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Epidemiological Report

Isolation and Characterization of *Candida* Spp. in Jordanian Cancer Patients: Prevalence, Pathogenic Determinants, and Antifungal Sensitivity

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SUMMARY: The presence of *Candida* spp. in the oral cavity was evaluated in 95 cancer patients (57 in-patients and 38 out-patients) and in 65 healthcare workers in Amman, Jordan. *Candida* carriage occurred in 72.6% of cancer patients and 33.8% of healthcare workers, with *Candida albicans* being the species most commonly recovered, followed by *C. glabrata*. In-patients were found to harbor *Candida* spp. at significantly higher levels than out-patients (P = 0.0044). The number of adhered *C. albicans* cells and the secretion of extracellular proteinase was significantly higher in the in-patient group than in the out-patient group (P = 0.0016 and 0.00007, respectively); this significant difference was not observed regarding phospholipase secretion. Antifungal sensitivity testing data suggest that isolates were most sensitive to amphotericin B and nystatin, and least sensitive to miconazole and fluconazole, which are commonly used antifungal agents in Jordan.

INTRODUCTION

Oropharyngeal candidiasis is a common infection in cancer patients and currently ranks as the most common human fungal disease. *Candida albicans*, the major human pathogen of the genus *Candida*, is a commensal yeast of the oral, gastrointestinal, and vaginal mucosa in healthy individuals and seems to be almost universally present. The use of broad-spectrum antibiotics, steroids, or other immunosuppressive agents; diabetes mellitus; AIDS; cancer chemotherapy and radiotherapy; and organ transplantation can increase the risk for opportunistic bacterial as well as fungal infections. *C. albicans*, *C. glabrata*, and *C. tropicalis* represent more than 80% of isolates from clinical infections (1). However, other species of the genus *Candida*, such as *C. parapsilosis*, *C. krusei*, *C. lusitaniae*, *C. stellatoidea*, *C. guilliermondii*, and *C. dubliniensis*, have been shown to increase the incidence of mycoses (1).

The prevalence of oral yeast carriage in various countries varies among studies according to location, age of the patients, and the site sample, and has been reported to range from 20-75% (1) without any symptoms. The incidence of *C. albicans* isolated from the oral cavity has been reported to be 45% in neonates (2), 45-65% in healthy children (3), 30-45% in healthy adults (4), 50-65% in people with removable dentures (5), 65-88% in those residing in acute and long term care facilities (6), 90% in patients with acute leukemia undergoing chemotherapy (7), and 95% in patients with HIV (8). In immunocompromised patients, infection can spread through the bloodstream or upper gastrointestinal tract, leading to severe infection with significant morbidity and mortality (1).

A number of putative virulence factors have been suggested in the enhancement of *C. albicans* pathogenesis. These include yeast-to-hyphal form transition, phenotype switching, molecular mimicry, adhesion factors or surface hydrophobicity, secretion of phospholipase and aspartyl proteinase (Sap) (9-13). Antifungal prophylaxis was indicated in the prevention of colonization and multiplication of *Candida* spp. in patients susceptible to primary and recurrent infections (14).

A routine oral examination of cancer patients has revealed a greater incidence of *Candida* infections than that in most other types of patients. Almost all surveys on fungal infections in cancer patients come from the United States, Europe, and other developed countries, and little is known about this problem in developing countries. For the first time, we report on the prevalence of *Candida* spp. in cancer patients in Jordan. The contribution of some putative virulence factors of *Candida* spp. and in vitro susceptibility to a number of antifungal agents were also evaluated.

MATERIALS AND METHODS

Patients: This study was conducted over a period of 9 months at the King Hussein Cancer Center and Al-Bashir Hospital in Amman (population about 1.5 million), Jordan. Ninety-five cancer patients (38 out-patients and 57 in-patients), undergoing radiotherapy, chemotherapy, and/or surgery, participated in this study. Patients were examined for signs or symptoms of oral candidiasis at baseline, independent of any signs or symptoms of oral candidiasis. The median age was 45.6 years and the mean age 41.4 years. Males represented 42.1% of patients studied, females 57.9%. In addition, 65 healthcare workers caring for the patients and administrative and hospital staff members were included as controls for comparison purposes. The two groups were matched for age and sex. Informed consent to participate was obtained from all patients and healthcare workers. The following clinical history was obtained for each patient and healthcare worker: age, sex, underlying diseases, prior surgical procedures, recent history of fungal infection, and antimycotic treatment. Only those patients and controls who had not used antimycotic agents in the previous 4 weeks were included.

Collection of samples: Samples were taken from two sites within the oral cavity, the gingival sulci and the pharyngeal

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portion, by using a swabbing method reported previously (15). A sterile cotton swab (Nippon Menbo, Tokyo, Japan) was immediately inoculated into a brain heart infusion broth (Oxoid, Basingstoke, UK) containing 50 μg/ml chloramphenicol (Sigma, St. Louis, Miss., USA). Tubes were incubated at 37°C for up to 48 h when streaking into Sabouraud dextrose agar (SDA) (Difco Laboratories, Detroit, Mich., USA) was performed to obtain isolated colonies. All plates were incubated at 35°C for 24 - 48 h, and yeast-like colonies were isolated. All isolates were grown on SDA slants and stored at 4°C.

Identification of isolates: The yeast-like colonies were identified by classical methods (5,16) using the following tests: formation of germinative tubes, study of micromorphology, assimilation of carbon and nitrogen sources, fermentation, and urea hydrolysis. The differential medium CHROMagar Candida (CHROMagar, Paris, France) was used to confirm the result by colony morphology and pigmentation according to the manufacturer's instructions and as has been previously described (17).

Phospholipase assay: Phospholipase secretion from various isolates of *C. albicans*, as well as from *C. albicans* ATCC 36082, kindly provided by Dr. M.A.Ghannoum (Center for Medical Mycology, Mycology Reference Laboratory, University Hospital of Cleveland, Ohio, USA), was used as a control strain and measured as described previously (9). In brief, the isolates were cultured for 18 h at 30°C in Sabouraud broth containing 2% (wt/vol) glucose in an orbital shaker. Cells were harvested by centrifugation (5,000 g, 30 min), washed twice with phosphate-buffered saline (PBS), and resuspended at a density of 1×10^8 cells^{-1}. Ten microliters of the yeast suspensions were spotted onto SDA plates (6.5% SDA, 5.84% [wt/vol] NaCl, 0.55% [wt/vol] $CaCl_2$, supplemented with 8% [vol/vol] egg yolk emulsion [Oxoid]), and the diameter of the precipitation zone around each colony was measured after incubation of the plates at 37°C for 72 h. Each assay was carried out in triplicate, and the results were expressed as the triplicate mean. Phospholipase activity (Pz) was determined as described previously (9).

Proteinase assay: Proteinase production was evaluated by the method of Staib (18). Three flasks per isolate, each containing 50 ml of Sabouraud broth, were incubated at 26°C in an orbital shaker for 24 h. Cells were harvested by centrifugation (5,000 g, 30 min), washed with PBS, and resuspended at a density of 1×10^8 cells^{-1}. Ten microliters of the cell suspension were placed onto test medium (1% bactoagar [Difco], 0.1% [wt/vol] KH_2PO_4, 0.5% [wt/vol] $MgSO_4$, and 1% [wt/vol] glucose, pH 5.0) containing 0.16 (wt/vol) bovine serum albumin (Sigma) as the sole nitrogen source. After 5 days of incubation at 37°C, the plates were fixed with 20% trichloroacetic acid and stained with 1.25% amido black (90% methanol, 10% acetic acid). Destaining was performed with 15% acetic acid, and the clear zones around each colony were measured and used in the determination of the Pz value as described previously (9). Each assay was carried out in triplicate on two separate occasions, and results were expressed as the triplicate mean.

Adherence assay: Overnight culture of *Candida* spp. was conducted at 37°C in a 0.67% (wt/vol) yeast nitrogen base (YNB) (Difco) supplemented with 2.5% (wt/vol) glucose. Flasks containing 50 ml of the same medium were inoculated with 1 ml of the overnight culture and grown for 24 h in a shaking water bath (180 rpm) at 37°C. The cells were harvested by centrifugation (1,200 g, 10 min) and washed twice with sterile PBS by repeated centrifugation.

Buccal epithelial cells (BECs) were collected from healthy human volunteers by gentle rubbing of the mucosal surface of the cheeks with a sterile tongue depressor. BECs were washed twice with sterile PBS and collected by centrifugation (500g, 10 min). Adherence assays were performed as previously described (19). Yeast cells were suspended in sterile PBS at 1×10^7 cells^{-1}; BEC were suspended in a sterile PBS to a concentration of 2×10^5 cells^{-1}. Two milliliters of the yeast suspension was mixed with 2 ml of the BEC suspension in a sterile screw-capped bottle. The mixture was shaken at 37°C for 2 h then filtered through a 20 μm filter (Retsch, Idar-Oberstein, Haan, Germany) to remove non-adherent yeast cells. The BECs on the filter were washed twice with 5 ml volumes of sterile PBS and finally suspended in 5 ml of the same buffer. A drop of this suspension was mounted on a glass slide, then air-dried, heat-fixed, and stained with crystal violet for 1 min. The adherence was determined microscopically by counting the mean number of yeast cells adhering to every 100 BECs. Each assay was carried out in duplicate, and Student's *t* test was used to evaluate the difference in the adherence values; a P value of <0.05 was considered significant.

In vitro antifungal susceptibility testing: Minimum inhibitory concentration (MIC) was measured by the broth microdilution method according to NCCLS guidelines (20). A serial dilution was made from the stock solutions of the antifungal agents to have final concentration ranges of 0.03 to 16 μg ml^{-1} for amphotericin B, ketoconazole, and miconazole; 0.125 to 64 μg ml^{-1} for fluconazole; and 0.7 to 18.5 μg ml^{-1} for nystatin (ICN Biomedicals Inc., Aurora, Ohio, USA). A hundred microliters of each concentration was dispensed into a labeled culture tube and kept in the freezer until use. The prepared inocula of clinical isolates of *Candida* spp. were incubated with the different drug concentrations at 35°C in an air-ambient incubator with positive (drug-free tube) and negative (broth tube) control. Tubes were read after 24 to 48 h. The MICs of amphotericin B and nystatin were defined as the lowest concentration at which there was 100% inhibition of growth. The MICs of ketoconazole, miconazole, and fluconazole were defined as the lowest concentrations at which there was 80% inhibition of growth (i.e., slightly hazy) compared with that in the drug-free controls.

Aliquot from each isolate showing inhibition of visible growth was inoculated on the surface of an SDA plate using a multipoint inoculator (Denley Instruments, West Sussex, UK). Inoculated plates were then incubated at 37°C for 24 - 48 h, and which conditions the lowest concentration of each antifungal tested for which the subculture was negative is the minimum fungicidal concentration (MFC) of the respective drug.

Statistical analysis: Statistically significant differences between the means of phospholipase and acid proteinase production, respectively, and the adherence of *Candida* spp. were determined by conducting Student's *t* tests using statistical analysis software (STATISTICA for Windows, version 5.0, StatSoft Inc., Oklahoma City, Okla., USA). Data are expressed as the means ± SE, and $P < 0.05$ was considered statistically significant.

RESULTS

Prevalence of *Candida* spp.: Yeasts were recovered

Table 1. *Candida* isolates from cancer patients (in- and out-patients) and healthcare workers

Patients	Number of individuals screened	Number (%) of positive *Candida*	*Candida* spp.			
			C. albicans no. (%)	*C. glabrata* no. (%)	*C. parapsilosis* no. (%)	*C. tropicalis* no. (%)
In-patients	57	49 (85.9)	22 (38.6)	15 (26.3)	8 (14.0)	4 (7.0)
Out-patients	38	20 (52.6)	8 (21.0)	7 (18.4)	4 (10.5)	1 (2.6)
Total	95	69 (72.6)	30 (31.5)	22 (23.2)	12 (12.6)	5 (5.3)
Healthcare workers	65	22 (33.8)	18 (27.7)	4 (6.2)	0 (0)	0 (0)

during the 9-month period of the study, and the numbers of patients from whom they were respectively isolated are shown in Table 1. There was no statistically significant difference between the total numbers of *Candida* spp. isolates obtained from the gingival or pharyngeal portions of the out-patients ($P > 0.551$) and those of in-patients ($P > 0.639$) or healthcare workers ($P > 0.364$). However, *Candida* spp. were detected more frequently in the pharyngeal portion than in the gingival one. The prevalence of positive yeast carriage in cancer patients was significantly higher for both in- and out-patients with cancer than for healthcare workers (72.6% as compared with 33.8%). *C. albicans* was the most frequent isolate in all the groups studied, accounting for 38.6%, 21.0%, and 27.7% of the yeast from in-patients, out-patients, and healthcare workers, respectively. *C. glabrata* was the second in prevalence, accounting for 26.3% and 18.4% in in- and out-patients, and 6.2% in healthcare workers. *C. parapsilosis* and *C. tropicalis* were isolated from cancer patients only.

Figure 1 shows the percentages of *Candida* spp. from patients with hematological and solid organ cancer. *C. glabrata* and *C. parapsilosis* rates were significantly higher in patients with hematological cancer than in solid organ cancer patients ($P = 0.0310$ and 0.00001, respectively). On the other hand, there was no statistically significant difference between the percentages of *C. albicans* ($P = 0.5812$) and *C. tropicalis* ($P = 0.3737$) obtained from hematological

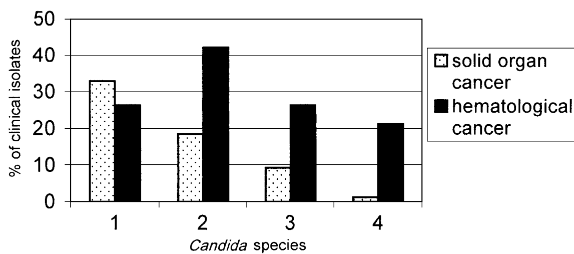

Fig. 1. The percentage of *Candida* spp. from patients with hematological cancer and solid organ cancer. 1, *C. albicans*; 2, *C. glabrata*; 3, *C. parapsilosis*; 4, *C. tropicalis*.

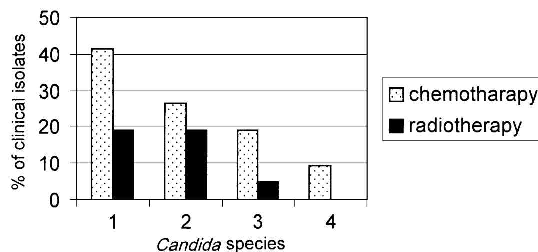

Fig. 2. The percentage of *Candida* spp. from patients receiving chemotherapy or radiotherapy.1, *C. albicans*; 2, *C. glabrata*; 3, *C. parapsilosis*; 4, *C. tropicalis*.

and solid organ cancer patients. Cancer patients were receiving chemotherapy (55.8%) or radiotherapy (44.2%) as a therapeutic solution for their underlying tumors. Chemotherapy patients were colonized more frequently by all types of *Candida* spp. ($P = 0.0189$, 0.0032, and <0.00001, respectively, for *C. albicans*, *C. parapsilosis*, and *C. tropicalis*) except for *C. glabrata* ($P = 0.3978$) than patients treated with radiation (Fig. 2).

Extracellular enzyme production: All the tested *C. albicans* isolates obtained from in- and out-patients with cancer were able to secrete proteinase and phospholipase (Table 2). The mean proteinase production (Pz) of 22 *C. albicans* isolates obtained from in-patients was 2.97 mm, whereas the mean proteinase production of 8 isolates obtained from out-patients was 1.71 mm. Acid proteinase production by the ATCC 36082 *C. albicans* control strain was 2.1 mm. As shown in Table 2, the Student's *t* test clearly indicated a statistically significant difference between the amount of proteinase secreted by *C. albicans* isolates obtained from in-patients and isolates obtained from out-patients ($P = 0.00007$).

The mean phospholipase production (Pz) of *C. albicans* isolates obtained from in-patients was 1.35 mm, whereas the mean phospholipase production of isolates obtained from out-patients was 1.5 mm. Overall, the level of phospholipase activities of all *C. albicans* isolates was consistently lower than that produced by the ATCC 36082 *C. albicans* control strain (Pz = 4.1mm). As shown in Table 2, no statistically significant differences were found among the 22 *C. albicans* isolates obtained from in-patients and the 8 isolates obtained from out-patients in terms of mean phospholipase production ($P = 0.471$).

Adherence of various isolates to BECs in vitro: The adherence ability of *Candida* spp. obtained from in- and out-patients with cancer as well as that of ATCC control *Candida* spp. was determined. The adherences of *C. albicans* ATCC 36082, *C. glabrata* ATCC 22553, and *C. parapsilosis* ATCC 22019 to human BECs were 484 ± 16, 240 ± 23, and 298 ± 29, respectively. There was a statistically significant difference between the mean number of adhered *C. albicans* isolates obtained from out-patients and that obtained from

Table 2. Extracellular secretion of proteinase and phospholipase by *C. albicans* isolates obtained from cancer patients

Patients	Total number of *C. albicans* isoltes tested	Proteinase Pz[1] ± SE[2]	Phospholipase Pz[1] ± SE[2]
In- patients	22	2.97 ± 0.15	1.35 ± 0.16
Out- patients	8	1.71 ± 0.20	1.5 ± 0.17
P value	30	> 0.00007	> 0.471

[1]: Pz = (A/B)−1; A, diameter of colony + zone diameter; B, diameter of colony.

[2]: SE, standard error.

Table 3. Adherence of *Candida* spp. to human buccal epithelial cells (BECs) in vitro

Isolate	Total number of isolates tested	Mean number of yeast cells adhere to 100 BEC ± SE[1)]	*P* value
C. albicans			
In-patients	22	549 ± 24	< 0.0016
Out-patients	8	365 ± 31	
C. glabrata			
In-patients	15	297 ± 23	> 0.916
Out-patients	7	301 ± 26	
C. parapsilosis			
In-patients	8	233 ± 32	> 0.496
Out-patients	4	200 ± 32	

[1)]: SE, standard error

in-patients (P = 0.0016) (Table 3). On the other hand, no statistically significant difference was observed for the adherence of *C. glabrata* and *C. parapsilosis* obtained from out- and in-patients with cancer (P > 0.916 and > 0.496, respectively).

By comparison of the adherences of the three species of *Candida* regardless of the group they were isolated from, the adherence of *C. albicans* was shown to be highest. *C. glabrata* adherence was lower than that of *C. albicans* but higher than that of *C. parapsilosis* (Table 3). Student's *t* test was carried out to determine the significant differences between the mean numbers of adhered cells. The test clearly indicated a statistically significant difference between the adherence of *C. albicans* and those of *C. glabrata* and *C. parapsilosis* (P < 0.0002 and < 0.0000002, respectively). The difference between the adherence of *C. glabrata* and *C. parapsilosis* was also statistically significant (P < 0.042).

Antifungal susceptibility: MIC and MFC ranges of amphotericin B, nystatin, fluconazole, miconazole, and ketoconazole for *Candida* isolates representing out- and in-patients with cancer are shown in Table 4. For *C. albicans* isolates, the MIC values range from 0.25 to 4.6 μg ml^{-1} for polyenes and from 0.25 to 8 μg ml^{-1} for azole antifungals. Similarly, the MIC for *C. glabrata* range from 0.06 to 2.3 μg ml^{-1} for polyenes and from 0.25 to 8 μg ml^{-1} for the azole group. However, *C. parapsilosis* was found to be the least sensitive; its isolates showed a range from 0.125 to 4.6 μg ml^{-1} for polyenes and from 0.25 to 2 μg ml^{-1} for the azole group. Fluconazole was the least active agent against *C. albicans* (1 - 8 μg ml^{-1}) and *C. glabrata* (2 - 8 μg ml^{-1}), although it was more effective against *C.albicans* (most species inhibited at 4 μg ml^{-1}) than against *C. glabrata* (most species inhibited at 8 μg ml^{-1}). For *C. parapsilosis*, nystatin was the least active (range 1.15 to 4.6 μg ml^{-1}).

By comparison of the MFC values to the five antifungals tested against the clinical isolates of *Candida* spp., all the tested species were killed at concentrations equal to or higher than the upper limits of the MIC ranges of the respective drugs (Table 4). All three *Candida* spp. had closely related MFCs values except for *C. glabrata* (MFC range 32 - 64 μg ml^{-1} for fluconazole), revealing the high resistance of *C. glabrata* to fluconazole.

Table 4. Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of the five antifungal agents for the different *Candida* spp.

Antifungal agents	Description (μg/ml)	*C. albicans*	*C. glabrata*	*C. parapsilosis*
Amphotericin B	MIC	0.25-2	0.06-0.25	0.125-1
	MFC	4-16	4-8	4-16
Nystatin	MIC	0.29-4.6	0.58-2.3	1.15-4.6
	MFC	4.6-9.25	4.6-18.5	2.3-18.5
Fluconazole	MIC	1-8	2-8	0.5-2
	MFC	8-16	32-64	8-32
Miconazole	MIC	0.5-4	0.25-2	0.25-2
	MFC	4-16	8-16	8-16
Ketoconazole	MIC	0.25-1	1-2	0.5-2
	MFC	4-16	4-16	2-8
Total number of isolates tested		48	26	12

DISCUSSION

Results obtained in this study establish several points pertinent to the prevalence of oral candidiasis in Jordanian cancer patients. Consistent with published data (21,22), oral *Candida* carriage was common in cancer patients. Four *Candida* spp. (*C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*) were isolated from the oral cavity of cancer patients, whereas only two species (*C. albicans* and *C. glabrata*) were isolated from the oral cavity of healthcare workers, a finding consistent with that noted by other investigators (23,24). There were many alterations in the frequency and distribution of *Candida* spp. among cancer patients, which may be ascribed, in part, to subject-based differences, the multitude of predisposing factors prevailing in cancer patients, the method used to obtain samples, and the site sampled within the oral cavity.

Previous work has indicated that fungal infections are more frequent in hematological malignancy patients than that in patients with solid tumors (25-28). This study clearly demonstrates that there is indeed a higher prevalence of *C. glabrata* (P = 0.031) and *C. parapsilosis* (P < 0.00001) species in hematological cancer patients compared with that in patients with solid tumors. Although the predominance of *C. glabrata* and *C. parapsilosis* in patients with hematological malignancies cannot be readily explained, it must be noted that fluconazole is the predominant medication utilized to treat fungal infections in patients receiving head and neck radiation (22). Hence, the use of fluconazole, which is strongly associated with changes in the *Candida* spp. isolated from the blood and vagina (23,27,28), may play a role in this regard.

It is well established that the number and diversity of yeast infections increase in patients irradiated for malignant lesions of the head and neck (22) and in patients receiving chemotherapy (24-26). Data obtained in this study demonstrate that, with the exception of *C. glabrata*, patients treated with chemotherapy harbor more *Candida* spp. than do those treated with radiotherapy. The variable range of side effects associated with these two therapeutic approaches may be responsible for this noted difference.

Adherence of *C. albicans* isolates obtained from in-patients to human BECs was significantly higher than that of isolates obtained from out-patients (P < 0.0016). This implies that in-patients harbor more virulent *C. albicans*, but not *C. glabrata* or *C. parapsilosis*, than do out-patients (29,30). The reported loss of adhesion molecules like B2 integrin from *C. glabrata* and *C. parapsilosis* (30) may hinder their ability to adhere to human BECs. All *C. albicans* isolates tested in this study, irrespective of their source, were capable of secreting phospholipase. Previous work has reported that blood isolates

of *C. albicans* produce higher extracellular phospholipase levels than do commensal isolates (12). Additionally, isolates with high extracellular phospholipase activity are invasive in the newborn mouse candidiasis model, whereas those with low extracellular phospholipase activity are not invasive (12). Proteinase production is believed to enhance the ability of *C. albicans* to colonize and penetrate host tissues and to evade the host immune system (31). The presence of a correlation between the level of proteinase activity in clinical *C. albicans* isolates (9) or *C. albicans* laboratory strains with altered proteinase levels, and the virulence of *C. albicans*, supports the contention that this enzyme may have some role in the degree of virulence of *C. albicans* (9,11,31).

Antifungal drug resistance of *Candida* spp. continues to increase in response to the widespread application of triazole therapeutics in treatment of immunosuppressed patients. MIC and MFC data for the two polyenes (amphotericin B and nystatin) and for the three azoles (fluconazole, miconazole, and ketoconazole) are in general agreement with previously reported data (32-34). Isolates tested were susceptible to amphotericin B, nystatin, miconazole, ketoconazole, and fluconazole, and *C. albicans* was more susceptible to azoles than was *C. glabrata*. These results are similar to those noted in other studies conducted in close geographical regions. In Lebanon (35), for example, few *C. albicans* isolates resistant to fluconazole (6%) were noted, and no resistance against amphotericin B was noted. On the other hand, a surveillance program (SENTRY) of bloodstream infections in the United States, Canada, Latin America, and Europe (1997 through 1999) has shown that isolates of *C. albicans*, *C. parapsilosis*, and *C. tropicalis* are all highly susceptible to fluconazole (MICs ≥ 8 μg ml^{-1}) (33,36). Moreover, Bille et al. (37) reported that 97% of 2,634 *C. albicans* isolates and 83.4% of non-*C. albicans* isolates were susceptible to fluconazole.

In conclusion, the frequent occurrence of *Candida* spp. in the oral cavity of cancer patients indicates a need for effective management of the infection prior to any anti-tumor treatment, as severe complications can otherwise result. Although our findings were obtained from a relatively small number of patients with severe underlying disease, they suggest that people receiving prophylactic or therapeutic antifungal drugs have to be carefully monitored to prevent and control the emergence of fungal isolates insensitive to available antifungals. Our findings also indicate that variations in phospholipase and proteinase activity as well as adherence properties may differentially contribute to the pathogenicity of *Candida* spp.

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