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Short Communication

In Vitro Susceptibility of *Candida* Species Isolated from Blood Culture to Some Antifungal Agents

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SUMMARY: Fungal infections are among the major causes of morbidity in cancer patients. In order to optimize the treatment of such patients, it is critical to determine the type of fungus causing infection as well as its susceptibility to antifungals. This study was undertaken to the study resistance of *Candida* spp. isolated from blood cultures of cancer patients to ketoconazole (KET), fluconazole (FLU), amphotericin B (AmpB), and flucytosine (FCU). A modified NCCLS M 27-A method was used to evaluate the activity of the species. Of the 56 *Candida albicans* isolates, 7 (12.5%) were resistant to FLU (MIC ≥ 64 μg/ml), 6 (10.7%) were resistant to KET (MIC ≥ 64 μg/ml) and 3 (5.3%) were resistant to FCU (MIC ≥ 32 μg/ml). One (14.3%) of 7 *C. parapsilosis* isolates was resistant to FLU (MIC ≥ 64 μg/ml). One (33.3%) of 3 *C. tropicalis* isolates was resistant to KET (MIC ≥ 64 μg/ml). None of the *C. guilliermondii* or *C. pelliculosa* isolates was resistant to KET, FLU, AmpB, or FCU. Based on these results, AmpB is an effective antifungal agent that can be used against all *Candida* isolates.

Candida bloodstream infections are important causes of morbidity and mortality in immunosuppressed cancer patients. *Candida* spp. are currently the most common pathogens isolated from blood cultures worldwide. *C. albicans* fungaemia is responsible for 25 to 60% of the total mortality from *Candida* bloodstream infection in cancer patients after major surgery. In addition, non-*C. albicans* spp. are responsible for 35 to 65% of all of the candidaemias occurring in cancer patients. In most cases, immunosuppressed cancer patients are infected with opportunistic fungi, and this fact has led to an increase in the use of antifungal agents, which in turn has resulted in the occurrence of resistant isolates (1,2). Antifungal sensitivity is critical in the treatment due to the fact that both *albicans* and non-*C. albicans* spp. are developing an increasing resistance against antifungal agents.

In the present study, blood samples obtained from cancer patients were cultured using BACTEC fungal medium (Becton-Dickinson Microbiology Systems, Cockeysville, Md., USA), and tested daily using a BACTEC 9240 system (BD Biosciences, Sparks, Md.,USA) as described by Özçelik et al. (3). Stock solutions were dissolved in dimethylsulphoxide (ketoconazole [KET], amphotericin B [AmpB]) and in water (fluconazole [FLU], flucytosine [FCU]). All *Candida* isolates were subcultured on Sabouraud Dextrose agar plates prior to antifungal susceptibility testing. Culture suspensions were prepared according to the guidelines of NCCLS M27-A (4). The inoculum suspension was prepared using the spectrophotometric method of inoculum preparation, and the final culture suspension was 2.5×10^3 cfu/ml. Broth microdilution testing was performed in accordance with the guidelines in NCCLS document M27-A. The final concentrations of all antifungal agents were 62.5 to 0.03 μg/ml. A 100 μl yeast inoculum was added to each well of the microdilution trays. The trays were incubated at 35°C in a humid chamber, and MIC endpoints were read after 48 h of incubation.

As shown in Table 1, the MIC values of the *Candida* isolates were as follows (μg/ml); KET ≤ 0.0312 - 64; FLU ≤ 0.0312 - 64; AmpB ≤ 0.0312 - 2; and FCU ≤ 0.0312 - 64. All *Candida* isolates were highly susceptible to AmpB (MIC < 1 μg/ml); followed by FCU (MIC ≤ 4 μg/ml), KET (MIC ≤ 8 μg/ml), and FLU (MIC ≤ 8 μg/ml).

Antifungal sensitivity tests are important to make a decision about treatment, because both albicans and non-*C. albicans* are resistant against antifungal agents. In addition, determination of the species of *Candida* is of increasing importance for understanding the antifungal resistance, the prognosis, and the appropriate management. *C. albicans* and non-*C. albicans* infections account for most (15 to 70%) of the infections occurring in cancer patients (5). Ayhan et al. (6) reported that 69% of cancer patients in Turkish hospitals were infected with *Candida* spp. In our study, *C. albicans* accounted for 81% of the total number of *Candida* spp. isolated, whereas, non-*C. albicans* spp. were less frequent (1.5 - 10.1%). These results are in agreement with those of Kovacicova et al. (7), who found that *C. albicans* was the most common isolate in the bloodstream of cancer patients. Similarly, *C. parapsilosis* was the second most common isolated pathogen from blood cultures (8). AmpB is a polyene antifungal with a fungicidal action. Antifungal agents with azole are primarily fungistatic. AmpB is indicated for disseminated candidosis, cryptococcosis, coccidioidomycosis, histoplasmosis, aspergillosis, mucormycosis, and sporotrichosis (9). In our study, all of the isolates were most susceptible to ampB (MIC < 1 μg/ml); followed by FCU (MIC ≤ 4 μg/ml), KET (MIC ≤ 8 μg/ml), and FLU (MIC ≤ 8 μg/ml) (Table 1). Despite this, the use of maximal doses of ampB may be needed against some *Candida* spp. (10). On the other hand, although azole antifungal agents have been shown

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Table 1. Minimum inhibitory concentrations (MICs) range of *Candida* spp. to tested antifungal agents

		KET	FLU	AmpB	FCU
		≤ 0.0312 - 64	≤ 0.0312 - 64	≤ 0.0312 - 2	≤ 0.0312 - 64
C. albicans (56)	S	≤ 0.125 - 4 (28)	≤ 1 - 8 (29)	≤ 0.0123 - 1 (48)	≤ 0.0312 (50)
	I	16 - 32 (22)	16 - 32 (20)	2 (8)	16 (3)
	R	≥ 64 (6)	≥ 64 (7)	–	32 - 64 (3)
C. parapsilosis (7)	S	≤ 0.125 - 4 (7)	8 (1)	1 (6)	≤ 0.0312 (7)
	I	–	16 - 32 (5)	2 (1)	–
	R	–	≥ 64 (1)	–	–
C. tropicalis (3)	S	≤ 0.0312 (2)	2 - 4 (2)	≤ 0.125 - 0.25 (3)	≤ 4 (3)
	I	–	16 (1)	–	–
	R	≥ 64 (1)	–	–	–
C. guilliermondii (2)	S	–	–	≤ 0.0312 (2)	≤ 4 (2)
	I	16 (2)	8 - 32 (2)	–	–
	R	–	–	–	–
C. pelliculosa (1)	S	2 (1)	–	≤ 0.0312 (1)	≤ 0.0312 (1)
	I	–	32 (1)	–	–
	R	–	–	–	–
C. parapsilosis ATCC 22019		≤ 0.03	≤ 0.03	≤ 0.03	≤ 0.03
C. krusei ATTC 6258		≤ 0.03	1.95	≤ 0.03	≤ 0.03

KET, Ketoconazole; FLU, Fluconazole; AmpB, Amphotericin B; FCU, Flucytosine.
S, Susceptible; I, Intermediate susceptibility; R, Resistant.

to be effective against invasive candidiasis in immunocompromised hosts, the emerging resistance to these agents gives rise to concerns about their future clinical usefulness. In addition, non-*C. albicans* spp. such as *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei* have been associated with a decrease in antifungal susceptibility in immunosuppressed patients (3,7,8,11). In the present study, *C. albicans* isolates were resistance to FLU, KET, and FCU. Similar results were also observed by Kovacicova et al. (7), who showed resistance to these antifungal agents in cancer patients. All *C. parapsilosis* isolates were sensitive to all antifungal agents, with the exception that one isolate was resistant to FLU. In contrast, Yang et al. (11) reported that none of the *C. parapsilosis* isolates collected from a hospital in Taiwan was resistant to FLU. *C. tropicalis* was initially regarded as a species susceptible to FLU and AmpB, displaying decreased susceptibility to KET (5). Also in the present study, only one *C. tropicalis* isolate was resistant to KET, and *C. guilliermondii* and *C. pelliculosa* showed no resistance against any of the antifungal agents used. Özçelik et al. (3) also reported that *C. guilliermondii* is not resistant to antifungal agents. On the other hand, Pfaller et al. (12) reported that one *C. guilliermondii* isolate was resistant to FLU.

In conclusion, fungal infections are among the major causes of morbidity in cancer patients. It is critical to know the type of fungus causing infection as well as its susceptibility to antifungals in order to optimize the treatment of the patients. AmpB has generally been the drug of choice to treat fungal infections in cancer patients, despite that fact that KET and FLU, which are equally effective, are less toxic.

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