ORIGINAL PAPER

The use of readily available laboratory tests for the identification of the emerging yeast *Candida auris* in Mexico

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Abstract

Identification of the emerging multidrug-resistant yeast *Candida auris* is challenging. Here, we describe the role of the Mexico national reference laboratory Instituto de Diagnóstico y Referencia Epidemiológicos Dr. Manuel Martínez Báez (InDRE) and the Mexican national laboratory network in the identification of *C. auris*. Reference identification of six suspected isolates was done based on phenotypic and molecular laboratory methods, including growth in special media, evaluation of isolate micromorphology, and species-specific PCR and pan-fungal PCR and sequencing. The four *C. auris* isolates identified were able to grow on modified Sabouraud agar with 10% NaCl incubated at 42 °C. With one exception, isolates of *C. auris* were spherical to ovoid yeast-like cells and blastoconidia, with no hyphae or pseudohyphae on cornmeal agar. *C. auris* isolates were resistant to fluconazole. Species-specific and pan-fungal PCR confirmed isolates as *C. auris*. Sequence analysis revealed the presence of two different *C. auris* clades in Mexico, clade I (South Asia) and clade IV (South America).

Keywords *Candida auris* · Laboratory identification · Antifungal resistance

Introduction

Candida auris is an emerging multidrug resistance yeast able to cause invasive fungal infections and hospital outbreaks. This species of *Candida* differs from other species in that it more readily spreads from patient to patient in

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healthcare settings through contact with contaminated environmental surfaces or equipment (Welsh et al. 2017; Jeffery-Smith et al. 2018). The laboratory identification of *C. auris* using commercially available methods for yeast identification is challenging due to frequent misidentification (Jeffery-Smith et al. 2018; Caceres et al. 2019; Kathuria et al. 2015; Carvajal-Valencia et al. 2020). Currently, the most reliable methods for identification of *C. auris* include the VITEK® 2 with the most current software update, MALDI-TOF, molecular methods using specific PCR assays (Kathuria et al. 2015; Kordalewska et al. 2017; Satoh et al. 2009), or by the amplification and sequencing of the ITS1-ITS2 or the D1/D2 region of the ribosomal cistron (Satoh et al. 2009; Lockhart et al. 2017a, b, c).

In Mexico, *C. auris* was reported for the first time in May 2020 in a woman with severe endometriosis (Ayala-Gaytán et al. 2021). Three months later, the same hospital reported a large outbreak of *C. auris* associated with high mortality in patients with severe COVID-19 (Villanueva-Lozano et al. 2021). The impact of the current COVID-19 pandemic resulted in overcrowded hospitals, with larger numbers of critically ill patients at increased risk of invasive candidiasis. These overcrowded conditions set the stage for an increase in hospital-associated infections, including those caused by *C. auris* (Chowdhary and Sharma 2020).

Here, we describe the role of the Mexico national reference laboratory, Instituto de Diagnóstico y Referencia Epidemiológicos Dr. Manuel Martínez Báez (InDRE), and the Mexican national laboratory network in the detection of emerging problems, and the identification of the emerging yeast *C. auris*. This report presents a summary of the microbiological characteristics, focused on the phenotypic and molecular characteristics of Mexican isolates proven to be *C. auris*.

Materials and methods

Isolates

Six isolates suspected to be *C. auris* following CDC recommendations for *C. auris* identification ((CDC) CfDCaP 2018a) were submitted to InDRE for species identification. One from blood and two from urine came from a private hospital at the state of Nuevo León (labeled MYC-28, MYC-29, and MYC-30). The other three isolates were referred from public hospitals: a sputum isolate from Tabasco state (MYC-43), a blood isolate from Querétaro state (MYC-121), and a urine isolate from Morelos state (MYC-221). Before remission to InDRE, MYC-28, MYC-29, and MYC-30 were identified as *C. auris* using MALDI-TOF MS Bruker, and MYC-43 as *C. auris*, MYC-121 as *C. krusei*, and MYC-221

as *C. parapsilosis* using the VITEK® 2 system, v8.01 software.

Phenotypic identification

Suspected isolates were grown on Sabouraud dextrose agar (SDA, Becton Dickinson), incubated at 30 °C for 120 h. In parallel, isolates were grown on a selective modified Sabouraud agar (Welsh et al. 2017), supplemented with 10% NaCl (Sab-10% NaCl) and dulcitol instead of dextrose as carbon source. Isolates on this media were incubated at 42 °C for 72 h. In addition, morphological characteristics of isolates were evaluated, including growth on Cornmeal agar (Becton Dickinson).

Isolates were also tested using the VITEK® 2 system. Identification was performed using VITEK® 2 YST cards and results were analyzed using the software version 8.01 (bioMérieux, Marcy, L'Etoile, France). Additionally, assimilation of the following carbohydrates was evaluated: glucose, galactose, cellobiose, raffinose, melezitose, rhamnose, trehalose, dulcitol, and xylose. Results of carbohydrate assimilation were extracted from the VITEK® 2 system.

Molecular identification

Genomic DNA (gDNA) extraction was performed using the FastDNA™ kit (MP Biomedicals, USA) and the FastPrep® equipment as suggested by the manufacturer. Lysed material was further purified using the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany).

Two PCR amplifications were used for molecular identification. The first was a pan-fungal PCR targeting the internal transcribed spacers (ITS1-ITS2) of the ribosomal cistron using primers ITS1F (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′), with a product size of 380 bp. The second amplification was a species-specific PCR for *C. auris* using primers ITS1F and CaurisR (5′-CCACCGCGAAGATTGGTG-3), with a product size of 280 bp. The PCR conditions have been previously described (Theill et al. 2018), and PCR products were sequenced by the Sanger method (Sanger et al. 1977). Sequence analysis was done using the nBLAST tool at the NCBI database (http://www.ncbi.nlm.nih.gov/blast). A phylogenetic tree of maximum-likelihood III of the ITS regions of 14 non-redundant sequences extracted from the GenBank database and four sequences obtained here were constructed using MEGA7.0 software and Jukes–Cantor nucleotide substitution model with 1000 bootstraps (Kumar et al. 2016).

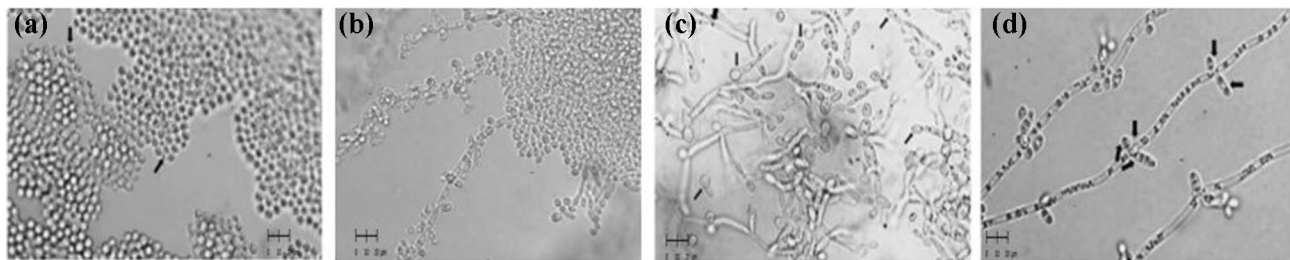### Fluconazole susceptibility testing

Fluconazole (FLU) susceptibility testing was performed using the agar dilution method, using Sigma-Aldrich FLU,

following InDRE standard operating procedure (SOP) (Pérez et al. 2019). Minimum inhibitory concentration (MIC) was assessed in concentrations ranging from 2 to 256 µg/mL. After 24 h incubation, the MIC was determined as the lowest concentration that inhibits yeast growth (CLSI 2017). For susceptibility testing, *Candida parapsilosis* (ATCC 22019) was used as a susceptible strain, and *Candida krusei* (PAHO-ARG13) as a resistant strain (CLSI-M60). *Candida auris* MIC values for were interpreted using the tentative cut-off values proposed by the CDC (MIC ≥ 32 µg/mL) ((CDC) CfDCaP 2018a).

Results

Four isolates were from men and two from women, and patients were presented with a median age of 59 years (range 54–78 years). All patients were diagnosed with COVID-19 and were hospitalized in the intensive-care unit (ICU). Median ICU stay was 35 days (range 23–71 days). Five of the six patients died, all of them infected with *C. auris* and *C. krusei*.

Using phenotypic methods, we found that isolates originally identified as *C. auris* (MYC-28, MYC-29, MYC-30, and MYC-43) grew on SDA at 37 °C and Sab-10% NaCl at 42 °C. Microscopic examination of these isolates revealed spherical and ovoid yeast-like cells and blastoconidia. Chlamydoconidia and hyphae or pseudohyphae were not observed on cornmeal agar for isolates MYC-28, MYC-29, and MYC-30 (Fig. 1A). In contrast, isolate MYC-43 displayed blastoconidia and hyphae or pseudohyphae on cornmeal agar (Fig. 1B). Isolates originally identified as *C. parapsilosis* and *C. krusei* did not grow in Sab-10% NaCl at 42 °C and produced pseudohyphae and chlamydoconidia on cornmeal agar (Fig. 1C and D).
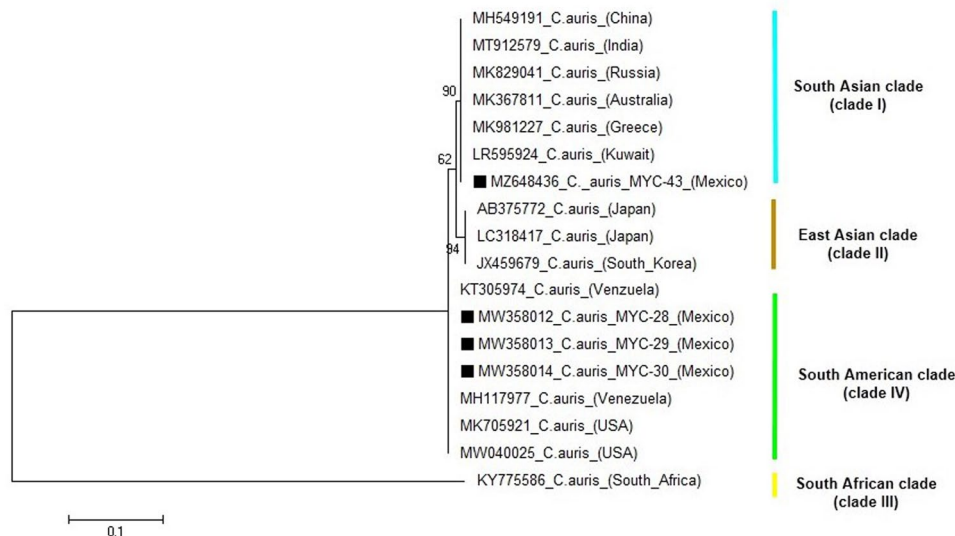
Assimilation of carbohydrates testing shows that isolates originally identified as *C. auris* (MYC-28, MYC-29, MYC-30, and MYC-43) assimilated five of the nine carbohydrates tested: glucose, raffinose, melezitose, trehalose, and dulcitol. Raffinose and dulcitol were only assimilated by *C. auris* isolates. Final species identification was performed using the species-specific *C. auris* PCR. An approximately 280 bp band was amplified for MYC-28, MYC-29, MYC-30, and MYC-43 isolates, while no amplification was observed for isolates MYC-121 and MYC-221 (Fig. 2). Using pan-fungal PCR sequencing, *C. auris* isolates had 99–100% identity with other *C. auris* sequences within GenBank. Phylogenetic analysis of the sequence of ITS1-ITS2 regions showed that Mexican *C. auris* isolates were clustered with the *C. auris* South American clade (clade IV) for isolates MYC-28, MYC-29, and MYC-30, and isolate MYC-43 grouped with *C. auris* South Asian clade (clade I) (Lockhart et al. 2017c) (Fig. 3). Sequences were deposited at the GenBank database under accession numbers MW358012 (MYC-28), MW358013 (MYC-29), MW358014 (MYC-30), MZ648436
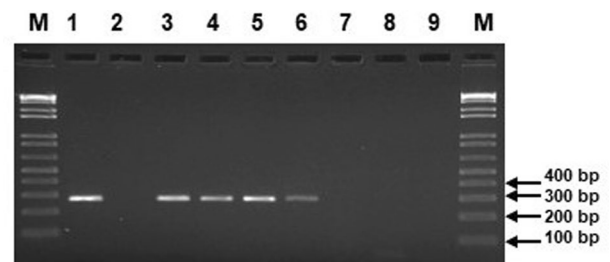
Fig.2 Products of the species-specific C. auris PCR. Positive amplification: #1 *C. auris* (isolate positive control), #3 (MYC-28), #4 (MYC-29), #5 (MYC-30), and #6 (MYC-43). Negative amplification: #2 *C. albicans* (isolate negative control), #7 (MYC-121), #8 (MYC-228), and #9 (negative control). M: DNA molecular weight marker 1 Kb Plus and its migration is shown on the right side

Fig. 1 Culture on cornmeal agar at 30 °C, for 3–5 days. 40X. **a** *Candida auris,* clade IV, Nuevo León, Mexico. Yeasts alone and some with blastoconidia, the size and spherical shape is a constant characteristic that differentiates it from other species (picture from isolate MYC-28). **b** *Candida auris,* clade I, Tabasco, Mexico. Yeasts alone, with blastoconidia and hyphae or pseudohyphae (picture from isolate MYC-43). **c** *Candida parapsilosis.* Yeasts alone, with blastoconidia, pseudohyphae alone, or branched. Incipient or immature chlamydoconidia. The cells adopt a characteristic irregular size and shape (picture from isolate MYC-221). **d** *Candida krusei.* Septate hyphae with spherical, ovoid, and elongated lateral blastoconidia on the sides. Bilateral symmetry seems to be a differential characteristic in this species (picture from isolate MYC-121). Blastoconidia and chlamydoconidia are indicated with arrow, respectively

Fig.3 *C. auris* phylogenetic analysis. The evolutionary tree was constructed with 18 nucleotide sequences of the Internal Transcribed Spacer (ITS) regions of the 18S rDNA gene using the maximum-likelihood method based on the Jukes–Cantor model with 1000 boot-straps. Analyses were conducted in MEGA v.7 software. The tree includes representative sequences of the four *Candida auris* clades and four Mexican sequences reported here; they were highlighted with a bold square. *C. auris* isolates from the state of Nuevo León (MYC-28, MYC-29 and MYC-30) matched with *C. auris* isolates corresponding to the South American clade (clade IV), and the isolate from Tabasco state (MYC-43) matched with *C. auris* isolates corresponding to the South Asian clade (clade I). The four clades of *C. auris* are indicated on the right side of the tree

(MYC-43), MZ020643 (MYC-121), and MZ020644 (MYC-221).

Fluconazole susceptibility testing of the four *C. auris* isolates showed high MIC values. Isolates MYC-28, MYC-43, MYC-29, and MYC-30 had MICs of 128 μg/mL; based on proposed breakpoints (MIC ≥ 32), these isolates were considered resistant to fluconazole ((CDC) CfDCaP 2018a).

Discussion

The national laboratory surveillance confirmed four cases of *C. auris* in Mexico belonging to two different clades; all cases were identified in patients in the ICU with severe COVID-19. With the identification of *C. auris* in several Mexican states and the increase in the number of patients hospitalized in ICUs as a result of the COVID-19 pandemic, the mycology reference service of the InDRE implemented national laboratory surveillance for *C. auris*. Several challenges had to be addressed during the implementation of this surveillance, especially the limited availability of reference methods, such as MALDI-TOF, for the confirmation of *C. auris*.

In combination with several other low-cost tests, we were able to successfully use species-specific PCR for definitive identification of *C. auris* in a resource-limited setting, obtaining reliable and comparable results as those obtained with the reference methods. *Candida auris*-specific PCR

tests have been used for the identification of colonized patients by the direct detection of *C. auris* DNA on skin swabs (Walchak et al. 2020). This type of non-culture based methodology has shown relevant utility in the early detection and control of *C. auris* outbreaks ((CDC) CfDCaP 2018b). Here, we have used this test to definitively identify isolates of *C. auris* using simple PCR and gel electrophoresis rather than real-time PCR. We have shown that this test could be employed in combination with other simple tests when MALDI-TOF and DNA sequencing are not available.

Without specialized *Candida* chromogenic media, the use of basic mycology methods, such as the evaluation of growth under different culture conditions, such as high salt and high temperature, were key to identifying potential isolates of *C. auris*. On cornmeal agar, *C. auris* generally has homogeneous size and shaped blastoconida and no hyphae and pseudohyphae. However, one of the four isolates (MYC-43) did present pseudohyphae, showing that is not a definitive characteristic of *C. auris*.

The evaluation of the profile of carbohydrates' assimilation is not widely used for identification of *Candida* spp, mainly because it is a complex and laborious laboratory method. We confirmed that dulcitol assimilation is key to identify *C. auris* to other closely related species of *Candida* (Welsh et al. 2017; Jeffery-Smith et al. 2018; Caceres et al. 2019). Despite the close genetic relationship between *C. auris* and the *C. haemolunii* complex, the biochemical assimilation profile between them is distinct, so that

a four carbohydrate assimilation combination (galactose, raffinose, rhamnose, and dulcitol) is decisive for the differentiation of *C. auris* from the *C. haemulonii* species complex (Jeffery-Smith et al. 2018).

Using the VITEK® 2 system, we found that all four isolates were correctly identified as *C. auris*. As reported previously, VITEK® 2 system using the software version 8.01 is able to identify *C. auris*, especially isolates from the South American clade (clade IV) (Ambaraghassi et al. 2019).

Here, we observed all isolates were resistant to fluconazole, with high MIC values (≥ 128 µg/mL). This high resistance to fluconazole is similar to others reports around the world. Unfortunately, our information about antifungal susceptibly testing (AFST) had the limitation of not being a gold standard methodology, but it is important to highlight that the method used is a viable alternative in settings where access to AFST using broth microdilution or gradient diffusion is not available.

Phylogenetic analysis of sequences showed that the three Mexican *C. auris* isolates from the north of the country belonged to the South American clade (clade IV). Surprisingly, the Mexican *C. auris* isolate from the south of the country clustered with the South Asian clade (Clade I), indicating that there have been multiple introductions of *C. auris* into Mexico and several clades are now circulating through the population.

With limited resources and limited access to reference methods for *Candida* species identification such as MALDI-TOF, we were able to confirm the species identification of isolates suspected to be *C. auris*. Here, we describe how conventional mycology laboratory methods, such as microscopy, growth on differential media, and growth at elevated temperatures, were key aspects to tentative identification of *C. auris*. The use of a simple species-specific PCR test confirmed the tentative identification. These methods can all be easily implemented in laboratories in countries with limited resources, or with logistical difficulties in accessing reference methods for *Candida* identification.

Declarations

Conflict of interest The authors declare that they have no conflict of interest. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the Instituto de Diagnóstico y Referencia Epidemiológicos Dr. Manuel Martínez Báez (InDRE).

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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