Prevalence and Antifungal Susceptibility Profile of Oral *Candida* spp. Isolates from a Hospital in Slovakia

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Abstract: Oral fungal infections are a worldwide healthcare problem. Although *Candida albicans* is still the most common yeast involved in the infections of oral cavity, non-*Candida albicans Candida* species (NCACs) have been highly related to these infections, particularly in older, immunosuppressed or patients with long exposure to antimicrobial drugs. The goal of this work was to perform a quick epidemiological and mycological study on the oral samples collected from a laboratory of a hospital in Slovakia, for 60 days. The samples' identification was performed by Germ-tube formation test, CHROMID® *Candida,* Auxacolor 2, ID 32C automated method, and the antifungal susceptibility testing determined by E-test®. Results confirm that comparing with bacteria, yeasts still occur in the lower number, but there is a high rate of antifungal resistance (81.6%)—to, at least one drug—among the collected samples, particularly to azoles and 5′-FC, which is clinically noteworthy.

Keywords: *Candida albicans*; NCAC; *Candida* spp.; resistance; antifungals; oral infection

1. Introduction

Oral infections are a heavy economic burden, a worldwide priority, and urgent problem, that can lead to pain, tooth loss, disfigurement, and death [1–3]. Since the 1980s, the studies on oral infections by *Candida albicans* have increased due to the escalation in HIV infection and the acquired immune deficiency syndrome (AIDS) epidemic [4]. Recent global estimates indicate an incidence of 2 million cases of oral candidiasis (i.e. thrush) annually, which, with skin, hair, and nail fungal infections, is the highest among fungi diseases [5]. In fact, oral candidiasis (OC) has been described throughout human history, since the times of Hippocrates (460–370 bC, "a disease of the diseased"), mirroring the opportunistic pathogenic nature of *Candida* spp. [6]. Later, in 1838, the infection was first described as directly linked specifically to *Candida albicans*, by pediatrician Francois Veilleux [7]. However, recently, non-*Candida albicans Candida* species (NCACs), such as *Candida glabrata, Candida tropicalis*, and *Candida krusei* have been highly related to these infections [8,9], colonizing mostly (but not only) older patients [10]. Oral candidiasis is diagnosed through differential patterns of mucosal changes (e.g., erythematous, pseudomembranous, and curd-like plaques—biofilms) [11,12].

Like other fungal infections, OC occurs as a consequence of other health problems, such as cancer, HIV, tobacco smoking, denture wearing, chemotherapy, and corticosteroid therapies, linked to immunosuppression states. The risk factors for oral candida infection are complex, but these factors clearly influence oral *Candida* sp. carriage and the upsurge of

oral candidiasis [13–19]. For example, in patients with diabetes, there is a higher *Candida* spp. colonization rate than on non-diabetic subjects (60–80% vs. 27%) [20]. It is, however, important to note that the colonization rate does not always lead to disease, but it is a run-up to infection when host immunity is compromised [21,22]. Hence, an early precise diagnosis can grant a quick and efficient antifungal therapy [23]. Conversely, this is repeatedly overdue or unavailable leading to death or serious chronic illness, nonetheless, they are still overlooked by public health authorities while most mortalities from these disorders are, in fact, preventable [5,22].

Additionally, up-to-date reports indicate an escalation of antimicrobial resistance in oral microbial species (oral microbiome) [24–28], and mounting evidence also supports a major role in systemic extra-oral infections and inflammation (e.g., cardiovascular, neurological) [29–31]. Not differently, it has been reported that, particularly in older adults, the oral colonization by *Candida* spp. increases and is related to other morbidities (e.g., Crohn's disease) [32–35]. This is mostly true for NCACs, which present a reduced response to fluconazole, raising the risk of recalcitrant local and disseminated candidiasis [36,37].

To our knowledge, this is the first retrospective re-examination study, evaluating and characterizing the clinical isolates of *Candida* spp. collected in the Department of Clinical Microbiology, Nitra Faculty Hospital in Slovakia. The collection of the samples was performed over a period of 60 days in 2019, relating them to wards, gender, ages, and antifungal susceptibility profiles.

2. Materials and Methods

2.1. Collection and Identification of the Clinical Isolates

Samples and clinical data of patients were collected between April and May 2019, from the laboratory of the Department of Clinical Microbiology, from several wards of the Nitra Faculty Hospital in Slovakia. Various tests were performed to confirm the identification of the samples.

2.1.1. Germ-Tube Formation Test, CHROMID® Candida, Auxacolor 2 (Bio-Rad®) Test

All clinical isolates were first plated on Sabouraud dextrose agar (SDA, Thermo Fisher Oxoid, UK) for 24 h at 37 °C. Afterwards, germ-tube test was performed to differentiate between *Candida* spp. Colonies of the cultivated yeasts were suspended in sterile saline and adjusted to the density of McFarland 2. For the morphological examination, human serum was used. Two drops of the yeast suspension were applied to wells of microtiter plates with 30 μL of human serum. After incubation for 2–3 h at 37 °C, the number of the germ tube and the total number of the cells was calculated in Bürker counting chamber. Production of chlamydospores, blastospores, true hyphae, and pseudohyphae was compared.

Parallelly, CHROMID® *Candida* (bioMérieux®, Craponne, France) was used for presumptive identification of *Candida strains*, based on the pigmentation of the developing colonies due to different enzyme activity. The results were interpreted after 48 h of incubation at 30 °C, according to colony's color and manufacturer's instructions. Subsequently, for identification of the medically important isolated yeasts, the colorimetric sugar assimilation test Auxacolor 2 (Bio-Rad®, Hercules, CA, USA) served for accurate identification. The method was applied as described in the kit's guideline and results were then evaluated.

2.1.2. The ID 32C Automated Method

The cultures were also analyzed macroscopically and microscopically and confirmed by the ID 32C automated method—ATB Expression (bioMérieux®, Craponne, France). For this, a single-use disposable plastic strip was used; 32 wells contain substrates for 29 assimilation tests (carbohydrates, organic acids, and amino acids), one susceptibility test (cycloheximide), one colorimetric test (esculin), and negative control. Testing was performed according to the guideline of the manufacturer.

2.2. Antifungal Susceptibility Testing

For all the isolates, the antifungal susceptibility for fluconazole (Flu), voriconazole (Vcz), 5′-fluorouracil (5FC), and amphotericin B (AmB) (i.e., the most common antifungal drugs used to treat oral infections [38]) was determined by the agar-based E-test® (bioMérieux, Craponne, France), with RPMI-1640, 8.4 g/L (Roswell Park Memorial Institute; Sigma Chemical Co., St. Louis, MO, USA), 2% glucose (Thermo Fisher Oxoid, UK) and 1.5% agar (NEOGEN, Lansing, MI, USA) buffered with 0.165 M morpholinepropanesulfonic acid buffer (MOPS; pH = 7; Sigma Chemical Co., St. Louis, MO, USA). The plates were inoculated by dipping a sterile swab into the inoculum suspension adjusted to the turbidity of a 0.5 McFarland standard and streaking it across the surface of the agar in four directions. The plates were dried at ambient temperature for 15 min before applying the E-test® strips. The minimum inhibition concentrations (MIC) endpoints were determined after 24 and 48 h of incubation at 35 °C. The MIC was read for Amphotericin B (AmB), as the drug concentration that zone determined the point of complete inhibition (100%), and for Voriconazole (Vcz), as required by the method [39].

2.3. Software and Statistical Analysis

The analysis was performed using GraphPad Prim v.7 (GraphPad Software, CA, USA). All experimental data are presented as mean ± standard error of the mean (SEM).

3. Results

Here, we evaluated the rate of antifungal resistance of *Candida* oral samples in our laboratory. For this, we collected oral isolates from different wards of the Department of Clinical Microbiology, Nitra Faculty Hospital in Slovakia, for 60 days. Among the 62 oral samples collected, 21 isolates were yeasts of *Candida* genus, from four different hospital wards: Infectious diseases (ID), Oncology, Otorhinolaryngology (ORL), and Intensive Care Unit (ICU). Not surprisingly, most patients came from ID ward, followed by Oncology and ORL (Figure 1, Tables 1 and 2). The average age of the cohort was 51.9 ± 19.7 years (female: 48.6 ± 21.0 and male: 52.3 ± 20.4). These results indicate that the oral *Candida* spp. prevalence (in this hospital and period) was higher among males than among females (61.9% vs. 38.1%). Men were also older than females, who came mostly from ID ward (50.0%), followed by Oncology (25.0%), ORL, and ICU (both with 12.5%). On the other side, male subjects came equally from ID, Oncology and ORL (30.7% each), followed by ICU (7.7%).
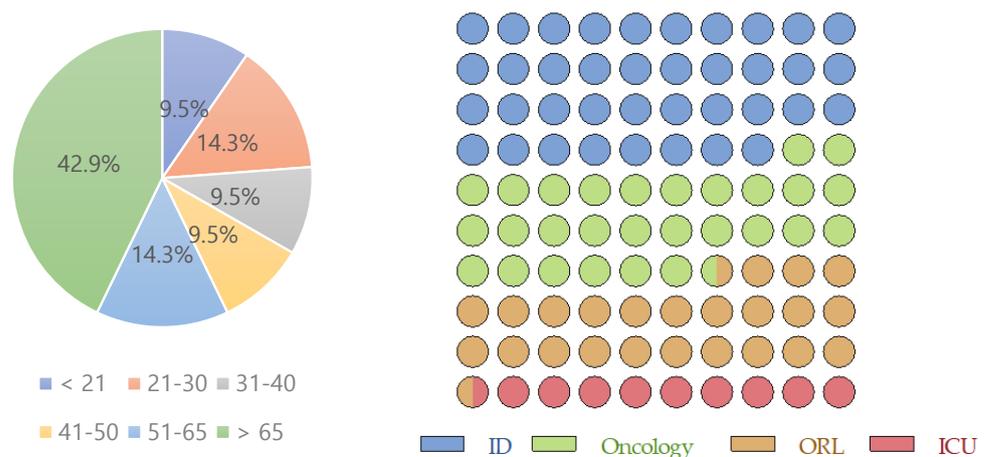

Figure 1. Distribution and percentage of ages and samples collected from specific hospital wards. (*n* = 21; Female: *n* = 8, 38.1%; Male: *n* = 13, 61.9%; ID—infectious diseases; ORL—otorhinolaryngology" (ORL) and ICU—intensive care unit).

Table 1. Gender and age distribution of the collected samples.

	<21	21–30	31–40	41–50	51–65	>65
Female	1 (4.8%)	1 (4.8%)	1 (4.8%)	1 (4.8%)	1 (4.8%)	3 (14.3%)
Male	1 (4.8%)	2 (9.5%)	1 (4.8%)	1 (4.8%)	2 (9.5%)	6 (28.6%)
Total	2 (9.5%)	3 (14.3%)	2 (9.5%)	2 (9.5%)	3 (14.3%)	9 (42.9%)

Table 2. Gender and ward distribution of the collected samples.

	ID	Oncology	ORL	ICU
Female	4 (19%)	2 (9.5%)	1 (4.8%)	1 (4.8%)
Male	4 (19%)	4 (19%)	4 (19%)	1 (4.8%)
Total	8 (38.1%)	6 (28.6%)	5 (23.8%)	2 (9.5%)

ID: infectious diseases; ORL: otorhinolaryngology; ICU: intensive care unit.

It is relevant to note that from all the identified samples of the hospital, the ratio of yeasts to bacteria is, in general, about 1/5. Nevertheless, taking into consideration only samples from the oral cavity, there is the prevalence of the yeast strains. Furthermore, of the collected *Candida* spp., the most prevalent was *C. albicans* (61.9%, $n = 13$), followed by *C. krusei* (14.3%, $n = 3$), *C. valida* (9.5%, $n = 2$), and *C. glabrata*, *C. tropicalis*, and *C. intermedia* (all: 4.8%, $n = 1$) (Table 3).

Table 3. Total number and percentage, identification of the collected oral *Candida* spp. isolates.

Candida spp.	n	%	Susceptibility Profile			
			Flu	Vcz	5FC	AmB
C. albicans	13	61.9	S	S	**R**	S
			S	S	S	S
			S	S	**R**	S
			S	S	**R**	S
			S	S	S	S
			S	S	**R**	S
			S	S	S	S
			S	S	**R**	S
			S	S	S	S
			S	S	**R**	S
			S	S	**R**	S
			S	S	S	S
			S	S	S	S
C. krusei	3	14.3	**R**	S	**R**	S
			R	S	**R**	S
			R	S	**S**	S
C. valida	2	9.5	**R**	**I**	S	S
			R	**I**	S	S
C. tropicalis	1	4.8	S	S	**R**	S
C. glabrata	1	4.8	S	S	S	S
C. intermedia	1	4.8	**R**	S	**R**	S

S: sensible; R: resistant; I: intermediate (**Bold**: intermediate and resistance to the antifungal drug).

After performing the antifungal susceptibility tests, all isolates were sensible to AmB, but several ($n = 11$) had resistance to 5FC (52.4%), to Flu (28.5%, $n = 6$) and Vcz (intermediate profile: 0.95%, $n = 2$). This means that 81.6% of the collected strains had some resistance to one or more antifungal drugs, which is clinically noteworthy.

4. Discussion

Even if *Candida* spp. is a member of the oral microbiota, in some circumstances, the increase of *Candida* spp. population can lead to dysbiosis, easily originating oral candidiasis. Among the more than 350 heterogeneous species of *Candida*, *C. albicans* is the

most prevalent in human OC [40]. For both *C. albicans* and NCACs, the transition from a harmless commensal to a pathogen is complex and poorly understood, although it seems to include different expression of virulence factors [6,41]. Moreover, it is known that NCACs have been progressively encountered and recognized as important agents of human oral infections [6,40,42–45]. This has also been aggravated by COVID-19 pandemic since an increase in oral candidiasis has been seen in these patients [46].

In this work, we performed a characterization of the antifungal profile of *Candida* oral samples from Nitra Faculty Hospital in Slovakia, collected for 60 days. It is pertinent to note that, although studies similar as this one (with short samples) have been published related to other countries (discussed in the next lines), we still do not know the status in Slovakia related to antifungal susceptibility/resistance of *Candida* clinical isolates, which made this study comprehensively relevant. The 21 *Candida* isolates were from several wards: ID, Oncology, ORL, and ICU, and most patients were from ID and Oncology wards (Figure 1, Table 2). The oral *Candida* spp. prevalence was higher among males when comparing with females. Besides, men were older than females. In fact, Naidu et al. documented similar results, with male predominance. It was indicated that, among HIV patients with oral lesions, including OC, 44% were male and 22% were female, aged between 20 and 55 years [47]. Even so, different gender balances also have occurred related to candidiasis in general [48–51].

With no surprises, *C. albicans* was the most common species (61.9%, *n* = 13), followed by *C. krusei* (14.3%), *C. valida* (9.5%) and *C. glabrata*, *C. tropicalis*, and *C. intermedia* (all: 4.8%) (Table 3). Similar epidemiological results have been reported. Arlsan and co-workers (Turkey) found 12 different genotypes and compared the virulence factors of several oral *Candida* spp. isolated from individuals with and without caries. The most isolated species was *C. albicans*, and different genotypes exhibited different virulence activities [52]. Correspondingly, Aitken-Saavedra et al. (Chile) revealed that 66% of the isolated yeasts were *C. albicans*, followed by *C. glabrata* (20.7%) [53]. In fact, in the last years, NCAC have been more identified in oral infections. In our case, around 27% of the isolates were NCAC, with *C. krusei* showing to be the most common (Table 3). In 2015, Indira et al. (India) [54] also reported that between 35 and 49% of oral candidiasis were related to *C. krusei*, which was, in fact, the most common *Candida* sp. isolated in people living with HIV with diabetes (50%). Other similar results (China and Iran) corroborate our results, with this species being the most or one of the most NCAC isolated (both in single and multi-fungal cultures) [55–57].

Regarding the antifungal susceptibility tests, half isolates presented resistance to 5FC, and almost 29% has resistance to Flu, hence almost 82% of the collected samples showed resistance to, at least, one antifungal class, which is clinically remarkable. All isolates were sensible to AmB and two samples had intermediate profile to Vcz. Previous research showed the effectiveness of Vcz and AmB *Candida* spp. isolates [25,26,55,58–62] (Portugal, Brazil, Korea, US, Japan), but also of both AmB and Flu (China) [57], as seen in this work. A recent study involving immunosuppressed patients (with diabetes mellitus), showed a predominance of *C. albicans*, but among denture wearers, *C. glabrata* was prevalent. Importantly, this report indicated that ketoconazole, Flu, and itraconazole were effective against the isolated *Candida* spp. (India) [63]. Correspondingly, Premkumar et al. (India) stated that, although *C. albicans* was the most predominantly isolated species, NCACs were also observed (e.g., *C. dubliniensis*, *C. tropicalis*, and *C. parapsilosis*) [64]. The authors indicated a variable resistance toward AmB and Flu [64]. Finally, it has been suggested that, in patients with oral infections and dentures, chlorhexidine (CHX) is an effective anti-*Candida* spp. CHX showed to have a good antiseptic effect on *Candida* sp. by killing it and preventing new adhesion [65]. This has also been showed in a recent work, with CHX being placed in a medical device [66] and in patients with long oral carriage of *Candida* spp. [61]. Hence, in the future, it might be important to start evaluating the susceptibility of this drug in *Candida* spp. oral isolates.

5. Conclusions

This preliminary work shows that, comparing with bacteria, yeasts are still isolated in a lower number in hospitalized patients in Slovakia. Nonetheless, and critically, there is a high rate of antifungal resistance to, at least one, drug (particularly to azoles and 5′-FC—around 82%), which is an important clinical finding.

It is, however, essential to state that the low number of samples is a limitation of this study, and more studies should be performed in the future to support our conclusions. Although echinocandins are not used to treat OC, adding information on the echinocandin could also improve the assumptions. Moreover, the fact that VITEK or mass spectrometry was not used could also be considered a limitation, since misidentification of species could have occurred. Nonetheless, using more than one identification method for each sample (germ-tube formation test, CHROMID® Candida, Auxacolor 2 Bio-Rad® test, ID 32C automated method—ATB Expression bioMérieux®), provided high confidence in the identifications.

Taking into consideration the increasing number of *Candida* spp. oral strains, at a significant rate of drug resistance, the identification of effective alternative therapies (such as photodynamic therapy or natural compounds) to the current antifungal agents is essential.

References

1. Peres, M.A.; Macpherson, L.M.D.; Weyant, R.J.; Daly, B.; Venturelli, R.; Mathur, M.R.; Listl, S.; Celeste, R.K.; Guarnizo-Herreño, C.C.; Kearns, C.; et al. Oral diseases: A global public health challenge. *Lancet* **2019**, *394*, 249–260. [CrossRef]
2. WHO. Oral Health—WHO Fact Sheets. Available online: https://www.who.int/news-room/fact-sheets/detail/oral-health (accessed on 15 January 2019).
3. Tonetti, M.S.; Jepsen, S.; Jin, L.; Otomo-Corgel, J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. *J. Clin. Periodontol.* **2017**, *44*, 456–462. [CrossRef] [PubMed]
4. Williams, D.; Lewis, M. Pathogenesis and treatment of oral candidosis. *J. Oral Microbiol.* **2011**, *3*, 5771. [CrossRef] [PubMed]
5. Bongomin, F.; Gago, S.; Oladele, R.; Denning, D. Global and Multi-National Prevalence of Fungal Diseases—Estimate Precision. *J. Fungi* **2017**, *3*, 57. [CrossRef] [PubMed]
6. Lewis, M.A.O.; Williams, D.W. Diagnosis and management of oral candidosis. *BDJ* **2017**, *223*, 675–681. [PubMed]
7. Taylor, M.; Raja, A. *Oral Candidiasis (Thrush)*; StatPearls Publishing: Treasure Island, FL, USA, 2019.
8. Andrade, J.C.; Kumar, S.; Kumar, A.; Černáková, L.; Rodrigues, C.F. Application of probiotics in candidiasis management. *Crit. Rev. Food Sci. Nutr.* **2021**, *2021*, 1–16. [CrossRef] [PubMed]
9. Salehi, B.; Kregiel, D.; Mahady, G.; Sharifi-Rad, J.; Martins, N.; Rodrigues, C.F. Management of *Streptococcus mutans-Candida* spp. Oral Biofilms' Infections: Paving the Way for Effective Clinical Interventions. *J. Clin. Med.* **2020**, *9*, 517. [CrossRef]
10. Benito-Cruz, B.; Aranda-Romo, S.; López-Esqueda, F.J.; de la Rosa-García, E.; Rosas-Hernández, R.; Sánchez-Vargas, L.O. Oral Candida isolates and fluconazole susceptibility patterns in older Mexican women. *Arch. Gerontol. Geriatr.* **2016**, *65*, 204–210. [CrossRef]
11. Neville, B.W.; Damm, D.D.; Allen, C.M.; Bouquot, J.E. Oral and maxillofacial pathology. In *Fungal and Protozoal Diseases*; Elsevier: London, UK, 2011; pp. 213–221.

12. Regezi, J.; Sciubba, J.; Jordan, R. Oral pathology clinical pathologic correlations. In *White Lesions*; Elsevier: St. Louis, MO, USA, 2008; pp. 98–102.

13. Javed, F.; Klingspor, L.; Sundin, U.; Altamash, M.; Klinge, B.; Engström, P.-E. Periodontal conditions, oral Candida albicans and salivary proteins in type 2 diabetic subjects with emphasis on gender. *BMC Oral Health* **2009**, *9*, 12. [CrossRef]

14. Al Mubarak, S.; Robert, A.A.; Baskaradoss, J.K.; Al-Zoman, K.; Al Sohail, A.; Alsuwyed, A.; Ciancio, S. The prevalence of oral Candida infections in periodontitis patients with type 2 diabetes mellitus. *J. Infect. Public Health* **2013**, *6*, 296–301. [CrossRef]

15. Lamey, P.J.; Darwaza, A.; Fisher, B.M.; Samaranayake, L.P.; Macfarlane, T.W.; Frier, B.M. Secretor status, candidal carriage and candidal infection in patients with diabetes mellitus. *J. Oral Pathol.* **1988**, *17*, 354–357. [CrossRef] [PubMed]

16. Mulu, A.; Kassu, A.; Anagaw, B.; Moges, B.; Gelaw, A.; Alemayehu, M.; Belyhun, Y.; Biadglegne, F.; Hurissa, Z.; Moges, F.; et al. Frequent detection of 'azole' resistant Candida species among late presenting AIDS patients in northwest Ethiopia. *BMC Infect. Dis.* **2013**, *13*, 82. [CrossRef] [PubMed]

17. Goregen, M.; Miloglu, O.; Buyukkurt, M.C.; Caglayan, F.; Aktas, A.E. Median rhomboid glossitis: A clinical and microbiological study. *Eur. J. Dent.* **2011**, *5*, 367–372. [CrossRef] [PubMed]

18. Arendorf, T.M.; Walker, D.M. Tobacco smoking and denture wearing as local aetiological factors in median rhomboid glossitis. *Int. J. Oral Surg.* **1984**, *13*, 411–415. [CrossRef]

19. Flaitz, C.M.; Nichols, C.M.; Hicks, M.J. An overview of the oral manifestations of AIDS-related Kaposi's sarcoma. *Compend. Contin. Educ. Dent.* **1995**, *16*, 136–138. [PubMed]

20. Kumar, B.V.; Padshetty, N.S.; Bai, K.Y.; Rao, M.S. Prevalence of Candida in the oral cavity of diabetic subjects. *J. Assoc. Physicians India* **2005**, *53*, 599–602.

21. Lott, T.J.; Holloway, B.P.; Logan, D.A.; Fundyga, R.; Arnold, J. Towards understanding the evolution of the human commensal yeast *Candida albicans*. *Microbiology* **1999**, *145*, 1137–1143. [CrossRef]

22. Rodrigues, C.; Rodrigues, M.; Henriques, M. Candida sp. Infections in Patients with Diabetes Mellitus. *J. Clin. Med.* **2019**, *8*, 76. [CrossRef]

23. Černáková, L.; Rodrigues, C.F. Microbial interactions and immunity response in oral *Candida* species. *Future Microbiol.* **2020**, *15*, 1653–1677. [CrossRef]

24. Panariello, B.H.D.; de Araújo Costa, C.A.G.; Pavarina, A.C.; Santiago, S.L.; Duarte, S. Advances and Challenges in Oral Biofilm Control. *Curr. Oral Health Rep.* **2017**, *4*, 29–33. [CrossRef]

25. Rodrigues, C.C.F.; Gonçalves, B.; Rodrigues, M.M.E.; Silva, S.; Azeredo, J.; Henriques, M. The Effectiveness of Voriconazole in Therapy of Candida glabrata's Biofilms Oral Infections and Its Influence on the Matrix Composition and Gene Expression. *Mycopathologia* **2017**, *182*, 653–664. [CrossRef] [PubMed]

26. Rodrigues, C.F.; Henriques, M. Liposomal and Deoxycholate Amphotericin B Formulations: Effectiveness against Biofilm Infections of *Candida* spp. *Pathogens* **2017**, *6*, 62. [CrossRef] [PubMed]

27. Sanz, M.; Beighton, D.; Curtis, M.A.; Cury, J.A.; Dige, I.; Dommisch, H.; Ellwood, R.; Giacaman, R.A.; Herrera, D.; Herzberg, M.C.; et al. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. *J. Clin. Periodontol.* **2017**, *44*, S5–S11. [CrossRef] [PubMed]

28. BDJ Team. Antibiotic resistance among patients with severe gum disease is increasing. *BDJ Team* **2018**, *5*, 18117. [CrossRef]

29. Vos, T.; Abajobir, A.A.; Abate, K.H.; Abbafati, C.; Abbas, K.M.; Abd-Allah, F.; Abdulkader, R.S.; Abdulle, A.M.; Abebo, T.A.; Abera, S.F.; et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **2017**, *390*, 1211–1259. [CrossRef]

30. Petersen, P.E.; Bourgeois, D.; Ogawa, H.; Estupinan-Day, S.; Ndiaye, C. The global burden of oral diseases and risks to oral health. *Bull. World Health Organ.* **2005**, *83*, 661–669.

31. Han, Y.W.; Wang, X. Mobile microbiome: Oral bacteria in extra-oral infections and inflammation. *J. Dent. Res.* **2013**, *92*, 485–491. [CrossRef]

32. Rodrigues, C.; Henriques, M. Oral mucositis caused by *Candida glabrata* biofilms: Failure of the concomitant use of fluconazole and ascorbic acid. *Ther. Adv. Infect. Dis.* **2017**, *4*, 10–17. [CrossRef]

33. Vanden Abbeele, A.; de Meel, H.; Ahariz, M.; Perraudin, J.-P.; Beyer, I.; Courtois, P. Denture contamination by yeasts in the elderly. *Gerodontology* **2008**, *25*, 222–228. [CrossRef]

34. Sendid, B.; Salvetat, N.; Sarter, H.; Loridant, S.; Cunisse, C.; François, N.; Aijjou, R.; Gelé, P.; Leroy, J.; Deplanque, D.; et al. A pilot clinical study on post-operative recurrence provides biological clues for a role of candida yeasts and fluconazole in Crohn's disease. *J. Fungi* **2021**, *7*, 324. [CrossRef]

35. Moorhouse, A.J.; Moreno-Lopez, R.; Gow, N.A.R.; Hijazi, K. Clonal evolution of *Candida albicans*, *Candida glabrata* and *Candida dubliniensis* at oral niche level in health and disease. *J. Oral Microbiol.* **2021**, *13*, 1894047. [CrossRef] [PubMed]

36. Černáková, L.; Light, C.; Salehi, B.; Rogel-Castillo, C.; Victoriano, M.; Martorell, M.; Sharifi-Rad, J.; Martins, N.; Rodrigues, C.F. Novel Therapies for Biofilm-Based *Candida* spp. Infections. *Adv. Exp. Med. Biol.* **2019**, *1214*, 93–123. [PubMed]

37. Doi, A.M.; Pignatari, A.C.C.; Edmond, M.B.; Marra, A.R.; Camargo, L.F.A.; Siqueira, R.A.; da Mota, V.P.; Colombo, A.L. Epidemiology and Microbiologic Characterization of Nosocomial Candidemia from a Brazilian National Surveillance Program. *PLoS ONE* **2016**, *11*, e0146909. [CrossRef] [PubMed]

38. Pappas, P.G.; Kauffman, C.A.; Andes, D.R.; Clancy, C.J.; Marr, K.A.; Ostrosky-Zeichner, L.; Reboli, A.C.; Schuster, M.G.; Vazquez, J.A.; Walsh, T.J.; et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2015**, *62*, e1–e50. [CrossRef]

39. European Committee on Antimicrobial Susceptibility Testing EUCAST Breakpoint Tables for Interpretation of MICs for Antifungal Agents v.10.0. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Clinical_breakpoints/AFST_BP_v10.0_200204_updatd_links_200924.pdf (accessed on 20 February 2022).

40. Pfaller, M.A.; Diekema, D.J. Epidemiology of invasive candidiasis: A persistent public health problem. *Clin. Microbiol. Rev.* **2007**, *20*, 133–163. [CrossRef]

41. Rodrigues, C.F.; Rodrigues, M.E.; Henriques, M.C.R. Promising alternative therapeutics for oral candidiasis. *Curr. Med. Chem.* **2018**, *25*, 2515–2528. [CrossRef]

42. Hajjeh, R.A.; Sofair, A.N.; Harrison, L.H.; Lyon, G.M.; Arthington-Skaggs, B.A.; Mirza, S.A.; Phelan, M.; Morgan, J.; Lee-Yang, W.; Ciblak, M.A.; et al. Incidence of bloodstream infections due to *Candida* species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. *J. Clin. Microbiol.* **2004**, *42*, 1519–1527. [CrossRef]

43. Horn, D.L.; Neofytos, D.; Anaissie, E.J.; Fishman, J.A.; Steinbach, W.J.; Olyaei, A.J.; Marr, K.A.; Pfaller, M.A.; Chang, C.; Webster, K.M. Epidemiology and Outcomes of Candidemia in 2019 Patients: Data from the Prospective Antifungal Therapy Alliance Registry. *Clin. Infect. Dis.* **2009**, *48*, 1695–1703. [CrossRef]

44. Krcmery, V.; Barnes, A.J. Non-albicans *Candida* spp. causing fungaemia: Pathogenicity and antifungal resistance. *J. Hosp. Infect.* **2002**, *50*, 243–260. [CrossRef]

45. Miranda, L.N.; van der Heijden, I.M.; Costa, S.F.; Sousa, A.P.I.; Sienra, R.A.; Gobara, S.; Santos, C.R.; Lobo, R.D.; Pessoa, V.P.; Levin, A.S. *Candida colonisation* as a source for candidaemia. *J. Hosp. Infect.* **2009**, *72*, 9–16. [CrossRef]

46. Katz, J. Prevalence of candidiasis and oral candidiasis in COVID-19 patients: A cross-sectional pilot study from the patients' registry in a large health center. *Quintessence Int.* **2021**, *52*, 714–718. [PubMed]

47. Naidu, G.S.; Thakur, R.; Singh, A.K.; Rajbhandary, S.; Mishra, R.K.; Sagtani, A. Oral lesions and immune status of HIV infected adults from eastern Nepal. *J. Clin. Exp. Dent.* **2013**, *5*, e1–e7. [CrossRef] [PubMed]

48. Da Silva Fay, V.; Schäffer Gregianini, T.; Gorini da Veiga, A.B.; Bottin Gonçalves, S.M.; Rodrigues, D.M.; Bonamigo, R.R. A 12-year study of fungal infections in Rio Grande do Sul, Southern Brazil. *Rev. Iberoam. Micol.* **2019**, *36*, 55–60.

49. Gupta, B.; Gupta, S.; Chaudhary, M.; Raj, A.T.; Awan, K.H.; Patil, S. Oral candida prevalence and species specificity in leprosy. *Disease Month* **2020**, *66*, 100920. [CrossRef] [PubMed]

50. Esebelahie, N.O.; Enweani, I.B.; Omoregie, R. Candida colonisation in asymptomatic HIV patients attending a tertiary hospital in Benin City, Nigeria. *Libyan J. Med.* **2013**, *8*, 20322. [CrossRef]

51. Álvaro-Meca, A.; Jensen, J.; Micheloud, D.; Díaz, A.; Gurbindo, D.; Resino, S. Rate of candidiasis among HIV-infected children in Spain in the era of highly active antiretroviral therapy (1997–2008). *BMC Infect. Dis.* **2013**, *13*, 115. [CrossRef] [PubMed]

52. Arslan, S.; Koç, A.N.; Şekerci, A.E.; Tanriverdi, F.; Sav, H.; Aydemir, G.; Diri, H. Genotypes and virulence factors of Candida species isolated from oralcavities of patients with type 2 diabetes mellitus. *Turk. J. Med. Sci.* **2016**, *46*, 18–27. [CrossRef]

53. Aitken-Saavedra, J.; Lund, R.G.; González, J.; Huenchunao, R.; Perez-Vallespir, I.; Morales-Bozo, I.; Urzúa, B.; Tarquinio, S.C.; Maturana-Ramírez, A.; Martos, J.; et al. Diversity, frequency and antifungal resistance of *Candida* species in patients with type 2 diabetes mellitus. *Acta Odontol. Scand.* **2018**, *76*, 580–586. [CrossRef]

54. Indira, P.; Kumar, P.M.; Shalini, S.; Vaman, K. Opportunistic infections among People Living with HIV (PLHIV) with Diabetes Mellitus (DM) attending a tertiary care hospital in coastal city of South India. *PLoS ONE* **2015**, *10*, e0136280. [CrossRef]

55. Wu, J.; Gan, C.; Li, J.; Liu, Y.; Chen, Z.; Zhang, Y.; Yi, G.; Sui, J.; Xu, J. Species Diversity and Antifungal Susceptibilities of Oral Yeasts from Patients with Head and Neck Cancer. *Infect. Drug Resist.* **2021**, *14*, 2279–2288. [CrossRef]

56. Maheronnaghsh, M.; Fatahinia, M.; Dehghan, P.; Teimoori, A. Identification of Candida Species and Antifungal Susceptibility in Cancer Patients with Oral Lesions in Ahvaz, Southern West of Iran. *Adv. Biomed. Res.* **2020**, *9*, 50. [PubMed]

57. Xiao, J.L.; Xu, G.C.; de Hoog, S.; Qiao, J.J.; Fang, H.; Li, Y.L. Oral Prevalence of Candida Species in Patients Undergoing Systemic Glucocorticoid Therapy and the Antifungal Sensitivity of the Isolates. *Infect. Drug Resist.* **2020**, *13*, 2601–2607. [CrossRef] [PubMed]

58. Rodrigues, C.; Silva, S.; Azeredo, J.; Henriques, M. Candida glabrata's recurrent infections: Biofilm formation during Amphotericin B treatment. *Lett. Appl. Microbiol.* **2016**, *63*, 77–81. [CrossRef] [PubMed]

59. Sanitá, P.V.; De Oliveira Mima, E.G.; Pavarina, A.C.; Jorge, J.H.; Machado, A.L.; Vergani, C.E. Susceptibility profile of a Brazilian yeast stock collection of *Candida* species isolated from subjects with Candida-associated denture stomatitis with or without diabetes. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* **2013**, *116*, 562–569. [CrossRef]

60. Song, Y.B.; Suh, M.K.; Ha, G.Y.; Kim, H. Antifungal susceptibility testing with etest for *Candida* species isolated from patients with oral candidiasis. *Ann. Dermatol.* **2015**, *27*, 715–720. [CrossRef]

61. LaFleur, M.; Qingguo, Q.; Lewis, K. Patients with long-term oral carriage harbor high-persister mutants of *Candida albicans*. *Antimicrob. Agents Chemother.* **2010**, *54*, 39–44. [CrossRef]

62. Suzuki, T.; Imamura, A. A case of chronic mucocutaneous candidasis cured with micafungin. *Kansenshogaku Zasshi.* **2005**, *79*, 143–148. [CrossRef]

63. Rajakumari, M.L.; Saravana Kumari, P. Prevalence of Candida species in the buccal cavity of diabetic and non-diabetic individuals in and around Pondicherry. *J. Mycol. Med.* **2016**, *26*, 359–367. [CrossRef]

64. Premkumar, J.; Ramani, P.; Chandrasekar, T.; Natesan, A.; Premkumar, P. Detection of species diversity in oral candida colonization and anti-fungal susceptibility among non-oral habit adult diabetic patients. *J. Nat. Sci. Biol. Med.* **2014**, *5*, 148. [CrossRef]

65. Mantri, S.S.; Parkhedkar, R.D.; Mantri, S.P. Candida colonisation and the efficacy of chlorhexidine gluconate on soft silicone-lined dentures of diabetic and non-diabetic patients. *Gerodontology* **2013**, *30*, 288–295. [CrossRef]

66. Alves, D.; Borges, P.; Grainha, T.; Rodrigues, C.F.; Pereira, M.O. Tailoring the immobilization and release of chlorhexidine using dopamine chemistry to fight infections associated to orthopedic devices. *Mater. Sci. Eng. C* **2021**, *120*, 111742. [CrossRef] [PubMed]