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Original article

Nosocomial fungemia by *Candida auris:* First four reported cases in continental Europe

Alba Cecilia Ruiz Gaitán[a], Ana Moret[b], José Luis López Hontangas[c], José Miguel Molina[c], Ana Isabel Aleixandre López[b], Alicia Hernández Cabezas[c], Juan Mollar Maseres[d], Rabat Chouman Arcas[c], María Dolores Gómez Ruiz[c], Miguel Ángel Chiveli[b], Emilia Cantón[a], Javier Pemán[a,c,*]

[a] *Severe Infection Research Group, Health Research Institute La Fe, Valencia, Spain*
[b] *Anesthesiology and Surgical Critical Care Department, La Fe University and Polytechnic Hospital, Valencia, Spain*
[c] *Microbiology Department, La Fe University and Polytechnic Hospital, Valencia, Spain*
[d] *Preventive Medicine Department, La Fe University and Polytechnic Hospital, Valencia, Spain*

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ABSTRACT

Background: Candida auris is an emerging multidrug-resistant yeast that can cause invasive infections and is associated with high mortality. It is typically resistant to fluconazole and voriconazole and, some cases, also to echinocandins and amphotericin B. This species, phylogenetically related *to Candida haemulonii*, is frequently misidentified by commercial identification techniques in clinical laboratories; therefore, the real prevalence of *C. auris* infections may be underestimated.
Aims: To describe the clinical and microbiological features of the first four cases of *C. auris* fungemia episodes observed in the European continent.
Methods: The four patients were hospitalized in the adult surgical intensive care unit. A total of 8 isolates (two per patient) from blood and catheter tip were analyzed.
Results: All isolates were misidentified as *Saccharomyces cerevisiae* by AuxaColor 2, and as *Candida sake* by API ID20C. VITEK MS technology misidentified one isolate as *Candida lusitaniae*, another as *C. haemulonii* and could not identify the other six. *C. auris* identification was confirmed by ITS rDNA sequencing. All isolates were fluconazole (MIC >256 mg/l) and voriconazole (MIC 2 mg/l) resistant and susceptible to posaconazole, itraconazole, echinocandins and amphotericin B.
Conclusions: C. auris should be regarded as an emerging pathogen, which requires molecular methods for definitive identification. Our isolates were highly resistant to fluconazole and resistant to voriconazole, but susceptible to the other antifungals tested, which emphasizes the importance of accurately identifying this species to avoid therapeutic failures.

Fungemia nosocomial por *Candida auris:* primeros cuatro casos en Europa continental

Palabras clave:
Candida auris
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Resistencia al fluconazol
Cuidados intensivos quirúrgicos

RESUMEN

Antecedentes: Candida auris es una levadura multirresistente de reciente aparición que puede causar infecciones invasivas asociadas con una elevada mortalidad. Habitualmente, *C. auris* es resistente al fluconazol y el voriconazol, y en algunos casos, también a las equinocandinas y la anfotericina B. Esta especie, relacionada filogenéticamente con *Candida haemulonii*, no se identifica por las técnicas comerciales habitualmente disponibles en los laboratorios clínicos, por lo que la prevalencia real de las infecciones causadas por *C. auris* puede estar subestimada.
Objetivos: Describir las características clínicas y microbiológicas de los cuatro primeros casos de fungemia por *C. auris* observados en el continente europeo.

* Corresponding author.
E-mail address: javier.peman@gmail.com (J. Pemán).

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Métodos: Los cuatro pacientes eran adultos y estaban en la unidad de cuidados intensivos quirúrgicos. Se analizaron un total de 8 aislamientos (dos por paciente), obtenidos a partir de un hemocultivo y de punta de catéter.
Resultados: Todos los aislamientos se identificaron erróneamente como *Saccharomyces cerevisiae* por AuxaColor 2 y como *Candida sake* por API ID20C. El sistema VITEK MS identificó erróneamente un aislamiento como *Candida lusitaniae*, otro como *C. haemulonii* y no pudo identificar los seis aislamientos restantes. La identificación de *C. auris* se confirmó mediante secuenciación de la región ITS del ADNr. Todos los aislamientos fueron resistentes al fluconazol (CMI > 256 mg/l) y el voriconazol (CMI 2 mg/l) y sensibles al posaconazol, el itraconazol, las equinocandinas y la anfotericina B.
Conclusiones: *C. auris* es un agente patógeno de reciente aparición que actualmente solo puede ser identificado mediante secuenciación molecular. Nuestros aislamientos fueron muy resistentes al fluconazol y resistentes al voriconazol, pero sensibles a los otros antifúngicos ensayados, lo cual destaca la importancia de identificar correctamente esta especie en la práctica asistencial para evitar fracasos terapéuticos.

Candida auris is an emerging multidrug-resistant yeast that can cause invasive infections and is associated with high mortality. Since the first description by Satoh in 2009 from the ear discharge in a Japanese patient,[18] several cases of nosocomial fungemia have been reported in critically ill patients from India, South Korea, South Africa, Kuwait, Venezuela and United Kingdom.[3,7,8,10,11,16,17] However, the real burden of *C. auris* infections could be underestimated due to the fact that this species is still frequently misidentified as *Candida famata*, *Candida haemulonii*, *Candida sake*, *Saccharomyces cerevisiae* or *Rhodotorula glutinis* by commercial identification techniques in clinical laboratories (Vitek 2, API, AuxaColor, and MALDI-TOF).[9] Additionally, *C. auris* is typically resistant to fluconazole and voriconazole and, in some cases, also to echinocandins and amphotericin B,[5] which could explain the therapeutic failures observed in deep-seated infections caused by this species.[14,16]

We report four cases of *C. auris* fungemia identified at Hospital Universitario y Politécnico La Fe in Valencia (Spain), all of them diagnosed in the adult surgical intensive care unit (SICU) between April and June 2016. To the best of our knowledge, we describe here the clinical and microbiological features of the first *C. auris* bloodstream episodes observed in continental Europe.

Case reports

Case 1

A 66-year-old man who underwent liver resection due to hepatocellular carcinoma was admitted to the SICU after surgery. He developed surgical wound complications, including surgical site infection, liver abscess and evisceration. Abdominal wires were put, a negative-pressure wound therapy system was applied and antibiotics were given. Despite the treatment, the patient's condition continued worsening with fever and pleural effusion. On day 32nd of SICU stay, empirical antifungal therapy with fluconazole (400 mg/day) was started. However, on day 42, *C. auris* was isolated from blood culture. Antifungal therapy was immediately switched to anidulafungin (200 mg on day 1 followed by 100 mg/day) and after 48 h liposomal amphotericin B (3 mg/kg/day) was added. The central venous catheter (CVC) was removed on day 43 and *C. auris* was recovered from the catheter tip. *C. auris* was also isolated from peritoneal fluid culture and from pharyngeal, rectal, and urine surveillance cultures during the first week after fungemia. Blood cultures became negative on day 57; liposomal amphotericin B was stopped on day 64 and anidulafungin 1 week after. The patient recovered completely and was discharged on day 84.

Case 2

A 39-year-old woman, with severe ventricular dysfunction after prosthetic mitral valve replacement, was admitted to SICU for extracorporeal membrane oxygenation. During SICU stay, she developed cardiac tamponade and a multiple organ dysfunction syndrome. The patient was treated with antibiotics, mechanical ventilation, cardiotomy and fluconazole (400 mg/day) as empirical therapy. On day 59 in the SICU *C. auris* was isolated from blood culture and antifungal therapy was switched to micafungin (100 mg/day). The CVC was removed on day 62 and *C. auris* was recovered from the catheter tip. *C. auris* was also isolated from pharyngeal, rectal, and urine surveillance cultures during the SICU stay but no abnormalities were found in the fundoscopic exam. Blood cultures became negative on day 75; however, the patient died on day 91due to multiple-organ failure caused by ventricular dysfunction.

Case 3

A 48-year-old man with polytrauma after a road traffic accident was admitted to SICU due to cerebral injuries and severe thoracic trauma. Cerebral damage required emergent decompressive craniectomy and the patient was intubated, sedated, and mechanically ventilated. On day 16, he recovered and was transferred to the trauma surgery ward to continue the treatment and start rehabilitation therapy. On day 18th after hospital admission *C. auris* was isolated from the blood cultures, and antifungal therapy with micafungin (100 mg/day) was immediately started. The CVC was removed on day 21 and *C. auris* was recovered from the catheter tip. *C. auris* was also isolated from pharyngeal, rectal, and urine surveillance cultures during the first week after fungemia. Blood cultures became negative on day 24 and micafungin treatment was maintained two weeks more. The patient responded satisfactorily to therapy and was discharged on day 44.

Case 4

A 26-year-old man with polytrauma and severe cranial injury resulting from a road traffic accident was admitted to the SICU after decompressive craniectomy and maxillo-facial emergency surgery due to extensive intraparenchymal hemorrhage and cerebral edema. On day 35 of SICU stay, *C. auris* was isolated from blood cultures and antifungal therapy with anidulafungin (200 mg on day 1 followed by 100 mg/day) was started. The CVC was removed on day 40, and *C. auris* was recovered from the catheter tip. *C. auris* was also isolated from pharyngeal, rectal, and urine surveillance

Table 1
Main clinical and epidemiological characteristics of the patients.

Clinical features	Case 1	Case 2	Case 3	Case 4
Sex/age (yr)	M/66	F/39	M/48	M/26
SICU stay before fungemia (days)	42	59	18	35
Fungemia date (dd/mm/yyyy)	20/04/2016	16/05/2016	13/06/2016	28/06/2016
Clinical status at time of positive culture				
Neutropenia ($<10^9$ L^{-1})	No	No	No	No
Mechanical ventilation	Yes	Yes	Yes	No
Presence of CVC	Yes	Yes	Yes	Yes
Broad-spectrum antibiotics	Yes	Yes	Yes	Yes
Parenteral nutrition	Yes	Yes	Yes	Yes
Indwelling urinary catheter	Yes	Yes	Yes	Yes
Previous antifungal agents	Fluconazole	Fluconazole	No	No
Underlying conditions	Abdominal surgery	CPE, cardiotomy	Polytrauma, craniotomy	Polytrauma, craniotomy
Candida score	5	4	5	5
Pittet colonization index	0.75	1	0.33	0.5
Fungemia episode treatment and outcome				
CVC removal	Yes	Yes	Yes	Yes
Antifungal therapy	Anidulafungin, AMB	Micafungin	Micafungin	Anidulafungin
Microbiological fungemia outcome	Cleared	Cleared	Cleared	Not cleared
Survival up to 30 days	Yes	No	Yes	No

CPE, cardiogenic pulmonary edema; AMB, liposomal amphotericin B.

cultures during the post-fungemia SICU stay. Despite treatment, the patient's condition continued worsening with renal and liver failure, which required renal replacement therapies. On day 56 the patient died due to septic shock, multiple-organ failure and neurological dysfunction.

Materials and methods

Patients' medical records were reviewed, and demographic, epidemiological, and clinical data were collected including age, gender, underlying conditions, previous exposition to antimicrobial drugs, invasive medical procedures, presence of CVC, dates of CVC removal and culturing, dates and dosages of antifungals administered, and outcome of fungemia. Mycological surveillance cultures from pharyngeal and rectal swabs, and urine were also performed weekly in all patients. Microbiological samples were processed according to the good clinical laboratory practice, including Maki and sonication techniques for catheter tip culture.

Blood cultures were collected under aseptic conditions and processed by conventional automated system (BacT/ALERT® VIRTUO™, bioMérieux, Marcy l'Etoile, France). All *Candida* isolates from blood cultures and catheter tips were identified by phenotypic and biochemical characteristics, proteomic profile and DNA sequencing technology. Phenotypic colony features were evaluated after 48 h of incubation in Sabouraud dextrose agar (Becton Dickinson, Baltimore, USA), CHROMagar Candida® (Difco™, Becton Dickinson) and BBL Mycosel agar (Becton Dickinson). Biochemical characteristics were analyzed using commercial tests including API ID20C (bioMérieux) and AuxaColor™ 2 (BioRad-Laboratories, Marnes-la-Coquette, France). Proteomic profiles were obtained and evaluated by VITEK MS (bioMérieux) according to manufacturer's instructions. Definitive identification was performed by sequencing the internal transcribed spacer (ITS) using the primers ITS3-ITS4 and ITS2-ITS5 previously described[1]; sequencing reaction was performed using GenomeLab™ GeXP (Beckman Coulter, Brea, USA) equipment and the sequences obtained were compared with those in Microbial Genomes BLAST (http://www.ncbi.nlm.nih.gov/guide/sequence-analysis/). A similarity of ≥97% was used as the criterion for species identification. ITS sequences (GenBank accession no. KJ126759, KC692045) of our isolates shared a 95–99% similarity with the ITS sequences of several *C. auris* strains. The similarity with the isolate from Japan (Satoh, K. 2009 GenBank accession no. AB375772)[18] was 94–96%.

In vitro antifungal susceptibility testing against amphotericin B, fluconazole, itraconazole, posaconazole, voriconazole, flucytosine, caspofungin, anidulafungin, and micafungin was performed by the colorimetric microdilution panel Sensititre Yeast One® Y010 (TREK Diagnostic Systems, Cleveland, USA) according to the manufacturer's instructions.

Results and discussion

The main clinical characteristics of the patients, the antifungal treatment administered and fungemia outcome are described in Table 1. All cases were observed between April and June 2016 in adult patients (26–66 years old) admitted to SICU, with an average length of stay before fungemia of 36 days (range, 18–59 days). All patients had been previously exposed to broad-spectrum antibiotics, parenteral nutrition and multiple invasive medical procedures including CVC, indwelling urinary catheter, and surgery, but none had neutropenia. Two patients received fluconazole before fungemia was diagnosed. *Candida* score was ≥4 in all patients at the onset of fungemia. Clinical management included catheter removal and prompt echinocandin therapy in all cases; in one case (Case 1) liposomal amphotericin B was administered concomitantly.

In all fungemia episodes yeast growth was detected in blood culture bottles after 7–32 h incubation. In the quantitative CVC tips culture of the 4 patients a significant yeast growth was also obtained (>15 CFU by the Maki technique and >100 CFU by sonication). Subcultures on Sabouraud dextrose agar revealed smooth white to cream-colored colonies. All isolates grew well at 37 °C, but not at 45 °C or on Mycosel agar. In Chromagar Candida all isolates formed smooth pinkish, creamy colonies after 48 h incubation. The identification of the isolates obtained by the different techniques are shown in Table 2. All isolates were initially identified as *S. cerevisiae* by AuxaColor 2 technique and as *C. sake* with the API ID20C yeast identification system. By means of VITEK MS technology, the blood isolate from patient 1 was identified as *Candida lusitaniae* and the CVC tip isolate from patient 2 as *C. haemulonii*; the other six isolates could not be identified by this technique. DNA identification, based on the ITS region sequences, classified all isolates as *C. auris*.

The real burden of *C. auris* infections could be underestimated since this species is currently misidentified in the clinical laboratories by commercially available identification tests based on biochemical characteristics. Consequently, it is necessary

Table 2
Results of identification testing for 8 isolates from 4 patients with *C. auris* fungemia.

Case	Sample	Identification results					
		API 20C	AuxaColor	Vitek-MS	ITS sequence analysis	Accession no.	Similarity with sequence no. AB375772
1	Blood	*C. sake* (99.8%)	*S. cerevisiae*	*C. lusitaniae* (78%)	*C. auris*	KJ126759.1	94%
	CVC	*C. sake* (99.8%)	*S. cerevisiae*	No identification	*C. auris*	KJ126759.1	94%
2	Blood	*C. sake* (99.8%)	*S. cerevisiae*	No identification	*C. auris*	KC692045.1	96%
	CVC	*C. sake* (99.8%)	*S. cerevisiae*	*C. haemulonii* (99.9%)	*C. auris*	KC692045.1	96%
3	Blood	*C. sake* (99.8%)	*S. cerevisiae*	No identification	*C. auris*	KC692045.1	95%
	CVC	*C. sake* (99.8%)	*S. cerevisiae*	No identification	*C. auris*	KC692045.1	95%
4	Blood	*C. sake* (99.8%)	*S. cerevisiae*	No identification	*C. auris*	KJ126759.1	96%
	CVC	*C. sake* (99.8%)	*S. cerevisiae*	No identification	*C. auris*	KJ126759.1	96%

CVC, central venous catheter.

to confirm the identification by DNA sequencing of all high fluconazole-resistant isolates of species usually susceptible to this antifungal agent.

Nowadays, two MALDI-TOF systems are commercially available for routine bacterial and fungal identification in the clinical microbiology laboratories: Vitek MS (bioMérieux) and MALDI Biotyper CA System (Bruker Daltonics Inc., Billerica, MA, USA). Vitek MS combines two software platforms: Vitek MS IVD for routine diagnosis (that not includes *C. auris* in its database), and Vitek MS Research Use Only (RUO) whose database has recently been implemented (May 2016) with the *C. auris* spectra only for research purposes.[12] MALDI Biotyper CA System has a database library that contains spectra of 3 strains of *C. auris*, two from Korea and one from Japan.[13] Usually, genomics studies include *C. auris* within the *C. haemulonii complex* due to striking similarities in biochemical characteristics; this may be the reason why *C. auris* is misidentified by Vitek MS.[4,11] Furthermore, Chatterjee et al. have recently reported the first draft genome of *C. auris* to explore its genomic basis of virulence.[6] The authors found that *C. auris* has a highly divergent genome and shares genes with *C. albicans* and *C. lusitaniae*, pointing out a common ancestry. These findings could explain the frequent misidentification of *C. auris* as *C. lusitaniae* using the MALDI-TOF databases.

The eight *C. auris* isolates showed the same antifungal MIC, independently of the sample or the patient. Fluconazole and voriconazole MICs were ≥256 mg/l and 2 mg/l, respectively, and those of the other antifungal agents ranged from ≤0.06 mg/l of flucytosine to 0.5 mg/l of amphotericin B. Although there are no defined breakpoints for this species, based on those established for other *Candida* species, our isolates were highly resistant to fluconazole and resistant to voriconazole, but susceptible to the other antifungals tested. Comparing these results with those reported by other authors, it seems that *C. auris* is a species which is globally resistant to fluconazole and voriconazole and susceptible to other antifungals, although resistance to echinocandins and amphotericin B has also been described.[2,3,7,13,16] In our cases, it seems that there is no relationship between previous fluconazole treatment and *C. auris* fungemia, as only two patients received fluconazole before candidemia onset.

C. auris is an emerging cause of invasive candidiasis included within the *C. haemulonii* complex (Group II) based on physiological characteristics and isoenzimatic profile.[4] *C. auris* fungemia is associated with a high mortality rate, therapeutic failure, and widespread resistance to different antifungal agents.[7,8,13,15–18] Outbreaks of nosocomial bloodstream infections by *C. auris* have been reported by other authors.[3,7,8,16,17] In our series, the coexistence of four cases of fungemia by *C. auris* in a short period of time, in the same hospitalization unit, and with identical susceptibility pattern suggests a common source for all cases; to confirm this fact, we are currently conducting phylogenetic studies with all *C. auris* strains isolated in the SICU since the first fungemia episode caused by this species.

In conclusion, *C. auris* is an emerging pathogen that is underreported because it is misidentified in routine diagnostic laboratories. Furthermore, its resistance to fluconazole and other antifungal agents could make the management of deep-seated infections caused by this species difficult.

Conflict of interest

None to declare.

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