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CANDIDEMIA IN A BRAZILIAN TERTIARY CARE HOSPITAL: SPECIES DISTRIBUTION AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS

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SUMMARY

Recent studies have shown differences in the epidemiology of invasive infections caused by *Candida* species worldwide. In the period comprising August 2002 to August 2003, we performed a study in Santa Casa Complexo Hospitalar, Brazil, to determine *Candida* species distribution associated with candidemia and their antifungal susceptibility profiles to amphotericin B, fluconazole and itraconazole. Antifungal susceptibility was tested according to the broth microdilution method described in the NCCLS (M27A-2 method). Only one sample from each patient was analyzed (the first isolate). Most of the episodes had been caused by species other than *C. albicans* (51.6%), including *C. parapsilosis* (25.8%), *C. tropicalis* (13.3%), *C. glabrata* (3.3%), *C. krusei* (1.7%), and others (7.5%). Dose-dependent susceptibility to itraconazole was observed in 14.2% of strains, and dose-dependent susceptibility to fluconazole was found in 1.6%. Antifungal resistance was not found, probably related to low use of fluconazole. Further epidemiological surveillance is needed.

KEYWORDS: Candidemia; *Candida* species; Antifungal resistance; Susceptibility tests.

INTRODUCTION

The term candidemia indicates the presence of *Candida* species in the blood. With the growing number of immunodepressed patients, antibiotic and antifungal use, epidemiology of candidemia has changed in recent years, with emergence of species with reduced susceptibility to antifungal agents[5]. Studies performed in different countries, however, have shown different susceptibility patterns[2], reinforcing the need of further research. In this study, we analyze the *Candida* species causing candidemia and their antifungal susceptibility patterns in a large Brazilian hospital.

MATERIAL AND METHODS

This transversal study included all bloodstream isolates of *Candida* spp. collected from patients admitted at Santa Casa Complexo Hospitalar, a tertiary care hospital with 1200 beds, from August 2002 to August 2003. To avoid the inclusion of several samples from the same patient, which should bias *Candida* species distribution, just one sample from each patient was analyzed (the first isolate). Germ tubes were performed, and negative strains were identified through kit ID 32C (BioMérieux SA, France).

Antifungal susceptibility was tested according to the broth microdilution method described in the National Committee for Clinical Laboratory Standards (M27-A2)[9]. In accordance to this protocol, the following antifungal drugs were tested: amphotericin B (Sigma Chemical Corporation, USA), fluconazole, (Pfizer Inc., USA) and itraconazole (Janssen Pharmaceutica, USA). Broth microdilution testing was performed in RPMI-1640 with L-glutamine, without bicarbonate (Gibco Invitrogen Corporation, USA), buffered with MOPS at pH 7.0. The plates were incubated at 35 ºC for 48 hours, visually read, and then corroborated through an ELISA reader. The minimum inhibitory concentration (MIC) for amphotericin B was defined as the lowest concentration that showed reduction in the turbidity. The MIC for itraconazole and fluconazole was defined as the concentration that showed prominent reduction in the turbidity when compared to a positive control.

Strain susceptibility to fluconazole and itraconazole were classified as susceptible (MIC ≤ 8 µg/mL and MIC ≤ 0.125 µg/mL), susceptible-dose dependent (MIC 16 - 32 µg/mL and MIC 0.25 - 0.5 µg/mL) and resistant (MIC > 32 µg/mL and MIC > 0.5 µg/mL, respectively)[9]. Although there are no established breakpoints for amphotericin B, most susceptible strains show MIC ≤ 1 µg/mL, and that concentration was used to define susceptibility. The control was performed by using standard strains ATCC (American Type Culture Collection): *C. krusei* ATCC® 6258 and *C. parapsilosis* ATCC® 22019. The protocol was approved by institutional review board/independent ethics committee, and the authors declare no conflict of interests to write this manuscript. To confirm species and susceptibility, strains were submitted to Department of Pathology, University of Iowa.

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RESULTS

From August 2002 to August 2003, a total of 120 isolates were recovered from patients with candidemia (Table 1). *C. albicans* (48.3%) was the most prevalent one, followed by *C. parapsilosis* (25.8%), and *C. tropicalis* (13.3%). No resistant strain was found. Dose-dependent susceptibility to itraconazole was observed in isolates of *C. krusei* (50%), *C. glabrata* (25%), *C. parapsilosis* (16%), C. *tropicalis* (12%), and *C. albicans* (7%). Dose-dependent susceptibility to fluconazole was found only in *C. krusei* isolates; all strains of *C. glabrata* were susceptible to fluconazole.

DISCUSSION

In the present study, we showed that most episodes of candidemia at our institution were caused by species other than *C. albicans* (51.6%). Added, *C. glabrata* and *C. krusei* accounted for only 5.0% of all cases. The distribution of species with reduced susceptibility to azoles, mainly *C. glabrata*, has shown large variations in different regions of the world. While *C. glabrata* is the second most prevalent species in the United States[2,10,11], it is infrequent in South America, contrasting with *C. parapsilosis* and *C. tropicalis*[2,6,12]. In Brazil, *C. albicans* has been the main agent of candidemia (20-50%), followed by *C. parapsilosis* (17-35%), *C. tropicalis* (12-27%), and *C. guilliermondii* (2-10%)[1,4,7,8]. *C. krusei* has been an infrequent cause of candidemia worldwide (1-2%)[1,10,12].

In accordance to our findings, previous studies in South America have shown low rate of antifungal resistance[3,6]. GODOY *et al*.[6] found that most *Candida* spp. isolates were susceptible to all antifungal agents. In a Brazilian study with 200 cases of candidemia, despite the high rate of infections caused by species other than *C. albicans* (60%, mainly *C. tropicalis* and *C. parapsilosis*), COLOMBO *et al*.[3] found that resistance to azoles occurred in only 1.5% of isolates. In the same study, two isolates of *C. krusei* and 11% of isolates of *C. glabrata* were resistant to fluconazole.

SENTRY studies[11] showed that there was low resistance to azoles in samples collected in Canada and South America when compared to samples collected in the United States. The less frequent use of fluconazole may explain the low resistance to azoles found in those studies. We have found only two isolates of *C. krusei* in this study, both of it were susceptible-dose dependent to fluconazole (confirmed by repetition). However, it is known that this species is intrinsically resistant to it[5].

Concluding, our study confirmed data from previous studies that demonstrated high prevalence of candidemia in Brazil caused by species other than *C. albicans*[1,4,7]. Resistance to antifungal agents was not found, and this finding may at least partially be explained by the low use of fluconazole. Epidemiological surveillance, however, is necessary, in order to follow the dynamics of candidemia.

RESUMO

Candidemia em hospital terciário brasileiro: distribuição das espécies e padrões de susceptibilidade aos antifúngicos

Estudos realizados em diferentes países têm mostrado diferença na epidemiologia das infecções invasivas por *Candida* spp. No período de agosto de 2002 a agosto de 2003, foi conduzido estudo na Santa Casa Complexo Hospitalar, Porto Alegre, Brasil, para determinar a distribuição das espécies de *Candida* associadas a candidemia e o perfil de susceptibilidade das mesmas aos antifúngicos anfotericina B, fluconazol e itraconazol. Os testes de susceptibilidade foram realizados de acordo

Table 1
Species distribution and antifungal susceptibility profile of *Candida* spp. bloodstream isolates from Santa Casa Complexo Hospitalar (n = 120) between August 2002 and August 2003

Species	n (%)	Antifungal agents	MIC Range (µg/mL)	Suscept. (%)	Susceptible dose dep. (%)	Resistant (%)
C. albicans	58	Amphotericin B	0.25 – 1.00	100%	–	–
	(48.3)	Fluconazole	0.12 – 8.00	100%	–	–
		Itraconazole	0.03 – 0.25	93%	7%	–
C. parapsilosis	31	Amphotericin B	0.12 –1.00	100%	–	–
	(25.8)	Fluconazole	0.12 – 2.00	100%	–	–
		Itraconazole	0.03 – 0.50	84%	16%	–
C. tropicalis	16	Amphotericin B	0.5 – 1.00	100%	–	–
	(13.3)	Fluconazole	0.12 – 2.00	100%	–	–
		Itraconazole	0.03 – 0.25	88%	12%	–
C. glabrata	4	Amphotericin B	0.50 – 1.00	100%	–	–
	(3.3)	Fluconazole	1.00 – 8.00	100%	–	–
		Itraconazole	0.12 – 0.25	75%	25%	–
C. krusei	2	Amphotericin B	0.50 – 1.00	100%	–	–
	(1.7)	Fluconazole	16.00 – 16.00	–	100%	–
		Itraconazole	0.06 – 0.50	50%	50%	–
Other species *	9	Amphotericin B	0.25 – 1.00	100%	–	–
	(7.5)	Fluconazole	0.25 – 4.00	100%	–	–
		Itraconazole	0.03 – 0.50	56%	44%	–

* Other species included *C. famata* (n = 2), *C. sake* (n = 2), C. *guillermondii* (n = 2), *C. lusitaniae* (n = 1), *C. dubliniensis* (n = 1), *C. lipolytica* (n = 1).

com a metodologia M27-A2 padronizada pelo NCCLS. Foi incluído no estudo o primeiro isolado de hemocultivo de cada paciente. A maioria dos episódios (51,6%) ocorreu por espécies outras que *C. albicans*, incluindo *C. parapsilosis* (25,8%), *C. tropicalis* (13,3%), *C. glabrata* (3,3%), *C. krusei* (1,7%) e outras espécies (7,5%). Não foi encontrada resistência aos antifúngicos testados, possivelmente devido ao baixo consumo de fluconazol na Instituição. Susceptibilidade dose-dependente ao itraconazol ocorreu em 14,2% e ao fluconazol 1,6%. Faz-se necessário monitoramento epidemiológico.

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