Molecular and Phylogenetic Analysis of *Candida* spp. from Vaginal Infections

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Abstract

Background: *Candida* species are the primary cause of vaginal candidiasis, a common gynecological infection. With the rising prevalence of non-*albicans Candida* species—often showing varying antifungal susceptibility profiles—accurate molecular identification has become increasingly imperative.
Objective: This study aimed to isolate and molecularly characterize *Candida* species from vaginal infections to provide insights into their genetic diversity and epidemiology.

Methods: Forty-five women presenting with symptoms of vaginal infections were screened, yielding 20 *Candida*-positive samples identified using morphological, biochemical, and chromogenic agar methods. Ten representative isolates were subjected to DNA sequencing of the internal transcribed spacer 1 (ITS-1) region of ribosomal RNA, while an additional 20 isolates were validated using polymerase chain reaction (PCR). Genomic DNA was extracted using a modified G-Spin DNA extraction kit, and DNA concentration and purity were measured using a Nanodrop spectrophotometer. PCR amplification was performed with ITS1/ITS4 primers, and products were analyzed with agarose gel electrophoresis. Phylogenetic analysis was conducted using the Maximum Composite Likelihood and Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

Results: The NCBI-BLAST database revealed strong homology between local isolates and *Candida glabrata, Candida albicans,* and *Candida krusei*, with sequence identity ranging from 99.12% to 99.96%. All ten isolates were successfully submitted to GenBank (accession numbers PV789394.1-PV789403.1).

Conclusion: This research provides detailed insights into the genetic diversity of Candida species causing vaginal infections, emphasizing the importance of molecular identification for effective clinical management and epidemiological surveillance. The findings contribute to a deeper understanding of their molecular epidemiology.

Keywords: Candidiasis, DNA sequencing, Genetic diversity.

Introduction

Vaginal candidiasis is a common gynecological condition affecting millions of women worldwide and is often referred to as a yeast infection. *Candida species* are the primary pathogens, with *Candida albicans* being the most frequently isolated. However, non-*albicans Candida* species, such as *C. glabrata* and *C. krusei*, are increasingly recognized as significant contributors to drug-resistant and recurrent infections, complicating treatment strategies. Accurate identification of *Candida* species is essential for epidemiological surveillance, understanding resistance patterns, and informing appropriate therapeutic interventions (Salih *et al., 2021).*
Recent advancements in molecular diagnostics have significantly improved the ability to accurately and rapidly identify *Candida* species, including those that are difficult to

distinguish or cultivate using conventional methods. Techniques such as real-time PCR, DNA microarrays, and next-generation sequencing (NGS) offer high specificity and sensitivity, enabling earlier and more accurate diagnosis (Jenks et al., 2023). These molecular methods are particularly valuable in cases of mixed infections or when dealing with emerging *Candida* species that may exhibit atypical morphological or anti-fungal resistance profiles (Arafa et al., 2023). Integrating such diagnostic tools into clinical practice is vital for optimizing patient outcomes and limiting the spread of resistant strains.

The rising incidence of antifungal resistance among *Candida* species, particularly non-*albicans* variants, poses a significant public health concern (Perlin et al., 2017). Resistance mechanisms may include efflux pumps, target enzyme alterations, or biofilm formation, all of which reduce the efficacy of widely used antifungal agents such as echinocandins and azoles (Lee et al., 2023).

Understanding the molecular mechanisms of resistance and tracking epidemiological trends in *Candida* species distribution are critical for developing new antifungal strategies and preserving the efficacy of existing treatments (Czajka et al., 2023). This evolving landscape highlights the need for continuous surveillance and research into the genetic characteristics of circulating *Candida* strains.
The present study aims to expand our understanding of the molecular epidemiology of vaginal *Candida* infections by isolating and genetically characterizing species from clinical samples using ITS-1 region sequencing. This study aims to improve diagnostic support and contribute to local and regional public health strategies.

3. Materials and Methods

2.1.1. Equipment and Instruments

All instruments and equipment used in this study were carefully selected to ensure the accuracy and reliability of the experimental procedures. Table 1 provides a detailed list of the specific equipment and instruments used, including their manufacturers and countries of origin, to support reproducibility.
All reagents, chemicals, and solutions used in the molecular study were of high purity and suitable for molecular biology applications. Their specific details, including manufacturers and countries of origin, are listed in Table 2.

3.1.5. Primers

Based on Fujita et al. (2001), universal primers targeting the ribosomal RNA ITS1 region were used for PCR amplification of *Candida* spp. These primers were synthesized and supplied by Macrogen (Korea). Table 3 presents the nucleotide sequences and expected PCR product sizes.

Table 3. PCR primers with their nucleotide sequences and product size.

Primers	Sequence (5'-3')		Size of the PCR product
Universal primers targeting the ribosomal RNA (ITS1) region gene	ITS1	TCCGTAGGTGAACCTGCG	500–900bp
	ITS4	TCCTCCGCTTATTGATATGC	

3.1.6. Molecular Study Kits

The kits selected for this study were chosen for their reliability in molecular biological applications. Table 4 details the PCR detection kits, their countries of origin and manufacturers.

2.2. Methods

3.2.1. Sample Collection and Initial Diagnosis

Forty-five women presenting with symptoms of vaginal infection were enrolled in this study. Twenty of the samples tested positive for *Candida* species. The preliminary diagnosis was conducted using morphological assessment, biochemical assays, and chromogenic agar culturing. Ten representative specimens were selected for accurate specie identification via DNA sequencing and PCR validation.

3.2.2. Fungal DNA Extraction

Genomic DNA was extracted from Candida isolates using a modified G-spin DNA Extraction Kit (iNtRON, Korea) according to the manufacturer's instructions with minor modifications. The extraction process involves the following steps:

1. Sample Preparation

One milliliter of cultured *Candida* cells (approximately 1 x 10^9 cells) was transferred into a 1.5mL microcentrifuge tube. The sample was centrifuged at 10,000 rpm for 1 minute. The supernatant was discarded, and the pellet was retained.

2. Cell Lysis

Twenty microliters of Proteinase K and 200µl of CL lysis buffer were added to the pellet. The mixture was vortexed to resuspend the cells completely and incubated at 60°C for 30 minutes to facilitate protein digestion and cell lysis.

3. DNA Binding

After lysis, 200 µl of BL buffer was added to each tube, followed by vortexing for 10 seconds. The tubes were incubated for an addditional 10 minutes at 60ºC, with intermittent inversion every three minutes. Then, 200 µl of absolute ethanol were added, and the mixture was vortexed to ensure adequate mixing. Pipetting was used to break up any visible precipitate.

4. Column Purification

A 2ml collection tube was fitted with a GD column. The entire mixture, including any precipitate, was transferred into the GD column. The column was centrifuged at 10,000 rpm for 1 minute. Subsequently, the GD column was transferred to a new 2mL collection tube, and the flow-through was discarded.

5. Washing Steps

a. First wash: 400 µL of WA buffer was added to the GD column, followed by centrifugation at 10,000rpm for 1 minute. The GD column was return to the 2mL collection tube after discarding the flow-through.

b. Second wash: 600 µL of WB buffer was added to the GD column, which was then centrifuged for 1 min at 10,000 rpm. After discarding the flow-through, the column was reinserted into the collection tube and centrifuged again for 2 minutes at 12,000 rpm to dry the column matrix and ensure complete ethanol removal.

6. DNA Elution

A sterile 1.5mL microcentrifuge tube has been gently filled with the dried GD column. 100 µL of preheated elution buffer (60°C) were added directly to the center of the column matrix. The column was left to stand for a minimum of 3 minutes to allow complete absorption of the buffer. It was then centrifuged for 1 minute at 10,000 rpm to elute the puried DNA.

3.2.3. Estimation of Extracted Total DNA

Purity and concentration of the extracted genomic DNA were assessed using the Nanodrop Lite UV-Vis spectrophotometer (Thermo Scientific, USA). The procedure included the following steps:

1. The Nanodrop software was launched, and the relevant application (DNA/nucleic acid) was selected.
2. The measurement pedestals were cleaned multiple times using a dry wipe. Two microliters of nuclease-free water were then applied to the lower pedestal surface.
3. One microliter of the extracted DNA sample was applied, and the Nanodrop sampling arm was lowered. DNA purity (A260/A280 ratio) and concentration (ng/µl) were then measured.

3.2.4. Polymerase Chain Reaction (PCR)

PCR targeting the ribosomal RNA (ITS1) was conducted for molecular detection of *Candida* species. The PCR primers used were previously described in Section 3.1.5. The GoTaq® Green PCR Master kit (Promega, USA) was used according to the manufacturer's instructions. Table 5 outlines the standard PCR master mix protocol:

Table 5: Standard PCR Master Mix Protocol:

PCR Master mixture	**Volume (µL).**
DNA template (5-100ng)	2.5
Forward primer (10.0 pmol)	2.0
Reverse primer (10.0 pmol)	2.0
GoTaq® Green PCR master	12.50
PCR water	3.50
Total	25

To ensure thorough mixing and collection of reagents at the bottom of each tube, the PCR tubes were vortexed and centrifuged using an Exispin vortex centrifuge for three minutes at 3,000 rpm. The tubes were then placed in a T100 Thermocycler (BioRad, USA) for amplification.

3.2.5. PCR Thermocycler Conditions

Amplification of the ITS1 gene was achieved using optimized PCR thermocycling settings. The protocol was generated using the Optimase ProtocolWriter™ web application and executed using a traditional thermocycler.

3.2.6. PCR Product Analysis

Agarose gel electrophoresis was used to assess the PCR amplification efficiency and verify the product size. The procedure was conducted as follows:
1. Gel Preparation: A 1.5% agarose gel was prepared by dissolving agarose in 0.5X TBE buffer. The solution was heated in a microwave for 5 minutes until fully dissolve, then allowed to cool to 50°C.
2. Ethidium Bromide Staining: After cooling, 3 µL of ethidium bromide was added to the agarose solution and gently mixed.
3. Gel Casting: The gel was poured into a tray with an appropriately positioned comb. After setting at room temperature for 15 minutes, the comb was carefully removed.
4. Electrophoresis Setup: The gel tray was placed in an electrophoresis chamber and fully submerged in 0.5X TBE buffer.
5. Sample Loading: 10 µL of each PCR product were loaded into separate wells. A DNA ladder was loaded into the first well as a reference. Electrophoresis was run at 100 V and 80 mA for 1 hour.
6. Visualization: A UV transilluminator was used to view and capture images of the PCR results [see Figure 1].

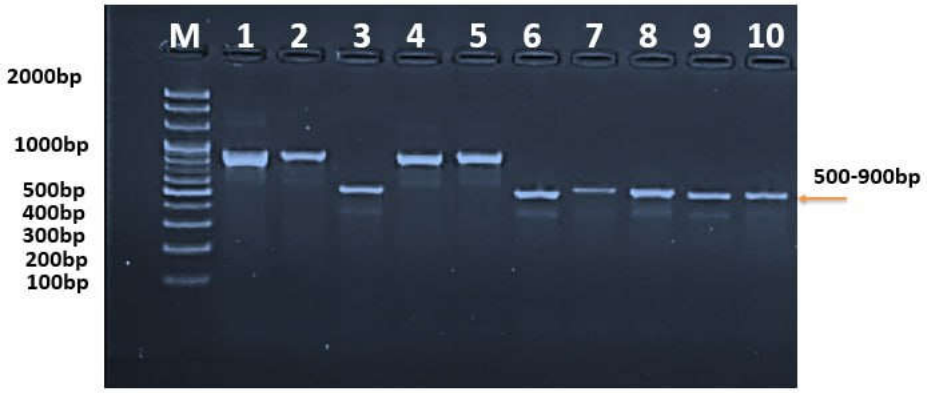

Figure 1: Candida spp. isolates in PCR.

3. Results

3.1. DNA Sequencing Results

The Internal Transcribed Spacer (ITS-1) ribosomal RNA gene was used to genetically identify local *Candida* species isolates via DNA sequencing. The obtained sequences were compared with similar *Candida* species available in the NCBI-BLAST database to determine homology and phylogenetic relationships.

3.1.1. Phylogenetic Analysis

Evolutionary distances were calculated using the Maximum Composite Likelihood approach in MEGA 6.0 software. was used to calculate evolutionary distances, and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). As shown in Figure 2, the analysis revealed clustering of local *Candida* isolates with known reference strains in the NCBI-BLAST database:

- Candida spp. isolates No. 1, 2, 5, 7, 9, and 10 were closely related to *Candida albicans*.
- Isolates No. 3, 4, and 6 showed high genetic similarity with *Candida glabrata*.
- Isolate No. 8 was closely related to *Candida krusei*.

The total genetic changes observed ranged from 0.60% to 0.10%, indicating high genetic similarity within the respective clusters.

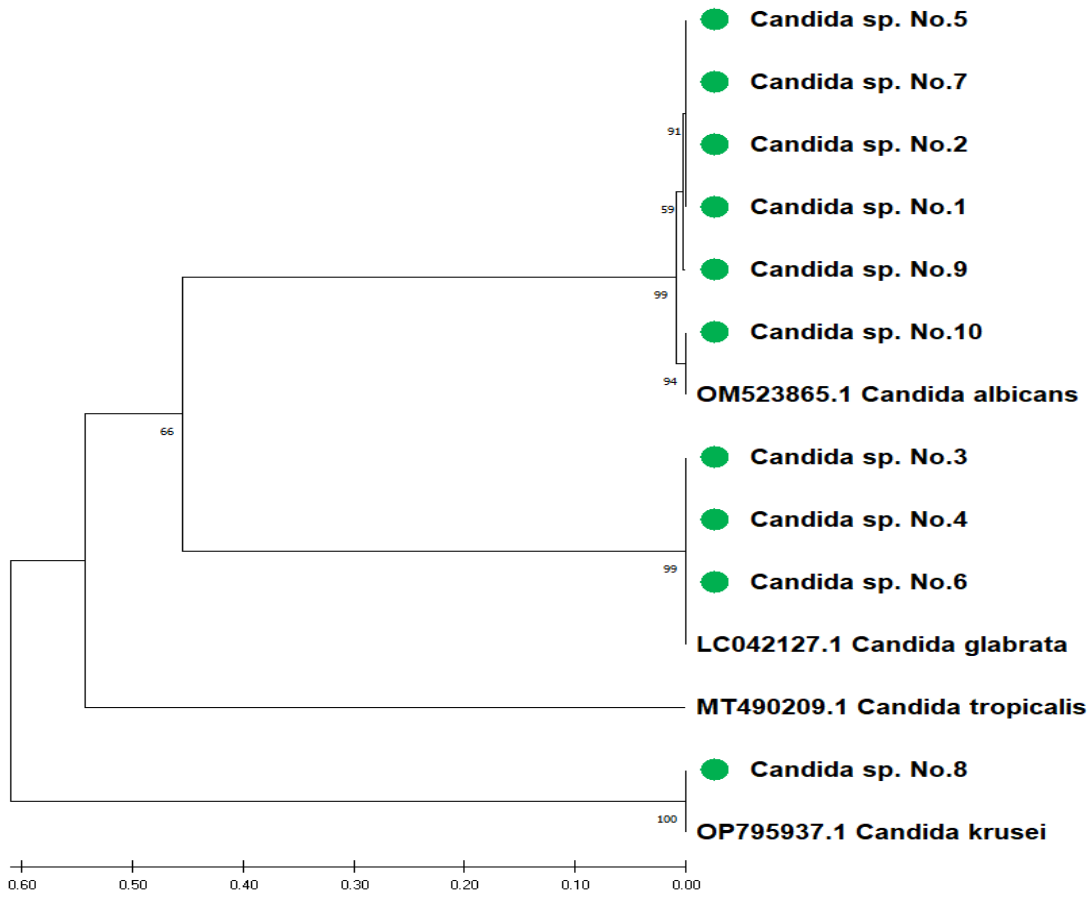

Figure 2. The partial sequence of the internal transcribed spacer ribosomal RNA gene in local isolates of Candida spp. was utilized to analyze genetic relations using phylogenetic tree analysis. The Maximum Composite Likelihood method in MEGA 6.0 was used to calculate the evolutionary distances, and the Unweighted Pair Group method with Arithmetic Mean (UPGMA tree method) was utilized to build the phylogenetic tree. NCBI-BLAST Candida albicans was found to be closely related to the local isolates of Candida species No.1, 2, 5, 7, 9, and 10. It was determined that the isolates of local Candida species No. 3, 4, and 6 were closely related to NCBI-BLAST Candida glabrata. At total genetic changes (0.60-0.10%), the local isolate of Candida spp. No.8 was shown to be closely linked to NCBI-BLAST Candida krusei.

3.1.2. Homology Sequence Identity

The percentage identity between local *Candida* isolates and their closest reference strains in the NCBI-BLAST database ranged from 99.12% to 99.96%, strongly supporting species identification. Details of the homology sequence identity are presented in Table 7.

Table 7. Homology sequence identity between local *Candida spp*. isolates and closely related NCBI BLAST reference sequences

Local Candida isolate	Accession No.	Homology sequence identity (%)		
		NCBI Blast identity	NCBI accession number	Identity (%)
Candida sp. No.1	PV789394.1	Candida albicans	OM523865.1	99.96%
Candida spp. No.2	PV789395.1	Candida albicans	OM523865.1	99.76%
Candida sp. No.3	PV789396.1	Candida glabrata	LC042127.1	99.63%
Candida sp. No.4	PV789397.1	Candida glabrata	LC042127.1	99.64%
Candida spp. No.5	PV789398.1	Candida albicans	OM523865.1	99.76%
Candida sp. No.6	PV789399.1	Candida glabrata	LC042127.1	99.60%
Candida spp. No.7	PV789400.1	Candida albicans	OM523865.1	99.24%
Candida sp. No.8	PV789401.1	Candida krusei	OP795937.1	99.12%
Candida sp. No.9	PV789402.1	Candida albicans	OM523865.1	99.12%
Candida spp. No.10	PV789403.1	Candida albicans	OM523865.1	99.64%

3.1.3. GenBank Submission

All *Candida* spp. isolates (No. 1 to No. 10) were successfully submitted to the GenBank database. Accession numbers assigned to each isolate range from PV789394.1 to PV789403.1, ensuring public accessibility and traceability of the sequences data.

3.1.4. Multiple Sequence Alignment

Multiple sequence alignment of the Internal Transcribed Spacer ribosomal RNA gene from local *Candida* spp. isolates from NCBI-GenBank was performed using the online ClustalW tool. Figure 3 illustrates the alignment results, highlighting nucleotide similarities (indicated

by asterisks *) and substitution mutations. The alignment visually confirms the genetic relationships identified in the phylogenetic analysis.

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Candida_tropicalis ACTTCAACAAACGGTGGGTTGGTGGGGGAATGGAGGAGGAAGGAAGCAAAATCCGTGGAC 257
Candida_krusei CTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAGCGCAGCGAAAT-GCGATAC 227
Candida.sp.No.8 CTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAGCGCAGCGAAAT-GCGATAC 227
Candida_glabrata CTTTCAACAATGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 461
Candida.sp.No.3 CTTTCAACAATGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 461
Candida.sp.No.4 CTTTCAACAATGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 461
Candida.sp.No.6 CTTTCAACAATGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 473
Candida.sp.No.9 CTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 218
Candida_albicans CTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 278
Candida.sp.No.10 CTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 278
Candida.sp.No.1 CTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 257
Candida.sp.No.2 CTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 257
Candida.sp.No.5 CTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 257
Candida.sp.No.7 CTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT-GCGATAC 257
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Candida_tropicalis GTAATAGGAGTGGCAAATTTCGGTGAATGATGGAATCTTTGACGGAAATTTGCGACTTTT 317
Candida_krusei CTAGTGTGAATTGCAGCCAT-CGTGAATCATCGAGTTCTTGAACGCACATTGCGCCCCTC 286
Candida.sp.No.8 CTAGTGTGAATTGCAGCCAT-CGTGAATCATCGAGTTCTTGAACGCACATTGCGCCCCTC 286
Candida_glabrata GTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 521
Candida.sp.No.3 GTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 521
Candida.sp.No.4 GTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 521
Candida.sp.No.6 GTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 533
Candida.sp.No.9 GTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 278
Candida_albicans GTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 338
Candida.sp.No.10 GTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 338
Candida.sp.No.1 GTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 317
Candida.sp.No.2 GTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 317
Candida.sp.No.5 GTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 317
Candida.sp.No.7 GTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT 317
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Candida_tropicalis GGTTTTCCAAGGGGCTTGCCGGTTTGAGGGTAATTTGTGCGTAAAACCCCGGGGTTGGGT 377
Candida_krusei GGCATTCCGGGGGGCATGCCTGTTTGAGCGTCGTTTCCATCTTGCGCGTGCGCAGAGTTG 346
Candida.sp.No.8 GGCATTCCGGGGGGCATGCCTGTTTGAGCGTCGTTTCCATCTTGCGCGTGCGCAGAGTTG 346
Candida_glabrata GGTATTCCGGGGGGCATGCCTGTTTGAGCGTCATTTCCTTCTCAAACACATTGTGTTTGG 581
Candida.sp.No.3 GGTATTCCGGGGGGCATGCCTGTTTGAGCGTCATTTCCTTCTCAAACACATTGTGTTTGG 581
Candida.sp.No.4 GGTATTCCGGGGGGCATGCCTGTTTGAGCGTCATTTCCTTCTCAAACACATTGTGTTTGG 581
Candida.sp.No.6 GGTATTCCGGGGGGCATGCCTGTTTGAGCGTCATTTCCTTCTCAAACACATTGTGTTTGG 593
Candida.sp.No.9 GGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTCTCCCTCAAACCGCTGGGTTTGGT 338
Candida_albicans GGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTCTCCCTCAAACCGCTGGGTTTGGT 398
Candida.sp.No.10 GGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTCTCCCTCAAACCGCTGGGTTTGGT 398
Candida.sp.No.1 GGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTCTCCCTCAAACCGCTGGGTTTGGT 377
Candida.sp.No.2 GGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTCTCCCTCAAACCGCTGGGTTTGGT 377
Candida.sp.No.5 GGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTCTCCCTCAAACCGCTGGGTTTGGT 377
Candida.sp.No.7 GGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTCTCCCTCAAACCGCTGGGTTTGGT 377
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Figure 3: Multiple sequence alignment analysis of the internal transcribed spacer (ITS) ribosomal RNA gene in local *Candida* spp. isolates and related *Candida* spp. Isolates from NCBI GenBank. The alignment was performed using the ClustalW online tool. Asterisks (*) indicate conserved nucleotides, while sequence variations represent substitution mutations among isolates.

4. Discussion

Accurate identification of *Candida* species causing vaginal infections is essential for effective clinical management and epidemiological surveillance. In this study, we employed molecular techniques—specifically ITS1 region sequencing—to precisely identify *Candida* species isolated from vaginal samples. The findings underscore the importance of molecular methods in differentiating between *Candida* species, especially given the increasing

prevalence of non-*albicans Candida* species and their variable antifungal susceptibility profiles (Pfaller & Diekema, 2007).

Although conventional phenotypic approaches remain foundational, they can be time-consuming and often lack the specificity required for precise species-level identification, particularly in the case of morphological similar or atypical isolates (E. Nejad *et al.,* 2020). In contrast, molecular methods such as PCR and DNA sequencing offer superior sensitivity, specificity, and speed, thus enabling more accurate diagnoses and informing targeted therapeutic interventions (Wong *et al.,* 2025).

Our phylogenetic analysis confirmed that *Candida albicans* remains the predominant species among local isolates, aligning with global trends in vulvovaginal candidiasis (Pappas et al., 2016). However, the significant presence of non-*albicans Candida* species—specifically *Candida glabrata* and *Candida krusei*—reflects an epidemiological shift with important clinical implications. These species often exhibit reduced susceptibility or intrinsic resistance to commonly used antifungal drugs.

C. glabrata is particularly known for its reduced susceptibility to fluconazole, largely due to mechanisms such as the overexpression of efflux pumps (e.g., Cdr1p, Cdr2p, Mdr1p) and alterations in the ergosterol biosynthesis pathway (Pfaller & Castanheira, 2023; Duggan & Usher, 2023). Similarly, *C. krusei* is intrinsically resistant to fluconazole, a resistance attributed to a natural mutation in its ERG-11 gene, which encodes the azole target enzyme, 14α-demethylase (Pristov et al., 2019). Therefore, timely and accurate identification of these non-*albicans* species is crucial to guide appropriate antifungal therapy and prevent therapeutic failure.

The high sequence identity (99.12%–99.96%) between local isolates and reference strains in the NCBI-BLAST database strongly supports the reliability and discriminatory capacity of ITS1 sequencing for *Candida* species identification. This high degree of homology reflects intra-species genetic conservation and confirms the accuracy of Candida strain identification in this study. As a highly variable yet conserved region of ribosomal DNA, the ITS region serves as an effective molecular barcode, offering superior resolution compared to other genetic markers (Schoch et al., 2012). Furthermore, the successful submission of our sequences to GenBank enriches the global *Candida* genetic repository, aiding future comparative genomic studies, epidemiological tracking, and diagnostic tool development.

The multiple sequence alignment provided visual confirmation of nucleotide similarities and identified substitution mutations within the ITS gene among different isolates. These genetic variations contribute to our understanding of genetic diversity, population structure, and evolutionary relationships among *Candida* strains. While this study primarily focused on species identification, the detected variations provide the foundation for future investigations into virulence factors, resistance markers, or geographical clustering patterns. Further research could assess the functional implications of these mutations and their associations with clinical phenotypes such as disease severity or recurrence.

Despite the strengths of molecular identification, certain limitations warrant consideration. Although ITS sequencing is highly effective at the species level, it may lack sufficient discriminatory power for subtyping closely related strains or for intra-species epidemiological tracking. More discriminatory methods—such as multilocus sequence typing (MLST) or pulsed-field gel electrophoresis (PFGE)—may be necessary in these contexts (Pfaller & Diekema, 2010). Additionally, mixed infections and low fungal biomass in clinical samples may compromise DNA extraction and amplification, potentially leading to false-negative results or skewed identification outcomes (Kidd et al., 2020). Future efforts should focus on improving assay sensitivity through quantitative PCR (qPCR), metagenomic sequencing, to overcome these limitations and achieve a more comprehensive characterization of the vaginal mycobiome.

5. Conclusion

This study successfully employed advanced molecular techniques for the precise identification of *Candida* species in vaginal infections. The findings highlight the growing clinical significance of non-*albicans Candida* species and underscore the necessity of accurate species identification for informed patient management and effective public health interventions. Our findings contribute valuable data to the molecular epidemiology of *Candida* infections and lay a strong foundation for future investigations into antifungal resistance mechanisms, virulence factors, and the development of novel diagnostic and therapeutic strategies.

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