Infection Due to Fluconazole-Resistant Candida in Patients with AIDS: Prevalence and Microbiology

Janine R. Maenza, William G. Merz, Mark J. Romagnoli, Jeanne C. Keruly, Richard D. Moore, and Joel E. Gallant

A cross-sectional study was conducted to assess the prevalence and microbiology of oral infection due to fluconazole-resistant Candida in patients with AIDS. Oral swab specimens for fungal cultures were obtained from 100 consecutive outpatients with CD4 lymphocyte counts of <200/mm³. At least one fungal organism demonstrating in vitro resistance to fluconazole (minimum inhibitory concentration, ≥8 μg/mL) was isolated from 26 (41%) of 64 patients for whom cultures were positive. When fluconazole-resistant C. albicans was isolated, in vitro resistance correlated with clinical thrush. None of 10 patients from whom only non-albicans species of Candida were isolated had active thrush. The patients from whom fluconazole-resistant Candida albicans was isolated had lower CD4 cell counts (median, 9/mm³), a greater number of treated episodes of thrush (median, 4.5), and a greater median duration of prior fluconazole treatment (231 days) than did patients from whom fluconazole-susceptible C. albicans was isolated (median CD4 cell count, 58/mm³ [P = .004]; median number of treated episodes of thrush, 2.0 [P = .001]; and median duration of prior fluconazole treatment, 10 days [P = .01]; respectively). In a multivariate analysis, the number of episodes and duration of fluconazole therapy were independent predictors of resistance.

Mucosal candidiasis affects up to 90% of patients with AIDS [1]. With the increasing use of oral azoles as prophylaxis for and treatment of mucosal candidiasis and invasive fungal infections, infection due to azole-resistant Candida is becoming recognized as a growing problem [2–11]. While fluconazole resistance has been associated with advanced immunosuppression and prolonged exposure to azoles [4, 11, 12], fluconazole therapy has been shown to reduce the frequency of cryptococcosis, esophageal candidiasis, and superficial fungal infections [13, 14]. It is difficult to weigh the benefits of antifungal prophylaxis against the risk of drug resistance without data on the prevalence of fluconazole resistance in Candida isolates recovered from patients with AIDS.

We carried out a cross-sectional study to investigate the epidemiology of oral infection due to fluconazole-resistant Candida in HIV-infected patients. Specifically, we assessed the prevalence and microbiology of infection due to fluconazole-resistant Candida in HIV-infected patients with CD4 cell counts of <200/mm³. We also examined clinical risk factors for the development of fluconazole resistance in organisms and assessed new laboratory techniques for the detection of fungal pathogens.

Methods

Collection of clinical data. This study was conducted at the outpatient clinic for HIV infection at The Johns Hopkins Hospital. The appointment schedule was reviewed before each day’s clinic session to identify all patients with previously documented CD4 cell counts of <200/mm³. From 14 November to 5 December 1994, oral specimens for cultures were obtained from 100 consecutive outpatients meeting this CD4 cell count criterion. If thrush was present, swabs were obtained from the affected areas. If thrush was not present, swabs were taken from the tongue, buccal mucosa, and tonsillar area.

The following data were collected for each patient: presence or absence of pseudomembranous thrush, symptoms attributable to oral or esophageal candidiasis, history of opportunistic infections, and duration and dose of prior and current antifungal therapy. Durations of antifungal therapy were determined by review of patients’ medical records with use of the assumption that each day of “as needed” or “prn” use contributed 0.5 day to the calculated duration of therapy. Sensitivity analyses were also performed in which “as needed” days of therapy were weighted at 10% and 90%.

Laboratory methods. All swabs were plated on standard fungal culture medium (Sabouraud dextrose agar with 100 μg of gentamicin/mL) and on CHROMagar Candida (CHROMagar Company, Paris) also with 100 μg of gentamicin/mL. CHROMagar Candida is a differential chromogenic
growth medium: species-specific enzymes react with a substrate in the medium to produce differently colored colonies [15, 16]. Identification to the fungal species level was determined by using the germ tube test, urease activity, carbohydrate fermentation reactions, and carbon assimilation patterns. Fluconazole susceptibility assays were performed by means of the broth macrodilution method recommended by the National Committee for Clinical Laboratory Standards [17]. MICs were determined at 48 hours. Isolates for which an MIC of fluconazole was ≥8 μg/mL were considered resistant [18].

**Statistical analysis.** The χ² or Wilcoxon signed-rank test was used to determine univariate associations. A multivariate analysis was performed by using a logistic regression model in which the dependent variable was the fluconazole susceptibility of *Candida albicans* isolates. All *P* values are two-tailed. Statistical analysis was performed by means of Epi-Info Version 6 (Centers for Disease Control and Prevention [CDC], Atlanta) and the SAS system (SAS Institute, Cary, NC).

**Results**

The demographic and clinical characteristics of 100 consecutive outpatients with AIDS are shown in tables 1 and 2. All patients met the 1993 CDC definition for AIDS: 38 on the basis of the CD4 cell count criterion alone and 62 on the basis of the diagnosis of an AIDS-defining condition. Most patients (86%) had a history of oral thrush, and 28% had a history of documented or presumed candidal esophagitis. The median number of previously treated episodes of oral and/or esophageal candidiasis was 2.0 (median duration from the first diagnosis of thrush, 611 days). Pseudomembranous thrush was noted in 26% of the patients at the time that specimens for cultures were obtained. Sixty-eight patients had a history of fluconazole treatment; 37 of these patients were taking fluconazole at the time that specimens for cultures were obtained.

Cultures of specimens from 64 of the patients were positive for fungi (table 3). With use of standard culture media, *C. albicans* was isolated alone from 45 patients and in combination with another organism from five patients. Additional organisms were identified by use of CHROMagar Candida in 16 cultures yielding *C. albicans*. Organisms identified in these mixed cultures included *C. albicans* and *Torulopsis* (Candida) *glabrata*, *Saccharomyces cerevisiae*, *Candida tropicalis*, and *Candida parapsilosis*.

Non-albicans *Candida* organisms that were isolated with use of standard media included five isolates of *T. glabrata* in pure cultures and *T. glabrata* and *C. tropicalis* isolates in one mixed culture. Six cultures with CHROMagar Candida yielded *T. glabrata* only, and two cultures with CHROMagar Candida yielded *T. glabrata* and *C. tropicalis*. With use of both media, *S. cerevisiae* and *Zygosaccharomyces rouxii* were cultured alone from one patient each. Cultures of specimens from three patients for whom results of physical examinations were clinically consistent with thrush were negative for fungi.

There was a trend toward more frequent isolation of non-albicans Candida species on CHROMagar Candida than on Sabouraud dextrose agar (*P* = .06). However, no patients from whom only non-albicans Candida organisms were isolated and

<table>
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<th><strong>Table 1.</strong> Clinical characteristics of 100 consecutive outpatients with AIDS.</th>
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<td><strong>Characteristic</strong></td>
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<td>Sex</td>
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<td>Intravenous drug use</td>
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<td>Intravenous drug use and homosexual</td>
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<td>Heterosexual</td>
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<tr>
<td>Transfusion</td>
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<td>Unknown</td>
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<td>Mean (SD)</td>
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<td>Median</td>
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<td>Symptoms at the time specimen obtained for culture</td>
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<td>None</td>
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<td>Oral</td>
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<td>Oral and esophageal</td>
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<td>Esophageal</td>
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<td>Thrush noted during examination at the time specimen obtained for culture</td>
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* Unless stated otherwise, numbers are the percent of patients with the indicated characteristic.
no patients for whom only cultures with CHROMagar Candida yielded Candida albicans had clinical thrush.

Fluconazole susceptibility testing was performed for 62 of the 64 fungal isolates (figure 1). At least one organism for which the MIC of fluconazole was ≥8 μg/mL was recovered from 26 patients (or 41% of those for whom cultures were positive). Highly resistant organisms for which MICs of fluconazole were ≥64 μg/mL were isolated from 11 patients (or 17% of those for whom cultures were positive).

Overall, 31% of the C. albicans isolates tested were resistant to fluconazole (MIC, ≥8 μg/mL). At least one fluconazole-resistant organism was isolated from 22 (32%) of the 68 patients with a history of fluconazole treatment, and fluconazole-resistant C. albicans was isolated from 15 (22%) of these 68 patients. When considering only patients with thrush, 11 (48%) of 23 patients were infected with fluconazole-resistant C. albicans, 11 (48%) were infected with fluconazole-susceptible C. albicans, and 1 (4%) was infected with both fluconazole-susceptible C. albicans and fluconazole-resistant C. tropicalis.

Clinical correlates of the microbiological data are listed in table 4. Patients from whom fluconazole-resistant C. albicans and/or fluconazole-resistant non-albicans Candida species were isolated had lower median CD4 cell counts (9/mm³ and 4/mm³, respectively) than did patients from whom fluconazole-susceptible C. albicans was isolated (58/mm³) (P = .004 and P = .002, respectively). Patients from whom fluconazole-resistant C. albicans was isolated also had a larger median number of previously treated episodes of thrush and/or esophagitis than did those patients from whom no organisms or fluconazole-susceptible C. albicans was isolated (4.5 vs. 2, 0 episodes, respectively) (P = .02 and P = .001, respectively).

Durations of treatment with specific antifungal medications for all 86 patients who had a history of thrush were compared. Fluconazole was used most extensively by those patients from whom fluconazole-resistant C. albicans and both fluconazole-susceptible and -resistant non-albicans Candida species were isolated (table 4). There were no changes in the statistical significance of any of the comparisons of treatment durations when the assumption that “as needed” medications were taken 50% of the time was changed to 10% or 90% of the time.

Although an MIC breakpoint of 8 μg/mL was used to define fluconazole resistance, use of a higher breakpoint of 64 μg/mL did not change the calculated duration of fluconazole therapy for the patients from whom C. albicans isolates for which MICs were “high” were recovered. For the group of patients from whom isolates for which MICs were ≥8 μg/mL were recovered, the median duration of prior fluconazole therapy was 231 days; for those from whom isolates for which MICs were ≥64 μg/mL were recovered, the median duration was 213 days.

In a multivariate analysis, CD4 cell count, number of treated episodes of thrush and/or esophagitis, and duration of fluconazole treatment were assessed as predictors of susceptibility of C. albicans to fluconazole. CD4 cell count was not a significant predictor in this analysis when it was modeled as a continuous variable or a categorical variable. However, the number of treated episodes of thrush and/or esophagitis and the duration of fluconazole therapy were independently associated with the development of fluconazole resistance. The relative odds of resistance associated with a median of treated episodes of >4.5 was 11.9 (95% CI = 1.9, 75.9; P = .009). The relative odds of resistance associated with a median duration of fluconazole treatment of >231 days was 7.5 (95% CI = 1.4, 41.2; P = .02).

The clinical relevance of in vitro fluconazole resistance was examined by assessing patients being treated with fluconazole at the time that specimens for cultures were obtained. Isolates demonstrating in vitro fluconazole resistance were recovered from 13 patients taking fluconazole therapy when specimens for cultures were obtained (C. albicans isolates from six and only non-albicans Candida isolates from seven). Five of the six patients from whom C. albicans was isolated had clinical thrush. The sixth patient was receiving 100 mg of fluconazole per day and was asymptomatic; the isolate from this patient showed only borderline resistance (MIC = 8 μg/mL).

Of the patients who failed to respond clinically to fluconazole therapy, 2 were taking 100 mg/d (isolates with MICs of 8...
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MIC of fluconazole (pg/mL) 	 MIC of fluconazole (pg/mL)
Figure 1. A. Results of fluconazole susceptibility testing for isolates of Candida albicans that were recovered from patients with AIDS. B. Results of fluconazole susceptibility testing for isolates of non-albicans Candida species that were recovered from patients with AIDS. Torulopsis glabrata = black bars; Saccharomyces cerevisiae = cross-hatched bars; Candida tropicalis = diagonal line bar; Zygosaccharomyces rouxii = white bar; Candida parapsilosis = vertical line bar.

\( \mu g/mL \) and 64 \( \mu g/mL \), 2 were taking 200 mg/d (MICs of 16 \( \mu g/mL \) and 32 \( \mu g/mL \)), and 1 was taking 400 mg/d (MIC of 64 \( \mu g/mL \)). The median duration of prior fluconazole therapy for these patients was 279 days (range, 204–1,215 days). No specific patterns of intermittent, continuous, or “as needed” use were apparent for this group of patients. Over the following 4 months, two of these two patients were treated with intravenous amphotericin B, and the other patients had persistent thrush while receiving fluconazole therapy.

All seven patients taking fluconazole therapy (100 or 200 mg/d) from whom fluconazole-resistant non-albicans Candida species were isolated were asymptomatic. The organisms isolated were T. glabrata, alone and in combination with C. tropicalis, and S. cerevisiae. The median duration of prior fluconazole treatment for these patients was 561 days (range, 133–1,025 days).

In addition to the above-described patients with clinical thrush due to fluconazole-resistant C. albicans at the time that specimens for cultures were obtained, there were seven patients who had a history of oral and/or esophageal infection due to fluconazole-resistant Candida prior to the time of this study. Five of these patients were being treated with itraconazole capsules (200–400 mg/d), and two were receiving intravenous amphotericin B as treatment of their mucosal candidiasis.

The median CD4 cell count in this group of patients was 6/mm\(^3\) (range, 1–19/mm\(^3\)), and the median duration of prior fluconazole treatment was 339 days (range, 151–1,493 days). Of the five patients taking itraconazole capsules, three had persistent thrush (two cases in which C. albicans [fluconazole MIC = 16 \( \mu g/mL \)] was isolated, and one case in which a mixed culture yielded C. albicans [fluconazole MIC = 32 \( \mu g/mL \)] and T. glabrata [fluconazole MIC = 16 \( \mu g/mL \)]). The two asymptomatic patients were colonized with C. albicans for which the MICs of fluconazole were low (1 \( \mu g/mL \)). In vitro itraconazole susceptibility testing was not performed.

A sixth patient taking itraconazole was being treated for histoplasmosis and had no history of infection due to azole-resistant Candida. She had no thrush, and a culture of an oral specimen was sterile. Of the two patients being treated with amphotericin B, one was asymptomatic and had a sterile oral specimen; the other patient had residual thrush, and a culture of a specimen from this patient yielded fluconazole-resistant C. albicans (MIC = 64 \( \mu g/mL \)).

Discussion

This study demonstrates a high prevalence of fluconazole-resistant candidiasis in patients with advanced HIV infection. Although there are many previous reports describing infection due to fluconazole-resistant Candida, there is little information on the magnitude of this problem. In this cross-sectional analysis of patients with CD4 lymphocyte counts of <200/mm\(^3\), fluconazole-resistant organisms were isolated from fully one-quarter of patients, and fluconazole-resistant C. albicans was recovered from 16%. Clinically significant disease was seen in patients from whom fluconazole-resistant C. albicans was isolated. In almost one-half of all patients with active thrush, disease was caused by fluconazole-resistant C. albicans.

Previous studies [4, 9, 11, 12] have demonstrated that infection due to fluconazole-resistant Candida is associated with advanced immunosuppression (as reflected by lower CD4 lymphocyte counts) and with greater exposure to azole therapy.
Consistent with these findings, patients in the current study from whom fluconazole-resistant *C. albicans* was isolated had both lower CD4 cell counts and greater exposure to systemic azoles than did patients from whom fluconazole-susceptible *C. albicans* was isolated.

The relative importance of these risk factors for the development of infection due toazole-resistant *Candida* has not been well defined. However, in this study, a multivariate analysis demonstrated that CD4 cell count was not an independent predictor of fluconazole resistance, whereas duration of fluconazole use and number of treated episodes of candidiasis were independently associated with fluconazole-resistant organisms. These data suggest that the association between fluconazole resistance and low CD4 cell count may simply reflect the duration of HIV infection that is required for adequate cumulative fluconazole exposure, rather than a causal relationship between advanced immunosuppression and resistance.

The high proportion of isolation of fluconazole-resistant *C. albicans* (31% of *C. albicans* isolates) and other non-*albicans Candida* species from our patients is likely reflective of these interrelated risk factors. Specifically, the extensive use of fluconazole in the group of patients with a median CD4 cell count of only 20/mm$^3$ is likely a substantial contributor to the high prevalence of fluconazole resistance that we documented.

In comparison, Chavenet and colleagues [6] found a prevalence of microbiologically resistant *C. albicans* isolates of only 14%. However, in their study, the mean CD4 cell count was >100/mm$^3$, and fluconazole resistance was defined by an MIC$_{50}$ of 1.56 mg/L.

In the AIDS Clinical Trials Group study of antifungal prophylaxis (ACTG 981) [14], in vitro susceptibility testing was not performed; however, 11% of patients receiving fluconazole therapy had at least one episode of "breakthrough" thrush, thereby suggesting the presence of clinical resistance to fluconazole. A similar proportion of patients in our study failed to respond clinically to treatment with fluconazole: it was previously demonstrated that seven patients failed to respond clinically to treatment with fluconazole, thereby suggesting the presence of clinical resistance to fluconazole. A similar proportion of patients in our study failed to respond clinically to treatment with fluconazole: it was previously demonstrated that seven patients failed to respond clinically, and there was evidence at the time of the study that five patients failed to respond.

In addition, results of in vitro testing in our study support a correlation between microbiological resistance and clinical resistance. Although fluconazole has good bioavailability (which makes it unlikely that problems with absorption are responsible for poor clinical responses), serum azole levels would be useful to further characterize reasons for treatment failure.

The correlation between in vitro resistance and clinically significant thrush due to *C. albicans* has previously been dem-
The practice of using long-term fluconazole prophylaxis is also of concern. Since resistance has been shown to occur in the setting of prolonged exposure to systemic azoles in this study and in other studies [11, 12], it is necessary to weigh the risks of drug resistance with the benefits of antifungal prophylaxis. Although our data cannot be used to answer the question of benefit from prophylaxis vs. risk of resistance, we believe that the high prevalence of fluconazole resistance in isolates from this patient population with advanced immunosuppression should add weight to the cautionary arguments against routine antifungal prophylaxis.

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References


