

Emergence of Resistance of *Candida albicans* to Clotrimazole in Human Immunodeficiency Virus-Infected Children: In Vitro and Clinical Correlations

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Oropharyngeal candidiasis (OPC) is a common opportunistic infection in human immunodeficiency virus (HIV)-infected patients and other immunocompromised hosts. Clotrimazole troches are widely used in the treatment of mucosal candidiasis. However, little is known about the potential contribution of clotrimazole resistance to the development of refractory mucosal candidiasis. We therefore investigated the potential emergence of resistance to clotrimazole in a prospectively monitored HIV-infected pediatric population receiving this azole. Adapting the National Committee for Clinical Laboratory Standards M27-A reference method for broth antifungal susceptibility testing of yeasts to clotrimazole, we compared MICs in macrodilution and microdilution assays. We further analyzed the correlation between these in vitro findings and the clinical response to antifungal therapy. One isolate from each of 87 HIV-infected children was studied by the macrodilution and microdilution methods. Two inoculum sizes were tested by the macrodilution method (10^3 and 10^4 CFU/ml) in order to assess the effect of inoculum size on clotrimazole MICs. The same isolates also were tested using a noncolorimetric microdilution method. Clotrimazole concentrations ranged from 0.03 to 16 $\mu\text{g/ml}$. Readings were performed after incubation for 24 and 48 h at 35°C. For 62 (71.2%) of 87 clinical isolates, the MICs were low ($\leq 0.06 \mu\text{g/ml}$). The MIC for 90% of the strains tested was 0.5 $\mu\text{g/ml}$, and the highest MIC was 8 $\mu\text{g/ml}$. There was no significant difference between MICs at the two inoculum sizes. There was 89% agreement (± 1 tube) between the microdilution method at 24 h and the macrodilution method at 48 h. If the MIC of clotrimazole for an isolate of *C. albicans* was $\geq 0.5 \mu\text{g/ml}$, there was a significant risk ($P < 0.001$) of cross-resistance to other azoles: fluconazole, $\geq 64 \mu\text{g/ml}$ (relative risk [RR] = 8.9); itraconazole, $\geq 1 \mu\text{g/ml}$ (RR = 10). Resistance to clotrimazole was highly associated with clinically overt failure of antifungal azole therapy. Six (40%) of 15 patients for whom the clotrimazole MIC was $\geq 0.5 \mu\text{g/ml}$ required amphotericin B for refractory mucosal candidiasis versus 4 (5.5%) of 72 for whom the MIC was $< 0.5 \mu\text{g/ml}$ ($P = 0.001$; 95% confidence interval = 2.3 to 22; RR = 7.2). These findings suggest that an interpretive breakpoint of 0.5 $\mu\text{g/ml}$ may be useful in defining clotrimazole resistance in *C. albicans*. The clinical laboratory's ability to determine MICs of clotrimazole may help to distinguish microbiologic resistance from the other causes of refractory OPC, possibly reducing the usage of systemic antifungal agents. We conclude that resistance to clotrimazole develops in isolates of *C. albicans* from HIV-infected children, that cross-resistance to other azoles may develop concomitantly, and that this resistance correlates with refractory mucosal candidiasis.

Oropharyngeal candidiasis (OPC) is the most common opportunistic fungal infection in human immunodeficiency virus (HIV)-infected patients and other immunocompromised hosts. Clotrimazole troches are widely used in the treatment and prevention of OPC in the growing immunocompromised population.

When progressive OPC develops in HIV-infected patients, the use of clotrimazole is discontinued and systemic antifungal agents, such as fluconazole, are utilized instead. There may be several reasons for such relapses of OPC in HIV-infected patients receiving clotrimazole: low absolute CD4⁺ T lymphocytes, concomitant utilization of antibiotics, noncompliance with antifungal therapy, and emergence of resistance to clotrimazole.

Despite the extensive usage of clotrimazole in HIV-infected patients, little is known about the emergence of mi-

crobial resistance to this topically administered imidazole. While considerable attention has been directed to the emergence of resistance to fluconazole and itraconazole in HIV-infected patients, there have been virtually no studies investigating clotrimazole. Whether emergence of resistance of *Candida* species to clotrimazole or cross-resistance to other antifungal azoles develops in HIV-infected patients with refractory OPC is not known. Moreover, no previous studies, to our knowledge, correlated the in vitro activity of clotrimazole in accordance with National Committee for Clinical Laboratory Standards (NCCLS) methods for antifungal susceptibility testing (15) with the clinical response of patients with OPC.

We therefore investigated the potential emergence of resistance to clotrimazole in a prospectively monitored HIV-infected pediatric population receiving this azole. Adapting the NCCLS M27-A reference method with modifications for broth antifungal susceptibility testing of yeasts to clotrimazole, we compared macrodilution and microdilution assays and correlated the in vitro findings with clinical response to antifungal therapy.

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TABLE 1. MICs of clotrimazole ($\mu\text{g/ml}$) in quality control isolates

Parameter	Macrodilution, 48 h	Macrodilution, 48 h	Microdilution, 24 h	Microdilution, 48 h
Inoculum size (CFU/ml)	10^3	10^4	10^3	10^3
<i>Saccharomyces cerevisiae</i> ATCC 9763				
Mean MIC ($\mu\text{g/ml}$)	0.25	0.5	0.25	0.5
MIC range	0.25	0.25–0.5	0.125–0.25	0.25–0.5
No. of trials	5	5	7	7
<i>Candida albicans</i> ATCC 90028				
Mean MIC ($\mu\text{g/ml}$)	0.06	0.06	0.03	0.125
MIC range	0.03–0.06	0.06	0.03–0.06	0.06–0.25
No. of trials	4	2	3	3

MATERIALS AND METHODS

Patient population. Oropharyngeal cultures were obtained from 87 HIV-infected children. There were 56 males and 31 females with a mean age of 5.9 years (range, 0.4 to 14 years). The mean CD4 cell count was $16 \pm 2.4/\mu\text{l}$. All children had some evidence of OPC during care, and all had received clotrimazole at some point in their care. For those young children who were unable to use the troche formulation, a clotrimazole suspension prepared by the Pharmacy Department at the National Institutes of Health Clinical Center was administered. When OPC progressed despite the use of clotrimazole, either ketoconazole or fluconazole was used for treatment. If progression of OPC developed in patients receiving ketoconazole, fluconazole was administered instead. Itraconazole was seldom used because of concerns about erratic bioavailability of the capsular formulation and lack of approval of the solution for pediatrics. When OPC progressed despite administration of all available regimens of oral systemic antifungal therapy, a short course (usually 1 to 3 days) of parenteral amphotericin B was used to treat these refractory infections. Thus, patients requiring amphotericin B had OPC that was clinically resistant to clotrimazole, as well as to all available oral systemic azoles.

Antifungal agent. Clotrimazole (>99% pure) was obtained from Bayer (Wuppertal-Elberfeld, Germany) as a fluffy white powder (28). Clotrimazole is insoluble in water but dissolves in organic solvents, including ether and propylene glycol (7). A stock solution of 10,000 $\mu\text{g/ml}$ was prepared by dissolving clotrimazole powder in sterile dimethyl sulfoxide (DMSO) as described by Shadomy (25). This solution was stored in dark glass bottles at room temperature for less than 2 months. The same stock solution was used for all of the experiments in this study.

Test organisms. Eighty-seven isolates of *Candida albicans*, each from the oral cavity of a different HIV-infected child, were collected prospectively at the National Cancer Institute in the Warren Grant Magnuson Clinical Center at the National Institutes of Health in Bethesda, Md. Using standard methods (30), each isolate was identified as *C. albicans* by the Clinical Microbiology Laboratory of the NIH Clinical Center. These isolates were stored subsequently on potato dextrose agar slants at -70°C . Quality control organisms included *C. albicans* ATCC 90028 and *Saccharomyces cerevisiae* ATCC 9763. The latter isolate was used in previous studies with clotrimazole (25). The quality control analysis for the two isolates is presented in Table 1.

Test medium. RPMI 1640 medium with L-glutamine, without sodium bicarbonate, and buffered with morpholinopropanesulfonic acid (MOPS) at 0.165 M (BioWhittaker, Inc., Walkersville, Md.) (RPMI) was used in this experiment as recommended by the NCCLS standard (15).

Drug dilution. The test concentrations of clotrimazole chosen were serial twofold dilutions of 0.03 to 16 $\mu\text{g/ml}$ in order to encompass the previously reported MIC for 90% of the isolates of *C. albicans* tested (MIC_{90}) (3, 8, 19, 24–26). In brief, a stock solution (10,000 $\mu\text{g/ml}$; 100% DMSO) was first diluted 1:6.25 with pure water to obtain an intermediate solution (160 $\mu\text{g/ml}$; 1.6% DMSO). This intermediate solution was then diluted with RPMI in twofold steps to obtain the desired 10-fold final concentration set for the macrodilution method. In the second part of the study (microdilution or plates), we diluted first the same intermediate solution (160 $\mu\text{g/ml}$) 1:5 with pure water to produce the first of the two times the final desired concentration (32 $\mu\text{g/ml}$; 0.32% DMSO). Then, from the latter solution, we proceeded to twofold dilution using RPMI to obtain the twofold serial solution for the microdilution studies.

Inoculum preparation. Prior to testing, all isolates were subcultured at least twice on Sabouraud glucose agar plates to ensure purity. Five colonies ≥ 1 mm in diameter from a 24- to 48-h growth at 35°C were suspended in sterile 0.85% saline and then adjusted spectrophotometrically to produce a 0.5 McFarland standard density giving a 1×10^6 to 5×10^6 -CFU/ml suspension. This suspension was first diluted 1:10 with a sterile 0.025% Tween 20 solution in 0.85% saline water to ensure optimal dispersion. This is not a component of the NCCLS methodology but in these studies appeared to enhance optical optimization of the inoculum preparation. The suspension was then further diluted at 1:200 for the macrodilution method and 1:100 for the microdilution method with RPMI

1640 in order to obtain final inoculum sizes of 0.5×10^3 to 2.5×10^3 CFU/ml, respectively. A macrodilution method was also used to test the effect of inoculum size using the solution diluted 1:10 to obtain inoculum sizes of 1×10^4 to 5×10^4 CFU/ml. Inoculum size was further verified by quantitative subculturing on Sabouraud glucose agar plates.

Microdilution. For each isolate, a series of sterile polystyrene plastic tubes (12 by 75 mm; Falcon 2058; Becton Dickinson, Lincoln Park, N.J.) containing 0.1 ml of each clotrimazole dilution was inoculated with 0.9 ml of the inoculum suspension and then carefully mixed. A separate tube with 0.1 ml of RPMI 1640 without clotrimazole was also inoculated at the same time to serve as the growth control. A series of uninoculated clotrimazole tubes was incubated with each experiment to verify the sterility of drug dilutions. The contents of the tubes were mixed and incubated in room air at 35°C for 48 h and then vortexed before the endpoint was read. The MIC of an azole drug is the lowest concentration that inhibits at least 80% of the control growth; thus, each tube was compared with a 1:5 dilution of the growth control tube and uninoculated RPMI.

Microdilution. Sterile flat-bottom 96-well cell culture plates (Costar 3596; Cambridge, Mass.) were used. We filled each of the first 10 columns of the plates with 100 μl of a different clotrimazole solution, dispensing the highest concentration of the drug into the first column and the lowest concentration of the drug into column 10. One hundred microliters of RPMI was dispensed into column 11 to serve as the growth control, and 200 μl of uninoculated RPMI was dispensed into column 12 for the sterility control of the growth media. All isolates were dispensed in duplicate into two consecutive rows with 100 μl of the appropriate inoculum suspension, from column 1 to column 11. Plates were incubated in air at 35°C without agitation and read at 24 and 48 h. The plates were visually inspected with the aid of a reading mirror (Dynatech Microtiter system), and each well was scored using the following criteria: 4+, bottom of well entirely covered; 3+, more than 50% but less than entirely covered; 2+, less than 50% covered; 1+, only small particles; 0, clear. The MIC was defined as the lowest concentration to give a score of $\leq 2+$.

Definitions. High off-scale MICs ($>16 \mu\text{g/ml}$) were converted to the next highest concentration (32 $\mu\text{g/ml}$), and low off-scale MICs ($\leq 0.03 \mu\text{g/ml}$) were converted to 0.03 $\mu\text{g/ml}$. Results obtained with the macrodilution method at 48 h (inoculum of 0.5×10^3 to 2.5×10^3 CFU/ml) served as our reference standard of comparison to the other methods. We used the term "agreement" to describe MICs that were within 1 twofold dilution of the reference MIC. Resistant OPC in the study population was defined as OPC occurring in those symptomatic patients who received clotrimazole in standard doses four or five times per day, who had documented compliance, and who, despite these measures, required the administration of amphotericin B to treat infection resistant to alternative oral antifungal agents (nystatin, ketoconazole, fluconazole, and itraconazole).

Statistics. Fisher's exact test was used to analyze the relationship between elevated clotrimazole MICs and clinical resistance to antifungal medications. A paired *t* test (with a two-tailed value) was used to compare MICs obtained by different methods. A *P* value of <0.05 was considered to be significant. Correlation between the MICs from different methods was determined by Spearman's correlation coefficient (*r*).

RESULTS

Eighty-seven isolates of *C. albicans* were analyzed for susceptibility to clotrimazole. For 71% of the isolates, the MICs were very low ($\leq 0.06 \mu\text{g/ml}$) (Fig. 1). The MIC_{90} was 0.5 $\mu\text{g/ml}$. Inoculum sizes ranging from 10^3 to 10^4 CFU/ml did not affect MIC results obtained employing the macrodilution method (Fig. 2). Ninety-three percent of the MICs (± 1 dilution) obtained by using inocula of 1×10^4 to 5×10^4 CFU/ml agreed with the results of the reference method obtained with inocula of 0.5×10^3 to 2.5×10^3 CFU/ml ($r = 0.914$).

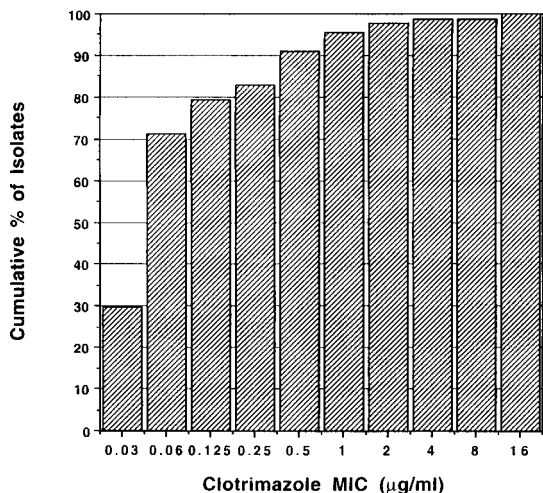


FIG. 1. Cumulative MICs of clotrimazole against *C. albicans*.

Comparison of the macrodilution reference method with the microdilution method showed good (88.5%) agreement for the results read at 24 h, with no significant difference ($P = 0.09$) (Fig. 3, upper panel). However, when the readings were taken at 48 h, the agreement was poor (54%) and the difference was statistically significant ($P < 0.0001$) (Fig. 3, lower panel).

Among the isolates used in this study, 10 were recovered from patients who fulfilled the definition of clinical resistance (Fig. 4). For 6 of these 10, the MIC of clotrimazole was ≥ 0.5 µg/ml, compared to the remaining 4 of 72 isolates, for which the MICs were < 0.5 µg/ml ($P = 0.001$) (Table 2). The relative risk (RR) that an isolate for which the MIC was ≥ 0.5 µg/ml would be recovered from a patient clinically resistant to oral azole therapy was 7.2 (95% confidence interval [CI], 2.3 to 22.4).

Isolates used in this study were previously tested for suscep-

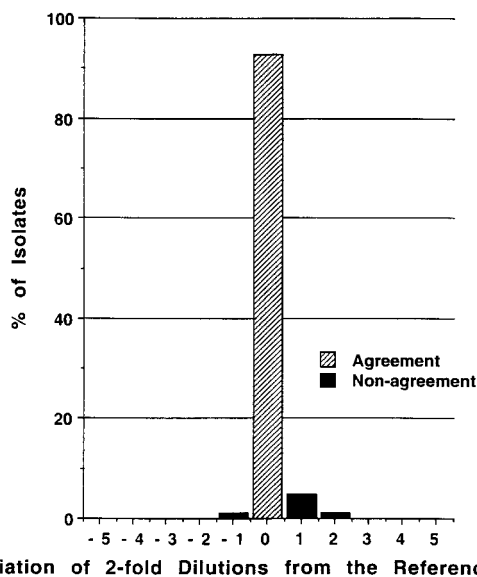


FIG. 2. Percentage of agreement (hatched) and magnitude of nonagreement (black) comparing an inoculum size of 10^4 CFU/ml to the standard inoculum size of 10^3 CFU/ml by the reference macrodilution method.

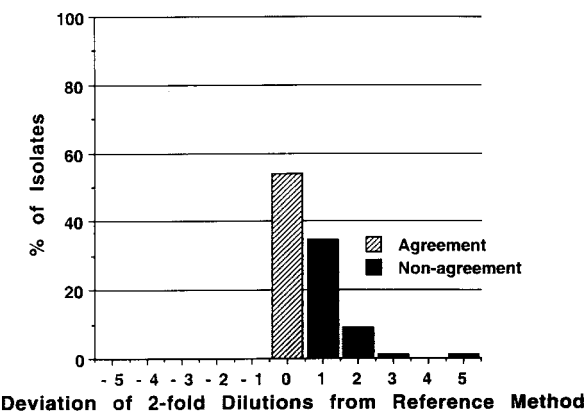
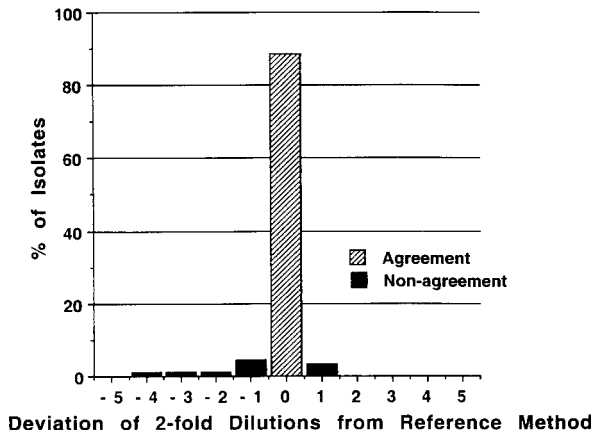


FIG. 3. Percentage of agreement (hatched) and magnitude of nonagreement (black) comparing the reference macrodilution method at 48 h of incubation with the reference microdilution method (10^3 CFU/ml) at 24 h of incubation (top panel) and 48 h of incubation (bottom panel).

tibility to fluconazole and itraconazole using the NCCLS macrodilution method (T. J. Walsh, S. Chanock, J. Peter, A. Francesconi, M. Kasai, T. Sein, S. Piscitelli, D. Sanglard, and P. A. Pizzo, Program Abstr. Am. Soc. Microbiol. Fifth Conf. Candida Candidiasis, abstr. B40). Among the patients fulfilling the definition of clinical resistance, we also found a significantly ($P = 0.03$) increased risk that other azole drugs would have an elevated MIC: fluconazole, ≥ 64 µg/ml (RR, 4.7; 95% CI, 1.5 to 14.1); itraconazole, ≥ 1.0 µg/ml (RR, 11.25; 95% CI, 3.8 to 33.1).

Significant cross-resistance developed between clotrimazole and other azoles ($P \leq 0.001$). In a comparison of isolates for which the clotrimazole MICs were ≥ 0.5 µg/ml to other azole MICs, for 6 (46%) of the 13 tested isolates the fluconazole MICs were ≥ 64 µg/ml (RR, 8.9; 95% CI, 4.2 to 19.2) and for 7 (58%) of 12 the itraconazole MICs were ≥ 1 µg/ml (RR, 10; 95% CI, 3.9 to 25.7) (Table 3).

DISCUSSION

Clotrimazole is a tritylimidazole derivative [bis-phenyl-(2-chlorophenyl)-1-imidazolylmethane]. Initially developed by Bayer in 1968, it was the first commercially available azole antifungal drug (8, 19, 25). Released in 1975 as a topical antifungal agent, clotrimazole has been a well-tolerated and frequently admin-

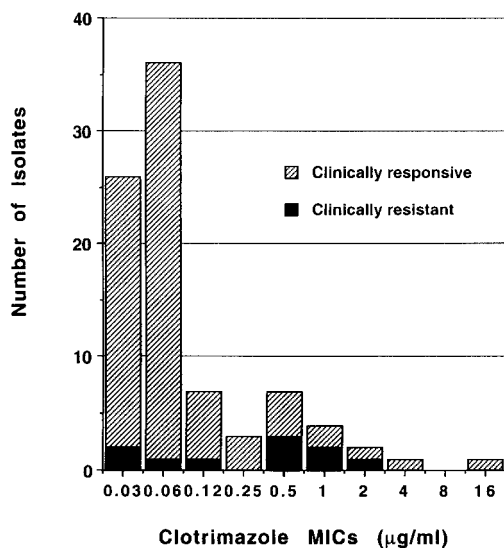


FIG. 4. Clinical resistance of isolates causing OPC and distribution of clotrimazole MICs for clinically responsive isolates (hatched bars) and clinically resistant isolates (black).

istered drug for mucocutaneous candidiasis. Clotrimazole is approved for both treatment and prevention of OPC.

Initial *in vitro* studies of clotrimazole used various methodologies (3–5, 8, 10, 16, 18, 19, 25, 26). Previous testing of the antifungal activity of clotrimazole disclosed several difficulties. As clotrimazole is highly insoluble in water, the drug must first be dissolved with an organic solvent such as dimethylformamide (3), ethanol (4, 6), chloroform, polyethylene glycol (8), or DMSO (10, 16, 25, 26). Further dilution of the drug can be completed by using an aqueous growth medium. Hoepflich and Huston demonstrated that using undefined growth media may affect the MICs of azoles such as clotrimazole and miconazole (5). As recommended by NCCLS standard M27-A, the use of a defined growth medium, such as RPMI, could obviate this problem. Inoculum size also was previously reported to influence MICs of clotrimazole (19, 25). However, in our studies using two separate inoculum sizes (10^3 and 10^4 CFU/ml), we were unable to find any significant difference between the MICs. Nonetheless, we cannot exclude the possibility that a substantially greater inoculum size would yield different MICs. Using the macrodilution method adapted from the NCCLS, we obtained a range of MICs and MIC₉₀s for *C. albicans* isolates similar to that reported in previous studies, which used different methods (3–5, 8, 10, 16, 18, 19, 25, 26). We also obtained similar results using a microdilution method after 24 h of

incubation. Since the completion of this study, Torres-Rodríguez et al. described the MICs of eberconazole, a new topical imidazole, versus clotrimazole and ketoconazole against a panel of yeast isolates consisting of *Candida* species and *Cryptococcus neoformans* using the NCCLS microdilution method with 2% glucose (27). The MIC₅₀ and MIC₉₀ of clotrimazole against *C. albicans* in the broth microdilution assay in that study ranged between 0.03 and 0.08 µg/ml, somewhat lower than those we observed in this study. However, a report on a recent survey employing NCCLS methodology without 2% glucose describes a geometric mean MIC of 2.1 µg/ml for a panel of azole-resistant *C. albicans* isolates (D. A. Stevens, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. F-125). This geometric mean MIC is consistent with the MICs of the resistant isolates reported here.

The use of clotrimazole has grown substantially since its discovery in 1968. Far from its initial limited use as a systemic antifungal agent, clotrimazole is now utilized extensively as a front-line topical antifungal for prevention or treatment of mucosal candidiasis in immunocompromised patients. While many studies were performed assessing the efficacy of clotrimazole for different uses over the past 3 decades, we examined clotrimazole in the contemporary context of emerging azole resistance. Patients with AIDS present quite a different venue for clotrimazole. Chronic and repetitive use of antifungal azoles to treat protracted OPC in HIV-infected patients predisposes to the development of resistance. The appearance of secondary azole resistance to this date has been demonstrated most commonly in HIV-positive patients who previously received fluconazole (14, 21).

Resistance of *C. albicans* to clotrimazole has not been previously well documented. Using different methods for antifungal susceptibility testing, neither Hamilton nor Holt found primary resistance to clotrimazole in *C. albicans* (4, 7). Holt and Azmi reported one case of *C. albicans* resistance to clotrimazole, miconazole, and econazole emerging in a pediatric patient previously treated for 2 months with miconazole for a chronic bladder infection (9). Lucatorto et al. described one case of an HIV-infected man developing clinical resistance during treatment of OPC with clotrimazole. This isolate of *C. albicans* showed decreased *in vitro* susceptibility to clotrimazole in a relative inhibition assay (13).

Therapeutic failure or recurrence of OPC can be attributed to the appearance of a different and more resistant *Candida* species but is more likely to be a consequence of antifungal resistance arising in the same isolate of the same species of *C. albicans* over the course of treatment (1, 2, 14, 29, 31; A. Vuffray, C. Durussel, P. Boerlin, P. F. Boerlin, J. Bille, M. P. Glauser, and J. P. Chare, Letter, AIDS 8:708–709, 1994). In addition to induction of azole resistance, other reasons for the lack of therapeutic response to clotrimazole include declining host response to *Candida* and antibacterial selection pressure caused by administration of broad-spectrum antibiotics.

The ability to determine MICs of clotrimazole may help to distinguish among the different causes of refractory OPC. Demonstration of a susceptible isolate could reduce the need for a systemic antifungal azole, thus reducing the risk of drug interactions and the development of resistance to these valuable compounds.

At present, there are no established interpretive breakpoint criteria by which to designate a *Candida* isolate as either susceptible or resistant to clotrimazole. Interpretive breakpoint criteria have been defined for fluconazole and itraconazole against *Candida* spp. (20). Logically, we must label an isolate as resistant to a drug if its MIC exceeds the amount of the drug attainable in the infected tissue of a patient. However, the

TABLE 2. Relationship between *in vitro* resistance to clotrimazole and refractory mucosal candidiasis^{a,b}

Patient group	No. (%) for which clotrimazole MICs were:		Total no. (%)
	≥0.5 µg/ml	<0.5 µg/ml	
Amphotericin B treated	6 (7)	4 (5)	10 (11)
Amphotericin B untreated	9 (10)	68 (78)	77 (89)
Total	15 (17)	72 (83)	87 (100)

^a The RR that an isolate with an MIC ≥ 0.5 µg/ml would be recovered from patient clinically resistant to oral azole therapy is 7.2 (95% CI: 2.3 to 22.4).

^b Fisher's exact test two-sided *P* value = 0.0014.

TABLE 3. Relationship between in vitro resistance to clotrimazole and cross-resistance to fluconazole and itraconazole

Group	No. (%) with clotrimazole MIC of:		% RR	95% CI	P value
	≥0.5 µg/ml	<0.5 µg/ml			
Isolates with fluconazole MICs of ≥64 µg/ml	6 (46)	7 (54)	8.9	4.2–19.2	≤0.001
Isolates with itraconazole MICs of ≥1.0 µg/ml	7 (58)	5 (42)	10.0	3.9–25.7	≤0.001

correlation between the antifungal activity of in vitro levels and levels of the drug in tissue is often variable. Clotrimazole troches, for example, may achieve salivary concentrations of 5.2 to 15 µg/ml for as long as 3 h after dissolution (25). Factors such as protein binding, physiologic temperature, and local pH and osmolality may alter the antifungal activity of the same drug concentration in vitro and in vivo. Correlation between in vitro concentrations and clinical response is a key variable in determining breakpoint criteria.

We found that for at least 80% of the isolates taken from a prospectively monitored HIV-infected population, the clotrimazole MICs were ≤0.25 µg/ml. Ninety-four percent of patients for whose isolates the drug demonstrated these lower MICs were treated successfully with azole antifungal drugs. In this study, a clinically resistant isolate was defined as one taken from the oral cavity of a symptomatic patient that required amphotericin B due to the failure of all other antifungal agents, including clotrimazole, nystatin, ketoconazole, fluconazole, and, where applicable, itraconazole. Assessment of each patient as having been unresponsive to antifungal therapy, and hence requiring amphotericin B, was made through consensus among the members of the clinical care team. This stringent criterion of failure obviated any doubt about clinical unresponsiveness. For those isolates that were classified as being obtained from clinically resistant cases, the MICs were significantly more likely to be ≥0.5 µg/ml ($P = 0.001$; 95% CI = 2.3 to 22; RR = 7.2). This clotrimazole MIC of ≥0.5 µg/ml suggests a possible interpretive resistance breakpoint.

This discrepancy between the higher achievable clotrimazole levels in the oral cavity when using troches and the lower MIC defining clinical resistance suggests that the measured clotrimazole level in saliva may not reflect the free and active part of the drug if, for example, clotrimazole was bound to protein in the saliva.

Whether resistance to clotrimazole develops as a consequence of previous exposure to clotrimazole itself or to other azole drugs as a cross-resistance phenomenon is not known. Sangeorzan et al. (23) studied an HIV population randomly using clotrimazole and fluconazole as the prophylactic treatment for OPC. Fluconazole MICs did not increase for isolates cultured from patients taking clotrimazole, whereas MICs of fluconazole increased for those from patients using fluconazole. These findings suggest that clotrimazole was a less potent inducer of azole resistance than was fluconazole. Nevertheless, cross-resistance could not be excluded because clotrimazole MICs were not determined for *C. albicans* isolates. By comparison, we found cross-resistance between clotrimazole and other azoles in the same isolate of *C. albicans*.

We conclude that resistance to clotrimazole develops in isolates of *C. albicans* from HIV-infected children, that cross-resistance to other azoles develops concomitantly, and that this resistance correlates with refractory mucosal candidiasis. The clinical laboratory's ability to determine MICs of clotrimazole may help to distinguish among the different causes of refractory OPC and possibly reduce the usage of systemic antifungal agents. Emergence of resistance of *C. albicans* to clotrimazole

may be a key link in the sequence of events leading to refractory OPC in HIV-infected patients.

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