Short Communication

Susceptibility of *Candida* Isolates from Denture-Related Stomatitis to Antifungal Agents In Vitro

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The aim of this study was to determine the susceptibility of *Candida* isolates obtained from patients with *Candida*-associated denture stomatitis to 4 antimycotics. Antifungal susceptibility was assayed using the ATB-Fungus-2INT test. A total of 120 *Candida* strains were identified: *C albicans* (59.2%), *C glabrata* (20%), *C tropicalis* (12.5%), and *C parapsilosis* (8.3%). Amphotericin B, 5-fluorocytosine, fluconazole, and itraconazole were effective against 100%, 98.6%, 88.7%, and 87.3% of *C albicans* and 79.6%, 77.6%, 71.4%, and 79.6% of the other *Candida* strains, respectively. The identification of candidal strains and determination of their susceptibility to antifungals may improve the management of *Candida*-associated denture stomatitis. *Int J Prosthodont 2007;20:504–506.*

Candida-associated denture stomatitis is a common disease, observed in 11% to 67% of complete denture wearers.[1] The etiology of the disease is multifactorial, but it is generally accepted that *Candida albicans* and non-*albicans* species play a major role in the pathogenesis of denture stomatitis.[2] Usually, treatment involves both topical antifungal therapy and improvement of oral and denture hygiene. Although new antifungals such as triazoles and echinocandins have significantly improved the treatment of oral candidiasis, therapeutic failure is frequently observed, and the identification of pathogens and susceptibility testing should precede antifungal therapy.[1] The aim of this study was to examine the susceptibility of *Candida* strains isolated from denture-related stomatitis to 4 antifungal agents: amphotericin B, 5-fluorocytosine, fluconazole, and itraconazole.

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Materials and Methods

A total of 120 *Candida* strains were isolated from patients with *Candida*-associated denture stomatitis. The strains were obtained from 105 patients (59 males and 46 females), aged 54 to 75 years, attending the Department of Prosthetic Dentistry, Poznan University of Medical Sciences, Poland, after informed consent was obtained under a protocol approved by the Bioethics Committee. All patients had worn acrylic resin complete dentures for more than 1 year. Apart from denture stomatitis, no other oral diseases were detected. Patients had not received any antibiotics, steroids, or immune therapy, and had not used an antiseptic mouthwash for the last 3 months before entering the study. Swabs, collected from areas of the palatal mucosa, were inoculated in Sabouraud's medium with chloramphenicol (bioMerieux). The yeasts were identified by a germ-tube formation test[3] and the carbohydrate assimilation patterns using the ID32 *Candida* identification kit.

The susceptibility to amphotericin B, 5-fluorocytosine, fluconazole, and itraconazole was examined using the ATB-Fungus-2INT test (bioMerieux), which permits testing under conditions similar to the reference method for microdilution. Antifungal activity was expressed as the minimum inhibitory concentration. The following resistance breakpoints were used according to the manufacturer's recommendations:

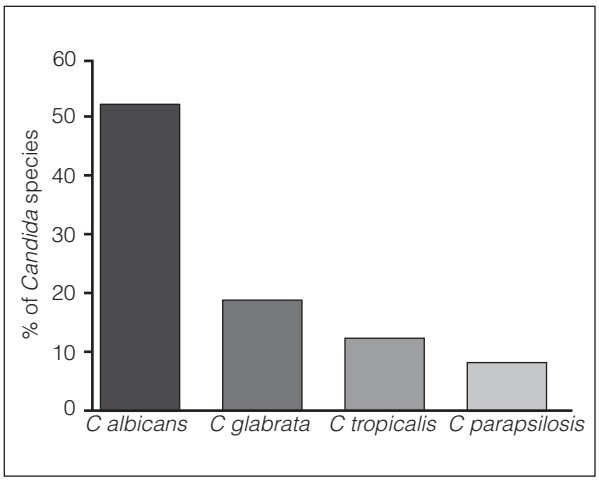

Fig 1 The identity of *Candida* species isolated from patients suffering from *Candida*-associated denture stomatitis given as the percentage of the particular *Candida* species.

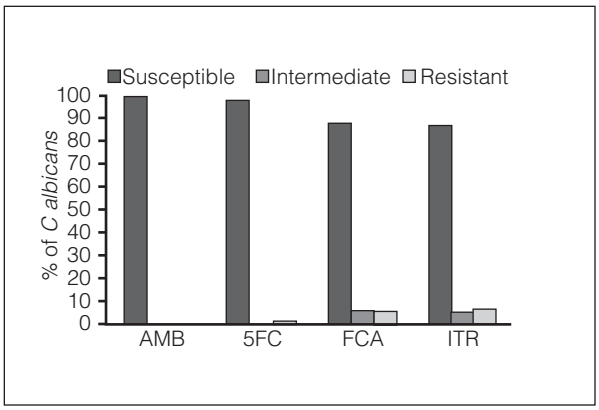

Fig 2 The in vitro susceptibility of *C albicans* isolates to antifungal agents given as the percentage of *C albicans* isolates that are susceptible or resistant (or with intermediate resistance) to the indicated antifungal agent. AMB = amphotericin B; 5FC = 5-fluorocytosine; FCA = fluconazole; ITR = itraconazole.

- Amphotericin B: resistant, ≥ 2 µg/mL
- 5-fluorocytosine: susceptible, ≤ 4 µg/mL; intermediate, susceptible dose dependent, 8 to16 µg/mL; resistant, ≥ 32 µg/mL
- Fluconazole: susceptible, ≤ 8 µg/mL; intermediate, susceptible dose dependent, 16 to 32 µg/mL; resistant, ≥ 64 µg/mL
- Itraconazole: susceptible, ≤ 0.125 µg/mL; intermediate, susceptible dose dependent, 0.25 to 0.5 µg/mL; resistant, ≥ 1 µg/mL

Results

The most prevalent yeast was *C albicans* (59.2%). Other species included *C glabrata* (20%), *C tropicalis* (12.5%), and *C parapsilosis* (8.3%) (Fig1). *C albicans* was most sensitive to all antifungal agents (Fig 2). All isolates were susceptible to amphotericin B and only 1 was resistant to 5-fluorocytosine. Four (5.6%) and 5 (7.0%) *C albicans* strains were resistant to fluconazole and itraconazole, respectively. The other 3 strains, *C glabrata*, *C tropicalis,* and *C parapsilosis*—designated as *Candida* spp—were more resistant to all tested antimycotics (Fig 3). Nevertheless, 79.6%, 77.6%, 71.4%, and 79.6% of *Candida* spp isolates were susceptible to amphotericin B, 5-fluorocytosine, fluconazole, and itraconazole, respectively. Five *Candida* spp strains (10.2%) were resistant to amphotericin B and itraconazole, 1 strain (2%) was resistant to 5-fluorocytosine, and 9 strains (18.4%) were resistant to fluconazole.

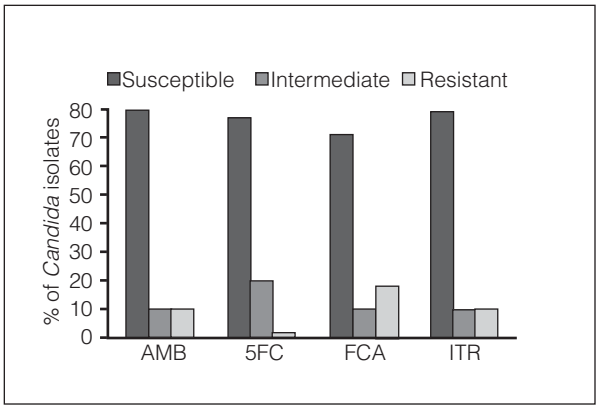

Fig 3 The in vitro susceptibility of *Candida* spp isolates to antifungal agents given as the percentage of *Candida* spp isolates that are susceptible or resistant (or with intermediate resistance) to the indicated antifungal agent. AMB = amphotericin B; 5FC = 5-fluorocytosine; FCA = fluconazole; ITR = itraconazole.

Discussion and Conclusions

The majority of isolates identified from patients with *Candida*-associated denture stomatitis represented *C albicans*, while the remaining strains included *C glabrata*, *C tropicalis*, and *C parapsilosis*. The results indicate that non-*albicans* species are involved in oral candidiasis, although *C albicans* is considered the most prevalent.[2] Amphotericin B and 5-fluorocytosine were effective against 100% and 98.6% of *C albicans* and 79.6% and 77.6% of *Candida* spp, respectively. The results confirmed infrequent resistance to amphotericin B, and high susceptibility to 5-fluorocytosine for *C albicans* strains in apical and marginal peridontitis.[4] The resistance to azoles was slightly higher: fluconazole and itraconazole were not effective against 5.6% and 7% of *C albicans* and 18.4% and 10.2 % of the other *Candida* strains, respectively. Nevertheless, the susceptibility to azoles was very high: 80% and 84.2% of all tested candidal strains were fully susceptible to fluconazole and itraconazole, respectively.

Overall, the results indicate that the high susceptibility to antifungals observed in vitro, particularly for *C albicans*, does not correlate with the outcomes of the in vivo antifungal therapy of *Candida*-related denture stomatitis. This may reflect the *(1)* multifactorial etiology of the disease, *(2)* complex interactions between *Candida* and the host, *(3)* formation of candidal biofilms on mucosal surfaces and dentures,[5] and *(4)* limitations of the in vitro susceptibility tests. Nevertheless, identification of the pathogen and determination of its in vitro susceptibility to antifungals should facilitate the choice of an effective drug and prevent the spreading of resistant fungal strains among the patient population. To obtain a permanent cure after antimycotic therapy of *Candida*-associated denture stomatitis and reduce the risk of relapse of the disease, the patient should be instructed in meticulous oral and denture hygiene and be advised to keep the dentures in a disinfectant solution overnight after treatment. Lack of denture cleanliness is one of the essential factors involved in the etiology of denture stomatitis, and the need to remove denture plaque, especially on the tissue-fitting surface of the denture, is an essential procedure in the therapy of the disease.

References

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Literature Abstract

Effect of implant support on distal extension removable partial dentures: In vitro assessment

The purpose of this study was to evaluate the effect of implants on the stability of mandibular distal extension removable partial dentures (RPDs). A commercial model of a partially edentulous mandible with 6 anterior teeth was modified by cutting 1- and 2-mm in height in the edentulous areas and replacing them with silicone impression materials as simulated soft tissues. The remaining 6 teeth had an artificial periodontal membrane made with silicone impression material (Fit-Checker). Pressure sensors were placed in the first molars and premolars bilaterally and at the medio-lingual alveolar crest. Five cobalt-chromium RPDs were fabricated. Two implants were placed in the second molar regions bilaterally with healing caps. To simulate implant-supported RPDs (ISRPD), denture bases were fitted to the healing caps with autopolymerized resin. To simulate conventional RPDs (CRPD), sealing screws were placed with no connection to the denture base. A brass plate was attached to the occlusion rim of RPD. Loads up to 5 kg were applied on the plate. The displacement sensor and load cell were set up on the loading rod in the apparatus. The pressure at 5 soft tissue areas and the displacement of the RPD were measured simultaneously. All paired data were analyzed using the Wilcoxen signed rank test at a significance level of .05. The results indicated that for the 1-mm soft tissue, the pressure in the molar of the ISRPD was less than on the CRPD. For a 2-mm soft tissue, the pressure in the ISRPD was about half at the molar than in the CRPD. No statistical differences in pressure were noted at the premolar positions in both soft tissue thicknesses. The results also indicated that greater pressure was found on the 1-mm soft tissue compared with the 2-mm tissue for all areas except for the median. The displacement of the denture was smaller for the ISRPD for both soft tissues thicknesses than for the CRPD, with a difference of about 40 μm. In this study, only vertically applied load was evaluated. Any other potential movement of the denture base was not assessed. The results indicated that implants can minimize partial denture displacement and decrease pressure on the soft tissues in the molar regions. It would be interesting to evaluate the long-term implant survival rate when implants are used to support an RPD.

Ohkubo C, Kurihara D, Shimpo H, Suzuki Y, Kokubo Y, Hosoi T. *J Oral Rehabil* 2007;34:52–56. **References:** 23. **Reprints:** Chikahiro Ohkubo, Department of Removable Prosthodontics, Tsurumi University School of Dental Medicine, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-9501, Japan. E-mail: Okubo-c@tsurumi-u.ac.jp—*Beatrice Leung, University of Toronto, Toronto, ON*