Original

Prevalence and *in vitro* antifungal susceptibility of *Candida* spp isolated from clinical specimens in São Paulo, Brazil

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Summary

We examined the prevalence and the *in vitro* susceptibility to antifungal drugs of *Candida* spp isolated from clinical specimens at our university hospital in São Paulo, Brazil. Among 6,417 samples studied, positive cultures, were obtained from 222 (3.5%) most of them (68%) from the pediatric unit and nursery. *Candida albicans* and *Candida parapsilosis* were the most frequent species and the susceptibility patterns of a panel of 130 isolates to amphotericin B, ketoconazole and fluconazole, showed that the order of antifungal efficacy was amphotericin B > ketoconazole > fluconazole.

Key words *Candida* spp, Prevalence, Antifungal susceptibility, Brazil

Prevalencia y sensibilidad antifúngica *in vitro* de cepas de *Candida* spp. aisladas de muestras clínicas en São Paulo, Brasil

Resumen

Se presenta la prevalencia de *Candida* y la actividad *in vitro* de tres antifúngicos (anfotericina B, ketoconazol y fluconazol) sobre 130 cepas aisladas de muestras clínicas tomadas de pacientes tratados en el Hospital de Clínicas de la Universidad Estatal Paulista, São Paulo, Brasil. En un total de 6.417 muestras estudiadas, 222 (3,5%) mostraron positividad para el aislamiento de hongos, que fue más frecuente en los pacientes de la Unidad de Cuidados Intensivos y de Pediatría. Las levaduras más prevalentes fueron *Candida albicans* y *Candida parapsilosis*, mientras que los estudios de actividad antifúngica demostraron una eficiencia mas elevada de la anfotericina B en relación a los demás fármacos frente a las cepas estudiadas.

Palabras clave *Candida* spp, Prevalencia, Sensibilidad antifúngica, Brasil

Mortality and morbidity due to candidiasis are frequently observed in immunocompromised patients such as those with AIDS, transplants, malignancies and so on [6]. Pires *et al*. (1996) [10] reported that oral candidiasis develops in 90 to 95% of symptomatic HIV-infected individuals and its prevalence increases in parallel with the severity of immune dysfunction. According to Dupont [3], oral candidiasis is also an important sign for clinical diagnosis and an indicator of the evolution of immunodeficiency among HIV carriers.

Since information on the prevalence of pathogenic yeasts and their antimicrobial susceptibility patterns is important for therapy, this survey was undertaken to evaluate the prevalence of *Candida* species in clinical specimens, as well as the *in vitro* efficacy of three antifungal agents commonly used in the treatment of candidiasis in our hospital.

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MATERIALS AND METHODS

Clinical specimens and characterization of Candida.

A total of 6417 clinical specimens was used in this study; they consisted of blood (n= 3,333-51.9%), indwelling medical devices (2,589-40.3%) and ascitic fluid (495-7,7%) taken from in-patients treated during January 1991 to December 1994 at the Hospital of the School of Medicine of São Paulo State University-UNESP, Botucatu-SP, Brazil, a 400-bed university nosocomial center.

The isolation and speciation of *Candida* were done at the Department of Microbiology and Immunology of the Institute of Biosciences, Botucatu-SP-UNESP by using standard methods [7], including tests of formation of germ tubes, filamentation and formation of chlamydospores and physiological and biochemical characteristics.

Antifungal susceptibility testing

Microorganisms. A panel of 130 strains of *Candida*, randomly selected among isolates from the above clinical specimens, was studied. This panel consisted of 75 strains of *Candida albicans*, 21 strains of *Candida parapsilosis*, 19 strains of *Candida tropicalis*, 10 strains of *Candida guilliermondi*i, three strains of *Candida krusei*, one strain of *Candida lusitaniae* and one strain of *Candida glabrata*.

Antimicrobials and susceptibility test method. The antifungal agents included amphotericin B [AMP-B (Bristol Myers-Squibb, Brazil)], fluconazole [FLU (Pfizer, France)] and ketoconazole [KTC Memo JCB \ 010, Brazil].

Susceptibility tests were performed by using a standardized macrodilution broth method proposed by the National Committee for Clinical Laboratory Standards [9]. Stock solutions of antifungal agents were serially diluted in sterile saline (0.85%) to give a final concentration of 0.03 mg/mL (AMP-B and KTC) or 0.01 mg/mL (FLU). Isolates were grown on Sabouraud Dextrose Agar-SDA (Oxoid) for 24-48 h at 37ºC and five colonies (1 mm diameter) of each strain were picked up and suspended in 5 ml of sterile saline. A portion of this suspension was streaked onto SDA plates to check for purity. The suspension was adjusted to match the turbidity of the 0.5 McFarland scale and diluted 1:100 in Yeast Nitrogen Base Broth-YNB (Difco), supplemented with 1% glucose and 0.15% asparagine to yield a final inoculum size of approximately 5 x 104 cells per ml. A similar suspension was also prepared with a control strain of *Saccharomyces cerevisiae* (ATCC 2601). Inocula (0.9 ml) were placed in YNB tubes (10 ml) containing various concentrations of an antifungal agent and as a growth control a drug-free tube was included in each run. All racks of tubes were incubated without agitation for 24-48 h at 37ºC. Following incubation, the turbidity was measured and the minimal inhibitory concentration (MIC) was calculated; it was defined as the lowest drug concentration that produced a significant turbidity reduction, i.e., macroscopically clear or slightly hazy as compared with the growth control, YNB drug-free tube.

RESULTS AND DISCUSSION

Among 6,417 clinical specimens, 222 (3.5%) yielded positive *Candida* cultures. Of these, 40 strains (1.2%) were isolated from blood, 161 strains (62.2%) from indwelling medical devices and 21 strains (4.2%) from ascitic fluid. Their sources were as follows: 151 strains (68.0%) from the pediatric unit and nursery, 22 strains (9.9%) from the intensive care unit and the remaining 49 strains (22.1%) from other nosocomial wards.

Established risk factors for candidiasis include low age and immunosuppression of patients [4]. In the present study, although the severity of the candidiasis was not specifically examined, many of the clinical cases occurred in young patients, who were often under catheterization or using other medical devices. These patients were mostly <10 days-8 years old and suffering from systemic infection. In the 30 positive blood cultures obtained from children, *C. albicans* (n=14, 46%), *C. parapsilosis* (n=10, 33.3%), *C. guilliermondi*i (n=4, 13.3%) and *C. tropicalis* (n=2, 6.6%) were the species characterized.

Identification to the species level of all 222 isolates of *Candida* showed that *C. albicans* (n=128, 57.6%) was the most frequent, followed by *C. parapsilosis* (n=42, 18.9%), *C. tropicalis* (n=31, 14%), *C. guilliermondii* (n=14, 6.3%), *C. krusei* (n=4, 1.8%), *C. lusitaniae* (n=1, 0.4%), *C. glabrata* (n=1, 0.4%) and C. obtusa (n=1, 0.4%). The high incidence of *C. albicans* is in accordance with reports of others, [4,10,12] indicating that this species is the most frequent causative agent of candidiasis in immunosuppressed patients. The incidences of *C. parapsilosis* and *C. tropicalis* were also quite high, confirming the observations of Fisher *et al.* [5], who stated that these species show increasing prevalence in the etiology of serious forms of candidiasis.

Results of MIC determinations are illustrated in table 1 and figure 1. The MIC50 and MIC90 for all isolates were 0.013-0.078 mg/ml, 0.93-2.65 mg/ml and 0.06-5.37 mg/ml for AMP-B, KTC and FLU, respectively. The order of drug efficacy was AMP-B > KTC > FLU. In general, *C. albicans*, *C. guilliermondi*i and *C. parapsilosis* were the most sensitive species to AMP-B, FLU and KTC, respectively. *C. albicans* was relatively unsusceptible to KTC and FLU.

The antifungal activity patterns of the test drugs are comparable with findings obtained elsewhere [1,2,5,11,13]. The *in vitro* efficacy of AMP-B is of special interest because the MIC 50 and 90 values were extremely low and similar to each other. However, the toxicological side effects [8] of AMP-B limit its use in debilitated patients. Further, in connection with the higher *in vitro* antifungal activity of AMP-B than KTC and FLU, it is necessary to take into account that when complex media are used in susceptibility testing, results can be falsely high and may not correlate with the *in vivo* efficacy [5]. Since FLU has been shown to be an effective, orally active antifungal agent with low toxicity, it may be considered an alternative choice for the therapy of candidiasis, including that caused by *C. albicans*.

Table 1. Susceptibility of 130 strains of *Candida* isolated from clinical specimens to amphotericin B, ketoconazole and fluconazole.

		MIC (mg/ml)								
		amphotericin B			ketoconazole			fluconazole		
Species	(Nº)	Range	MI 50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90
C. albicans	(75)	0.003-0.78	0.012	0.059	0.003-6.25	1.37	2.83	0.078 - > 20	0.45	7.43
C. parapsilosis	(21)	0.003-0.78	0.02	0.093	0.003-6.25	0.046	0.38	0.078 - > 20	0.32	3.25
C. tropicalis	(19)	0.003-0.78	0.02	0.097	0.003-6.25	0.93	2.6	0.078 - > 20	0.28	3.25
C. guilliermondii	(10)	0.003-0.78	0.1	0.134	0.003-6.25	0.3	1.3	0.078 - > 20	0.62	2.25
C. krusei	(03)	0.024-0.097	ND	ND	0.39-3.12	ND	ND	2.5 - > 20	ND	ND
C. lusitaniae	(01)	0.012	ND	ND	1.56	ND	ND	1.25	ND	ND
C. glabrata	(01)	0.048	ND	ND	1.56	ND	ND	> 20	ND	ND

ND- not determined

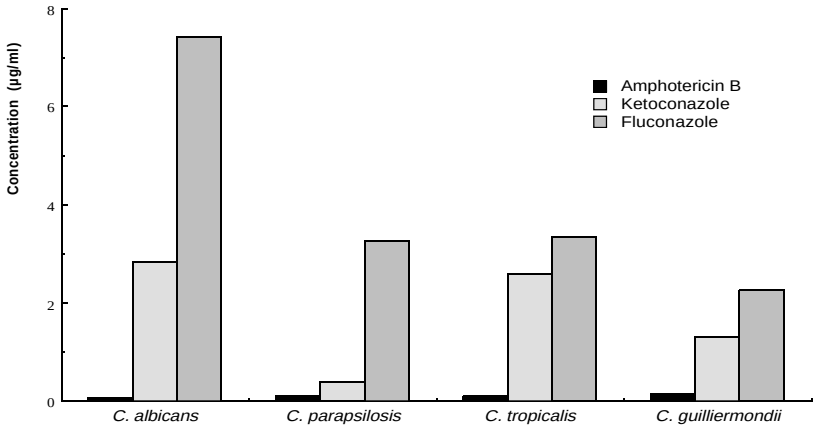

Figure 1. Distribution of MIC_{90} values of amphotericin B, ketoconazole and fluconazole for 130 strains of *Candida* isolated forma clinical specimens.

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