JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2005, p. 4159–4162 Vol. 43, No. 8
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.8.4159–4162.2005
Copyright © 2005, American Society for Microbiology. All Rights Reserved.

Oral *Candida* Isolates Colonizing or Infecting Human Immunodeficiency Virus-Infected and Healthy Persons in Mexico

Luis Octavio Sánchez-Vargas,[1,2] Natalia Guadalupe Ortiz-López,[1] María Villar,[1] María Dolores Moragues,[3] José Manuel Aguirre,[4] Miguel Cashat-Cruz,[5] Jose Luis Lopez-Ribot,[6] Luis Alberto Gaitán-Cepeda,[2] and Guillermo Quindós[1]*

Laboratorio de Micología Médica, Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco-Euskal Herriko Unibertsitatea, Bilbao, Spain[1]; Facultad de Odontología, Universidad Nacional Autónoma de México, México DF, México[2]; Laboratorio de Micología Médica, Departamento de Enfermería I, Universidad del País Vasco-Euskal Herriko Unibertsitatea, Bilbao, Spain[3]; Cátedra de Medicina Bucal, Departamento de Estomatología, Facultad de Medicina y Odontología, Universidad del País Vasco-Euskal Herriko Unibertsitatea, Bilbao, Spain[4]; Departamento de Epidemiología, Hospital Infantil de México Dr. Federico Gómez, México DF, Mexico[5]; and Department of Medicine, Division of Infectious Diseases, South Texas Centers for Biology in Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, Texas[6]

Received 9 February 2005/Accepted 17 April 2005

Oral yeast carriage was studied in 312 Mexican subjects. *Candida albicans* was the most frequent species, but other *Candida* spp. were isolated from 16.5 to 38.5% of patients. Colonization did not correlate with $CD4^+$ number or viral load, but highly active antiretroviral therapy reduced the frequency of candidiasis. Most isolates were susceptible to fluconazole, but 10.8% were resistant to one or more azoles.

It is estimated that the oral cavity is colonized by *Candida* in 40 to 60% of persons (39). Oropharyngeal candidiasis is the most prevalent complication in human immunodeficiency virus-infected patients with AIDS (HIV/AIDS) (1, 13, 21). The introduction of highly active antiretroviral therapy (HAART) has reduced the prevalence of opportunistic infections, including candidiasis (5, 7, 11). However, HAART is not worldwide available, and the emergence of antifungal-resistant isolates of *Candida albicans* has been reported (20).

In Mexico, most clinical laboratories do not routinely perform antifungal susceptibility testing, and little is known about the antifungal susceptibility profiles of *Candida* isolates in this country. To elucidate the status of oral *Candida* carriage and antifungal susceptibility patterns in Mexico, we conducted a 3-year prospective study of 111 adults infected with HIV/AIDS and 201 non-HIV-infected persons at the General Hospital of Mexico, the Federico Gómez Child Hospital, and the Odontology Clinics at the Facultad de Odontología (UNAM) in México DF. Persons were distributed into four groups: 51 HIV/AIDS-infected adults, 109 non-HIV-infected adults, and 60 HIV/AIDS-infected and 92 non-HIV-infected children.

Data on patient demographics, history of prior mycoses, antifungal treatment, and medications were collected at enrolment. Patients without antifungal treatment in the preceding 6 months were eligible. All patients were subjected to clinical evaluation, including HIV RNA and $CD4^+$ measurements for HIV-infected patients (Tables 1 to 3). Healthy volunteers were selected from persons seeking dental care at the UNAM. Informed consent was obtained from all persons. Approval was obtained from the Human Ethics, Research, and Publications Committee of the UNAM and from the rest of medical institutions involved.

Swabs were taken from oral lesions when present, the buccal mucosa, the floor of the mouth, and the dorsal surface of the tongue. Swabs were plated onto Sabouraud dextrose agar with chloramphenicol and incubated at 36°C for 2 days (and 7 days at 30°C). Isolates were identified by mycological standard methods (3, 22). Antifungal susceptibility was assessed by Fungitest (Bio-Rad, Paris, France) according to the manufacturer's instructions. *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) were used as quality controls. Fungitest has shown high to excellent correlation compared to the NCCLS M-27A method (9, 23–25). However, its qualitative approach could be a main limitation because it does not provide MICs, in contrast to the quantitative M-27A or EUCAST methods. The two-tailed Student *t* test was used to compare differences between continuous variables, and the chi-square test was used for categorical variables.

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 (8) and more than 200,000 Mexicans were infected by HIV. Little is known about the etiological importance of *Candida* oral colonization and infection among the Mexican people (12). In the present study (Table 1), 66.7% (74 patients) of HIV/AIDS

* Corresponding author. Mailing address: Laboratorio de Micología Médica, Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco-Euskal Herriko Unibertsitatea, Bilbao, Spain. Phone: 34946012854. Fax:34946013400. E-mail: guillermo.quindos@ehu.es.

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

Characteristic	Group			
	HIV/AIDS adults	Non-HIV adults	HIV/AIDS children	Non-HIV children
No. of persons (%)	51 (100)	109 (100)	60 (100)	92 (100)
Age (mean/range in yr)	36.4/22–65	64.4/42–89	5.18/1–13	6.93/1–13
No. of colonized or infected individuals (%)	38 (74.5)	67 (61.5)	36 (60)	37 (40.2)
No. of patients with candidiasis (%)	38 (74.5)	53 (48.6)	7 (11.7)	0 (0)
No. of patients on HAART (%)	22 (43.2)	0 (0)	50 (83.3)	0 (0)
Mean no. of $CD4^+$ T cells/μl	484.1	ND[a]	673.4	ND
No. of persons (%) with 200 to 500 $CD4^+$ T cells/μl	23 (45.1)	ND	25 (41.6)	ND
No. of persons (%) with <200 $CD4^+$ T cells/μl	14 (27.4)	ND	8 (13.3)	ND
Mean no. of HIV RNA copies/ml	65,945.1	ND	49,343.6	ND
No. of persons (%) with <10,000 HIV RNA copies/ml	17 (33.3)	ND	20 (33.3)	ND
No. of persons (%) with >10,000 HIV RNA copies/ml	34 (66.6)	ND	40 (66.6)	ND

[a] ND, not done.

were colonized or infected by yeasts, and 27% of them (10 patients) were colonized by non-*C. albicans* species. In addition, 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-*C. 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), *Saccharomyces cerevisiae* from 3 (1.6% of all yeast isolates), and *C. parapsilosis* from 2 (1.1% of all yeast isolates). However, neither *C. dubliniensis* nor *C. krusei* were isolated from any of the oral specimens. There were nine episodes of infection or colonization by two different yeast species. Non-*C. albicans* isolates were associated mainly with oral colonization of persons without HIV infection ($P < 0.05$). This distribution was similar to that observed in other American and European studies (16–18, 26, 27). Overall, yeasts were isolated from 59.9% of the subjects; of these, 32.7% presented with oral lesions by yeasts. This rate coincides with the 31.6% prevalence reported for oral candidiasis in Mexico during the last 12 years in HIV-infected patients (19). Moreover, only 12% of children infected with HIV/AIDS with oral colonization suffered from candidiasis. Most cases of oral candidiasis are caused by *C. albicans* (63.7% of isolates in the present study); however, the emergence of other species have been reported (2). Moreover, HAART influenced the carriage of *Candida* spp., reducing the frequency of oral candidiasis. For HIV patients, the status of yeast carrier was not associated with $CD4^+$-cell count or viral load in the present study as has been described by others (1, 5, 11).

Antifungal resistance to fluconazole (FCZ), itraconazole (ITZ), and ketoconazole (KTZ) has been described and particularly in the setting of oral candidiasis in HIV/AIDS patients (6, 14, 15, 25, 26). Cross-sectional surveys have indicated a prevalence of FCZ-resistant oral *C. albicans* isolates of 12 to 19% (20). Before the use of HAART, Martins et al. (14, 15) found a prevalence of *Candida* spp. resistant to FCZ of 22% in North Americans with oral candidiasis and of 14% in asymptomatically colonized patients. After the introduction of HAART, the same cohort showed a declining rate of oral candidiasis and carriage of *Candida* spp., as well as a trend toward less-frequent in vitro resistance to FCZ. In the present study, 18.7% of isolates were resistant at least to one azole antifungal (KTZ and ITZ were the less active). The majority of resistant isolates were *C. glabrata* from patients without HIV infection and *C. albicans* from patients infected with HIV/AIDS. This susceptibility did not differ from previously pub-

TABLE 2. Mycological characteristics of 312 persons included in this study

Group	Species of yeast	No. of isolates (%)	Group (no. [%])			
			HIV/AIDS adults	Non-HIV adults[a]	HIV/AIDS children[b]	Non-HIV children
Colonized	*Candida albicans*	71 (83.5)	0 (0)	12 (66.7)	27 (90)	32 (86.5)
	Candida glabrata	6 (7.1)	0 (0)	3 (16.7)	1 (3.4)	2 (5.4)
	Candida parapsilosis	1 (1.2)	0 (0)	0 (0)	1 (3.4)	0 (0)
	Candida 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)
Candidiasis	*Candida albicans*	65 (63.7)	32 (84.2)	27 (47.4)	6 (100)	0 (0)
	Candida glabrata	29 (28.4)	3 (7.9)	26 (45.6)	0 (0)	0 (0)
	Candida parapsilosis	1 (0.9)	0 (0)	1 (1.8)	0 (0)	0 (0)
	Saccharomyces cerevisiae	3 (2.9)	3 (7.9)	0 (0)	0 (0)	0 (0)
	Candida tropicalis	4 (3.9)	0 (0)	3 (5.2)	1 (0)	0 (0)
	Total	102 (100)	38 (100)	57 (100)	7 (100)	0 (100)

[a] Mixed cultures: five cultures yielding *C. albicans* plus *C. glabrata*, one culture yielding *C. albicans* plus *C. tropicalis*, one culture yielding *C. glabrata* plus *C. tropicalis*, and one culture yielding *C. glabrata* plus *C. parapsilosis*.

[b] Mixed cultures: one culture yielding *C. albicans* plus *C. glabrata*.

TABLE 3. In vitro antifungal susceptibility of isolates[a]

Species	No. of isolates (%)	Susceptibility (no. of isolates [%])[a]				
		Amphotericin B (I/R)	Miconazole (I/R)	Ketoconazole (I/R)	Itraconazole (I/R)	Fluconazole (I/R)
C. albicans	136 (100)	2 (1.5)/0 (0)	14 (10.3)/1 (0.7)	25 (18.4)/14 (10.3)	29 (21.3)/11 (8.1)	8 (5.9)/3 (2.2)
C. glabrata	35 (100)	0 (0)/0 (0)	11 (31.4)/3 (8.6)	12 (34.3)/4 (11.4)	18 (51.4)/7 (20)	11 (31.4)/2 (5.7)
C. parapsilosis	2 (100)	1 (50)/0 (0)	1 (50)/0 (0)	0 (0)/0 (0)	2 (100)/0 (0)	0 (0)/0 (0)
C. tropicalis	11 (100)	1 (9.1)/0 (0)	7 (63.6)/1 (9.1)	2 (18.2)/1 (9.1)	5 (45.5)/2 (18.2)	2 (18.2)/1 (9.1)
S. cerevisiae	3 (100)	0 (0)/0 (0)	0 (0)/0 (0)	0 (0)/0 (0)	0 (0)/0 (0)	0 (0)/0 (0)
Total	187 (100)	4 (2.1)/0 (0)	33 (17.6)/5 (2.7)	39 (20.8)/19 (10.2)	54 (28.9)/20 (10.7)	21 (11.2)/6 (3.2)

[a] I, intermediate; R, resistant.

lished data of the American SENTRY program (10, 17). The overall resistance of *C. albicans* isolates to ITZ was 8.1%. However, nearly one-third of the isolates from patients with HIV/AIDS with candidiasis were intermediate susceptible. Importantly, resistance to FCZ was very low: only 2.8% of isolates from colonized persons were resistant, and all isolates from patients with HIV/AIDS with candidiasis were susceptible. The FCZ-intermediate isolates represented 5.9% of all *C. albicans* isolates. We did not observe resistance to amphotericin B or 5-fluorocytosine. A lower number of *C. glabrata* isolates (20%) from Mexican subjects were resistant to ITZ than in the SENTRY program (32.8 to 34.9%) (10, 17). Among the 35 isolates of *C. glabrata*, 5.7% were resistant to FCZ, a figure similar to that observed in the SENTRY studies (5.7 to 8.7%). *C. tropicalis* isolates showed a different susceptibility pattern since two of these isolates were resistant (18.2%) to ITZ and one was resistant to FCZ (9.1%). Both isolates of *C. parapsilosis* were susceptible to ITZ and FCZ. *S. cerevisiae* was susceptible to all of the antifungals tested (4, 10, 17).

L.O.S.-V. was a visiting Scholar at the Universidad del País Vasco from the Programa de Becas MAE Curso 2002-2003 de la Agencia Española de Cooperación Internacional. This study was in part supported by grants from the Universidad del País Vasco-Euskal Herriko Unibertsitatea (project 1/UPV 00093.327-E-14645/2002), from the Fondo de Investigaciones Sanitarias del Ministerio de Sanidad (project PI030662/2003), and PAPIIT 214300 DGAPA-UNAM (México).

We thank Patricia Pérez Ríos, Hilda Hidalgo Loperena, and Javier Romo García for assistance with clinical data from the Department of Stomatology and the Unit of Infectology of the Hospital General de México, México DF, Mexico.

REFERENCES

1. **Barchiesi, F., M. Maracci, B. Radi, D. Arzeni, J. Baldassarri, A. Giacometti, and G. Scalise.** 2002. Point prevalence, microbiology and fluconazole susceptibility patterns of yeast isolates colonizing the oral cavities of HIV-infected patients in the era of highly active antiretroviral therapy. J. Antimicrob. Chemother. **50:**999–1002.
2. **Barchiesi, F., V. Morbiducci, F. Ancarani, and G. Scalise.** 1993. Emergence of oropharyngeal candidiasis caused by non-albicans species of *Candida* in HIV-infected patients. Eur. J. Epidemiol. **9:**455–456.
3. **Bikandi, J., R. San Millán, M. D. Moragues, G. Cebas, D. C. Coleman, G. Quindós, and J. Pontón.** 1998. Rapid identification of *Candida dubliniensis* by indirect immunofluorescence based on differential localization of antigens on *C. dubliniensis* blastospores and *Candida albicans* germ tubes. J. Clin. Microbiol. **36:**2428–2433.
4. **Blignaut, E., S. Messer, R. J. Hollis, and M. A. Pfaller.** 2002. Antifungal susceptibility of South African oral yeast isolates from HIV/AIDS patients and healthy individuals. Diagn. Microbiol. Infect. Dis. **44:**169–174.
5. **Ceballos-Salobreña, A., L. Gaitán-Cepeda, L. Ceballos-García, and L. P. Samaranayake.** 2004. The effect of antiretroviral therapy on the prevalence of HIV-associated oral candidiasis in a Spanish cohort. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. **97:**345–350.
6. **Ceballos-Salobreña, A., L. A. Gaitán-Cepeda, F. Orihuela-Cañada, D. Barrionuevo Olea, L. Ceballos-García, and G. Quindós.** 1999. Resistencia in vitro a los antifúngicos en *Candida albicans* de pacientes infectados por el VIH con y sin candidiasis oral. Rev. Iberoam. Micol. **16:**194–197.
7. **Ceballos-Salobreña, A., G. Quindós-Andrés, and L. Ceballos-García.** 2000. The influence of different risk behaviors in the appearance of oral candidiasis in AIDS patients. Med. Oral **5:**71–80.
8. **CONASIDA.** 2003. Panorama epidemiológico del VIH/SIDA e ITS en México. Consejo Nacional para la Prevención y control del VIH/SIDA, Secretaría de Salud, México. Dirección General de Epidemiología, Mexico City, Mexico. [Online.] http://www.salud.gob.mx/conasida.
9. **Davey, K. G., A. D. Holmes, E. M. Johnson, A. Szekely, and D. W. Warnock.** 1998. Comparative evaluation of Fungitest and broth microdilution methods for antifungal drug susceptibility testing of *Candida* species and *Cryptococcus neoformans*. J. Clin. Microbiol. **36:**926–930.
10. **Diekema, D. J., M. A. Pfaller, S. A. Messer, A. Houston, R. J. Hollis, G. V. Doern, R. N. Jones, et al.** 1999. In vitro activities of BMS-207147 against over 600 contemporary clinical bloodstream isolates of *Candida* species from the SENTRY antimicrobial surveillance program in North America and Latin America. Antimicrob. Agents Chemother. **43:**2236–2239.
11. **Diz-Dios, P., A. Ocampo, I. Otero, I. Iglesias, and C. Martínez.** 2001. Changes in oropharyngeal colonization and infection by *Candida albicans* in human immunodeficiency virus-infected patients. J. Infect. Dis. **183:**355–356.
12. **Gaitan-Cepeda, L., M. Cashat-Cruz, J. J. Morales-Aguirre, L. Sanchez-Vargas, S. Aquino-Garcia, R. Fragoso-Rios, V. Cuairan-Ruidiaz, C. Avila-Figueroa, J. Morales, and C. A. Figueroa.** 2002. Prevalence of oral lesions in Mexican children with perinatally acquired HIV: association with immunologic status, viral load, and gender. AIDS Patient Care STDS **16:**151–156.
13. **Gottfredsson, M., G. M. Cox, O. S. Indridason, G. M. D. De Almeida, A. E. Heald, and J. R. Perfect.** 1999. Association of plasma levels of human immunodeficiency virus type 1 RNA and oropharyngeal *Candida* colonization. J. Infect. Dis. **180:**534–537.
14. **Martins, M. D., M. Lozano-Chiu, and J. H. Rex.** 1997. Point prevalence of oropharyngeal carriage of fluconazole-resistant *Candida* in human immunodeficiency virus-infected patients. Clin. Infect. Dis. **25:**843–846.
15. **Martins, M. D., M. Lozano-Chiu, and J. H. Rex.** 1998. Declining rates of oropharyngeal candidiasis and carriage of *Candida albicans* associated with trends toward reduced rates of carriage of fluconazole-resistant *C. albicans* in human immunodeficiency virus-infected patients. Clin. Infect. Dis. **27:**1291–1294.
16. **Odds, F. C.** 1988. *Candida* and candidiasis, 2nd ed. Baillière Tindall, London, United Kingdom.
17. **Pfaller, M. A., R. N. Jones, G. V. Doern, H. S. Sader, R. J. Hollis, S. A. Messer, et al.** 1998. International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and antifungal susceptibilities of isolates collected in 1997 in the United States, Canada and South America for the SENTRY program. J. Clin. Microbiol. **36:**1886–1889.
18. **Quindós, G., M. T. Ruesga, E. Martín-Mazuelos, R. Salesa, R. Alonso-Vargas, A. J. Carrillo-Muñoz, S. Brena, R. San-Millán, and J. Pontón.** 2004. In-vitro activity of 5-fluorocytosine against 1,021 Spanish clinical isolates of *Candida* and other medically important yeasts. Rev. Iberoam. Micol. **21:**63–69.
19. **Ramirez-Amador, V., L. Esquivel-Pedraza, J. Sierra-Madero, G. Anaya-Saavedra, I. Gonzalez-Ramirez, and S. Ponce-de-Leon.** 2003. The changing clinical spectrum of human immunodeficiency virus-related oral lesions in 1,000 consecutive patients: a 12-year study in a referral center in Mexico. Medicine **82:**39–50.
20. **Sanglard, D., and F. C. Odds.** 2002. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. Lancet Infect. Dis. **2:**73–85.
21. **Vargas, K. G., and S. Joly.** 2002. Carriage frequency, intensity of carriage, and strains of oral yeast species vary in the progression to oral candidiasis in human immunodeficiency virus-positive individuals. J. Clin. Microbiol. **40:**341–350.
22. **Williams, D. W., and M. A. O. Lewis.** 2000. Isolation and identification of *Candida* from the oral cavity. Oral Dis. **6:**3–11.

23. **Willinger, B., P. Apfalter, A. M. Hirschl, A. Makristathis, M. Rotter, and M. Seibold.** 2000. Susceptibility testing of *Candida* species: comparison of NCCLS microdilution method with Fungitest®. Diagn. Microbiol. Infect. Dis. **38:**11–15.
24. **Willinger, B., E. Engelmann, H. Hofmann, S. Metzger, P. Apfalter, A. M. Hirschl, A. Makristathis, M. Rotter, B. Raddatz, and M. Seibold.** 2002. Multicenter comparison of Fungitest for susceptibility testing of *Candida* species. Diagn. Microbiol. Infect. Dis. **44:**253–257.
25. **Witthuhn, F., D. Toubas, I. Béguinot, D. Aubert, C. Rouger, G. Remy, and J. M. Pinon.** 1999. Evaluation of the Fungitest kit by using strains from human immunodeficiency virus-infected patients: study of azole drug susceptibility. J. Clin. Microbiol. **3:**864–866.
26. **Wroblewska, M. M., E. Swoboda-Kopec, A. Rokosz, E. Krawczyk, H. Marchel, and M. Luczak.** 2002. Epidemiology of clinical isolates of *Candida albicans* and their susceptibility to triazoles. Int. J. Antimicrob. Agents **20:** 472–475.
27. **Xu, J., and T. G. Mitchell.** 2003. Geographical differences in human oral yeast flora. Clin. Infect. Dis. **36:**221–224.