Candidiasis in HIV-positive patients in Greece: Conventional epidemiological data and evaluation of the clinical resistance of Candida species to seven antifungal agents

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Abstract

The purpose of this study was the determination of the resistance of different *Candida* species isolated from oropharengeal and esophageal lesions of HIV positive and AIDS patients over a ten year period, from patients in a Special Infection Unit. Clinical resistance of culture-identified isolates to ketoconazole, itraconazole, fluconazole, voriconazole, 5-flucytosine, amphotericin B and capsofungin showed variation according to the administered antiretroviral regimen (Protein Inhibitors, Other HAART or no HAART) and different incidence and resistance values were clearly observed among both *C. albicans* and non –*C. albicans* species depending on the antifungal compound used.

Keywords: *Candida species, resistance, susceptibility, antifungal, HIV*

Introduction

Candida species colonize the gastrointestinal tract of even healthy individuals, which invariably precede hematogenous or systemic disease (4). There is also an important correlation between patients who develop candidemia, compromised immune status, and oropharyngeal and esophageal candidiasis (2).
Although *Candida albicans* is the main etiologic agent of oropharyngeal candidiasis in HIV-positive patients, other species, like *C. dubliniensis, C. glabrata, C. krusei, C. tropicalis, C. cerevisiae* can be detected, in mixed cultures with *Candida albicans*. *C. guilliermondii, C. parapsilosis* and *C. kefyr* are rarely encountered.
Thus, the present study was designed to assess the molecular epidemiology and resistance of the different *Candida* species to antifungal agents in HIV-positive patients and in patients who have developed AIDS.

Materials and methods

This prospective study was conducted over 10 years (November 1995 – November 2005) in "G. Gennimatas" General Hospital in Athens, Greece and its protocol was approved by the hospital's ethics committee. Informed consent was obtained from the patients. Samples were collected from patients during the initial phase of oropharyngeal colonization with *Candida,* as well as during episodes of recurrent candidiasis.

Specimens were cultured in Corn Meal Agar (Dalmau - CMA), Malt Extract Agar, Sabouraud Dextrose Agar with cloramphenicol. 2,3,5-triphenyltetrazolium chloride (TTC) agar and CHROMAgar candida® (CHROMAgar, Paris, France) were also used. The cultures were deemed positive if there was growth on these plates. Yeasts were identified using the API ID 32C system (BioMeriéux, Marcy l'Etoile, France). The patients were compared ($p < 0.05$) for selected parameters by the Chi-Square test and the Fisher Exact Test. Resistant strains were further analyzed with Pulsed Field Gel Electrophoresis (PFGE), Polymerase Chain Reaction (PCR), and sequencing of the internal transcribed spacer region (ITS) (unpublished results).

Results

Of 665 samples analyzed 237 were culture- positive, producing 247 isolates. *C. albicans* was the most common etiologic agent (68.9%). The results were in accordance with similar international data (6) except *C. parapsilosis* and *C. cerevisiae*, their relative incidences being generally reversed. The **Table 1** shows the different species isolated from the cultures and their incidence. In 23 patients, more than one species were isolated (**Table 2).**

Table 1. Incidence of different *Candida* species in 247 isolates from 237 oropharyngeal samples

	N	(%)
C. albicans	186	68.9
C. glabrata	18	6.7
C. tropicalis	16	5.9
C. dubliniensis	12	4.4
C. krusei	10	3.6
C. parapsilosis	4	1.4
C. lusitaniae	3	1.1
C. sake	2	0.7
Cryptococcus neoformans	2	0.7
T. cutaneum	2	0.7
Zygosaccharomyces	2	0.7
Blastoschizomyces capitalus	1	0.4
C. cerevisiae	2	0.7
C. globosa	1	0.4
C. curvata	1	0.4
C. guilliermondii	1	0.4
C. holmii	1	0.4
C. lambica	1	0.4
C. norvegensis	1	0.4
C. pelliculosa	1	0.4
C. pulhcerimma	1	0.4
C. valida	1	0.4
Geotrichum fermentans	1	0.4
C. carsonii	1	0.4
Pichia fermentans	1	0.4
Pichia sp.	1	0.4

Table 2. Distribution of multiple *Candida* isolates in oropharyngeal samples of 23 patients

Patient No	Sample No	Fungal species isolated		
1	3	*C. albicans*	*C. krusei*	
2	5	*C. albicans*	*C. dubliniensis*	
	6	*C. albicans*	*C. dubliniensis*	
3	1	*C. albicans*	*C. norvegensis*	
4	2	*C. albicans*	*C. glabrata*	*C. tropicalis*
5	2	*C. albicans*	*C. glabrata*	*C. tropicalis*
	4	*C. albicans*	*C. glabrata*	
6	1	*C. albicans*	*P. fermentans*	
7	3	*C. tropicalis*	*B. capitatus*	
8	1	*C. albicans*	*C. tropicalis*	
9	3	*C. albicans*	*C. glabrata*	
10	1	*C. albicans*	*C. lambica*	*C. pulhcerimma*
11	1	*C. albicans*	*C. glabrata*	
12	1	*C. albicans*	*C. tropicalis*	
13	1	*C. albicans*	*C. gulliermondii*	
14	2	*C. albicans*	*C. lusitaniae*	
15	1	*C. albicans*	*C. glabrata*	
16	1	*C. albicans*	*C. glabrata*	*C. krusei*
17	3	*C. sake*	*Zygosacharomyces*	
	5	*C. albicans*	*C. glabrata*	*C. tropicalis*
	7	*C. albicans*	*C. glabrata*	*C. parapsilosis*
18	2	*C. albicans*	*C. parapsilosis*	
19	2	*C. albicans*	*C. valida*	
20	3	*C. albicans*	*C. glabrata*	
21	4	*C. albicans*	*C. cerevisiae*	
22	1	*C. albicans*	*C. glabrata*	*C. tropicalis*
23	1	*C. albicans*	*C. tropicalis*	

Contrary to the non-*Candida albicans* species causing candidiasis to HIV-negative patients (incidence 30-70%), in the HIV-positive hosts the incidence tends to be lower (28.5%), with *C. glabrata* (6.7%) being the second most common etiologic agent after *C. albicans*.

Table 3. *C. albicans* resistance (%) in various agents by HAART regimen

Agent*	PI	Other HAART	No HAART
KZ	8,8	11,1	-
FL	33,1	22	22,2
ITR	12,8	17,1	11,1
VO	-	1,1	-
5FC	17,9	19,6	11,1
AB	-	-	-
Cas	-	-	-

*ketoconazole (KZ); fluconazole (FL); itraconazole (ITR); voriconazole (VO); 5-flucytosine (5FC); amphotericin B (AB); caspofungin (CAS).

The susceptibility data were in accordance with relevant international studies (1). *C. albicans* was mostly susceptible to all antifungal agents, while the administered HAART makes no significant difference.
To ketoconazole, clinical resistance was observed in 3 of 34 patients (8.8%) subjected to protease inhibitors (PIs), in 3 of 27 (11.1%) subjected to other treatment and in none of the six untreated patients.
To itraconazole, clinical resistance was observed in 5 of 39 patients (12.8%) subjected to PIs, in 7 of 41 (17.1%) subjected to other treatment (2 being dose-dependent resistance) and in 1 of 9 (11.1%) untreated patients.
To voriconazole, clinical resistance was observed in no patient of the 39 subjected to protease inhibitors (PIs), in 1 of 40 (1.1%) subjected to other treatment and in none of the nine untreated patients.
To fluconazole, clinical resistance was observed in 9 of 39 patients (33,1 %) subjected to PIs (two being dose-dependent), in 9 of 41 (22%) subjected to other treatment (three being dose-dependent) and in two of the nine untreated patients (22,2 %), (one being dose-dependent).
To 5-flucytosine, clinical resistance was observed in 7 of 39 patients (17,9 %) subjected to PIs, (5 being dose-dependent), in 8 of 41 (19.6%) subjected to other treatment (four being dose-dependent) and in 1 of the 9 untreated patients (dose-dependent).
To amphotericin-B and capsofungin, clinical resistance was observed in none of the 89 and 40 patients respectively, regardless the HAART treatment.
As for the *non-C. albicans* species, although they are mainly susceptible to the tested antifungal agents, their resistance is much higher, especially to the classical azoles (ketoconazole, itraconazole, and fluconazole), although 9.1% were resistant to amphotericin B.

Table 4. Non-*C. albicans* resistance (%) in various agents by HAART regimen

Agent	PI	Other HAART	No HAART
KZ	33,3	75	22,2
FL	42.9	60	50
ITR	42,9	60	50
VO	14,3	10	-
5FC	19	10	-
AB	4,8	20	-
Cas	-	-	-

*ketoconazole (KZ); fluconazole (FL); itraconazole (ITR); voriconazole (VO); 5-flucytosine (5FC); amphotericin B (AB); caspofungin (CAS).

To ketoconazole clinical resistance was observed in 5 of 15 patients (33,3%) subjected to PIs (two being dose-dependent), in 6 of 8 (75%) subjected to other treatment (one being dose-dependent) and in two of the nine untreated patients (22,2%), (one being dose-dependent).
To itraconazole, clinical resistance was observed in 9 of 21 patients (42,9 %) subjected to PIs (one being dose-dependent), in 6 of 10 (60%) subjected to other treatment (one being dose-dependent) and in one of the two untreated patients (50%), (dose-dependent).
To voriconazole, clinical resistance was observed in 3 of 21 patients (14,3 %) subjected to PIs, in 1 of 10 (10%) subjected to other treatment and in none of the two untreated patients.
To fluconazole, clinical resistance was observed in 9 of 21 patients (42,9 %) subjected to PIs (one being dose-dependent), in 6 of 10 (60%) subjected to other treatment (five being dose-dependent) and in one of the two untreated patients (50%).
To 5-flucytosine, clinical resistance was observed in 4 of 21 patients (19%) subjected to PIs (two being dose-dependent), in 1 of 10 (10 %) subjected to other treatment) and none of the two untreated patients.
To amphotericin-B, clinical resistance was observed in 1 of 21 patients (4,8 %) subjected to PIs, in 2 of 10 (20%) subjected to other treatment and in none of the 2 untreated patients.
To capsofungin clinical resistance was not observed at any of the 12 patients

Discussion

The use of CHROMagar contributed significantly in the differentiation of *C. albicans* from *C. dubliniensis*, in accordance to published data (3, 5). *C. lusitaniae* presented pink colonies before treatment, and grey-red colonies during treatment with amphotericin B, while developing resistance, a rather useful feature to monitor almost in real-time the development of resistance in recurring cases and regularly treated patients.
C. albicans, C. tropicalis and *C. parapsilosis* are generally susceptible to all azoles (including the newer, wider-spectrum triazoles, voriconazole, ravuconazole, posaconazole), to echinocandins (capsofungin, micafungin), to 5-flucytosine, and to the classic or liposomic amphotericin-B. For *C. glabrata* resistant isolates to triazoles, increasing the dose usually resolves the problem. *C. krusei* is considered resistant to fluconazole and often to other azoles.
A number of *non-C. albicans* species are now resistant to fluconazole, one of the most effective and widely used antifungal agents, both for superficial and systemic infections. *C. krusei, C. inconspicua* and *C. norvegensis*, have endogenous resistance, whereas *C. dubliniensis* and *C. tropicalis* become resistant through exposure to the drug. It is very possible that widespread and frequent use of fluconazole and itraconazole may have caused the emergence of new strains and species of *Candida*, with low susceptibility to common antifungal medication (1).
In the clinical setting, resistance against the polyene amphotericin-B is not common for *Candida albicans* but rather more so for *C. glabrata* and *C. krusei* and even more for *C. lusitaniae*, a less common pathogen.

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ΚΑΝΤΙΝΤΙΑΣΗ ΣΕ ΟΡΟΘΕΤΙΚΟΥΣ ΑΣΘΕΝΕΙΣ ΣΤΗΝ ΕΛΛΑΔΑ: ΣΥΜΒΑΤΙΚΑ ΕΠΙΔΗΜΙΟΛΟΓΙΚΑ ΔΕΔΟΜΕΝΑ ΚΑΙ ΑΞΙΟΛΟΓΗΣΗ ΤΗΣ ΚΛΙΝΙΚΗΣ ΑΝΤΟΧΗΣ ΕΙΔΩΝ *Candida* ΣΕ ΕΠΤΑ ΑΝΤΙΜΥΚΗΤΙΑΚΟΥΣ ΠΑΡΑΓΟΝΤΕΣ

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Περίληψη

Σκοπός της εργασίας είναι ο καθορισμός της αντοχής διαφορετικών ειδών *Candida* που απομονώθηκαν από στοματοφαρυγγικές και οισοφαγικές βλάβες οροθετικών HIV ασθενών και ασθενών με σύνδρομο επίκτητης ανοσοανεπάρκειας μιας εξειδικευμένης Μονάδας Ειδικών Λοιμώξεων σε μια περίοδο 10 ετών. Η κλινική αντοχή των στελεχών, που ταυτοποιήθηκαν καλλιεργητικά, σε 7 αντιμυκητιακούς παράγοντες (κετοκοναζόλη, ιτρακοναζόλη, φλουκοναζόλη, βορικοναζόλη, αμφοτερικίνη-Β, κασποφουνγκίνη, 5-φθοριοκυτοσίνη), έδειξε διαφοροποιήσεις σχετιζόμενες με την αντιρετροϊική θεραπεία (αναστολείς πρωτεασών, άλλη αντιρετροϊκή θεραπεία, καμία αντιρετροϊκή θεραπεία). Επίσης, διαφορετικές τιμές επίπτωσης και αντοχής εμφανίστηκαν μεταξύ τόσο των στελεχών του είδους *C. albicans* όσο και μεταξύ των στελεχών των λοιπών ειδών του γένους, ανάλογα με το αντιμυκητιακό σκεύασμα που χρησιμοποιήθηκε.

Λέξεις-κλειδιά: *Είδη Candida, αντοχή, ευαισθησία, αντιμυκητιακά, HIV.*