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Colonization of Human Immunodeficiency Virus-Infected Outpatients in Taiwan with *Candida* Species

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To understand the *Candida* colonization of human immunodeficiency virus (HIV)-infected outpatients in Taiwan, we have conducted a prospective cohort study of *Candida* colonization and its risk factors at the National Taiwan University Hospital from 1999 to 2002. More than 50% of the patients were colonized with *Candida* species, and 12% developed symptomatic candidiasis. Patients colonized with fluconazole-resistant strains of *Candida* species had a higher prevalence of candidiasis than those colonized with susceptible strains. Our analysis found that antibiotic treatment and lower $CD4^+$ counts (<200 cells/mm^3) increased the rate of oropharyngeal candidiasis in HIV-infected patients, while antiretroviral therapy protected patients from the development of candidiasis.

Mucosal candidiasis, including oropharyngeal, esophageal, and vaginal candidiasis, is common among human immunodeficiency virus (HIV)-infected patients (4, 11). In particular, oropharyngeal candidiasis occurs in up to 90% of patients during the course of HIV infection (17). Progressive cell-mediated immunodeficiency, with $CD4^+$ lymphocyte counts less than 200 cells/mm^3, is a risk factor for colonization with *Candida* species and the development of candidiasis (3). The widespread use of azole antifungal agents for the treatment of mucosal candidiasis results in colonization with less susceptible organisms and the development of resistance (4, 15). Thus, oropharyngeal candidiasis due to drug-resistant fungi is an emerging problem for patients infected with HIV (18).

The overall prevalence of known HIV infection in Taiwan remains relatively low (0.01%) (9). As in most other industrialized countries, the majority of HIV-infected patients in Taiwan receive care in the outpatient setting. Therefore, to better understand the epidemiology of *Candida* species carriage among HIV-infected outpatients in Taiwan, we undertook a study to determine the prevalence of oropharyngeal colonization. Our objectives were to assess the colonization status and the risk factors for colonization and the development of candidiasis in HIV-infected outpatients in Taiwan. The susceptibilities of those *Candida* isolates to antifungal drugs were also determined.

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MATERIALS AND METHODS

Study population and data collection. HIV-infected patients were monitored regularly in the outpatient infectious diseases clinic of National Taiwan University Hospital, a major referral hospital for the management of HIV-related complications. The patients were enrolled after they provided informed verbal consent. This was a prospective study performed by the use of three surveys, conducted from May to June 1999, May to September 2001, and January to April 2002. A standardized data collection form was used to retrieve demographic information, the most recent $CD4^+$ lymphocyte count, and the highly active antiretroviral therapy (HAART) prescribed. In addition, clinical information for the previous 3 months was obtained and included information on whether the patient had a history of oral or esophageal candidiasis or hospitalization and the antibacterial and antifungal drugs received.

Sampling and microbiologic processing. Oropharyngeal swab specimens for culture were obtained from all patients by using a dry sponge swab (EZ Culturette; Becton Dickinson, Sparks, Md.). All swabs were maintained at room temperature and were transported to the laboratory within 24 h. They were then plated on solid medium within 12 h of arrival. The swabs collected in 1999 were plated on Sabouraud dextrose with chloramphenicol and gentamicin (BBL), and those collected in 2001 and 2002 were plated on Chromagar Candida (BBL). All plates were incubated at 30°C. Three independent colonies were selected from each positive culture. Additional colonies were selected from cultures with more than one morphotype. All isolates were first subjected to the germ tube assay. For germ tube assay-positive isolates, a temperature sensitivity assay was performed to differentiate *Candida albicans* from *Candida dubliniensis* (growth

TABLE 1. Numbers of patients in three surveys

Characteristic[a]	No. of patients enrolled in:							
	1999	2001	2002	1999 and 2001	1999 and 2002	2001 and 2002	Any two surveys	All three surveys
Total patients	122	243	276	9	10	108	127	51
All positive	69	130	137	2	2	32	36	11
All negative	53	113	139	1	2	29	32	11
Positive once				6	4	47	59	13
Positive twice								16

[a] Positive, positive for yeast by culture; negative, negative for yeast by culture.

TABLE 2. Distribution of *Candida* species

Species	No. of isolates collected				No. of isolates tested			
	1999	2001	2002	Total	1999	2001	2002	Total
Candida albicans	61	111	121	293	61	91	66	218
Candida glabrata		5	5	10		5	5	10
Candida parapsilosis	3	3	4	10	3	3	4	10
Candida tropicalis		4	3	7		4	3	7
Candida lusitaniae		1	4	5		1	4	5
Candida famata	2		1	3	2		1	3
Candida guilliermondii	1	2		3	1	2		3
Candida sake			2	2			2	2
Others		3	2	5		3	1	4
Total	67	129	142	338	67	109	86	262

defect at 42°C). The VITEK yeast biochemical card (YBC; bioMerieux, St. Louis, Mo.) was used to identify those isolates that failed to form germ tubes and isolates that formed germ tubes but that failed to grow at 42°C. We used the API 32C system (bioMerieux) to assess our results when the VITEK YBC yielded results of less than 90% certainty.

Antifungal susceptibility testing. The MICs of antifungal drugs were determined by in vitro antifungal susceptibility testing according to the M27-A guidelines published in 1997 by the National Committee of Clinical Laboratory Standards (NCCLS) (10). RPMI 1640 medium (31800-022; Gibco BRL) was used for dilution and growth of the yeast culture. The growth end point of each isolate was determined with a spectrophotometer (Spectra MAX Plus; Molecular Devices Corp., Sunnyvale, Calif.). The MICs were also interpreted according to NCCLS guidelines. The amphotericin B MIC was determined, as the MIC of amphotericin B is needed to completely inhibit the growth of isolates after 48 h of incubation at 35°C. Isolates for which the amphotericin B MIC was ≥2 μg/ml were considered amphotericin B resistant.

The fluconazole MIC was defined as the concentration of fluconazole needed to reduce the turbidity to 50% after 48 h of incubation at 35°C. Isolates for which fluconazole MICs were ≥64 μg/ml, 16 to 32 μg/ml, and ≤8 μg/ml were defined as resistant, susceptible-dose dependent, and susceptible to fluconazole, respectively.

Statistical analysis. All clinical laboratory data were entered into a relational database designed in Access 97 software (Microsoft, Redland, Wash.). The chi-square test was used to study the association of factors with incident or persistent oral yeast species colonization. Risk factors for patients with oropharyngeal colonization were identified by multiple logistic regression.

RESULTS

Patients. A total of 122, 243, and 276 patients were enrolled in this study in 1999, 2001, and 2002, respectively (Table 1). The majority (91%) of the patients were men. The $CD4^+$ counts were available for 599 patients, and the average $CD4^+$ count was 279.5 cells/mm^3. Only 15.3% of the patients had $CD4^+$ counts greater than 500 cells/mm^3, while 43.9% of the patients had $CD4^+$ counts less than 200 cells/mm^3 and 13% of the patients had $CD4^+$ counts less than 50 cells/mm^3. Consequently, 84.4% of the patients were receiving HAART. A total of 35.3% of patients also received antibiotics as treatment or primary or secondary prophylaxis for opportunistic infections.

A total of 127 patients were enrolled in two of the three surveys. Of these, 32 were negative for yeasts by culture, 36 were positive for yeasts in both surveys, and 59 were positive for yeasts by culture only once. Of 51 patients who were enrolled in all three surveys, 11 patients were negative for yeasts by culture and 11 patients were positive for yeasts by culture in all three surveys. Of the remaining 29 patients, 13 patients were positive for yeasts by culture once and 16 patients were positive for yeasts by culture twice.

Distribution of yeasts. Yeast culture positivity rates in 1999, 2001, and 2002 were 56.6% (69 of 122 patients), 53.5% (130 of 243 patients), and 49.6% (137 of 276 patients), respectively. One isolate of each *Candida* species from each patient in each survey was analyzed (Table 2). *C. albicans* was the most common species and accounted for 91, 86, and 85.2% of the isolates in 1999, 2001, and 2002, respectively. A total of 338 isolates were recovered; and these consisted of 293 (86.7%) *C. albicans* isolates, 10 (3%) *Candida glabrata* isolates, 10 (3%) *Candida parapsilosis* isolates, 7 (2.1%) *Candida tropicalis* isolates, 5 (1.5%) *Candida lusitaniae* isolates, and 13 (3.7%) other isolates. Four, nine, and nine different *Candida* species were isolated from patients in the 1999, 2001, and 2002 surveys, respectively.

Antifungal susceptibilities of yeasts. The susceptibilities to antifungal agents of one *Candida* species isolate among multiple isolates collected from each patient were analyzed (Table 2). Of 262 isolates, the amphotericin B MICs for 12 (4.6%), 188 (71.8%), 61 (23.3%), and 1 (0.4%) isolates were ≤0.25, 0.5, 1, and 2 μg/ml, respectively. The only resistant isolate was a *Candida famata* isolate. A total of 244 (93.1%), 12 (4.6%), and 6 (2.3%) isolates were susceptible, susceptible-dose dependent, and resistant to fluconazole, respectively (Table 3). Of six fluconazole-resistant isolates, five were *C. albicans*. The prevalence of candidiasis was higher among patients colonized with fluconazole-resistant *Candida* species than among those colonized with fluconazole-susceptible isolates ($P < 0.05$).

On the basis of multivariate analysis, antibiotic treatment and lower $CD4^+$ counts (<200 cells/mm^3) were independent risk factors for oropharyngeal colonization among the patients. In contrast, treatment with HAART and antifungal drugs decreased the odds of oropharyngeal colonization. There were 83 episodes of candidiasis among 641 patients within the 3 months prior to the oropharyngeal swab specimen culture, and 12.3% of the patients had received antifungal therapy during that period. On the basis of the multivariate analysis, antibiotic treatment and lower $CD4^+$ counts (<200 cells/mm^3) were also independent risk factors for the development of oropharyngeal

TABLE 3. Susceptibilities of the *Candida* species to fluconazole

Yr	No. (%) of isolates for which fluconazole MIC (μg/ml) was:									
	0.125	0.25	0.5	1	2	4	8	16–32	≥64	Total
1999	13 (19.4)	38 (56.7)	3 (4.4)	2 (3)	4 (6)	2 (3)	2 (3)	2 (3)	1 (1.5)	67
2001	6 (5.5)	39 (35.8)	26 (23.8)	12 (11)	9 (8.2)	4 (3.7)	7 (6.4)	3 (2.8)	3 (2.8)	109
2002	4 (4.7)	26 (30.2)	16 (18.6)	17 (19.8)	2 (2.3)	6 (7)	6 (7)	7 (8.1)	2 (2.3)	86
Total	23 (8.8)	103 (39.3)	45 (17.2)	31 (11.8)	15 (5.7)	12 (4.6)	15 (5.7)	12 (4.6)	6 (2.3)	262

TABLE 4. Multiple regression for candidiasis versus noncandidiasis

Risk factor	Factor value	Odds ratio	95% Confidence limit	*P* value
Antiretroviral therapy	False vs true	5.249	1.815, 15.18	0.0022
Antifungal received	False vs true	0.023	0.009, 0.058	<0.0001
Antibiotics received	False vs true	0.254	0.086, 0.745	0.0125
CD4 counts	0–199 vs ≥200	6.095	2.038, 18.229	0.0012

candidiasis among the patients (Table 4). In contrast, the odds of oropharyngeal candidiasis decreased 5.2-fold for HIV-infected patients receiving antiretroviral therapy.

DISCUSSION

In this study 52.4% of HIV-infected outpatients were colonized with yeasts. This rate is slightly lower than those found in previous surveys (i.e., 60 to 63%) (1, 11). Symptomatic oral candidiasis has been reported to occur in 7 to 48% of HIV-infected patients and in 43 to 93% of patients with progressive immunodeficiency (8, 16). In the present study, 12.9% of HIV-infected patients had developed candidiasis within the 3 months before the surveys.

Fluconazole is widely used for the treatment of mucosal candidiasis, resulting in colonization with less susceptible organisms and the development of resistance among usually susceptible species, such as *C. albicans* (4, 15). *Candida krusei* was isolated once in the present study. Surprisingly, the fluconazole MIC for this species was 0.25 μg/ml, even though *C. krusei* is considered less susceptible to fluconazole than other species (12). Another interesting result was the finding that all five *C. lusitaniae* isolates were susceptible to amphotericin B, even though *C. lusitaniae* has been reported to be relatively resistant to amphotericin B (6). The overall resistance rates to amphotericin B and fluconazole were 0.4 and 2.3%, respectively, which are lower than those indicated in previous reports from Taiwan (7, 19).

Oral colonization with yeasts is known to be significantly higher among HIV-infected patients than healthy individuals (11). In addition to a reduction in the HIV load and restoration of the immune system, antiretroviral therapy may have a more intrinsic role in the elimination of *Candida* species in HIV-infected patients (2). Thus, the frequency of oropharyngeal candidiasis decreased with antiretroviral therapy in the present study, as well as in previous studies (2, 5). Our findings of a significantly increased risk of oropharyngeal colonization and candidiasis in HIV-infected patients with progressive immunodeficiency ($CD4^+$ count less than 200 cells/mm^3) is consistent with the findings described in previous reports (3, 11). In a previous report by Ohmit et al. (11), antibiotic treatment was associated with incident or persistent oral *Candida* species colonization but not oropharyngeal candidiasis. In contrast, we found that antibiotic treatment was associated not only with oropharyngeal colonization but also with candidiasis. The difference between these two studies may lie in the populations studied.

Systemic candidemia is recognized as an important, albeit uncommon, cause of mortality (14). Up to 47% of HIV-infected patients with candidemia may succumb to their infection (13). In contrast, mucosal candidiasis does not contribute to morbidity or a significant reduction in quality of life but, rather, contributes to increased medical costs for the treatment of HIV infection. We therefore recommend that HIV-infected patients who are receiving antibiotics and who have $CD4^+$ counts below 200 cells/mm^3 be carefully monitored for candidiasis.

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