

Molecular Identification and Phylogenetic Characterization of *Candida* Species Isolated from Oral and Nasal Samples of COVID-19 Patients in Duhok, Iraq

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ABSTRACT

The COVID-19 pandemic has been associated with an increased risk of opportunistic fungal infections, particularly candidiasis, in hospitalized and immunocompromised patients. This study aimed to determine the prevalence and molecular identity of *Candida* species isolated from oral and nasal swabs of COVID-19 patients in Duhok, Iraq. A total of 45 isolates were recovered from 14 of 100 patients. *Candida albicans* was the predominant species (17 isolates, 37.7%), followed by *C. krusei* (13 isolates, 28.8%), *C. glabrata* (9 isolates, 20.0%), and *C. tropicalis* (6 isolates, 13.3%). Although CHROMagar provided rapid preliminary identification, several isolates were misclassified phenotypically and required confirmation through ITS sequencing. Molecular analysis demonstrated clear species-level discrimination and revealed genetic diversity among clinical isolates. These findings highlight the importance of integrating molecular diagnostics into routine clinical workflows to improve the accuracy of *Candida* identification in COVID-19 settings, enabling timely management and reducing the burden of fungal co-infections.

Keywords: COVID-19; *Candida*; molecular identification; ITS sequencing; phylogenetic analysis; fungal co-infection; CHROMagar; candidiasis; COVID-19-associated candidiasis (CAC)

INTRODUCTION

Opportunistic fungal infections represent a major cause of morbidity and mortality in immunocompromised individuals, particularly those with chronic diseases, malignancies, prolonged hospitalization, or exposure to invasive medical procedures. During the COVID-19 pandemic, the incidence of fungal co-infections increased substantially, with *Candida* species emerging as one of the most frequent agents associated with poor clinical outcomes among critically ill patients¹. Studies have reported that patients with severe COVID-19 exhibit impaired immune responses, mucosal barrier disruption, and extensive use of broad-spectrum antibiotics and corticosteroids, all of which contribute to a higher susceptibility to candidiasis^{2,3}.

C. albicans remains the leading cause of candidiasis worldwide, accounting for the majority of mucosal and systemic infections. However, a progressive shift toward non-*albicans Candida* species—such as *C. glabrata*, *C. tropicalis*, and *C. krusei*—has been documented in recent years, particularly in hospital settings and in patients exposed to antifungal pressure or prolonged ICU stays⁴. This trend gained further relevance during the COVID-19 pandemic, during which several studies reported altered species distributions and increased incidence of mixed infections^{5,6}.

Accurate identification of *Candida* species is essential for clinical management because virulence traits, antifungal susceptibility profiles, and epidemiological behaviors vary among species. Conventional phenotypic methods—microscopy, CHROMagar culture, germ-tube formation, and chlamyospore production—remain widely used due to their rapidity and low cost. However, these methods frequently misidentify closely related species and cannot resolve cryptic taxa⁷. This challenge became especially evident during the pandemic, when laboratory overload and limited diagnostic resources increased reliance on rapid—but less accurate—phenotypic assays.

In this context, molecular techniques targeting the internal transcribed spacer (ITS) region of fungal rDNA have become the gold standard for species-level identification. ITS sequencing provides high discriminatory power and reveals phylogenetic relationships that are undetectable by traditional methods, improving diagnostic accuracy and enabling early detection of clinically relevant species^{8,9}. Integrating molecular diagnostics with phenotypic approaches is therefore crucial for reducing misclassification and guiding effective antifungal therapy.

Despite the global rise in COVID-19-associated candidiasis, data from Iraq—particularly from the Kurdistan Region—remain limited. Only isolated reports have documented the presence and distribution of *Candida* species in specific patient groups, and comprehensive analyses combining phenotypic and molecular identification remain scarce¹⁰. Given the clinical importance of fungal co-infections in COVID-19 patients, updated regional data are essential for strengthening surveillance and informing treatment strategies.

Therefore, the present study aimed to investigate the prevalence and molecular identity of *Candida* species isolated from oral and nasal samples of COVID-19 patients in Duhok, Iraq, using both conventional laboratory methods and ITS sequencing. Additionally, the study evaluated discrepancies between phenotypic and molecular identification techniques and analyzed the phylogenetic relationships of the isolates using reference sequences from multiple countries. Understanding the local epidemiology of *Candida* in COVID-19 patients will help improve diagnostic practices and guide targeted clinical management.

MATERIAL AND METHODS

Study Design and Population

This cross-sectional study included 100 patients diagnosed with COVID-19 by RT-PCR and admitted to hospitals or outpatient units in Duhok, Iraq, between September 2020 and June 2021. Patients of all ages and both sexes were eligible. Clinical data—including age, sex, comorbidities, and hospitalization status—were retrieved from medical records. The study was approved by the Research Ethics Committee of the Duhok Directorate General of Health (Reference No. 1503/20/2/1), and written informed consent was obtained from all participants.

Sample Collection

Oral (buccal mucosa) and nasal swabs were collected aseptically using sterile cotton swabs and immediately transported to the laboratory in appropriate transport media. Samples were processed within 30 minutes of collection to minimize overgrowth or loss of viability.

Phenotypic Identification of *Candida* Species

Direct Microscopy

Each specimen was examined using 10% KOH wet mounts and Lactophenol Cotton Blue staining to identify yeast cells, pseudohyphae, and other morphological features¹¹.

Culture on Sabouraud Dextrose Agar

Samples were inoculated onto Sabouraud Dextrose Agar (SDA) supplemented with 0.05 mg/mL chloramphenicol to suppress bacterial growth and incubated at 37°C for 48 hours¹². Yeast colonies were evaluated based on texture, color, and morphology.

Germ Tube Test

Single colonies were inoculated into human serum and incubated for 2–3 hours at 37°C. Formation of germ tubes was assessed microscopically and used as a presumptive indicator of *C. albicans* or closely related species¹³.

Chlamydospore Production

The Dalmau plate technique was performed using Corn Meal Agar supplemented with 1% Tween 80 to assess chlamydospore formation, aiding differentiation between *C. albicans* and related taxa¹⁴.

Chromogenic Medium (CHROMagar Candida)

All isolates were subcultured onto CHROMagar Candida (CHROMagar™, France) and incubated at 35°C for 48 hours. Colony color and morphology were used for presumptive species identification. However, ambiguous profiles were expected, given the limitations of phenotypic chromogenic methods¹⁵.

Molecular Identification

DNA Extraction

Genomic DNA was extracted from 24-hour SDA cultures using the Animal and Fungi DNA Preparation Kit (Gena Bioscience, Germany) according to the manufacturer's protocol.

PCR Amplification of the ITS Region

The ITS region was amplified using universal fungal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3')¹⁶.

PCR reactions (25 µL) contained:

- 3 µL template DNA (0.1 µg),
- 12.5 µL GoTaq Green Master Mix (Promega, USA),
- 1 µL ITS1 and 1 µL ITS4 primers (25 µM),
- 7.5 µL nuclease-free 3'áter.

Cycling conditions: initial denaturation at 95°C for 5 min; 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min; final extension at 72°C for 7 min. Amplicons were visualized on 2% agarose gels stained with ethidium bromide.

Sequencing and Data Processing

PCR products were purified using the UltraClean PCR Clean-Up Kit (Qiagen) and bidirectionally sequenced on an Applied Biosystems 3500 Genetic Analyzer at MacroGen Inc. The resulting chromatograms were inspected and assembled in FASTA format before submission to GenBank for accession numbers.

Phylogenetic Analysis

Sequences were compared using BLASTn against the NCBI GenBank database, retaining only matches with $\geq 90\%$ identity. Multiple sequence alignments were performed using MUSCLE. Phylogenetic trees were constructed using the Maximum Likelihood method under the Tamura 3-parameter model, selected based on the lowest Bayesian Information Criterion (BIC). Bootstrap support was calculated using 100 replicates. Isolates were compared with reference sequences from geographically diverse regions to identify clustering patterns and infer genetic relatedness.

Statistical Approach

Frequencies and percentages were calculated for all species. No inferential statistics were applied due to the study's descriptive nature and the limited number of isolates. Mixed colonization events were documented qualitatively.

RESULTS

Prevalence of *Candida* spp. among COVID-19 patients

Of 100 COVID-19 patients, *Candida* spp. were isolated from 14 (14%), yielding 45 isolates from oral and nasal swabs. *C. albicans* was the most common species (17/45; 37.7%), followed by *C. krusei* (13/45; 28.8%), *C. glabrata* (9/45; 20.0%), and *C. tropicalis* (6/45; 13.3%). Mixed colonization (≥ 2 species in the same patient) was observed in 6 of 14 colonized individuals (42.8%).

Species	No. of isolates	Percentage (%)	No. of positive patients	Mixed colonization (Y/N)
<i>Candida albicans</i>	17	37.7	10	Yes
<i>Candida krusei</i>	13	28.8	7	Yes
<i>Candida glabrata</i>	9	20.0	6	Yes
<i>Candida tropicalis</i>	6	13.3	5	Yes
Total	45	100%	14	6/14 (42.8%)

Table 1. Frequency and distribution of *Candida* isolates recovered from COVID-19 patients (n = 45).

Demographic and clinical characteristics

Among the 14 *Candida*-positive patients, 8 were female (57.1%), and 6 were male (42.9%), with a median age of 43 years (range: 30–75). Comorbidities included hypertension (21.4%), pregnancy (21.4%), asthma (14.3%), and autoimmune or inflammatory disease (14.3%). Four patients (28.6%) had no underlying conditions.

Variable	Value
Sex	6 males (42.9%), 8 females (57.1%)
Median age (range)	43 years (30–75)
Comorbidities	Hypertension (21.4%), Pregnancy (21.4%), Asthma (14.3%), Autoimmune disease (14.3%), None (28.6%)
Mixed colonization	6 patients (42.8%)

Table 2. Demographic and clinical characteristics of *Candida*-positive COVID-19 patients (n = 14).

Phenotypic identification of isolates

Microscopy confirmed yeast cells and pseudohyphae consistent with *Candida*.

On SDA plates, all isolates formed smooth, creamy colonies. CHROMagar Candida allowed preliminary differentiation: light green (*C. albicans*), pink (*C. krusei*), blue (*C. tropicalis*), and white-to-cream (*C. glabrata*).

Germ-tube production and chlamydo-spore formation supported presumptive identification of *C. albicans*.

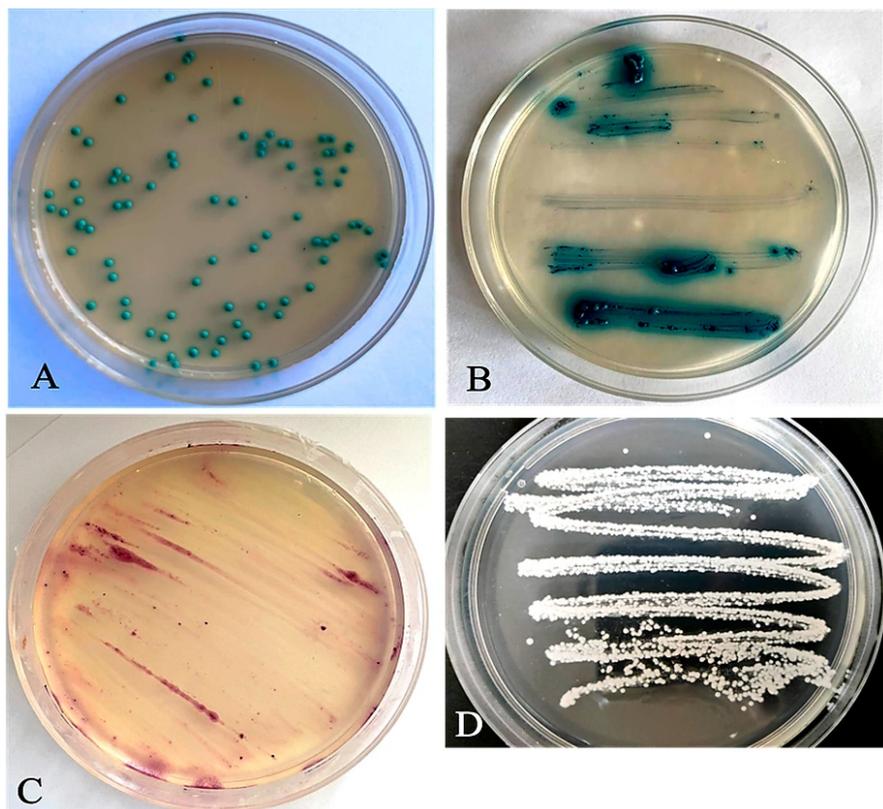


Figure 1. CHROMagar Candida pigmentation of clinical isolates (enhanced).

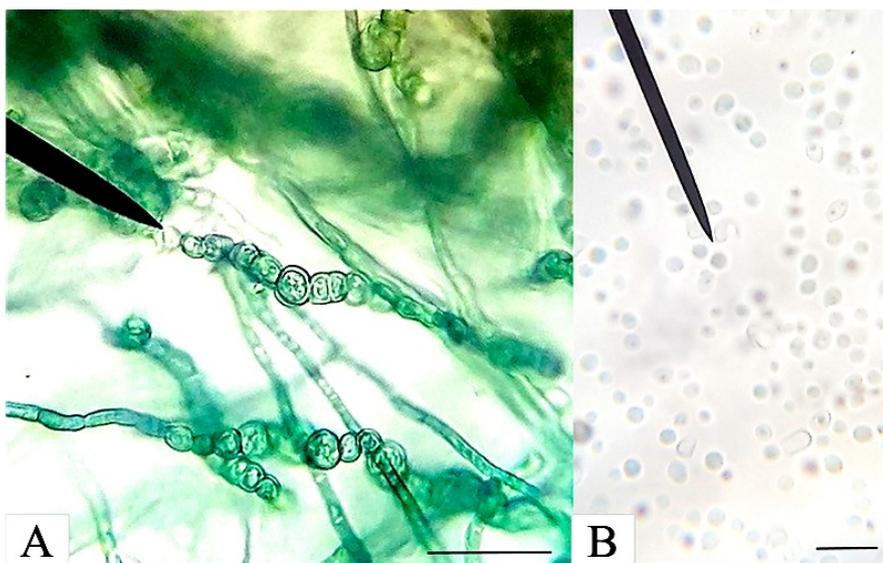


Figure 2. Microscopic structures of *C. albicans*: (A) chlamydo-spores; (B) germ tubes (enhanced).

Concordance between phenotypic and molecular identification

ITS sequencing was used as the reference method to confirm species-level identity. Phenotypic methods correctly identified 33 of the 45 isolates (73.3%), while 12 isolates (26.7%) were misidentified. Misclassifications included *C. albicans* incorrectly assigned as *C. africana* or *C. dubliniensis*, *C. tropicalis* and *C. krusei* misidentified as *C. albicans*, and several non-*Candida* yeasts—such as *Rhodotorula mucilaginosa*, *Cyberlindnera fabianii*, *Kluyveromyces marxianus*, and *Purpureocillium lilacinum*—incorrectly attributed to *C. glabrata* or *C. krusei*.

These discrepancies highlight the limited specificity of chromogenic media and basic morphological assays, particularly in the presence of mixed colonization or atypical colony pigmentation.

Phenotypic ID	CHROMagar color	ITS-confirmed identity	No. of isolates	Interpretation
<i>C. albicans</i>	Light green	<i>C. albicans</i>	3	Correct
<i>C. albicans</i>	Light green	<i>C. africana</i>	2	Misidentified—cryptic species
<i>C. albicans</i>	Light green	<i>C. dubliniensis</i>	1	Misidentified—closely related
<i>C. tropicalis</i>	Blue	<i>C. albicans</i>	1	Misidentified
<i>C. krusei</i>	Pink	<i>C. albicans</i>	3	Misidentified
<i>C. krusei</i>	Pink	<i>Rhodotorula mucilaginosa</i>	1	Misidentified—non- <i>Candida</i> yeast
<i>C. glabrata</i>	White/cream	<i>Rhodotorula mucilaginosa</i>	2	Misidentified
<i>C. glabrata</i>	White/cream	<i>Cyberlindnera fabianii</i>	1	Misidentified
<i>C. glabrata</i>	White/cream	<i>Kluyveromyces marxianus</i>	1	Misidentified
<i>C. krusei</i>	Pink	<i>Kluyveromyces marxianus</i>	1	Misidentified
<i>C. tropicalis</i>	Blue	<i>Purpureocillium lilacinum</i>	1	Misidentified

Table 3. Comparison of phenotypic identification with ITS-based molecular identification for all isolates (n = 45). The table shows correct identifications and the full spectrum of misclassifications, including cryptic species, closely related *Candida* taxa, and non-*Candida* yeasts, as revealed by ITS sequencing.

ITS amplification and sequencing

PCR amplification using ITS1/ITS4 primers AI-generated a ~550 bp product in all isolates. Amplicons were resolved by electrophoresis (Figure 3), and sequencing produced high-quality reads deposited in GenBank (OK030631–OK030639).

Reference sequences used for phylogenetic comparison included strains from Brazil¹⁷, Sudan¹⁸, India¹⁹, and the Netherlands²⁰.

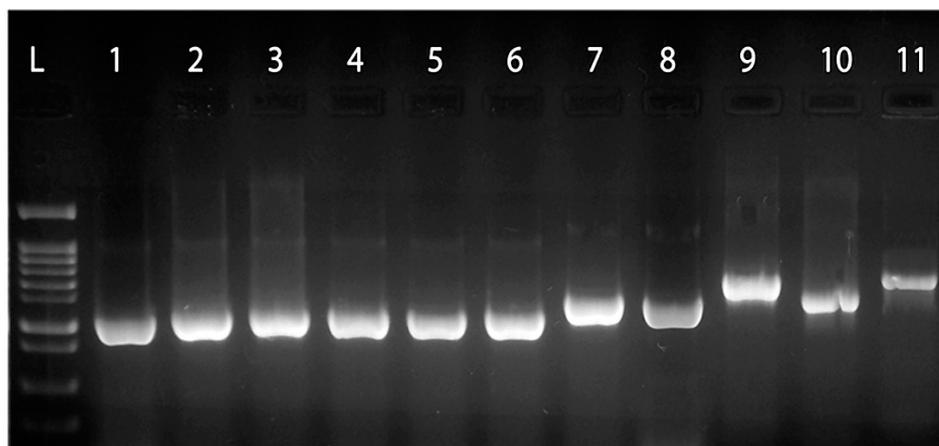


Figure 3. PCR amplification of the ITS region for selected isolates (enhanced).

Strain	Host	Country	GenBank Accession No.	Reference No.
<i>Candida albicans</i>	Human swab	Iraq	OK030631	Current study
<i>Candida albicans</i>	Human swab	Iraq	OK030632	Current study
<i>Candida albicans</i>	Human swab	Iraq	OK030633	Current study
<i>Candida albicans</i>	Human swab	Iraq	OK030634	Current study
<i>Candida albicans</i>	Human swab	Iraq	OK030635	Current study
<i>Candida albicans</i>	Human swab	Iraq	OK030636	Current study
<i>Candida albicans</i>	Human swab	Iraq	OK030637	Current study
<i>Candida albicans</i>	Human swab	Iraq	MN508374	GenBank direct submission
<i>Candida albicans</i>	Human urine	Iran	MH854530	GenBank direct submission
<i>Candida albicans</i>	Tissue	Vietnam	MN263159	GenBank direct submission
<i>Candida albicans</i>	Human blood	Malaysia	MH748591	GenBank direct submission
<i>Candida albicans</i>	Human swab	Nigeria	MH084830	GenBank direct submission
<i>Candida albicans</i>	Human blood	Brazil	MG599180	17
<i>Candida albicans</i>	Human swab	Sudan	MH016331	18
<i>Candida albicans</i>	Human sputum	India	MG757726	19
<i>Candida albicans</i>	CBS	Netherlands	KY101883	20
<i>Candida albicans</i>	Human swab	Egypt	MZ674383	GenBank direct submission
<i>Candida africana</i>	Human swab	Iraq	OK030638	Current study
<i>Candida africana</i>	Human swab	Iraq	OK030639	Current study

Table 4. Reference ITS sequences used for phylogenetic analysis, including local isolates and global GenBank entries^{17–20}.

Phylogenetic analysis

Maximum Likelihood analysis grouped isolates into well-resolved clades corresponding to their ITS identity.

Local *C. albicans* clustered closely with sequences from Brazil¹⁷, Sudan¹⁸, India¹⁹, and the Netherlands²⁰, indicating strong global relatedness.

Non-albicans isolates formed distinct clades with high bootstrap support. Mixed colonization was reflected by phylogenetic diversity within patients.

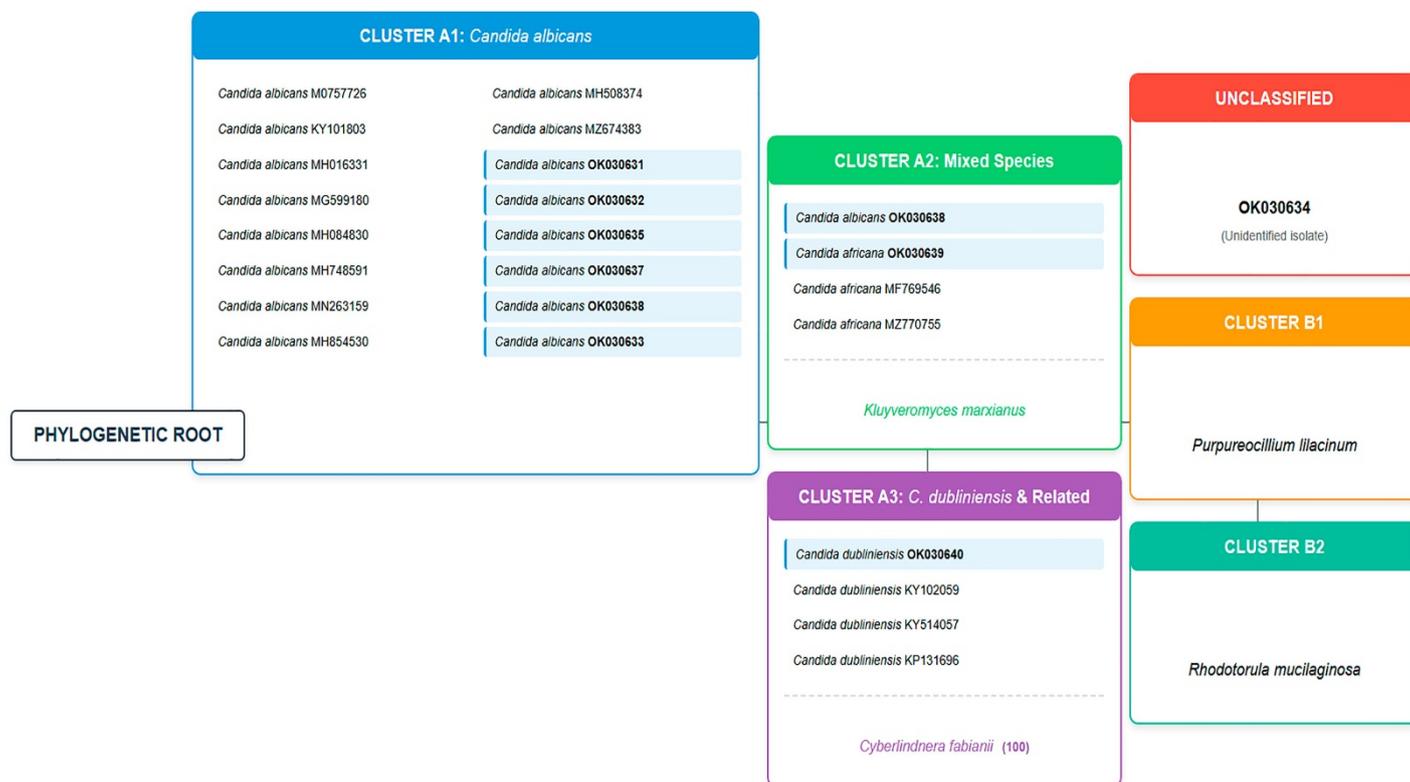


Figura 4. Maximum Likelihood phylogenetic tree based on ITS region sequences of *Candida* isolates from COVID-19 patients in Duhok, Iraq, compared with global reference strains. The tree was constructed using the Tamura 3-parameter model with 100 bootstrap replicates. **Cluster A1** comprises predominantly *C. albicans* strains, including local isolates (OK030631-OK030633, OK030635, OK030637-OK030638; highlighted in blue) alongside reference sequences from Brazil¹⁷, Sudan¹⁸, India¹⁹, and the Netherlands²⁰. **Cluster A2** contains mixed *C. africana* and *C. albicans* isolates, with *Kluyveromyces marxianus* forming a distinct subclade. **Cluster A3** groups *C. dubliniensis* strains with *Cyberlindnera fabianii* (bootstrap value 100). **Clusters B1 and B2** represent phylogenetically distinct non-*Candida* yeasts: *Purpureocillium lilacinum* and *Rhodotorula mucilaginosa*, respectively. Isolate OK030634 remained unclassified. Scale bar indicates genetic distance (substitutions per site)

DISCUSSION

Candidiasis remains one of the most frequent opportunistic fungal infections in hospitalized and immunocompromised patients, and its incidence increased substantially during the COVID-19 pandemic¹. Several studies have shown that critically ill COVID-19 patients exhibit immune dysregulation, extensive corticosteroid exposure, and prolonged hospitalization, all of which potentiate fungal colonization and infection^{3,21}. In the present study, *Candida* colonization occurred in 14% of the sampled COVID-19 patients, a prevalence comparable to previous reports from Iran, India, and Lebanon, where COVID-19-associated candidiasis ranged between 10% and 18%^{22,23}.

Consistent with global trends, *C. albicans* remained the most frequently isolated species. However, non-*albicans* species accounted for more than 60% of isolates (*C. krusei*, *C. glabrata*, *C. tropicalis*), reflecting the progressive epidemiological shift described in several regions during and after the pandemic²⁴. Non-*albicans* species are of particular clinical relevance due to reduced susceptibility to azoles, the ability to form robust biofilms, and high persistence in mucosal niches⁷. The presence of mixed colonization in 42.8% of patients further underscores the complexity of fungal ecology in COVID-19, where dysbiosis of respiratory and oral mucosae has been widely documented⁷.

One of the most significant findings of this study is the high discordance between phenotypic and molecular identification. Although CHROMagar and germ-tube testing remain widely used due to their low cost and rapid turnaround¹⁵, these methods misidentified 26.7% of isolates. The phylogenetic reconstruction (Figure 4) provides crucial insights into why these misidentifications occur. Misclassification most frequently involved species closely related to *C. albicans*, such as *C. dubliniensis* and *C. africana*—organisms that cluster separately yet remain phenotypically ambiguous (Figure 4, Clusters A2 and A3). For instance, Cluster A2 contains both *C. africana* (OK030639, MF769546, MZ770755) and *C. albicans* (OK030638), explaining why CHROMagar failed to differentiate them. Similarly, *C. dubliniensis* (OK030640) forms a distinct clade (Cluster A3) with *Cyberlindnera fabianii*, highlighting how phenotypically similar yet phylogenetically distant organisms can be misclassified as single species. A substantial proportion of isolates corresponded to non-*Candida* yeasts, including *Rhodotorula mucilaginosa*, *Cyberlindnera fabianii*, and *Kluyveromyces marxianus*—all of which occupy phylogenetically distinct positions (Clusters B1, B2, and subclade A2, respectively) far removed from *Candida* species in the tree topology. Similar diagnostic discrepancies have been reported previously²⁵, particularly in settings where colony pigmentation is atypical or mixed cultures are present. These findings reinforce the need to integrate molecular diagnostics—especially ITS sequencing—which remains the gold standard for yeast identification due to its discriminatory power and species-level accuracy^{8,9}.

Phylogenetic analysis (Figure 4) demonstrated that the *C. albicans* isolates from this study clustered closely within a single, well-supported monophyletic group (Cluster A1) alongside reference strains from multiple countries, including Brazil¹⁷, Sudan¹⁸, India¹⁹, and the Netherlands²⁰. This cohesive clustering indicates not merely sequence similarity but shared evolutionary ancestry and high genetic conservation across geographic regions. The tight phylogenetic grouping of Iraqi isolates (OK030631, OK030632, OK030635, OK030637, OK030638, OK030633) with globally distributed references suggests limited intraspecies divergence in *C. albicans* populations, aligning with previous molecular barcoding studies showing worldwide homogeneity²⁰. Conversely, non-*albicans* isolates and non-*Candida* yeasts formed distinct, well-supported clades (Clusters A2, A3, B1, B2), reflecting their phylogenetic distance from *C. albicans* and supporting the accuracy of molecular identification. The clear separation between clusters visually validates why phenotypic methods fail: organisms with convergent phenotypic traits (e.g., similar colony morphology) can be phylogenetically distant, while genetically close relatives may exhibit divergent phenotypes.

Clinically, these phylogenetic findings have important implications. First, misidentification of *C. krusei* or *C. glabrata* could lead to inappropriate empirical therapy, given their intrinsic or frequent resistance to fluconazole. Second, the detection of non-*Candida* yeasts in separate phylogenetic clusters (Figure 4, Clusters B1 and B2) highlights the risk of overlooking emerging opportunists—especially in vulnerable COVID-19 patients—because they may be dismissed as contaminants when in fact they are phylogenetically distinct organisms capable of causing invasive disease¹⁰. Third, the high prevalence of mixed colonization calls for improved diagnostic strategies, as phenotypic media often fail to differentiate minor populations within polymicrobial samples. The mixed composition of Cluster A2—containing both *C. albicans* and *C. africana*—illustrates how co-colonization can be phylogenetically resolved but remain phenotypically opaque, potentially masking clinically relevant polymicrobial interactions.

The study has certain limitations, including the relatively small number of colonized patients and the absence of antifungal susceptibility testing, which would have provided additional clinical insight. From a phylogenetic perspective, while Figure 4 robustly resolves species-level relationships using ITS sequences, future studies should incorporate multi-locus sequence typing (MLST) or whole-genome sequencing to examine intra-cluster diversity and detect fine-scale population structure within Clusters A1–A3^{8,20}. Additionally, including clinical metadata alongside phylogenetic positions could reveal correlations between genetic clusters and patient outcomes, treatment responses, or comorbidity profiles—an integrated approach that would transform phylogenetic trees from descriptive tools into predictive clinical aids. Nonetheless, the combination of phenotypic, molecular, and phylogenetic methods offers a comprehensive view of local species distribution and diagnostic performance.

Novel Contributions Of This Study

This study provides the first integrated phenotypic–molecular–phylogenetic characterization of *Candida* species colonizing COVID-19 patients in the Kurdistan Region of Iraq, revealing three key novel contributions:

1. A high rate of diagnostic misclassification (26.7%) using standard phenotypic methods, including the misidentification of non-*Candida* yeasts, is an underreported issue in COVID-19 cohorts. Phylogenetic analysis visually demonstrates how phenotypically similar organisms (e.g., *C. albicans* and *C. africana* in Cluster A2) are routinely conflated, while phylogenetically distant non-*Candida* yeasts are misassigned to *Candida* species.

2. Molecular confirmation and phylogenetic placement of local *C. albicans* isolates, demonstrating their close relationship with global strains via ITS sequencing. The tight clustering within the monophyletic Group A1 (Figure 4) provides visual evidence that Iraqi isolates are integrated into the global *C. albicans* population rather than representing regionally restricted variants.
3. The identification of complex mixed colonization patterns in nearly half of colonized patients underscores the need for molecular surveillance in clinical mycology during respiratory viral pandemics. The phylogenetic tree reveals that what appears phenotypically as single-species colonization can represent multiple phylogenetically distinct lineages coexisting within the same patient, a finding with direct implications for treatment and infection control.
4. *Additional contribution:* Phylogenetic validation of non-*Candida* yeasts as distinct clinical entities. The clear separation of *Rhodotorula mucilaginosa* (Cluster B2) and *Purpureocillium lilacinum* (Cluster B1) from all *Candida* clusters (Figure 4) provides taxonomic confirmation that these are not *Candida* contaminants but phylogenetically independent opportunists requiring specific clinical consideration^{26,27}.

Collectively, these findings—supported by the phylogenetic evidence in Figure 4—highlight the critical importance of integrating molecular diagnostics into routine practice and contribute valuable regional data to the global understanding of COVID-19–associated fungal colonization. The phylogenetic framework established here serves as a reference for future surveillance studies tracking the evolution and spread of fungal pathogens in post-pandemic settings.

CONCLUSIONS

In this study, the combined use of phenotypic methods, ITS sequencing, and phylogenetic analysis allowed for a comprehensive characterization of *Candida* species colonizing COVID-19 patients in the Kurdistan Region of Iraq. *C. albicans* remained the predominant species; however, non-*albicans Candida* and non-*Candida* yeasts accounted for a substantial proportion of isolates, and nearly half of colonized patients exhibited mixed colonization. These findings demonstrate that conventional phenotypic identification alone is insufficient, as more than one quarter of isolates were misclassified, including several clinically relevant species that may require different therapeutic approaches.

Molecular identification based on ITS sequencing provided accurate species resolution and revealed strong phylogenetic relatedness between local isolates and global reference strains. This underscores the value of molecular surveillance for improving diagnostic precision, guiding antifungal therapy, and monitoring the emergence of uncommon or cryptic species that routine laboratory methods may overlook.

Overall, the results highlight the importance of implementing molecular diagnostics in clinical laboratories, particularly for COVID-19 and other conditions that predispose patients to fungal colonization and infection. Strengthening diagnostic workflows in this manner may reduce misdiagnosis, improve patient management, and enhance fungal surveillance at regional and global levels.

Author Contributions

Conceptualization: Wazeera R. Abdullah (W.R.A.), Asia A. M. Saadullah (A.A.M.S.), Th. T. Mohammed (T.T.M.) **Methodology:** W.R.A., A.A.M.S. **Sample collection:** W.R.A., A.A.M.S., **Laboratory analysis:** W.R.A., A.A.M.S. **Molecular analysis and sequencing:** W.R.A. **Data curation:** A.A.M.S. **Formal analysis:**

W.R.A., A.A.M.S. **Visualization:** W.R.A. **Writing—original draft preparation:** W.R.A. **Writing—review and editing:** W.R.A., A.A.M.S., T.T.M. **Supervision:** A.A.M.S., T.T.M.

All authors have read and approved the final version of the manuscript.

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Institutional Review Board Statement

The study protocol was approved by the Research Ethics Committee of the Duhok Directorate General of Health, Iraq (Reference No. 1503/20/2/1). All procedures were conducted in accordance with national ethical regulations, the standards for human experimentation, and the principles of the Declaration of Helsinki.

Informed Consent Statement

Written informed consent was obtained from all participants before sample collection and laboratory analysis.

Data Availability Statement

The datasets generated and analyzed during the study—including all ITS sequences—have been deposited in GenBank (accessions OK030631–OK030639).

All additional data supporting the findings of this study are available within the article and its accompanying tables and figures.

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Conflicts of Interest

The authors declare no conflicts of interest.

The authors alone are responsible for the content and writing of this manuscript.

Data and AI Disclosure

All figures and tables in this article were created by the authors using data obtained through clinical sampling, laboratory analysis, and sequence-based identification described in this study. Figures 1–4 were enhanced using author-supervised AI-assisted workflows (OpenAI) solely for visualization and image quality improvement.

Generative artificial intelligence was used solely for language polishing, grammatical correction, and formatting standardization, always under full human supervision. No AI tool was used for data generation, data analysis, sequence interpretation, or scientific inference. The authors independently verified all scientific results and conclusions in accordance with BioNatura Journal's policy on AI-assisted content (<https://bionaturajournal.com/artificial-intelligence--ai-.html>).

REFERENCES

1. Arastehfar A, Carvalho A, Nguyen MH, Hedayati MT, Netea MG, Perlin DS, et al. COVID-19–associated candidiasis: An underestimated complication in the absence of immunological predispositions? *J Fungi*. 2020;6(4):211.
2. Babamahmoodi F, Rezai MS, Ahangarkani F, Mohammadi Kali A, Alizadeh-Navaei R, Alishahi A, et al. Multiple *Candida* strains causing oral infection in COVID-19 patients under corticosteroid and antibiotic therapy. *Front Cell Infect Microbiol*. 2022;12:1103226.
3. El Zakhem A, Mahmoud O, Zakhour J, El Sheikh WG, Tamim H, Kanj SS. Impact of COVID-19 on the epidemiology and outcomes of candidemia. *J Fungi*. 2023;9(7):769.
4. Araujo JM, de Almeida Junior JN, Magri MMC, Costa SF, Guimarães T. Epidemiological assessment and risk factors for mortality of *Candida* bloodstream infections during the COVID-19 pandemic. *J Fungi*. 2024;10(4):268.
5. Froidefond M, Sevestre J, Chaudet H, Ranque S. COVID-19 is a confounder of increased *Candida* airway colonisation. *Pathogens*. 2023;12(3):463.
6. Blaize M, Raelina A, Kornblum D, Kamus L, Lampros A, Berger M, et al. Occurrence of candidemia in COVID-19 patients admitted to ICUs. *J Fungi*. 2022;8(7):678.
7. Calderone RA, Fonzi WA. Virulence factors of *Candida albicans*. *Trends Microbiol*. 2001;9(7):327–35.
8. Singh A, Goering RV, Simjee S, Foley SL, Zervos MJ. Application of molecular techniques to the study of hospital infections. *Clin Microbiol Rev*. 2006;19(3):512–30.
9. Ciardo DE, Schär G, Böttger EC, Altwegg M, Bosshard PP. ITS sequencing versus biochemical profiling for identification of medically important yeasts. *J Clin Microbiol*. 2006;44(1):77–84.
10. Kakehi A, Hagiya H, Iio K, Nakano Y, Ihoriya H, Taira Y, et al. *Candida dubliniensis* fungemia in severe COVID-19: a case report. *J Infect Chemother*. 2022;28(10):1433–5.
11. Ellis D, Davis S, Alexiou H, Handke R, Bartley R. *Descriptions of Medical Fungi*. 2nd ed. Adelaide: Mycology Unit; 2007.
12. Bhavan PS, Rajkumar R, Radhakrishnan S, Seenivasan C, Kannan S. Culture and identification of *Candida albicans* from vaginal ulcer samples. *Int J Biol*. 2010;2(1):84–92.
13. Sheppard DC, Locas MC, Restieri C, Laverdiere M. Utility of the germ tube test for direct identification of *Candida albicans*. *J Clin Microbiol*. 2008;46(10):3508–9.
14. Trost A, Graf B, Eucker J, Sezer O, Possinger K, Göbel UB, et al. Identification of clinical yeasts by PCR/RFLP. *J Microbiol Methods*. 2004;56(2):201–11.
15. Tornai-Lehoczki J, Péter G, Dlačny D. CHROMagar *Candida* as a tool for presumptive identification of yeasts. *Int J Food Microbiol*. 2003;86:189–200.
16. White TJ, Bruns T, Lee S, Taylor J. Amplification and sequencing of fungal rRNA genes for phylogenetics. In: Innis M, et al., editors. *PCR Protocols*. Academic Press; 1990. p. 315–22.
17. Sturaro D, Góis-Filho IRT, Alonso GC, Oliveira ML, Matsumoto MT, Svidzinski TIE, et al. Molecular identification and antifungal susceptibility of *Candida albicans* bloodstream isolates in Brazil. *Rev Iberoam Micol*. 2018;35(2):71–5.
18. Elhag NA, Elbasheer MA, Elhusein MA, Osman IA, Elhadi N. Molecular identification of *Candida albicans* in Sudanese patients using PCR-RFLP. *J Pure Appl Microbiol*. 2021;15(2):789–97.
19. Padmavathi AR, Bakkiyaraj D, Thajuddin N, Pandian SK. Antibiofilm activity of *Citrus sinensis* extracts against *Candida albicans*. *Biofouling*. 2018;34(6):588–605.

20. Vu D, Groenewald M, Szöke S, Cardinali G, Eberhardt U, Stielow B, et al. DNA barcoding analysis of >9000 yeast isolates. *Stud Mycol.* 2016;85:91–105.
21. Oufi Z, Ahmed B, Mohammed A. Conventional and molecular identification of *Candida* associated with vaginal candidiasis. *Al-Kindy Coll Med J.* 2022;38(1):1–8.
22. Khalil MAF, El-Ansary MRM, Bassyouni RH, Mahmoud EE, Ali IA, Samir TM. Oropharyngeal candidiasis in Egyptian COVID-19 patients. *Diagnostics.* 2022;12(7):1719.
23. Katz J. Prevalence of oral candidiasis in COVID-19 patients. *Quintessence Int.* 2021;52(8):714–8.
24. Moser D, Biere K, Han B, Hoerl M, Schelling G, Choukér A, et al. COVID-19 impairs immune response to *Candida albicans*. *Front Immunol.* 2021;12:640644.
25. Liguori G, Gallé F, Lucariello A, Di Onofrio V, Albano L, Mazzarella G, et al. Comparison between multiplex PCR and phenotypic systems for *Candida* identification. *New Microbiol.* 2010;33(1):63–9.
26. Romeo O, Tietz HJ, Criseo G. *Candida africana*: Is it a fungal pathogen? *Curr Fungal Infect Rep.* 2013;7:192–7.
27. Gutierrez J, Morales P, Gonzalez MA, Quindos G. *Candida dubliniensis*, a new fungal pathogen. *J Basic Microbiol.* 2002;42(3):207–27.

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