WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 13, 1303-1316. Research Article ISSN 2277– 7105

CLINICAL ISOLATES OF *Candida Lusitaniae* DURING THE COVID-19 PANDEMIC IN MEXICO

Cudberto Contreras Pérez[1], Elizabeth González Durán[2], Patricia Gutiérrez García[1], M. Dolores Téllez Saucedo[2], Edgar S. Suro Marín[3], Liset Pérez Vázquez[2], Claudia Ríos-Rosas[1], Claudia E. Wong-Arámbula[4] and José D. Méndez[5]*

[1]Micology Laboratory. Instituto de Diagnóstico y Referencia Epidemiológicos "Dr. Manuel Martínez Báez". Secretaría de Salud. México City. México.

[2]Molecular Bacteriology Laboratory. Instituto de Diagnóstico y Referencia Epidemiológicos "Dr. Manuel Martínez Báez". Secretaría de Salud. México City. México.

[3]Chagas Laboratory. Instituto de Diagnóstico y Referencia Epidemiológicos "Dr. Manuel Martínez Báez". Secretaría de Salud. México City. México.

[4]Molecular Sequencing Laboratory. Instituto de Diagnóstico y Referencia Epidemiológicos "Dr. Manuel Martínez Báez". Secretaría de Salud. México City. México.

[5]Medical Research Unit in Metabolic Diseases. Cardiology Hospital. Mexican Institute of Social Security. México City. México.

Article Received on
29 July 2022,

Revised on 19 August 2022,
Accepted on 09 Sept. 2022

DOI: 10.20959/wjpr202213-25555

***Corresponding Author**
Dr. José D. Méndez
Medical Research Unit in Metabolic Diseases. Cardiology Hospital. Mexican Institute of Social Security. México City. México.

ABSTRACT

During the COVID-19 pandemic, opportunistic yeast infections of the *Candida* genus have increased. In a period of eight months (September 2021 to April 2022), 102 clinical yeast isolates were received, of which 7 were identified as *Candida lusitaniae* (6.86 %), but only one strain of this pathogen was associated with a patient of COVID-19. Another 10 patients had COVID-19 (10 %) and they had yeast infections, with 2 fatal cases occurring, one due to *Candida auris* and the other due to *Candida parapsilosis*. *Candida auris* was associated in 4 cases, *Candida albicans* in 4 and *Candida duobushaemulonii* in 1 case. The seven *Candida lusitaniae* isolates came from 5 immunocompromised patients with different invasive co-infections. 2 patients were adults, one with COVID-19 and the other with decompensated type 2 diabetes mellitus. The other 3 cases were premature neonates with neonatal bacterial sepsis. In the five hospital units in different geographical areas, control measures were established to prevent the spread of the

pathogen and for an additional period of three months, no additional cases were recorded. Isolates were identified by phenotypic tests, biochemical tests (Vitek 2, version 9.1) and molecular tests (PCR and sequencing of the internal spacer region (ITS) of ribosomal DNA (rDNA). Phylogenetic analysis of this region allowed determining the evolutionary relationship among *Candida lusitaniae* isolates. The excellent reliability of the Vitek system stands out, with a probability of 94 to 97% for the identification of the species involved. No resistant strain was detected, all were sensitive to the 6 antifungals: fluconazole, voriconazole, caspofungin, micafungin, amphotericin B and fluocitosina Special importance is given, in the microscopic characteristics found, as well as the existence of subgroups, which may be related, with the variable behavior of the biochemical characteristics and geographic areas.

KEYWORDS: Humans, COVID-19, *Candida lusitaniae*, *Candida parapsilosis*, *Candida auris*, *Candida albicans*, *Candida duobushaemulonii*, Antifungals.

1. INTRODUCTION

Candida lusitaniae is an opportunistic yeast that frequently develops resistance to amphotericin B.[1-3] It was described in 1989 as an emerging pathogen, affecting immunocompromised patients.[4-6] In 2015, the hospital epidemiological surveillance network (RHOVE) in Mexico registered 5 species of medical importance of the genus *Candida*, isolated from cases of fungemia.[7] *Candida albicans* ranked first with 1,830 cases, followed by *Candida tropicalis* with 393, *Candida glabrata* with 203, *Candida parapsilosis* with 186, and *Candida krusei* with 107 cases. *Candida lusitaniae* is not among the registered species and publications on this pathogen are scarce.[8]

A review in 2015, on invasive candidiasis, did not register any clinical case due to *Candida lusitaniae*, indicating a frequency of 0.65% as an agent of onychomycosis.[9] In a recent study in 2019, a global frequency of 3.7 %[10] was reported, similar to that found in other countries.[11] In recent years, there seems to be an increase in clinical cases. There is evidence of nosocomial transmission in neonatal intensive care units and it is suggested that these neonates are at increased risk of infection with this pathogen.[12]

The purpose of this work is to communicate the findings of seven isolates of *Candida lusitaniae*, obtained from five clinical cases, highlighting the morphological, biochemical, and molecular characteristics, as well as the susceptibility to antifungals, to contribute with

timely information, which allows establishing measures of control, which prevent its dispersion and transmissibility in hospital units.

2. MATERIAL AND METHODS

2.1. Biological material and information on isolates and patients

The Institute of Epidemiological Diagnosis and Reference (InDRE) in a period of 8 months (September 2021 to April 2022), received 102 clinical isolates of yeasts, from different geographical areas of the country. Seven strains arrived with the determination of *Candida lusitaniae*, identified by Vitek 2, version 8.1, which were obtained from five patients with fungemia. Two cases corresponded to adults, one with COVID-19 infection and the other with decompensated type 2 diabetes mellitus with a serum glucose of 1066 mg/100 mL. The other 3 cases were premature neonates with various concomitant bacterial infections. The patients were treated with fluconazole. The epidemiological data of these cases are presented in Table 1.

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

Isolation key	No. Case/ Diagnosis	Age and sex	Source	Entity	Specie*
MYC-124-21	1/ Covid-19	45 M	Urine culture	México City	*Candida lusitaniae*
MYC -126-21	2/ Type 2 diabetes mellitus. Metabolic acidosis.	23 M	Urine culture	México City	*Candida lusitaniae*
MYC -44-22	3/ R/N. Premature 34.6 Weeks	37 Days F	Peripheral heme	Guanajuato	*Candida lusitaniae*
MYC -94-22	4/R/N.Premature 31.2 Weeks. Sepsis	31 Days F	Catheter tip	Jalisco	*Candida lusitaniae*
MYC -95-22	4 / R/N. Premature. 31.2 Weeks. Sepsis	31 Days F	Peritoneal fluid	Jalisco	*Candida lusitaniae*
MYC -112-22	5/R/N. Premature. Gastroschisis	40 Days F	Blood culture	Tabasco	*Candida lusitaniae*
MYC -113-22	5/ R/N. Premature. Gastroschisis	40 Days F	Catheter tip	Tabasco	*Candida lusitaniae*
ARG-0PS-12	Reference strain	-	Quality control program	Malbran Institute	*Candida lusitaniae*

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

2.2. Vitek 2 system version 9.01 (identification and susceptibility to antifungals)

For identification, a cell suspension was prepared for each isolation, from colonies isolated with 48 hours of incubation in Sabouraud dextrose agar (aSd), adjusted to a turbidity

equivalent to a range of 1.8 to 2.0 units on the McFarland scale. For antifungal susceptibility tests, a dilution of the previous suspension was made, adding 288 ul to a volume of 3 ml of 0.5% isotonic saline solution. Two control strains were used: sensitive strain (*Candida parapsilosis* ATCC 22019) and resistant strain (*Candida krusei*, OPS-ARG-13-2021).

2.3. Molecular identification

Genomic DNA (gDNA) was extracted from the reference strain (OPS-ORG-12) and from 7 isolates (MYC-124-21, MYC-126-21, MYC-44-22, MYC-94-22, MYC-95- 22, MYC-112-22 and MYC-113-22), from 1 ml of a cell suspension in saline solution, adjusted to an optical density of 3.0 on the MacFarland scale. Additionally, the lysed material was purified with the QIAamp® DNA Mini kit extraction kit (QIAGEN, Hilden, Germany), as previously published.[13]

The molecular identification of *Candida lusitaniae* was carried out by means of a simple and low-cost pan-fungal PCR, directed at the internal transcribed spacers (ITS1-ITS2) of the 18S, 5.8S and 28S ribosomal cistron, using the primers ITS1F (5´-TCCGTAGGTGAACCTGCGG -3´) and ITS4R (5'-TCCTCGCTTATTGATATGC-3'), to obtain an amplification product of approximately 400 bp. The reaction and cycling conditions were the same as those previously described.[13,14] The PCR products were sequenced using the Sanger method.[15] The identity of the isolates was determined with the nBLAST tool, available in the NCBI database (http://www.ncbi.nlm.nih.gov/blast). The intra-species variability of *Candida lusitaniae* was determined by a phylogenetic analysis of the ITS region (ITS1-ITS4). The maximum likelihood phylogenetic tree of the ITS region was built with nine non-redundant sequences extracted from GenBank, a reference sequence from *Candida lusitaniae* and 7 sequences obtained in this work, using the Jukes-Cantor nucleotide substitution model and 1000 replicas, within the MEGA 6.0 program.[16]

3. RESULTS

3.1. Morphological characteristics

The seven strains of *Candida lusitaniae* developed well in the culture media used, except in mycosel agar, where they were inhibited by cycloheximide. The morphology in (aSd) and Biggy (Ab) agar was similar in both media, consistent with spherical, white and/or black colonies with a wet appearance and smooth surface (Figure 1A). The microscopic image alone reveals single cells (yeast), some with blastoconidia, without formation of pseudohyphae or hyphae.

In maize flour agar, the strains have a similar behavior and present two reproduction phases: 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 pear-shaped blastoconidia sides (Figure 1B, 1C). Advanced stage, which occurs between 72 and 96 hours, is characterized by greater branching and abundant formation of lateral blastoconidia, which cover most of the hyphae, with the appearance of clusters (Figure 1C).

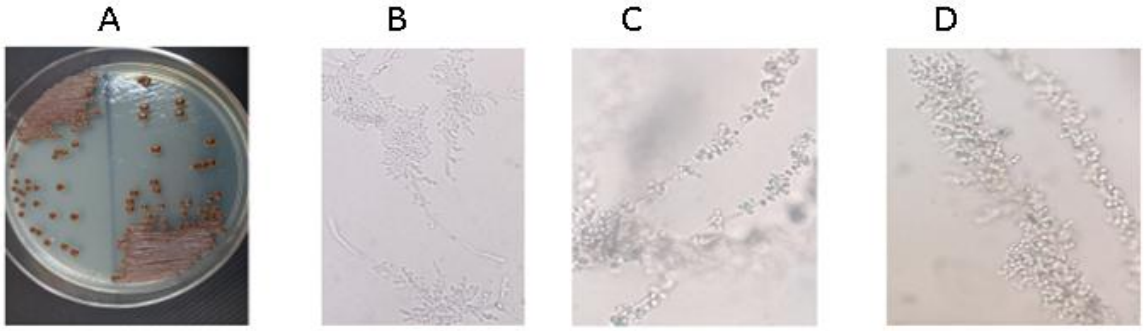

Figure 1: A. *Candida lusitaniae*. Colonies on Biggy agar, 48 hours, incubated at 30 °C. B. *Candida lusitaniae*, initial reproduction at 24 o'clock, with formation of pseudohyphae and branching hyphae, with occasional lateral blastoconidia, 40 X. C. *Candida lusitaniae*, reproduction at 48 o'clock hours, a greater formation of lateral blastoconidia occurs, 40 X. D. *Candida lusitaniae*, late reproduction between 72 and 96 hours, with the presence of abundant blastoconidia, forming lateral clusters, covering all the hyphae, 40 X.

3.2. Biochemical characteristics

The results of the biochemical tests with the Vitek 2 system are shown in Table 2. The seven clinical strains were identified as *Candida lusitaniae*, with a reliability of identification from very good to excellent and a probability of 94 to 97%. The reference strain, *Candida lusitaniae* (OPS-ARG-12), from Argentina showed acceptable reliability and a probability of 88%. Among the 46 substrates (sources of carbon, nitrogen, and enzymatic activities) handled by the team, sixteen substrates allow us to highlight differential characteristics in the biochemical behavior between these seven isolates and the reference strain (Table 2). *Candida lusitaniae* exhibits moderate metabolic activity, hydrolyzing in general about 30 of these substrates. Some of them are decisive for the identification and differentiation with other *Candida* species, such as rhamnose, raffinose, cellobiose, N-acetyl glucosamine and esculin.

Table 2: Variability in the biochemical behavior of *Candida lusitaniae*, in relation to the different isolation sources.

Substrate Number of case	MYC-124-211	MYC-126-212	MYC-44-223	MYC-94-224*	MYC-95-225*	MYC-112-226**	MYC-113-227**	OPS-ARG-128
Tyrosine	-	-	-	+	+	-	-	+
L-Malate	-	-	+	+	+	+	+	+
Xylitol	+	+	+	-	-	(+)	(-)	+
D-Sorbitol	+	+	+	+	+	+	+	-
DL-Lactate	+	+	+	-	-	(+)	+	+
Arginine	+	+	+	-	-	-	-	+
D-Galactose	-	-	+	-	-	-	(+)	-
Citrate	+	+	-	+	+	-	-	+
Glycerol	-	-	(-)	-	-	+	+	+
Gentiobiosa	+	+	+	+	+	+	+	-
L-Sorbose	+	+	+	+	+	+	+	-
Esculin	+	+	+	+	+	+	+	-
L-Rhamnose	+	+	+	+	+	+	+	+
Raffinose	-	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	+	+	+	+
N-Acetyl glucosamine	+	+	+	+	+	+	+	+

(*) Isolates 4 and 5 come from case 4.; (**) Isolates 6 and 7 come from case 5.

3.3. Susceptibility to antifungals

The seven clinical isolates of *Candida lusitaniae* were markedly sensitive to the 6 conventional antifungals: fluconazole (0.5 ug/mL), voriconazole (0.12 ug/mL), caspofungin (0.25 ug/mL), micafungin (0.12 ug/mL), amphotericin B (0.5 ug/mL), and fluorocytosine (1 ug/mL). The sensitive reference strain, *Candida parapsilosis* (ATCC 22019), was consistent with the criteria established by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antibiotic Susceptibility Testing (EUCAST).

The minimum inhibitory concentration (MIC) was also determined by the agar dilution method for the control strains: *Candida parapsilosis* ATCC 22019, a sensitive strain, and *Candida krusei* (OPS ARG 13), a resistant strain, in accordance with the conditions previously described.[10] The sensitive strain *Candida parapsilosis* showed an MIC for fluconazole of 4 ug/ml, while the resistant strain *Candida krusei* showed an MIC of 16 ug/ml, results that are consistent with the cut-off points established for fluconazole by the CLSI and the EUCAST.[10]

Table 3: Results of susceptibility to conventional antimycotics, Mexican isolates of *Candida lusitaniae*.

Isolation key	Fluconazole ug/mL MIC	Voriconazole ug/mL MIC	Caspofungin ug/mL MIC	Micafungin ug/mL MIC	Amphoteri-cin B, ug/mL MIC	5-fluocytosine ug/mL MIC
MYC -124-21	</= 0.5 S	</= 0.12 S	0.25 S	0.12 S	0.5 S	</= 1 S
MYC -126-21	</= 0.5 S	</= 0.12 S	0.25 S	0.12 S	0.5 S	</= 1 S
MYC -44-22	</= 0.5 S	</= 0.12 S	0.25 S	0.12 S	0.5 S	</= 1 S
MYC -94-22	</= 0.5 S	</= 0.12 S	0.25 S	0.12 S	0.5 S	</= 1 S
MYC -95-22	</= 0.5 S	</= 0.12 S	0.25 S	0.12 S	0.5 S	</= 1 S
MYC -112-22	</= 0.5 S	</= 0.12 S	0.25 S	0.12 S	0.5 S	</= 1 S
MYC -113-22	</= 0.5 S	</= 0.12 S	0.25 S	0.12 S	0.5 S	</= 1 S
ARG-0PS 12	</= 0.5 S	</= 0.12 S	0.25 S	0.12 S	0.5 S	</= 1 S
Candida parapsilosis ATCC 22019 (*)	2 S	</= 0.2 S	0.5 S	0.5 S	0.5 S	</= 1 S
Candida krusei OPS-ARG-12 (**)	NA	</= 0.12 S	</= 0.12 S	0.12 S	0.5 S	NA

(*) *Candida parapsilosis*. Determination of sensitivity to Fluconazole by the agar dilution method. MIC = 4 ug/mL, sensitive.

(**) *Candida krusei*. In the fluconazole and flucytosine columns, no values appear, because this species has intrinsic natural resistance to fluconazole. In the case of fluorocytosine, the species may acquire resistance during treatment. Determination of sensitivity to Fluconazole by the agar dilution method. MIC = 16 ug/mL, resistant. NA = Not applicable.

3.4. Molecular characteristics

Pan-fungal PCR targeting the ITS region of the rDNA cistron allowed amplification of the expected product of approximately 400 bp for isolates MYC-124-21, MYC-126-21, MYC-44-22, MYC-94- 22, MYC-95-22, MYC-112-22 and MYC-113-22 (Figure 2). The identity of the amplicon was confirmed by sequencing. The sequences of the seven isolates exhibited between 99 and 100 % similarity, with respect to the identity of the reference sequence of *Candida lusitaniae* (OPS-ARG-12), also sequenced in this work. The maximum identity with other sequences of the same species deposited in the Gen Bank was also observed. The obtained sequences were deposited in the Gen Bank database (GB accession numbers: OP080715 to OP080721, respectively).

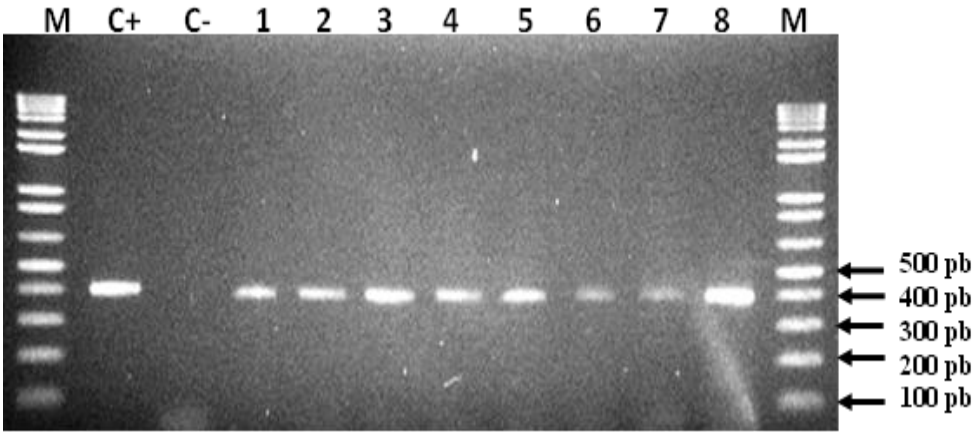

Figure 2: Pan-fungal PCR amplification product (ITS1-ITS2) of *Candida lusitaniae*.

Positive amplification: C+ (positive control, *Candida auris*), #1 (MYC-124-21), #2 (MYC-126-21), #3 (MYC-44-22), #4 (MYC-94-22), #5 (MYC-95-22), #6 (MYC-112-22), #7 (MYC-113-22) and #8 (positive control, *Candida lusitaniae*, panel, OPS-ARG-12). Negative amplification: C- (negative control), **M**: 1Kb Plus DNA molecular weight marker, its migration is shown on the right side in base pairs (bp).

The comparison of the seven sequences, by means of a multiple local alignment, within the MEGA 6.0 program, showed the genetic variability among the clinical isolates of *Candida lusitaniae* studied. This intra-species variability was corroborated by phylogenetic analysis of the ITS1-ITS4 region with the construction of a Maximum Likelihood tree. The phylogenetic tree showed that the seven *Candida lusitaniae* isolates were distributed into three sub-groups (Figure 3). The first group is shared by the sequences of case 5 (MYC-112-22 and MYC-113-22) from Tabasco and case 1 (MYC-124-21) from CDMX; the isolates from case 1 belong to the same patient but come from different sources, which suggests that they originate from the same strain and share identity with the sequence isolated in the center of the country, belonging to an adult who had COVID-19. In the second group, the sequence of case 2 ((MYC-126-21) from México City that presented with severe decompensated ketoacidosis was grouped together with the reference strain from Argentina (OPS-ARG-12), used in this study. While Like group 3, it is shared by the sequences of case 4 (MYC-94-22 and MYC-95-22) isolated in Jalisco and the sequence of case 3 (MYC-44-22) identified in Guanajuato, both regions located geographically to the west of the Mexican Republic (Figure 3).

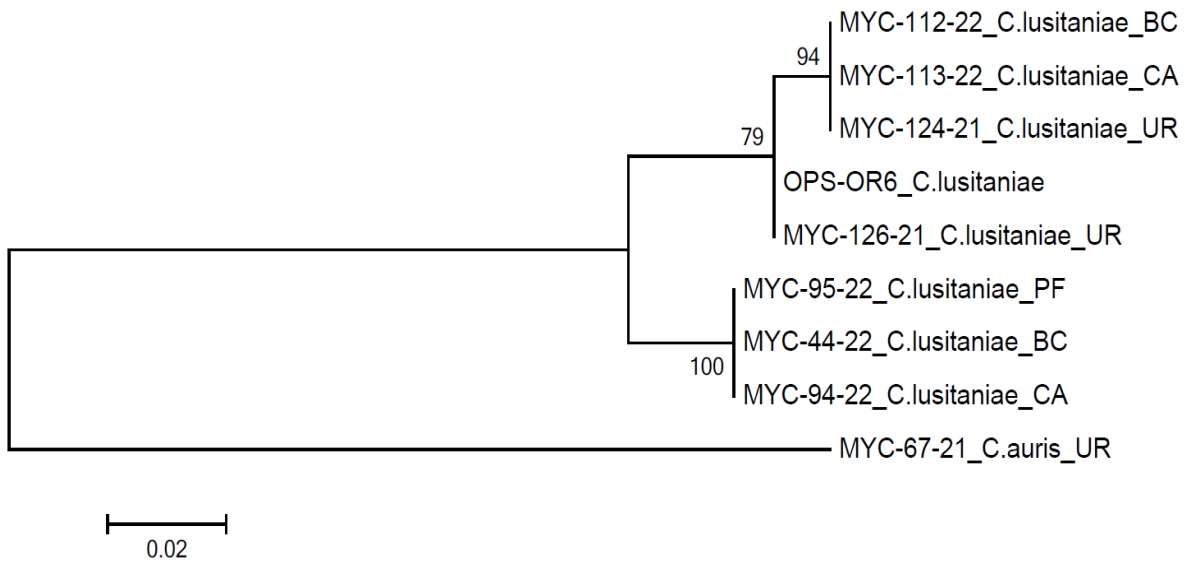

Figure 3: Phylogenetic analysis of the ITS region of the rDNA of *Candida lusitaniae* in Mexico.

Maximum likelihood phylogenetic tree of the ITS1-ITS2 regions of the 18S gene, 5.8S and the partial region of the 28S gene (320nt), built with the Jukes and Cantor evolutionary model with 1000 replicates, within the MEGA6.0v program. In the tree, the key to the isolates is indicated, followed by the name of the microorganism and the source of isolation (Blood culture (BC); catheter (CA); urine culture (UR); peritoneal fluid PF)). *Candida lusitaniae* reference sequence (OPS-AR6-12). *Candida auris* (MYC-67-21 with GB accession number: MZ648437) was used as an outgroup. Three different groups are observed within *Candida lusitaniae*, coming from isolates of human origin from different entities of the Mexican Republic.

4. DISCUSSION

In Mexico, recent studies on the etiology of candidiasis,[9,17-18] indicate a low frequency of *Candida lusitaniae* of 0.14 % in invasive conditions,[9] occasionally it has been isolated from onychomycosis,[9] esophageal candidosis,[17] one case of fungemia, in a hospitalized patient[18] and one case of lower respiratory tract infection.[8] In the last two years, during the COVID-19 pandemic, infections by this species seem to have increased. In a period of eight months (September 2021 to April 2022), 102 clinical yeast isolates were received, of which seven were identified as *Candida lusitaniae*, with a frequency of 6.86 %.

However, of the five patients with *Candida lusitaniae* isolation, only one had COVID-19. Of the 102 patients, 11 patients had COVID-19 pneumonia and all of them had opportunistic

yeast infections of the *Candida genus* (10.78 %), with 2 fatal cases occurring. The *Candida* species isolated in these patients were: *Candida auris*, 4 cases; *Candida parapsilosis*, 1 case; *Candida duobushaemulonii*, 1 case, and as already mentioned, *Candida lusitaniae* in another case.

It is likely that the appearance of the first case of infection by the emerging species *Candida auris*,[19] and the detection of the first outbreak, which occurred in Monterrey, Nuevo León,[20] affecting several private medical units, resulted in an alert on cases of fungemia,[21] to monitor the dispersion and control of *Candida auris* in the country. These prevention measures in the Health Sector have led to strict medical surveillance in the medical units, causing greater communication between the medical area and the laboratory. In the case of *Candida auris*, it has been described that some strains have been misidentified as *Candida lusitaniae*.[22] For this reason, clinical isolates of this species must be sent to the reference laboratory to confirm their existence. determinative and avoid mistaken identifications of *Candida auris.*

In this work, we have had the opportunity to study the morphological, biochemical, physiological, and molecular characteristics of *Candida lusitaniae*. These characteristics are useful for early and timely identification. The results are highly reliable, to establish preventive and control measures for its transmissibility and dispersion in medical units. This yeast grew excellent in conventional culture media. In Biggy agar it presents differential characteristics, with other species of *Candida*, particularly with *Candida auris*, *Candida lusitaniae* significantly reduces ammonium citrate and bismuth, producing frank amounts of bismuth sulfide, forming dark brown colonies, while *Candida auris* reduces weakly these salts, first forming white colonies, which later present a light brown button in the center. Both species are inhibited by cycloheximide, present in mycosel agar, *Candida lusitaniae* does not grow at 42 ^{o}C, while the two clades of *Candida auris* (I and IV) that circulate in Mexico, grew well at this temperature.

The phylogenetic analysis of the ITS region of the rDNA in this study also indicates that the two species *Candida auris* and *Candida lusitaniae* are clearly different from each other. This information agrees with that previously reported in phylogenetic analyzes by other authors.[20] This analysis allowed to confirm once again the genetic variability observed between this same species, which was reported in a previous study.[10]

Therefore, the analysis of genetic variability allowed the identification of three sub-groups within the Mexican isolates of *Candida lusitaniae*, of human origin and from different entities of the Mexican Republic, in accordance with what was previously reported.[10, 11] Particularly, for the sequences corresponding to premature neonates, from the states of Tabasco and Jalisco, México, respectively, spatio-temporal variability was observed between the studied strains of this species, even when the source of isolation is different, peritoneal fluid (very rare) and the other blood culture, it was possible to identify different subgroups, in accordance with what was reported by other authors.[11] It is noteworthy that the isolates of group 3, corresponding to the west of the country, behave differently, compared to the other two groups from the center and south of Mexico. However, the isolates from Mexico City were not grouped within the same branch in the phylogenetic tree, evolutionarily they are close to each other.

The automated Vitek 2 equipment identified the seven isolates of *Candida lusitaniae* without any problem, with a very good level of identification and a probability between 94 and 97 %. Regarding the reliability of the identification methods, it is probable that erroneous identifications have occurred in automated equipment with previous versions.[23] The update of Vitek 2, in its versions 8.1 and 9.1, has revealed a highly accurate identification, in addition to advances in mycology (phenotypic methods)[24] and molecular biology,[24] allow a greater knowledge of this species, establishing clear differences taxonomic, with the species *Candida parapsilosis*, *Candida albicans*,[10] *Candida tropicalis*[25] and *Candida auris*.

No antifungal resistant strain was found, the seven strains showed high susceptibility to fluconazole, voriconazole, caspofungin, micafungin, amphotericin B and 5-flucytosine. The five patients were treated with fluconazole alone with good response and there were no deaths. In a previous study by us,[10] which included three clinical fungemia isolates and two environmental ones, the strains were highly sensitive to itraconazole. Other authors[26] also found that all their isolates were sensitive to amphotericin B, the rapid and accurate identification of *Candida* species will contribute to taking the appropriate measures for its control.[27]

5. CONCLUSIONS

Candida lusitaniae is currently considered a strict sensu species. The phylogenetic analysis of the seven isolates clearly separates three subgroups; We had already observed this behavior in a previous study.[10] The subgroups are probably related to the geographical region, as well

as the variations observed in the biochemical and molecular characteristics. The prevention of infections associated with health care requires reliable, timely and comprehensive results between the medical area and the laboratory studies.

6. REFERENCES

1. Merz GW. *Candida lusitaniae.* Frequency of recovery, colonization; infection, and amphotericin B resistance. J. Clin. Microbiol, 1984; 20: 1194-1195.
2. 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.
3. Peyron F, Favel A, Michel-Nguyen A, Gilly M, Regli P, Bolmström A. Improved detection of amphotericin B-resistant isolates of *Candida lusitaniae* by Etest. J. Clin. Microbiol, 2001; 39(1): 339-42.
4. Runco R, Raquel Salim R. *Candida lusitaniae* en un paciente pediátrico inmunocomprometido: éxito terapéutico del voriconazol. Boletín Micológico, 2005; 20: 97-102.
5. Blinkhorn RJ, Adelstein D, Spagnuolo PJ. Emergence of a new opportunistic pathogen, *Candida lusitaniae*. J. Clin. Microbiol, 1989; 27(2): 236-40.
6. 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.
7. Informe anual 2015. Red Hospitalaria de Vigilancia Epidemiológica RHOVE. Dirección General de Epidemiología. Secretaría de Salud, México, 2016.
8. 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.
9. 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 Iberoam. Micol, 2017; 34(4): 203–210.
10. Contreras PC, Gutiérrez GP, Beltrán PLG, Pastén SS, Méndez JD. *Candida lusitaniae.* Isolation, Identification and Clinical Relevance. www.wjpr.net, 2019; 8(5): 606-21.

11. 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).
12. 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): 343-345.
13. González-Durán E, Contreras-Pérez CU, Caceres-Diego H, Ríos-Rosas C, Piñón-Ortega JJ, Téllez-Saucedo MD, Marín-Suro ES, Wong-Arámbula CE, Moreno-Escobar AE, Ramírez-González JE, Ramírez-Barrios J G, Montes-Colima NA, Lockhart Shawn R, Martínez-Montiel N, Martínez-Contreras R, García-Ruíz P, Salazar-Sánchez MI, Hernández-Rivas L, López-Martínez I. The use of readily available laboratory tests for the identification of the emerging yeast *Candida auris* in Mexico. Arch. Microbiol, 2022; 204: 592.
14. Theill L, Dudiuk C, Morales-Lopez S, Berrio I, Rodríguez JY, Marin A, Gamarra S, Garcia-Effron G. Single-tube classical PCR for *Candida auris* and *Candida haemulonii* Identification. Rev. Iberoam. Micol, 2018; 35(2): 110–112.
15. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences of the United States of America, 1977a; 74: 5463-5467.
16. Kumar S, Stecher G, Tamura K. MEGA 7: Molecular Evolutionary Genetics Analysis Version 7.0for Bigger Datasets. Mol. Biol. Evol, 2016; 33(7): 1870-1874.
17. Méndez-Tovar LJ, Rodríguez-Sánchez JF, Manzano-Gayosso P, Hernández-Hernández F, Blancas-Valencia JM, Silva-González I. Candidiasis esofágica en pacientes de un hospital de especialidades. Revista Médica del Instituto Mexicano del Seguro Social, 2019; 57(2): 1-15.
18. Reséndiz–Sánchez j, Morales–Aguirre JJ. Factores asociados a mortalidad por fungemias causadas por *Candida* sp. en niños. Bol. Med. Hosp. Infant. Mex, 2007; 64(2).
19. Ayala‐Gaytan JJ, Montoya AM, Martinez‐Resendez MF, Guajardo‐Lara CE, Treviño‐Rangel R de J, Salazar‐Cavazos L, Llaca‐Diaz JM, González GM. First case of *Candida auris* isolated from the bloodstream of a Mexican patient with serious gastrointestinal complications from severe endometriosis. Infection, 2020; 49(3): 523-525.

20. Villanueva-Lozano H, Treviño-Rangel RdJ, González GM, Ramírez-Elizondo MT, Lara-Medrano R, Alemán-Bocanegra MC, Guajardo-Lara CE, Gaona-Chavéz N, Castilleja-Leal F, Torre-Amione G, Martínez-Reséndez MF, Outbreak of *Candida auris* infection in a COVID-19 hospital in Mexico, Clin. Microbiol. Infect, 2021; S1198-743X(20)30790-4.
21. Aviso Epidemiológico. CONAVE /13/ 2020/*Candida auris*. 09 de diciembre de 2020. México.
22. Spivak ES, Hanson KE. 2018. *Candida auris*: an emerging fungal pathogen. J. Clin. Microbiol, 2018; 56(2): e01588-17.
23. Hadfield TL, Smith MB, Winn RE, Rinaldi MG, Guerra C. Mycoses caused by *Candida lusitaniae*. Rev. Infect. Dis., 1987; 9(5): 1006-12.
24. *Candida lusitaniae*. Lloret Sos C, Gutiérrez O, Borrell Solé N. SEIMC. Control Calidad. Servicio de Microbiología, Hospital Universitario Son Dureta, Palma de Mallorca.
25. Camacho-Cardoso JL, Martínez-Rivera MA, Manzano-Gayosso P, Méndez-Tovar LJ, López-Martínez R, Hernández-Hernández F. Detección molecular de especies de *Candida* en especímenes de pacientes hospitalizados. Gac. Méd. Méx., 2017; 153: 581-589.
26. 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.
27. Franco-Curiel DL, De La Fuente I, Ribacoba L, Fernández-Rodríguez M, Guridi A, Sevillano E, Eraso E, Quindós-Andrés G. Estudio comparativo de la Candidiasis Invasiva en México y España. Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU), Bilbao. 2018. México: Universidad de Guadalajara; 2018. https://sciforum.net/manuscripts/4596/slides.