

Oral *Candida* Speciation using HiCrome Agar with Antifungal Susceptibility Patterns in Patients with Malignancy Undergoing Treatment

Authors:

Tripti H Uchil^{1,2}, Pavan Chand Attavar², Prajna Sharma³, Manjula Shantaram⁴, Megha L. Bhat⁴, Deeksha KS⁴, Sreeraj Surendran⁴, Rachan Shetty⁵, Reshma Kolar⁶

¹Department of Microbiology, AJ Institute of Allied Health Sciences, Mangalore, Karnataka, India.

²Department of Microbiology, Srinivas Institute of Medical Sciences & Research Centre, Srinivas University, Mukka, Mangalore, Karnataka, India.

³Department of Microbiology, AJ Institute of Medical Sciences & Research Centre, Mangalore, Karnataka, India.

⁴AJ Research Centre, AJ Institute of Medical Sciences & Research Centre, Mangalore, Karnataka, India.

⁵Department of Medical Oncology, AJ Hospital & Research Centre, Mangalore, Karnataka, India.

⁶Department of Community Medicine, AJ Institute of Medical Sciences & Research Centre, Mangalore, Karnataka, India.

Corresponding Author:

Pavan Chand Attavar, Department of Microbiology, Srinivas Institute of Medical Sciences & Research Centre, Srinivas University, Mukka, Mangalore, Karnataka, India.

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ABSTRACT

Background: Oral candidiasis is a frequent opportunistic fungal infection in patients with malignancy undergoing chemotherapy and radiotherapy. Species identification and antifungal susceptibility testing are crucial to effective management. The aim of the study was to identify oral *Candida* species using HiCrome Candida agar and evaluate antifungal susceptibility patterns among patients with malignancy undergoing treatment. **Methods:** A cross-sectional study was conducted among patients with malignancy receiving chemotherapy and/or radiotherapy. Saliva samples were cultured on Sabouraud dextrose agar and presumptively identified using HiCrome Candida agar. Antifungal susceptibility testing was performed using the disk diffusion method. **Results:** *Candida albicans* was the predominant species identified by HiCrome agar (50%), followed by *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata* and *Candida guilliermondii* among cancer patients. Amphotericin B and Nystatin showed 100% susceptibility by all *Candida* species, whereas Itraconazole exhibited lowest susceptibility among all the azole compounds. **Conclusion:** Although HiCrome agar is used as a cost effective, presumptive identification of *Candida* species, it is unable to differentiate other species of *Candida*. Other conventional methods or molecular methods are recommended for the accurate species identification. The study emphasises the importance of AST as drug resistance is commonly seen in *Candida* species.

Keywords: Oral candidiasis, *Candida*, HiCrome agar, antifungal susceptibility, AST, malignancy.

INTRODUCTION

Oral candidiasis is one of the most frequent opportunistic oral fungal infections and source of morbidity in immunocompromised people throughout the world. There has been recent evidence showing high levels of genotypic diversity in clinical candida isolates and geographic differences in susceptibility patterns to antifungals. Hence continuous monitoring of the epidemiology of these species and clinical isolates is important [1]. Oral *Candida* has become a common problem in patients with advanced malignancies, and a prompt diagnosis and targeted treatment has led to increased incidence and thus

accounted for the impact on nutrition, quality of life and overall treatment outcome [2]. A systematic review of oral fungal infections in cancer patients also confirmed the significant burden of *Candida* in this group of patients and highlighted the importance of evidence-based diagnosis and prevention strategies [3]. *Candida* species are commensals present in the oral cavity without causing disease but can cause disease when host defence is compromised. Several factors contribute to the transition from commensalism to pathogenicity, such as immune dysfunction, disturbance of normal oral flora, mucosal damage, systemic diseases, etc., [4]. *Candida* species

interaction with the innate immune system is an essential theme in the determination of colonization, persistence and mucosal infection [5]. Therefore, early and effective diagnosis and treatment of oral candidiasis are necessary to avoid local complications, local spread to other areas, and to prevent dissemination into the system, especially in vulnerable groups of patients [6].

Chemotherapy patients are one of the most vulnerable category of patients for opportunistic fungal infections because of the host immunity compromise both in relation to the malignancy and to the treatments given to the cancer patient. Recent clinical studies support the notion that regulation of the oral microbial ecosystem may impact the colonization of *Candida* during cancer therapies and be involved in the progression of the disease [7]. Structural and functional alterations of the salivary glands post-radiotherapy are recognised as substantial risk factors for oral fungal infections, as salivary dysfunction impairs the bulk of the body's defence mechanisms in the oral cavity [8]. Total colonization of *Candida* has been observed in large numbers, and the need for routine *Candida* carriage monitoring during radiotherapy in patients with head and neck cancer in some studies [9].

Oncology patients who have received chemotherapy suffer from an enormous burden of disease caused by *Candida*. It can manifest as pseudomembranous lesions, erythematous patches, angular cheilitis, a burning sensation, dysphagia, impaired taste and nutrition problems. Despite increasing evidence on the availability of an expanding number of different antifungal drugs and cancer treatment modalities, oral fungal infections continue to be a supportive care issue of importance, as revealed by recent evidence from systematic reviews and network meta-analyses [10]. Generally high acquisition of *Candida* colonization has been also reported in previous studies investigating patients undergoing oral and pharyngeal radiation therapy, and the role of the mucosal alterations induced by this therapy and its effect on fungal proliferation has been underlined [11]. The importance of early diagnosis and management of oral *candida* infections in the cancer care environment is underlined by these observations [12].

Dry mouth and decreased saliva production (also called hypo-salivation) are among the most common side effects experienced during cancer treatment, especially during treatment with radiotherapy to the head and neck. Dry mouth symptoms are well established in epidemiological studies within adults who have salivary gland dysfunction, and salivary gland disease is a risk factor for oral infections [13]. Two aspects of the pathophysiology of xerostomia are quantitative changes in salivary secretion, which lead to diminished lubrication, buffering, and antibacterial properties, and qualitative changes [14]. This promotes a fungal environment that favours adherence,

colonization and persistence, leading to changes that can create the oral environment that favours fungal adherence, colonization and persistence [15]. Numerous clinical studies have continually shown a correlation between decreased salivary flow rates and higher numbers of *Candida* in the oral cavity, making salivary dysfunction a large contributing risk factor to candidiasis disease [16].

Candida albicans has been known historically as the major cause of oral candidiasis. In recent years, however, epidemiological studies have reported a trend that the epidemiology of non-*albicans Candida* species is increasing as well in immunocompromised patients. In a study of immunocompromised patients in India, considerable species diversity was reported, and it was highlighted that correct speciation is important as some of the *candida* species are intrinsically resistant to azoles. These findings are in support with what has been reported at the district hospital level for large series of clinical isolates that showed a significant representation of non-*albicans Candida* species [17]. These species are clinically relevant as many of them are less susceptible to most antifungal drugs and could hold the key to specific virulence properties [18].

Fungal resistance to antifungals is an emerging issue in the treatment of candida infections. Oral candida isolates from immunocompromised patients have been increasingly reported as resistant to azole antifungals, especially fluconazole. Resistance testing for different *Candida spp.* has shown significant differences in geographic regions and species [19]. This variability underscores the need to conduct susceptibility testing and facilitate decision-making for proper therapy to avoid an outbreak of treatment failure. Moreover, keeping track of antifungal resistance patterns is critical to aid antifungal stewardship programs and patient outcomes [20].

The identification of *Candida* species is therefore very important for epidemiological surveillance and clinical management. Currently used methods of diagnostics (germ tube test and biochemical characterization) suffer from several drawbacks in terms of both turnaround time and discriminatory power. Recently, chromogenic culture media, like HiCrome *Candida* agar, have been proposed as useful media for the rapid presumptive identification of clinically relevant *Candida* species [21]. Comparative studies have shown the ability of chromogenic media to identify multiple *Candida* species from the different sizes and color colony by early diagnosis and detection of mixed infections [22]. Furthermore, chromogenic methods are also found to be useful for routine laboratory applications as they are less expensive, and interpretation of the results is less difficult [23].

MATERIALS AND METHODS

The present cross-sectional study was designed to isolate, identify, and characterise oral *Candida* species in cancer patients being treated and normal healthy

human beings. This study was conducted at AJ Cancer Institute at Mangalore, Karnataka, India. There were 100 patients diagnosed with any malignancy having undergone at least 2 cycles of chemotherapy and/or radiotherapy. Hundred healthy individuals were taken as a control without a major systemic disease and not treated for a major chronic illness. Patients who had received antifungal treatment within the previous two weeks were excluded from the study. Saliva samples were collected by the spitting method in an aseptic way. Collected saliva sample of 1-2ml was kept in sterile containers. Samples were promptly taken to the microbiology lab and processed within 2 hours.

Isolation of *Candida* Species

Collected saliva samples were cultured on Sabouraud Dextrose Agar (SDA) with Chloramphenicol and incubated at 37°C for 24-48 hours. Culture plates were then checked for the presence of colonies growing as yeast-like forms, indicating the presence of *Candida* species. All colonies were Gram-stained for microscopic examination in SDA. Budding yeast was used as a criterion for identification of *Candida* spp. Those colonies were chosen for further speciation.

Speciation Using HiCrome *Candida* Agar

All the presumptive *Candida* isolates from SDA were sub cultured in HiCrome *Candida* Agar medium and allowed to incubate at 37°C for 24-48 hours. Species were identified by the colour of the colonies and the colony morphology according to the HiMedia manufacturers' guidelines.

Presumptive species identification was interpreted as follows:

1. Light green colonies – *Candida albicans*
2. Blue colonies – *Candida tropicalis*

RESULTS

The distribution and identification of *Candida* species from HiCrome *Candida* agar and antifungal susceptibility and resistance of *C. albicans* and non-*albicans* *Candida* isolates to commonly used antifungal agents.

Table 1: Distribution of *Candida* Species Identified by HiCrome *Candida* Agar in Healthy Controls and Patients with Malignancy

<i>Candida</i> species	Healthy n (%)	Malignancy n (%)	Total n (%)
<i>Candida albicans</i>	2 (66.7)	18 (50.0)	20 (51.3)
<i>Candida tropicalis</i>	0 (0.0)	6 (16.7)	6 (15.4)
<i>Candida parapsilosis</i>	0 (0.0)	8 (22.2)	8 (20.5)
<i>Candida krusei</i>	1 (33.3)	2 (5.6)	3 (7.7)
<i>Candida glabrata</i>	0 (0.0)	1 (2.8)	1 (2.6)
<i>Candida guilliermondii</i>	0 (0.0)	1 (2.8)	1 (2.6)
Total	3 (7.7%)	36 (92.3%)	39 (100.0)

A total of 39 *Candida* isolates were obtained from HiCrome *Candida* agar, 36 (92.3%) from patients with malignancy and 3 (7.7%) from healthy controls. *Candida albicans* is the most common species, and the malignancy group had 18 isolates (50.0%), whereas the healthy group had 2 isolates (66.7%). Non-*albicans* *Candida* species, which were most prevalent among patients with malignancy, were *C. parapsilosis* (22.2%) and *C. tropicalis* (16.7%). A single isolate (2.8%) was positive for each of the *C. glabrata* and *C. guilliermondii* species. In contrast, only *C. albicans* and *C. krusei* were found in the healthy controls. The study results indicate a higher level of *Candida* species diversity in cancer patients under treatment.

3. Pink to purple colonies – *Candida krusei*
4. Cream to mauve colonies – *Candida parapsilosis*
5. Cream to white – *Candida glabrata*
6. Pink to red - *Candida guilliermondii*

When more than one colony colour was found within the same culture plate, it was determined that the culture was a “mixed” *Candida* infection.

Antifungal Susceptibility Testing (AST)

All *Candida* isolates were identified as susceptible to the fungicides by the disk diffusion method on the medium of Mueller–Hinton agar supplemented with 2% glucose and methylene blue. After each antifungal disc was put in place, the zone of inhibition was measured in millimetres after incubation. The antifungal disks were ordered from HiMedia Laboratories. Zone interpretation were measured after 24hrs of incubation and interpreted as per M44 CLSI guidelines. Throughout the study, *Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019 were used as quality control strains.

Statistical Analysis

The statistical analysis was performed using SPSS 31.0. Descriptive statistics was expressed using frequency and percentage. Heat map analysis was used to visually represent the detection rate and resistance patterns across different *Candida* species and antifungal agents.

Sample size

It was observed that 51% of carriage of oral *Candida* in cancer patients and 20% carriage of oral *Candida* in healthy individuals [20]. Based on this study, sample size was calculated as 200.

A predominance of *C. albicans* is seen, suggesting that it continues to be the most common etiological species seen in the setting of malignancy being treated. An increased number of non-*albicans* *Candida* species were also detected, further underscoring the importance of adequate species-level identification to direct appropriate antifungal therapy.



Figure 1: *Candida albicans* (light green), *Candida tropicalis* (blue), *Candida krusei* (pink to purple), *Candida glabrata* (cream), *Candida parapsilosis* (cream to mauve) and *Candida guilliermondii* (pink) showing distinct coloured colonies on HiCrome Candida agar.

Table 2: Interpretations of various antifungal agents

Antifungal Agent	Disc concentration	Diameter of zone of inhibition (mm)	
		Sensitive	Resistant
Fluconazole	10mcg	≥19mm	≤14mm
Itraconazole	10mcg	≥17mm	≤13mm
Clotrimazole	10mcg	≥20mm	≤11mm
Miconazole	30mcg	≥26mm	≤22mm
Amphotericin B	20mcg	≥15mm	≤10mm
Nystatin	50mcg	≥17mm	≤13mm

Zone interpretation was done using CLSI guidelines. Antifungal agents that do not have CLSI established breakpoints, interpretations were made using epidemiological cut off values proposed in previously published articles for analysis [24-26]. The zone diameters obtained were within the acceptable range provided by manufacturers and thus validated the test.

Table 3: Heat Map Representation of Antifungal Susceptibility Patterns (%) Among Different *Candida* Species from malignant patients

Detection rate	<i>Candida albicans</i>	<i>Candida tropicalis</i>	<i>Candida parapsilosis</i>	<i>Candida krusei</i>	<i>Candida glabrata</i>	<i>Candida guilliermondii</i>
Fluconazole(10mcg)	77.8%	33.3%	75.0%	0.0%	0.0%	100.0%
Itraconazole (10mcg)	44.4%	83.3%	75.0%	0.0%	0.0%	100.0%
Miconazole (30mcg)	94.4%	66.7%	87.5%	50.0%	100.0%	100.0%
Clotrimazole (10mcg)	83.3%	66.7%	75.0%	100.0%	100.0%	100.0%
Amphotericin B (20mcg)	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Nystatin (50mcg)	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Among cancer patients, Amphotericin B and Nystatin showed complete susceptibility (100%) against all *Candida* species tested. The azole drugs were highly susceptible in *Candida albicans* (94.4%) and *Candida parapsilosis* (87.5%), and the maximum susceptibility for *Candida tropicalis* with Itraconazole was 83.3%. *Candida krusei* were not susceptible to Fluconazole and Itraconazole (intrinsic resistant to fluconazole). *C.guilliermondii* was completely sensitive (100%) to all the antifungal agents (Table 3).

Table 4: Heat Map Representation of Antifungal Resistance Patterns (%) Among Different *Candida* Species

Resistance	<i>Candida albicans</i>	<i>Candida tropicalis</i>	<i>Candida parapsilosis</i>	<i>Candida krusei</i>	<i>Candida glabrata</i>	<i>Candida guilliermondii</i>
Fluconazole (10mcg)	22.2%	66.7%	25.0%	100.0%	100.0%	0.0%
Itraconazole	55.6%	16.7%	25.0%	100.0%	100.0%	0.0%

(10mcg)						
Miconazole (30mcg)	5.6%	33.3%	12.5%	50.0%	0.0%	0.0%
Clotrimazole (10mcg)	16.7%	33.3%	25.0%	0.0%	100.0%	0.0%
Amphotericin B (20mcg)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Nystatin (50mcg)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 4 shows the antifungal resistance profile of different *Candida* species. The highest resistance was seen in *Candida krusei* with 100% resistance to Fluconazole & Itraconazole and *Candida glabrata* developed 100% resistance to Fluconazole, Itraconazole and Clotrimazole. *Candida tropicalis* was also found to be resistant to Fluconazole (66.7%), and *Candida albicans* was resistant to Itraconazole (55.6%). Generally, the resistance levels were lower to Miconazole and Clotrimazole. Interestingly, there was no resistance against Amphotericin B and Nystatin in any of the *Candida* species tested, indicating that they are active against a wide spectrum of fungi and have excellent antifungal activity.

Table 5: Overall Antifungal Susceptibility Rate Pattern (%) in *Candida albicans* and Non-*albicans Candida*

Detection rate	<i>Candida albicans</i>	Non- <i>albicans Candida</i>
Fluconazole (10mcg)	78.0%	50.0%
Itraconazole (10mcg)	44.0%	66.7%
Miconazole (30mcg)	94.0%	77.8%
Clotrimazole (10mcg)	83.0%	72.2%
Amphotericin B (20mcg)	100.0%	100.0%
Nystatin (50mcg)	100.0%	100.0%

Table 5 shows that there was no notable difference between the overall antifungal susceptibility patterns of strains of *C. albicans* and non-*albicans Candida*. *Candida albicans* isolates were more susceptible to Miconazole (94%), Clotrimazole (83%), Fluconazole (78%) and Non-*albicans Candida* to Miconazole (77.77%), Clotrimazole (72.22%) and Itraconazole (66.66%) than *Candida albicans* (44%). There was no difference in susceptibility to azole antifungals, but susceptibility to the polyene antifungals was not affected.

Table 6: Overall Antifungal Resistance Pattern (%) in *Candida albicans* and Non-*albicans Candida*

Resistance	<i>Candida albicans</i>	Non- <i>albicans Candida</i>
Fluconazole (10mcg)	22.0%	50.0%
Itraconazole (10mcg)	55.5%	33.3%
Miconazole (30mcg)	5.5%	22.2%
Clotrimazole (10mcg)	16.6%	27.8%
Amphotericin B (20mcg)	0.0%	0.0%
Nystatin (50mcg)	0.0%	0.0%

Table 6 shows the overall patterns of antifungal resistance of *Candida albicans* and non-*albicans Candida* isolates. Non-*albicans Candida* were more resistant to Fluconazole than *Candida albicans* (50.0% vs. 22.0%), suggesting that the non-*albicans* species are less susceptible to Fluconazole. In case of Itraconazole, resistance was more apparent in *Candida albicans* (55.5%) than in non-*albicans Candida* (33.33%).

The resistance to Miconazole and Clotrimazole was relatively low, but slightly higher in non-*albicans Candida* isolates. Most importantly, no resistance was found against Amphotericin B and Nystatin in either group, which shows that they are effective and consistent against all the tested isolates.

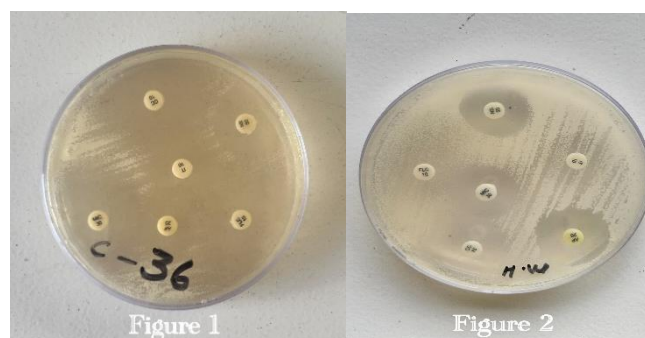


Figure 2: AST plate showing 100% sensitivity to all the antifungal agents (1) and *Candida* species showing resistance to Fluconazole and Itraconazole (2).

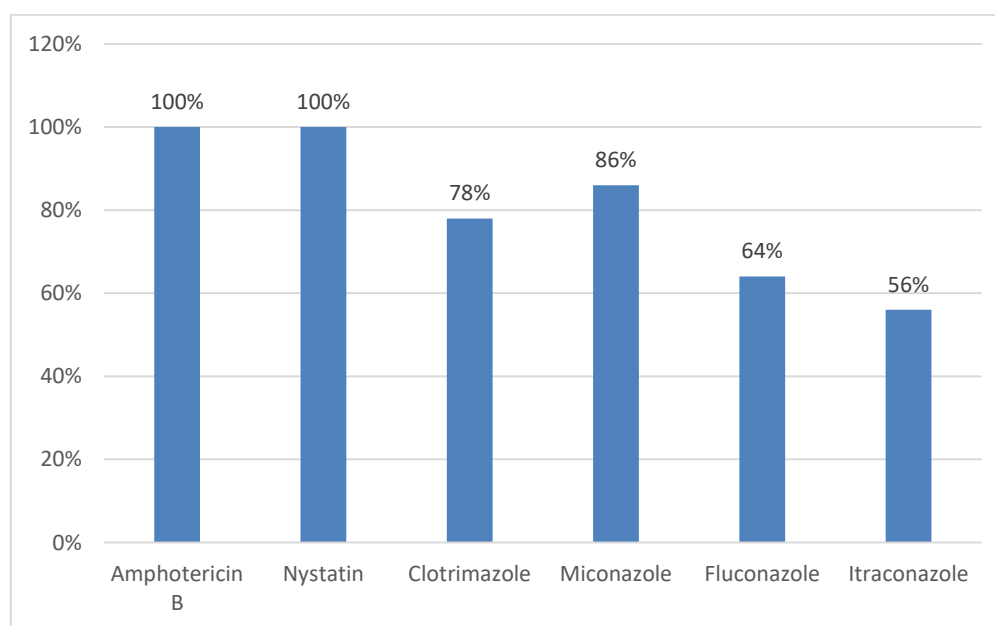


Figure 3: Antifungal Susceptibility of *Candida* Species

Complete susceptibility (100%) was observed for Amphotericin B and Nystatin. Miconazole was most susceptible (86%), followed by Clotrimazole at 78%. Lower susceptibility was observed for Fluconazole (64%) and Itraconazole (56%). In general, Amphotericin B and Nystatin showed the greatest antifungal activity, while susceptibility to the azole antifungal agents were comparatively low (fig 3).

In the control group, all the *Candida* species showed 100% sensitivity to all the antifungal agents.

DISCUSSION

Oral candidiasis is an opportunistic fungal infection that is common in those with malignancy and is often linked to changes in the oral microbiota and/or an impaired immune response due to cancer treatment. The predominant species identified in the present study was *Candida albicans* using HiCrome Candida agar. This finding agrees with other studies in which *C. albicans* was the most common *Candida* species isolated from the mouths of cancer patients [17,18]. However, the presence of a significant number of non-*albicans* *Candida* species, particularly *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. guilliermondii* was also detected. Varying antifungal susceptibility profiles and the ability to cause treatment failure have been documented for non-*albicans* *Candida* species, which are clinically important and have been increasingly identified worldwide [18]. HiCrome agar

is still useful as a screening tool; however, it is rapid, inexpensive, and easily used in the routine diagnostic laboratory. The results of the present study indicate that chromogenic media may be used as good preliminary identification tools only. For accurate species level identification, molecular methods like PCR may be recommended.

The present study also showed that there were substantial differences in the susceptibility to antifungal agents between the *Candida* species. The results showed that all isolates were fully susceptible to Amphotericin B and Nystatin, and still these polyene antifungals are highly effective against *Candida albicans* and non-*albicans* *Candida* species. This is consistent with previous studies on the antifungal susceptibility profile of oral *Candida* isolates [19]. Susceptibility to the azole antifungal agents, on the other hand, was found to be quite

variable among species. The susceptibility was high for *Candida albicans* and low for *Candida tropicalis* with Miconazole and Clotrimazole. The susceptibility was high for *Candida albicans* and low for *Candida tropicalis* with Fluconazole. The observations highlight the need to identify the species before starting any antifungal treatment. A single isolate of *C. guilliermondii* was pan-susceptible.

A particular resistance pattern for *Candida krusei* was also revealed. The species was completely resistant to Fluconazole and Itraconazole, as *C. krusei* which has intrinsic resistance to Fluconazole [18]. In addition, other non-*albicans* *Candida* species were also found to be resistant to Fluconazole than *C. albicans*. Recent studies have reported similar findings and suggest a rise in azole resistance among non-*albicans* *Candida* species obtained from immunocompromised patients [16,18]. This new resistance pattern could make empirical antifungal therapy more difficult and highlight the need for regular antifungal susceptibility testing in oncology clinics.

The large proportion of *C. albicans*, the rising prevalence of non-*albicans* *Candida* species, and the diversity of antifungal susceptibility patterns emphasize the need for *C. albicans* species identification and susceptibility testing. This is because Amphotericin B and Nystatin have been shown to remain effective on a long-term basis, and the growing resistance to azole antifungals highlights the importance of ongoing monitoring of antifungal resistance in the context of malignancy treatment.

STRENGTHS OF THE STUDY

1. The study included both patients with malignancy and healthy controls, allowing comparison of oral *Candida* carriage and species distribution between the two groups.
2. The study evaluated multiple clinically relevant *Candida* species rather than focusing only on *Candida albicans*, providing a broader understanding of non-*albicans* *Candida* species among patients with malignancy.
3. Antifungal susceptibility testing was performed for multiple commonly used antifungal agents, providing clinically relevant information regarding susceptibility and resistance patterns.
4. The use of quality control strains, including *Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019, strengthened the reliability and quality assurance of the antifungal susceptibility testing.
5. The study combined species identification with antifungal susceptibility profiling, which provides useful information for laboratory diagnosis and antifungal stewardship in oncology settings.
6. The study was conducted using standardized laboratory procedures and included ethical approval and informed consent from all participants.

LIMITATIONS OF THE STUDY

1. The study was conducted at a single centre, which may limit the generalizability of the findings to other geographical regions and healthcare settings.
2. The cross-sectional study design does not allow assessment of temporal changes in *Candida* colonization or establishment of causal relationships between cancer treatment and antifungal susceptibility patterns.
3. Species identification was based primarily on colony colour and morphology using HiCrome *Candida* agar. As acknowledged in the study, this provides presumptive identification and may not reliably differentiate closely related *Candida* species or mixed infections.
4. Longitudinal follow-up was not performed; therefore, changes in *Candida* species distribution or antifungal resistance during the course of cancer treatment could not be assessed.

CONCLUSION

According to the current study, the most frequent species found in oral collections of individuals undergoing cancer treatment was *Candida albicans*. However, non-*albicans* *Candida* species were also discovered albeit in considerable quantities, including *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, *C. glabrata* and *C. guilliermondii*. The vulnerability of these species to antifungals, which varies depending on the species, makes them clinically significant. While HiCrome agar is an inexpensive, rapid and convenient preliminary identification method, which is often used in the lab, it has limitations in differentiating closely related species and mixed *Candida* species. Molecular methods can be combined for use, and these methods might help improve the routine clinical diagnosis.

While susceptibility to azole antifungal drugs varied by species, all *Candida* isolates were fully sensitive to Amphotericin B and Nystatin. Sensitivity to Fluconazole and Itraconazole was very less by the *Candida* species. Overall, the study emphasises the importance of accurate species identification, appropriate use of antifungals and routine testing for antifungal susceptibility to effectively treat oral *Candida* infections in cancer patients. In clinical laboratories, the application of molecular diagnostic assays may facilitate prompt diagnosis and ultimately improve patient care and treatment results.

DECLARATIONS

Ethics Approval and Consent to Participate

The study was conducted in accordance with applicable ethical principles and was approved by the Institutional Ethics Committee of AJ Institute of Medical Sciences and Research Centre, Mangalore, Karnataka, India (Approval No. AJEC/REV/215/2024). Written informed consent was obtained from all participants before enrolment.

Participant information was collected and analysed anonymously to maintain confidentiality.

Consent for Publication

Written informed consent was obtained from the participants for publication of the study findings in an anonymised form. No personally identifiable information is reported in this manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author Contributions

All authors contributed to the conception and design of the study, data collection, laboratory investigation, analysis and interpretation of the findings, manuscript preparation, and critical revision of the manuscript. All authors read and approved the final version of the manuscript.

Data Availability Statement

The data generated and/or analysed during the present study are available from the corresponding author on reasonable request, subject to appropriate ethical and confidentiality considerations.

Clinical Trial Registration

Not applicable. This was a cross-sectional study and did not involve a clinical trial.

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REFERENCES

1. Andersen KM, Kristoffersen AK, Ingebreetsen A, Vikholt KJ, Örtengren UT, Olsen I, *et al.*, Diversity and antifungal susceptibility of Norwegian *Candida glabrata* clinical isolates. *Journal of Oral Microbiology*. 2016 Jan 1;8(1):29849.
2. Monsen RE, Kristoffersen AK, Gay CL, Herlofson BB, Fjeld KG, Hove LH, *et al.*, Identification and susceptibility testing of oral candidiasis in advanced cancer patients. *BMC Oral Health*. 2023 Apr 18;23(1):223.
3. Chitapanarux I, Wongsrita S, Sripan P, Kongsupapsiri P, Phakoetsuk P, Chachvarat S, *et al.*, An underestimated pitfall of oral candidiasis in head and neck cancer patients undergoing radiotherapy: an observation study. *BMC oral health*. 2021 Jul 16;21(1):353.
4. Taylor M, Brizuela M, Raja A. Oral Candidiasis. 2023 Jul 4. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. PMID: 31424866.
5. Richardson JP, Moyes DL, Ho J, Naglik JR. *Candida* innate immunity at the mucosa. *Semin Cell Dev Biol*. 2019; 89:58–70.
6. Lewis MAO, Williams DW. Diagnosis and management of oral candidiasis. *Br Dent J*. 2017;223(9):675–81.
7. Doppalapudi R, Vundavalli S, Prabhat MP. Effect of probiotic bacteria on oral *Candida* in head and neck-radiotherapy patients: A randomized clinical trial. *J Cancer Res Ther*. 2020;16(3):470–7.
8. Wu VW, Leung KY. A review on the assessment of radiation induced salivary gland damage after radiotherapy. *Frontiers in Oncology*. 2019 Oct 17; 9:1090.
9. Shrestha M, Boaz K, Srikant N, Shakya A. An assessment of *Candidal* colonization and species differentiation in head and neck cancer patients receiving radiation. *J Nepal Health Res Counc*. 2014; 12:156–61.
10. de Lima AF, Fagundes VL, Marques NB, Borba HL, Domingos EL, Tonin FS, *et al.*, The efficacy and safety of antifungal agents for managing oral candidiasis in oncologic patients: A systematic review with network meta-analysis. *Cureus*. 2024;16(9): e69340.
11. Chitapanarux I, Wongsrita S, Sripan P, Kongsupapsiri P, Phakoetsuk P, Chachvarat S, *et al.*, An underestimated pitfall of oral candidiasis in head and neck cancer patients undergoing radiotherapy: an observation study. *BMC oral health*. 2021 Jul 16;21(1):353.
12. Morita I, Morioka H, Abe Y, Nomura T, Nakashima S, Sugiura I, *et al.*, Discordance between hypo salivation and xerostomia among community-dwelling older adults in Japan. *PLoS One*. 2023 Mar 3;18(3): e0282740.
13. So JS, Chung SC, Kho HS, Kim YK, Chung JW. Dry mouth among the elderly in Korea: A survey of prevalence, severity, and associated factors. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;110(4):475–83.
14. Turner MD. Hyposalivation and xerostomia: etiology, complications, and medical management. *Dental Clinics*. 2016 Apr 1;60(2):435-43.
15. Tarapan S, Matangkasombut O, Trachootham D, Sattabanasuk V, Talungchit S, Paemuang W, *et al.*, Oral *Candida* colonization in xerostomic post radiotherapy head and neck cancer patients. *Oral diseases*. 2019 Oct;25(7):1798-808.

16. Zhou PR, Hua H, Liu XS. Quantity of *Candida* colonies in saliva: a diagnostic evaluation for oral candidiasis. *Chin J Dent Res*. 2017 Jan 1;20(1):27-32.
17. Anita, Kumar S, Pandya HB. Speciation and antifungal susceptibility testing of *Candida* isolated from immunocompromised patients of a tertiary care centre in Gujarat, India. *J Pure Appl Microbiol*. 2023;17(1):427–33.
18. Eddouzi J, Lohberger A, Vogne C, Manai M, Sanglard D. Identification and antifungal susceptibility of a large collection of yeast strains isolated in Tunisian hospitals. *Med Mycol*. 2013;51(7):737–46.
19. Alkhars N, Gaca A, Zeng Y, Jallad NI, Rustchenko E, Wu TT, *et al.* Antifungal susceptibility of oral *Candida* isolates from mother-infant dyads to nystatin, fluconazole, and caspofungin. *J Fungi (Basel)*. 2023;9(5):580.
20. Sankari L, Mahalakshmi K, Kumar VN. Chromogenic medium versus PCR-RFLP in the speciation of *Candida*: A Cross-sectional study. *BMC Res Notes*. 2019; 12:681.
21. Mohammed S, Shekhany K, Jalal P, Fattah C. Identification and genotyping of candida species involved in oral candidiasis among diabetic patients. *Sulaimani Dental Journal*. 2022 Jun 1;9(1):9.
22. Abharian PH, Dehghan P, Hassani-Abharian P, Jabalameli Z. Frequency of *Candida* species in the oral cavity of narcotics and stimulants smokers in Isfahan, using polymerase chain reaction-restriction fragment length polymorphism method. *Advanced biomedical research*. 2020 Jan 1;9(1):30.
23. Daef E, Moharram A, Eldin SS, Elsherbiny N, Mohammed M. Evaluation of chromogenic media and semi nested PCR in the identification of *Candida* species. *Brazilian Journal of Microbiology*. 2014; 45:255-62.
24. Gupta J, Shah H. Isolation, Speciation and antifungal drug susceptibility of *Candida* species from clinically suspected infections in a tertiary care hospital. *Trop J Path Micro* 2016;2(3):183-191.
25. Ali M, Edrees WH, Al-Shehari WA, Xue G, Al-Hammadi S, Qasem EA, *et al.*, Antifungal susceptibility pattern of *Candida* species isolated from pregnant women. *Front Cell Infect Microbiol*. 2024 Aug 7; 14:1434677.
26. Kumar D, Bhattacharyya S, Gupta P, Banerjee G, Singh M. Comparative Analysis of Disc Diffusion and E-test with Broth Micro-dilution for Susceptibility Testing of Clinical *Candida* Isolates Against Amphotericin B, Fluconazole, Voriconazole and Caspofungin. *J Clin Diagn Res*. 2015 Nov;9(11): DC01-4.