EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 13
Issue: 8
Year: 2026

Candida Lusitaniae, ISOLATION AND CLINICAL IMPORTANCE IN MEXICO DURING THE FINAL STRECH OF THE COVID-19 PANDEMIC

Cudberto Contreras Pérez[1], Elizabeth González Durán[2], Patricia Gutiérrez García[3], Claudia Ríos-Rosas[3], José D. Méndez[4*]

[1]Mycobacteria Laboratory. Instituto de Diagnóstico y Referencia Epidemiológicos "Dr. Manuel Martínez Báez". Secretaría de Salud. México City. México.
[2]Diagnostic Test Evaluation Management. Department of Molecular Biology and Technique Validation. Instituto de Diagnóstico y Referencia Epidemiológicos "Dr. Manuel Martínez Báez". Secretaría de Salud. México City. México.
[3]Micology Laboratory. Instituto de Diagnóstico y Referencia Epidemiológicos "Dr. Manuel Martínez Báez". Secretaría de Salud. México City. México.
[4]Medical Research Unit in Metabolic Diseases. Cardiology Hospital. Instituto Mexicano del Seguro Social. México City. México.

***Corresponding Author: José D. Méndez**
Medical Research Unit in Metabolic Diseases. Cardiology Hospital. Instituto Mexicano del Seguro Social. México City. México. **DOI:** https://doi.org/10.5281/zenodo.21884415

How to cite this Article: Cudberto Contreras Pérez[1], Elizabeth González Durán[2], Patricia Gutiérrez García[3], Claudia Ríos-Rosas[3], José D. Méndez[4*] (2026). *Candida lusitaniae*, Isolation And Clinical Importance In Mexico During The Final Strech Of The Covid-19 Pandemic. European Journal of Biomedical and Pharmaceutical Sciences, 13(8), 383–391.

Article Received on 15/07/2026 **Article Revised on 05/08/2026** **Article Published on 10/08/2026**

ABSTRACT
During the COVID-19 pandemic, opportunistic yeast infections of the genus Candida increased. During the final phase of the pandemic in Mexico, nine isolates of *Candida lusitaniae* were identified. The cases came from nine hospitalized patients: five neonates, one adolescent, and three adults. From August 2025 to July 2026, no additional cases were reported. The identification of *Candida lusitaniae* by phenotypic tests proved rapid, reliable, and timely. The use of CHROMagar™ Orientation represents an important differentiating factor compared to other species of the genus *Candida*, particularly those grouped in the CTG clade, with which erroneous identifications occurred in the past. Regarding primary or acquired resistance in this species, the results in the literature have been controversial. Of the 11 *Candida lusitaniae* isolates, only one was resistant to voriconazole, amphotericin B, caspofungin, and micafungin, but sensitive to 5-fluorocytosine. The resistant strain was isolated from a 16-year-old patient diagnosed with pneumonia, from the state of Michoacán, Mexico, which maintains reliable coverage of systemic candidiasis cases in that region. Currently, *Candida lusitaniae* is considered a species sensu stricto. Sequencing of PCR products from the ITS1 region reveals significant genetic variability among clinical isolates of *Candida lusitaniae*. Phylogenetic analysis of the studied isolates clearly distinguishes three subgroups: two stable subgroups and one subgroup with intraspecific variability. No relationship was found between patients, medical institutions, or geographic region. The results obtained are related to the low transmissibility and dispersion of this species in medical facilities. The prevention of healthcare-associated infections (HAIs) requires reliable, timely, and comprehensive results between the medical field and the clinical laboratory. The rapid and accurate identification of *Candida lusitaniae* will contribute to taking more effective measures for its control.

KEYWORDS: *Candida lusitaniae*, Phenotypic tests, Fungigram, PCR/Sequencing.

1. INTRODUCTION
Clavispora lusitaniae (*Candida lusitaniae*) is an opportunistic yeast-like fungus. The first cases of human infection occurred between 1979 and 1988. In 1989, it was described as an emerging pathogen associated with nosocomial infections and severe systemic infections in immunocompromised patients.[1-4] In some patients, treatment failure has been reported due to acquired resistance, primarily to amphotericin B[5-6], as well as to imidazoles and 5-fluorocytosine.[7-8]

Laboratory tests used for its identification include the analysis of morphological and reproductive characteristics[9-10], biochemical characterization[11-13] using commercial kits[14-15] and automated equipment (Phoenix System, Vitek 2, Compac, and Maldi-TOF)[16-18], as well as molecular biology tests (PCR/sequencing) of the ITS region.[19] Occasionally, misidentification occurs for this species, as has happened with *Candida tropicalis*, *Candida parapsilosis*, *Candida albicans*, and *Saccharomyces cerevisiae*.[7]

In Mexico, *Candida lusitaniae* has been little studied[20-21] and has been isolated from various clinical samples.[22] During the COVID-19 pandemic, there was an increase in clinical cases, where *Candida lusitaniae* was isolated mainly from cases of fungemia in neonates.[23] There is evidence of nosocomial transmission in neonatal intensive care units, which is associated with a higher risk of infections by this pathogen.[24] Recently, a high frequency of isolation in oral mucosa was found in a group of students in the city of Monterrey, Nuevo León, Mexico.[25]

The purpose of this work is to reveal the epidemiological behavior of *Candida lusitaniae* during the last 3 years (2023-2025), now that COVID-19 has become an endemic disease in Mexico, as in other countries, which will allow strengthening of control measures in Hospital Units to decrease the spread and transmissibility of this opportunistic pathogen, due to the history of resistance to antifungals, in particular the appearance of resistant isolates.

2. BIOLOGICAL MATERIAL AND METHODS

During the late stage of COVID-19 (2022-2025), the Institute of Epidemiological Diagnosis and Reference (InDRE) received 10 clinical isolates corresponding to cases of fungemia (MYC-141-22, MYC-46-23, MYC-72-23, MYC-89-23, MYC-92-23, MYC-94-23, MYC-95-23, MYC-236-23, MYC-240-23 and MYC-88-25) from various regions of Mexico, collected through the National Network of State Public Health Laboratories (LESP). The study included the reference strain ARG-OPS-12-2021 and two *Candida lusitaniae* isolates from the InDRE Collection (MYC-308-05 and MYC-97-17), initially identified as Candida spp. by biochemical tests, and later identified as *Candida lusitaniae* using the Vitek 2 Compact automated instrument, version 9.1. Their inclusion is important because strain MYC-308-05 corresponds to the first *Candida lusitaniae* isolates, obtained in 2005, while strain MYC-97-17 is a strain isolated in 2017, both prior to the COVID-19 pandemic. The metadata of the studied isolates is concentrated in Table 1. Overall, the isolates were recovered from five adults, six neonates and one two-month-old infant, with a predominant diagnosis of sepsis and/or pneumonia.

Table 1: General information on key, diagnosis, age, sex, sample type and Candida species identified.

Key to isolation	Diagnosis	Age and sex	Source	Entity	Species
MYC-97-17	Pneumonia	16 months, M	Blood culture	Michoacán	*Clavispora lusitaniae*
MYC-308-05	Pneumonia	R/N 2 months, M	Blood culture	Chiapas	*Clavispora lusitaniae*
MYC-141-22	Neonatal sepsis	R/N 24 days, M	Blood culture	Tabasco	*Clavispora lusitaniae*
MYC-46-23	Pneumonia	16 a. F	Pleural fluid	Michoacán	*Clavispora lusitaniae/Merozyma guilliermondii*
MYC-72-23	Generalized peritonitis	53 a. F	Peritoneal fluid	Estado de México	*Clavispora lusitaniae*
MYC-89-23	Pneumonia	2 a. F	Catheter tip	Estado de México	*Clavispora lusitaniae*
MYC-92-23	Premature. Sepsis	R/N 2 months, M	Blood culture	Jalisco	*Clavispora lusitaniae*
MYC-94-23	Abdominal sepsis, pancreatitis, nosocomial pneumonia	65 a. F	Peritoneal fluid	Estado de México	*Clavispora lusitaniae*
MYC-95-23	Metabolic acidosis, type 2 DM decompensated	59 a. F	Urine culture	Estado de México	*Clavispora lusitaniae*
MYC-236-23	Premature. Neonatal sepsis	R/N 18 days. F		Tabasco	*Clavispora lusitaniae*
MYC-240-23	Premature. Neonatal sepsis	R/N 44 days. F	Blood culture	Tabasco	*Clavispora lusitaniae*
MYC-88-25	Pneumonia	74 a. M	Expectoration	Sonora	*Merozyma guilliermondii/ Clavispora lusitaniae*
ARG-0PS-12	Reference strain	-	Quality control program	Malbrán Institute	*Clavispora lusitaniae*

(*) The strains were identified by biochemical tests (Vitek 2, version 9.1).

2.1. Laboratory Identification

2.1.1. Morphological characteristics

The isolates were cultured on Sabouraud dextrose agar, Mycosel agar, Biggy agar, CHROMagar OrientationTM, and corn flour agar, supplemented with Tween 80 and trypan blue. Morphological and sporulation characteristics (formation of pseudohyphae, hyphae, and

blastoconidia) were reviewed, in accordance with the findings reported by Contreras-Pérez et al.[19,23]

2.1.2. Biochemical characteristics and susceptibility to antifungals (YST cards; identification and AST-YS08 susceptibility card)

For identification and susceptibility testing, a cell suspension was prepared for each isolate from colonies incubated for 48 hours on Sabouraud dextrose agar (aSd), adjusted to a turbidity equivalent to a range of 1.8 to 2.0 McFarland units, and analyzed using the Vitek 2 System, version 9.01.

For antifungal susceptibility testing, a dilution of the suspension was prepared by adding 288 µl to another tube containing 3 ml of 0.5% isotonic saline solution.

The susceptibility card includes the following antifungals: Voriconazole, Amphotericin B, and 5-Fluyocytosine (Vitek 2, version 9.1).

2.1.3. Molecular identification

Genomic DNA from both the reference strain ARG-OPS-12 (*Candida lusitaniae*) and the 12 clinical isolates associated with fungemia collected between 2005 and 2025 was extracted according to the methodology previously described by González et al., 2022.[19, 26] Molecular identification was performed by amplification of the ITS region using panfungal PCR, and the amplified product was subsequently sequenced. The identity of the obtained sequences was confirmed by BLASTn analysis against the NCBI database.

To assess the intraspecific variability of *Candida lusitaniae*, a phylogenetic analysis based on the ITS region was performed, including the 12 isolates characterized in this study, the reference strain, and seven clinical isolates previously reported during the early stages of the COVID-19 pandemic in Mexico by Contreras-Perez et al., 2022.[19] The phylogenetic tree was constructed using the maximum likelihood method, with the General Time Reversible (GTR) evolutionary model, with 1,000 replicates, using the MEGA version 7.0 program.[26] This analysis allowed for comparison of the genetic stability and intraspecific variability of *Candida lusitaniae* among isolates recovered from both early and late stages of the COVID-19 pandemic in Mexico.

3. RESULTS

3.1 Morphological characteristics

The thirteen strains of *Candida lusitaniae* grew well on the culture media used, except on Mycosel agar, where they were inhibited by cycloheximide. Morphology on (aSd) and Biggy (Ab) agar was similar in both media, consistent with spherical colonies that appeared moist and had a smooth surface (Figure 1A). On CHROMagar Orientation™ bacteriological medium, all strains formed green colonies, rapidly hydrolyzing (24 hours) some of the chromogens in the medium. The microscopic image shows single cells (yeasts), some with blastoconidia and the formation of pseudohyphae or hyphae, while on corn flour agar (CFA), the strains exhibit similar behavior, presenting two phases of reproduction: an initial stage, where the yeasts, between 24 and 48 hours of growth, form pseudohyphae and hyphae, some branched and with the presence of spherical or pyriform lateral blastoconidia (Figure 1). The advanced stage occurs between 72 and 96 hours, which is characterized by greater branching and abundant formation of lateral blastoconidia, which cover most of the hyphae, giving them a clustered appearance. Sporulation on corn flour shows slight variations. Notably, 12 strains formed abundant pseudohyphae and lateral blastoconidia on both sides, small, ovoid to spherical in shape, in short chains with a tendency to branch. One strain, isolate MYC-88-25, unlike the rest, revealed different microscopic characteristics. This strain was identified by PCR sequencing as *Meyerozyma guilliermondii* (formerly *Candida guilliermondii*), whose sporulation pattern is very different from that of *Candida lusitaniae*, which was characterized by the formation of pseudohyphae with elongated lateral blastoconidia, tending towards a rectangular shape (Figure 2).

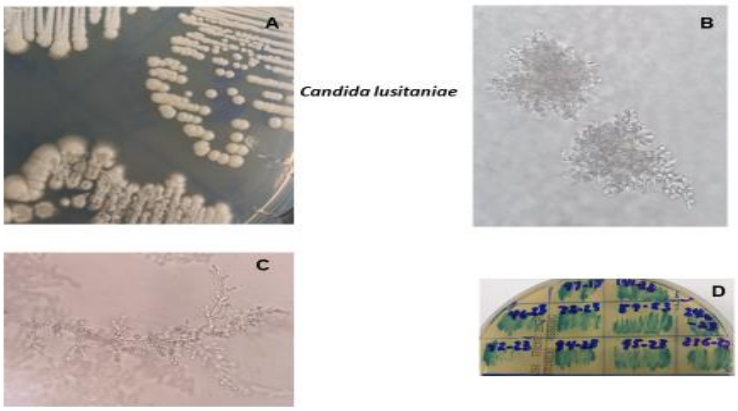

Figure 1: Macro and microscopic characteristics of *Clavispora lusitaniae*. A. *Candida lusitaniae* on Sabouraud dextrose agar. White, smooth colonies are observed in different stages of pseudohyphal and hyphal formation (1

to 7 days). B. Microscopic image in the early stage (24-48 hours) of pseudohyphal formation (400X). C. Microscopic image in the late stage (72-96 hours), with frank formation of pseudohyphae, mycelium, and ovoid to pyriform lateral blastoconidia (480X). D. *Candida lusitaniae* on CHROMagar Orientation, green colony formation (24-48 hours).

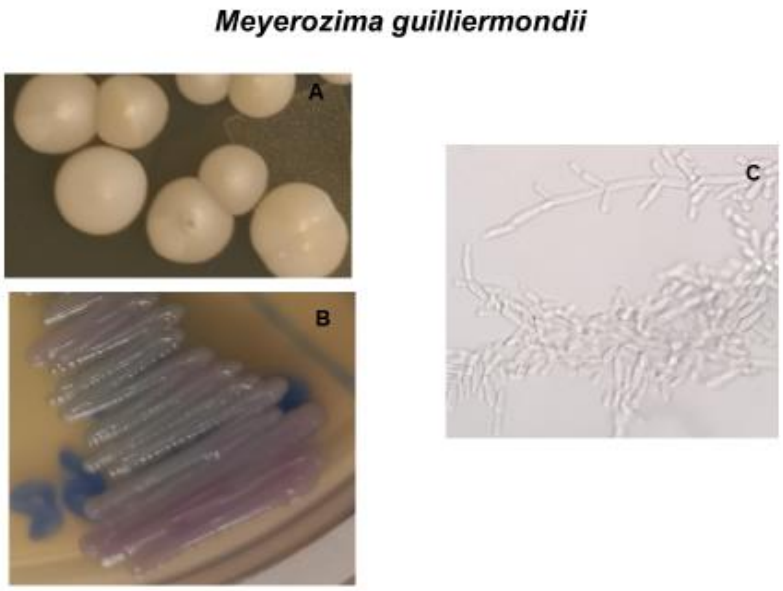

Figure 2. Macro and microscopic characteristics of *Meyerozyma guilliermondii.* A. *Meyerozyma guilliermondii* on Sabouraud dextrose agar. Growth of smooth, white colonies with little tendency to form pseudohyphae and hyphae (1 to 7 days). B. *Meyerozyma guilliermondii* on CHROMagar *Candida*. Growth of smooth, pinkish-white colonies between 24 and 72 hours (600X). C. Microscopic image (72-96 hours), with formation of pseudohyphae, mycelium, and lateral blastoconidia, tending toward a rectangular shape (480X).

3.2. Biochemical characteristics and susceptibility to antifungals (Fungigram)

The results of the main biochemical tests are shown in Table 2. *Candida lusitaniae* exhibited moderate metabolic activity, generally hydrolyzing approximately 28 of the 46 substrates on the Vitek 2 identification cards. Some substrates are crucial for identification and differentiation from other Candida species, such as tyrosine, D-galactose, rhamnose, raffinose, cellobiose, xylose, arabinose, N-acetylglucosamine, and esculin. The results of the main biochemical tests using the Vitek 2 system are shown in Table 2. Eleven clinical isolates were identified as *Candida lusitaniae*, with a very good to excellent identification reliability and a probability of 94% to 97%. The reference strain of *Candida lusitaniae* (ARG-OPS-12), of Argentinian origin, showed acceptable reliability and a probability of 88%.

This strain, unlike the other isolates, differs in that it does not hydrolyze sorbitol, xylose, sorbose, and esculin, while it is the only one that hydrolyzes glycerol; nevertheless, the Vitek System confirmed its identification as *Candida lusitaniae*. Among the 46 substrates (carbon sources, nitrogen sources, and other substrates) handled by the instrument, sixteen substrates highlight differential characteristics in the biochemical behavior between these twelve isolates and the reference strain.

Table 2: Biochemical characteristics of *Candida lusitaniae.* 16 differential substrates with other *Candida* species.

Substrate	MYC-97-17	MYC-308-05	MYC-141-22	MYC-46-23	MYC-72-23	MYC-89-23	MYC-92-23	MYC-94-23	MYC-95-23	MYC-236-23	MYC-240-23	MYC-88-25	ARG-OPS-12
Tyrosine	-	-	-	-	(+)	-	+	+	-	-	-	-	+
L Malate	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylitol	+	+	+	-	+	+	+	+	+	+	+	+	+
D Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	-
DL Lactate	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine	(+)	+	+	+	+	+	-	+	+	+	+	+	+
D Galactose	-	+	+	-	+	-	-	+	-	-	-	-	-
Citrate	-	+	+	+	+	-	+	+	-	+	+	+	+
Glycerol	(-)	-	-	-	(-)	-	-	-	-	-	(-)	-	+

D Xilose	-	-	-	-	-	-	-	-	-	-	-	-	-
L Sorbose	+	+	+	+	+	+	+	+	+	+	+	+	-
Escolaina	+	+	+	+	+	+	(+)	+	+	+	+	+	-
L Ramnose	+	+	+	+	+	+	+	+	+	+	+	+	+
Rafinose	-	-	-	-	-	-	-	-	-	-	-	-	-
Celobiose	+	+	+	+	+	+	+	+	+	+	+	+	+
N-Acetyl glucosamine	+	+	+	+	+	+	+	+	+	+	+	+	+

Regarding antifungal susceptibility, ten clinical isolates of *Candida lusitaniae* (MYC-141-22, MYC-72-23, MYC-89-23, MYC-92-23, MYC-94-23, MYC-95-23, MYC-236-23, MYC-240-23) were susceptible to all three antifungals tested: voriconazole (0.12 µg/mL), amphotericin B (0.5 µg/mL), and flucytosine (1 µg/mL). One isolate (MYC-46-23) was resistant to two of the three antifungals tested. In this case, the isolation was repeated using a Vitek 2 v 8.1 card, which included, among the antifungals, the two echinocandins, caspofungin (0.25 µg/mL) and micafungin (0.12 µg/mL). The results confirmed resistance to voriconazole and amphotericin B, and the isolate was also resistant to both echinocandins but susceptible to 5-fluorocytosine. One isolate (MYC-308-05) of the 10 susceptible to voriconazole and amphotericin B was resistant to 5-fluorocytosine. Table 3 summarizes the susceptibility profile observed for the isolates during the study period.

Table 3: Susceptibility profile of clinical isolates of *Candida lusitaniae*.

Strain, key	Voriconazole	Amphotericin B	5- Flucytosine	Echinocandins
M-97-17	<=0.12 S	<=0.25 S	<=1 S	NP
M-308-05	<=0.12 S	<=0.5 S	>=64 R	NP
M-141-22	<=0.12 S	<=0.25 S	<=1 S	NP
M-46-23	>=4 R	>=8 R	<=1 S	Caspof >= 8 R Micaf >= 8 R
M-72-23	<=0.12 S	<=0.25 S	<=1 S	NP
M-89-23	<=0.12 S	<=0.25 S	<=1 S	NP
M-92-23	<=0.12 S	<=0.25 S	<=1 S	NP
M-94-23	<=0.12 S	<=0.25 S	<=1 S	NP
M-95-23	<=0.12 S	<=0.5 S	<=1 S	NP
M-236-23	<=0.12 S	<=0.5 S	<=1 S	NP
M-240-23	<=0.12 S	<=0.5 S	<=1 S	NP
M-88-25 (6)	(&)	<=0.5 S	(&)	Caspofungin <=1 S Micafungin <=2 S

NP = Not tested, because the isolates were susceptible to all three antifungals tested.

Note: Isolate M-46-23, which showed resistance to two types of antifungals (voriconazole and amphotericin B), was tested against a third type of antifungal (echinocandins: caspofungin and micofungin) using the Vitek version 8.1 card and, interestingly, also showed resistance to the tested echinocandins (Caspof ≥ 8; Micaf ≥ 8).

(&) Global Copy CLSI-based +Natural Resistance 2026.

3.3. Molecular characteristics

Pan-fungal PCR targeting the ITS region of the rDNA cistron allowed amplification of the expected product of approximately 400 bp for all 11 isolates (MYC-308-05, MYC-97-17, MYC-141-22, MYC-46-23, MYC-72-23, MYC-89-23, MYC-92-23, MYC-94-23, MYC-95-23, MYC-236-23, MYC-240-23, MYC-88-25) and the reference strain (MYC-OPS-ARG-12) (Figure 3).

The amplicon identity of the 11 isolates, approximately 400 bp in length, including the reference sequence of *Candida lusitaniae* (ARG-OPS-12), was confirmed by sequencing, exhibiting between 99 and 100% similarity to *Candida lusitaniae.* Meanwhile, the sequence of isolate MYC-88-25 (approximately 500 bp) exhibited 100% similarity to *Meyerozyma guilliermondii.*

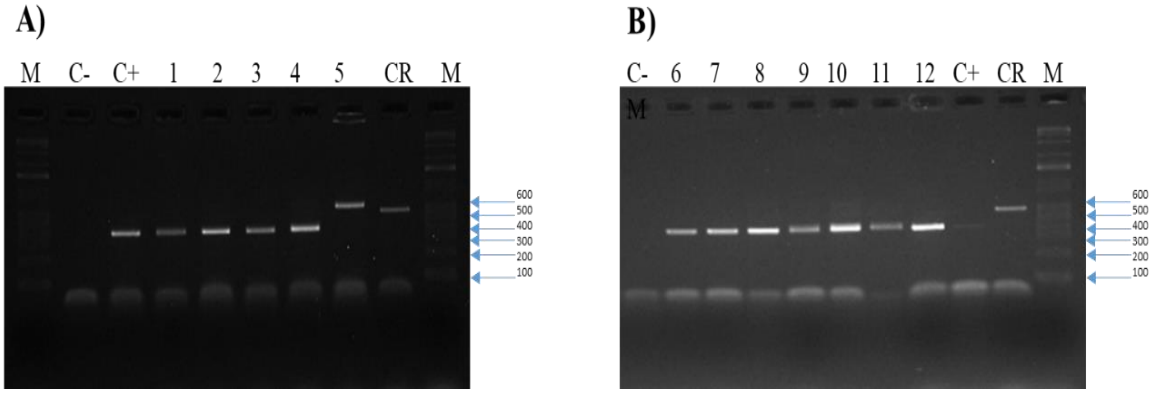

Figure 3. *Candida lusitaniae* panfungal-STI PCR products. A) C-: Negative control; C+: Reference *Candida lusitaniae* (ARG-OPS-12); CR: Reaction control (*Candida albicans*); #1 (MYC-97-17); #2 (MYC-46-23); #3 (MYC-72-23); #4 (MYC-141-22); #5 (MYC-88-25, *Meyerozyma guilliermondii*). B) C-: Negative control; #6 (MYC-89-23); #7 (MYC-92-23); #8 (MYC-94-23); #9 (MYC-95-23); #10 (MYC-236-23); #11 (MYC-24-23); #12 (MYC-308-05); C+: Reference *Candida lusitaniae* (ARG-OPS-12); CR: Reaction control (*Candida albicans*); M: 1Kb Plus DNA molecular weight marker; product migration is shown on the right side of the gel in base pairs (bp). Isolates in lanes 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, and C+ showed a ~400 bp product corresponding to *Candida lusitaniae*, lane 5 of ~550 bp to *Meyerozyma guilliermondii,* and lane CR of *Candida albicans* of ~500 bp.

Phylogenetic analysis based on the ITS region revealed limited intraspecific variability among Mexican isolates of *Candida lusitaniae*. Isolates recovered during the early and late stages of the COVID-19 pandemic were distributed indiscriminately into two groups (Figure 4). The most abundant and conserved group (branch 4), supported by a high statistical support value (100%), included three sequences corresponding to isolates from the early stage of COVID-19 (MYC-44-22, MYC-94-22, and MYC-95-22), four isolates recovered in the late stage (MYC-72-23, MYC-95-23, MYC-236-23, and MYC-240-23), and one isolate recovered in 2005 (MYC-308-05), prior to the pandemic.

The second group (high statistical support, 99%) was subdivided into three subgroups (Branch 1, Branch 2, and Branch 3) (Figure 4). In Branch 3 (high statistical support, 93%), three early-stage sequences (MYC-124-21, MYC-112-22, MYC-113-22), one late-stage isolate (MYC-89-23), and one pre-pandemic isolate recovered during 2017 (MYC-97-17) were grouped. In Branch 2 (moderate statistical support, 53%), only the MYC-94-23 isolate, corresponding to the late stage of COVID-19, was grouped. Finally, in branch 1 (high statistical support, 83%) five isolates were grouped, of which one early stage isolate (MYC-126-21), the reference strain OPS-ARG-12 and three late-stage isolates (MYC-141-22, MYC-46-23, MYC-92-23) were grouped.

The genetic distances between the clustered sequences were short, including the reference strain and the sequences from the early and late stages of COVID-19, with no apparent association with the isolation period or geographic origin. Interestingly, the MYC-94-23 isolate clustered independently (R2). This clustering had not been observed in the early stage of the pandemic, unlike the other isolates, which clustered in the same way, as previously reported by Contreras et al. (2022) (Figure 4). The sequences corresponding to the early stage were previously published in 2022.[19] The sequences from the late stage and those from the pre-pandemic isolates obtained here were deposited in GenBank (Accession numbers GB: PZ654859 - PZ654870).

The maximum likelihood phylogenetic tree of 20 sequences from the ITS region (18S and 5.8S rRNA genes), including Mexican isolates of *Candida lusitaniae* recovered in the early (dark triangle, published by Contreras et al. (2022)) and late (dark rectangle, characterized here) stages of COVID-19. The maximum likelihood tree was constructed using the General Time Reversible (GTR) evolutionary model with 1000 replicates, using the MEGA 7.0 program. The tree defines two groups: a well-defined and conserved group of *Candida lusistaniae* (R4) and a second group, which is further subdivided into three subgroups (R1, R2, R3). The isolate of *Meyerozyma guillermondii* (MYC-88-25) was used as an outgroup.

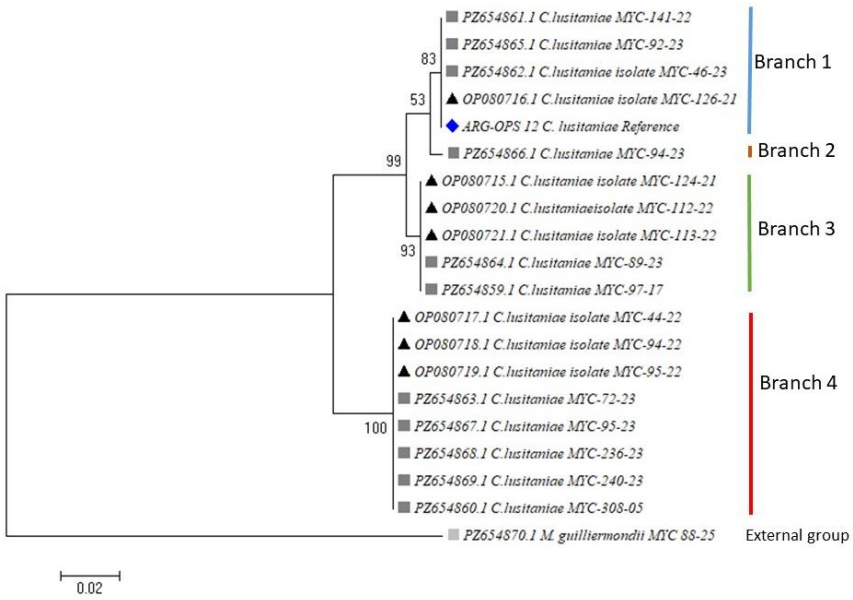

Figure 4: Phylogenetic analysis of *Candida lusitaniae* during the early and late stages of COVID-19 in Mexico.

4. DISCUSSION AND CONCLUSION

The identification of *Candida lusitaniae* by phenotypic tests (colony morphology, sporulation characteristics, and automated biochemical tests (Vitek 2)) proved rapid, reliable, and timely. The automated Vitek 2 system identified all 11 *Candida lusitaniae* isolates with a very good identification rate and a probability between 94% and 97%. Past misidentifications of *Candida lusitaniae* with *Candida parapsilosis* and *Candida albicans* were clarified in a recent study.[23]

Other *Candida* species involved have been *Meyerozyma guilliermondii* and *Metschnikowia pulcherrima*.[27-28] In our experience with Vitek 2, versions 8.1 and 9.1, the identification of *Candida pulcherrima* in 5 isolates was correct and confirmed by PCR/sequencing. Regarding *Meyerozyma guilliermondii*, version 8.1 correctly identified this species, without showing low discrimination results. With version 9.1, the system showed discrepancies in two isolates; one of them (MIYC-46-23) showed low discrimination power between the two species, *Candida lusitaniae* (50%) and *Meyerozyma guilliermondii* (50%), were identified by PCR/sequencing of the ITS region, confirming *Candida lusitaniae* as the species involved. The second isolate (MYC-88-25) was identified with 97% probability as *Candida lusitaniae*, but PCR/sequencing identified it as *Meyerozyma guilliermondii*. These results confirm a close taxonomic relationship between the two species, as observed in the grouping included in Clade CTG.[8] This phenomenon had already been detected previously since 2000.[28] Some *Candida lusitaniae* isolates with late sporulation have also been erroneously identified as presumptive strains of *Candida auris*.[26] *Candida lusitaniae* exhibits excellent growth in conventional culture media. However, the use of the CHROMagarTM Orientation bacteriological medium does not provide the same results for the cultivation of yeast-like fungi, so it was an important differential contribution, with other species of the genus *Candida*, in particular those species grouped in the CTG Clade.[8]

Regarding antifungal susceptibility testing, there is a marked discrepancy in the results. While some *Candida lusitaniae* isolates have shown acquired resistance to amphotericin B, fluconazole, and 5-fluorocytosine, this behavior has not been consistent, and variable results have been found.[29-30] Unexpectedly, high susceptibility to imidazoles, particularly itraconazole, fluconazole, and voriconazole, has been found. Only one isolate out of 80 strains tested was resistant to amphotericin B.[29-30] In two previous publications.[18,23], we studied twelve isolates, which were found to be susceptible to itraconazole and fluconazole. Only two non-clinical isolates showed dose-dependent susceptibility to itraconazole, a behavior already described for this antifungal.[30]

In this study, 11 additional isolates of *Candida lusitaniae* were analyzed, of which only one was resistant to voriconazole, amphotericin B, caspofungin, and micafungin, but sensitive to 5-fluorocytosine. The resistant strain was isolated from a 16-year-old patient diagnosed with pneumonia, from the state of Michoacán, which maintains reliable data on cases of systemic candidiasis in that region.

Currently, *Candida lusitaniae* is considered a species sensu stricto. Cytoplasmic protein analysis[23] and sequencing of PCR products from the ITS1 region reveal significant genetic variability among clinical isolates of *Candida lusitaniae.* Phylogenetic analysis of the studied isolates clearly distinguishes three subgroups; this behavior has already been observed by other authors.[31] Comparison with previously reported *Candida lusitaniae* isolates suggests differences in the degree of genetic

variability detected in the Mexican isolates through analysis of the ITS region. This comparison revealed that the Kuwaiti isolates were distributed across multiple well-defined phylogenetic clusters, while the Mexican isolates showed a more conserved distribution and less intraspecific variability. These findings suggest lower genetic diversity within the analyzed population in Mexico. The conserved nature of the ITS region limits the resolution of this marker for inferring fine evolutionary relationships or transmission patterns. Therefore, the observed variability was interpreted exclusively within the context of this locus. To overcome this limitation, studies based on whole-genome sequencing or multilocus sequencing are necessary to characterize and infer the genetic diversity of *Candida lusitaniae* in the country. Apparently, no relationship was found between patients, medical institutions, or geographic region with this distribution.

At InDRE, the first isolations of *Candida lusitaniae* occurred in 2002, and over a 21-year period, up to 2025, 23 clinical isolates were identified. These included 14 cases in neonates and 7 in adults, of which 3 were isolated from peritoneal fluid, 3 from urinary tract infections, and 1 from pleural fluid. The isolations in neonates occurred in hospitalized patients, suggesting that *Candida lusitaniae* can be acquired directly in hospitals, as demonstrated by several authors.[32] Although the incidence of colonization is low and infections are infrequent[33], the presence of *Candida lusitaniae* in the hospital environment constitutes a potential risk as a source of transmission for immunocompromised patients.[34-35]

In this study, we have had the opportunity to investigate the morphological, biochemical, physiological, molecular, and susceptibility characteristics of *Candida lusitaniae*. These characteristics are useful for early and timely identification. The results are related to the low transmissibility and spread of this species in medical facilities. Preventing healthcare-associated infections requires reliable, timely, and comprehensive results from both the medical and clinical laboratory settings. Rapid and accurate identification of *Candida* species and their antifungal susceptibility profiles will contribute to more effective control and treatment measures.

5. REFERENCES

1. Merz GW. *Candida lusitaniae*. Frequency of recovery, colonization; infection, and amphotericin B resistance. J. Clin. Microbiol, 1984; 20: 1194-95.
2. Blinkhorn RJ, Adelstein D, Spagnuolo PJ. Emergence of a new opportunistic pathogen, *Candida lusitaniae*. J. Clin. Microbiol, 1989; 27(2): 236-40.
3. Runco R, Raquel Salim R. *Candida lusitaniae* en un paciente pediátrico inmunocomprometido: éxito terapéutico del voriconazol. Boletín Micológico, 2005; 20: 97-102.
4. Viudes A, Pemán J, Cantón E, Salavert M, Ubeda P, López-Ribot JL, Gobernado M. Two cases of fungemia due to *Candida lusitaniae* and a literature review. Eur. J. Clin. Microbiol. Infect. Dis., 2002; 21(4): 294-9.
5. Peyron F, Favel A, Nguyen A, Gilly M, Regli P, Bolmstrom A. Improved detection of amphotericin B-resistant isolates of *Candida lusitaniae* by etest. J. Clin. Microbiol., 2001; 39: 339-42.
6. Kovacicova G, Hanzen J, Pisarcikova M, Sejnova D, Horn J, Babela R, Svetlansky I, Lovaszova M, Gogova M, Krcmery V. Nosocomial fungemia due to amphotericin B-resistant *Candida spp*. in three pediatric patients after previous neurosurgery for brain tumors. J. Infect. Chemother., 2001; 7(1): 45-8.
7. Francois F, Noel T, Pepin R, Brulfert A, Chastin Ch, Favel A, Villard J. Alternative identification test relying upon sexual reproductive abilities of *Candida lusitaniae* strains isolates from hospitalized patients. J. Clin. Microbiol., 2001: 3906-14.
8. Mendoza-Reyes FD, Gómez-Gaviria M, Mora-Montes MH. *Candida lusitaniae*: Biology, pathogenicity, virulence factors, diagnosis, and treatment. Infection and Drug Resistance, 2022; 15: 5121-5135.
9. Gargeya YB, Pruitt WR, Simmons RB, Meyer SA, Ahearn DG. Occurrence of *Clavispora lusitaniae*, the teleomorph of *Candida lusitaniae*, among clinical isolates. J. Clin. Microbiol., 28: 2224-27.
10. Runco R, Salim R. *Candida lusitaniae* en un paciente pediátrico inmunocomprometido: Éxito terapéutico del Voriconazol. Bol. Micol., 2005; 20: 97-102.
11. Kwon-Chung KJ, Bennett J E. Medical Mycology. Lea & Febiger, Philadelphia, USA, 1992; 308: 318-19.
12. Mariat F, Drouhet E. Las levaduras de importancia médica y veterinaria. Dermatología Rev. Mex., 1996; 40: 31-42.
13. Hazen KC, Howell SA. *Candida*, *Cryptococcus*, and other yeast of medical importance. In: Manual of Clinical Microbiology. Murray R P, Jorgensen H J, Pfaller A M, Volizen H R, editor. Eight Edition, Washington; DC, USA, 2003; 1696.
14. Heelan JS, Sotomayor E, Coon KD, Arezzo JB. Comparison of the rapid yeast plus panel with the API20C yeast system for identification of clinically significant isolates of *Candida* species. J. Clin. Microbiol., 1998; 36: 1443-45.
15. Michel-Nguyen A, Favel A, Chastin C, Selva M, Regli P. Comparative evaluation of a commercial system for identification of *Candida lusitaniae*. Eur. J. Clin. Microbiol. Dis., 2000; 19: 393-5.
16. Portillo-Miño JD, Cerón-Muñoz E, Toro.Zapata C, Chaucanez-Bastidas Y. Endocarditis infecciosa debida a *Candida lisitaniae* en un lactante menor: Reporte de caso. Infection, 2020; 24(4): 266-69.
17. Vitek systems, Inc. Vitek Senior Reference Manual. Technical Bulletins. BioMériux, USA, 1993; 1-23.

18. Siller-Ruíz M, Hernández-Egido S, Sánchez-Juanes F, González-Buitriago JM, Muñoz-Bellido JL. (2017). Métodos rápidos de identificación de bacterias y hongos. Espectrometría de masas MALDI-TOF, medios cromogénicos. Enferm. Infecc. Microbiol. Clin, 35(5): 303-313.
19. Contreras-Pérez C, González-Durán E, Gutiérrez-García P, Téllez-Saucedo MD, Suro-Marín ES, Pérez-Vázquez L, Ríos-Rosas C, Wong-Arámbula CE, Méndez JD. Clinical isolates of *Candida Lusitaniae*. during the COVID-19 pandemic in México. WJPR, 2022; 11 (13): 1303-1316.
20. Martínez EI, González IM, Haydee K. Torres GHK. Identificación molecular de *Candida lusitaniae* en infección de tracto respiratorio inferior. Rev. Argent. Microbiol., 2014; 46(4): 1-8.
21. Carreto-Espinoza C, Duck-Hernández E, Carranco-Dueñas JA, Oldak-Skvirsky. Sepsis neonatal tardía por *Candida lusitaniae*. An Med ABC, 2023; 68(2): 115-19.
22. Reyes-Montes MR, Duarte-Escalante E, Martínez-Herrera E, Acosta-Altamirano G, Frías-De León MG. Current status of the etiology of candidiasis in Mexico. Rev. Iberoamer. Micol, 2017; 34(4): 203–210.
23. Contreras PC, Gutiérrez GP, Beltrán PLG, Pastén SS, Méndez JD. *Candida lusitaniae*. Isolation, identification and clinical relevance, WJPR. 2019; 8(5): 606-21.
24. Caballero-Trejo A, Aguirre-Morales CE, González-González GM, Cortés-Palma D, Miranda-Novales MG. Colonización por *Candida* en una unidad de cuidados intensivos neonatales. Rev. Med. Inst. Mex. Seguro Soc. 2014; 52(2): S16-S23.
25. Rojas AC, Montesino AC, Carrión AD, Olazarán MS, Montoya MA, González AR. Isolation of *Clavispora lusitaniae* from the oral cavity of immunocompetent young adults from the north of Mexico. Ind. J. Microbiol., 2023; 64: 475-81.
26. González-Durán E, Contreras-Pérez C et al. The use of readily available laboratory tests for the identification of the emerging yeast *Candida auris* in Mexico. Arch. Microbiol., 2022; 204: 592.
27. Noel T, Nguyen MA, GoumarA, Fallage K, Chastin C, Leclerc F, Villard J. Differentiation between atypical isolates of *Candida lusitaniae* and *Candida pulcherrima* by determination of mating type. J. Clin. Microbil., 2005; 43(3): 1430-32.
28. Graf B, Zill E, Adam T, Goebel UB. Evaluation of the Vitek 2 System for rapid identificaction of yeasts and yeast-like organisms. J. Clin. Mirobiol., 2000; 38(5): 1782-5.
29. Favel A, Michel-guyen A, Chastn C, Trousson F, Penaud A, Regli P. *In-vitro* susceptibility pattern of *Candida lusitaniae* and evaluation of the Etest method. J. Antimicrob. Chemoter, 1997; 39: 591-6.
30. Favel A, Michel-Guyen A, Datry A, Challier S, Leclere F, Chastin CH, Fallage K, Regli P. Susceptibility of clinical isolates of *Candida lusitaniae* to five systemic antifungal agents. J. Antimicrob. Chemoter, 2004; 53: 526-9.
31. Khan Z, Ahmad S, Al-Sweih N, Khan S, Joseph L. *Candida lusitaniae* in Kuwait: Prevalence, antifungal susceptibility and role in neonatal fungemia. PLoS One, 2019; 14(3): 1-14.
32. Caballero-Trejo A, Aguirre-Morales CE, González-González GM, Cortés-Palma D, Miranda-Novales MG. Colonización por *Candida* en una unidad de cuidados intensivos neonatales. Rev. Med. Inst. Mex. Seguro Soc, 2014; 52(2): S16-23.
33. Pertuz-Meza YB, González-Rubio Altamar C, Cabas-De la Cruz L. Sepsis por *Candida lusitaniae* en un paciente con adenocarcinoma gástrico: reporte de un caso. Duazary, 2023; 20(829): 145-51.
34. Fowler SL, Rhoton B, Springer SC, Messer SA, Hollis RJ, Pfaller MA. Evidence for person-to-person transmission of *Candida lusitaniae* in a neonatal intensive-care unit. Infect. Control Hosp. Epidemiol., 1998; 19(5): 343-5.
35. Sánchez V. Vázquez AJ, Barth-Jones D, Dembry L, Sobel DJ, Zervos JM. Epidemiology of nosocomial acquisition of *Candida lusitaniae*. J Clin Microbiol, 1992; 30: 3005-3008.