

Molecular Identification of Candida Species and Evaluation of Antifungal Susceptibility Patterns among Clinical Isolates from Immunocompromised Patients

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Abstract

Background: Candida isolated from immunocompromised patients must be correctly identified as species because the activity and susceptibility interpretive criteria vary by species. Objective: To typify Candida isolates using internal transcribed spacer (ITS) sequencing, and to compare the identification with the standard phenotypic methods and to investigate species-specific antifungal susceptibility.

Methods: One isolate of Candida from each of 180 immunocompromised adults was used in a hospital-based cross sectional laboratory study. Colony morphology and biochemical identification were compared to ITS sequencing, while fluconazole, micafungin and amphotericin B MICs were determined by reference broth microdilution. Susceptibility categories were based on species appropriate criteria and where required, provisional guidance.

Results: Candida albicans accounted for 62/180 isolates (34.4%), followed by C. tropicalis (26.7%), C. parapsilosis (18.9%), C. glabrata (12.2%), C. auris (5.6%), and C. krusei (2.2%). Phenotypic and molecular species identification matched in 163/180 isolates (90.6%) with 6/10 C. auris isolates being misidentified or not resolved using the standard workflow. Fluconazole resistance was found in 40/180 isolates (22.2%) (9/10 C. auris and 12/48 C. tropicalis); micafungin resistance was found in 5/180 (2.8%). Higher fluconazole resistance was found in patients with prior azole exposure (36.8% vs 13.4%, $p < 0.001$). Molecular identification took 21.4 ± 5.9 hours compared with 44.6 ± 12.1 hours for routine identification ($p < 0.001$).

Conclusions: ITS-based species identification confirmed clinically relevant species identification including C. auris and assisted with susceptibility interpretation in an immunocompromised patient population.

Keywords: Candida; Immunocompromised Host; ITS Sequencing; Fluconazole; Micafungin; Antifungal Susceptibility; Candida Auris.

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Introduction

There are several overlapping risk factors for candidiasis in immunocompromised patients: disruption of mucosal barriers, central venous devices, prolonged hospital stay, cytotoxic chemotherapy, transplant-related immunosuppression, and broad-spectrum use of antibacterials.

The epidemiology of invasive Candida infections has also become more heterogeneous with the growing clinical importance of non-albicans species [1]. The significance of a positive laboratory result will vary based on the compartment sampled and the clinical status of the patient: Candida in blood or a normally sterile site is not the same as yeast in respiratory secretions or

urine. Therapeutic guidelines include the need to identify the fungus quickly, to control the source (where appropriate), and to choose the appropriate antifungal agent based on species identification and measured susceptibility [2]. The 2025 international guideline also highlights the emergence of new species, antifungal resistance and the importance of accurate diagnosis in all care settings [3].

Candida albicans is still the most common yeast, but other less common yeasts, such as C. tropicalis, C. parapsilosis, C. glabrata, and others, may have different susceptibility patterns and epidemiological associations [4]. Thus, a general diagnosis of "Candida spp." may mask clinically

relevant information. When phenotypic identification is inconclusive or rare or cryptic species are suspected, molecular fungal barcoding is a complementary approach. The internal transcribed spacer (ITS) region of the ribosome has been shown to be a universal fungal barcode [5]. Molecular techniques are especially important for the identification of *Candida auris*, as older biochemical databases and associated phenotypes may result in misidentification [6]. It is a global emerging pathogen, often resistant to fluconazole, and has the potential to be transmitted in healthcare settings [7].

Local isolate collections can also provide information on the percentage of fluconazole-resistant non-albicans isolates, such as fluconazole-resistant *C. parapsilosis*, which have been reported in surveillance in India [8]. The aim of the present laboratory study was to describe the distribution of species among *Candida* isolates from immunocompromised adults, compare the results of sequencing-based identification with the results of routine biochemical identification, and explore the antifungal MIC-based resistance patterns and their relationship with previous azole exposure.

Materials and Methods

A cross-sectional laboratory assessment was requested at a tertiary-care referral centre for 12 months. Eligible specimens included a *Candida* isolate from a patient ≥ 18 years of age who had a primary immunocompromising condition, such as hematological malignancy, solid-organ malignancy being treated, solid-organ transplantation, or sustained systemic corticosteroid/biologic immunosuppression. The first isolate from each patient was kept. Duplicate isolates, nonviable specimens, non-*Candida* yeasts and non-complete clinical records were not included. Mixed colonies were purified and tested separately.

Clinical parameters were age, sex, and immunocompromising status, admission to the intensive care unit, specimen type and use of systemic azoles in the last 90 days. Isolates from blood and from sterile sites were recorded separately from those from urine, respiratory and mucosal sites.

Isolation from nonsterile sites was not considered to be an invasive infection, and the investigation was not intended to determine causality of candidiasis, but rather organism identity and in vitro susceptibility. A sample of 180 isolates was chosen to provide a reasonable description of the prevalence of a species group commonly resistant to the drugs, and subgroup analyses were exploratory.

Culture, phenotypic methods and sequencing: Clinical specimens were cultured on Sabouraud

dextrose agar and chromogenic yeast medium and representative colonies were identified by microscopy and germ-tube testing as appropriate. The laboratory biochemical identification platform and the available identification database and confirmatory phenotypic tests were used for routine species identification. A fresh pure colony was used to extract fungal DNA from an independent technician who was blind to the phenotypic call. Conserved fungal primers ITS1 and ITS4 were used to amplify the ITS1-5.8S-ITS2 region, which was then sequenced in both directions using the Sanger sequencing method and quality checked for sequencing accuracy. Consensus sequences were compared to curated reference sequences, and a good species-level match and separation from the closest species was necessary for assignment. Sequences that were discordant or ambiguous were re-extracted and reamplified and, if required, sequenced in the D1/D2 ribosomal-region.

The time to identification was calculated from the time a pure colony was documented until the time it was confirmed as a species release. Discordance consisted of either incorrect identification or a genus level/inconclusive phenotypic result that was resolved by sequencing. Nomenclature was matched with the laboratory reference database for uncommon taxa and familiar clinical *Candida* names were used for clarity. Identification comparison was done in a blinded manner prior to MIC analysis.

Antifungal susceptibility testing and interpretation: Broth microdilution testing was conducted according to the CLSI M27 reference workflow with RPMI 1640 medium, reference quality-control strains, and 24-hour endpoints as appropriate. The MICs of fluconazole, micafungin and amphotericin B were reported in $\mu\text{g/mL}$. When no species-specific CLSI clinical breakpoints were available, the *C. glabrata* fluconazole results were classified as susceptible-dose dependent or resistant. *C. krusei* was considered intrinsically fluconazole resistant. Contemporary CDC tentative resistance thresholds were used for fluconazole ($\geq 32 \mu\text{g/mL}$), micafungin ($\geq 4 \mu\text{g/mL}$) and amphotericin B ($\geq 2 \mu\text{g/mL}$) for *C. auris*. Other species: If the MIC was $\geq 2 \mu\text{g/mL}$, it was considered an elevated MIC and not a clinical resistance category.

Statistical Analysis: The species and resistance frequencies were presented as n (%), and age and identification intervals as mean $\pm$ SD. Phenotypic identification agreement was determined with respect to the sequencing defined identity. Categorical comparisons were made by Pearson chi-square or Fisher exact testing based on the expected cell counts. An unadjusted odds ratio (95% CI) was used to compare previous azole exposure with fluconazole resistance, and a causal

interpretation was not intended because of the possibility of confounding between drug exposure and *Candida* species. Differences were assessed and compared using paired t-test for paired turnaround times. Two-sided significance level of 0.05 was used for analyses.

Results

The evaluated collection comprised 180 isolates from 180 immunocompromised patients (mean age,

49.8 ± 15.6 years; 102 male, 56.7%). Hematologic malignancy and ongoing treatment for solid malignancy were the two largest host categories, accounting together for 104 patients (57.8%). Seventy isolates (38.9%) originated from blood; the remainder came from urine, respiratory specimens, tissue/sterile fluids, and mucosal material.

A history of azole exposure in the prior 90 days was documented for 68 patients (37.8%) (Table 1).

Table 1: Patient characteristics and specimen distribution for *Candida* isolates (N=180)

Characteristic	Value
Mean age, years	49.8 ± 15.6
Male / female patients	102 (56.7%) / 78 (43.3%)
Hematological malignancy	60 (33.3%)
Solid malignancy receiving treatment	44 (24.4%)
Solid-organ transplantation	30 (16.7%)
Long-term corticosteroid/biologic therapy	46 (25.6%)
Admitted to intensive care	62 (34.4%)
Prior azole exposure within 90 days	68 (37.8%)
Blood / urine specimens	70 (38.9%) / 50 (27.8%)
Respiratory specimens	30 (16.7%)
Deep tissue or sterile body fluid	18 (10.0%)
Oral/esophageal specimens	12 (6.7%)

The four immunocompromising-condition categories are mutually exclusive primary classifications; specimen categories collectively include all 180 isolates.

ITS sequencing identified six *Candida* species. Non-albicans yeasts represented 118/180 isolates (65.6%), led by *C. tropicalis* (48 isolates) and *C. parapsilosis* (34). Routine phenotypic methods agreed with sequence-defined identity for 163

isolates (90.6%). Discordance was greatest for *C. auris* (6/10) and *C. glabrata* (5/22); *C. auris* was most commonly reported as a closely related yeast or left unresolved by routine biochemical identification.

Sequencing-based identification was completed in 21.4 ± 5.9 hours from pure colony versus 44.6 ± 12.1 hours with the phenotypic workflow (paired $p < 0.001$) (Table 2).

Table 2: Sequence-defined *Candida* species and agreement with conventional phenotypic identification

Sequence-defined species	Isolates, n (%)	Phenotypic correct, n	Agreement
<i>Candida albicans</i>	62 (34.4%)	61	98.4%
<i>Candida tropicalis</i>	48 (26.7%)	46	95.8%
<i>Candida parapsilosis</i>	34 (18.9%)	31	91.2%
<i>Candida glabrata</i>	22 (12.2%)	17	77.3%
<i>Candida auris</i>	10 (5.6%)	4	40.0%
<i>Candida krusei</i>	4 (2.2%)	4	100.0%
Total	180 (100.0%)	163	90.6%

ITS sequencing, with confirmatory D1/D2 analysis for ambiguous cases, supplied the reference species identity. A genus-level or inconclusive routine identification was classified as discordant.

Forty isolates (22.2%) met fluconazole resistance criteria including intrinsic *C. krusei* resistance. Resistance differed markedly across species (exact omnibus test, $p < 0.001$) and was particularly frequent in *C. auris* (9/10) and *C. tropicalis* (12/48).

Of 22 *C. glabrata* isolates, 6 were fluconazole resistant and the remaining 16 were susceptible-dose-dependent rather than categorically susceptible. Micafungin resistance was found in five isolates overall (2.8%).

Nine isolates exhibited amphotericin B MICs ≥ 2 $\mu\text{g/mL}$, including six *C. auris* isolates; only the *C. auris* MICs were interpreted using the specified tentative species threshold (Table 3).

Table 3: Antifungal resistance and elevated amphotericin B MIC by sequence-defined species

Candida species (n)	Fluconazole resistant, n (%)	Micafungin resistant, n (%)	Amphotericin B MIC ≥ 2 , n (%)
<i>C. albicans</i> (62)	5 (8.1%)	0 (0.0%)	1 (1.6%)
<i>C. tropicalis</i> (48)	12 (25.0%)	1 (2.1%)	0 (0.0%)
<i>C. parapsilosis</i> (34)	4 (11.8%)	0 (0.0%)	1 (2.9%)
<i>C. glabrata</i> (22)	6 (27.3%)	2 (9.1%)	1 (4.5%)
<i>C. auris</i> (10)	9 (90.0%)	2 (20.0%)	6 (60.0%)
<i>C. krusei</i> (4)	4 (100.0%)	0 (0.0%)	0 (0.0%)
Total (180)	40 (22.2%)	5 (2.8%)	9 (5.0%)

Fluconazole-resistant *C. krusei* represents intrinsic resistance; 16 additional *C. glabrata* isolates were susceptible-dose-dependent (not susceptible). *C. auris* categories use tentative CDC criteria. Amphotericin B MIC ≥ 2 for other *Candida* is descriptive, not a uniform resistance breakpoint.

Fluconazole resistance occurred in 25/68 previously azole-exposed patients (36.8%) compared with 15/112 without such exposure (13.4%). The association was significant by Fisher exact test ($p=0.00039$; unadjusted odds ratio 3.76; approximate 95% CI 1.80-7.84), although species composition, indication for prophylaxis, and prior healthcare exposure may explain part of this association. No patient survival endpoint was evaluated; species recovered from nonsterile compartments were not presumed to represent proven invasive infection.

Discussion

The species-level results show that molecular identification may be of significant value to a traditional clinical mycology workflow in immunocompromised patients. Non-*albicans* *Candida* accounted for nearly two-thirds of the isolates and a clinically relevant sub-group was identified by sequencing that had not been fully identified by routine biochemical testing. *C. auris* and *C. glabrata* were the two species with the biggest diagnostic discrepancies. These results justify specific escalation to molecular identification where phenotypic results are unusual, do not match the clinical context, or are not consistent with susceptibility results.

When interpreting the observed proportion of fluconazole resistance, it is important to consider the species-specific breakpoints. The CLSI harmonization project has demonstrated that the same fluconazole MIC does not always correspond to the same category for each *Candida* species [9]. Notably, there is no normal fluconazole-susceptible category for *C. glabrata*; reporting all 16 non-resistant isolates as “susceptible” would overestimate the activity of the drug. Intrinsic resistance of *C. krusei* also requires species identification prior to interpretation of an azole report. Therefore, the ability to name and have a believable MIC is an integral part of antifungal

stewardship. The discovery of *C. auris* is not just about choosing the right antifungal. Genomic studies have reported several geographically distinct outbreaks, and highlight the importance of accurate identification in hospital networks [10]. Its global distribution has been reviewed, and it has been reported to be frequently resistant to azoles, with problems in diagnosis and environmental contamination [11]. Resistant phenotypes can also occur by different molecular mechanisms as demonstrated by a recent genetic synthesis of *C. auris* [12]. The high percentage of isolates with elevated amphotericin B MICs in this subgroup should not be interpreted as evidence of resistance to other *Candida* species, but rather as a reason to confirm the susceptibility platform and to communicate species-specific interpretations.

The 90.6% concordance of the phenotypic and molecular identification should be evaluated in the context of the methodology used. The present comparison does not prove that sequencing is always better than contemporary MALDI-TOF identification, as a well-maintained contemporary mass-spectrometry database might perform differently. Furthermore, good quality pure cultures, well established reference databases, trained interpretation and consideration of closely related species are required for ITS-based sequencing. A review of modern fungal diagnostics highlights the significant progress made in molecular diagnostics, and the continuing difficulties with sampling and interpretation [13].

In general, resistance to micafungin was rare, consistent with the ongoing clinical use of echinocandins for many types of invasive candidiasis. However, the four of five resistant isolates were *C. glabrata* and *C. auris*, which would warrant careful species-level review. The pharmacodynamic studies of *C. glabrata* and fks alterations illustrate the importance of using appropriate MIC testing when evaluating emerging echinocandin resistance and, if necessary, molecular investigation of the resistance mechanism [14]. The denominator for *C. auris* is very small, and the distribution of the species is highly skewed, making it difficult to make inferences and to compare the micafungin resistance between the species.

The cross-sectional comparison was unadjusted, and prior azole exposure was associated with fluconazole resistance at the isolate level, but it is not possible to draw a causal inference. Selective pressure is possible but patients receiving azole prophylaxis could be systematically different with regard to diagnosis, length of hospital stay, and colonization history. Clinical specimens from blood and nonsterile sites were also collected. Laboratory results from recovery of respiratory samples or urine may only indicate colonization and are not a measure of the incidence or mortality of invasive candidiasis. Patient-level adjudication would be needed for that question.

Data from surveillance confirm the need to report antifungal profiles by species rather than a single pooled percentage. The SENTRY program analyzed over 20,000 invasive *Candida* isolates during 20 years and showed that the distribution of non-albicans species and significant geographical differences in fluconazole resistance have occurred [15]. The results of this study are not directly comparable to the current collection of isolates from both sterile and nonsterile sites of selected immunocompromised patients. An Indian tertiary-care evaluation revealed that *C. auris* might not be identified by standard identification methods and that sequencing could identify isolates with clinically relevant azole resistance [16]. This offers a useful justification for molecular identification of yeast isolates with inconclusive or epidemiologically high-risk clinical significance.

Another limitation is the categorization of minimum inhibitory concentrations of antifungals. CLSI and EUCAST methods were used to compare *C. auris* MICs, which resulted in MIC distributions that were method dependent and proposed tentative epidemiological cutoff values [17].

These cutoffs are not the same as validated outcome-based clinical breakpoints and local laboratory reports should not suggest that susceptibility to all agents can be inferred from the numerical MIC. When using tentative thresholds for *C. auris*, reports should include the interpretive criteria used and suggest confirmatory testing if the report does not align with the clinical course.

This study design has several strengths: one isolate per person, sequencing confirmation of all isolates, explicit handling of *C. glabrata* and *C. auris* breakpoints and a clinically relevant exposure variable. Limitations are that it is a single-centre referral study, has limited power for rare species, does not genotype the resistance mechanism, has no standardized longitudinal outcome assessment, and relies on reported prior azole exposure. Future prospective studies should integrate strong organism identification, MIC, treatment history, invasive-disease classification, and molecular

resistance analysis to assess if faster identification leads to better patient care.

Conclusion

In this *Candida* collection, molecular identification by fungal ITS sequencing provided better species resolution than routine phenotypic identification, especially for *C. auris*. The distinct susceptibility pattern of fluconazole and micafungin highlight the need for species-specific interpretation, and high amphotericin B MICs should be interpreted with caution. Validation of molecular identification, along with reference MIC testing and antimicrobial stewardship, may enhance microbiology support for immunocompromised patients.

References

1. Bays DJ, Jenkins EN, Lyman M, et al. Epidemiology of invasive candidiasis. *Clin Epidemiol.* 2024;16:549-566. doi:10.2147/CL.EP.S459600.
2. Pappas PG, Kauffman CA, Andes DR, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2016;62(4):e1-e50. doi:10.1093/cid/civ933.
3. Cornely OA, Sprute R, Bassetti M, et al. Global guideline for the diagnosis and management of candidiasis: an initiative of the ECMM in cooperation with ISHAM and ASM. *Lancet Infect Dis.* 2025;25(5):e280-e293. doi:10.1016/S1473-3099(24)00749-7.
4. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev.* 2007;20(1):133-163. doi:10.1128/CMR.00029-06.
5. Schoch CL, Seifert KA, Huhndorf S, et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc Natl Acad Sci U S A.* 2012;109(16):6241-6246. doi:10.1073/pnas.1117018109.
6. Kordalewska M, Zhao Y, Lockhart SR, et al. Rapid and accurate molecular identification of the emerging multidrug-resistant pathogen *Candida auris*. *J Clin Microbiol.* 2017;55(8):2445-2452. doi:10.1128/JCM.00630-17.
7. Chowdhary A, Sharma C, Meis JF. *Candida auris*: a rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. *PLoS Pathog.* 2017;13(5):e1006290. doi:10.1371/journal.ppat.1006290.
8. Singh A, Singh PK, de Groot T, et al. Emergence of clonal fluconazole-resistant *Candida parapsilosis* clinical isolates in a multicentre laboratory-based surveillance study in India. *J Antimicrob Chemother.* 2019;74(5):1260-1268. doi:10.1093/jac/dkz029.
9. Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D; CLSI Subcommittee for

- Antifungal Susceptibility Testing. Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and *Candida*: time for harmonization of CLSI and EUCAST broth microdilution methods. *Drug Resist Updat*. 2010;13(6):180-195. doi:10.1016/j.drug.2010.09.002.
10. Lockhart SR, Etienne KA, Vallabhaneni S, et al. Simultaneous emergence of multidrug-resistant *Candida auris* on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. *Clin Infect Dis*. 2017;64(2):134-140. doi:10.1093/cid/ciw691.
 11. Forsberg K, Woodworth K, Walters M, et al. *Candida auris*: the recent emergence of a multidrug-resistant fungal pathogen. *Med Mycol*. 2019;57(1):1-12. doi:10.1093/mmy/myy054.
 12. Chowdhary A, Jain K, Chauhan N. *Candida auris* genetics and emergence. *Annu Rev Microbiol*. 2023;77:583-602. doi:10.1146/annurev-micro-032521-015858.
 13. Pemán J, Ruiz-Gaitán A. Diagnosing invasive fungal infections in the laboratory today: It's all good news? *Rev Iberoam Micol*. 2025;42(1):1-14. doi:10.1016/j.riam.2025.01.004.
 14. Lepak A, Castanheira M, Diekema D, Pfaller M, Andes D. Optimizing echinocandin dosing and susceptibility breakpoint determination via in vivo pharmacodynamic evaluation against *Candida glabrata* with and without fks mutations. *Antimicrob Agents Chemother*. 2012;56(11):5875-5882. doi:10.1128/AAC.01102-12.
 15. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty years of the SENTRY Antifungal Surveillance Program: results for *Candida* species from 1997-2016. *Open Forum Infect Dis*. 2019;6(Suppl 1):S79-S94. doi:10.1093/ofid/ofy358.
 16. Ninan MM, Sahni RD, Chacko B, Balaji V, Michael JS. *Candida auris*: clinical profile, diagnostic challenge and susceptibility pattern: experience from a tertiary-care centre in South India. *J Glob Antimicrob Resist*. 2020;21:181-185. doi:10.1016/j.jgar.2019.10.018.
 17. Arendrup MC, Prakash A, Meletiadis J, Sharma C, Chowdhary A. Comparison of EUCAST and CLSI reference microdilution MICs of eight antifungal compounds for *Candida auris* and associated tentative epidemiological cutoff values. *Antimicrob Agents Chemother*. 2017;61(6):e00485-17. doi:10.1128/AAC.00485-17.