difficult to compare obtained results between studies due to the variation in the choice of standard strains and mouthrinses.

The present study examines the effect of CHX and 12 commercially available mouthrinses of eight different species of standard *Candida* strains. Five mouthrinses (Azulan, Dentosept, Eludril Classic,

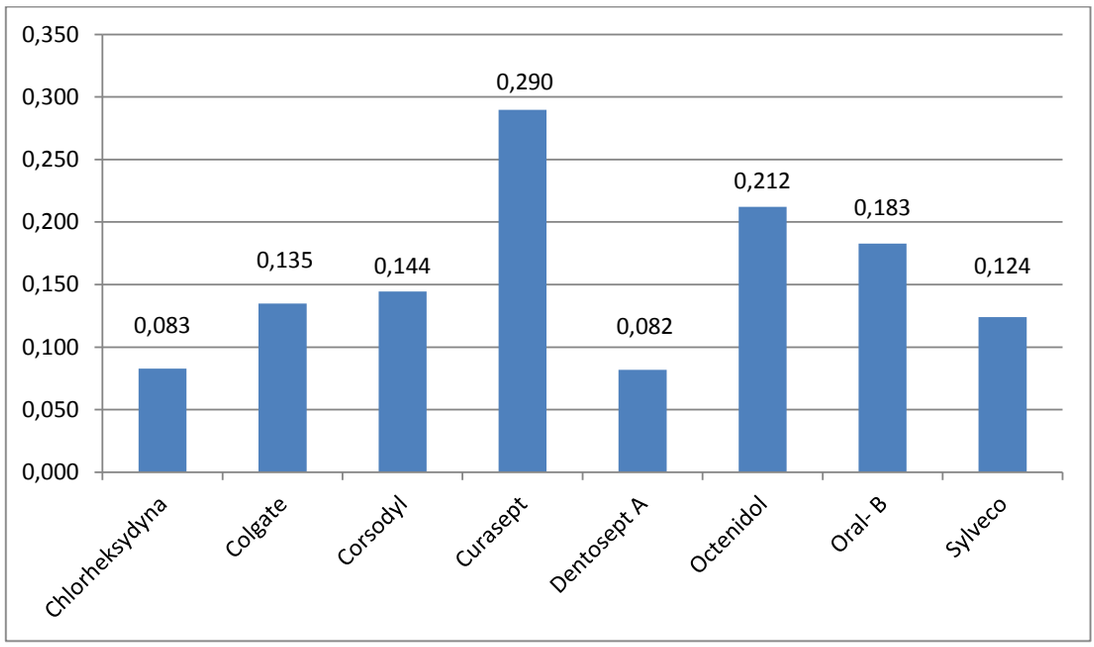

Fig. 1. The value of MIC for particular mouthrinses

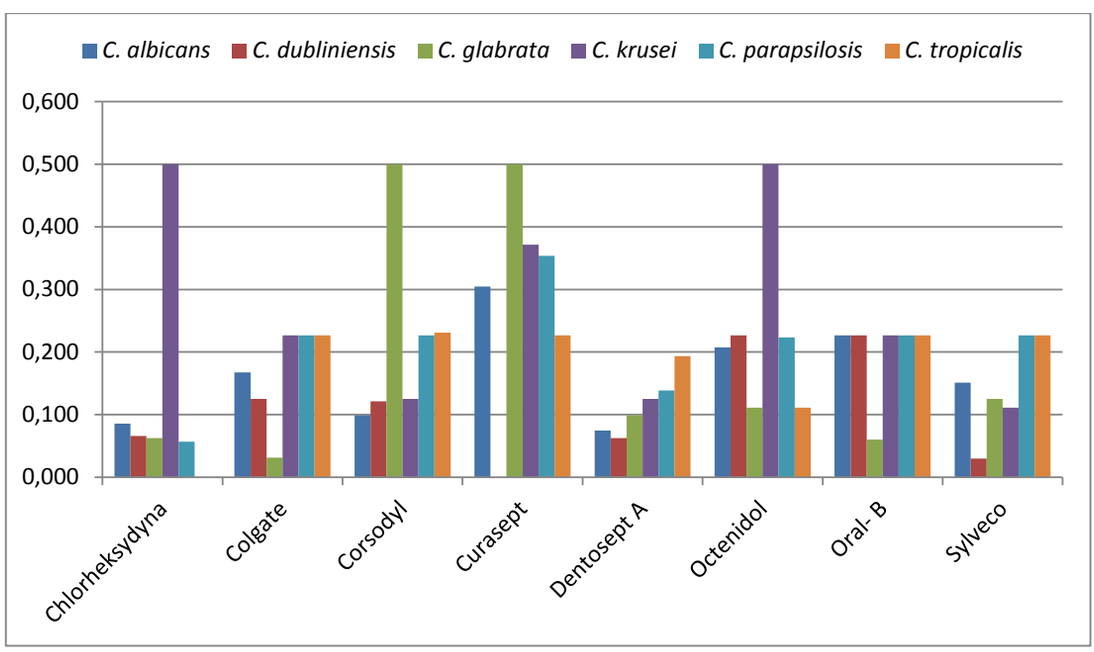

Fig. 2. The value of MIC for particular species of reference strains

Listerine Total Care, *Tinctura salviae*) had no antifungal activity, while of the seven commercially-available mouthrinses which demonstrated antifungal properties, only *C. tropicalis* was resistant to CHX and to Colgate, while *C. dubliniensis* was resistant to Curaspet.

Ronanki et al. [6] performed an *in vitro* agar well-diffusion study of the efficacy of 10 commercially available CHX mouthrinses at different concentrations (0.2%; 0.12% and 0.1%), either diluted or diluted 1:1 with sterile water, on *C. albicans* standard strain MTCC 183. All the examined mouthrinses demonstrated activity against *C. albicans*: their mean inhibitory zones ranged from 22.6 mm to 26.8 mm, irrespective of CHX concentration. In the present study, the mean

inhibitory zones ranged from 10 mm to 30 mm, but they were dependent on CHX and mouthrinse concentration.

Talebi et al. [7] evaluated the antifungal activities of 16 mouthrinses, including Oral B, Sensodyne, CHX and Colgate against standard strain of *C. albicans* PTCC 5027 using the agar diffusion method. The largest inhibition zone was found for Oral B (23.25 mm) followed by Sensodyne (19.87 mm) and CHX (14.21 mm); the strain was resistant to Colgate. The MIC was 0.015 mg/L for CHX, 0.007 mg/L for Sensodyne, and 0.003 mg/L for Oral B.

Our present findings found Oral B to have a weaker effect (13 mm), but CHX and Colgate were stronger (20.9 mm); the MIC values for these mouthrinses were higher than those found by Talebi et al. [7]. In contrast, a similar *in vitro* study based on the agar dilution technique by Eick et al. [8] examined the susceptibility of *C. albicans* ATCC 76615 to CHX as well as to various commercially-available mouthrinses containing 0.06 to 1% of chlorhexidine. The MIC of CHX against a standard strain of *C. albicans* was found to be 0.5%.

In study conducted by Prasanth et al. [9] eight mouthrinses were tested against *C. albicans* MTCC 854 standard strain using the agar diffusion assay. Four mouthrinses had 0.2% CHX, while two contained 0.03% Triclosan and another two potassium nitrate and sodium fluoride. The highest growth inhibitory zones were for Triclosan (27.67 mm and 23.67 mm, for the two mouthwashes), with much lower areas being observed for CHX (14.67–18.0 mm). The mouthrinses containing potassium nitrate and sodium fluoride had no antifungal activity. The activity of CHX was found to be stronger in our present study than in these investigations.

Rohrer et al. [10] compared the antifungal activity of octenidol with that of chlorhexidine against *C. albicans* ATCC 90028 strain using the agar diffusion assay. The results were similar for the two examined mouthrinses, with the mean inhibitory zones being 16.44 mm for octenidol and 20.9 mm for CHX.

A similar study by Mruthyuenjaya et al. [11] examined the antimycotic efficacy of Listerine, Colgate Plax, Freshclor, Guard-OR, Amflor, Rexidin Plus and CHX against *C. albicans* ATCC 10231. The greatest inhibition of *C. albicans* growth was demonstrated by CHX and Listerine at a dilution of 1:16, with inhibition zones of respectively 22.7 mm and 23.1 mm; Colgate Plax and Fresclor had no effect. It should be noted that Listerine did not demonstrate any antifungal activity in the present study.

Fu et al. [12] investigated the antifungal ability of seven mouthrinses, including Listerine, Colgate Plax, Crest, CHX and fluconazole against *C. albicans* ATCC90028 and *C. krusei* ATCC6258. An antifungal susceptibility test was carried out according to CLSI M44-A (disk diffusion test) and CLSI M-27A (broth microdilution method). For *C. albicans* ATCC90028, CHX was found to be the most effective mouthwash (disk diffusion testing) with an inhibition zone diameter of 18.6 mm, which is similar to our present findings, followed by CPC, Colgate (13.4 mm), Crest, PV-I and Listerine (8.0 mm); the inhibition zone was 30.1 mm for fluconazole. For *C. krusei* ATCC6258, CPC was the most effective mouthwash (19 mm) followed by Colgate (18.2 mm) and CHX (14.8 mm). In the broth microdilution method, both strains were susceptible to all of the mouthrinses tested.

Radwan-Oczko et al. [13] evaluated the activity of Dentosept A against six standard strains (*C. albicans* ATCC 10231, *C. albicans* ATCC 90028, *C. glabrata* ATCC 66032, *C. krusei* ATCC 14234, *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 750) using the dilution technique for the determination of MIC. Dentosept A was found to be very effective against fungi, which was in accordance with our present findings, which showed that the average zone of inhibition ranged from 20 to 28.78 mm in the case of undiluted Dentosept A, depending on the species of fungus.

Bugno et al. [14] examined the influence of CHX on *C. albicans* ATCC 10231. The study used a linear regression method to evaluate microbial reduction obtained as a function of the exposure time, considering a 99.999% reduction in the count of the stabarized microbial population within 60 s as an indicator of effectiveness; they found that a 0.12% solution of CHX had very strong activity against *C. albicans* ATCC 10231.

Meiller et al. [15] compare the efficacy of 0.2% CHX and Listerine Antiseptic, Tatar Control Listerine Antiseptic and Peridex mouthrinses against three isolates of *Candida* ATCC species and one NCPF. The susceptibility of isolates to mouthrinses was determined by broth macrodilution according to the National Committee for Clinical Laboratory Standards. All mouthrinses were effective against the fungal species at commercially-

available concentrations. Unfortunately, the authors do not provide detailed data concerning the examined strains.

The differences observed between the studies can be explained by the use of different strains of fungi: although they are standard strains, they have different origins and properties. It should be underlined that the tests were conducted *in vitro*; therefore, the results concerning antimycotic efficacy cannot be directly translated into clinical effectiveness.

In conclusions, some of the tested mouthrinses have antimycotic properties at commercially available concentrations. In spite of the fact that chlorhexidine is thought to be the gold standard for mouthrinse use, Dentosept has stronger antimycotic activity and acts on a wider spectrum of fungi species. Chlorhexidine and Colgate do not appear to act against *C. tropicalis*, neither does Curaspet against *C. dubliniensis;* therefore, the determination of the fungus species is necessary.

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