MYCOSES 41, 203–211 (1998) ACCEPTED: DECEMBER 10, 1997

Cross-sectional study of oral *Candida* carriage in a human immunodeficiency virus (HIV)-seropositive population: predisposing factors, epidemiology and antifungal susceptibility

Querschnittstudie über orale *Candida*-Besiedlung in der HIV-seropositiven Bevölkerung: Prädispositionsfaktoren, Epidemiologie und Antimyzetika-Empfindlichkeit

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Key words. *Candida*, HIV infection, epidemiology, predisposing factors, azole resistence, susceptibility testing.

Schlüsselwörter. *Candida*, HIV-Infektion, Epidemiologie, Prädispositionsfaktoren, Azolresistenz, Empfindlichkeitsprüfung.

Summary. The *Candida* species isolated from oral rinses of 130 human immunodeficiency virus (HIV) infected patients were compared with those of 130 healthy non-matched volunteers. The oral rinses were plated on CHROMagar *Candida* medium (CAC) and on CAC supplemented with 10 μg (CF10) and 100 μg (CF100) of fluconazole per ml. The prevalence of non-*albicans Candida* spp. in oral rinses of HIV-infected patients and their correlation with the clinical and epidemiological characteristics of the patients were studied. Susceptibility of the *Candida* spp. isolated was determined by a microbroth dilution method based on the NCCLS reference procedure. Results of susceptibility tests of the yeast isolates were compared with their growth at the time of isolation on CAC supplemented with fluconazole. Thirty-five (30.7%) strains of non-*albicans Candida* spp. were isolated from the HIV-positive population, *vs.* seven (15.9%) from the immunocompetent population. Growth on CF10 correlated in 96% of the cases with fluconazole minimum inhibitory concentration (MIC) >8 μg ml^{-1}. Smoking and use of azoles were significantly associated with oral carriage of non-*albicans Candida* spp. ($P<0.05$). The prevalence of non-*albicans Candida* spp. in HIV-positive persons in oral rinse samples is twice as high as in the HIV-negative population. Smoking and treatment with azoles are risk factors for the oral carriage of non-*albicans Candida* spp. The isolation of yeasts on CAC plates supplemented with fluconazole allows combination of presumptive yeast identification and fluconazole susceptibility testing.

Zusammenfassung. Die *Candida*-Isolate aus den Mundspülproben von 130 HIV-infizierten Personen wurden mit denen von 100 gesunden, unausgelesenen Freiwilligen verglichen. Die Mundspülproben wurden auf CHROMagar *Candida* (CAC) und auf CAC mit 10 μg (CF10) und 100 μg (CF100) Fluconazol pro ml angelegt. Die Häufigkeit von Nicht-*albicans Candida*-Stämmen in den Mundspülproben der HIV-Infizierten und ihre Korrelation mit den klinischen und epidemiologischen Besonderheiten der Patienten wurde erfaßt. Die Empfindlichkeit der *Candida*-Isolate wurde im Mikrodilutionstest gemäß NCCLS-Standard bestimmt. Die Ergebnisse der Empfindlichkeitsprüfung wurden mit ihrem Wachstum zum Zeitpunkt der Isolierung auf CAC mit Fluconazol-Zusatz verglichen. In der HIV-positiven Population wurden 35 Nicht-*albicans Candida*-Stämme (30.7%) gefunden gegenüber 7 Stämmen (15.9%) bei der immunkompetenten Vergleichsgruppe. Das

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Wachstum auf CF10 korrelierte in 96% der Fälle mit der Fluconazol MHK $>8\ \mu g\ ml^{-1}$. Rauchen und Azol-Medikation waren signifikant mit oraler Besiedlung durch Nicht-*albicans Candida*-Arten assoziiert ($P<0.05$). Die Prävalenz von Nicht-*albicans Candida*-Arten in Mundspülproben HIV-positiver Personen war zweimal so hoch wie die in der HIV-negativen Population. Rauchen und Azol-Behandlung sind Risikofaktoren für die orale Besiedlung mit Nicht-*albicans Candida*-Arten. Die Hefeisolierung auf CROMagar, supplementiert mit Fluconazol, erleichtert die Hefeidentifizierung und die Empfindlichkeitsprüfung für Fluconazol.

Introduction

Oropharyngeal *Candida* infection is the most common opportunistic disease in individuals infected with the human immunodeficiency virus (HIV) [1]. In recent years, the incidence of infections apparently involving multiple yeast species is increasing, and changing trends in the epidemiology of oral *Candida* infections have been noted [2]. The most common opportunistic yeast pathogen when yeast mixtures are isolated is still *C. albicans* but other *Candida* species, such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. dubliniensis*, are encountered with increasing frequency, particularly because of the widespread use of fluconazole as a therapeutic and prophylactic antifungal agent [2]. In addition to *C. glabrata* and *C. krusei*, fluconazole-resistant strains of *C. albicans* have emerged [3, 4]. Consequently, oral *Candida* infections are a growing medical problem requiring prompt diagnosis and early antifungal susceptibility results to guarantee suitable therapy.

Recently, Patterson *et al.* [5] described the use of the differential medium CHROMagar *Candida* (CAC) with fluconazole added in different concentrations for the isolation of yeasts from oral samples, thus obtaining both a presumptive yeast identification and indications concerning their fluconazole susceptibility. A 96% concordance was found between predicted susceptibility results obtained on CAC and the standard macrobroth dilution technique proposed by the National Committee for Clinical Laboratory Standards (NCCLS) [6].

Although the causes for the emergence of *Candida* species other than *C. albicans* are unknown, an important factor may be a relative lack of susceptibility to azole antifungals [2]. Use of antibiotics and i.v. drugs, denture wearing, smoking and low CD4 count change the environmental conditions of the oral mucosa and promote its colonization by *C. albicans* [7]. Whether these factors also predispose to colonization with non-*albicans Candida* species has been poorly investigated.

The objectives of the present study were to determine the spectrum of the yeast mouth flora in 130 HIV-infected individuals and to correlate the findings with the clinical and epidemiological characteristics of these patients. We further sought to compare the susceptibility results obtained on fluconazole-containing CAC with those of the microbroth dilution method. For comparison, oral rinses from a non-matched group of healthy, HIV-seronegative volunteers were also examined.

Material and methods

Study population

Between December 1994 and September 1995, 35 HIV-infected individuals and 95 AIDS patients, attending the outpatient clinic of the Institute of Tropical Medicine, Antwerp, or hospitalized in the University Hospital Antwerp were included in the study after verbal consent. Epidemiological, clinical and laboratory data, including sex, age, demographic origin, HIV risk factors, concomitant antibiotic and antifungal therapy, CD4 cell count, smoking habits, denture wearing, hospitalization and number of previous episodes of oral *Candida* infection treated with antifungal agents, were registered. Particular attention was given to the presence of erythematous or white plaques and symptoms such as sore mouth or tongue, odynophagia and dysphagia.

Because it proved to be impractical to create a substantial group of matched HIV-seronegative control individuals, 130 healthy young volunteers, mostly medical students or laboratory assistants, were sampled between February and April 1996. These controls were not hospitalized and were free of any clinical symptoms at the time of sampling. They had no underlying disease known to be associated with immunosuppression. A questionnaire was completed, recording epidemiological and clinical data, including sex, age, demographic origin, smoking habits, denture wearing, concomitant antibiotic or antifungal therapy and number of previous periods of treatment of antifungal agents.

Specimen collection and culture

Each person rinsed his or her mouth with 10 ml of sterile saline and returned the mouthwash sample into a sterile container. From the HIV-seropositive group, three 9-cm Petri dishes with CHROMagar Candida (CAC; CHROMagar,

Paris, France) were surface inoculated with 100 µl of the samples and incubated at 37 °C in ambient air for 48 h. One plate had no additions, a second contained 10 µg of fluconazole (Pfizer Central Research, Sandwich, UK) per ml (CF10) and a third contained 100 µg of fluconazole per ml (CF100) to select for populations of potentially fluconazole-resistant yeasts. Specimens from the HIV-seronegative group were inoculated only on CAC without fluconazole added.

The number of colony-forming units (CFU) on the isolation plates was determined using a Laser colony counter (Spiral System Instruments, Led Techno, Eksel, Belgium).

Yeast identification

Presumptive identifications of yeast isolates were based on the colours of the colonies on CAC and were confirmed by standard morphological and physiological criteria. Colony colours on CAC were not influenced by addition of fluconazole to the medium. Light-green colonies were confirmed as *C. albicans* by production of germ tubes in fetal calf serum after 3 h at 37 °C and the production of chlamydospores on 1% rice-cream 1% Tween 80 agar after 48 h at 25 °C. Dark-green colonies were tested for β-glucosidase activity and by DNA fingerprinting with the oligonucleotide probe Ca3, to differentiate *C. dubliniensis* from *C. albicans* [8]. Colonies with other colours were identified on the basis of their morphologies on 1% rice-cream 1% Tween 80 agar and their assimilation patterns with the API ID32C yeast identification system (bioMérieux, Charbonnières les Bains, France).

Antifungal susceptibility

From each isolation plate (CAC, CF10 and CF100), one colony of each species present was tested for fluconazole and itraconazole susceptibility by a previously described microbroth dilution method with spectrophotometric determination of end points [9]. The IC_{50} values (lowest antifungal concentration that reduced growth below 50% of control) of fluconazole and itraconazole were recorded. Recently, break points for antifungal susceptibility testing have been proposed by the NCCLS antifungal subcommittee: MICs of 16 and 32 µg ml^{-1} for fluconazole and 0.25 and 0.5 µg ml^{-1} for itraconazole, indicating reduced ('dose-dependent') fluconazole and itraconazole susceptibility; while MICs $\geq$64 µg ml^{-1} for fluconazole and $\geq$1 µg ml^{-1} for itraconazole have been suggested as the break points for resistance for these antifungal agents [10].

Statistical analysis

Patient characteristics were compared with the isolation frequencies of the yeast species on CAC. Analysis was performed with Statistica Windows. A multiple logistic regression model was used (Egret software) to define which clinical characteristic correlated best with carriage of *Candida albicans* and non-*albicans Candida* species in the mouth of HIV-seropositive patients. χ^2 was used to define an association between mixtures of *Candida* spp. and growth of yeasts on CF10 and CF100. $P<0.05$ was considered to be statistically significant.

Results

Study population

Of the 130 seropositive patients, 95 (73.1%) were male. Mean age was 38.5 years (SD = 10). Seventy-six (58.5%) had a CD4 count <200 cells ml^{-1}. Twenty-eight (21.5%) seropositive patients were of non-European origin, including 25 of African, two of American and one of Asian origin. Sixty-three (48.5%) patients were currently using antibiotics. Twenty-two (16.9%) were hospitalized, 21 (16.1%) had symptoms of oropharyngeal candidosis, 76 (58.5%) were previously and 16 (12.3%) were currently under treatment with azoles, *viz.* 71 with fluconazole and 22 with itraconazole.

Six (4.6%) patients had a symptomatic oropharyngeal or oesophageal *Candida* infection despite treatment with 100 mg of fluconazole per day for at least 10 days, and were considered to have *Candida* infections clinically resistant to fluconazole. None of the patients treated with itraconazole showed clinical resistance to this agent at the time of sampling. For some patients, some information was lacking. Of 127 patients, 60 (47.2%) were homosexuals, 67 (52.7%) were heterosexuals, eight (6.3%) were intravenous drug users; in one (0.8%) case, the cause of the HIV infection was a blood transfusion and in three (2.4%) cases the risk factor for seropositivity was unknown. Of 126 patients, 64 (50.8%) were smokers, 26 of 119 (21.8%) wore a denture.

The immunocompetent population consisted of 72 medical students, 49 persons working in a microbiology laboratory and nine other healthy volunteers. Fifty-one (39.2%) were men, mean age was 27.3 years (SD 9.1). Of the four (3.1%) seronegative individuals of non-European origin, two were of African and two were of Asian origin. Thirteen (10%) were smokers and 13 (10%) wore a denture. Seven (5.4%) were currently using antibiotics. Sixteen (12.3%) had previously taken antifungal agents. The seronegative population

clearly differed from the HIV-positive population in this study in many of the characteristics recorded. This group was therefore regarded as a group for comparison, not as a control population.

Isolation of Candida *species*

From the seropositive population, 114 different *Candida* isolates were obtained from 81 (62.3%) of 130 oral rinses. In 56 (43%) cases, only a single species was isolated; 54 were *C. albicans*, one *C. glabrata* and one *C. inconspicua*. The remaining 25 (19.2%) oral rinses yielded mixtures of yeast species, every one of which included *C. albicans* plus at least one other *Candida* sp. In 18 (13.8%) mixed isolates *C. albicans* was combined with one non-*albicans Candida* species, *viz. C. glabrata* (seven instances), *C. dubliniensis* (five), *C. tropicalis* (four), *C. krusei* (one) and *C. guilliermondii* (one). The most common combination was *C. albicans* and *C. glabrata*, followed by *C. albicans* and *C. dubliniensis*, seen in, respectively, 38.9% and 27.8% of specimens containing two species. In six (4.6%) mixtures, two other non-*albicans Candida* spp. were present, *viz. C. glabrata* + *C. krusei* (two), *C. dubliniensis* + *C. inconspicua* (two), *C. dubliniensis* + *C. tropicalis* (one) and *C. krusei* + *C. dubliniensis* (one). In one (0.8%) mixture, three non-*albicans Candida* species were combined with *C. albicans*, *viz. C. glabrata* + *C. dubliniensis* + *C. tropicalis*.

In total, 114 *Candida* isolates were obtained from 81 patients, *viz.* 79 (69.3%) *C. albicans*, 11 (9.6%) *C. glabrata*, 10 (8.8%) *C. dubliniensis*, six (5.3%) *C. tropicalis*, four (3.5%) *C. krusei*, three (2.6%) *C. inconspicua* and one (0.9%) *C. guilliermondii*. No isolates contained *C. parapsilosis*.

In 29 instances, *C. glabrata* (11 isolates), *C. albicans* (eight isolates), *C. krusei* (four isolates), *C. inconspicua* (three isolates), *C. tropicalis* (two isolates) and *C. guilliermondii* (one isolate) were isolated on CF10. Among these isolates, 12, comprising *C. glabrata* (seven isolates), *C. albicans* (three isolates) and *C. tropicalis* (two isolates), were also recovered from CF100.

From the 130 immunocompetent persons, who were sampled only on CAC without fluconazole, *Candida* species were isolated from 40 (30.8%) persons. In 37 (28.5%) cases, only a single species was isolated including 34 *C. albicans*, two *C. parapsilosis* and one *C. guilliermondii*. Mixtures of *Candida* species were isolated from just 3 (2.3%) of 130 oral rinses, including two (1.5%) persons infected with *C. albicans* and *C. glabrata* and one (0.8%) person with a mixture of *C. albicans*, *C. glabrata* and *C. tropicalis*. Overall, 44 *Candida* isolates were obtained from 40 positive specimens, comprising 37 (84.1%) *C. albicans*, three (6.8%) *C. glabrata*, two (4.5%) *C. parapsilosis*, one (2.3%) *C. tropicalis* and one (2.3%) *C. guilliermondii*, *C. dubliniensis*, *C. krusei* and *C. inconspicua* were not isolated from any sample from this comparative population.

Clinical characteristics associated with Candida *species isolated*

Candida isolations from oral rinses of 130 HIV-seropositive patients and patient characteristics are shown in Table 1. Multiple regression analysis with outcome variable positive *vs.* negative cultures showed a highly significant relation between positive cultures and hospitalization ($P<0.01$). Isolations were also significantly higher ($P<0.05$) among Europeans *vs.* non-Europeans, smokers *vs.* non-smokers and patients with CD4 counts <200 cells ml^{-1}.

In this study, the presence of oral symptoms could not be analysed because of multicolinearity. When the multiple regression model was used with outcome variable *C. albicans vs.* non-*albicans Candida* spp., only smoking and previous or current use of azoles were significantly associated with the presence of non-*albicans Candida* spp. ($P<0.05$).

Growth of yeasts on CF10 and CF100 occurred primarily with mixed isolates

There was a significant association between the number of specimens containing *Candida* species other than *C. albicans* and the number of specimens containing at least one species isolated on CF10 and/or CF100 ($\chi^2=30$; $P<0.001$). From the 54 oral rinses containing only *C. albicans*, 48 isolates grew only on CAC, three on CAC and CF10, and three on CAC, CF10 and CF100. From the 27 oral rinses containing *Candida* spp. other than *C. albicans* (all but two in the form of mixtures of species), 20 (74.1%) harboured at least one yeast growing on CF10 including 21 non-*albicans Candida* spp. and two *C. albicans*. Thus, the growth of a yeast on CAC in the presence of fluconazole was primarily but not exclusively a property of species other than *C. albicans*, and the growth of non-*albicans Candida* species rarely occurred in the absence of *C. albicans*.

Colony counts

Colony counts of the *Candida* species growing on CAC supplemented with fluconazole ranged from 10 to 52 600 CFU ml^{-1} (median 6160 CFU ml^{-1}) for 29 isolates on CAC, from 30 to 9640 CFU ml^{-1} (median 1550 CFU ml^{-1}) for 29 isolates on CF10, and from 30 to 1720 CFU ml^{-1} (median

Table 1. *Candida* isolations from oral rinses of 130 HIV-secropositive patients in relation to patient characteristics. Yeast isolations are split between those containing *C. albicans* ($n=54$) alone and those containing other and mixed *Candida* spp. ($n=27$)

Characteristic	Number of patients for which characteristic known	Isolation of yeast species		Multiple regression model		Positive isolations		Multiple regression model	
		Negative *n* (%)	Positive *n* (%)	β	*P*	*C. albicans* alone *n* (%)	Other[a] or mixed *Candida* spp. *n* (%)	β	*P*
Male	95	31 (32.6)	64 (67.4)	−0.7022	NS	44 (68.7)	20 (31.3)	0.4747	NS
Female	35	18 (51.4)	17 (48.6)			10 (58.8)	7 (41.2)		
European	102	29 (28.4)	73 (71.6)	1.5150	<0.05	47 (64.4)	26 (35.6)	−0.9794	NS
Non-European	28	20 (71.4)	8 (28.6)			7 (87.5)	1 (12.5)		
CD4 count <200 cells ml^{-1}	76	22 (28.9)	54 (71.1)	−0.3085	<0.05	38 (70.4)	16 (29.6)	−0.5977	NS
CD4 count ≥200 cells ml^{-1}	54	27 (50)	27 (50)			16 (59.2)	11 (40.8)		
Smoking	64	16 (25)	48 (75)	1.2880	<0.05	27 (56.2)	21 (43.8)	−1.5330	<0.05
Non-smoking	62	30 (48.4)	32 (51.6)			26 (81.2)	6 (18.8)		
Denture wearing	26	8 (30.8)	18 (69.2)	0.4906	NS	13 (72.2)	5 (17.8)	0.2655	NS
No denture wearing	93	37 (39.8)	56 (60.2)			36 (64.3)	20 (35.7)		
Current antibiotics	63	23 (36.5)	40 (63.5)	−0.1857	NS	27 (67.5)	13 (32.5)	−0.5427	NS
No antibiotics	67	26 (38.8)	41 (61.2)			27 (65.8)	14 (34.2)		
Hospitalized	22	9 (40.9)	13 (59.1)	−1.7840	<0.01	5 (38.5)	8 (61.5)	−0.9889	NS
Not hospitalized	108	40 (37)	68 (63)			49 (72)	19 (28)		
Oral symptoms	21	0 (0)	21 (100)	[c]		13 (61.9)	8 (38.1)	0.6123	NS
No oral symptoms	109	49 (45)	60 (55)			41 (68.3)	19 (31.7)		
Previous or current azole treatment	92	28 (30.4)	64 (69.6)	0.3902	NS	42 (65.6)	22 (34.4)	−1.7740	<0.05
No azole treatment	38	21 (55.3)	17 (44.7)			12 (70.6)	5 (29.4)		

[a] *C. glabrata* and *C. inconspicua* were isolated single, the 25 other samples comprised mixed species.
[b] NS, not statistically significant.
[c] Not included in the model because of multicollinearity problems.

455 CFU ml^{-1}) for 12 isolates on CF100. Colony counts were thus often considerably higher on the medium without fluconazole than on isolation plates containing fluconazole. This was the case for 22 of the 29 yeasts growing on CAC and CF10 and for 11 of the 12 yeasts growing on CAC and CF100. In 12 of the 29 cases in which the same species was isolated on CAC and CF10, the CFU ml^{-1} on CAC was more than three times higher than on CF10. A >threefold difference in CFU ml^{-1} was also noted for 8 of the 12 yeasts grown on CAC and CF100. This finding indicated a possible selection of less susceptible subpopulations on the fluconazole-containing media. However, identical species from the same sample isolated on CAC, CF10 and CF100 did not show obvious differences in their fluconazole susceptibilities; in all instances, the IC_{50} for fluconazole against a randomly chosen colony varied by no more than two twofold dilutions between the various isolation plates.

Antifungal susceptibilities of isolates from seropositive and seronegative populations

The *in vitro* fluconazole and itraconazole susceptibilities of *Candida* species isolated on CAC from the HIV-seropositive population are summarized in Table 2. The data are reported as the IC_{50} concentrations of fluconazole and itraconazole required to inhibit 50% and 90% of the isolates tested.

From the 114 isolates from the HIV-positive population, 15 (13.2%) *Candida* isolates (of which 12 were non-*albicans Candida* spp.) were considered to be resistant for fluconazole according to NCCLS break points ($IC_{50} \geq 64$ μg ml^{-1}); 11 (9.6%) isolates (of which seven were non-*albicans Candida* spp.) showed a reduced ('dose-dependent') fluconazole susceptibility ($IC_{50} = 16$, 32 μg ml^{-1}). The remaining 88 (77.2%) isolates were susceptible to fluconazole ($IC_{50} \leq 8$ μg ml^{-1}). Eighteen (15.8%) *Candida* isolates, including 14 non-*albicans Candida* spp., were considered to be resistant to itraconazole ($IC_{50} \geq 1$ μg ml^{-1}); 10 (8.8%) isolates, including six non-*albicans Candida* spp., were of intermediate susceptibility ($IC_{50} = 0.25$, 0.5 μg ml^{-1}). The remaining 86 (75.4%) isolates were susceptible to itraconazole ($IC_{50} \leq 0.13$ μg ml^{-1}). Overall, 85 (74.6%) isolates, including 14 non-*albicans Candida* spp., were cross-susceptible to fluconazole and itraconazole. Eleven (9.6%) isolates, including six non-*albicans Candida* spp., were cross-resistant to fluconazole and itraconazole.

The yeast isolates from the six patients considered to be clinically resistant to fluconazole included two monocultures of *C. albicans* and four mixtures, comprising *C. albicans* + *C. glabrata* (three isolates) and *C. albicans* + *C. glabrata* + *C. krusei* (one isolate). Of the two *C. albicans* monocultures, one was resistant *in vitro* and one had intermediate susceptibility to fluconazole with IC_{50}, respectively, of >64 μg ml^{-1} and 32 μg ml^{-1}. Of the four mixtures, three contained one or two *Candida* spp. resistant to fluconazole (three *C. glabrata* and one *C. krusei*). One mixture had a *C. glabrata* isolate with intermediate susceptibility to fluconazole ($IC_{50} = 32$ μg ml^{-1}). The four *C. albicans* spp. in the mixtures were all susceptible to fluconazole.

Among the 44 isolates from the HIV-negative

Table 2. *In vitro* susceptibility of different *Candida* species to itraconazole and fluconazole isolated from 130 HIV-secropositive persons

Isolate	Agent	No.	Range	IC_{50} (μg ml^{-1})[a] for 50% of strains	IC_{50} (μg ml^{-1})[a] for 90% of strains
C. albicans	Fluconazole	79	≤0.13–>64	0.25	4.0
	Itraconazole	79	≤0.032–4.0	≤0.032	0.025
C. glabrata	Fluconazole	11	16–>64	32	>64
	Itraconazole	11	1.0–>16	1.0	>16
C. dubliniensis	Fluconazole	10	≤0.13–8.0	1.0	8.0
	Itraconazole	10	≤0.032–0.13	0.063	0.13
C. tropicalis	Fluconazole	6	0.5–>64	4.0	>64
	Itraconazole	6	0.063–>16	0.5	>16
C. krusei	Fluconazole	4	64	64	64
	Itraconazole	4	0.25–1.0	0.5	1.0
C. inconspicua	Fluconazole	3	8.0–6.4	16	64
	Itraconazole	3	≤0.032–0.5	0.13	0.5
C. guilliermondii	Fluconazole	1	1.0	1.0	1.0
	Itraconazole	1	0.13	0.13	0.13

[a]Microbroth dilution test with RPMI–2% glucose.

population, two (4.4%) *C. glabrata* isolates were resistant to fluconazole, and one (2.3%) *C. tropicalis* isolate each had intermediate fluconazole susceptibility. The remaining 40 isolates (90.9%) were susceptible to fluconazole. Four (9%) *Candida* isolates, namely three *C. glabrata* and one *C. tropicalis*, were resistant to itraconazole, while one (2.3%) *C. parapsilosis* and one (2.3%) *C. guilliermondii* isolate each had a dose-dependent susceptibility to itraconazole. The remaining 38 (86.4%) isolates were susceptible to itraconazole. Overall, two *C. glabrata* isolates were cross-resistant and 38 other yeasts were cross-susceptible.

Comparison of microdilution test results from the seropositive population with results of strains isolated on CHROMagar Candida *medium supplemented with fluconazole*

Among 88 yeasts with $IC_{50} \leq 8$ μg ml^{-1}, 84 grew only on CAC, two grew on CF10 and two on CF100. Twenty-five of the 26 yeasts with $IC_{50} > 8$ μg ml^{-1} grew on CAC containing 10 or 100 μg of fluconazole per ml. One *C. tropicalis* isolate with an $IC_{50} \geq 64$ μg ml^{-1} was not isolated on fluconazole-containing media. There was no correspondence between the fluconazole IC_{50} at the intermediate susceptibility levels of 16 and 32 μg ml^{-1} and growth on CF10 but not on CF100, as 6 of 11 isolates with intermediate IC_{50} values grew on CF100 and, conversely, 10 of 15 isolates with $IC_{50} \geq 64$ μg ml^{-1} did not grow on CF100.

However, isolation of yeasts on CF10 or CF100 corresponded in 25 out of 26 cases (96.1%) with a fluconazole susceptibility $IC_{50} > 8$ μg ml^{-1}. Yeasts with fluconazole broth microdilution IC_{50}s ≤ 8 μg ml^{-1} were correctly predicted in 84 (95.5%) of 88 cultures by absence of growth on media containing fluconazole at 10 or 100 μg ml^{-1}.

Discussion

As expected, we found a significant difference in the oral yeast carriage between HIV-seropositive (62.3%) and healthy persons (30.8%) [11–13]. From 32 studies on yeast isolations from the oral cavity in normal individuals, the median frequency was 34.4% for all *Candida* spp. [13], and thus very close to our findings. Previous reports on the isolation of *Candida* from the oral cavity of HIV-infected persons, in the presence or absence of clinical symptoms mention frequencies ranging from 11% to 96% [14].

We found a significantly higher prevalence of carriage of oral *Candida* in hospitalized *vs.* non-hospitalized patients, corresponding with the findings of studies in settings other than HIV infection [13, 15]. All patients with symptoms and/or signs of active oropharyngeal candidosis had positive *Candida* cultures, but 38.1% were mixtures of *C. albicans* and other species (Table 1).

As expected, patients with T-helper cell counts < 200 cells ml^{-1} produced more *Candida*-positive samples [7, 16, 17].

Hitherto, the majority of published data about the isolation of yeasts from HIV-positive patients concerned patients of European and North-American origin. Further studies are necessary to confirm our data showing a particularly low rate of *Candida* carriage in the non-European subpopulation of our HIV-positive group.

In some previous studies, no relation was found between smoking and *Candida* carriage [18, 19], while we, like others, found that smoking is associated with 30–70% higher *Candida* carriage [20, 21]. Smoking was significantly related to oral *Candida* carriage and to a higher prevalence of non-*albicans Candida* spp. This novel observation could now be easily followed up by using the commercially available culture medium that differentiates on isolation *C. albicans* from other species. Previous or current azole treatment favoured the carriage of non-*albicans Candida* species [2].

Non-*albicans Candida* spp. were detected twice as often in the HIV-seropositive population (30.7%) compared with the immunocompetent population (15.9%). The distribution of the non-*albicans Candida* species was also different in both groups. The recently discovered species *C. dubliniensis* represented 9% of the cultures and was therefore the most prevalent non-*albicans* species, after *C. glabrata*, in the seropositive population [28]. So far, all reports of *Candida dubliniensis* appear to associate the species specifically with HIV-positive individuals [22–25].

We found mixtures of *Candida* spp. in 19.2% of the oral rinses of the seropositive population compared with only 2.3% in the healthy population. Other surveys in various populations showed that 7.8–27.8% of clinical samples from HIV-negative persons contained mixtures of *Candida* species on CAC [26–30].

In previous studies, samples from HIV-seropositive patients contained mixtures of yeast species in 15% [26] and 19% [29] of cases. Our own finding was a prevalence of 21%. Like others who questioned the role of non-*albicans Candida* species in mixed oropharyngeal *Candida* infections [16, 26–30] or as sole colonizers [31], we are unable to determine the part played by non-*albicans*

Candida spp. as causes of clinical disease. Although isolation of such species was significantly associated with previous azole antifungal treatment, and although many such species showed reduced azole sensitivity *in vitro*, there was only a low level of clinical resistance to azole therapy, and it was not significantly associated with the presence of non-*albicans Candida* spp.

Growth of yeast isolates on CF10 correlated with fluconazole $IC_{50} > 8$ μg ml^{-1} in 96% of cases. These results corroborate those of Patterson *et al.* [5], who found a correlation of 98% when comparing fluconazole MICs with growth on CAC media supplemented with 8 and 16 μg ml^{-1} of fluconazole. Nevertheless CAC supplemented with fluconazole was not able to discriminate fluconazole 'dose-dependent' strains from fluconazole-resistant strains, according to break points proposed by the NCCLS [10].

Detection of resistance of a yeast isolate to an antifungal agent should be predictive of clinical failure of treatment with that agent. We followed the proposed NCCLS break points (Rex *et al.*, *J. Inf. Dis.*, in press) in classifying isolates as resistant to fluconazole, and we found 15 yeasts, from 14 patients, with IC_{50} values at or above the resistance break point. Of these 14 patients, only five were regarded as being clinically resistant to fluconazole treatment. A sixth patient who showed clinical resistance to fluconazole treatment was infected with a *C. albicans* isolate of intermediate ('dose-dependent') fluconazole susceptibility. None of the 28 yeast isolates with intermediate susceptibility or resistance to itraconazole *in vitro* were associated with clinical resistance to itraconazole treatment. These results indicate that a mycological finding of azole resistance in an isolate cannot be used for prediction of therapeutic outcome. A similar conclusion might be drawn from the data used to set the azole resistance break points [10], in which more than 50% of patients infected with yeasts that showed resistance to fluconazole and itraconazole were successfully treated with these agents.

The overall prevalence of fluconazole-resistant yeast isolates that we found in this study (13%) was slightly but not exceptionally greater than the figures just below 10% that have been reported by others in HIV-positive patients [3, 32]. Most, but not all, of the isolates with decreased fluconazole susceptibility also showed decreased itraconazole susceptibility, a finding similar to that of others [26, 33].

We regard as intriguing the frequent finding of substantially decreased counts of yeasts exposed to fluconazole in the isolation medium compared with counts for the same sample inoculated on fluconazole-free medium. This observation suggests that intraspecies heterogeneity in azole susceptibility of oral *Candida* populations may be more common than usually supposed. Variability in results of MIC tests with different yeast colonies in fresh isolates has been mentioned by others [34, 35], which corroborates our supposition of inhomogeneity.

Acknowledgements

We thank the many physicians of the Tropical Institute of Antwerp and the University Hospital of Antwerp who contributed to the collection of the clinical oral rinses. We gratefully acknowledge the technical assistance of S. Verhelst and the statistical help of J. C. Van der Auwera.

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