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Yeast Oropharyngeal Colonization in Human Immunodeficiency Virus-Infected Patients in Central Taiwan

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Abstract A prospective, cross-sectional study was conducted at a medical center in central Taiwan to understand the prevalence, associated factors, and microbiologic features for oropharyngeal yeast colonization in human immunodeficiency virus-infected outpatients. Oral yeast colonization was detected in 127 (45 %) patients, including 21 (16.5 %) colonized by more than one species. Of the 154 isolates, *Candida albicans* was the most common species (114, 74 %), followed by *Candida dubliniensis* (10, 6.5 %), *Candida glabrata* (10, 6.5 %), *Candida tropicalis* (7, 4.5 %), and 13 others. We found that receiving antituberculous drug ($p = 0.046$) or atazanavir ($p = 0.045$) was two predictors for patients colonized by non-*C. albicans* species ($p = 0.005$) and risking mixed yeast colonization ($p = 0.009$). Even though our data showed that clinical antifungal drugs remained effective in vitro against the colonizing yeasts, the increased mixed yeast colonization indicates a potential issue for controlling mixed infections in hospital settings.

Keywords Human immunodeficiency virus · HIV · Colonization · Mixed yeast

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Introduction

Human immunodeficiency virus (HIV)-infected patients are prone to opportunistic infections with the progression of cell-mediated immunodeficiency. More than 80 % of patients had *Candida* oropharyngeal colonization during the course of HIV infection [1, 2]. Despite the introduction of highly active antiretroviral therapy (HAART) since 1996, *Candida* species continue to be a major pathogen for those patients [3, 4].

The overall prevalence of HIV infection in Taiwan has increased dramatically in past decades [5]. According to Centers for Diseases Control (CDC), Taiwan, the annual newly reported cases of HIV infections increased from 342 in 1997 to 1967 in 2011.

Moreover, there was a dramatic increase in the population of HIV-infected intravenous drug users (IDUs) between 2004 and 2006 in Taiwan [6]. Consequently, the overall prevalence of HIV infections increased from 0.00675 % in 1997 to 0.09697 % in 2011. Coincidentally, the number of patients receiving HAART has increased in the recent decade in Taiwan after the government started the treatment for free in 1997.

Commensal microbiota in humans are usually the etiological agents causing infections [7–10]. Hence, oropharyngeal colonization by yeast pathogens is useful for predicting subsequent development of yeast infections in HIV-infected patients [11]. The risk factors and species distribution of oropharyngeal yeast colonization in HIV-infected patients in northern and southern Taiwan had been reported [12–15]. To obtain a more comprehensive understanding of the status of oropharyngeal yeast colonization in HIV-infected outpatients throughout Taiwan, we conducted a separate prospective, cross-sectional study at a medical center in central Taiwan. We also determined the in vitro drug susceptibilities of these isolates.

Materials and Methods

Study Setting

This study was carried out at China Medical University Hospital (CMUH), which serves more than 2 million residents and is a major referral hospital for HIV care in central Taiwan. This study was approved by the Institutional Review Board of CMUH.

Study Protocol

From November 2009 through February 2010, consecutive HIV-infected adult patients who returned for follow-up regularly at the outpatient infectious disease unit of CMUH were recruited for study after informed consents were obtained. Demographic information of age and sex, underlying illnesses, clinical conditions, history of antibiotics, and antifungal therapy in the past 6 months, status of hospitalization in the past 12 months, and the use of antiretroviral agents were collected from a standardized data form. Plasma HIV viral load and $CD4^+$ lymphocyte count were also determined at the time of oropharyngeal specimen collection.

Sample Collection and Culture

Oropharyngeal samples were obtained by swabbing the oropharyngeal mucosa of patients with a dry cotton swab (EZ Culturette; Becton–Dickinson, Sparks, MD, USA). All swabs were streaked onto Chromagar Candida medium (CHROMagar Microbiology, Paris, France) and incubated at 30 °C for 72 h. Three (if there were) individual colonies from each positive culture were collected. Additional colonies were selected when the cultures had more than one morphotype present. To distinguish *Candida albicans* from *Candida dubliniensis*, all isolates were subjected to VITEK Yeast Biochemical Card (bioMérieux, Marcy l'Etoile, France). For those identified as *C. dubliniensis* and randomly selected 27 *C. albicans* by VITEK were further assessed by examining *HWP1* gene PCR products [16]. When the VITEK identification probability was less than 90 % or when uncommon species were identified, the sequences of internal transcribed spacer (ITS) regions of ribosomal DNA amplified by primers ITS1, 5′-TCCGTAGGTGAACCTGCGG-3, and ITS4 5′-TCCTCCGCTTATTGATATGC-3′ and the D1/D2 region of ribosomal DNA amplified by primers NL1 5′-GCATATCAATAAGCGGAGGAAAAG-3′ and NL4 5′-GGTCCGTGTTTCAAGACGG-3′ were used for further assessment [17, 18].

Antifungal Susceptibility Tests

Minimal inhibitory concentrations (MICs) of antifungal drugs were determined by the broth microdilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [19]. Briefly, in-house prepared microplates contained amphotericin B (0.0313–16 μg/mL), fluconazole (0.125–64 μg/mL), and voriconazole (0.0156–8 μg/mL) in RPMI medium 1,640 with L-glutamine without bicarbonate. After incubation at 35 °C for 24 h, the growth of each isolate was measured by Biotrak II plate spectrophotometric reader (Amersham Biosciences, Biochrom Ltd., Cambridge, England). Based on the guidelines of CLSI document M27-S4 [20] and a previous report [21], the epidemiological cutoff values for amphotericin B were 2 mg/L for all species [22]. For fluconazole, the clinical breakpoints of *C. albicans*, *Candida tropicalis*, and

Candida parapsilosis are as following: MICs ≤2 mg/L were considered to be susceptible, ≥8 mg/L resistant, and 4 mg/L susceptible-dose dependent (SDD); for *Candida glabrata*, MICs ≤32 mg/L were SDD, ≥64 mg/L resistant. For voriconazole, the clinical breakpoints of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* were MICs ≤0.125 mg/L susceptible, ≥1 mg/L resistant, and 0.25–0.5 mg/L intermediate. The MICs of 50 and 90 % of the total population were defined as MIC_{50} and MIC_{90}, respectively. Standard control strains included *C. albicans* ATCC 90028, *Candida krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 750.

Data Analysis

The results were analyzed with SPSS software for Windows, version 12.0. The χ^2 test was applied for categorical variables, and the Student's *t* test for continuous variables. The factors, found *p* values less than 0.1 in the univariate analysis, were assessed for their independent effects by logistic regression. A *p* value less than 0.05 was considered significant.

Results

Patients

During the study period, 282 patients, approximately one-third of HIV-infected patients taken care in CMUH, were enrolled. Their demographic data are shown in Table 1. They were predominately male (255, 90.4 %), and 34 (12.1 %) patients were IDUs. Average known period of HIV infections was 5 years. Overall, 239 (84.8 %) patients had a $CD4^+$ cell count >200 cells/mm^3, resulting from high proportion of patients (73.8 %) receiving HAART.

Status and risk factors for yeast colonization

There were 45 % (127/282) patients colonized by yeasts. Receiving antituberculous drugs was a risk for yeast colonization ($p = 0.048$) (Table 1). A high proportion of patients with high HIV viral loads ($p = 0.052$) or low $CD4^+$ cell counts (0.068) were colonized by yeasts. Receipt of efavirenz-protected patients from colonization ($p = 0.097$). However, based on multivariate analysis, none of the factors had significant effect on colonization.

Species Distribution of Yeasts

One isolate of each species from the same patient was analyzed, and 13 different species were recovered (Table 2). Of the 154 yeast isolates, eight isolates needed to be speciated by ribosomal DNA sequencing. There were three *Candida guilliermondii*, two *C. glabrata*, one *C. dubliniensis*, and one *C. parapsilosis*, identified by ITS sequencing. The remaining isolate, of *Trichosporon* sp., could not be identified to species level. *C. albicans* was the most common species (114) accounting for 74 % of the 154 isolates, followed by *C. dubliniensis* (10, 6.5 %), *C. glabrata* (10, 6.5 %), *C. tropicalis* (7, 4.5 %), *C. guilliermondii* (3, 1.9 %), *C. parapsilosis* (3, 1.9 %), and seven others (Table 2). Overall, *Candida* species accounted for 96.8 % of total isolates. Furthermore, a higher proportion of patients receiving antituberculous drug (OR 3.995, 95 % CI 1.024–15.578, $p = 0.046$) or atazanavir (3.061, 1.024–9.152, $p = 0.045$) was colonized by non-*C. albicans* species (Table 3).

Among the 127 patients colonized by yeasts, 106 (83.5 %), 16 (12.6 %), and 5 (3.9 %) were by single, two, and three or more species, respectively. There was one patient colonized by four species, four by three species, 16 by two species (Table 2). *C. albicans* was the most common species co-colonized with other species, accounting for 76.2 % of the 21 mixed yeast colonization cases, followed by *C. glabrata* (38.1 %), *C. tropicalis* (33.3 %), and *C. dubliniensis* (28.6 %). By univariate analysis, culture positive for non-*C. albicans* species ($p = 0.04$), prior exposure of antituberculous drug ($p = 0.007$), and receiving atazanavir in prior 6 months ($p = 0.019$) were at risk for mixed colonization, whereas a $CD4^+$ cell count >200 cells/mm^3 protected patients from mixed colonization ($p = 0.03$) (Table 4). Furthermore, receipt of antituberculous drug (OR 17.244, 95 % CI 2.38–124.74, $p = 0.005$) or atazanavir (5.716, 1.54–21.21, $p = 0.009$), and colonized by non-*C. albicans* species (4.595, 1.13–18.64, $p = 0.033$) were three predictors for mixed colonization based on multivariate analysis (Table 4).

Antifungal Susceptibility of Yeasts

The MIC_{50} were 0.5, 0.125, and 0.0156 μg/mL, and the MIC_{90} were 0.5, 2, and 0.0313 μg/mL, for amphotericin

Table 1 Characteristics of 282 HIV-infected patients enrolled for oropharyngeal fungal cultures and predictors of positive culture for yeasts

Characteristic	All	Yeast culture		Univariate	Multivariate
	($N = 282$)	Positive ($N = 127$)	Negative ($N = 155$)	p value	p value, OR (95 % CI)
Age (years)	37.2 ± 10.8[a]	37.8 ± 11.5	36.6 ± 10.3	0.364	
$CD4^+$ (cells/mm^3)	471.5 ± 261.6	452.5 ± 286.6	487.1 ± 239.0	0.27	
HIV viral load, log (copies/mm^3)	2.4 ± 1.45	2.6 ± 1.57	2.2 ± 1.33	0.052	0.267, 1.10 (0.93–1.31)
Known period of HIV infection (years)	5 ± 4.16 ($N = 274$)	5.1 ± 4.84 ($N = 122$)	4.8 ± 3.54 ($N = 152$)	0.591	
Type of transmission					
Men having sex with men or bisexual	178 (63.1)[b]	81 (63.8)	97 (62.6)	0.901	
Heterosexual	68 (24.1)	26 (20.5)	42 (27.1)	0.21	
Intravenous drug user	34 (12.1)	18 (14.2)	16 (10.3)	0.361	
Males	255 (90.4)	115 (90.6)	140 (90.3)	1	
$CD4^+$ counts >200 cells/mm^3	239 (84.8)	102 (80.3)	137 (88.4)	0.068	0.201, 0.63 (0.32–1.28)
Underlying diseases					
Diabetic mellitus	2 (0.7)	1 (0.8)	1 (0.6)	1	
Chronic kidney diseases	0	0	0	–	
Hospitalization within 6 months	2 (0.7)	0	2 (1.3)	0.503	
Residence in a jail or rehabilitation center within 6 months	2 (0.7)	1 (0.8)	1 (0.6)	1	
Medications					
Antiretroviral therapy within 6 months	208 (73.8)	93 (73.2)	115 (74.2)	0.892	
Lamivudine/zidovudine (Combivir)	114 (40.4)	52 (40.9 %)	62 (40)	0.903	
Lamivudine or lamivudine/zidovudine (3TC with combivir)	218 (77.3)	100 (78.7)	118 (76.1)	0.669	
Stavudine (d4T)	11 (3.9)	3 (2.4)	8 (5.2)	0.355	
Abacavir	94 (33.3)	44 (34.6)	50 (32.3)	0.704	
Efavirenz	69 (24.5)	25 (19.7)	44 (28.4)	0.097	0.067, 0.58 (0.32–1.04)
Nevirapine	33 (11.7)	15 (11.8)	18 (11.6)	1	
Atazanavir	30 (10.6)	15 (11.8)	15 (9.7)	0.698	
Antibacterials within 6 months[c]	17 (6)	10 (7.9)	7 (4.5)	0.316	
Antituberculosis drugs	7 (2.5)	6 (4.7)	1 (0.6)	0.048	0.08, 7.05 (0.79–62.60)
Antifungals within 6 months	4 (1.4)	3 (2.4)	1 (0.6)	0.33	
Fluconazole	3(1.1)	2 (1.6)	1 (0.6)	0.59	
Amphotericin B	1 (0.4)	1 (0.8)	0	0.45	

Variables entered in the multivariate analysis are in bold

OR odds ratio

[a] Number ± standard deviation

[b] Number (percentage)

[c] Antibacterial agents including penicillin derivatives, cephalosporins, trimethoprim/sulfamethoxazole, macrolides, clindamycin, or antituberculosis drugs

Table 2 The distribution of species and mixed yeast colonization

	No. of isolate	%	No. of patients with mixed yeast colonization												
			1	1	1	1	1	4	4	3	1	1	1	1	1
Candida albicans	114	74.0	+	+	+			+	+	+	+	+	+		
Candida dubliniensis	10	6.5		+			+	+							
Candida glabrata	10	6.5			+		+		+					+	+
Candida tropicalis	7	4.5		+	+		+			+				+	
Candida guilliermondii	3	1.9				+					+				
Candida parapsilosis	3	1.9	+			+									
Candida famata	1	0.6													+
Candida intermedia	1	0.6										+			
Saccharomyces cerevisiae	1	0.6											+		
Stephanoascus ciferrii	1	0.6													
Trichosporon asahii	1	0.6	+												
Trichosporon faecale	1	0.6				+									
Trichosporon sp.	1	0.6	+												
Total	154														

No Number

B, fluconazole, and voriconazole, respectively. All tested isolates were susceptible to amphotericin B and voriconazole. Only one *C. albicans* isolate had fluconazole MIC 16 μg/mL, considered as resistant.

Discussion

According to the data collected by CDC, Taiwan (http://www.cdc.gov.tw/info.aspx?treeid=1f07e8862ba550cf&nowtreeid=6c5ea6d932836f74&tid=DC84CFE0D875EC06), there were 18,378 HIV-infected patients, including 16,862 (91.75 %) males and 1,516 (8.25 %) females in Taiwan by the end of 2009. A total of 706 HIV-infected patients including 551 (90.92 %) males and 55 (9.08 %) females received medical care from CMUH in 2009. In the present study, the enrolled patients were predominately male (255, 90.4 %), which is consistent with the ratio in Taiwan and in CMUH.

The colonization rate among HIV-infected patients varied from 44 to 88 %, which is significantly higher than those in non-HIV-infected individuals [11, 23–27]. Many factors, such as clinical course of HIV infections, underlying diseases, medications, homosexual activity, drug use, and geography affect yeast colonization [28]. We have found two significant differences between the populations in the present study and the study conducted in southern Taiwan [13]. One is the rate of hospitalization (0.7 vs. 33.9 % $p < 0.0000001$), and the other is the proportion of patients with diabetic mellitus (0.7 vs. 3.4 % $p = 0.03$). Whether the difference in the rates of yeast colonization (45 vs. 59 % $p = 0.0006$) is due to these two factors solicits further investigation.

Our previous findings [13, 15] and others [2, 23, 26, 29, 30] showed that HIV-infected patients with progressive immunodeficiency (low $CD4^+$ cell counts) have higher prevalence of yeast colonization. In the present study, we found that HIV-infected patients with $CD4^+$ cell counts $\leq$200 cells/mm^3 ($p = 0.068$) and high viral load ($p = 0.052$) appeared to have higher prevalence of yeast colonization even though they have not reached statistically significance.

The prevalence of non-*C. albicans* species yeast colonization varied from 5 to 64.2 % [28]. Among the isolates from HIV-infected patients, 87 % in 1999–2002 [15], 70 % in 2005 [14], and 74 % in the present study were *C. albicans*. Hence, the prevalence of non-*C. albicans* species has indeed increased in the past decade, consistent with previous findings by different groups (5–30.8 % before 2002 vs. 13–64.2 % after 2002) [28]. A total of 10 *C. dubliniensis* isolates were recovered in the present study, whereas none was found in our previous two nationwide surveys of non-HIV-infected

Table 3 Characteristics of HIV-infected patients colonized by Non-*Candida albicans*

Characteristic	*C. albicans* ($N = 114$)	Non-*C. albicans* ($N = 40$)	Univariate p value	Multivariate p value, OR (95 % CI)
Age (years)	38.0 ± 11,57[a]	37.67 ± 10.36	0.866	
$CD4^+$ (cells/mm^3)	449.4 ± 283.1	425.75 ± 296.85	0.654	
HIV viral load, log (copies/mm^3)	2.6 ± 1.56	2.3798 ± 1.59	0.54	
Known period of HIV infection (years)	5.2 ± 4.92 ($N = 110$)	4.8 ± 5.85 ($N = 39$)	0.717	
Intravenous drug user	15 (13.2)[b]	10 (25)	0.088	0.201, 2.479 (0.616–9.977)
Bisexual	75 (65.8)	20 (50)	0.09	0.396, 0.652 (0.243–1.749)
$CD4^+$ >200 cells/mm^3	92 (80.7)	28 (70)	0.186	
Medications				
Antiretroviral therapy within 6 months				
Combivir	50 (43.9)	11 (27.5)	0.091	0.749, 0.808 (0.218–2.993)
d4T	3 (2.6)	1 (2.5)	1	
3TC	40 (35.1)	22 (55)	0.039	0.551, 1.96 (0.215–17.841)
Abacavir	36 (31.6)	20 (50)	0.055	0.804, 1.286 (0.177–9.358)
Efavirenz	21 (18.4)	11 (27.5)	0.259	
Nevirapine	14 (12.3)	3 (7.5)	0.562	
Kaletra	42 (36.8)	9 (22.5)	0.119	
Indinavir	1 (0.9)	1 (2.5)	0.453	
Atazanavir	14 (12.3)	10 (25)	0.075	0.045, 3.061 (1.024–9.152)
Antibacterials[c] within 6 months	8 (7)	7 (17.5)	0.067	
Antituberculosis drugs	5 (4.4)	6 (15)	0.035	0.046, 3.995 (1.024–15.577)
Antifungals within 6 months				
Fluconazole	2 (1.8)	0	1	
Amphotericin B	1 (0.9)	0	1	

Variables entered in the multivariate analysis are in bold

OR odds ratio

[a] Number ± standard deviation

[b] Number (percentage)

[c] Antibacterial agents including penicillin derivatives, cephalosporins, trimethoprim/sulfamethoxazole, macrolides, clindamycin, or antituberculosis drugs

patients in 2002 and 2006 [31, 32], consistent with the generally accepted view that this species preferentially colonizes HIV-infected populations. Interestingly, half the patients colonized by *C. dubliniensis* were also colonized by *C. albicans*. It has been reported that the prevalence of *C. dubliniensis* colonization is higher among HIV-infected patients of European descent (9 %) than African descent (1.5 %), suggesting that *C. dubliniensis* carriage may be influenced by ethnicity [33]. Nevertheless, we did not identify factors contributing to *C. dubliniensis* colonization in HIV-infected patients in the present study.

Protease inhibitors were the first reported to be associated with a lower rate of oropharyngeal *Candida* colonization and candidiasis [34]. In contrast, our previous study found that protease inhibitor-containing antiretroviral therapy predisposed the patients to oropharyngeal yeast colonization [12]. In the present study, receiving efavirenz-containing regimen appeared to be negatively associated with yeast colonization, a finding consistent with our previous study [13]. Furthermore, in the present study, we found that receiving atazanavir was a predictor for patients colonized by non-*C. albicans* species as well as mixed yeast colonization. This is the first study showing the potential effect of atazanavir on oropharyngeal mixed yeast colonization. Further clinical investigations with larger

Table 4 Characteristics of HIV-infected patients colonized by more than one species

Characteristic	More than one species ($N = 21$)	One species ($N = 106$)	Univariate p value	Multivariate p value, OR (95 % CI)
Age (years)	38.8 ± 9.79[a]	37.6 ± 11.9	0.668	
$CD4^+$ (cells/mm^3)	379.1 ± 281.76	467.1 ± 286.6	0.2	
HIV viral load log (copies/mm^3)	2.5 ± 1.66	2.6 ± 1.6	0.731	
Known period of HIV infection (years)	5.1 ± 7.34	5.12 ± 4.19	0.984	
Intravenous drug user	6 (28.6)[b]	12 (11.3)	0.1	0.054, 3.499 (0.98–12.52)
$CD4^+$ >200 cells/mm^3	13 (61.9)	89 (84)	0.033	0.198, 0.445(0.13–1.53)
Non-*C. albicans*	5 (23.8)	8 (7.5)	0.040	0.033,4.595 (1.13-18.64)
Medications				
Antiretroviral therapy within 6 months	16 (76.2)	77 (72.6)	0.796	
Combivir	8 (38.1)	44 (41.5)	0.813	
d4T	1 (4.8)	2 (1.9)	0.421	
3TC	10 (47.6)	38 (35.8)	0.333	
Abacavir	8 (38.1)	36 (34)	0.803	
Efavirenz	6 (28.6)	19 (17.9)	0.366	
Nevirapine	1 (4.8)	14 (13.2)	0.463	
Kaletra	6 (28.6)	39 (36.8)	0.619	
Indinavir	0	2 (1.9)	1.000	
Atazanavir	6 (28.6)	9 (8.5)	0.019	0.009, 5.716 (1.54–21.21)
Antibacterials[c] within 6 months	4 (19)	6 (5.7)	0.060	
Trimethoprim/sulfamethoxazole/Baktar	2 (9.5)	5 (4.7)	0.326	
Fluoroquinolone	1 (4.8)	0	0.165	
Macrolides	1 (4.8)	0	0.165	
Antituberculosis drugs	4 (19)	2 (1.9)	0.007	0.005, 17.244 (2.38–124.74)
Antifungal drugs	0	3 (2.8)	1.000	

Variables entered in the multivariate analysis are in bold

OR odds ratio

[a] Number ± standard deviation

[b] Number (percentage)

[c] Antibacterial agents including penicillin derivatives, cephalosporins, trimethoprim/sulfamethoxazole, macrolides, clindamycin, or antituberculosis drugs

sample sizes to determine the impact of the drug on mixed yeast colonization are warranted.

In conclusion, the association between oropharyngeal mixed yeast colonization and receiving atazanavir and antituberculous drugs need further investigations. Antifungal drugs remain effective against the colonized yeasts. We found that 45 % of HIV-infected outpatients were colonized by yeasts and 16.5 % of them by more than one species. The observation of increased mixed yeast colonization indicates a potential issue for controlling mixed infections in hospital settings.

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Conflict of interest The authors report no conflicts of interest.

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