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First report of sporadic cases of *Candida auris* in Colombia[☆]

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ABSTRACT

Background: *Candida auris* is a recently reported *Candida* species that is phenotypically similar to *Candida haemulonii* and related to hospital outbreaks. This organism can be misidentified as *Candida haemulonii*, *Candida famata*, *Candida catenulata*, or *Rhodotorula glutinis* by phenotypic approaches. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and DNA sequence analysis using internal transcribed spacer rDNA bar-coding provide an accurate identification.

Case reports: Three cases of *C. auris* infection in patients with risk factors for fungal infection (one admitted to the intensive care unit, one with lymphoma, and one with HIV; all three with previous antibiotic use) are reported; these infections were not epidemiologically related. Yeast isolates were recovered from blood, ocular secretion, and bronchoalveolar lavage and were misidentified as *C. catenulata* and *Candida albicans* by the phenotypic MicroScan method. The isolates were confirmed to be *C. auris* by means of MALDI-TOF MS and DNA sequence analysis. Antifungal susceptibility testing was performed on these *C. auris* isolates, which exhibited high minimum inhibitory concentrations to triazoles and amphotericin B. One patient survived and the other two died. Only one of these deaths was related to fungemia.

Conclusions: *C. auris* is an emerging and opportunistic multidrug-resistant human pathogen. It is necessary to strengthen measures to achieve an accurate and quick identification and also to avoid its dissemination. This will require improvements in health and infection control measures, as well as the promotion of antifungal stewardship in healthcare facilities.

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Introduction

Uncommon *Candida* species are increasingly recognized as causes of *Candida* infections and are being associated with hospital outbreaks (Chen et al., 2009; Pfaller et al., 2006; Miranda-Zapico et al., 2011). *Candida auris* is a recently described *Candida* species, which is phenotypically and phylogenetically closely related to the *Candida haemulonii* species complex (*Candida duobushaemulonii* and *Candida pseudohaemulonii*). *C. auris* was first recovered in 2009 from the external ear canal of a 70-year-old female Japanese patient in Tokyo (Satoh et al., 2009). Subsequently, three more cases of nosocomial candidemia were diagnosed in Korea

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(Lee et al., 2011a). These *C. auris* isolates, identified by internal transcribed spacer (ITS) regions and D1/D2 rRNA sequencing, had previously been misidentified as *C. haemulonii* and *Rhodotorula glutinis* by the commercial Vitek 2 and API 20C phenotyping systems, respectively. Since then, *C. auris* infections have been described in India, South Africa, Kuwait, Brazil, the UK, Pakistan, and most recently in Venezuela and the USA (Chowdhary et al., 2013; Magobo et al., 2014; Emara et al., 2015; Prakash et al., 2016; Schelenz et al., 2016; Calvo et al., 2016; Vallabhaneni et al., 2016).

The accurate identification of *Candida* species has important implications in the treatment of invasive candidiasis, since *C. auris* is often described as a multidrug-resistant species. Indeed, antifungal susceptibility studies have demonstrated that *C. auris* generally exhibits high minimum inhibitory concentrations (MICs) to fluconazole, but less frequently to voriconazole, amphotericin B, or echinocandins (Chowdhary et al., 2014; Chatterjee et al., 2015).

The existence of *C. auris* in Colombia was first identified by the present authors during a prospective study (Ceballos et al., 2015). More recently, 17 new cases have been diagnosed in the city of Valledupar, a location geographically distant from Bogotá (Morales-López et al., 2017). This article reports the clinical and biological characteristics of the three *Candida* cases caused by the species *C. auris* that occurred during the prospective study, which was performed over a 4-year period in a hospital in Bogotá, Colombia.

Cases reports

Case 1

In November 2013, a 74-year-old male was admitted with alteration of his mental status and a Glasgow coma scale score of 7/15. He had a history of major depression, hypertension, mitral valve replacement, and hypothyroidism. On physical examination the patient had an oxygen saturation (SO₂) of 88%, hyporesponsive pupils, rales in both pulmonary bases, fever, and high creatinine phosphokinase. He was intubated and admitted to the intensive care unit (ICU) where acute myocardial infarction and stroke were ruled out. A neuroleptic malignant syndrome was diagnosed on the basis of his clinical course. He developed rhabdomyolysis and acute kidney failure. Nosocomial pneumonia caused by an extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* was diagnosed during his stay in the ICU. He received empirical treatment with cefepime and then with ertapenem. However, the patient's mental status deterioration persisted during his in-hospital stay, and 15 days after admission he developed multiple organ failure. Since blood culture results reported yeasts, anidulafungin (100 mg/day) was administered; in spite of this, the patient died 2 days later.

Case 2

A 45-year-old man with a history of cutaneous T cell non-Hodgkin lymphoma that was non-responsive to multiple

chemotherapy regimens, and who had received the last cycle with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) 17 days previously, was admitted to the hospital in December 2014. He presented to the emergency room for a 3-day fever associated with signs of infection in tumoral skin lesions on his face and parietal region, which were erythematous and desquamating. The patient also presented palpebral swelling and abundant purulent conjunctival and skin secretions, so a diagnosis of preseptal cellulitis was made. Treatment was started with cefepime plus vancomycin. Topical mupirocin 2% with oxytetracycline hydrochloride, fluorometholone, and polymyxin B sulfate in an ophthalmic ointment was also prescribed. One set of blood cultures showed negative results 5 days after hospitalization. The patient did not show any improvement and the treatment was switched to meropenem and linezolid. Conjunctival secretion cultures and skin biopsy cultures of the parietal lesion were positive for *Candida catenulata* and *Candida parapsilosis*, respectively, as identified using the MicroScan system. Treatment with caspofungin (50 mg/day) was started for 15 days and samples were subsequently negative for *Candida*. During his inpatient treatment, the malignancy progressed and the patient's condition continued to deteriorate. He died 2 months after admission.

Case 3

A 51-year-old woman was admitted to the emergency room in February 2015 for respiratory failure and oral candidiasis. She had previously been diagnosed with HIV (in 2000, during pregnancy screening) and had a history of poor adherence to her antiretroviral therapy, which she had not been taking for a year before her admission (her last absolute CD4 lymphocyte count was 115 cells/mm³). Bronchoalveolar lavage (BAL) confirmed pneumonitis by *Pneumocystis jirovecii* on Gomori–Grocott stain. She received treatment with trimethoprim–sulfamethoxazole and steroids for 21 days and fluconazole for 7 days. Orotracheal intubation was required in the ICU for 7 days due to acute respiratory distress syndrome (ARDS). She made favorable progress and was discharged after 23 days. The results of BAL cultures taken at admission reported *Candida albicans* by phenotypic method.

Yeast identification

Clinical isolates were initially seeded on CHROMagar *Candida* medium (Becton Dickinson, Meylan, France) and Sabouraud dextrose agar (Difco, St Louis, MO, USA) at 35 °C for 24–36 h. Subsequently, identification was conducted using both the automated MicroScan WalkAway 96 Plus system (Siemens, Deerfield, IL, USA) and the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) Bruker Microflex LT Biotyper system (Bruker, Bremen, Germany). The isolates exhibited pink colonies on CHROMagar *Candida* medium. The phenotypic MicroScan method failed to identify the first isolate; however, this was identified by MALDI-TOF MS as *C. auris*. The ocular secretion sample from the second patient showed isolates

Table 1
Antifungal drug susceptibility profiles of *C. auris* isolates.

Isolate	Source	MIC (μg/ml) of drug								
		AMB	FLC	ITR	VOR	POS	ISA	CAS	MYC	ANI
0001 PUJ/HUSI	Blood	0.75	24	0.25	0.64	0.023	0.125	0.47	0.19	0.12
0435 PUJ/HUSI	Ocular secretion	0.75	12	0.25	0.047	0.023	0.19	0.094	0.125	0.004
00537 PUJ/HUSI	BAL	>32	>256	2	0.75	0.5	1.5	0.016	0.094	0.016

AMB: amphotericin B, FLC: fluconazole; ITC: itraconazole; VRC: voriconazole; POS: Posaconazole; ISA: Isavuconazole; CAS: caspofungin; MYC: Miconazole; ANI: anidulafungin.

identified as *C. catenulata* and *C. parapsilosis* by MicroScan, but these were then identified as *C. auris* and *Candida metapsilosis*, respectively, by MALDI-TOF MS. The BAL isolate was

phenotypically misdiagnosed as *C. albicans*. Identification of these three clinical isolates was finally obtained by ITS rDNA bar-coding using ITS1 and ITS4 primer pairs (Irinji et al., 2015).

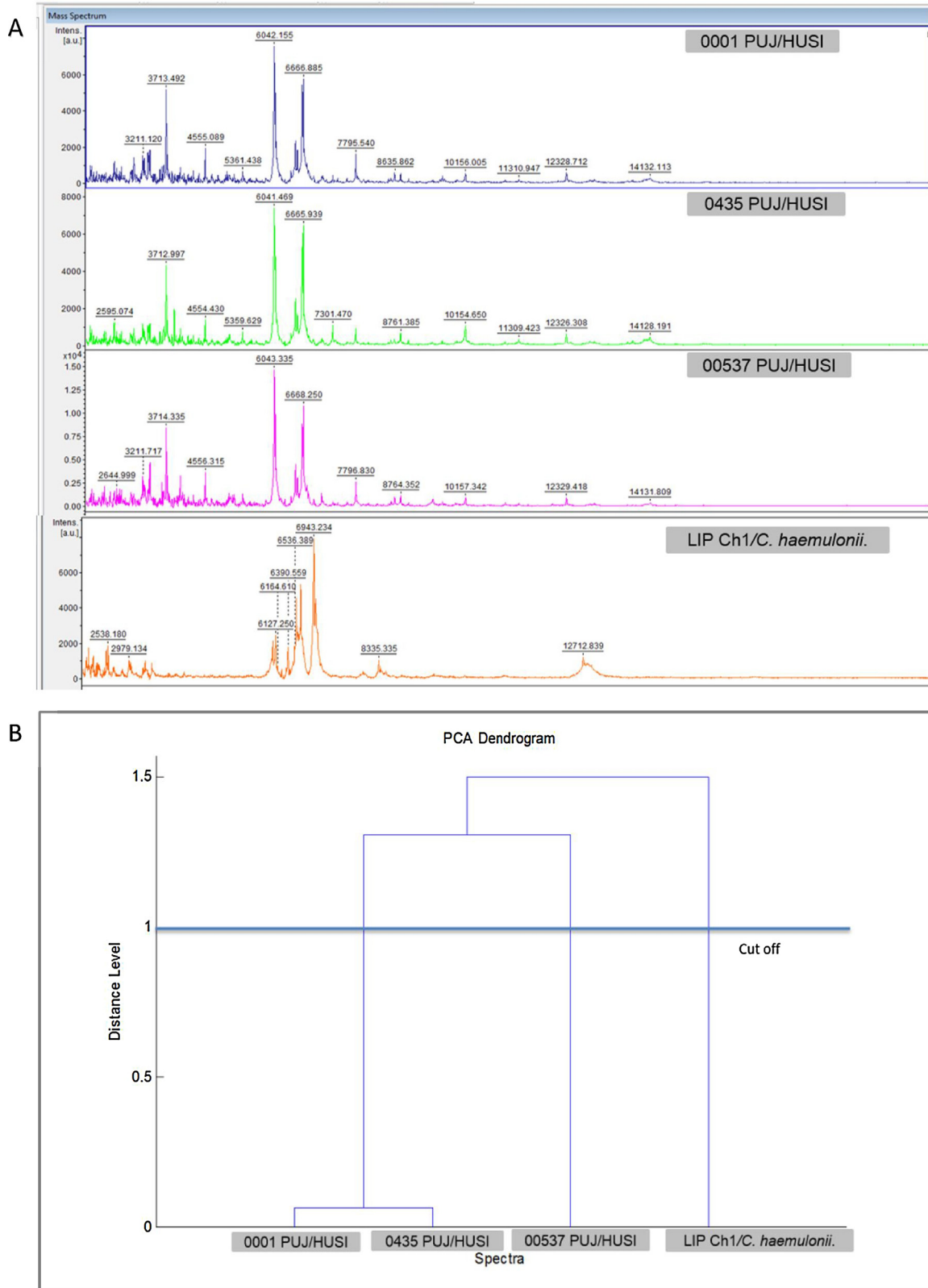


Figure 1. (A) Mass spectra for the *Candida auris* isolates (upper three panels, PUJ/HUSI) compared with a *Candida haemulonii* spectrum from a clinical isolate (lower panel). (B) Bruker Biotyper PCA (analysis of principal components) dendrogram clustering of the MSPs (mass spectrum) representative spectra from each isolate/strain, with distances displayed in relative units on the y-axis.

Antifungal susceptibility assay

The in vitro antifungal susceptibility profile of the three isolates was determined using the commercial Etest method (bioMérieux, Marcy l'Etoile, France), according to the manufacturer's instructions. Susceptibility to echinocandins (anidulafungin, caspofungin, and micafungin), triazoles (fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole), and a polyene (amphotericin B) was assessed.

Briefly, the yeast inoculum was adjusted with a spectrophotometer by adding sterile 0.85% NaCl to match 0.5 McFarland. The MICs were read after 24–48 h of incubation at 35 °C. The MIC values were determined at the point of intersection of the inhibition growth ellipse with the Etest strip. The MICs of the quality control strains *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were all within the reference ranges (data not shown).

Table 1 shows the MICs of the nine antifungals tested against *C. auris*. Fluconazole demonstrated no activity against the three isolates (MIC 12 to >256 µg/ml), whereas the third isolate of *C. auris* was fully resistant to amphotericin B and itraconazole. All three echinocandins were the most efficient antifungals.

Mass spectrometry phylogenetic analysis

For phylogenetic analysis of the mass spectra of the three *C. auris* isolates, the dendrogram was generated using the respective functionality of the MALDI-TOF MS Biotyper 3.1 offline client. The mass spectra of each quadruplicate of the respective isolates with a score value of >2 were considered for the preparation of the dendrogram. The spectra of all of the isolates tested were analyzed as a core-oriented dendrogram using an arbitrary distance level of 1000 as the cut-off (Kathuria et al., 2015a; Rodríguez-Leguizamón et al., 2015). The dendrogram obtained showed two clades when compared to the mass spectrum of each isolate (Figure 1).

Discussion

Concerning the epidemiology of candidemia, the Latin America Invasive Mycosis Network estimated an incidence of 1.18/1000 hospital admissions for the region. Colombia and Argentina showed the highest incidences (1.96 and 1.95/1000 hospital admissions, respectively) and Chile the lowest (0.33/1000 hospital admissions) (Nucci et al., 2013).

Increasing numbers of emerging or cryptic *Candida* species in human infections have been reported in recent years, including species such as *Candida orthopsilosis*, *C. metapsilosis*, *Candida bracarenis*, and *Candida nivariensis*, which are difficult to identify by phenotypic methods (Miranda-Zapico et al., 2011; Angoulvant et al., 2016). Among them, *C. auris* was described in 2009 to be genetically close to species of *C. haemulonii* complex and was suggested to be a new opportunistic fungal pathogen implicated in candidemia (Angoulvant et al., 2016).

The patients with *C. auris* isolates in this report had similar risk factors to those described in the literature, such as immunosuppression, diabetes, chronic renal disease, use of broad-spectrum antibiotics, hospitalization in the ICU, urinary catheters, recent surgery, and central venous catheters (Lee et al., 2011a; Emará et al., 2015; Calvo et al., 2016; Lockhart et al., 2016). While most reports worldwide are of patients with fungemia (28.6%), there are a few reports on isolation from other sites such as wounds, urine, peritoneal fluid, and pleural effusion (Vallabhaneni et al., 2016; Angoulvant et al., 2016; Lockhart et al., 2016). This appears to be the first report of *C. auris* isolated from ocular secretion and the second from BAL; it is important to emphasize that the BAL isolate was not interpreted as infection but only as colonization. Currently, there is

no information about the ecological niche of *C. auris*, or about the colonization rate in the general population.

The cases presented here were clearly not related to epidemiological outbreaks, as described previously in India, Venezuela, the UK, Spain, and Oman (Schelenz et al., 2016; Calvo et al., 2016; Chowdhary et al., 2014; Lee et al., 2011b; Ruiz Gaitán et al., 2017; Mohsin et al., 2017). Each case in this report presented independently with no time relationship between them. The proteomic dendrograms, which show different spectra, seem to be in agreement with this epidemiological finding. However, caution should be used when interpreting MALDI-TOF MS relatedness models; these preliminary data will need to be completed through a molecular biology approach. The absence of an outbreak could be due to the strong infection control program at the Hospital Universitario San Ignacio, where adherence to hand hygiene in healthcare workers was very high (90%) during the study period. On the other hand, it could be hypothesized that the appearance of *C. auris* was a consequence of an increase in the antifungal selection pressure; however, these patients had not received previous treatment, which reduces this possibility. Nevertheless, a cross infection cannot be ruled out completely.

Difficulties in mycological identification of *C. auris* using conventional phenotypic systems and the limited access to MALDI-TOF MS and molecular biology tools in routine laboratories may contribute to underestimation of this organism. According to the literature, Phoenix and Vitek technologies can misidentify *C. auris* as *C. haemulonii*, *R. glutinis*, *Candida famata*, and *C. catenulata* (Chowdhary et al., 2013; Schelenz et al., 2016). Using MicroScan, the study isolates were initially identified as *C. catenulata* and *C. albicans*. It is also possible that some systems cannot identify any species from the sample. Misidentification may lead to inadequate treatment of these patients due to the multidrug resistance patterns of this yeast species.

The susceptibility profile of *C. auris* exhibited high MICs for fluconazole and other triazoles. Overestimation of MICs to amphotericin B and caspofungin has been described using the Vitek system compared to the Clinical and Laboratory Standards Institute (CLSI) reference methods (Kathuria et al., 2015b; Killian et al., 2014). According to the Etest, both of the first two isolates were susceptible to all antifungal agents except for fluconazole. The third isolate had high MICs for fluconazole, itraconazole, and amphotericin B.

In conclusion, it is important to actively seek and achieve a correct identification of *Candida* species by MALDI-TOF MS or molecular biology methods considering the increased circulation of cryptic species with resistance to multiple antifungal agents reported in Latin America, as well as the alert from the US Centers for Disease Control and Prevention (Anon, 2016) related to the dissemination of *C. auris* (Calvo et al., 2016; Ceballos et al., 2015; Morales-López et al., 2017). It is necessary to strengthen measures to achieve not only accurate and rapid identification, but also to avoid its dissemination by improving infection control measures and promoting antifungal stewardship in healthcare facilities.

Author contributions

SLV, JRG, AH, CH, and CAA analyzed the clinical data and GC, MYL, and BEA performed the antifungal susceptibility experiments. AC and CMPG analyzed microbial data from MALDI-TOF MS. FM and PLP performed the molecular identification and SLV, CAA, CMPG, and PLP conceived and designed the experiments.

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Ethical approval and consent to participate

This study was approved by the Research and Ethics Committee of Hospital Universitario San Ignacio (HUSI).

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Conflict of interest

The authors declare that they have no competing interests.

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