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Oropharyngeal Colonization of HIV-Infected Outpatients in Taiwan by Yeast Pathogens▽

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Among 234 isolates comprising 26 different *Candida* species colonizing the oropharynx of 181 (54.3% of 399 surveyed) HIV-infected outpatients, 27 (11.7%) were fluconazole resistant. Antibacterial treatment was associated with increased rates of yeast colonization, while antiretroviral therapy and pneumococcal vaccination protected patients from yeast colonization.

In human immunodeficiency virus (HIV)-infected patients, oropharyngeal colonization by *Candida* pathogens predicts subsequent development of yeast infections (18), which may lead to systemic candidemia, a recognized major cause of mortality (15). Widespread use of azole antifungal agents for the treatment and prophylaxis of candidiasis results in colonization by less susceptible organisms and development of resistance (8, 16). Therefore, oropharyngeal candidiasis due to drug-resistant fungi has become an emerging problem for patients infected with HIV (17).

The overall prevalence of HIV infection in Taiwan increased dramatically in recent years (2). Thus, we conducted a prospective cohort study to determine the prevalence of yeast oropharyngeal colonization in HIV-infected patients who were seen regularly at the outpatient infectious disease clinic of National Taiwan University Hospital. The study was conducted between April and June 2005. Verbal informed consent was obtained beforehand, and a total of 399 patients were screened, of whom complete relevant information was available for 201 patients. A standardized data collection form was used to retrieve demographic as well as clinical information and laboratory data. Statistical analysis was performed following our previous report with modest modifications (19). The chi-square test was used to study the association to transient or persistent oropharyngeal yeast colonization. The Student *t* test was applied for continuous variables, and logistic regression was applied to assess the independent effect of factors, including employment, shelter stay, low $CD4^+$ counts ($\leq$200 cells/mm^3), and receipt of antibacterial treatment, highly active antiretroviral therapy (HAART), or a pneumococcal vaccine. A probability (*P*) of <0.05 was considered significant.

Oropharyngeal samples were obtained using dry sponge swabs (EZ Culturette; Becton Dickinson, Sparks, MD). All swabs were streaked onto CHROMagar *Candida* agar medium, and the isolates were subjected to the ID 32C system (bioMérieux, Hazelwood, MO) for species identification first. The Vitek yeast biochemical card (bioMérieux) and the sequence of the internal transcribed spacer (ITS) regions and the D1/D2 region of ribosomal DNA (11, 12) were used for further identification when ID 32C failed to reach greater than 90% confidence and when uncommon species were identified. All but four of the isolates were successfully identified to the species level.

Oropharyngeal colonization with yeasts is known to be significantly higher among HIV-infected patients than among healthy individuals, and the rate of colonization varied from 44% to 88% (1, 6, 14, 18). More than half of the HIV-infected patients (53.4%) enrolled in this study were colonized by yeast pathogens, a rate similar to that in our previous report (10). The patient characteristics are summarized in Table 1. HIV-infected patients with progressive immunodeficiency ($CD4^+$ counts $\leq$ 200 cells/mm^3) appear to have higher prevalence of yeast colonization ($P = 0.17$), a result supporting our previous finding showing that low $CD4^+$ count is a risk factor (10, 23). Furthermore, we found that high HIV viral load ($P = 0.003$) increased the rate of yeast colonization, whereas receipt of HAART ($P = 0.044$) protected these patients from yeast colonization. Thus, reduction of viral load and restoration of the immune system resulting from antiretroviral therapy eliminated *Candida* species from HIV-infected patients (7). Interestingly, pneumococcal vaccination also protected patients from yeast colonization. In addition, receipt of antibacterial therapy was highly associated with yeast oropharyngeal colonization ($P = 0.002$). This is consistent with other studies (10, 14). Based on multivariate analysis, antibacterial therapy was the only independent risk factor for oropharyngeal yeast colonization (odds ratio, 2.7; 95% confidence interval, 1.27 to 5.84). Interestingly, all six injecting (i.v.) drug users were colonized by yeasts (five by *Candida albicans* and one by *Candida dubliniensis*) even though the $CD4^+$ counts of five

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TABLE 1. Characteristics of 339 HIV-infected patients

Characteristic	Value for group			*P* value[a]
	All (n = 339)	Yeast culture		
		Positive (n = 181)	Negative (n = 158)	
Age (mean ± SD)	40.1 ± 11.61	40.6 ± 13.63	39.6 ± 12.25	0.45
CD4 (mean ± SD)	403.7 ± 239.97	391 ± 276.49	418 ± 206.94	0.3
logHIV[b] (mean ± SD)	2.4 ± 1.24	2.6 ± 1.54	2.2 ± 1.15	**0.003**
No. of subjects with indicated transmission type (%)				
Homosexual	241 (71.1)	128 (70.7)	113 (71.5)	0.098
Heterosexual	86 (25.4)	44 (24.3)	42 (26.6)	
i.v. drug	6 (1.8)	6 (3.3)	0	
Other	6 (1.8)	3 (1.7)	3 (1.3)	
No. of males (%)	305/336 (90.8)	162/179 (90.5)	143/162 (91.1)	1
No. of subjects with $CD4^+$ counts of ≤200 cells/mm^3 (%)	67/323 (20.7)	41/172 (23.8)	26/151 (17.2)	0.1693
No. of subjects with clinic visits for other diseases (%)	79/339 (23.3)	47/181 (26)	32/158 (20.3)	0.247
No. of employed (%)	236/339 (69.6)	120/181 (66.3)	116/158 (73.4)	0.1582
No. of subjects with chronic disease (%)	38/339 (11.2)	23/181 (12.7)	15/158 (9.5)	0.3912
No. of subjects hospitalized within 1 year (%)	52/339 (12.4)	29/181 (16)	13/158 (8.2)	0.323
No. of subjects receiving (%):				
Antibacterial within 6 mo.	57/201 (28.3)	41/108 (38)	16/93 (17.2)	**0.002**
Flu vaccine	62/339 (18.3)	35/181 (19.3)	27/158 (17.1)	0.6731
HAART	290/339 (85.6)	148/181 (81.8)	142/158 (89.9)	**0.044**
Pneumococcal vaccine	238/316 (75)	119/170 (70)	118/146 (80.8)	**0.037**
No. of subjects who have ever stayed in a shelter (%)	12/339 (3.5)	9/181 (5)	3/158 (1.9)	0.15
No. of subjects in a jail or rehabilitation center (%)	3/339 (0.9)	2/181 (1.1)	1/158 (0.3)	1

[a] Values in boldface type indicate significance.
[b] logHIV, $\log_{10}$ value of HIV load.

of them were greater than 450 cells/mm^3. Whether i.v. drug users are at risk for yeast colonization requires further investigation with a larger sample size.

One isolate of each species from the same patient was analyzed, and 26 different species were identified (Table 2). Even though *C. albicans* was still the most common species recovered, its proportion decreased from 86.7% in our previous study (10) to 68.5% in the present study. A total of 10 *C. dubliniensis* isolates were recovered in the present study, whereas no *C. dubliniensis* was found in our previous two nationwide surveys (Taiwan Surveillance of Antimicrobial Resistance of Yeasts [TSARY]) of non-HIV-infected patients in 2002 and 2006 (22, 24).

The MICs of antifungal agents were determined according to the procedures in our previous study (24), which followed the guidelines of the Clinical and Laboratory Standards Institute (5). Growth was measured by the Biotrak II plate reader (Amersham Biosciences, Biochrom Ltd., Cambridge, England) after 24 and 48 h of incubation at 35°C. The overall rates of resistance to amphotericin B (MICs ≥ 2 μg/ml) and fluconazole (MICs ≥ 64 μg/ml) after 48 h of incubation were 0.9% and 11.5%, respectively (Table 2), which are higher than our previous study on candidemia patients (4) and lower than those on non-HIV-infected patients in Taiwan (9, 21, 22, 24). The prevalence of *Candida tropicalis* isolates resistant to fluconazole was high (63.7%), similar to our previous finding in the 2006 TSARY (20).

Even though fluconazole is widely used for the treatment and prophylaxis of mucosal candidiasis in many areas (8, 16), a very low proportion of patients in the present study received antifungal therapy, which is consistent with our previous report on candidemia patients in another hospital in Taiwan (3, 4). Among the 201 patients with available complete relevant information, only four had symptoms of candidiasis, and only these four received antifungal (fluconazole) therapy. This result is a good example of the general policy in Taiwan, which states "not to administer fungal prophylaxis for HIV-infected patients." We failed to recover yeast isolates from one of the four patients. The remaining three patients were colonized by *C. albicans*. These isolates had fluconazole MICs of 0.25, 0.5, and 16 μg/ml, and were from patients who received fluconazole 6, 0, and 27 days, respectively, prior to isolate recovery. However, the number is not enough to determine whether prior antifungal exposure contributed to higher MIC in these isolates.

A significantly higher portion of the patients in the present study was colonized by more than one species of yeast, compared to that in our previous report (36 of 181 versus 25 of 316; $P = 0.0002$). Interestingly, patients who were colonized by multiple species of yeast had a higher unemployment rate than did those colonized by a mere one species (29.5% versus 15%; $P = 0.03$). Thus, to investigate polyfungal colonization/infections, it is better to employ CHROMagar *Candida* medium, well demonstrated to detect a higher diversity of yeasts than routine culture media (13). Furthermore, molecular methods such as sequencing of ITS and/or D1/D2, which provide rapid and accurate identification of various fungal pathogens, were

TABLE 2. Species distribution and drug susceptibilities of isolates recovered from HIV-infected patients

Species	No. of isolates (%) with indicated MIC(s) (μg/ml) for:											Total no. of isolates (%)
	Fluconazole						Amphotericin B					
	24 h			48 h			24 h		48 h			
	≤8	16 to 32	≥64	≤8	16 to 32	≥64	≤0.5	1	≤0.5	1	2	
Candida albicans	141	3	10 (6.5)	136	3	15 (9.7)	154	0	146	8	0	154 (68.5)
Candida glabrata	13	1	0	11	3	0	13	1	13	1	0	14 (6)
Candida parapsilosis	13	0	0	13	0	0	12	1	10	2	1 (7.7)	13 (5.6)
Candida tropicalis	9	0	2 (18.2)	4	0	7 (63.7)	10	1	8	2	1 (9.1)	11 (4.7)
Candida dubliniensis	10	0	0	10	0	0	10	0	10	0	0	10 (4.3)
Candida guilliermondii	4	0	0	3	1	0	4	0	4	0	0	4 (1.7)
Candida krusei	0	3	0	0	1	2 (66.7)	2	1	1	2	0	3 (1.3)
Saccharomyces cerevisiae	3	0	0	3	0	0	3	0	2	1	0	3 (1.3)
Candida famata	2	0	0	2	0	0	2	0	1	1	0	2 (0.9)
Candida intermedia	2	0	0	2	0	0	2	0	2	0	0	2 (0.9)
Candida lusitaniae	2	0	0	2	0	0	2	0	2	0	0	2 (0.9)
Candida pelliculosa	2	0	0	2	0	0	2	0	2	0	0	2 (0.9)
Candida lambica	0	0	1 (100)	0	0	1 (100)	1	0	1	0	0	1 (0.4)
Candida inconspicua	0	1	0	0	0	1 (100)	1	0	1	0	0	1 (0.4)
Candida metapsilosis	1	0	0	1	0	0	1	0	1	0	0	1 (0.4)
Candida santamariae	1	0	0	1	0	0	1	0	1	0	0	1 (0.4)
Candida sojae	1	0	0	1	0	0	1	0	1	0	0	1 (0.4)
Geotrichum gigas	1	0	0	1	0	0	1	0	1	0	0	1 (0.4)
Kodamaea species	1	0	0	1	0	0	1	0	1	0	0	1 (0.4)
Pichia species	0	1	0	0	0	1 (100)	1	0	1	0	0	1 (0.4)
Lodderomyces elongisporus	1	0	0	1	0	0	1	0	1	0	0	1 (0.4)
Saccharomyces species	1	0	0	1	0	0	1	0	1	0	0	1 (0.4)
Trichosporon faecale	1	0	0	1	0	0	0	1	0	1	0	1 (0.4)
Trichosporonoides species	1	0	0	1	0	0	1	0	1	0	0	1 (0.4)
Yarrowia lipolytica	1	0	0	1	0	0	1	0	0	1	0	1 (0.4)
Zygoascus hellenicus var. *hellenicus*	1	0	0	0	1	0	1	0	1	0	0	1 (0.4)
Total	212 (90.6)	9 (3.8)	13 (5.6)	198 (84.6)	9 (3.8)	27 (11.5)	229 (97.9)	5 (2.1)	213 (91)	19 (8.1)	2 (0.9)	234

helpful in identifying rare emerging species in the present study.

In conclusion, periodical surveys of oropharyngeal yeast colonization in high-risk patient cohorts, such as HIV-infected patients, are useful in the clinical arena, especially in a region where few such studies have been conducted. The data obtained from the surveys may provide helpful information for better care of high-risk populations.

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