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Prevalence of Fungi Species and Oral Candida Carriage in Libyans

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Background: Approximately 40% of healthy individuals harbor oral fungi as commensals. However, Candida albicans can cause oral fungal infections. **Methods**: This study comprised 310 routine dental patients, aged 18 years and above, examined in the dental clinic over 9 months at the Central Dental Clinic in Benghazi city, presented with routinely encountered dental complaints such as dental caries, gingivitis, periodontal disease, dental prosthesis problems.
Each patient's mouthwash was collected and sent to the lab for routine mycological culturing in Sabouraud's Dextrose Agar. **Results**: There was no association between clinical factors and candida carriage, with the exception of the use of dental prosthesis, and the prevalence of oral yeast carriage in 310 cases was 32% (100 patients; 54 females). It was possible to isolate twelve strains of both candidal and non-candidal fungi. *Candida magnolia, Candida pelliculosa, Cryptococcus humicola complex, Cryptococcus Laurentii, Geotrichum capitatum,* and *Rhodotorula rubra* were found in one instance each, while 68 isolates had *Candida albicans*, 15 isolates had *Candida dubliniensis*, 5 isolates had Trichosporon spp., and 2 isolates had *Candida krusei*. Remarkably, no resistance to fluconazole was found in this investigation; the isolates were either susceptible (67%) or intermediately susceptible (33%).
Conclusions: The two most common species of oral fungi isolated are *Candida albicans* and *Candida dubliniensis*. They are highly resistant to amphotericin, flucytosine, and clotrimazole, but less to voriconazole and itraconazole. Remarkably, no fluconazole-resistant strains were discovered.

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1 Introduction

Approximately 40% of healthy people have fungi in their mouths, which are innocuous commensals, however, they can contribute to oral mucosal diseases such as dental caries and oral lichen planus, particularly in immunocompromised individuals. Furthermore, Candida species that colonize the mouth asymptomatically may cause oral sores or spread infections. (Lourenço et al, 2017). Those with diabetes, cigarette smokers, and users of electronic cigarettes have higher rates of Candida carriage. [1,2&3].

All the methods of candidal characterization such as growth characteristics and hybridization of nucleic acid sequence indicated that *Candida albicans* is the most prevalent yeast species [4].
besides *Candida dubliniensis, Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis,* and *Candida guillermondi,* although many other types of fungi were also isolated. *Candida albicans* is the main causative agent for most clinical types of oral candidiasis [5].

The collection of oral samples is usually done by hard swabbing or oral rinse methods. Then the fungi can be cultured on Sabouraud's agar dextrose (SAD) medium containing an antibiotic such as chloramphenicol, gentamicin, or cefotaxime to inhibit bacterial growth, then the agar plates are generally incubated aerobically at 30 °C for 48 hours [6].
The resistance to antifungal agents can be microbiological or clinical [7].
The latter describes a fungal infection that persists even after receiving proper therapy. Clinical resistance can be influenced by microbiological resistance, but other factors such as weakened immunity, underlying disease, decreased drug bioavailability, and elevated drug metabolism may also be involved [8].
Clinical manifestations of oral candidiasis, a frequent oral fungal infection, include pseudomembranous, erythematous, and plaque-like/nodular forms. Fungal infections are the primary cause of angular cheilitis and median rhomboid glossitis. Since 40% of healthy people from several demographics might harbor Candida yeasts without exhibiting any symptoms, the simple isolation of the yeast from intraoral surfaces cannot be taken as a warning sign of illness [9].
Candida species among healthy and immunocompromised individuals were studied. The study highlights the antifungal susceptibility profiles of isolates, particularly *C. albicans* [10].
A study in Dhaka, Bangladesh, involving 286 children, identified Candida albicans as the predominant species (86.34%), followed by *C. tropicalis* (8.68%), and *C. krusei* (3.12%) [11]

2 Material and Methods

Each patient with 15 ml mouthwash for about 3 minutes was collected and sent immediately to the lab for cultivation. SDA is used to isolate, grow, and maintain both pathogenic and non-pathogenic yeast and fungal species. To promote the growth of fungus, particularly dermatophytes, and to marginally restrict the growth of bacteria in clinical specimens, the pH was set at about 5.6. One liter of distilled water was used to suspend 65 g of the medium, which was then heated with constant stirring and boiled for one minute to dissolve it fully. The medium was then autoclaved for 15 minutes at 121 °C. The medium was supplemented with 1g of cefotaxime dissolved in sterile water. After cooling in a water bath to a temperature of 45 to 50 °C, the medium was transferred into petri dishes. The cultures were examined macroscopically, paying particular attention to the colony's color, growth type, texture, and dimensions Figure 1.

Fig. 1 growth of yeasts on (SAD) medium, 125 is the sample number

Two drops of lactose phenol cotton blue were applied to microscope slides to create thin smears of the organisms. The slides were then covered with a coverslip and examined under a light microscope set to X40. At 100 oil magnification, a Slides Gram stain was applied. Species-related differences can also be seen in the microscopic characteristics of Candida species. Blastoconidia are produced by all species either alone or in tiny clusters. Both circular and elongated blastoconidia are possible. The pseudohyphae produced by the majority of species can be lengthy, branching, or curved. Certain strains of Candida spp. develop chlamydospores and true hyphae. A germ tube is carried out, which is one of the virulence factors of *C. albicans*. This is a rapid test for the presumptive identification of *C. albicans,* as shown in Figure 2.

The identifying method ERBA SCAN 21 was applied. In the USA, Erba Diagnostics (formerly Immunovision and Diamedix Corporation) offered it. The CANDIDAtest 21 kit (Cat. No.: 10010220) was created to regularly identify the yeasts that are most clinically important Figure 3.
Using 21 biochemical assays (chromogenic substrates, decarboxylase, and assimilations), the kit allows for the identification of 34 strains. The tests were set up in microtitration plate wells. The purpose of the three rows, each with eight wells, was to identify a single strain. To aid in accurate readings of positive results, negative tests were put on the plate for every group of reactions.

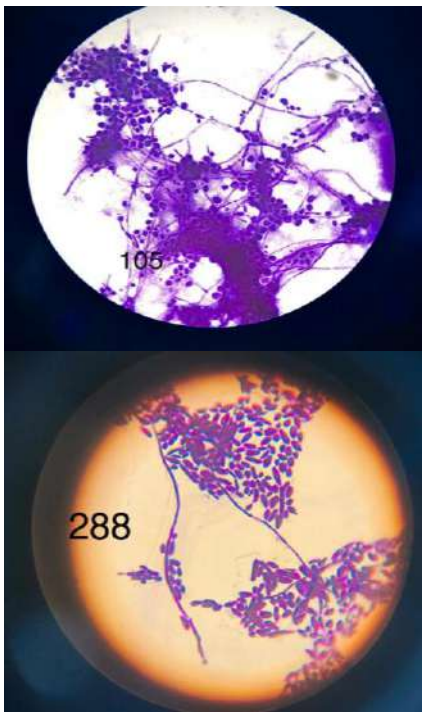

Fig. 2 Germ tube formation *C. albicans*, 288 is the sample number

Fig. 3 Erba Scan device for the identification of yeasts

The standardized filter paper disk agar diffusion method, the Kirby-Bauer method, was used to determine the sensitivity or resistance of yeasts to various antifungal compounds, and it uses the SDA. The colonies were emulsified in 5 mL of sterile saline until the turbidity was similar to the McFarland No. 0.5 turbidity standard drug Figure 4,5,6, and 7.

Fig. 4 McFarland standards used to adjust the turbidity of yeast suspensions

Fig. 5 *Candida dubliniensis* sensitive to coltri., itracon., flucon., & vorico

Fig. 6 Fluconazole, itraconazole, and voriconazole can all affect *C. dubliniensis*

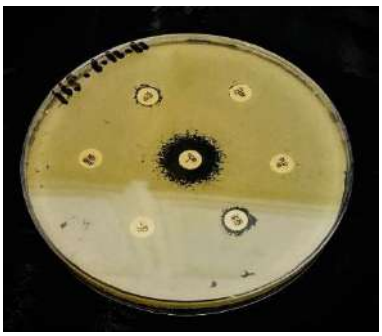

Fig. 7 *C. magnoliae* is intermediate to voriconazole and resistant to all antifungals

All the obtained information was tabulated validated and statistically analyzed by SPSS v28. Descriptive statistics was used to describe demographic characteristics in terms of the mean, median, mode, standard deviation, and range. Comparisons were made through the chi-square test for all variables of the study. Statistical significance was assumed at a p-value<0.05. Correlations are tested by the Spearman Chi-square test. The ethical approval was obtained by the research ethics review committee of the College of Dentistry, Benghazi University.

3 Results

A total of 310 routine dental patients were examined over 9 months at the central dental clinic in Benghazi. The sample comprised adult patients, aged from 20-71 years (45 males and 55 females) with dental complaints such as dental caries, gingivitis, periodontal disease, and dental prosthesis problems, excluding those patients currently receiving antifungal agents, antibiotics, chemotherapy, radiotherapy, or treatment for malignancy. The majority of

patients are healthy, except for a few patients with common medical illnesses such as diabetes mellitus, hypertension, asthma, paranasal sinusitis, hyperthyroidism, hypothyroidism, or anemia, plus a few numbers of patients who smoke or are alcoholics—generally, Candida oral carriage peaks at middle age groups. *C. albicans* is most prevalent among the age group (20-49 years), while *C. dubliniensis* was prevalent in the age group (20-29 years). There is no correlation between the kind of candida isolate and the patient's age group. (Pearson Chi-Square=54.352, df=55, p-value=0.499).

One hundred positive growths for fungi and yeasts could be isolated in this study, categorized into 12 strains of fungi. *C. albicans* was the most prevalent species (68 isolates), *Candida dubliniensis* (15 isolates), *Trichosporon spp* (5 isolates), *C. catenulata* (2 isolates), *Candida glabrata* (2 isolates), *C. krusei* (2 isolates), and there was only 1 isolate for *C. magnolia*, *C. pelliculosa*, *Cryptococcus humicola complex*, *Cryptococcus Laurentii*, *Geotrichum capitatum*, and *Rhodotorula rubra*. Blastoconidia are produced by all species either alone or in tiny clusters. Both circular and elongated blastoconidia are possible. The pseudohyphae produced by the majority of species can be lengthy, branching, or curved. Certain strains of Candida produce chlamydospores and true hyphae.

Amphotericin, flucytosine, and clotrimazole have high resistance rates of 98%, 97%, and 83%, respectively; fluconazole and nystatin have moderate resistance rates of 67% and 60%, respectively; while voriconazole has high sensitivity rates of 91% and 71%. The microbes that were isolated exhibit a high level of amphotericin resistance. Only one (1%) of the 68 Candida albicans displayed an intermediate susceptibility to amphotericin, whereas 67 (99%), were resistant to it. The isolated fungi, one from each species, were resistant to amphotericin antimycotic *(Candida magnoliae Candida pelliculosa, Cryp.* complex *Cryp. Laurentii Geot. Capitatum Rhod. rubra*). Two strains of *Candida catenulata* exhibit amphotericin resistance; of the 15 strains of *Candida dubliniensis*, 14 (93%) were resistant and one (7%) intermediate. For each of the five microbes, Tricho spp. demonstrated resistance to the agent, Pearson Chi-Square=2. 111, df =11, p-value= 0. 998.

Table 1: Number of fungal species isolated

Isolated M.O ErbaScan	Number of cases
Candida albicans	68
Candida dubliniensis	15
Trichosporon spp	5
Candida catenulata	2
Candida glabrata	2
Candida krusei	2
Candida magnoliae; *Candida pelliculosa*	1 each
Cryptococcus Laurentii; Geotrichum capitatum; Rhodotorula rubra	1 each

4 Discussion

The prevalence of oral candida carriage in healthy individuals has been found to range from 18% in a Japanese study [5].

to higher rates, especially in patients with diabetes and immunocompromised conditions. A study in the neighboring country of Kuwait [12].

also revealed a higher oral candidal carriage rate (46%) than the 30.6% observed in the United Arab Emirates [13].

The prevalence of oral Candida species varies based on age, health status, and external factors. Studies indicate that healthy individuals exhibit a Candida carriage rate of approximately 30-50%, whereas this percentage significantly increases in immunocompromised patients, such as those with diabetes, HIV, or cancer [14].

There was no correlation between the strains of fungus species and either gender or age, and candidate isolates could be grown from samples of both sexes and varying age groups. Additionally, there was no statistically significant difference between the current study's results and those of healthy people of the same gender [15].

There was no correlation between oral candidal carriage and systemic disorders in this group. This could be because of the small number of patients in this study and a few cases of these systemic illnesses seeking dental care. There were numerous reports of a rise in oral fungal infections among people with HIV and other immunosuppressive diseases worldwide, but there were no patients with these conditions in this study [16].

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According to the study's findings regarding the distribution of various Candida species among the patients who produced Candida in culture, Candida albicans accounted for 68% of all isolates, making it the most prevalent yeast species in the sample. According to earlier research, *Candida albicans* was the most common oral Candida species isolated in Kuwait, accounting for up to 63.7% of all isolates tested [12].

, and in Saudi Arabia, it accounted for 55.6% of all isolates tested. *C. glabrata* and *C. krusei* are thought to be the most common non-Albicans oral Candida species after *C. albicans* [16].

On the other hand, *C. dubliniensis* was the most prevalent species among the non-albicans Candida species isolated in this investigation, making up 15% of all examined species.

Another study conducted in the western region of Libya in 2018 produced nearly identical results, with *Candida albicans* accounting for the highest prevalence (41.7%), followed by *C. glabrate* (27.1%) and *C. dubliniensis* (11.5%) [17].

The results of additional investigations [18,19], were consistent with the isolation of *C. glabrata* and *C. dubliniensis* as the most significant non-albicans pathogens in the oral cavity. This observation can be linked to the fact that both species have become critical pathogens in immunocompromised patients in recent years, and oral candidiasis is caused by either virulence factors in *C. Dubliniensis* or antifungal resistance in *C. glabrata* [20].

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Most isolated species are resistant to amphotericin, the most potent antifungal drug. The highest resistance was to it, then to nystatin, then to clotrimazole. However, most fungal species are generally either moderately or highly susceptible to the antifungal medicines that have been tried. To guarantee population representation, the study samples had to have been collected from a variety of locations. In this situation, multicenter studies are better since they can use the same approach and cover more areas. The technique for susceptibility testing of yeast differs from traditional antibiotic susceptibility testing, and requires more experience, especially when reading the zone sizes.

It is believed that the information gathered from this study will help determine better antifungal treatment approaches and create a suitable prophylactic program for these patients.

The findings of a systematic review on oral fungal infections in patients undergoing cancer therapy for solid tumors are consistent with the observed statistically significant increase in the prevalence of oral Candida carriage during cancer therapies, as reported by [10].

More research should be done to develop a suitable system for tracking the appearance of more harmful fungi and assessing their resistance to antifungals.

Despite their expense, more advanced and contemporary laboratory techniques ought to be used to obtain more dependable findings with less work and effort.

Table 2 Microorganism strains susceptibility to all antimycotic agents

Species	amphotericin			Nystatin			Clotrimazole			Itraconazole			Voriconazole			fluconazole			fluocytosine		
Grade ►	S	I	R	S	I	R	S	I	*R	***S	**I	R	S	I	R	S	I	R	S	I	R
C. albicans	0	1	67	3	48	17	1	11	56	53	9	6	64	2	2	25	43	0	1	1	66
C.catenulata	*0*	*0*	*2*	*0*	*1*	*1*	*0*	*0*	*2*	*1*	*1*	*0*	*2*	*0*	*0*	*0*	*2*	*0*	*0*	*0*	*2*
C. dubliniens	*0*	*1*	*14*	*2*	*6*	*7*	*0*	*5*	*10*	*12*	*3*	*0*	*15*	*0*	*0*	*8*	*7*	*0*	*0*	*0*	*15*
C. glabrata	*0*	*0*	*2*	*0*	*2*	*0*	*0*	*0*	*2*	*0*	*0*	*2*	*2*	*0*	*0*	*0*	*2*	*0*	*0*	*0*	*2*
C. krusei	0	0	2	0	0	2	0	0	2	0	0	2	2	0	0	0	2	0	0	0	2
C. magnoliae	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	1
C. pelliculosa	0	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	1	0	0	0	1
Cryp. h comp	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	1	0	0	0	1
Cryp. Laurent	0	0	1	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1
Geot.capitatu	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1
Rhod. Rubra	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	1	0
Tricho. Spp	0	0	5	1	2	2	0	0	5	3	0	2	3	0	2	0	5	0	0	0	5
Total	0	2	98	8	60	32	1	16	83	71	13	16	91	3	6	33	67	0	1	2	97

**R= Resistant* ***I= Intermediate* ****S=Sensitiv*

Conflict of interest: The authors certify that there are no conflicts of interest.

References

[1] S.A. Mokeem, T. Abduljabbar, A.A..Al-Kheraif, M.N. Alasqah, D. Michelogiannakis, L.P. Samaranayake et al, Oral Candida carriage among cigarette- and waterpipe-smokers, and electronic cigarette users. *Oral Dis*. 25(1):319-26. 2019.

[2] A.G. Lourenço, A. Ribeiro, C. Nakao, A.C.F. Motta, L.G.L. Antonio, A.A. Machado, et al., Oral Candida spp carriage and periodontal diseases in HIV-infected patients in Ribeirão Preto, *Brazil. Rev Inst Med Trop Sao Paulo*. 59:e29. 2017.

[3] N.A. Alaizari, J.R. Al-Anazi,. OralCandida carriage in smokers and tobacco users: Asystematic review and meta-analysis of observational studies*, J Oral Biosci* 62(4):342-8. 2020.

[4] Z. Akram, A.A.Al-Kheraif, S.V. Kellesarian, F. Vohra, F. Javed. Comparison of oral Candida carriage in waterpipe smokers, cigarette Smokers & non-smokers*. J. Oral Sci*. 60(1):115-2018.

[5] L. Hu, C. He, C. Zhao, X. Chen, H. Hua, Z. Yan. Characterization of oral candidiasis and the Candida species profile in patients with oral mucosal diseases. Microbial Pathogenesis Volume 134, 103575 September 2019.

[6] P. Madhavan, F. Jamal, S. Chong PPJJoa. Laboratory isolation and identification of Candida species. 11(16):2870-7. 2011.

[7] Z.A. Kanafani, and J. R. Perfect. "Antimicrobial resistance: resistance to antifungal agents: mechanisms and clinical impact." *Clin Infect Dis* 46(1): 120-128. 2008.

[8] J. H. Rex, M. A. Pfaller, J. N. Galgiani, M. S. Bartlett, A. Espinel-Ingroff, M. A. Ghannoum et al. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of in vitro-in vivo correlation data for fluconazole, itraconazole, and candida infections. *Subcommittee on Antifungal Susceptibility Testing of the National Committee for Clinical Laboratory Standards. Clin Infect Dis*. 24(2):235-47. 1997.

[9] E. Rosa. oral candidosis: physiopathology, decision making, and therapeutics id tu digital 12184. isbn 9783662471944, 2015.

[10] Matti Mauramo, Nurgül Tonoz, Jörg Halter, Betsy Joseph, Tuomas Waltim. Oral Candida carriage and resistance against common antifungal agents in hematopoietic stem cell transplantation recipients. Supportive Care in Cancer 32:185 https://doi.org/10.1007/s00520-024-08396-4, 2024.

[11] T. Rawnuck, Md. Selim Reza, R. Ahmed, Mohammad Fatteh Ul Islam, A. I. Hossain, N. Sultana, S. Monwar. (2022) Prevalence of Oral Candidiasis among Children Caused by Different Candida Species, Medicine Today, DOI:10.3329/medtoday. v34i1.58678

[12] S. H. Al-Amad, B. Rahman, N. Khalifa, M.A. Awad. Oral candidal carriage and its association with dental carious lesions in asymptomatic adults: a cross-sectional study from the UAE. *BMC Oral Health.* 21(1):197. 2021.

[13] A.N. Ellepola, Z. U. Khan, B. Joseph, R. Chandy, L. Philip. (2011). Prevalence of Candida dubliniensis among oral Candida isolates in patients attending the Kuwait University Dental Clinic. Med Princ Pract. 20(3):271-6. doi: 10.1159/000323440. Epub 2011 Mar 29. PMID: 21454999.

[14] B. J. Kullberg and M. C. Arendrup. (2015) Invasive Candidiasis, N Engl J Med Vol. 373 Issue 15 Pages 1445-56 AccessionNumber:26444731DOI:10.1056/NEJMra1315399 https://www.ncbi.nlm.nih.gov/pubmed/26444731.

[15] M. Mun, T. Yap, A. D. Alnuaimi, G. G. Adams, M. J. McCullough. Oral candidal carriage in asymptomatic patients. *Aust Dent J.* 61(2):190-5. 2016.

[16] S. Alrayyes, H. Alruwaili, I. Taher, Elrahawy K, Almaeen A, Ashekhi A, et al. Oral Candidal carriage and associated risk indicators among adults in Sakaka, Saudi Arabia. *BMC Oral Health*. 19. 2019.

[17] Z. A. Krema, E.E.M. Trfas, M.S. Ellabib, M. Cogliati. Oral carriage of candidiasis in patients with oral dental diseases: predisposing factors, species, and their antifungal susceptibility patterns. *J Bacteriol Mycol Open Access.* 6(3):223–227. 2018.

[18] R. Pelletier, I. Alarie, R. Lagacé, T.J. Walsh. Emergence of disseminated candidiasis caused by Candida krusei during treatment with caspofungin: Case report and review of literature. *Medical Mycology*. 43(6):559-64. 2005.

[19] C.R. Cooper. Chapter 2 - Yeasts Pathogenic to Humans. In: Kurtzman CP, Fell JW, Boekhout T, editors. The Yeasts (5th Ed). *London: Elsevier*; 2011. p. 9-19.

[20] M. Radosavljevic, H. Koenig, V. Letscher, J. Waller, F. Maloisel, B. Lioure et al. Candida catenulata Fungemia in a Cancer Patient. *Journal of clinical microbiology*. 37:475-7. 1999.