The use of essential oils based antiseptic solution in the treatment of denture stomatitis

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SUMMARY

Introduction Local therapy of denture stomatitis (DS) associated with Candida species fungi infection usually involves the application of nystatin and miconazole. Due to the fact that these drugs may be less efficient against biofilm and possible resistance development, a new approach in the treatment includes the use of antiseptic agents.

The aim of the study was to compare clinical and microbiological therapeutic outcomes of antiseptic solution Listerine® and Daktanol® antifungal oral gel in the treatment of DS associated with Candida species fungi.

Material and Methods The study included 30 patients affected by DS, divided into the two treatment groups, control group (n=15) treated by Daktanol® gel and experimental group (n=15) treated by the antiseptic solution Listerine®. Successful treatment was evaluated based on palatal mucosa inflammation reduction classified according to the Newton classification and the difference in the number of fungal colony- forming units (CFU) isolated by smears before and after the treatment that lasted 14 days.

Results Reduction in inflammation intensity and fungal CFU number on palatal mucosa (p<0.01) as well as on denture base (p<0.01) were observed in both groups of subjects after the treatment.

Conclusion Antiseptic solution Listerine® and Daktanol® antifungal gel both reduced palatal mucosal inflammation and CFU number of fungi in mouth without significant differences among them. CFU number of fungi isolated from denture base was significantly lower after the treatment with Listerine® (p<0.05).

Keywords: Candida albicans; denture stomatitis; Listerine, Daktanol gel

INTRODUCTION

Denture stomatitis (DS) is an inflammation of oral mucosa covered by the denture base with reported incidence of 15-70% [1]. It is linked to a number of non-infectious aetiologies such as poor quality of dentures, poor hygiene and nocturnal denture wearing as well as infectious etiological factor, Candida species fungi [2]. Number of fungal cells in the saliva and on dentures is leading etiological factor for DS development [3]. The degree of Candida albicans (C.albicans) denture base contamination is directly correlated to inflammation intensity [4].

This disease is characterized by erythema of oral mucosa covered by the denture base; it is usually asymptomatic and often associated with angular cheilitis [5]. Its intensity is clinically evaluated according to the Newton classification criteria [6,7]. DS treatment most frequently includes local and systemic administration of antifungals, reduction and eradication of biofilm, change of bad habits related to nocturnal denture wearing and poor denture hygiene as well as replacement of inadequate dentures. The most frequently used local antifungal drugs in dental practice are nystatin and miconazole [8,9]. Although effectively act against fungi, these drugs have no effect on biofilm matrix, therefore well-protected pathogens can survive in extracellular matrix. Also, their use comes with the risk of resistance development [10, 11]. Commercially available oral antiseptic Listerine® acts directly against fungal cell, chemically, causing damage to the cell wall structure and membrane permeability. Also, it disrupts metabolic processes dependant on microorganism membrane enzymes, and, as other phenolic products, exerts anti-inflammatory effect [12]. Listerine® acts against free unbound Candida cells as well as against formed biofilm and it is shown to be more efficient than the azole antifungal drugs [13]. This Listerine® solution property has not been studied in a clinical trial yet.

The aim of this study was to compare clinical and microbiological outcomes of antiseptic agent Listerine® and antifungal Daktanol® gel in the treatment of DS associated with Candida species fungi.

MATERIAL AND METHODS

This prospective clinical study was conducted at the Department of Dental Medicine in Foca and the Microbiological laboratory of the University Hospital Foca. The
research was conducted in accordance with the principles of the Helsinki Declaration of 2008. Prior to inclusion in the study subjects were informed about the aim and the protocol of research and gave their consent to participation. The study included 30 patients, maxillary acrylic dentures wearers, affected by DS. Criteria for inclusion of patients in the study were good general health, or chronic well-controlled disease and dental acrylic dentures wearing for at least one year. Also, swabs of palatal mucosa and denture basal surface had to be positive on *Candida* species fungi. Exclusion criteria were as follows: the use of corticosteroids and immunosuppressive therapy, tumours of the maxillofacial region and radiotherapy in the head and neck area, chemotherapy in the last year, blood disorders, local surgical therapy in the last 3 months, local or systemic use of antibiotics and antifungal drugs in the last 3 months, the use of hormones for therapeutic purposes, the existence of other diseases in the oral cavity and also functionally, prophylactically and cosmetically inadequate dentures that are indicated for replacement with new dentures.

By the means of random numbers table, participants who joined the study under even-number were classified into the control group and received standard treatment with *Daktanol* oral gel (2% miconazole, Galenika a.d. Belgrade, Serbia). The participants included in the study under odd-number were classified into the experimental group and were treated with antiseptic agent *Listerine* cool mint™ (Johnson&Johnson, S.p.A. Rome, Italy). Each group consisted of 15 patients, and therapy was administered according to the following protocol:

1. Control group of patients (n = 15) received standard local antifungal therapy in the form of *Daktanol* gel containing 2% miconazole, used at the dose of 1-2 teaspoons, 4 times per day for 14 days. Basal surface of dentures were coated with gel overnight and rinsed with water in the morning prior to use.

2. Experimental group of patients (n = 15) used an antiseptic agent *Listerine* also for 14 days as follows: 4 times a day 1 minute long mouth swish with antiseptic solution that was spitted afterward. Dentures were immersed in this solution overnight with base facing upward in a glass container, and completely overflowed by the solution. In the morning dentures were rinsed with water.

Parameters used for the evaluation of inflammation and denture contamination, as well as the presence of expected improvement were clinical and microbiological. Clinical improvement implied palatal mucosa inflammation intensity reduction after the treatment compared to the inflammation intensity prior to the therapy.

### 1. Clinical parameters

The intensity of palatal mucosa inflammation was assessed by Newton classification which distinguishes three clinical types of denture stomatitis [6,7]: type *Newton I*: dotted hyperaemic shape and size of a pinhead lesion (pin-point) that present localized areas of poorly expressed inflammation; type *Newton II*: diffuse erythema of palatal mucosa covered by the denture base – generalized inflammation; type *Newton III*: granular surface of the mucosa - inflammatory papillary hyperplasia.

### 2. Microbiological parameters

Swabs of palatal mucosa surfaces and denture bases were taken without prior mouth and dentures rinsing, in the morning, before any food intake. Sabouraud dextrose agar was used to grow fungi and it was incubated at 37 °C, for 48 hours. The number of fungal colonies (CFU - colony forming units) was counted after 48 hours. The following criteria were used for CFU quantification: <10 colonies present after incubation – smear is negative; 10-25 colonies present after incubation – fungi present in small numbers; >25 colonies present after incubation – fungi are present in large numbers [14]. Two days after the treatments, control examinations were conducted and swabs for samples cultivation were taken again.

The obtained data were statistically analyzed in the SPSS software (SPSS for Windows, version 11.5, Chicago, Ill.). Description of the sample was carried out by descriptive statistics methods. Therapeutic results within a group (paired samples) were evaluated by the Wilcoxon Signed Rank test. The effect of the therapy on the clinical improvement was assessed using the Fisher's Exact Test, and therapeutic results between observed groups were compared using χ² test. The relationship between certain characteristics and inflammation intensity as well as therapy outcomes were estimated using χ² test and Fisher's Exact Test. Results are presented in tables.

### RESULTS

The study included 30 patients, 19 (63.3%) female and 11 (36.7%) male, with an average age of 56.1 years (SD = 7.126). The largest number of dentures, 17 (56.7%), was between 5 and 10 years old, 9 dentures (30%) were aged over 10 years, while 4 dentures (13.3%) were used less than 5 years. Most of patients, 27 (90%) had clinically present denture stomatitis classified as type II *Newton*, while 3 patients (10%) had denture stomatitis *Newton* type III.

In both groups, significant reduction in the CFU number at the palate (p <0.01) as well as at the denture base was observed after the treatments (p <0.01) (Table 1). Inflammation intensity reduction was observed in most patients, but significant difference after treatments between the two applied therapeutic modalities was not observed (Table 2). There was no significant difference in palate smear CFU number reduction in relation to the applied therapy, but significant difference (p <0.05) was observed in denture smear CFU number reduction in patients treated with antiseptic agent *Listerine* compared to patients treated by *Daktanol* oral gel (Table 3).

Gender, age of patients or age of dentures, had no statistically significant effect on clinical improvement after the treatment, as well as on the reduction in the CFU numbers isolated from palatal mucosa and denture basal surface after the treatment (Table 4).
Table 1. Reduction of isolated fungal colony forming unit (CFU) number after the treatment

Tabela 1. Smanjenje broja izolovanih gljivih kolonija nakon za-vršene terapije

| CFU number before and after the treatment | Listerine* | Daktanol* |
|------------------------------------------|-----------|-----------|
| Broj CFU pre i posle terapije            | Palate    | Denture base | Palate | Denture base |
| Increased CFU number                     | 0         | 0          | 0      | 0           |
| Decreased CFU number                     | 15        | 15         | 14     | 15          |
| Unchanged CFU number                     | 0         | 0          | 1      | 0           |
| \( \chi^2 = 6.136 \); df = 1; p = 0.013 |           |            |        |             |

Paired sample, Wilcoxon Signed Rank test
Vezani uzorak, Viličkovo tisto eksperimentalnih parova

Table 2. The influence of applied treatments on the clinical improvement

Tabela 2. Eфикасност применијеног тремтана на клинико побољшанje

| Clinical improvement | Applied therapy | Total Ukupno (n; %) |
|----------------------|-----------------|---------------------|
| Kliničko poboljšanje | Daktanol*       | Listerine*          |
| Yes                  | 11; 60.0        | 13; 86.7            | 24; 80.0 |
| Da                   | 11; 60.0        | 13; 86.7            | 24; 80.0 |
| Ne                   | 9; 60.0         | 12; 80.0            | 21; 70.0 |
| Total                | 20; 100.0       | 25; 100.0           | 45; 100  |

Fisher’s Exact Test =0.241
Fisherov test tačne verovatnoće =0.241

Table 3. Reduction of fungal colonies number isolated from palate and denture base after the treatment

Tabela 3. Сmanjenje broja gljivih kolonija izolovanih sa nepca i baze proteze nakon terapije

| Reduction of the number of fungal colonies | Applied treatment | Total Ukupno (n; %) |
|-------------------------------------------|------------------|---------------------|
| Smanjenje broja gljivih kolonija          | Daktanol*        | Listerine*          |
| Palate* Nepece*                           | 9; 60.0          | 12; 80.0            | 21; 70.0 |
| Partially Delimično                      | 5; 33.3          | 3; 20.0             | 8; 26.7  |
| No                                        | 1; 6.7           | 0; 0.0              | 1; 33.3  |
| Total                                     | 15; 100.0        | 15; 100.0           | 30; 100.0 |

Palate* Nepece* = 3.929; df = 2; p = 0.381
** \( \chi^2 = 6.136 \); df = 1; p = 0.013

Table 4. Influence of gender, age of respondents and age of dentures on the treatment outcome

Tabela 4. Uticaj pola, starosti ispitanika i starosti proteza na ishod terapije

| Clinical improvement | Reduction of CFU in palate smear | Reduction of CFU in denture base smear |
|----------------------|----------------------------------|---------------------------------------|
| Kliničko poboljšanje | Smanjenje CFU izolovanih u brisu nepca | Smanjenje CFU izolovanih u brisu proteza |
| Gender               | Fisher’s Exact Test =0.417        | \( \chi^2 = 0.695 \); p = 0.706         |
| Pol                  |                                 | \( \chi^2 = 8.504 \); p = 0.203         |
| Age of respondents   | \( \chi^2 = 6.622 \); p = 0.157   | \( \chi^2 = 4.451 \); p = 0.348         |

DISCUSSION

The present study evaluated the effect of oral antiseptic Listerine* on palatal mucosa inflammation and Candida species CFU number isolated from palatal mucosa and denture base among denture wearers affected with DS.

Literature review couldn’t identify other similar clinical studies. Yet, there are indirect evidences of Listerine* antifungal properties that justify its use in DS treatment. Meiller et al. conducted an in vitro study in which they observed the effect of Listerine* on clinically isolated Candida species, British and American strains of the same species. Fungal cells were incorporated into an experimental biofilm. Authors reported that after 60 seconds of experimental biofilm exposure to this antiseptic, no living fungal cells were observed in the sample [15]. In a study conducted with clinically isolated Candida species, Listerine* showed very good antimicrobial activity in laboratory testing. After 60 minutes, there was no living cell of Candida species in the sample [16]. Listerine* was also efficient against experimental biofilm composed of one laboratory and 34 clinically isolated C. albicans strains where it reduced metabolical activity of fungi for 75-80% [13]. The effect of Listerine* against C. albicans clinical isolates was confirmed in a recent study where Listerine*reduced C. albicans growth on Sabouraud dextrose agar [17]. The results presented in this study clearly confirmed findings of previous in vitro studies.

In the present study, Listerine* showed better efficacy in reducing the number of CFU isolated from denture basis compared to the Daktanol* gel. This finding could be the result of different viscosity of used agents. Miconazole was applied in a form of gel, what might hinder its denture base coating. However, due to its physical characteristics, Listerine* solution, could reach rugged, porous acrylic surface more easily. Beside therapy, improved hygiene and avoiding nocturnal dentures wearing could have positive impact on palatal mucosa inflammation reduction.

CONCLUSION

Therapeutic outcomes after the use of antiseptic agent Listerine* in DS treatment are similar to the therapeutic outcomes obtained by standard Daktanol* oral gel therapy. Therefore, Listerine* can be used in the treatment of DS associated with Candida species.
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Primena antiseptičnog sredstva na bazi esencijalnih ulja u terapiji proteznog stomatitisa

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Primena antiseptičnog sredstva na bazi esencijalnih ulja u terapiji proteznog stomatitisa

UVOD
Protezni stomatitis (PS) je zapaljeno stanje oralne sluzokože po-krivenebazomproteze,kojasamoguhišćunaobičnimost. Zbog osećenja neophodnosti, kao i zbog razljivosti, moguće rezistencije, parazitna je usmerava na terapijske efekte koji se mogu postići primenom antiseptičkih sredstava. Cilj rada je da se uporede klinički i mikrobiološki ishodi primene antiseptičkog sredstva u lečenju PS udruženog sa pojavom gljivica iz roda Candida.

Materijal i metode rada
Studija je uključivala 30 ispitanika obolelih od PS, podeljenih u dve terapijske grupe: kontrolnu (n = 15) lečenu gelom Daktanol® i eksperimentalnu (n = 15) – antiseptikom Listerine®. Uspešnost terapije procenjivana je na osnovu smanjenja intenzitetaprapkinje palatinalne sluzokože, preko početka lečenja i razlike u broju izolovanih gljivica iz roda Candida albicans. Rezultati: Kod obe grupe ispitanika došlo je do smanjenja intenziteta zapaljenja i smanjenja broja gljivica iz roda Candida albicans.

Zaključak
Cilj rada bio je da se uporede klinički i mikrobiološki ishodi primene antiseptičkog sredstva u lečenju PS udruženog sa pojavom gljivica iz roda Candida.

Materijal i metode rada
Ova prospektivna klinička studija sprovedena je na Klinici za stomatologiju Medicinskog fakulteta u Foči i u Mikrobiološkoj laboratoriji Univerzitetske bolnice Foča. Istraživanje je sprovedeno u skladu sa principima Helsinške deklaracije iz 2008. godine, a na kliničkih ispitnicama u obuhvat primenom gljivica iz roda Candida.

Kratki sadržaj
Uvod
Lokalna terapija proteznog stomatitisa (PS) udruženog sa infekcijom gljivica iz roda Candida najčešće se sprovodi upotrebom nistatina i mikonazola. Zbog osećanja neophodnosti, kao i zbog razljivosti, moguće rezistencije, pažnja se usmerava na terapijske efekte koji se mogu postići primenom antiseptičkih sredstava. Cilj rada je da se uporede klinički i mikrobiološki ishodi primene antiseptičkog sredstva Listerine® i oralnog gela sa antimikotskim dejstvom Daktanol® u lečenju PS udruženog sa pojavom gljivica iz roda Candida. Metode rada
Studija je uključivala 30 ispitanika obolelih od PS, podeljenih u dve terapijske grupe: kontrolnu (n = 15) lečenu gelom Daktanol® i eksperimentalnu (n = 15) – antiseptikom Listerine®. Uspešnost terapije procenjivana je na osnovu smanjenja intenzitetaprapkinje palatinalne sluzokože, preko početka lečenja i razlike u broju izolovanih gljivica iz roda Candida albicans. Rezultati: Kod obe grupe ispitanika došlo je do smanjenja intenziteta zapaljenja i smanjenja broja gljivica iz roda Candida albicans.

Zaključak
Cilj rada bio je da se uporede klinički i mikrobiološki ishodi primene antiseptičkog sredstva Listerine® i antimikotika Daktanol® u lečenju PS udruženog sa pojavom gljivica iz roda Candida.
Pomoću tablice slučajnih brojeva određeno je da se ispitanici koji su se u studiju priključili pod parnim rednim brojem srušili u kontrolnu grupu i primaju standardnu terapiju oralnim gelom Daktanol® (2% mikonazol, Galenika a.d. Beograd, Srbija). Ispitanici uključeni u studiju pod neparnim rednim brojem srušili su u eksperimentalnu grupu i primili su terapiju antiseptičnog sredstvom Listerine™ cool mint™ (Johnson & Johnson, S.P.A. Rim, Italija). Svaka grupa se sastojala od po 15 ispitanika, a terapija je primenjivana po sledećem protocolu:

1. kontrolna grupa ispitanika (n = 15) primala je standardnu lokalnu antifungalnu terapiju u vidu gela Daktanol®, koji sadrži 2% mikonazolu, u dozi od 1–2 kafene kašičice, četiri puta dnevno, tokom 14 dana. Proteze su se preko noći sa bazalne strane premazivale gelom, a ujutro ispirale vodom pre upotrebe.

2. eksperimentalna grupa ispitanika (n = 15) koristila je antiseptično sredstvo Listerine™ i to po sledećem režimu: četiri puta dnevno jednomomentno mućkanje antiseptičnog rastvora, koji se nakon tog vremena ispljune, a proteze su preko noći potapali u ovaj rastvor tako što su ih postavljali u čašu, bazom okrenutom ka gore i prelivali rastvorom Listerine™ do potpunog prekrivanja svih njenih površina. Ujutro su se proteze ispirale vodom, a terapija je takode trajala 14 dana.

Parametri na osnovu kojih se procenjavaju stepeni inflamacije i kontaminacije nadoknada, kao i naslage očekivano pojavljenih pošto je načina chelerte uzorak. Parametri na osnovu kojih se procenjavaju stepeni inflamacije i kontaminacije nadoknada, kao i naslage očekivano pojavljenih pošto je načina chelerte uzorak.

1. Klinički parametri

Intenzitet zapaljenja palatinalne sluzokože procenjavao se klasifikacijom Newton, prema kojoj razlikujemo tri klinička tipa proteznog stomatitisa [6, 7]: tip Newton I – tačkaste hiperepителиne lezije oblika i veličine glode koje predstavljaju lokalizovane područje slab izraženog zapaljenja; tip Newton II – difuzni eritem na dole izolovane sa bazalarne površine proteza nakon terapije u odnosu na intenzitet utvrđen pre terapije.

2. Mikrobiološki parametri

Uzimanje brisa inflamatorno promenjene nepčane sluzokože i bazne površine proteza vršeno je ujutro, natašte, bez prethodnog očekivanog polobljavanja, bili su klinički i mikrobiološki. Klinički polobljavanje podrazumevalo je smanjenje intenziteta zapaljenja sluzokože nakon terapije u odnosu na intenzitet utvrđen pre terapije.

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U studiju je bilo uključeno 30 ispitanika, 19 (63,3%) ženskog i 11 (36,7%) muškog pola, prosečne starosti 56,1 godine (SD = 7,126). Najveći broj proteza – 17 (56,7%) bio je star između pet i deset godina, devet proteza (30%) bile su stare više od deset godina, dok su četiri proteze (13,3%) korišćene kraće od pet godina. Najveći broj pacijenata – 27 (90%) imao je klinički izražen protezni stomatitis klasifikovan kao tip Newton II, dok su tri pacijenta (10%) imala protezni stomatitis tipa Newton III.

Kod obe grupe ispitanika došlo je do visoko značajnog smenanja broja CFU i na nepcu (p < 0,01) i na bazi proteze nakon terapije (p < 0,01) (Tabela 1).

Smanjenje intenziteta zapaljenja nastupilo je kod većine ispitanika, bez uočenog postojanja značajne razlike nakon terapije između dva primenjena terapijska modaliteta (Tabela 2). Nije pronađena značajna razlika u smanjenju broja CFU dobijenih brisom nepca u odnosu na primenjenu terapiju, ali je nađena značajna razlika (p < 0,05) u smanjenju broja CFU izolovanih brisom baze proteza kod pacijenata lečenih antiseptičnim sredstvom Listerine™ u odnosu na pacijente lečene oralnim gelom Daktanol™ (Tabela 3).

Pol i starost pacijenata, kao ni starost proteza, nisu imali statistički značajnog uticaja na smanjenje intenziteta zapaljenja nakon terapije, kao ni na smenanju broja CFU izolovanih sa palatinalne sluzokože i bazalnih površina proteza nakon terapije (Tabela 4).

DISKUSIJA

U našoj studiji procenjivan je uticaj oralnog antiseptika Listerine™ na stepen inflamacije palatinalne sluzokože i broj kolonija gljivica iz roda Candida na nepcu i proteznoj ploči kod nosilaca totalnih proteza obolelih od proteznog stomatitisa.

Druge kliničke studije koje su koristile isti tretman, po istom ili sličnom protokolu kao u našem istraživanju, nisu nađene pregledom literature. Ipak, postoje posređeni dokazi o antiinflamatornom dejstvu ovog sredstva koji opravdavaju njegovu primenu u terapiji proteznog stomatitisa. Meiller i saradnici su sprovedeni u studiju u kojoj je proučavano dejstvo antiseptika Listerine™ na kliničke izolate iz roda Candida i britanske i američke laboratorijske oseove istog roda. Posmatrajući antimikrobno dejstvo na gljivice čelične u sastavu eksperimentalnog biofilma, utvrđeno je da nakon 60 sekundi izlaganja ovom antiseptiku više nije bilo živih gljivica čelična u uzorku [15]. U studiji u kojoj su uzorci mikroorganizama poticali od pacijenata sa Candida-pozitivnim brisom oralne sluzokože, Listerine™ je pokazao veoma dobru antimikrobnu aktivnost u in vitro uslovima. Nakon 60-minutnog izlaganja, u uzorku nije bilo živih gljivica C. albicans [16]. U studiji u kojoj je ispitivano dejstvo antiseptika Listerine™ na biofilm sastavljen od jednog laboratorijskog i 34 klinički izolovana soja vrste C. albicans, utvrđeno je da je Listerine™ re-
Dukovao postojeću metaboličku aktivnost gljivica za 75–80% [13]. Efekat sredstva Listerine® na kliničke izolate C. albicans u biofilmu laboratorijski je potvrdjen i u skorijem istraživanju, gde je Listerine® inhibisao rast C. albicans na saburo agaru [17]. Rezultati prikazani u našem istraživanju klinički su potvrđili nalaze navedenih in vitro studija, što se ogleda u smanjenju broja brisom izolovanih gljivicih kolonija sa nepca i baze proteza nakon terapije proteznog stomatitisa.

Listerine® je u našoj studiji pokazao veću efikasnost u smanjenju broja gljivicih kolonija izolovanih brisom baze proteza u odnosu na rezultat dobijen u kontrolnoj grupi. Na ovakav način je mogla uticati različita viskoznost primenjenih sredstava. Mikonazol je bio primenjen u formi gela, pa je samim tim teže oblagao površinu proteza. Listerine® je zbog fizičkih karakteristika rastvora lakše dopirao do neravnih, poroznih prostora i mikropukotina koje se prisutne na akrilnoj baznoj površini zabednih nadoknada, što je mogući uzrok pronađene razlike. Pored korišćene terapije, smanjenju infilmatije palatinale snuzokeže mogli su doprineti poboljšani higijenski režim usne dupla i proteza, kao i noćno nosenje proteza.

ZAKLJUČAK

Terapijski ishodi primene antiseptičkog sredstva Listerine® u lečenju proteznog stomatitisa slični su terapijskim ishodima dobijenim primenom standardne terapije oralnim gelom Dak-tanol®. Oralni antiseptik Listerine® može se koristiti u terapiji proteznog stomatitisa udruženog sa pojavom gljivica iz roda Candida kod nosilaca totalnih akrilnatih proteza.