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Correspondence

Species distribution & antifungal susceptibility of oral *Candida* colonising or infecting HIV infected individuals

Sir,

Oropharyngeal candidiasis (OPC) occurs in up to 90 per cent of HIV infected cases during the course of infection[1] and is considered as an important marker of the disease and its progression. *Candida* spp. that colonize the oral cavity are the source of yeasts that cause oral candidiasis[2]. Although many studies have been conducted on oral carriages of *Candida* species-both in healthy and immuno compromised individuals, but because of different criteria in recruitment and yeast assessment methods, results from these studies are not comparable[3]. Though *Candida albicans* is the most frequently isolated species as coloniser and pathogen of the oral mucosa, other *Candida* species, such as *C. tropicalis*, *C. krusei*, and *C. glabrata* are recovered increasingly[4]. OPC increases morbidity and reduces the quality of life of HIV/AIDS patients and therefore requires prompt diagnosis and adequate therapy[5]. The azoles, particularly fluconazole, remain among the most common antifungal drugs, but their intensive clinical use for both therapy and prophylaxis has favoured the emergence of resistant strains. Thus, the present study was carried out to determine the species distribution and antifungal susceptibility profile of oral *Candida* isolates colonizing or infecting HIV infected individuals.

A total of 210 consecutive HIV positive individuals, attending National AIDS Research Institute (NARI) clinic at Pune, during January 2007 - March 2009 were included in the study. During the same period, 125 consecutive HIV negative individuals who came for detection of their HIV serostatus without oral mucosal lesions were included as the control group. The study was approved by the Ethics committee of the institute. All participants were included in the study after obtaining their informed written consent. Individuals with diabetes mellitus or other systemic diseases, pregnant women, smokers, denture/orthodontic device users, and those under treatment with antimicrobials during the preceding three months were excluded.

HIV serostatus of the patients was determined by commercially available ELISA and Rapid antibody tests (Genetic system, Biorad Labs, USA and Tridot, J Mitra & Co., New Delhi), using National AIDS Control Organisation (NACO) recommended algorithm[6]. CD4 cell counts were measured by using a FACS count system (Becton Dickinson, Singapore BD).

Oral swabs were obtained by firmly swabbing the lesion site with sterile cotton swabs in case of symptomatic individuals and the dorsum of tongue and buccal mucosa in case of asymptomatic ones. The samples were immediately inoculated on Sabouraud dextrose agar (SDA) with chloramphenicol (0.05 g/l) and incubated at 37^0 C for 2 days, and for additional seven days at 30^0 C before being considered as negative. All isolates were identified by standard mycological techniques[7]. Identification of all isolates was confirmed by API 20 C AUX (BioMerieux, France). The minimum inhibitory concentrations (MICs) of all *Candida* isolates from HIV positive individuals to fluconazole, ketoconazole and itraconazole were determined by the E test (AB Biodisk, Sweden) as per manufacturers' instructions. Breakpoint definitions for fluconazole and itraconazole MICs were based on Clinical Laboratory Standards Institute (CLSI) M27-A2[8] standard guidelines. Due to lack of consensual breakpoints for MICs of ketoconazole, arbitrary values based on an earlier study were used[9].

Statistical analysis was performed using SPSS software version 15.0 (SPSS Inc, USA). Chi square test was used to check the association between categorical variables. $P<0.05$ was considered statistically significant.

Of the 210 HIV positive individuals, 60 had lesions of oral candidiasis (symptomatic) and isolates were obtained from all while, of the 150 asymptomatic individuals, 88 (58.7%) were positive for oral carriage of *Candida* spp. Twenty eight (22.4%) of the HIV negative individuals showed candidial colonisation.

The Table shows the *Candida* spp. isolated from HIV positive individuals. *C. albicans* was the predominant isolate. Higher frequencies of non-albicans species were observed in HIV infected individuals with oral candidiasis (35%) as compared to the asymptomatic HIV positive (19.1%). In HIV positive asymptomatic group mixed candidial colonization was seen in 6 patients (6.81%). The *Candida* spp. isolated from the HIV negative individuals were *C. albicans* 21 *(75%), C. tropicalis* 1 (3.6%), *C. krusei* and *C. glabrata* 3 isolates (10.7% each). The range of CD4 in carrier and non-carrier HIV positive patients was 19-987 cells/mm^3 (median 194.5 cells/mm^3) and 61-1105 cells/mm^3 (median 365 cells/mm^3) respectively. All HIV positive individuals with oral candidiasis had CD4 counts less than 250 cells/mm^3. There was a statistically significant association between oral candidiasis and low CD4 counts, (P<0.001). Of the 154 isolates obtained from HIV positive individuals, the MIC ranges were 0.032-256 µg/ml for fluconazole, 0.004-32 µg/ml for ketoconazole and 0.016-8 µg/ml for itraconazole. The *Candida* spp. showed an overall resistance of 14 per cent to one of the three azoles tested (Table). Of the 210 HIV positive patients, 36 (17.1%) had history of prior exposure to antifungals and 38 isolates were obtained from them (2 patients had mixed infection). Isolates from these patients exhibited greater resistance to azoles (26.3%) compared to isolates from the unexposed group (7.8%) (P< 0.006).

Table. *Candida* species and their *in vitro* antifungal susceptibility pattern from HIV positive individuals

Candida species	HIV positive (symptomatic) N (%)	HIV positive (asymptomatic) N (%)	*In vitro* antifungal susceptibility pattern*	
			S N (%)	R N (%)
Candida albicans	39 (65)	76 (80.9)	93 (83.0)	14 (12.5)
Candida non-albicans	21 (35)	18 (19.1)	26 (68.4)	7 (18.4)
C. tropicalis	6 (10.0)	5 (5.3)	7 (70.0)	1 (10.0)
C. lusitaniae	5 (8.3)	-	4 (80)	1 (20)
C. parapsilosis	2 (3.3)	5 (5.3)	7 (100)	-
C. glabrata	2 (3.3)	4 (4.3)	2 (33.3)	3 (50)
C. krusei	2 (3.3)	3 (3.2)	1(20)	2 (40)
C. kefyr	1 (1.7)	-	1(100)	-
C. boidinii	1 (1.7)	-	1(100)	-
C. guillermondii	1 (1.7)	-	1(100)	-
C. norvegensis	1 (1.7)	-	1(100)	-
C. sphaerica	-	1 (1.1)	1(100)	-
Total	60	94	119 (79.3)	21(14.0)

*S, susceptible to all three azoles; R, resistant to one of the three azoles. Four isolates were susceptible for one or two of the azoles and susceptible dose dependent for others, thus data not included

Various studies have shown the isolation rate of *Candida* ranging from 61-100 per cent in HIV infected individuals with oral candidiasis[10-13], while a wide range of oral carriage rates have been reported for asymptomatic HIV infected (11.0 - 96.0%) as well for HIV uninfected (10.0 - 68.0%) subjects[3]. In our study, *Candida* was isolated from all symptomatic HIV positive individuals, whereas the oral colonization in the asymptomatic HIV infected and uninfected group was 58.7 and 22.4 per cent respectively. This variability in carriage rates reported may be caused by geographic differences, time of sampling or the different methods used for yeast recovery and quantitation. *C. albicans* was the predominant isolate, while *C. tropicalis* was the commonest non-albicans spp. in HIV infected, a finding similar to previous studies[4,14]. A significant association between oral candidiasis and low CD4 count was noted. Thus, this clinical condition could be useful in estimating immunosuppression in resource limited settings. Overall 14 per cent of *Candida* species were resistant to at least one of the three azoles tested. *Candida* non-albicans species were more resistant to azoles compared to albicans, information that can be useful for clinicians dealing with non - responding cases. Thus, eliciting history of exposure to these drugs may be important in choosing appropriate therapy.

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