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Activity of Dentosept on Yeastlike Fungi Responsible for Infections of Oral Cavity

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Abstract

The aim of this study was to determine the susceptibility to Dentosept (Phytopharm, Klęka) of the yeastlike fungi isolated from oral cavity infections. Investigations included 128 strains of fungi from genus Candida (105 strains), Geotrichum (16), Rhodotorula (5) and Saccharomyces (2). The susceptibility (MIC) of fungi was determined by means of a plate dilution technique in Sabouraud's agar. Obtained results revealed that Dentosept was the most active towards the strains of Candida mesenterica and C.parapsilosis (MIC $\leq$ 5,0 mg/ml) and the least active towards strains of C.dubliniensis, C.guilliermondii and C.krusei (MIC 15,0 – $\geq$ 20,0 mg/ml).

Keywords: infections, oral cavity, yeastlike fungi, Dentosept

Introduction

The yeastlike fungi occur in the oral cavity of about 40-60% of population [1-5]. They belong to opportunistic microorganisms. Most often they cause endogenous infections. The infection is initiated with colonization connected with the ability of yeastlike fungi to adhere to mucous membrane of oral cavity. Specially yeasts easily adhere to surfaces covered with saliva. They can also adhere to some bacteria (coaggregation) occuring in the oral cavity, like: Actinomyces viscosus, Fusobacterium nucleatum, Lactobacillus salivarius, Porphyromonas gingivalis, Staphylococcus epidermidis, Streptococcus gordonii, Streptococcus mitis, Streptococcus mutans, Streptococcus salivarius, Streptococcus oralis and Streptococcus pyogenes [6]. The pathogenicity of yeastlike fungi from genera Candida is connected with its dimorphic character, which means the ability to produce 2 forms: a yeastlike form (blastospores) and a hyphae formation. The results of the studies indicate that the hyphae formation of fungus is more pathogenic than blastospores [2, 4]. It was also documented that the hyphae formation can penetrate into tissues easier, and it has proteins present on its surface binding with components C_3 of complement: C_3b and C_3d [4, 7]. The important factor of virulence is molecular mimicking, which helps to pass over the host's immune system [6]. Additionally, Candida albicans species, produce a toxine called candidotoxine. The pathogenicity of fungi from genera Candida is specially conditioned with proteinases production that participate in the adhesion and colonization of host cells and are connected with tissue invasion and destruc-

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tion [8-11]. Moreover, fungi produce various hydrolitic enzymes, among which are lipases, proteases, phosphomannoesterases, and asparagin proteinases [2, 12-17]. The most important enzymes seem to be phospholipases and aspartyl proteinases [13, 14]. In laboratory experiments it was confirmed that phospholipases contribute to pathogenicity through the destruction of the host's epithelial cells which allows fungi to invade tissues. Aspartyl proteinases enhance the destruction of proteins: such as collagen, keratin, fibronectin, laminin, mucin and some components of cytokines and antibodies [15, 16].

It was proven experimentally, that Candida albicans species, which exhibit high phospholipases activity adhere with ease to epithelial cells beeing more pathogenic to man [18, 19]. It was found, that the ending of hyphae exhibited the highest activity of phospholipases enabeling tissue penetration of fungi [13, 21]. Samaranayake at al. [20] revealed, that progression of Candida albicans, Candida tropicalis, Candida krusei and Candida glabrata species has an influence on pH of saliva. In their study, the fungi species mentioned above, decreased pH values of saliva from 7.5 to 3.3 in 72 hours. Such conditions are favourable to activity of hydrolitic enzymes produced by fungi, with aspartyl proteinase among them [20]. The study conducted by Kurnatowska et al. [19] revealed, that the highest activity of aspartyl proteinase was characteristic of species Candida albicans, Candida tropicalis and Candida krusei, which were isolated from patients with leucoplakia and juvenile periodontitis.

The yeastlike fungi ferment different carbohydrates. Candida albicans species ferment, among other, glucose, galactose, maltose and saccharose with pyruvic and acetic acids production, which leads to pH reduction. The diet containing carbohydrates contributes to the development of yeastlike fungi in the oral cavity.

The saliva contains different antimycotic agents among which is lysozyme, lactoferrin, lactoperoxidase and proteins containing histidine, called histamines. The in vitro study revealed antimycotic activity of histatin. It also revealed that this activity is 50-200 times stronger as compared with lysozyme [22-24]. Another important factor of fungal virulence is the biofilm. It is produced on surfaces and facilitates adhesion of microorganisms. It also makes fungi difficult to achieve for antifungal disinfectants and weakens the natural host's defence system [25-29]. The fungi from genera Candida producing biofilm are a causative agent of prosthetic stomatitis [30]. Moreover yeastlike fungi are present in dental plaque which can be its reservoir in oral cavity causing the reccurence of infections [24, 26, 28, 31]. Fungi from genera Candida can be also present in root canals, carious lesions, and the periapical surface of teeth. This too, was confirmed in microbiological, histological and electron-microscopy studies [31]. They often occur in pathologic pockets of patients with periodontal diseases [31]. Some authors consider carious lesions and periodontal pockets as an important ecological niche from where yeastlike fungi can spread causing infections of various organs (lungs, alimentary canal, genital duct or skin) [32, 33]. Factors predisposing to infections caused by yeastlike fungi are states of decreased self defence system (also AIDS), carcinoma, age (infants and people over 70 years old), pregnancy, cytostatic and steroid therapy, transplantation, radiotherapy, antibiotic therapy, diet reach with carbohydrates, diabetes, tuberculosis, endocrinal disorders, invasive diagnostic procedures [2, 24, 27, 34, 35]. The progression of candidiosis is favoured by: periodontal diseases, carious lesions, xerostomy, using dentures and wrong hygiene of oral cavity (dentures) [34, 36]. It is known that the number of species resistant to antimicotic agents is still growing up. Therefore, new fungicidal disinfectants, especially of herbal origin, are still in demand. One of such preparation is Dentosept (Phytopharm, Klęka). It is a multicomponent preparation containing the following liquid extracts: Matricaria chamomile (flavers), Quercus robur (cortex), Salvia officinalis (leaves), Arnicae montana (plant) – each 13,0 g; Acorus calamus (rhizome), Mentha piperita (plant), Thymi vulgaris (plant) – each 6,5 g. Contents of essential oils is > 0,25% and tanning agents > 1,5 g. This preparation reveals antiseptic and antiphlogistic effects. Its most important ingredients are tanning agents, flavonoids, essential oils (particularly thymol and menthol). Dentosept is used for irrygation of oral cavities in periodontal diseases, inflammations and ulcerations of mucous membrane, tongue and candidiosis.

Materials and Methods

Materials were obtained by swabbing with sterile swabs from oral cavity of 116 patients with various infections. Among these were infections of the oral mucous membrane (23 persons), the tongue (15 persons), gingiva (17 persons), dental pulp (32 persons) and periodontal disease (29 persons). Each swab was placed in a sterile containment tube and was moistened with a sterile salt solution. All containment tubes were transported within 1-2 hrs of the sampling from the place of collection to the microbiology laboratory. The specimens were streaked directly on Sabouraud's agar. The incubation was performed aerobically for 24-48 hrs at 37°C. All yeast isolates were cultured on CHROMagar Candida from tentative identification. The individual platers were then incubated for 48 hrs at 37°C in anaerobic conditions. The fungi species were identified with the use of Gram-staining smears, by assessing colony morphology, the germ tube test in normal human serum and a commercially available yeast identification system API 20C AUX (bio Mericux).

Table 1. Susceptibility of 128 strains yeastlike fungi to Dentosept.

Yeastlike fungi	No. of strains	MIC (mg/ml)					
		>20,0	20,0	15,0	10,0	5,0	<5,0
Candida albicans	37	8	7	9	5	4	4
Candida dubliniensis	7	7					
Candida glabrata	12	4	3	1	2	2	
Candida guilliermondii	2	1	1	1			
Candida kefyr	5	2			1	1	1
Candida krusei	8	3	5				
Candida mesenterica	3				1	1	1
Candida parapsilosis	10			2	2	2	4
Candida tropicalis	21	6	8	4		1	2
Candida total	105	31	23	17	11	11	12
Geotrichum candidum	16	2	4	1	1	2	6
Rhodotorula rubra	5		1		2	1	1
Saccharomyces cerevisiae	2					1	1
Total	23	2	5	1	3	4	8
All total	128	33	28	18	14	15	20

Table 2. Susceptibility of standard strains to Dentosept.

Yeastlike fungi	No. of strains	MIC (mg/ml)					
		>20,0	20,0	15,0	10,0	5,0	<5,0
Candida albicans PCM 1490 PZH	1			1			
Candida albicans ATCC90028	1		1				

The following yestlike fungi strains were tested: Candida albicans (37 strains), Candida dubliniensis (7 strains), Candida glabrata (12 strains), Candida guilliermondii (2 strains), Candida kefyr (5 strains), Candida krusei (8 strains), Candida mesenterica (3 strains), Candida parapsilosis (10 strains), Candida tropicalis (21 strains), Geotrichum candidum (16 strains), Rhodotorula rubra (5 strains), Saccharomyces cerevisiae (2 strains) and 2 reference strains: Candida albicans PCM 1409 PZH and Candida albicans ATCC 90028. The susceptibility (MIC) of fungi was determined by means of a plate dilution technique in the agar. Dentosept was dissolved in sterille distilled water (immediately before the experiment) to adequate concentrations. The following concentrations of herbal preparation were used: 20,0; 15,0; 10,0; 5,0 mg/ml. Adequate concentrations were added to Sabouraud's agar. The agar plate without Dentosept was the control for the experiment. Inoculum containing 10^6 CFU (colony forming units) per spot were seeded with Steers replicator upon the surface of the agar. Incubation was performed under the aerobic conditions at 37°C. The MIC was interpreted as the lowest concentration of Dentosept that inhibited growth of the yeastlike fungi strains.

Results

The results of the investigations indicated that the strains belonging to species of Candida mesenterica and Candida parapsilosis were the most susceptible to Dentosept. The MIC's in concentrations of ≤ 5,0 mg/ml inhibited growth of 67% and 60% of the strains respectively. The strains of Candida kefyr species showed the lower susceptibility. Within the range of ≤ 5,0-10,0 mg/ml, Dentosept inhibited growth of 60% of the strains of the species. The remaining strains were less susceptible (MIC in ranges 10,0 – ≥ 20,0 mg/ml). The least susceptible were the strains from Candida glabrata and Candida tropicalis species. The growth of only 33% and 14% respectively of the strains responsible were inhibited by ≤ 5,0-10,0 mg/ml. The yeastlike fungi strains of Candida albicans species, the most commonly present within the oral cavity and responsible for candidiasis therein occured less susceptible (MIC ≤ 5,0 mg/ml, 22% of susceptible strains). Dentosept in concentrations 10,0-15,0 mg/ml inhibited the growth of 38% of the strains, and 20,0 mg/ml inhibited the growth of the next 19% of the strains. However, about 22% of Candida albicans strains

were not susceptible to the concentrations (MIC > 20,0 mg/ml) tested.

Dentosept showed the lowest activity within the strains from species of Candida guilliermondii (MIC in ranges 15,0 – > 20 mg/ml), Candida krusei (MIC ≥ 20,0 mg/ml) and Candida dubliniensis (MIC > 20,0 mg/ml).

The fungi strains of Saccharomyces genus were more susceptible. The growth of all strains was inhibited by concentrations ≤ 5,0 mg/ml. The strains belonging to the Geotrichum and Rhodotorula genus were less sensitive to this drug. The low concentrations within the range of ? 5,0 mg/ml inhibited the growth of 40% and 50% strains out of all yeastlike fungi examined.

The antifungal properties of essentials oils and their active components which are present in Dentosept have been reported by numerous authors [37-42]. Pauli [39] showed that α-bisabolol from essentials oils of chamomile revealed growth inhibitory properties towards yeast and filamentous fungi as well as destruction of the strains of Candida albicans and Saccharomyces cerevisiae species.

Other authors [43] showed that Candida albicans strains was sensitive to 0,1 mg/ml of α-bisabolol. Yousef et al. [37] showed that the growth of Candida albicans strains was inhibited by Chamomile oil by 50 mg/l, and Peppermint oil by 0,78 mg/l. Kalemba et al. [38] described that Candida albicans strain was sensitive to thymol (MIC = 50 μg/g, thyme oil (MIC = 5 mg/ml) and chamazulene (from Chamomile oil) (MIC = 500 μg/ml). However the strains of Candida utilis species were susceptible to concentration = 5 ppm majoram oil [41].

Conclusions

It should be emphasized that from all 105 strains of Candida genu low concentrations of Dentosept (≤ 5,0 mg/ml) inhibited the growth of 22% strains, and 22% of strains were not susceptible to the tested concentrations within the ranges of 5,0 – 20,0 mg/ml (MIC > 20,0 mg/ml).

1. Most susceptible to Dentosept were the strains of Candida mesenterica and Candida parapsilosis.

2. Yeastlike fungi strains of Candida albicans, most commonly present in infections of the oral cavity occured less susceptible.

3. Dentosept was least active towards the strains of Candida dubliniensis, Candida guilliermondii and Candida krusei.

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