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Identification of *Candida parapsilosis Sensu Lato* in Pediatric Patients and Antifungal Susceptibility Testing

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ABSTRACT A total of 59 *Candida parapsilosis sensu stricto* and 1 *Candida orthopsilosis* recovered from catheters and blood cultures of pediatric patients from the northeastern region of Argentina were studied. Susceptibility to azoles, amphotericin B, and echinocandins was tested by the broth microdilution method. According to CLSI clinical breakpoints, >91% of the strains were azole susceptible, whereas 15% showed high amphotericin B MICs.

KEYWORDS *C. orthopsilosis*, *C. parapsilosis sensu stricto*, PCR-REA, amphotericin B, blood culture, echinocandin

Candidemia is the fourth most prevalent nosocomial bloodstream infection and the most frequent nosocomial fungal infection (1–3). *Candida parapsilosis sensu lato* is the second most frequently isolated species from blood in Asia, Latin America, and some European countries, surpassing the *Candida albicans* frequency in some pediatric hospitals (4–10). *C. parapsilosis sensu lato* includes 3 closely related species: *C. parapsilosis sensu stricto*, *Candida orthopsilosis*, and *Candida metapsilosis* (11).

The aims of this study were to determine the prevalence of the *C. parapsilosis* species complex as a candidemia agent in pediatric patients from the northeastern region of Argentina and to evaluate its susceptibility.

Between 2009 and 2014, 60 isolates from 40 neonates (<1 month old) and pediatric patients (<6 years old) admitted to Resistencia and Corrientes, Argentina, hospitals were studied. All patients had proved invasive fungal disease according to EORTC/MSG (European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group) criteria (12). Twenty-nine isolates were recovered from blood and 31 from catheters with positive blood cultures. All isolates were referred for identification and antifungal susceptibility studies to the Mycology Department of the Instituto de Medicina Regional, Universidad Nacional del Nordeste, and deposited in its culture collection.

The isolates were identified as *C. parapsilosis sensu lato* using API ID 32C (bioMérieux, Marcy l'Etoile, France) and micromorphology (13). Molecular identification was performed using a PCR-based restriction endonuclease analysis (PCR-REA), where an *FKS1* region was amplified by PCR and subsequently digested with EcoRI (14). DNA extraction was performed following the protocol of Bosco-Borgeat et al. (15).

The *in vitro* antifungal activity of fluconazole (FLC), voriconazole (VRC), and anidulafungin (ANF) (Pfizer, USA); caspofungin (CSF) (Merck & Co., USA); and itraconazole (ITC) and amphotericin B (AMB) (Sigma-Aldrich) were evaluated according to CLSI documents M27-A3 and M27-S4 (16, 17). All but one isolate were molecularly identified

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TABLE 1 Geometric mean, concentration range, MIC_{50}, and MIC_{90} obtained from 59 isolates of *C. parapsilosis sensu stricto*

Drug[a]	Geometric mean (μg/ml)	Range (μg/ml)	MIC_{50}	MIC_{90}	Susceptibility (% [*n*])[b]				
					ECV		CBP		
					WT	Non-WT[d]	S	SDD/I	R[d]
AMB[c]	1.00	0.50–2.00	1	2	100 (59)				
FLC	1.50	0.25–4.00	2	2			91.5 (54)	8.5 (5)	
ITC[c]	0.10	≤0.03–0.25	≤0.03	≤0.03	100 (59)				
VRC	0.06	≤0.03–0.125	≤0.03	0.06			100 (59)		
ANF	1.10	0.015–4.00	1	2			97 (57)	3 (2)	
CSF	1.00	0.125–2.00	1	1			100 (59)		

[a]AMB, amphotericin B; FLC, fluconazole; ITC, itraconazole; VRC, voriconazole; CSF, caspofungin; ANF, anidulafungin.
[b]ECV, epidemiological cutoff values; CBP, clinical breakpoint; WT, wild type; S, susceptible; SDD, susceptible dose dependent (for azole drugs); I, intermediate (for ANF and CSF); R, resistant.
[c]No clinical breakpoints are available. Thus, epidemiological cutoff values were used as susceptibility criteria (18).
[d]No resistant or non-wild type isolate was obtained.

as *C. parapsilosis sensu stricto*. The single *C. orthopsilosis* isolate identified showed the following MIC values: AMB 1 μg/ml, FLC 1 μg/ml, ITC 0.06 μg/ml, VRC 0.06 μg/ml, ANF 0.5 μg/ml, and CSF 1 μg/ml. For AMB, *C. parapsilosis sensu stricto* isolates showed a narrow range of MIC values (geometric mean [GM], 1 μg/ml; range, 0.5 to 2 μg/ml) and an unexpectedly high MIC_{90} of 2 μg/ml, with nine strains showing AMB MIC values of 2 μg/ml (Table 1). Azoles and echinocandins showed good activity. The majority of the strains showed a low FLC MIC, and only five strains (8.5%) had MIC values that were considered susceptible dose dependent (SDD). All of the strains were susceptible to VRC (range, ≤0.03 to 0.125 μg/ml) and considered wild type when ITC was tested (MIC GM, 0.1 μg/ml) following the clinical breakpoints and epidemiological cutoff values, respectively (18). For the echinocandins, MIC values were lower for CSF than for ANF. However, all of the strains were considered susceptible to CSF, and all but two were susceptible to ANF (considered intermediate) (Table 1).

Spanish studies in neonates and pediatric populations showed a *C. parapsilosis sensu stricto* prevalence of between 72% and 91% and a *C. orthopsilosis* presence of between 9% and 27% (6, 19, 20). Moreover, in a global study, the rate of *C. orthopsilosis* in South America was 10.9%, with variations between 16.5% in Venezuela and 3.1% in Argentina (1, 6, 19, 20). Only one *C. orthopsilosis* isolate (2%) and no *C. metapsilosis* isolate was found in our study population.

Most studies report *C. parapsilosis sensu stricto* as the species in the complex with the highest MICs to antifungal agents (19–21). Although an AMB tolerance in *C. parapsilosis sensu lato* isolates has been described (8, 22, 23), most authors reported an AMB MIC_{90} of <1 μg/ml (1, 6, 19–21, 24–28). In our study, nine isolates (15%) had an MIC of 2 μg/ml, which was similar to the MICs obtained by Lockhart et al. (20) and Cantón et al. (21).

Echinocandins were not used as treatment or prophylaxis in our patients. However, we obtained high CSF MIC values. To circumvent the described problems related to CSF susceptibility testing (27), ANF susceptibility was added to the study. As described before, wide MIC ranges were found (0.015 to 4 μg/ml for ANF and 0.125 to 2 μg/ml for CSF) (19, 21, 29–34). Also, the MIC_{90} for ANF was similar to those obtained by other authors (1, 6, 19–21, 24–28, 30).

Regarding azoles, FLC presented the highest MIC ranges (0.25 to 4 μg/ml). However, 90% of the isolates were susceptible and showed MIC values similar to those presented elsewhere (1, 6, 19–21, 24–28, 30). Five isolates were categorized FLC-SDD (MIC, 4 μg/ml), and two of them had the highest ITC and VRC MIC values in this series (0.25 and 0.125 μg/ml, respectively), probably due to cross-tolerance mechanisms among azoles (35). No differences in susceptibility profiles were observed in patients with more than one isolate, except for one patient on FLC treatment with six successive isolates (2 from catheter and 4 from blood). The last isolate recovered from blood was categorized as FLC-SDD, whereas the previous five isolates were susceptible to all azoles.

A high percentage of the studied strains were susceptible to FLC, but an unexpectedly high number of isolates showed high AMB MIC values. These are important results for our region, since FLC and AMB are sometimes the only drugs available in most Latin American hospitals due to economic factors.

No conclusions can be drawn about the *C. orthopsilosis* antifungal susceptibility profile because only one strain was isolated; however, we observed low MICs to all the tested drugs. The discrimination of cryptic species within the *C. parapsilosis* species complex would not have a considerable clinical utility. However, surveillance is important to determine the epidemiology of this complex, observe changes in species distribution, and detect resistant strains, especially in pediatric patients whose treatment options are limited. To our knowledge, this is the first study in Argentina on the prevalence of *C. parapsilosis sensu lato* and its susceptibility profile in neonates and pediatric patients with candidemia.

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We have no conflicts of interest to declare.

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