Point prevalence, microbiology and antifungal susceptibility patterns of oral *Candida* isolates colonizing or infecting Mexican HIV/AIDS patients and healthy persons

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Summary

We have conducted a longitudinal study over a 3-year period to address the point prevalence, microbiological characteristics and antifungal susceptibility patterns of yeast isolates colonizing or infecting the oral cavities of 111 HIV-infected (51 adults, 60 children) and 201 non HIV-infected (109 adults, 92 children) Mexican persons. Regarding the epidemiology of oral candidiasis, *Candida albicans* was the most frequent species isolated. Seventy-one out of 85 isolates from colonized persons were *C. albicans* (83.5%), 27 isolates of them were from HIV-infected children and 44 from non HIV-infected patients. Sixty-two isolates belonged to serotype A which was the most prevalent serotype of *C. albicans*. Non-*albicans* species (*Candida glabrata*, *Candida tropicalis* and *Candida parapsilosis*, and *Saccharomyces cerevisiae*) were isolated from 16.5% of colonized patients and from 38.5% patients with candidiasis or *Candida*-related lesions. There were nine episodes of infection or colonization by at least 2 different yeast species. In the case of HIV/AIDS patients, it was determined that yeast carriage was not associated with the number of CD4+ cells or the viral load, but HAART reduced the prevalence of oral candidiasis. Overall, most patients harbored strains *in vitro* susceptible to fluconazole, however 10.8% of the yeasts were resistant to one or more azole antifungal agents and 29% were intermediate susceptible to them. On the contrary, 5-fluorocytosine was very active against all isolates tested, and amphotericin B was active against 97.9% of them.

Key words

Oral, *Candida*, Antifungal, HIV, Mexico

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Prevalencia, microbiología y patrones de sensibilidad a los antifúngicos de los aislamientos orales de *Candida* que colonizaban o infectaban a pacientes mexicanos con infección por VIH o sida y a personas sanas

Resumen

Hemos realizado un estudio longitudinal durante tres años para valorar la prevalencia, la microbiología y los patrones de sensibilidad in vitro a los antifúngicos de los aislamientos de levaduras que colonizaban o infectaban la cavidad oral de 111 pacientes mexicanos con infección por VIH (51 adultos y 60 niños) y de 201 personas sanas no infectadas por el VIH (109 adultos, 92 niños). *Candida albicans* fue la especie más frecuentemente aislada. Setenta y uno de los 85 aislamientos de personas colonizadas eran *C. albicans* (83,5%); veintisiete de estos aislamientos procedían de niños con infección por VIH y 44 se aislaron de personas no infectadas por VIH. Sesenta y dos aislamientos de *C. albicans* pertenecían al serotipo A siendo el serotipo más prevalente. Se aislaron especies no *C. albicans* (*Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* y *Saccharomyces cerevisiae*) en el 16,5% de los pacientes colonizados y en el 38,5% de los pacientes con candidiasis o enfermedades asociadas a *Candida*. Hubo nueve episodios de infección o colonización mixta. En el caso de los pacientes infectados por VIH o con sida, la colonización por levaduras no estaba asociada con el número de CD4+ o la carga viral; sin embargo, el tratamiento anti-retroviral de alta actividad (HAART) reducía la prevalencia de candidiasis oral. La mayoría de los pacientes portadores de levaduras, estaban colonizados o infectados por cepas sensibles a fluconazol. Sin embargo, un 10,8% de las levaduras eran resistentes a uno o más antifúngicos azólicos y un 29% mostraban una sensibilidad intermedia. Por el contrario, la 5-fluorocitosina fue muy activa contra todos los aislamientos estudiados y la anfotericina B lo fue contra el 97,9% de éstos.

Palabras clave

Oral, *Candida*, Antifúngicos, VIH, México

Oral cavities are colonized by *Candida albicans* or other yeast species in 40% to 60% of healthy persons [39]. When local or general predisposing factors are present, *Candida* may cause acute or chronic oral infections such as pseudomembranous (oral thrush), atrophic (erythematous) or hyperplastic candidiasis or angular cheilitis. Other oral pathologies, such as denture stomatitis (chronic atrophic candidiasis), leukoplakia or lichen planus have been associated to the presence of *Candida* [31,46]. The frequency of these clinical presentations is variable and in many patients combinations of two or more of them have been reported [22-23]. Oral candidiasis is the most prevalent oral complication in HIV-infected and AIDS (HIV/AIDS) patients [51] and the risk is considered to be higher in patients with a CD4+ cell count of < 200 cells/mm^3 and high plasma HIV RNA loads [2,26]**.** *C. albicans* represents the most common causative agent of oral candidiasis; however, other species of *Candida* have begun to emerge [2]. The presence of *Candida* in the oral cavities of HIV/AIDS patients predicts the subsequent development of oral candidiasis. Topical nystatin or miconazole (MCZ) are used in the initial treatment of oral candidiasis, but most patients suffer more than one episode of oral candidiasis and they are treated with fluconazole (FCZ) or itraconazole (ITZ) [41]. The common use of FCZ and other azole antifungal agents for treating this infection has been associated with the emergence of azole-resistant isolates of *C. albicans* in these HIV/AIDS patients in many countries [40,41]. The introduction of highly active antiretroviral therapy (HAART) has reduced the prevalence of most opportunistic infections, including oral candidiasis [14,23]. Recovery of immune function associated to HAART is an important factor for the declining of oral candidiasis but, with independence of CD4+ cell count, low plasma HIV RNA loads have been correlated with low oral colonization by *Candida* spp., and with a reduction of the risk of symptomatic oral infections [23,26].

In Mexico, most clinical laboratories do not routinely perform antifungal testing, and little is known about the antifungal susceptibility profiles among Mexican clinical yeast isolates. To elucidate the status of oral *Candida* colonization and candidiasis and the antifungal susceptibility patterns of this fungal genus in Mexico, we have conducted a prospective study in 111 HIV/AIDS pediatric and adult patients (72 of them undergoing HAART) and in 201 HIV-non infected persons. This study represents one of the first reports on the antifungal susceptibility profiles of oral yeast isolates from confirmed HIV/AIDS patients in Mexico D.F., Mexico.

Patients, materials and methods

Studied population. A prevalence study over a 3-year period was conducted on 111 HIV/AIDS and 201 non HIV-infected persons attending the General Hospital of Mexico, the "Federico Gómez" Child Hospital and/or the Odontology Clinics at the Facultad de Odontología (Universidad Nacional Autónoma de México, México D.F.). These persons were distributed in four different groups: HIV/AIDS adults, non HIV-infected adults, HIV/AIDS children and non HIV-infected children, including 51, 109, 60 and 92 persons, respectively. Data on patient demographics, history of prior fungal infections, antifungal treatments and medications, such as if they were undergoing a stable HAART regimen (combination of the same three drugs in the three months before study entry) were collected at the time of enrolment. Patients were eligible for the

study if they had no topical or systemic antifungal treatment in the preceding six months. All patients were subjected to clinical evaluation, HIV RNA and CD4+ cell measurements. Healthy volunteers were selected from persons seeking dental care at the Odontology Clinics at the Universidad Nacional Autónoma de México. Informed consent was obtained from all persons. Prior to initiation of this study, approval was obtained from the Human Ethics, Research, and Publications Committee at the Universidad Nacional Autónoma de México and from the rest of medical institutions involved. Preliminary oral clinical and mycological aspects, including prevalence of candidiasis, have been partially published previously [25,47].

Clinical specimens, microbiological assessment and identification of isolates. Swabs were taken from oral lesions when present, the buccal mucosa, the floor of the mouth, and the dorsal surface of the tongue, as this has been found to be the most frequently colonized site in the oral cavity. Briefly, each sample was collected by passing a sterile cotton swab several times across the particular oral surface. Immediately after sampling, each swab was replaced in its sterile container with transportation medium and taken to the laboratory within two hours. Oral specimens were coded according to clinical group and patient. Oral swabs were plated onto Sabouraud dextrose agar supplemented with chloramphenicol, and incubated at 36 °C (± 1 °C) for two days and for a seven days additional period at 30 ºC (± 1 ºC) before being discharged as negative. Yeasts were identified to the species level by standard methods, such as germ tube test, microscopic morphology on Corn meal agar-Tween 80 (blastoconidia, pseudohyphae and true hyphae formation, as well as chlamydoconidia production) and carbohydrate assimilation patterns by means of API 20 C system (BioMerieux, France) [52]. All *C. albicans* isolates were serotyped by an indirect immunofluorescence assay with the IgM monoclonal antibody B9E [4]. In addition, for presumptive identification of *Candida dubliniensis*, all isolates identified as *C. albicans* were also tested for growth at 45 °C (± 1 ºC) for 48 h on Sabouraud dextrose agar [43], chlamydoconidia production on Casein agar [37] and by an indirect immunofluorescence assay with a polyclonal antiserum against this species [6]. Isolates were stored in sterile water at room temperature until use.

Antifungal susceptibility testing. Prior to antifungal susceptibility testing, each isolate was cultured on Sabouraud dextrose agar and CHROMagar Candida (CHROMagar, France) to ensure purity and viability. Antifungal susceptibility was assessed by means of Fungitest panel (Bio-Rad, France), according to the manufacturer's instructions. This is a 16-well microplate which allows susceptibility testing of yeasts for 5-fluorocytosine (5FC), amphotericin B (AMB), MCZ, ketoconazole (KTZ), FCZ and ITZ in modified RPMI 1640 in the presence of a redox indicator. Each antifungal drug was tested at two different concentrations (2 and 32 µg/ ml for 5FC, 0.5 and 8 µg/ ml for AMB and MCZ, 0.5 and 4 µg/ ml for KTZ and ITZ, and 8 and 64 µg/ ml for FCZ) selected in order to distinguish resistant isolates from susceptible ones. Briefly, each isolate, grown overnight on Sabouraud dextrose agar at 36 °C (± 1 ºC), was suspended in sterile distilled water and adjusted to 1 McFarland standard with a spectrophotometer. The final inoculum concentration was approximately 0.5–2.5 x10^3 cells/ml. The sealed Fungitest panels were incubated at 36 °C (± 1 ºC) and read after 48 h. *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were included as quality control strains. According to the manufacturer, isolates inhibited by both concentrations were defined as susceptible, those inhibited by the higher concentration as intermediate, and those inhibited by neither of them as resistant [53,54].

Statistical analysis. Differences between continuous variables were assessed by means of two-tailed Student's t-test for differences in means and percentages. Differences between categorical variables were evaluated by chi square test. Values of $p < 0.05$ were considered statistically significant.

Results

Clinical and microbiological characteristics. Table 1 shows the clinical characteristics of the studied patients. Yeasts were isolated from the oral cavities of 178 out of 312 persons (57.1%). Colonization or infection by yeasts was higher in adults both with and without HIV infection (74.5% and 61.5%, respectively) than in children with and without HIV infection (60% and 40.2%). For HIV-infected patients, oral carriage of yeast was not associated with the number of $CD4^+$ cell (median $CD4^+$ cell count was 623 cells/µl in colonized patients and 643 cells/µl in non-colonized patients; $p = 0.184$) or the viral load (median HIV RNA was 52,275 copies/ml in colonized patients versus 55,173 copies/ml in non-colonized patients; $p = 0.319$). Similarly, the antiretroviral regimen did not influence the status of yeast carrier. We found that 69.4% of patients undergoing HAART were colonized and/or infected with yeasts, and 59.5% of patients without HAART were colonized or infected by yeasts. Overall, we found that HIV/AIDS children with $CD4^+$ cell counts < 500 cells/µl had significantly more yeast colonization than healthy control children.

Ninety-eight (45 HIV/AIDS patients) out of 312 persons developed oral candidiasis. As for colonization, active oral candidiasis in HIV/AIDS patients was not associated with the number of $CD4^+$ cell (median $CD4^+$ cell count was 775 cells/µl in HIV/AIDS patients with oral candidiasis and 643 cells/µl in HIV/AIDS patients without candidiasis; $p = 0.326$) or the viral load (median HIV RNA was 60,995 copies/ml in HIV/AIDS patients with oral candidiasis and 55,173 copies/ml in HIV/AIDS patients without candidiasis; $p = 0.442$). However, HAART had influence on the presence of oral candidiasis reducing the frequency of oral infection. We found that 36.1% of patients undergoing HAART suffered from oral candidiasis compared with 45.9% of patients without antiretroviral treatment ($p < 0.05$). Moreover, we found that HIV/AIDS children with $CD4^+$ cell counts of < 500 cells/µl and treated with HAART had significantly less oral candidiasis ($p < 0.05$) than HIV/AIDS children not treated with antiretrovirals.

We found four different clinical presentations of oral candidiasis including pseudomembranous, erythematous, a combination of both, and denture stomatitis. The latter lesion was most frequent in persons without HIV infection. Pseudomembranous type was the commonest clinical presentation of oral candidiasis in HIV/AIDS adults, as 32 patients had this clinical presentation alone and other five patients suffered from it in combination with erythematous candidiasis ($p < 0.05$). One patient in this group presented erythematous candidiasis. Conversely, all HIV/AIDS children suffering from oral candidiasis presented erythematous lesions. Among the control (non-HIV-infected) adults, forty-two presented with denture stomatitis, six showed a combination of pseudomembranous

and erythematous oral candidiasis, other four suffered from pseudomembranous candidiasis and 1 presented erythematous lesions. None of the studied healthy children presented oral candidiasis.

Epidemiology of oral Candida *isolates recovered from the study population. C. albicans* was the most frequent species isolated from colonized and infected patients (Table 2). Seventy-one out of 85 isolates from colonized persons were *C. albicans* (83.5%). Sixty-two isolates belonged to serotype A which was the most prevalent serotype of *C. albicans* in the 4 groups of patients. Moreover, *C. albicans* was the most frequently isolated yeast in all clinical presentations of oral candidiasis: 77.8% of isolates from patients with pseudomembranous candidiasis, 77.8% of isolates from erythematous candidiasis and 72.7% of isolates from patients with combined, pseudomembranous and erythematous, candidiasis. However, *C. albicans* represented only 48.8% of isolates from persons with denture stomatitis and *C. glabrata* represented 41.3% of isolates. Non-*albicans* species were also isolated from 14 colonized patients (16.5%), being 6 *C. glabrata*, 7 *C. tropicalis* and 1 *C. parapsilosis*. They were also isolated from 42 patients (38.5%) with candidiasis or *Candida*-related lesions, as denture stomatitis, being 29 *C. glabrata*, 4 *C. tropicalis*, 1 *C. parapsilosis* and 3 *Saccharomyces cerevisiae* (all of them from patients with pseudomembranous lesions). There were 9 episodes of infection or colonization by at least 2 different yeast species, yielding 6 cultures *C. albicans* + *C. glabrata*, and one each *C. albicans* + *C. tropicalis*, *C. glabrata* + *C. tropicalis*, and *C. glabrata* + *C. parapsilosis*.

Antifungal susceptibility patterns of oral yeast isolates. Overall 10.8% of the yeasts were resistant to one or more azole antifungal agents and 29% were intermediate susceptible to them, being ITZ (10.7% and 28.9%, resistant or intermediate isolates, respectively) and KTZ (10.2% and 20.8%, resistant or intermediate isolates, respectively) the less active of the antifungal agents tested (Table 3). Lower degree of resistance was observed against MCZ (2.7% and 17.6%, resistant or intermediate isolates, respectively) and FCZ (3.2% and 11.2%, resistant or intermediate isolates, respectively). Conversely, 5FC was very active against all isolates tested, and AMB was active against 97.9% of them (two *C. albicans*, and one each *C. parapsilosis* and *C. tropicalis* were classified as intermediate). Most resistant isolates were *C. glabrata* from persons without HIV infection or *C. albicans* from HIV infected patients with pseudomembranous candidiasis.

Of 136 *C. albicans* isolates tested, 14 were resistant to KTZ (10.3%), and 11 were resistant to ITZ (8.1%). A minor resistance was observed to MCZ (0.7%) or FCZ (2.2%). The percentage of intermediate isolates to azole agents was higher: 21.3% to ITZ, 28.7% to MCZ or KTZ, and 5.9% to FCZ. No resistance was observed to AMB or 5FC. It was significant ($p < 0.05$) that 5 out of 14 KTZ resistant isolates belonged to serotype B of *C. albicans*. All serotype B isolates resistant to KTZ were from HIV/AIDS patients, three from adults with pseudomembranous candidiasis and two from children without candidiasis. Most resistant isolates to any of the azole antifungals tested showed at least intermediate susceptibility to other azole antifungal agent. Three isolates from non HIV-infected adults (one of them with denture stomatitis) were

Table 1. Clinical characteristics of 312 persons included in this study.

		Groups of studied persons			
		HIV adults	Non-HIV adults	HIV children	Non-HIV children
No. of persons (%)		51 (100)	109 (100)	60 (100)	92 (100)
Age (years)					
Mean		36.4	64.4	5.18	6.93
Range		22-65	42-89	1-13	1-13
$CD4^+$ T cells / µl					
Mean		484.1	ND	673.4	ND
No. of persons (%)	> 500	14 (27.4)	ND	25 (41.6)	ND
	200-500	23 (45.1)	ND	25 (41.6)	ND
	< 200	14 (27.4)	ND	8 (13.3)	ND
Virus load (HIV RNA copies / ml)					
Mean		65,945.1	ND	49,343.6	ND
No. of persons (%)	< 10,000	17 (33.3)	ND	20 (33.3)	ND
	≥ 10,000	34 (66.6)	ND	40 (66.6)	ND
No. of colonized or infected individuals (%)		38 (74.5)	67 (61.5)	36 (60)	37 (40.2)
No. of patients with candidiasis (%)					
Pseudomembranous		32 (62.7)	4 (3.7)	0 (0)	0 (0)
Erythematous		1 (1.9)	1 (0.9)	7 (11.7)	0 (0)
Denture stomatitis		0 (0)	42 (38.5)	0 (0)	0 (0)
Pseudo. + Erythem.		5 (9.8)	6 (5.5)	0 (0)	0 (0)
No. of non-colonized individuals (%)		13 (25.5)	42 (38.5)	24 (40)	55 (59.8)
No. of patients on antiretroviral therapy (%)					
AZT		2 (3.9)	0 (0)	0 (0)	0 (0)
HAART		22 (43.2)	0 (0)	50 (83.3)	0 (0)
None		27 (52.9)	109 (100)	10 (16.6)	0 (0)

AZT= Zidovudine; HAART= Highly active antiretroviral therapy; ND= Not done

resistant to ITZ and FCZ. Other two isolates from HIV/AIDS children without candidiasis were resistant to ITZ and KTZ.

Among the 35 isolates of *C. glabrata*, 20% were resistant (7 isolates) and 51.4% were intermediate (18 isolates) to ITZ, 11.4% were resistant (4 isolates) and 34.3% were intermediate (12 isolates) to KTZ, 8.6% were resistant (3 isolates) and 31.4% intermediate (11 isolates) to MCZ, 5.7% were resistant (2 isolates) and 31.4% intermediate (11 isolates) to FCZ, and none was resistant to 5FC or AMB. Most resistant isolates were from non HIV-infected persons with denture stomatitis and without a recent treatment with azoles. Some of these *C. glabrata* isolates showed in vitro cross-resistance among different azole agents: two isolates were resistant to ITZ, FCZ and KTZ, other isolate (from a woman with diabetes and erythematous candidiasis) was resistant to MCZ and KTZ, and other isolate was resistant to ITZ and MCZ. Most *C. glabrata* isolates with resistance to any of the azole antifungal agents showed at least intermediate susceptibility to other azole.

Among the 11 isolates of *C. tropicalis*, 2 were resistant (18.2%) and 5 were intermediate (45.5%) to ITZ, 1 was resistant (9.1%) and 2 were intermediate (18.2%) to KTZ or FCZ, 1 was resistant (9.1%) and 7 intermediate (63.6%) to MCZ, and none was resistant to 5FC or AMB. One of the *C. tropicalis* isolates resistant to ITZ (from a child without HIV infection and without oral candidiasis) was also resistant to FCZ. Isolates of *C. parapsilosis* were susceptible to most antifungal tested, however both isolates were intermediate to ITZ and one was intermediate to AMB and MCZ. The unique non-*Candida* species isolated, *S. cerevisiae* showed 100% susceptibility to all antifungal agents tested.

Discussion

In Europe, North America and Australia, *C. albicans* usually accounts for 60-80% of yeasts isolated from the mouth of healthy persons. However oral mycobiota varies from country to country and *C. albicans* is not always the predominant species. Little is known about the etiological importance of the different yeast species in the oral colonization and infection among Mexican people. In the present study, *C. albicans* was isolated from 66.7% to 86.5% of the oral cavities of healthy Mexican adults and children, respectively. Two other species of *Candida* were

Table 2. Distribution of clinical isolates among the different groups of individuals studied.

Clinical status	Species of yeast	No. isolates (%)	HIV adults	Non-HIV adults[a]	HIV children[b]	Non-HIV children
Colonized	*C. albicans*	71 (83.5)	0 (0)	12 (66.7)	27 (90)	32 (86.5)
	C. albicans A	62 (72.9)	0 (0)	8 (44.4)	24 (80)	30 (81)
	C. albicans B	9 (10.6)	0 (0)	4 (22.2)	3 (10)	2 (10.5)
	C. glabrata	6 (7.1)	0 (0)	3 (16.7)	1 (3.4)	2 (5.4)
	C. parapsilosis	1 (1.2)	0 (0)	0 (0)	1 (3.4)	0 (0)
	C. tropicalis	7 (8.2)	0 (0)	3 (16.7)	1 (3.4)	3 (8.1)
	Total	85 (100)	0 (0)	18 (100)	30 (100)	37 (100)
PSC	*C. albicans*	28 (77.8)	26 (81,3)	2 (50)	0 (0)	0 (0)
	C. albicans A	20 (55.6)	19 (59.4)	1 (25)	0 (0)	0 (0)
	C. albicans B	8 (22.2)	7 (21.9)	1 (25)	0 (0)	0 (0)
	C. glabrata	5 (13.9)	3 (9.4)	2 (50)	0 (0)	0 (0)
	S. cerevisiae	3 (8.3)	3 (9.4)	0 (0)	0 (0)	0 (0)
	Total	36 (100)	32 (100)	4 (100)	0 (0)	0 (0)
EC	*C. albicans*	7 (77.8)	1 (100)	0 (0)	6 (85.7)	0 (0)
	C. albicans A	5 (55.6)	1 (100)	0 (0)	4 (57.1)	0 (0)
	C. albicans B	2 (22.2)	0 (0)	0 (0)	2 (28.6)	0 (0)
	C. glabrata	1 (11.1)	0 (0)	1 (100)	0 (0)	0 (0)
	C. tropicalis	1 (11.1)	0 (0)	0 (0)	1 (14.3)	0 (0)
	Total	9 (100)	1 (100)	1 (100)	7 (100)	0 (0)
DS	*C. albicans*	22 (48.8)	0 (0)	22 (48.8)	0 (0)	0 (0)
	C. albicans A	20 (44.4)	0 (0)	20 (44.4)	0 (0)	0 (0)
	C. albicans B	2 (4.4)	0 (0)	2 (4.4)	0 (0)	0 (0)
	C. glabrata	20 (42.2)	0 (0)	20 (42.2)	0 (0)	0 (0)
	C. parapsilosis	1 (2.2)	0 (0)	1 (2.2)	0 (0)	0 (0)
	C. tropicalis	3 (6.5)	0 (0)	3 (6.5)	0 (0)	0 (0)
	Total	46 (100)	0 (0)	46 (100)	0 (0)	0 (0)
PSC+EC	*C. albicans*	8 (72.7)	5 (100)	3 (60)	0 (0)	0 (0)
	C. albicans A	6 (54.5)	3 (60)	3 (50)	0 (0)	0 (0)
	C. albicans B	2 (18.2)	2 (40)	0 (0)	0 (0)	0 (0)
	C. glabrata	3 (27.3)	0 (0)	3 (50)	0 (0)	0 (0)
	Total	11 (100)	5 (100)	6 (100)	0 (0)	0 (0)

PSC= Pseudomembranous candidiasis; EC= Erythematous candidiasis; DS= Denture stomatitis; PSC+EC= Pseudomembranous and erythematous candidiasis.

[a] Mixed cultures: 5 cultures yielding *C. albicans* + *C. glabrata*, 1 *C. albicans* + *C. tropicalis*, 1 *C. glabrata* + *C. tropicalis*, and 1 *C. glabrata* + *C. parapsilosis*.

[b] Mixed cultures: 1 culture yielding *C. albicans* + *C. glabrata*.

Table 3. Antifungal susceptibility of isolates classified according to their clinical presentation in the individuals studied.

Clinical status	Species of yeast[a]	No. of isolates (%)	AMB			MCZ			KTZ			ITZ			FCZ		
			S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Colonized	*C. albicans*	71 (100)	69 (97.2)	2 (2.8)	0 (0)	63 (88.7)	7 (9.9)	1 (1.4)	50 (70.4)	12 (16.9)	9 (12.7)	50 (70.4)	13 (18.3)	8 (11.3)	63 (88.7)	6 (8.5)	2 (2.8)
	C. albicans A	62 (100)	60 (96.8)	2 (3.2)	0 (0)	55 (88.7)	6 (9.7)	1 (1.6)	44 (71)	11 (17.7)	7 (11.3)	44 (71)	11 (17.7	7 (11.3)	55 (88.7)	6 (9.7)	1 (1.6)
	C. albicans B	9 (100)	9 (100)	0 (0)	0 (0)	8 (88.9)	1 (11.1)	0 (0)	6 (66.7)	1 (11.1)	2 (22.2)	6 (66.7)	2 (22.2)	1 (11.1)	8 (88.9)	0 (0)	1 (11.1)
	C. glabrata	6 (100)	6 (100)	0 (0)	0 (0)	4 (66.7)	0 (0)	2 (33.3)	3 (50)	2 (33.3)	1 (16.7)	2 (33.3)	2 (33.3)	2 (33.3)	4 (66.7)	2 (33.3)	0 (0)
	C. parapsilosis	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)
	C. tropicalis	7 (100)	6 (85.7)	1 (16.7)	0 (0)	1 (14.3)	5 (71.4)	1 (14.3)	4 (57.1)	2 (28.6)	1 (14.3)	1 (14.3)	5 (71.4)	1 (14.3)	5 (71.4)	1 (14.3)	1 (14.3)
	Total	85 (100)	81 (95.3)	4 (4.7)	0 (0)	68 (80)	13 (15.3)	4 (4.7)	58 (68.3)	16 (18.8)	11 (12.9)	53 (62.4)	21 (24.7)	11 (12.9)	73 (85.9)	9 (10.6)	3 (3.5)
PSC	*C. albicans*	28 (100)	28 (100)	0 (0)	0 (0)	26 (92.9)	2 (7.1)	0 (0)	20 (71.4)	5 (17.9)	3 (10.7)	21 (75)	7 (25)	0 (0)	27 (96.4)	1 (3.6)	0 (0)
	C. albicans A	20 (100)	20 (100)	0 (0)	0 (0)	18 (90)	2 (10)	0 (0)	16 (80)	4 (20)	0 (0)	16 (80)	4 (20)	0 (0)	20 (100)	0 (0)	0 (0)
	C. albicans B	8 (100)	8 (100)	0 (0)	0 (0)	8 (100)	0 (0)	0 (0)	4 (50)	1 (12.5)	3 (37.5)	5 (62.5)	3 (37.5)	0 (0)	7 (87.5)	1 (12.5)	0 (0)
	C. glabrata	5 (100)	5 (100)	0 (0)	0 (0)	4 (80)	1 (20)	0 (0)	3 (60)	2 (40)	0 (0)	0 (0)	5 (100)	0 (0)	3 (60)	2 (40)	0 (0)
	Saccharomyces	3 (100)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)
	Total	36 (100)	36 (100)	0 (0)	0 (0)	33 (91.7)	3 (8.3)	0 (0)	26 (72.2)	7 (19.4)	3 (8.3)	24 (66.7)	12 (33.3)	0 (0)	33 (91.7)	3 (8.3)	0 (0)
EC	*C. albicans*	7 (100)	7 (100)	0 (0)	0 (0)	7 (100)	0 (0)	0 (0)	4 (57.1)	3 (42.9)	0 (0)	5 (71.4)	2 (28.6)	0 (0)	7 (100)	0 (0)	0 (0)
	C. albicans A	5 (100)	5 (100)	0 (0)	0 (0)	5 (100)	0 (0)	0 (0)	3 (60)	2 (40)	0 (0)	3 (60)	2 (40)	0 (0)	5 (100)	0 (0)	0 (0)
	C. albicans B	2 (100)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
	C. glabrata	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
	C. tropicalis	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
	Total	9 (100)	9 (100)	0 (0)	0 (0)	9 (100)	0 (0)	0 (0)	5 (55.6)	3 (33.3)	1 (11.1)	7 (77.8)	2 (22.2)	0 (0)	9 (100)	0 (0)	0 (0)
DS	*C. albicans*	22 (100)	22 (100)	0 (0)	0 (0)	17 (76.3)	5 (23.8)	0 (0)	16 (72.7)	4 (18.2)	2 (9.1)	14 (63.6)	5 (22.7)	3 (13.6)	20 (90.9)	1 (4.5)	1 (4.5)
	C. albicans A	20 (100)	20 (100)	0 (0)	0 (0)	15 (75)	5 (25)	0 (0)	14 (70)	4 (20)	2 (10)	12 (60)	5 (25)	3 (15)	18 (90)	1 (5)	1 (5)
	C. albicans B	2 (100)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
	C. glabrata	20 (100)	20 (100)	0 (0)	0 (0)	11 (57.9)	7 (36.8)	1 (0)	12 (60)	7 (35)	1 (5)	7 (35)	9 (45)	4 (20)	13 (65)	6 (30)	1 (5)
	C. parapsilosis	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)
	C. tropicalis	3 (100)	3 (100)	0 (0)	0 (0)	1 (25)	2 (50)	1 (0)	3 (100)	0 (0)	0 (0)	2 (66.7)	1 (33.3)	0 (0)	2 (66.7)	1 (33.3)	0 (0)
	Total	46 (100)	46 (100)	0 (0)	0 (0)	30 (65.3)	14 (30.4)	2 (4.3)	32 (69.6)	11 (23.9)	3 (6.5)	23 (50)	16 (34.8)	7 (15.2)	36 (78.3)	8 (17.4)	2 (4.3)
PSC+EC	*C. albicans*	8 (100)	8 (100)	0 (0)	0 (0)	8 (100)	0 (0)	0 (0)	7 (87.5)	1 (12.5)	0 (0)	6 (75)	2 (25)	0 (0)	8 (100)	0 (0)	0 (0)
	C. albicans A	6 (100)	6 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	5 (83.3)	1 (16.7)	0 (0)	4 (66.7)	2 (33.3)	0 (0)	6 (100)	0 (0)	0 (0)
	C. albicans B	2 (100)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
	C. glabrata	3 (100)	3 (100)	0 (0)	0 (0)	1 (33.3)	2 (66.7)	0 (0)	1 (33.3)	1 (33.3)	1 (33.3)	2 (66.7)	1 (33.3)	0 (0)	1 (33.3)	1 (33.3)	1 (33.3)
	Total	11 (100)	11 (100)	0 (0)	0 (0)	9 (81.8)	2 (18.2)	0 (0)	8 (72.7)	2 (18.2)	1 (9.1)	8 (72.7)	3 (27.3)	0 (0)	9 (81.8)	1 (9.1)	1 (9.1)
Total	*C. albicans*	136 (100)	134 (98.5)	2 (1.5)	0 (0)	121 (89)	14 (10.3)	1 (0.7)	97 (71.9)	25 (18.4)	14 (10.3)	96 (70.6)	29 (21.3)	11 (8.1)	125 (91.9)	8 (5.9)	3 (2.2)
	C. albicans A	113 (100)	111 (98.2)	2 (1.8)	0 (0)	99 (87.5)	13 (11.5)	1 (0.9)	82 (72.6)	22 (19.5)	9 (8)	79 (69.9)	24 (21.2)	10 (8.8)	104 (92)	7 (6.2)	2 (1.8)
	C. albicans B	23 (100)	23 (100)	0 (0)	0 (0)	22 (95.6)	1 (4.3)	0 (0)	15 (65.2)	3 (13)	5 (21.7)	17 (73.9)	5 (21.7)	1 (4.3)	21 (91.3)	1 (4.3)	1 (4.3)
	C. glabrata	35 (100)	35 (100)	0 (0)	0 (0)	21 (60)	11 (31.4)	3 (8.6)	19 (54.3)	12 (34.3)	4 (11.4)	10 (28.6)	18 (51.4)	7 (20)	22 (62.9)	11 (31.4)	2 (5.7)
	C. parapsilosis	2 (100)	1 (50)	1 (50)	0 (0)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)
	C. tropicalis	11 (100)	10 (90.9)	1 (9.1)	0 (0)	3 (27.3)	7 (63.6)	1 (9.1)	8 (72.7)	2 (18.2)	1 (9.1)	4 (36.4)	5 (45.5)	2 (18.2)	8 (72.7)	2 (18.2)	1 (9.1)
	Saccharomyces	3 (100)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)
	Total	187 (100)	183 (97.9)	4 (2.1)	0 (0)	149 (79.7)	33 (17.6)	5 (2.7)	129 (69)	39 (20.8)	19 (10.2)	113 (60.4)	54 (28.9)	20 (10.7)	160 (85.6)	21 (11.2)	6 (3.2)

[a] All the isolates were resistant to 5-fluorocytosine.

PSC= Pseudomembranous candidiasis; EC= Erythematous candidiasis; DS= Denture stomatitis; PSC+EC= Pseudomembranous and erythematous candidiasis.

AMB= Amphotericin B; MCZ= Miconazole; KTZ= Ketoconazole; ITZ= Itraconazole; FCZ= Fluconazole.

S= Susceptible; I = Intermediate; R= Resistant.

recovered, representing *C. glabrata* between 5.4% and 16.7% (children and adults, respectively) and *C. tropicalis* between 8.1 and 16.7% (children and adults, respectively). *Candida* species distribution in healthy Mexicans is very similar to the observed distribution in other North American and European countries [39,57]. Overall, yeasts were isolated from 59.9% of the persons studied, of them 32.7% presented oral lesions produced by or associated to yeasts. *C. albicans* was the most frequent species (63.7% of isolates), with a clear predominance of serotype A (78.5% of *C. albicans* isolates) over serotype B. The majority of oral candidiasis is caused by *C. albicans*; however, reports on the emergence of other species of *Candida* have been published [3,36]. Barchiesi et al. [3] described an increase in the frequency of isolation of non-*albicans* yeast species from 3 to 4% of isolates in 1988-1989 to 16 to 18% of isolates in 1990 and 1991. Similarly, Morace et al. [36] found that 25% of the yeast species isolated from persons with HIV infection/AIDS were non-*C. albicans* species. The distribution of the two serotypes of *C. albicans* differs geographically: *C. albicans* serotype A has been detected significantly more frequently in subjects from Europe (75 to 94%) and Turkey (100%) than from the United States (45 to 68%) [5,8-9,24,27-29,49,50]. In oral candidiasis, *C. albicans* serotype A is the most commonly recovered [5,32,35] and it has been related to a higher virulence, but this fact has not been conclusively elucidated. On the other hand, researchers agree that the occurrence of *C. albicans* serotype B isolates is associated with declining immune status [5,8,9]. Serotype A is practically the only one observed in isolates from patients with denture stomatitis [32]. However, the proportion between serotypes A and B is more balanced in other clinical types of candidiasis. In this aspect, it could be interesting to underline the high incidence of serotype B in oral candidiasis in children with pseudomembranous candidiasis (oral thrush) [18] or in HIV/AIDS patients with angular cheilitis, pseudomembranous or erythematous oral candidiasis [9].

The ecology of the human mouth can be altered by different factors and HIV infection is associated to an increase in the carriage rates of yeasts and to a higher predisposition to oral candidiasis. The Mexican National Council of Prevention and Control of AIDS (CONASIDA) reported that 69,795 Mexican persons suffered from AIDS in the period from 1983 to 2003 [17] and more than 200,000 Mexicans were infected by HIV. The prevalence of oral candidiasis reported for Mexico during the last 12 years in HIV-infected patients has been 31.6% [45]. Symptom-free HIV patients with $CD4^+$ cell counts < 400 cells/µl have a 50% risk of progression to full-blown AIDS within 3 years. However, such patients who also have oral candidiasis have a 90% risk of progression in the same period, being a strong association between low $CD4^+$ cell count (i.e. < 200 cells/µl) and the development of candidiasis before HAART treatment was employed [51]. In the present study, 66.7% (74 patients) of HIV/AIDS patients were colonized or infected by yeasts, 27% of them (10 patients) were colonized by non-*C. albicans* species. Additionally, 51.7% (104 patients) of persons without HIV infection were also colonized or infected by yeasts, and 42.3% of them (44 patients) were colonized by non-*C. albicans* species. In both groups, *C. glabrata* was the most frequent non-*C. albicans* species. Overall, non-*albicans* species were isolated from 28.7% of persons: *C. glabrata* from 35 patients (18.7% of all yeast isolates), *C. tropicalis* from 11 (5.9% of all yeast isolates), *S. cerevisiae* from 3 (1.6% of all yeast isolates) and *C. parapsilosis* from 2 (1.1% of all yeast isolates). There were nine episodes of infection or colonization by two different yeast species. This high percentage of non-*C. albicans* isolates was associated mainly to oral colonization of persons without HIV infection ($p < 0.05$). However, neither *C. dubliniensis* nor *C. krusei* were isolated from any of the oral specimens studied with independence of the age of the patients and of their HIV infection status.

Gottfredsson et al. [26] found that the presence and intensity of asymptomatic oral carriage of yeasts were more significantly correlated with viral load than $CD4^+$ cell count and suggested the probable association to the suppression of oral mucosa immunity. Klein et al. [30] pointed out that HIV replication might have a direct effect on the virulence of *Candida* species and that HIV protease inhibitors also might play an important role in colonization by *Candida* species. In the present study, the status of yeast carrier (with or without oral candidiasis) was present in 74.5% and 60% of HIV/AIDS adults and children, respectively. This carriage rate was not associated with $CD4^+$ cell count or viral load. This lack of correlation has also been observed by other authors [2,23]. In the present study, only 12% of HIV/AIDS children with oral colonization suffered from oral candidiasis and the clinical presentation was erythematous. This form was present in only six out of 38 HIV/AIDS adults with oral candidiasis, and 5 of these patients presented a combined clinical presentation of pseudomembranous and erythematous candidiasis. The pseudomembranous clinical presentation was prevalent in the Mexican adults (97.4%), as it has been described in patients from other studies before and after the introduction of HAART [11-16,23]. This finding and the fact that 28 HIV/AIDS adults and seven children with $CD4^+$ cell count > 200 suffered from oral candidiasis, suggest, as Diz Dios et al. [23] proposed, that some other individual host characteristics must play a major role in determining the status of yeast carrier, which, eventually, could lead to a symptomatic infection.

HAART reduces HIV virus load, which favors immune reconstitution processes. Recent experimental data [10] have shown that some antiretroviral molecules belonging to the class of antiretroviral protease inhibitors can also directly interfere with *Candida* infection by inhibiting the fungal secretory aspartyl proteinases. Some of these enzymes are similar to HIV proteases and had been shown to play a pathogenic role in mucosal invasion. Probably, secretory aspartyl proteinases are produced at constitutively lower levels by commensal and not infecting strains than clinical isolates from patients with oral candidiasis [20].

The presence of antifungal resistance over the past decade has been related with resistance to triazole antifungals, especially FCZ, ITZ and KTZ, and particularly in oral candidiasis in HIV/AIDS patients [1,33-34,48,55,56]. The emergence of FCZ resistance in *C. albicans* isolates in this setting was alarming. In the HIV setting, cross-sectional surveys of oral yeast carriage indicated a prevalence of FCZ-resistant *C. albicans* isolates in HIV/AIDS people of 12-19% [48]. Before the widespread use of HAART, Martins et al. [33-34] found that the point prevalence carriage of *Candida* spp. resistant to FCZ was 22% in North American patients with oral candidiasis and 14% in asymptomatically colonized patients. A re-evaluation of the same cohort of patients after the introduction of HAART, showed a declining rate of oral candidiasis and oral carriage of *Candida* spp. as well as a trend toward less frequent in vitro resistance to FCZ. Barchiesi et al. [2] found that 93% of the isolates were susceptible in vitro to FCZ and the remaining 7% was susceptible dose dependent.

In the present study, we have tested antifungal susceptibility using Fungitest, a new commercially available

and easy-to-perform breakpoint test system. This method has been compared with the standard method described by the National Committee for Clinical Laboratory Standards by different authors [19,38,53-55] showing a high to excellent correlation of qualitative results (97% agreement with FCZ and 84% agreement with ITZ). Overall, 18.7% of the yeasts were resistant at least to one or more azole antifungal agents, being KTZ and ITZ the less active. Most resistant isolates were *C. glabrata* from patients without HIV infection or *C. albicans* from HIV/AIDS patients. Non-HIV infected persons exhibited a much broader diversity of oral yeast species, but antifungal susceptibility profiles were not different from those of isolates from HIV/AIDS patients. The susceptibility for the majority of species in this study against the mentioned antifungals did not differ from previously published data for corresponding American species of the SENTRY program [21,42].

In the present study, the overall resistance of *C. albicans* isolates to ITZ was 8.1%, ranging from the absence of resistance in HIV/AIDS patients with oral candidiasis to 12.9% in colonized patients (mostly healthy people and HIV/AIDS patients). However, nearly one third of isolates from HIV/AIDS patients with candidiasis were intermediate susceptible by Fungitest. Importantly, resistance to FCZ was very low: only 2.8% of isolates from colonized persons were resistant and all isolates from HIV/AIDS patients with candidiasis were susceptible. FCZ intermediate isolates represented 5.9% of all *C. albicans* isolates. No differences were observed between both serotypes of *C. albicans* in their susceptibility to both azoles. We did not observe resistance to AMB or 5FC in any *C. albicans* isolates and only 1.5% of isolates showed an intermediate susceptibility to AMB. The absence of resistance to 5FC in *C. albicans* serotype B isolates contrasts with the previously higher resistance to this antifungal compound observed in other studies [44].

C. glabrata was the second most common (18.7% of all isolates) species in the present study. The most notable difference between *C. glabrata* isolates from this study and isolates from the SENTRY program is that 20% of Mexican isolates were resistant to ITZ, compared to 32.8-34.9% of the isolates from the SENTRY Program. The resistance was higher (33.3%) in isolates from colonized people. Among the 35 isolates of *C. glabrata*, 5.7% were resistant to FCZ. This resistance to FCZ is similar to that observed in the SENTRY studies with isolates from the USA, Canada and the rest of America, being 5.7 to 8.7% of them resistant to this triazole.

Both isolates of *C. parapsilosis* were susceptible to ITZ and FCZ, as were the isolates from the SENTRY program. *C. tropicalis* isolates showed a different susceptibility pattern as two of them were resistant (18.2%) to ITZ and one to FCZ (9.1%). For the non-*Candida* isolates, *S. cerevisiae* was the most frequently isolated species and all isolates were susceptible to the antifungal agents tested. This finding coincides with previously published data on American and African isolates of *S. cerevisiae* [7,21,42].

Overall, the susceptibility patterns of the Mexican isolates does not differ from isolates obtained from the U.S., Canada, the rest of America and Europe, but for the Mexican isolates being somewhat more susceptible. It will be interesting to follow the species distribution and profile of antifungal susceptibility among yeast isolates from Mexican HIV/AIDS patients, as periodic analysis is critical in determining the rapidly evolving susceptibility trends among *Candida* species, especially at centers caring for patients at risk. These surveillance studies have indicated the importance of knowing geographic variations in the distribution of *Candida* species and differences in the prevalence of azole-resistance. Centers caring for HIV/AIDS patients and those patients with an underlying malignancy may have higher frequencies of azole-resistant non-*albicans Candida* isolates. The significant cross-resistance among azole antifungal agents should be an additional cause for concern.

In conclusion, our data revealed important characteristics of carriage of yeasts in the oral cavity of HIV/AIDS patients and healthy people in Mexico, such as that the status of yeast carrier was not associated with the number of $CD4^+$ cells or the viral load. However, HAART showed influence on the carriage of *Candida* spp., reducing the frequency of oral candidiasis observed. Moreover, most patients harbored strains that were susceptible to FCZ. Finally, the presence of *C. glabrata* associated to denture stomatitis was an interesting finding and further studies could clarify the role of this species in the etiology of oral candidiasis.

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