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Non-*albicans* oral candidosis in HIV-positive patients

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Specimens from HIV-positive patients with oral candidosis were taken for culture, species identification and azole susceptibility testing, which was correlated with treatment outcome. Of 921 specimens, 95 yielded non-*albicans* species, mainly from patients with low CD4 lymphocyte counts and extensive previous azole exposure. Most non-*albicans* isolates were from specimens co-infected with *Candida albicans*, complicating the interpretation of in-vitro susceptibility results, which accurately predicted antifungal failure when the non-*albicans* species was isolated alone. Eighty-five non-*albicans* isolates were resistant to fluconazole *in vitro*. Of 149 courses of azole therapy prescribed, 115 failed to clear non-*albicans* candidosis clinically. Culture media that discoloured in the presence of non-*albicans* colonies might, therefore, guide therapy.

Introduction

Oral candidosis frequently occurs in HIV infection and initially is easily treated with azole antifungals.[1,2] However, later in disease, failure of standard azole therapy is not uncommon in fluconazole-treated patients. Clinically resistant candidosis is most commonly associated with *Candida albicans* infection but other species have been isolated. Although some of these non-*albicans* species have reduced azole susceptibility *in vitro*, their pathogenic importance has been questioned;[3] their significance would be clarified if responses to treatment of patients infected with these organisms could be correlated to their in-vitro susceptibility. We have reviewed our patients with candidosis yielding non-*albicans* species, determined the in-vitro antifungal susceptibilities of these organisms and related this to clinical response. The susceptibility test chosen[4] has been found accurately to predict clinical fluconazole,[5] itraconazole[6] and ketoconazole[7] failure for HIV-positive patients infected with *C. albicans.*

Materials and methods

HIV-positive patients with oral candidosis gave mouth swabs and rinses for culture, species identification and susceptibility testing. Patients were treated with a 7 day standardized azole regime (ketoconazole 200 mg bd, itraconazole solution 200 mg bd, or fluconazole 100 mg od) and the outcome was assessed on day 7, classified as either success (clearance of all signs and symptoms) or failure (where there was persistent candidosis). Patients receiving hepatic enzyme inducers were excluded from this analysis. Where patients failing after a 7 day course of one azole were given a different agent, the susceptibility of the original isolate was correlated with the result of the second agent also.

Mycology

Species identification. Clinical specimens were inoculated on to Sabouraud dextrose agar and incubated for 48 h at 37°C; sample colonies of each morphology present were selected and identified at species level using commercially available API kits (BioMérieux, Basingstoke, UK), examination for chlamydospore production on Rice Tween Agar (BioMérieux) medium and assessment of germ tube production in horse serum.

Susceptibility testing. The relative growth method of azole susceptibility testing, developed by Odds,[7] was employed. In three previous studies response to standardized azole treatment (as above) has been correlated with in-vitro susceptibility to establish the degree of resistance for *C. albicans* that resulted in failure of a particular drug at the standard dose. Having established the best cut-off points for predicting treatment failure for *C. albicans* (relative growth of 80% for fluconazole, 68% for itraconazole and

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75% for ketoconazole) it was possible in the current study to assess whether the same degree of in-vitro resistance carried the same clinical consequences with non-*albicans* species. Where the non-*albicans* isolate was mixed with *C. albicans* on the plate, the relative growth of the non-*albicans* isolate was used in the assessment of the test's predictive value.

Results

Of our 921 clinical isolates identified at species level, 526 (57%) were fluconazole-susceptible *C. albicans* and 300 (32%) were fluconazole-resistant *C. albicans*. Ninety-five (10%) clinical specimens from 52 HIV-positive patients with pseudomembranous candidosis yielded non-*albicans* isolates. A total of 97 non-*albicans* isolates were grown, as two species yielded a mixture of two different non-*albicans* species. *Candida glabrata* was the commonest non-*albicans* species isolated, followed by *Candida krusei* (Table I). Most of the specimens yielding a non-*albicans* isolate also grew *C. albicans*.

Patient characteristics

All the patients infected with non-*albicans* species had low CD4 counts (median, 15 cells/mm^3; range, 2–230 cells/mm^3), and extensive previous azole treatment for recurrent candidosis (median, 24 months; range, 2–94 months). The majority of non-*albicans* species were isolated from patients with clinical azole resistance and <3 months after first fluconazole failure.

Correlation of in-vitro susceptibility results and clinical response

Twenty-five treatment episodes were assessed for patients whose specimens yielded a pure growth of a non-*albicans* species, and in 23 of these cases the in-vitro susceptibility correctly predicted clinical response, achieving a sensitivity of 90% and specificity of 100%. Most specimens, however, yielded a mixed infection, often with discordant susceptibility to the treatment used. If the susceptibility of the non-*albicans* isolate was used to predict outcome for all treatment episodes, including those where mixed infections with discordant susceptibility were isolated, the sensitivity of the test was 93% and the specificity 76%.

All 57 *C. glabrata* isolates were resistant to fluconazole and ketoconazole *in vitro* and 12 (22%) were cross-resistant to itraconazole. In general, clinical response followed that suggested by the in-vitro susceptibility result, with 90 of the 95 treatment episodes for patients infected with *C. glabrata* having the outcome predicted by the susceptibility of that organsism (Table II). Of the 79 treatment episodes where *C. glabrata* was present as part of a mixed infection, the in-vitro susceptibility of the *C. glabrata* isolate accurately predicted clinical response in 75 cases. In 22 cases there was disagreement between the susceptibility of the *C. glabrata* isolate and the co-infecting *C. albicans* isolate—19 where the former was resistant and the latter susceptible, and three with the reverse situation—clinical outcome followed the result of the *C. glabrata* in 18 of these 22 cases.

All *C. krusei* isolates were resistant to fluconazole and ketoconazole but susceptible to itraconazole *in vitro*. Fluconazole failed in 16 of 20 patients infected with *C. krusei*, all four where it did work being co-infected with fluconazole-susceptible *C. albicans*. Itraconazole solution was successful in all four infected with *C. krusei* who took it (Table II).

Only two specimens yielded pure cultures of other non-*albicans* species, *Candida tropicalis* and *Candida guilliermondi*, both with resistance to fluconazole *in vitro* and both from fluconazole-unresponsive patients (Table II).

Seven isolates of *Saccharomyces* spp. were resistant to fluconazole *in vitro*, five to ketoconazole and two to

Table I. Frequency of isolation of each non-*albicans* species from clinical specimens

Species	No. of specimens yielding species	No. of individual patients from whom species was obtained
C. glabrata	54 (17* + 37)	30 (12* + 18)
C. krusei	20 (2* + 28)	8
Saccharomyces spp.	10	7
C. tropicalis	6 (1*+5)	3
Candida kefyr	2	1
Candida lusitaniae	1	1
Candida norvagensis	1	1
C. guillermodi	1*	1*
Total	96	52

In the majority of cases the non-*albicans* species was obtained from a specimen that also yielded *C. albicans* except where indicated by an asterisk, where the non-*albicans* species was isolated alone.

Table II. Treatment outcome of patients with pure or mixed non-*albicans* infection (figures for pure alone are in brackets) with non-*albicans* species resistant (R) or susceptible (S) *in vitro* to the antifungal given

	C. glabrata		*C. krusei*		*Saccharomyces*		*C. tropicalis*		*C. kefyr*		*C. norvagensis*		*C. guillermondii*		*C. lusitaniae*	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Fluconazole 100 mg/day for 7 days																
number treated	50 (12)	0	20 (1)	0	7	2 (1)	3 (1)	1	0	2	1	0	1 (1)	0	0	0
number cleared	4 (0)	–	4	–	0	1 (1)	0	1	–	2	0	–	0	–	–	–
number failed	46 (12)	–	16 (1)	–	7	1	3 (1)	0	–	0	1	–	1 (1)	–	–	–
Itraconazole solution 200 mg/day for 7 days																
number treated	10 (3)	16 (5)	0	4	1	4 (1)	0	1	0	1	0	0	0	0	0	1
number cleared	0	1 (1)	–	4	0	0	–	1	–	1	–	–	–	–	–	0
number failed	10 (3)	15 (4)	–	0	1	4 (1)	–	0	–	0	–	–	–	–	–	1
Ketoconazole 200 mg bd for 7 days																
number treated	19	0	2	0	1	0	0	2	0	0	0	0	0	0	0	0
number cleared	0	–	0	–	0	–	–	2	–	–	–	–	–	–	–	–
number failed	19	–	2	–	1	–	–	0	–	–	–	–	–	–	–	–

itraconazole. All patients treated with a drug to which the *Saccharomyces* strain was resistant failed therapy, including four cases where the patient was co-infected with a susceptible *C. albicans* isolate (Table II). Of the six isolates of *C. tropicalis*, three were fully susceptible to azoles *in vitro* and the remaining three (all resistant to fluconazole *in vitro*) were from fluconazole-unresponsive patients (Table II).

Discussion

It has been suggested that, in the context of HIV-related oral candidosis, isolates of non-*albicans* species of *Candida* are commensals, rather than pathogens and that their isolation is of no clinical relevance.[3] All the isolates in our study were obtained from patients with symptomatic pseudomembranous oral candidosis, and in 25 cases a non-*albicans* species was the only fungus grown. In 23 of these 25 cases the patient's response was accurately predicted by the in-vitro azole resistance profile of the non-*albicans* isolate, further supporting the clinical significance of these organisms. Furthermore, in 31 of 39 cases where a resistant non-*albicans* isolate was cultured together with a susceptible *C. albicans* isolate, the patient failed to respond.

Ten percent of the specimens obtained from patients attending our candida clinic yielded a non-*albicans* species, though since referral to the clinic was more likely if the patient had unresponsive candidosis, this may overestimate the prevalence of these organisms. Of the 100 non-*albicans* isolates, 88 were fluconazole-resistant, which represented almost one quarter of the total fluconazole-resistant *Candida* isolates obtained from our whole cohort. The HIV-positive patients infected with non-*albicans* species of *Candida* were severely immunosuppressed, had long histories of recurrent candidosis requiring treatment and, in many cases, had candidosis unresponsive to standard azole therapy.

C. glabrata was the commonest non-*albicans* species isolated, then *C. krusei*, *Saccharomyces* spp. and *C. tropicalis* (Table I). With regard to individual species, our findings would support a pathogenic role for *C. glabrata* and *C. krusei,* which were detected as pure infections in some of our cases and, when mixed with susceptible *C. albicans* isolates, were associated with non-response in keeping with their resistance *in vitro* to the treatment used.

Pure cultures of *C. tropicalis* and *C. guillermondi* were isolated from single patients, suggesting a possible, though uncommon, role for these organisms in AIDS-related candidosis. Also, where resistant isolates of *Saccharomyces* spp. were mixed with susceptible *C. albicans*, treatment failure was observed in four cases, suggesting that this organism might also be clinically relevant.

The pathogenic role of these non-*albicans* species can only be suggested from our data, since our clinical

specimens may not have detected all of the isolates present and pathogenic *C. albicans* isolates may have been missed. However, both *C. glabrata* and *C. krusei* have been associated with clinical disease in other patient populations.[8,9] Even if the presence of non-*albicans* species is not causally related to treatment failure, their detection predicts non-response to standard azole therapy. The use of culture media which readily identify non-*albicans* species by differences in colony colour (e.g. Chromagar (Becton Dickinson, Oxford, UK)) could assist clinical decision-making since 85 of our 95 non-*albicans* isolates were fluconazole-resistant *in vitro.* In centres with the potential for antifungal susceptibility testing, the relative growth method[4] appears applicable to non-*albicans* isolates as well as *C. albicans* with the same cut-off values being predictive of clinical failure of standard azole treatment regimen.

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