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Note

Candida orthopsilosis fungemias in a Spanish tertiary care hospital: Incidence, epidemiology and antifungal susceptibility

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ABSTRACT

Background: Few studies exist on prevalence of fungemia by *Candida orthopsilosis*, with variable results.
Aims: To study the incidence, epidemiology and antifungal susceptibility of *C. orthopsilosis* strains isolated from fungemias over two years at a tertiary hospital.
Methods: Candidemia episodes between June 2007 and June 2009 in a university hospital (Puerta del Mar, Cádiz, Spain) were studied. The strains initially identified as *Candida parapsilosis* were genotypically screened for *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *Candida metapsilosis*, and their antifungal susceptibility was evaluated.
Results: In this period 52 cases of candidemia were documented. Of the 19 strains originally identified as *C. parapsilosis*, 13 were confirmed as *C. parapsilosis* sensu stricto and 6 as *C. orthopsilosis*. Of the 52 isolates, the most frequent species were *Candida albicans* (30.8%), *C. parapsilosis* sensu stricto (25%), *C. orthopsilosis*, *Candida tropicalis* and *Candida glabrata* in equal numbers (11.5%). *C. orthopsilosis* isolates were susceptible to amphotericin B, caspofungin, voriconazole and fluconazole, with no significant differences in MIC values with *C. parapsilosis* sensu stricto. The source of isolates of *C. orthopsilosis* were neonates (50%) and surgery (50%), and 100% were receiving parenteral nutrition; however *C. parapsilosis* sensu stricto was recovered primarily from patients over 50 years (69.2%) and 46.1% were receiving parenteral nutrition.
Conclusions: These findings show that *C. orthopsilosis* should be considered as human pathogenic yeast and therefore its accurate identification is important. Despite our small sample size our study suggests that a displacement of some epidemiological characteristics previously attributed to *C. parapsilosis* to *C. orthopsilosis* may be possible.

Fungemias por *Candida orthopsilosis* en un hospital terciario español: incidencia, epidemiología y sensibilidad a antifúngicos

Palabras clave:
Candida orthopsilosis
Candida parapsilosis
Candidemia
Factores epidemiológicos
Sensibilidad a antimicóticos

RESUMEN

Antecedentes: Apenas se han publicado estudios sobre la prevalencia de los episodios de fungemia por *Candida orthopsilosis*, y sus resultados han sido variables.
Objetivos: Examinar la incidencia, epidemiología y sensibilidad a antifúngicos de las cepas de *C. orthopsilosis* aisladas de fungemias en un periodo de 2 años en un hospital de asistencia terciaria.
Métodos: Entre junio de 2007 y junio de 2009, en el Hospital Universitario Puerta del Mar (Cádiz, España) se estudiaron todos los episodios de fungemia. Las cepas identificadas inicialmente como *Candida parapsilosis* se genotipificaron para su clasificación como *C. parapsilosis* sensu stricto, *C. orthopsilosis* y *Candida metapsilosis*, y se testó su sensibilidad a los antifúngicos.
Resultados: Durante este periodo, se documentaron 52 episodios de fungemia. De las 19 cepas identificadas originalmente como *C. parapsilosis*, 13 fueron *C. parapsilosis* sensu stricto, y 6 *C. orthopsilosis*. De los 52 aislamientos, las especies más frecuentes fueron *Candida albicans* (30,8%), *C. parapsilosis* sensu stricto (25%) y *C. orthopsilosis* (11,5%), y *Candida tropicalis* y *Candida glabrata* fueron

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aisladas en igual número. Todos los aislamientos de *C. orthopsilosis* fueron sensibles a anfotericina B, caspofungina, voriconazol y fluconazol, sin diferencias significativas en las concentraciones inhibitorias mínimas obtenidas con *C. parapsilosis* sensu stricto. Los aislamientos de *C. orthopsilosis* procedían de recién nacidos (50%) y de pacientes sometidos a cirugía (50%). El 100% de los pacientes recibía nutrición parenteral; sin embargo, el foco de *C. parapsilosis* sensu stricto procedía, ante todo, de pacientes de más de 50 años de edad (69,2%), y el 46,1% recibía nutrición parenteral.

Conclusiones: Los resultados del presente estudio revelan que *C. orthopsilosis* debe considerarse una levadura patogénica para el ser humano y, por esta razón, es importante su identificación. A pesar del pequeño tamaño de la muestra, el presente estudio evidencia el desplazamiento a *C. orthopsilosis* de algunas características epidemiológicas atribuidas previamente a *C. parapsilosis*.

Candidemia has emerged as a serious threat to hospitalized patients,[1,2] and non-*Candida albicans Candida* species are becoming increasingly prevalent.[14–16] *Candida parapsilosis* is one of the most common yeasts involved in invasive candidiasis[10] and is frequently transmitted horizontally via contaminated external sources such as medical devices or fluids, the hands of health care workers, prosthetic devices, and catheters.[24] Recent studies have reported an association between *C. parapsilosis* fungemias and the use of indwelling devices and parenteral nutrition, found frequently in patients admitted to neonatal or surgical ICUs.[17]

Candida orthopsilosis and *Candida metapsilosis* are recently described species phenotypically indistinguishable from *C. parapsilosis*.[21] Retrospective epidemiological studies have been undertaken to screen for *C. orthopsilosis* and *C. metapsilosis* among isolates previously identified as *C. parapsilosis*,[8,12,13,22] in which important epidemiological variations have been found. Studies on these species are necessary to clarify their incidence and epidemiology.

The aim of this work is to analyze the incidence, epidemiological data and sensitivity to antifungals of *C. orthopsilosis* and *C. parapsilosis* sensu stricto isolates from fungemia and the relation with all the *Candida* species isolated from blood cultures over a two-year period in a Spanish tertiary care hospital.

Material and methods

All the fungemia episodes between June 2007 and June 2009 in a tertiary university Hospital (Puerta del Mar, Cádiz, Spain) were studied and some variables were analyzed according to a pre-established protocol (Table 1).

Blood culture samples were processed using the Wider System (Wider, Francisco Soria Melguizo, Madrid, Spain) and subcultured on Sabouraud and CHROMagar Candida plates (CHROMagar, Paris, France). A total of 52 *Candida* spp. were isolated from 52 different patients and 19 of these strains were originally identified as *C. parapsilosis* by API ID 32C (bioMérieux, Madrid, Spain).

Molecular identification of *C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis* was carried out in the Microbiology laboratory, Faculty of Medicine, and assisted by the Facility of Bioscience Applied Techniques (STAB, Extremadura University, Spain), according to Chen et al.[5] DNA of the 19 isolates and the control strains *C. parapsilosis* sensu stricto ATCC 1449, *C. orthopsilosis* CECT 13011 and *C. metapsilosis* CECT 13010 was extracted using a MasterPure™ Yeast DNA Purification Kit (EPICENTRE, Madison, WI). PCR with the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) was used to amplify the 5.8S RNA gene and the flanking ITS1 and ITS2 region. All ITS sequences were submitted to the BLAST program of National Center for Biotechnology Information (NCBI) Web site (http://blast.ncbi.nlm.nih.gov/).

Antifungal susceptibility testing was performed using the Sensititre YeastOne panel-YO-7 (TREK Diagnostic Systems, Izasa, Madrid, Spain).

Results

During the study period, 52 *Candida* spp. were isolated from blood cultures. The most frequently isolated species was the *C. parapsilosis* complex (36.5%) – *C. parapsilosis* sensu stricto (25%) and *C. orthopsilosis* (11.5%) – followed by *Candida albicans* (30.8%), and with the same number *Candida tropicalis* and *Candida glabrata* (11.5%) (Table 1); no cases of *C. metapsilosis* were isolated. Episodes of fungemia were most frequently observed in patients from the following departments: ICUs (21.2%), general surgery (21%), neonatology (15.4%) and internal medicine (7.7%).

Gender, age and associated conditions of the 52 patients with candidemia are shown in Table 1. Prior antibacterial therapy was administered in 100% of the cases of candidemia; presence of intravascular catheter fluctuated from 77 to 100%, except in *Candida krusei*, a species in which the presence of hematological malignancies is noted. *C. parapsilosis* complex fungemias share several risk factors but with some differences: *C. orthopsilosis* fungemia was related mainly with parenteral nutrition (100%) and neonates (50%); *C. parapsilosis* sensu stricto, however, was recovered primarily from patients aged at least 50 years (69.2%) and only 46.1% were receiving parenteral nutrition ($P = 0.02$).

Antifungal susceptibility testing results of *C. parapsilosis* sensu stricto and *C. orthopsilosis* for fluconazole, voriconazole, amphotericin B and caspofungin are summarized in Table 2. All isolates were found to be susceptible to the four antifungal agents tested, followed MIC breakpoints by established CLSI guidelines.[6] There were no significant differences in MIC values between both species. However, by using the new breakpoints of fluconazole proposed by the EUCAST,[20] there is one isolate of *C. parapsilosis* sensu stricto which is susceptible-dose dependent to fluconazole (MIC = 4).

Discussion

Nosocomial infections by *C. parapsilosis* have increased considerably and high rates of candidemia by *C. parapsilosis* can be attributed to nosocomial transmission.[9,24] *C. parapsilosis* is notorious for its capacity to grow in total parenteral nutrition, for nosocomial spread by hand carriage, and for its persistence in the hospital environment.[24] Incidence varies from one study to another, due fundamentally to the type of the population studied. In children's hospitals incidence reaches up to 60% of the candidiasis isolated,[7,18] whereas in adults the incidence is lower, with references of 20–30%.[1,16] In our study we found an incidence of fungemia by *C. parapsilosis* complex of 36.5%, which reached 54.5% in neonates, percentages which vary with the identification

Table 1
Comparison of sex, age and associated conditions of 52 patients with candidemia by *C. orthopsilosis*, *C. parapsilosis*, *C. albicans*, and other *Candida* species.

	C. parapsilosis complex			Other *Candida* species			
	C. orthopsilosis	*C. parapsilosis* sp.	*C. albicans*	*C. glabrata*	*C. tropicalis*	*C. krusei*	Others[a]
Patients (N)	6	13	16	6	6	3	2
Sex							
Male	5	8	12	2	3	1	1
Female	1	5	4	4	3	2	1
Age group							
Neonate[b]	3	3	2	0	1	1	1
Children[c]	1	0	1	1	1	1	0
15–29	0	0	0	0	0	0	0
30–49	0	1	3	2	1	0	0
50–69	2	6	4	1	1	1	1
>70	0	3	6	2	2	0	0
Associate conditions							
Parenteral nutrition	6	6	6	5	3	1	1
Intravascular catheter	6	10	15	6	6	1	2
Surgery (previous 3 months)	3	7	8	3	1	1	2
Prior antifungal therapy	3	0	4	2	0	0	0
Prior antimicrobial therapy	6	13	16	6	6	3	2
Patients exitus	1	2	7	2	4	0	0

[a] *C. utilis* and *C. lipolytica*.
[b] <1 month old, all low-weight premature.
[c] 1 month to 14 years old.

Table 2
In vitro susceptibilities of *C. orthopsilosis* and *C. parapsilosis* sensu stricto bloodstream isolates to four antifungal agents.

Species (no. of isolates)	Antifungal agents[a]	Minimum inhibitory concentration (μg/ml)		
		MIC range	MIC_{50}	MIC_{90}
C. orthopsilosis (6)	ABD	0.03–1	0.25	0.5
	CAS	0.03–1	0.125	0.5
	VCR	<0.008–0.06	0.03	0.06
	FLC	0.25–4	0.5	2
C. parapsilosis sensu stricto (13)	ABD	0.06–1	0.5	1
	CAS	0.06–0.5	0.25	0.5
	VCR	<0.008–0.03	0.015	0.03
	FLC	0.125–4	1	2

[a] ABD: amphotericin B deoxycholate; CAS: Caspofungin; VCR: voriconazole; FLC: fluconazole.

of new species. In neonates *C. orthopsilosis* provided 50% of the isolates and *C. parapsilosis* sensu stricto 23%, the possible source of infection being exogenous colonization by neonatology staff. On the other hand, 69.2% of *C. parapsilosis* sensu stricto was recovered from patients aged at least 50 years as opposed to 33% of *C. orthopsilosis*. Our results indicate that the frequency of the species differ depending on the age of the patients, in agreement with Tay et al.[23] who found a higher frequency of *C. orthopsilosis* candidemia in pediatric patients compared to adult patients.

The existence of three genetically different groups in *C. parapsilosis* was described by Lin et al.[11] and the groups were subsequently classified as different species by Tavanti et al.[21] as *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis*. In the present study we found, after molecular identification of 19 *C. parapsilosis* complex isolates of fungemia, that 31.5% of the isolates phenotypically identified as *C. parapsilosis* were *C. orthopsilosis*. Studies performed on incidence of *C. orthopsilosis* within the complex group presented variable results[3,13,19,21,23] fluctuating from 2.37%[18] to 25.9%[19,21], which is similar to our results. Our findings show a high prevalence of fungemia by *C. parapsilosis* sensu stricto, and by *C. orthopsilosis*, second and third species in frequency after *C. albicans*. In one case of fungemia by *C. orthopsilosis*, identical isolation was obtained on repeating the hemoculture a week after treatment. No isolates of *C. metapsilosis* were obtained, a species which has been reported to be rarely recovered from clinical samples.[17]

The results of antifungal susceptibility testing showed that the *C. parapsilosis* sensu stricto and *C. orthopsilosis* isolates tested in this study were susceptible to amphotericin B, caspofungine, fluconazole and voriconazole. According to previous studies,[4,15,17] all the isolates show a high level of susceptibility to all the agents tested. Some authors find in *C. orthopsilosis* greater resistance to voriconazole[3], and greater susceptibility to echinocandins[3,8]; in the case of our strains, a slight displacement of MICs in this sense was observed. In general, there was no statistically significant difference between the susceptibility profile of the two species either with the azoles (fluconazole, voriconazole) or with amphotericin or caspofungin.

In this study we report a notable prevalence of the recently described species *C. orthopsilosis* from blood culture, and we point to the clinical relevance of these newly described yeasts. Although molecular methods are not routinely available, identification is easily performed by ITS sequencing. Differentiation of the new species allowed us to define the epidemiology of candidemia by *C. parapsilosis* sensu stricto and *C. orthopsilosis*. The low number of *C. orthopsilosis* isolates found during the period studied merits additional investigation concerning its epidemiology, since some

of the characteristics previously attributed to infections by *C. parapsilosis*, such as presence in neonates, may be displaced toward *C. orthopsilosis*.

Ethical responsibilities

Confidentiality of data. The authors declare that they have followed the protocols of their workplace on the publication of the data from the patients, and that all patients included in the study have received sufficient information and have given their written informed consent to participate in the study.

Conflicts of interest

The authors report no conflicts of interest.

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