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Species distribution and antifungal susceptibility profiles of oral *Candida* isolates from immunosuppressed cancer patients in maysan governorate

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Abstract
Background: Oral candidiasis is one of the most prevalent opportunistic fungal infections among immunocompromised cancer patients.
Objective: The aim of this study was to assess the distribution and in *vitro* antifungal susceptibility patterns of oral *Candida* species isolated from immunocompromised cancer patients at southern Iraq.
Methodology: This study involved a prospective cross-sectional review of 172 cancer patients receiving chemotherapy while admitted in a medical Centre between June to October 2023. Methods: Oropharyngeal swabs were cultured on Sabouraud Dextrose Agar and *Candida* Chromogenic Agar. Identification of *Candida albicans* was established with the germ tube test and antifungal susceptibility testing (AFST). We evaluated susceptibility to fluconazole, voriconazole, caspofungin, micafungin, amphotericin B and flucytosine.
Results: Five species of *Candida* were detected. The most prevalent isolate was *C. albicans* at 59.9% followed by *C. glabrata* (16.9%), *C. parapsilosis* (12.2%), *C. tropicalis* (7.6%) plus a total of 3,5% with result for the both latter species group identified as *C. krusei*. The overall susceptibility rates to amphotericin B, flucytosine, caspofungin, voriconazole, micafungin and fluconazole were 78.5%, 72.7%, 70.9%, 70.3%, 66.9% and a lowest rate of 53.5%. Fluconazole, voriconazole, caspofungin and flucytosine exhibited significantly different susceptibilities according to the species of *Candida* (all $p<0.05$). This was most clearly the case for *C. glabrata* and *C. krusei* isolates, which had low sensitivity to fluconazole.
Conclusion: *C. albicans* is the most common species isolated, but a considerable prevalence of non-albicans *Candida* species with high azole resistance represents a clinical challenge among immunosuppressed cancer patients.

Keywords: Oral candidiasis, non-albicans *Candida*, antifungal susceptibility, cancer patients

Introduction

Among factors contributing to human health are the emergence of fungal infections that have become a major global public health problem with millions of people affected every year, and many contribute to morbidity or mortality. These infections are more common in immunocompromised patients such as patients on chemotherapy, organ transplant recipients and with chronic diseases. Notably, opportunistic fungal pathogens, mainly *Candida* species are among the major etiological agents of these infections in clinics. Some may colonize human mucosal surfaces, and under certain conditions cause disease, classifying them as significant medical pathogens [1]. *C. albicans* is still the most common pathogen among the various *Candida* species. Normally a commensal organism of the mucosal surfaces in the oral cavity, gastrointestinal tract and genitourinary system, disturbances of host immunity or alterations in normal microbial flora can readily convert *C. albicans* from a populating organism to an opportunistic pathogen. Clinical forms of candidiasis vary from localized mucosal infections, such as oral candidiasis, to severe invasive infections of the bloodstream and internal organs, which elicit high mortality rates [2]. The most common type of yeast that is responsible for the majority of the worldwide infections. Although many different species of microorganisms live the life of commensals or endosymbionts as innocuous partners with their hosts (e.g. humans), some forms still have the potential to exist parasitically under certain circumstances, so are potentially harmful. These are called opportunistic infections. *Candida*, a fungus found on different mucosal surfaces especially of skin and the digestive system. Candidiasis, commonly called thrush, is an infection with *C. albicans*, the species of *Candida* most frequently isolated. Some species of *Candida* can spoil the process of winemaking [3]

Epidemiological observations have recently shown an increase in non-albicans *Candida* species, such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*. Such species are of specific concern due to their often-reduced susceptibility to antifungal agents in common clinical use. Nevertheless, *C. albicans* still represents the vast majority of clinical isolates and is the most common cause for oral and gastrointestinal candidiasis in those patients with reduced immune function [4]. Among the populations of individuals most at risk to fungal infections are cancer patients. Endogenous malignancy and anticancer treatment modalities, for example, chemotherapy and radiotherapy can lead to immune dysfunction and breach of the mucosa. Moreover, other factors, such as an extended period of hospitalization, broad-spectrum antibiotics usage and oncological care (e.g. corticosteroid therapy or invasive procedures) presented an additional risk for fungal colonization and infection. Candidiasis is thus a common clinical event in cancer patients, and can lead to delays in treatment, longer hospital stays, excess health care costs,

and deleterious effects on the overall outcome [5]. The ability of *C. albicans* to cause disease can largely be attributed to its virulence factors. Morphological transitions between yeast and hyphal forms, which are fundamental to its overall pathogenesis regulation, is perhaps the most important characteristic. Such morphological plasticity allows the organism to grow successfully in diverse host environments, invade tissues and evade immune responses. *C. albicans* also has specialized adhesion molecules to help it adhere to human tissues and medical devices [6], which aids colonization and pathogenesis.

Biofilm formation is another significant virulence factor of *C. albicans*. Biofilms are structured microbial communities that are protected from the environment as well as antifungal agents by an extracellular matrix. Among these, cells growing in biofilms show increased resistance to antifungal therapy and host immune defenses rendering them particularly difficult to treat thus biofilm associated infections are very hard to eradicate. In addition, *C. albicans* secretes hydrolytic enzymes, such as proteinases and phospholipases that aid in tissue invasion as well as host cell injury and dissemination [7]. While antifungal drugs for clinical use can be classified as azoles, polyenes and echinocandins, the incidence of antifungal resistance has been a growing problem on global level. These mechanisms of resistance that include changes in the drug targets themselves, overexpression of efflux pumps and general biofilm mediated protection reduce treatment efficacy, which makes clinical management more complicated. Thus, Antifungal susceptibility surveillance is needed to inform adequate therapeutic strategies and improve patient outcomes [8]. The clinical relevance of candidiasis in immunocompromised patients, particularly cancer patients, necessitates genus and species identification of these fungi followed by determination of antifungal susceptibility profiles. Examining the epidemiology and distribution of *Candida* isolates, including virulence characteristics and resistance patterns will yield useful information pertinent to infection control strategies, guidance on empirical therapy of invasive infections, as well as inform future antifungal interventions [9]. The aim of this study was to assess the distribution and in *vitro* antifungal susceptibility patterns of oral *Candida* species isolated from immunocompromised cancer patients at southern Iraq.

Methodology

Study Design

This was a prospective, cross-sectional observational study to determine the prevalence, species distribution and antifungal susceptibility profiles of oropharyngeal *Candida* isolates among cancer patients.

Study Population and Setting

The duration of the study was six months, January 2025 to June 2025. Patients and methods: The clinical group included cancer patients who were hospitalized for immunosuppressive chemotherapy at Al-Shifaa Oncology Center, part of the Maysan Health Directorate, Iraq. Inclusion criteria patients were included based on the epidemiological situation in Al-Bahadily *et al.* [5], and specific inclusion criteria, including confirmed malignancy diagnosis (solid tumors, hematologic tumor), ongoing hospital stay at the survey time point, active treatment with chemotherapeutic regimens, clinical suspicion or manifestation of associated opportunistic fungal infections in a subset of cases (oropharyngeal thrush). In order to avoid masking fungal growth or changing susceptibility profiles, patients were also excluded if they received systemic or topical antifungal prophylaxis or treatment up to 2 weeks prior to sampling. Of these, 172 patients were eligible for inclusion in final analysis. Demographic characteristics including age, gender and underlying malignancies were systematically documented.

Materials, Reagents, and Equipment

All chemicals, culture media and laboratory instruments used in this study were of analytical or microbiological grade, obtained from leading international manufacturers.

1. Culture Media and Diagnostic Reagents

(Table 1) Culture media and reagents used in Nodules for the isolation, purification and presumptive identification of *Candida* species.

Table 1: Culture media and diagnostic reagents

No.	Item	Purpose	Manufacturer	Origin
1	Sabouraud Dextrose Agar (SDA)	Primary isolation and purification	HiMedia Laboratories	India
2	*Candida* Chromogenic Agar	Presumptive species identification	HiMedia Laboratories	India
3	Human Serum	Germ tube test for *C. albicans*	Local Blood Bank	Iraq
4	Vitek 2 AST-YS08 Cards	Antifungal susceptibility testing	bioMérieux	France
5	Normal Saline (0.9% NaCl)	Inoculum preparation	Pioneer Pharmaceuticals	Iraq
6	McFarland Standard (0.5)	Turbidity standardization	bioMérieux	France

2. Laboratory Instruments and Consumables

The primary laboratory instruments and general consumables employed during the experimental procedures are summarized in (Table 2). All glassware was of borosilicate composition (Pyrex, UK).

Table 2: Laboratory instruments and consumables

No.	Instrument/Consumable	Model/Type	Manufacturer
1	Automated Identification System	Vitek 2 Compact	bioMérieux, France
2	Microbiological Incubator	IN55	Memmert, Germany
3	Autoclave Sterilizer	HV-50	Hirayama, Japan
4	Light Microscope	CX23	Olympus, Japan
5	Analytical Balance	CP224S	Sartorius, Germany
6	Sterile Cotton Swabs	Without transport gel	Citotest, China
7	Sterile Petri Dishes	90 mm, disposable	Citotest, China

Clinical Sample Collection and Transportation

A trained health care worker obtained an oropharyngeal specimen from the enrolled cancer patients. Specimens were collected with sterile and dry cotton swabs without transport gel. Swabs were applied to the buckling mucosa, palate, and any visible white pseudomembranous plaques or erythematous lesions consistent with oral thrush in a firm

but gentle fashion. After collection, swabs were immediately inserted into sterile tubes and sent to the microbiology lab of the mentioned center, with a time frame not exceeding 30 min to preserve viability of viable fungal cells [10, 11].

Microbiological Procedures

1. Primary Isolation and Cultivation

At the laboratory, the clinical swabs were inoculated directly on Sabouraud Dextrose Agar (SDA) plates. SDA was supplemented with chloramphenicol to inhibit the outgrowth of commensal oral bacteria. A microbiological incubator (Memmert, Germany) at 37 °C for 48 to 72 h was used to aerobic grow in the case of inoculated plates. Characteristic yeast-like colonies are apparently visible on the plates inspected daily, typical pleased with sorted smoothness-creamy- convex [12, 13].

2. Phenotypic Identification of *Candida* Species

Identification of the isolated *Candida* strains was performed presumptively by the combination of chromogenic media and morphological procedures.

2.1. Chromogenic Agar Method

Isolated and distinct yeast colonies from one of the primary SDA plates were subcultured on *Candida* Chromogenic Agar (HiMedia, India) for species differentiation according to colony pigmentation. The plates were then incubated aerobically at 37°C for two days. The species were presumptively identified based on the color chart furnished by the manufacturer: *C. albicans* forms pale green colonies, *C. tropicalis* blue to bluish metallic colonies and so forth (*C. glabrata* cream to white colonies; *C. krusei* pink -fuzzy colonies or clumps; *C. parapsilosis* grows pale- green to cream-colonies [14, 15].

2.2. Germ Tube Test

Germ tube test was done to further establish the presence of *C. albicans*. One hundred (100) µL of a pure yeast colony was emulsified in 0.5 mL of human serum in a sterile test tube. The suspension was maintained at 37 °C for exactly 2.5 to 3 h. A drop of suspension was put on a glass slide, and covered with a coverslip after incubation for the microscopic examination using light microscope (Olympus, Japan) at 40x magnification. The emergence of the short, slender, tube-like projections that arise from the yeast cell without any constriction at the point of origination (germ tubes) was considered a positive confirmatory test for *C. albicans* [16].

3. Antifungal Susceptibility Testing

In *vitro* antifungal susceptibility profiles of the confirmed *Candida* isolates were determined against fluconazole, voriconazole, and amphotericin B using the Vitek 2 Compact automated system (bioMérieux, France) with AST-YS08 yeast susceptibilities cards. This method performs Minimal Inhibitory Concentrations (MICs) for a panel of antifungal agents including Fluconazole, Voriconazole, Caspofungin, Micafungin, Amphotericin B, Flucytosine and Nystatin. A yeast suspension containing standardized CFUs was prepared by inoculating morphologically similar colonies from a 24-h pure culture into 3.0 mL of sterile 0.45% aqueous sodium chloride solution. Using a DensiCHEK Plus instrument, the turbidity of the suspension was standardized to that associated with an inoculum of 0.5 McFarland standard. The AST-YS08 card and the standardised suspension were next loaded into the Vitek 2 instrument. Filling and sealing of the cards along with incubation is automatically performed by the system with continuous monitoring of the growth kinetics. The Vitek 2 software automatically interpreted the MIC values and clinical categorizations as Susceptible, Susceptible-Dose Dependent, Intermediate or Resistant according to the most recent Clinical and Laboratory Standards Institute (CLSI) M27 guidelines [17, 18].

Statistical Analysis

For statistical analysis, all demographic and microbiological data were gathered together in a spreadsheet and analyzed using the Statistical Package for the Social Sciences (SPSS) software version 26.0 (IBM Corp., Armonk, NY, USA). Patient demographics, frequencies and percentages were obtained via descriptive statistics the distribution of using descriptive statistics to present patient demographics frequencies and percentage for different *Candida* species Antifungal susceptibility pattern was alsopresented using descriptive statistics. The Chi-square (χ^2) test or Fisher's exact test (when appropriate) was performed to determine the statistical significance of associations between qualitative variables, such as that between species of *Candida* and their resistance profiles. Statistical significance was defined as having a p-value less than 0.05.

Results and Discussion

Distribution of *Candida* Species among Cancer Patients

The current study retrieved 172 positive oropharyngeal swab samples from hospitalized cancer patients receiving immunosuppressive chemotherapy. Of these isolates, five distinct *Candida* species were identified phenotypically and biochemically. The distribution of the species is shown in (Table 3), and in (Figure1) *C. albicans* was the most common isolated species (59.9%; n=103). The second most common species were non-albicans *Candida* (NAC) with a total of 40.1% of all isolates. *C. glabrata* (16.9% [n=29]) was the most frequent isolate among NAC species, followed by *C. parasitosis* (12.2% [n=21]), *C. tropicalis* (7.6% [n=13]), and *C. krusei* 3-5%) (n = 6).

Table 3: Frequency and Percentage Distribution of *Candida* Species (n=172)

***Candida* Species**	**Number of Isolates (n)**	**Percentage (%)**
C. albicans	103	59.9%
C. glabrata	29	16.9%
C. parasitosis	21	12.2%
C. tropicalis	13	7.6%
C. krusei	6	3.5%
Total	172	100.0%

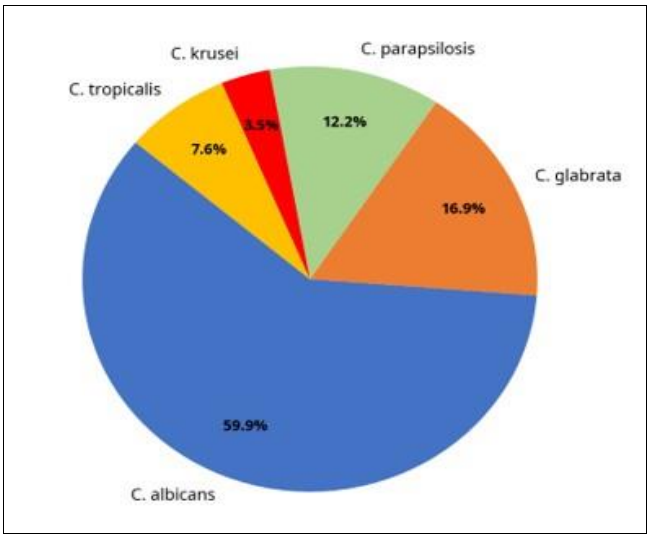

Fig 1: Distribution of *Candida* species among cancer patients (n=172)

Discussion on Species Distribution

The high frequency of *C. albicans* (59.9%) in the oral cavity of immunocompromised cancer patients is consistent with recent epidemiological data. A study by Alshahrani *et al*., reported the other similar trend, in which *C. albicans* was responsible for 64.8% of isolates from chemotherapy patients [19], our study in the eastern Iran (Units Data, Ara) the high isolation rate of *C. albicans* is mainly due to its powerful virulence factors, especially the germ tube-forming ability, strong adhesion to epithelial cells and a certain degree of reversible conversion between yeast and hyphal forms (the latter provides the conditions for tissue invasion in hosts with impaired mucosal immunity) [20]. Nonetheless, a notable result of this work is the increased proportion of non-albicans *Candida* species (40.1 %). Interestingly, the isolation of *C. glabrata* with a proportion of 16.9% as the second most common species is especially remarkable. This changing pattern of NAC species in oncology ward is a worrisome worldwide phenomenon. One study illustrated the consumption of NAC species in addition to *C. albicans* among patients with oropharyngeal candidiasis (OPC) [21]. In cancer patients, where azole antifungals are routinely and prophylactically used as long-term empirical treatment, the emergence of *C. glabrata* and less susceptible species such as *C. krusei* has been associated with selective suppression of *C. albicans* colonization in favor of intrinsically resistant or otherwise less susceptible NAC [22].

Antifungal Susceptibility Patterns

Antifungal susceptibility against six antifungals was tested in 172 *Candida* isolates using the Vitec 2 automated system in *vitro*. (Table 4) summarizes the results as the number and percentage of sensitive isolates and (Figures 2&3) visualizes these data.

Table 4: Antifungal Susceptibility Profile of ***Candida*** Isolates (Sensitive Counts and Percentages)

***Candida* Species (n)**	**Fluconazole**	**Voriconazole**	**Caspofungin**	**Amphotericin B**	**Micafungin**	**Flucytosine**
C. albicans (103)	71 (69%)	80 (78%)	82 (80%)	80 (78%)	74 (72%)	80 (78%)
C. glabrata (29)	1 (3%)	16 (55%)	16 (55%)	21 (72%)	17 (59%)	13 (45%)
C. parapsilosis (21)	13 (62%)	16 (76%)	14 (67%)	18 (86%)	14 (67%)	19 (90%)
C. krusei (6)	0 (0%)	3 (50%)	4 (67%)	6 (100%)	4 (67%)	4 (67%)
C. tropicalis (13)	7 (54%)	6 (46%)	6 (46%)	10 (77%)	6 (46%)	9 (69%)
Total (172)	92 (53.5%)	121 (70.3%)	122 (70.9%)	135 (78.5%)	115 (66.9%)	125 (72.7%)

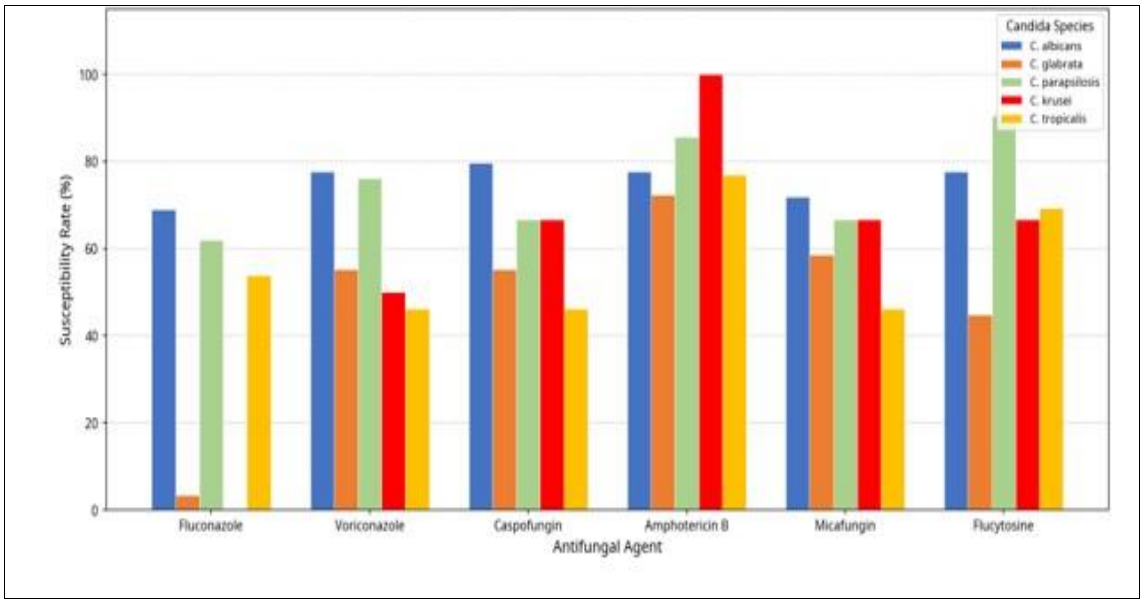

Fig 2: Antifungal susceptibility rates by *Candida* species

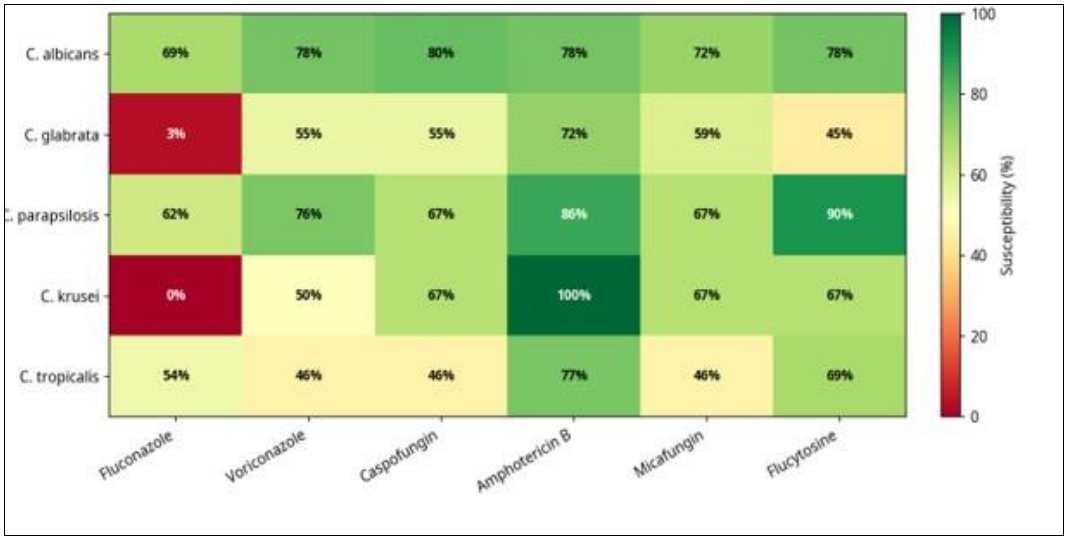

Fig 3: Susceptibility heat map (%) by species and antifungal agent

1. Overall Susceptibility and Statistical Significance

Overall, Amphotericin B showed the highest overall activity (78.5% susceptible among all isolates). Conversely, Fluconazole had the lowest total susceptibility rate at 53.5%. The susceptibility patterns were then compared across different *Candida* species using chi-square (χ^2) analysis. The individuals demonstrated a significant difference in resistance to fluconazole by species (χ^2=46.562, df=4, p<0.001). Accoutrements for Voriconazole (χ^2=11.032, p=0.026), Caspofungin (χ^2=11.366, p=0.023) and Flucytosine (χ^2=16.156, p=0.003) were also significantly different. There was not statistically significance difference in susceptibility to Amphotericin B (χ^2=2.987, p=0.560) and Micafungin (χ^2=4.560, p=0.336) of the different species also indicates a more uniform action of these agents against any type of *Candida* respectively.

2. Clinical Significance of Antifungal Resistance Profiles

2.1. Azole Resistance (Fluconazole and Voriconazole)

Perhaps the most worrying finding from this study is the fluconazole resistance rates, where they were apparently very high among these non-albicans species. *C. glabrata* showed near-total resistance while only 3% (1/29) of isolates were susceptible to *C. glabrata*. In fact, all (100%) *C. krusei* isolates were resistant to Fluconazole. Fluconazole susceptibility was only 69% even among dominant *C. albicans*. These observations are well corroborated by recent literature. A reduced affinity of its target enzyme, 14-α-demethylase [23] is the mechanism that gives *C. krusei* intrinsic (natural) resistance to Fluconazole. The strong resistance seen in *C. glabrata* (97% resistant) correlates with the fast and easy development of resistance mechanisms, which occur mostly by upregulation of membrane multidrug efflux transporters (CDR1, CDR2 and SNQ2). Study by Zamanian *et al.*, reported a higher rate (more than 73%) of azole resistance in oral *Candida* isolates from cancer patients, reiterating the decreasing clinical value of Fluconazole as the emergency first-line empiric therapy in oncology settings [24].

2.2. Echinocandins (Caspofungin and Micafungin)

Moderate efficacy as echinocandins which specifically targets the fungal cell wall synthesis (1,3-β-D-glucan synthase). Of note, caspofungin was active in 80% of *C. albicans* but only 55% and 46% of *C. glabrata* and *C. tropicalis* isolates, respectively. The total susceptibility rate to Micafungin was 66.9%. *C. parapsilosis* has reduced susceptibility to echinocandins (67% for both drugs), due to naturally occurring polymorphisms in the FKS1 gene of this species, which results in intrinsically higher Minimum Inhibitory Concentration (MIC) against this class of drugs [25, 27].

2.3. Amphotericin B and Flucytosine

Although amphotericin B (78.5% overall susceptibility) was still the most active agent against all strains (100% efficacy *C. krusei*, 86% *C. parapsilosis*), Result: There was a non-significant difference in susceptibility of all the species to amphotericin B (p=0.560), emphasising its broad-spectrum reliability. Flucytosine was active, but more notably active against *C. parapsilosis* (90% sensitive), than *C. albicans* (78% sensitive). Flucytosine is seldom employed as a single-agent therapy because secondary resistance occurs quickly; it is usually given in combination with Amphotericin B for acute systemic infections [28, 30].

Clinical Implications

These data have important clinical implications for the management of oropharyngeal candidiasis in cancer patients. The high proportion of non- albicans species, combined with their very high intrinsic resistance against Fluconazole, suggest that empirical azole therapy may fail clinically in large proportions of this patient population. Thus, routine species identification and antifungal susceptibility testing as in Vitek 2 system are crucial prior to starting therapy [5]. In the case of *C. glabrata* or *C. krusei* infections, an alternative class like Amphotericin B or the proper Echinocandins should be preferable [31, 33].

Conclusion

The current study proved that *C. albicans* is still the dominant oral candidal species in southern Iraq immunosuppressed cancer patients, but a large number of isolates have belonged to non-albicans *Candida* species specially *C. parapsilosis*, it has aroused alarm people, sometimes there may cause emerging opportunistic infection especially with rising rates using azole antifungal

medications in clinical practices which increases selection Cannon 90 resistant *Candida* creating new pathogenic strains Antifung Infect Dis.o with high antimicrobial resistancetrend;45863. This is clinically relevant because of these species having reduced susceptibility to widely used antifungal agents. Amphotericin B was the most active antifungal agent tested, with fluconazole being the least active, with significant resistance seen in *C. glabrata* and intrinsic resistance to fluconazole observed by *C. krusei*. Such results highlight obstacles to the practice of empirical fluconazole therapy in immunocompromised oncology patients. Routine identification of *Candida* species and antifungal susceptibility testing should be evaluated as a part of patient management strategy before starting treatment with an antifungal agent. This approach would enable the integration of evidence into therapeutic decision-making, prevent treatment failures, and ultimately lead to enhanced clinical outcomes.

Ethically Approval

The study protocol was ethically approved by the Ethical Review Committee, College of Pharmacy at University of Basrah (Approval No. UOB/CP/2025/ETH-042). Ethics statement the study was performed in strict accordance with the ethical principles of the declaration of Helsinki. All patients (or their legal representatives) gave written informed consent prior to sample collection, and explicit approval was provided by the attending oncologists.

Conflict of Interest

All authors acknowledge that there is no conflict of interest in this article.

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