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Article

Determinants for the Development of Oropharyngeal Colonization or Infection by Fluconazole-Resistant *Candida* Strains in HIV-Infected Patients

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Abstract A point prevalence study to document oral yeast carriage was undertaken. Risk factors for the development of oropharyngeal colonization or infection by fluconazole-resistant *Candida* strains in HIV-infected patients were investigated with a case-control design. Cases included all patients with fluconazole-resistant strains (MIC $\geq$ 64 μg/ml), and controls were those with susceptible (MIC $\leq$ 8 μg/ml) or susceptible-dependent-upon-dose (MIC 16–32 μg/ml) strains. One hundred sixty-eight *Candida* strains were isolated from 153 (88%) patients, 28 (16%) of whom had oropharyngeal candidiasis. Overall, 19 (12%) of the patients harbored at least one resistant organism (MIC $\geq$ 64 μg/ml). Among patients with resistant strains, tuberculosis ($P<0.001$), esophageal candidiasis ($P=0.001$), clinical thrush ($P<0.001$), and a CD4+ cell count $<200/mm^3$ ($P=0.03$) were more frequent. These patients had also been treated more commonly with antituberculous drugs (adjusted odds ratio [OR] 6.13; 95% confidence interval [CI] 2.11–17.80), ciprofloxacin (OR 6.0; 95% CI 1.23–29.26), fluconazole (OR 4.59; 95% CI 1.55–13.52), and steroids (OR 4.13; 95% CI 1.11–15.39). Multivariate analysis showed that the determinants for fluconazole resistance were therapy with antituberculous drugs (OR 3.61; 95% CI 1.08–12.07; $P=0.03$) and one of the following: previous tuberculosis (OR 3.53; 95% CI 1.08–14.57; $P=0.03$) or fluconazole exposure (OR 3.41; 95% CI 1.10–10.54). Findings from this study indicate that treatment with antituberculous drugs, previous tuberculosis, and fluconazole exposure are the strongest determinants for development of oropharyngeal colonization or infection by fluconazole-resistant *Candida* strains in HIV-infected patients.

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Introduction

Although the introduction of highly active antiretroviral therapy (HAART) has had a major impact on the infectious complications of AIDS [1, 2], oroesophageal candidiasis remains a common opportunistic infection in HIV-infected patients [3] as well as in other immunocompromised individuals [4, 5]. Fluconazole is considered the drug of choice for mucosal candidiasis in AIDS patients [6, 7]. Unfortunately, fluconazole resistance has become an increasing problem in the last few years. Despite numerous previous reports [8–16], only a

few studies have focused on the investigation of risk factors associated with this phenomenon [9], and thus far, the determinants for the development of fluconazole-resistant *Candida* strains have not been well characterized.

In AIDS patients fluconazole resistance has been associated with advanced immunosuppression and prolonged exposure to azoles. However, most previous studies had methodologic limitations, such as small samples and selected populations, usually patients with advanced immunosuppression [8, 9, 11]. In fact, the importance of immunosuppression as a risk factor for acquisition of fluconazole resistance in this setting remains controversial. In two previous studies, a low CD4+ cell count was not found to be an independent risk factor predicting fluconazole resistance in a multivariate analysis [10, 16]. Although it is clear that exposure to fluconazole is a risk factor for the development of resistance, fluconazole-resistant strains have also been isolated from HIV-infected patients who were never exposed to the drug [8, 11, 12], and a subset of patients, usually the least immunosuppressed, do not acquire fluconazole-resistant *Candida* isolates after having been given fluconazole [8, 17, 18]. The role of fluconazole in the acquisition of resistant strains of *Candida* in other immunocompromised patients has also been questioned [8, 19].

Fluconazole is an effective and well-tolerated antifungal agent. Therefore, it is essential to know if fluconazole indeed induces the emergence of resistant strains of *Candida*, or if different and potentially modifiable factors are also involved. The current study was designed to evaluate the determinants for the development of fluconazole resistance in HIV-infected patients either colonized or infected by *Candida* spp. Specifically, we wished to assess the importance of the use of azoles and the degree of immunosuppression and to investigate other risk factors for colonization or infection by fluconazole-resistant *Candida* strains. In order to document oral yeast carriage, a point prevalence study was conducted in a cohort of HIV-infected patients with a wide range of CD4+ cell counts.

Patients and Methods

Patient Population and Study Design. HIV-infected patients were evaluated prospectively to assess whether colonization or infection by *Candida* spp. was present. Thereafter, patients with fluconazole-resistant strains were compared with those with fluconazole-susceptible strains to investigate the determinants of resistance.

The study was conducted in two hospitals in Alicante, Spain (Hospital General Universitario de Elche and Hospital de Area Marina Baixa). Both are 700-bed community institutions that provide care for 350,000 inhabitants in the eastern area of Spain. The study was conducted between July 1996 and January 1997. At the time the study began, only antiretroviral agents belonging to the nucleoside reverse transcriptase inhibitor category (zidovudine, stavudine, lamivudine, didanosine, and zalcitabine) were available. Protease inhibitors (saquinavir, ritonavir, and indinavir) became available in September 1996, and at the time the study was performed, only 13 patients were on HAART.

To investigate risk factors for oropharyngeal colonization or infection by fluconazole-resistant *Candida* strains in HIV-infected patients, a case-control study was designed.

Clinical Evaluation. All consecutive HIV-infected patients attending either of the two hospitals for any reason, either as outpatients or inpatients, during the study period (July–August 1996 in Hospital Marina Baixa, December 1996–January 1997 in Hospital de Elche) were included. The only entry requirement for the study was HIV seropositivity.

A standard data sheet was employed for the evaluation. The medical history of each patient was taken and he/she was examined clinically, with particular attention being paid to the oropharynx. Clinical records were also reviewed. Oral candidiasis was considered only in its pseudomembranous form. On the day of the clinical evaluation, blood tests were taken, and an oral swab was obtained from the patients with candidiasis by brushing the affected areas. If no thrush was present, swabs from the tongue, jaws, palate, and tonsillar regions were taken.

The following data were recorded for each patient: age, sex, HIV transmission category, time since diagnosis of HIV infection, Centers for Disease Control (CDC) category (CDC classification system for HIV infection, 1993) [20], CD4+ cell count, previous or present opportunistic infections (detailing the infection in each case), previous or present oral/esophageal candidiasis, recurrent candidiasis, treatment of opportunistic infections, and antifungal therapy. Details of antifungal treatment, including the number of episodes treated, number of days of therapy, time elapsed since the last episode treated, and the indication for treatment (prophylaxis or eradication) were also recorded. Since the investigation of antifungal therapy for oropharyngeal candidiasis was not a purpose of the study, the clinical course of such patients was not specifically recorded. To minimize recall bias, all the available commercial formulations of both antifungal and antiviral agents were shown to the patients in order to help them to remember which drugs they had been given. To facilitate the filling in of the data sheet, tables containing the most common opportunistic infections, the drugs usually employed for the treatment of these infections, and the antifungal agents and other medications or processes that could potentially increase the rates of mucosal fungal colonization (such as diabetes, steroids, histamine H_2 receptor antagonists, etc.) were also drawn up.

Culture Procedure and Susceptibility Testing. The swabs were inoculated onto Sabouraud dextrose agar plates (Oxoid, UK) containing chloramphenicol; plates were incubated at 30 °C. Strain identification was carried out using the germ tube test and standard biochemical testing with the API 20 C AUX system (bioMérieux, France). Colonies presenting different morphology were studied separately, and for those with a single morphology, 3–5 colonies were randomly selected.

The microdilution method, following the National Committee for Clinical Laboratory Standards (NCCLS) recommendations, was performed for fluconazole susceptibility testing [21] with the use of RPMI 1640 buffered to pH 7.0 with 0.165 M MOPS. Fluconazole was prepared as a stock solution of 640 μg/ml and subsequently diluted to obtain a final concentration ranging from 0.125 to 64 μg/ml. A spectrophotometric standardized inoculum to a turbidity of a 0.5 McFarland standard was employed. Following incubation at 35 °C, plates were shaken for 1–2 min and turbidity was read visually at 24 h and 48 h. The minimal inhibitory concentration (MIC) was defined as the lowest drug concentration that caused an 80% reduction in turbidity compared with growth

control. Readings were done at 24 h and 48 h. MICs were measured at 24 h, except in cases of delayed growth, when MICs were measured at 48 h.

Definitions. Strains were classified as resistant (MIC $\geq$ 64 μg/ml), susceptible dependent upon dose (MIC 16–32 μg/ml) and susceptible (MIC $\leq$ 8 μg/ml) according to the recently proposed NCCLS breakpoints [22]. To investigate determinants for oropharyngeal colonization and infection by fluconazole-resistant *Candida*, cases were defined as those patients from whom resistant strains were isolated. Controls included all patients colonized or infected by susceptible or susceptible-dependent-upon-dose strains.

Statistical Analysis. Statistical analysis was performed by means of Epi Info version 6 (Centers for Disease Control and Prevention, USA) and SPSS version 6.0.1 (SPSS, USA) with chi-square analysis and Fisher's exact test where appropriate. The strength and precision of the associations were assessed using the odds ratios (OR) and their 95% confidence intervals (CIs). A stratified analysis was conducted to determine the presence or absence of confounding factors and was controlled by fitting a multiple logistic regression model. The model was constructed based on all the variables that were significantly associated with fluconazole resistance upon univariate analysis. With these variables and those considered of interest, a step-by-step model was designed.

Multivariate analysis was carried out using the Egret software package (Statistics and Epidemiology Research and Cytel Software, USA).

Results

A total of 179 consecutive patients were evaluated. The sample comprised all HIV-positive patients attending the two hospitals during the study period. Complete records were available for 174 patients; these patients constituted the study group. In 21 (12%) patients culture was negative. Overall, 48% of the patients had CD4+ cell counts above 200/mm^3 and 10% had counts above 500/mm^3.

One hundred sixty-eight fungal strains were isolated from 153 (88%) patients. Only 28 (16%) of them had oropharyngeal candidiasis. In 121 (79%) patients, *Candida albicans* alone was isolated. Yeast other than *Candida albicans* (YOCA) was isolated from 32 (21%) patients, 19 of them from pure culture. Five of the 19 patients from whom YOCA was the only fungus isolated had clinical thrush. In four cases the isolate of YOCA recovered was *Candida glabrata*, and in the other patient it was *Candida tropicalis*. The most frequent YOCA was *Candida glabrata* (45%), followed by *Candida inconspicua* (18%). In 13 patients two different yeasts were isolated from the culture, in 11 (7%) of them it was a mixed culture of *Candida albicans* and a YOCA, and in the remaining two patients, two different YOCA isolates were identified.

Fluconazole Susceptibility. Fluconazole susceptibility is shown in Table 1. Overall, 12% of the patients with a positive culture carried a resistant strain (MIC $\geq$ 64 μg/ml) and 18% carried a strain with decreased susceptibility to fluconazole (MIC $>$ 8 μg/ml). The MICs for the *Candida albicans* isolates were lower than those for the YOCA isolates (MIC50, 0.25 μg/ml and MIC50, 16 μg/ml, respectively) ($P = 0.0054$).

Table 1 Fluconazole susceptibility of the *Candida* isolates in HIV-infected patients (*Candida albicans* vs. yeast other than *Candida albicans* [YOCA])

MIC of fluconazole (μg/ml)	No. (%) of patients		
	C. albicans ($n = 121$)	YOCA ($n = 32$)	Total ($n = 153$)
≤ 8	111 (92)	11 (34)	122 (80)
16–32	1 (1)	9 (28)	10 (7)
≥ 64	9 (7)	10 (31)*	19 (12)
Unknown	0	2 (6)	2 (1)

*$P = 0.0001$ for the comparison of fluconazole-resistant YOCA isolates and fluconazole-resistant*Candida albicans* isolates

Risk Factors Associated with Colonization or Infection by Fluconazole-Resistant Candida Isolates. Univariate analysis showed no statistical differences between patients with susceptible or susceptible-dependent-upon-dose isolates and those with resistant isolates with respect to demographic characteristics, HIV transmission category and duration of HIV infection (Table 2). A CD4+ cell count $<$ 200/mm^3 was significantly more frequent among patients with resistant isolates ($P = 0.03$), who were also more often included in the C category of the CDC classification system ($P = 0.02$).

Either previous or active opportunistic infections at the time of assessment were more frequent in patients with resistant strains ($P = 0.02$ in both cases). Tuberculosis was the most common major opportunistic disease.

Cases and controls were compared with respect to leukocyte, neutrophil, and monocyte counts and immunoglobulin levels. Only the mean IgA level was significantly lower in patients with resistant isolates ($P = 0.05$). The monocyte count was also lower in these cases, although no statistical significance was reached ($P = 0.07$).

Patients with resistant isolates had more frequently received antituberculous drugs, ciprofloxacin (which was employed in seven patients as an antituberculous agent due to toxicity associated with conventional treatment), other antibiotics for the treatment of nonopportunistic infections, and steroids. No differences with respect to the antiretroviral therapy were found (Table 3).

Azole therapy differed significantly between the two groups. Compared with controls, a significantly higher proportion of cases had received fluconazole ($P = 0.01$), any azole compound or any antifungal drug ($P = 0.03$ in both cases). Recent fluconazole treatment (within the previous 3 months) and recent and greater exposure to

Table 2 Demographic and clinical characteristics of patients with fluconazole-resistant and fluconazole-susceptible isolates

Characteristic	No. (%) of patients		*P* value
	Resistant isolates, MIC ≥ 64 μg/ml (*n* = 19)	Susceptible isolates, MIC < 64 μg/ml (*n* = 132)	
Sex			
Male	15 (79)	109 (83)	0.7
Female	4 (21)	23 (17)	
Age			
Median	36 years	38 years	
Range	26–31 years	24–31 years	0.17
Risk factor for HIV infection			
Intravenous drug use	13 (68)	68 (52)	
Homosexuality	4 (21)	28 (21)	0.52
Other	2 (11)	36 (27)	
Time since diagnosis of AIDS			
Median	84 months	84 months	0.86
Range	1–84 months	0–72 months	
CDC category[a]			
A	4 (21)	48 (36)	
B	1 (5)	32 (24)	0.02
C	14 (74)	52 (39)	
CD4+ count[b]			
>200 (cells/mm^3)	4 (21)	63 (48)	0.03
<200 (cells/mm^3)	15 (79)	66 (50)	
Median count (range)	72 cells/mm^3 (3–651)	191 cells/mm^3 (2–840)	0.01
Previous opportunistic infections	15 (79)	52 (39)	0.02
Tuberculosis	8 (42)	14 (11)	<0.001
Pneumocystis carinii pneumonia	3 (16)	20 (15)	0.5
Active opportunistic infections	10 (53)	29 (22)	0.02
Tuberculosis	3 (16)	8 (6)	0.1
Oropharyngeal candidiasis	9 (47)	19 (14)	<0.001
Previous esophageal candidiasis	6 (32)	10 (8)	0.001
Recurrent candidiasis	10 (53)	44 (33)	0.08
Candidiasis in partner	1 (5)	16 (12)	0.3

[a] A, asymptomatic; B, symptomatic, not A or C conditions; C, major AIDS-defining opportunistic infections

[b] Because of missing values, percentages may not total 100
CDC, Centers for Disease Control

fluconazole (more than 21 days of treatment) were also more common among the cases (Table 4).

When the 32 patients with YOCA strains were analyzed separately, the only variable associated with fluconazole resistance was treatment with antituberculous agents ($P = 0.03$). No association was found with CD4+ cell count, antiretroviral treatment, opportunistic infections, previous tuberculosis, or use of antifungal agents, including fluconazole (Table 5).

In a multivariate analysis, the independent significant risk factors for resistance to fluconazole were treatment with antituberculous drugs (OR 3.61; 95% CI 1.08–12.07; $P = 0.009$) and one of the following: previous tuberculosis (OR 3.53; 95% CI 1.08–14.57; $P = 0.03$) or fluconazole therapy (OR 3.41; 95% CI 1.10–10.54; $P = 0.03$). The link existing between the three factors did not allow construction of a single model that included all of them simultaneously.

Since most of the patients exposed to antituberculous drugs had also been exposed to fluconazole, in order to clarify the association between fluconazole resistance and both antituberculous agents and fluconazole exposure, we performed a stratified analysis (Table 6). As the table shows, the highest frequency of fluconazole resistance was observed in patients treated with antituberculous agents, independent of fluconazole use.

After adjusting treatment with ciprofloxacin for fluconazole exposure, its relation with fluconazole resistance was close to statistical significance (OR 4.49; 95% CI 0.86–23.51; $P = 0.07$).

Discussion

This study confirms that fluconazole therapy is a risk factor for the acquisition of fluconazole-resistant *Candida*, but exposure to this drug is not the only

Table 3 Drugs taken by patients with fluconazole-resistant and -susceptible isolates

Drug	No. (%) of patients		OR	95% CI	*P* value
	MIC ≥ 64 μg/ml ($n = 19$)	MIC < 64 μg/ml ($n = 132$)			
Antituberculous agents[a]					
Yes	8 (42)	14 (11)	6.13	2.11–17.80	
No	11 (58)	118 (89)			
Ciprofloxacin					
Yes	3 (16)	4 (3)	6.0	1.23–29.26	0.02
No	16 (84)	128 (97)			
Antituberculous agents[a] or ciprofloxacin					
Yes	8 (42)	14 (11)	6.13	2.11–17.80	
No	11 (58)	118 (89)			
TMP-SMX					
Yes	2 (11)	4 (3)	3.76	0.43–27.28	0.1
No	17 (89)	128 (97)			
Steroids[b]					
Yes	4 (21)	8 (6)	4.13	1.11–15.39	0.03
No	15 (79)	121 (92)			
Other antibiotics[b]					
Yes	8 (42)	23 (17)	3.38	1.09–10.42	0.02
No	11 (58)	107 (81)			
Pentamidine					
Yes	4 (21)	23 (17)	1.26	0.32–4.66	0.4
No	15 (79)	109 (83)			
H-2 receptor antagonists[b]					
Yes	4 (21)	15 (12)	2.04	0.50–7.84	0.2
No	15 (79)	115 (87)			
Antiretroviral agents					
Yes	16 (84)	103 (78)	1.50	0.37–7.07	0.39
No	3 (16)	29 (22)			
AZT + DDC					
Yes	10 (53)	48 (36)	1.94	0.66–5.72	0.17
No	9 (47)	84 (64)			
AZT + DDI					
Yes	1 (5)	31 (23)	0.18	0.01–1.38	0.07
No	18 (95)	101 (77)			
Protease inhibitors					
Yes	2 (11)	11 (8)	1.29	0.0–1.29	0.7
No	17 (89)	121 (92)			

[a] Includes any of the following: isoniazid, rifampin, pyrazinamide, and ethambutol

[b] Because of missing values, percentages may not total 100
TMP-SMX, trimethoprim-sulfamethoxazole; AZT, zidovudine; DDC, zalcitabine; DDI, didanosine

Table 4 Characteristics of antifungal treatment in patients with fluconazole-resistant or -susceptible *Candida* isolates

Characteristic	No. (%) of patients		OR	95% CI	*P* value
	Resistant isolates, MIC ≥ 64 μg/ml ($n = 19$)	Susceptible isolates, MIC < 64 μg/ml ($n = 132$)			
Antifungal therapy					
No therapy	4 (21)	69 (52)	1.33	0.02–14.87	
Antifungal agent other than fluconazole	1 (5)	13 (10)	3.23	0.51–19.73	
Remote[a] fluconazole treatment	3 (16)	16 (12)	5.03	1.18–22.82	< 0.001
Recent[b] and short-term[c] fluconazole treatment	7 (37)	24 (18)	7.39	1.04–53.19	
Recent[b] and long-term[d] fluconazole treatment	3 (16)	7 (5)			
Unknown	1 (5)	3 (3)			

[a] More than 3 months before assessment
[b] Within the previous 3 months of assessment
[c] Less than 3 weeks' duration
[d] More than 3 weeks' duration

Table 5 Clinical characteristics of patients with isolates of yeast other than *Candida albicans* (YOCA), compared according to fluconazole susceptibility of the isolates

Characteristic	No. (%) of patients		OR	95% CI	*P* value
	MIC ≥ 64 μg/ml ($n=10$)	MIC < 64 μg/ml ($n=20$)			
CD4+ count					
>200 cells/mm^3	2 (20)	7 (35)	0.46	0.05–3.56	0.34
<200 cells/mm^3	8 (80)	13 (65)			
Previous opportunistic infections	8 (80)	12 (60)	2.67	0.35–24.17	0.25
Tuberculosis	2 (20)	4 (20)	1	0.10–8.90	0.67
Active opportunistic infections	7 (70)	8 (40)	3.5	0.55–24.60	0.12
Antiretroviral agents	8 (80)	17 (85)	0.71	0.07–7.69	0.55
Antituberculous agents	4 (40)	1 (5)	12.67	0.96–373.3	0.03
Antifungal therapy	8 (80)	12 (60)	2.67	0.35–24.17	0.25
Fluconazole therapy	8 (80)	9 (45)	4.89	0.66–44.45	0.07

Table 6 Association between antituberculous treatment and fluconazole resistance, stratified by exposure to fluconazole

Drug exposure	No. (%) of patients		OR	95% CI
	MIC ≥ 64 ($n=19$)	MIC < 64 ($n=132$)		
Exposure to fluconazole				
Antituberculous treatment				
Yes	6 (38)	10 (62)	3.0	0.71–12.9
No	8 (17)	40 (83)		
No exposure to fluconazole				
Antituberculous treatment				
Yes	2 (33)	4 (67)	13	1.1–158.4
No	3 (4)	78 (96)		

Mantel-Haenszel odds ratio for the association between antituberculous treatment and fluconazole resistance adjusted for exposure to fluconazole = 4 (95% CI, 1.3–11.9)

determinant for the development of fluconazole resistance. Moreover, our analysis demonstrates that previous tuberculosis and exposure to antituberculous antibiotics are the strongest determinants for development of colonization or infection by fluconazole-resistant *Candida*. In addition, our data also indicate that a low CD4+ cell count is not a determinant for fluconazole resistance.

Although a larger number of patients might have allowed better statistical precision and possibly an integral explanatory model for fluconazole resistance, in this study the number of patients evaluated was larger than that in previous reports, and the influence of many potential risk factors for the development of resistance was thoroughly investigated, including opportunistic infections and their therapies. Its cross-sectional design, which covers all stages of HIV infection, also supports the significance of the results. In fact, the point-prevalence nature of the study resulted in a small number of patients with oral infection and identified fewer than half of those with resistant strains as having clinical thrush. Risk factors for fluconazole-resistant oropharyngeal candidiasis have been evaluated before [9, 11] in patients with advanced immunosuppression. The purpose of the present study was to further investigate the determinants for fluconazole-resistant *Candida* carriage by covering a wider range of the HIV-infected population. Another limitation of the present study could be that it was conducted mostly in the era previous to HAART, and the prevalence of colonization or infection by *Candida* spp. might have been different at that time. Nevertheless, the main objective of this study was the investigation of the risk factors for resistance rather than its frequency. The limited number of patients with thrush found in this study and the fact that more than half of the resistant isolates were colonizing in nature precluded us from obtaining information about the clinical management of fluconazole-resistant oropharyngeal candidiasis.

Previous tuberculosis and antituberculous drugs were found to be the factors that best explain colonization or infection by fluconazole-resistant strains. Despite the small number of patients with previous tuberculosis and antituberculous therapy found in this study, the statistical association with fluconazole resistance was the strongest of all the variables studied. It is difficult to determine if the tuberculosis itself or its therapy leads to fluconazole resistance, because they are usually closely related and cannot be analyzed separately. Both variables were independently associated with fluconazole resistance. After adjusting for one of these factors, fluconazole also proved to be an independent predictor of resistance, but with a lower explanatory power.

Selective pressure from antibiotics is an established explanation for the development of bacterial resistance to antibiotics [23]. The relationship between antibiotics and candidal infections is also well known [24–26], and in a previous report, therapy with the quinolone norfloxacin was found to be, along with fluconazole

therapy, an independent risk factor for colonization by *Candida krusei*, an intrinsically fluconazole-resistant *Candida* spp. [27]. Ciprofloxacin was employed in the current study as an alternative antituberculous agent when toxicity developed with the standard drugs, so both variables cannot be analyzed in the same model in different stratums.

Antituberculous agents and ciprofloxacin were taken for prolonged periods of time in our patients, allowing the occurrence of profound changes in the ecosystem of the oropharynx that favored the proliferation of multiple *Candida* spp., including the resistant ones. An alternative explanation could be a selective antifungal effect of these antibiotics, as suggested by a murine model of invasive candidiasis [28] in which quinolones given in combination with fluconazole or amphotericin B prolonged survival in vivo compared to treatment with either antifungal agent alone. Nevertheless, it should be acknowledged that this is the first study in which the potential role of antituberculous therapy as a risk factor for antifungal resistance has been evaluated, and our findings need to be confirmed by further investigations.

Immunosuppression associated with tuberculosis and antituberculous therapy could also facilitate the emergence of fluconazole-resistant isolates. Nevertheless, the CD4+ cell count was not shown to be a determinant for fluconazole resistance in the present study. However, other immune mechanisms might be involved in the development of tuberculosis and fluconazole resistance. In fact, a link between mycobacterial infections and oropharyngeal candidiasis had been suggested previously [29, 30]. Moreover, in a recent report, *Mycobacterium avium* infection was found to predispose patients to oropharyngeal *Candida* infection that was refractory to fluconazole therapy [31]. It has also been recently reported that antibodies previously characterized as being candidacidal also exert a bactericidal activity in vitro against a multidrug-resistant isolate of *Mycobacterium tuberculosis*, and that even the molecules most active against *Candida albicans* were also the most active against *Mycobacterium tuberculosis* [32]. This would indirectly support the existence of a common receptor for *Candida* spp. and mycobacteria and, consequently, a common immune pathway for both infections.

Apart from the mentioned risk factors, we found different clinical predictors of fluconazole resistance. Most of them can be easily assessed during the clinical visit and provide useful information for a therapeutic decision in an individual patient. One of these factors is a low CD4+ cell count, which did not turn out to be an independent risk factor for resistance in our study, as had been stated by other authors [10, 16]. Other predictors of resistance not yet described are high IgA levels, any previous or present opportunistic infection, previous candidal esophagitis and treatment with steroids or antibiotics in the previous month. Low levels of monocytes were nearly of statistical significance. Peripheral blood monocytes have demonstrated a phagocytic activity in vitro against *Candida* spp. In fact, it has been suggested that conditions that alter monocytic function, such as steroid treatment or cytomegalovirus infection, might predispose patients to infection by yeasts [33].

Management of resistant oroesophageal candidiasis poses a challenge to clinicians because therapeutic options are limited and usually of low efficacy. Several studies have found a reduction in the prevalence and the recurrence of oropharyngeal candidiasis in patients treated with HAART [34–36]. In fact, low CD4+ cell counts are a risk factor for the development of thrush [14, 37], so it is not surprising that there is a disappearance of the mucosal lesions in parallel to the restoration of the immune function. However, advanced immunosuppression is not a determinant of resistance to fluconazole, as present and previous studies of *Candida* have demonstrated [10, 16], so clinical resolution of oropharyngeal candidiasis may be accompanied by persistent colonization by resistant strains of *Candida*. Such species might account for the development of fluconazole-resistant mucosal candidiasis when antiretroviral therapy fails [38].

Resistant isolates were common in patients with advanced HIV disease in the era previous to HAART [8, 10–12, 16]. Our study was carried out at the beginning of this era, and only a few patients were in treatment with protease inhibitors. Overall, we found a prevalence of 17% of patients carrying *Candida* isolates with reduced fluconazole susceptibility, even though many of them were asymptomatic colonized patients with no advanced HIV disease. Many of the resistant species were YOCA isolates, which have also been isolated with increasing frequency in HIV-infected and other immunocompromised patients in previous reports [8, 10, 13, 14, 39, 40]. Although their pathogenic role in oropharyngeal candidiasis has been questioned in the absence of concomitant infection with *Candida albicans* [12, 14, 41], some of these species have been recovered as single isolates from the oropharynx of patients with well-documented clinical thrush in the absence of concomitant infection with *Candida albicans* [8, 9], as verified by our study. In fact, severe infections in immunocompromised patients by such species have also been described [40, 42].

Although HAART has been documented to reduce the incidence of major opportunistic infections, a high proportion of therapeutic failures has been described in relation to the development of viral resistance and low adherence to treatment, mainly in severely immunocompromised patients [43]. In addition, an incomplete and deferred immune reconstitution despite adequate

CD4+ cell levels, which may explain the early and late development of opportunistic diseases, has been reported [3]. This proportion of failures might even increase in the future, as more experience with the new antiretroviral agents accumulates.

On the basis of our data, we conclude that previous tuberculosis or antituberculous treatment, as well as fluconazole exposure, might selectively change the mucosal flora to allow fluconazole-resistant *Candida* strains to emerge. These findings suggest a link between bacterial and *Candida* infections that warrants further investigations.

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