Polish Journal of Microbiology
2016, Vol. 65, No 1, 123–129

SHORT COMMUNICATION

Etiologic Agents and Antifungal Susceptibility of Oral Candidosis from Romanian patients with HIV-infection or type 1 *diabetes mellitus*

BOGDAN MINEA[1], VALENTIN NASTASA[2], ANNA KOLECKA[3], MAGDALENA MARES[4], NARCISA MARANGOCI[1], IRINA ROSCA[1], MARIANA PINTEALA[1], MONICA HANCIANU[5] and MIHAI MARES[2]*

[1] Institute of Macromolecular Chemistry "Petru Poni", Advanced Research Centre for Bionanoconjugates and Biopolymers, Iasi, Romania
[2] "Ion Ionescu de la Brad" University, Laboratory of Antimicrobial Chemotherapy, Iasi, Romania
[3] CBS Fungal Biodiversity Centre (CBS-KNAW), Yeast and Basidiomycete Research, Utrecht, Netherlands
[4] Third School Health Centre, Department of Dentistry, Piatra Neamt, Romania
[5] University of Medicine and Pharmacy "Gr. T. Popa", Faculty of Pharmacy, Iasi, Romania

Submitted 11 March 2015, revised 15 April 2015, accepted 21 April 2015

Abstract

This is the first Romanian investigation of oral candidosis in patients suffering of HIV-infection or type 1 *diabetes mellitus* (T1DM). *Candida albicans* was the dominant species in both types of isolates: n = 14 (46.7%) in T1DM, n = 60 (69.8%) in HIV. The most frequent non-*albicans Candida* spp. were *Candida kefyr* (n = 6; 20%) in T1DM and *Candida dubliniensis* (n = 8; 9.3%) in HIV. Resistance to fluconazole was detected only in the HIV non-*albicans Candida* group (n = 8; 9.3%). All isolates were susceptible to VOR. The experimental drug MXP had MIC values equal or close to the ones of VOR. Echinocandin resistance was more frequent than azole resistance.

K e y w o r d s: antifungal susceptibility, MXP-4509, oral candidosis, Romanian HIV and diabetes patients

The presence of oral *Candida* yeasts is considered a biomarker indicative of immune system impairment and, in immunodeficiency disorders, can be correlated with a progressive disease (Vargas and Joly, 2002). Oral candidosis (OC) is the most frequent type of yeast infection and occurs especially in denture wearers and individuals with severe conditions, such as HIV-infected patients, those under antibiotic or chemotherapy, organ transplantation recipients and patients with systemic diseases such as diabetes (Vergani *et al.*, 2013).

HIV-infected patients are susceptible to opportunistic mycoses as cell-mediated immunity decays (Sangeorzan *et al.*, 1994). Before the era of highly active antiretroviral therapy (HAART), oropharyngeal candidosis (OPC) occurred in as many as 90% of patients, at some point during the course of HIV infection (Lortholary *et al.*, 2012). Since the initiation of HAART in 1996, there has been a decrease in the incidence of OPC (Leigh *et al.*, 2004) while oropharingeal colonization varies from 44% to 62% (Lin *et al.*, 2013a).

To the best of our knowledge, the present study is the first Romanian investigation providing data regarding the etiologic spectrum and the antifungal susceptibility profile of OC isolates from patients with either HIV-infection or diabetes.

The 116 clinical yeast isolates included in this study were collected in three tertiary hospitals from different regions of Romania (*i.e.* Iasi, Timisoara and Brasov), from patients with overt OC. Of these patients, 30 were suffering from type 1 (insulin-dependent) *diabetes mellitus* (T1DM), while the other 86 were HIV infected (CD4+ T lymphocytes count $< 500/mm^3$). The final identification was performed using ID32C strips (bioMérieux, France). Isolates identified as *Candida albicans* or *Candida dubliniensis* were further verified with duplex PCR (Romeo and Criseo, 2011). The isolates for which the ID32C strips gave inconclusive results were sent to the CBS-KNAW Fungal Biodiversity Centre, Utrecht (The Netherlands), where they were identified by MALDI-TOF MS or the sequence analyses of the ITS

* Corresponding author: M. Mares, Laboratory of Antimicrobial Chemotherapy, "Ion Ionescu de la Brad" University, Iasi, Romania; **e-mail: mycomedica@gmail.com**

(Internal Transcribed Spacer) and the D1/D2 domains of the LSU (Large SubUnit) of the ribosomal DNA, as previously reported (Kolecka *et al.*, 2013).

In vitro susceptibility testing was performed following the EUCAST E. Def 7.1 guidelines (Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST), 2008), for six antifungal agents: fluconazole (Sigma – St. Louis, USA), voriconazole (Pfizer Ltd. – Sandwich, UK), caspofungin (Merck & Co, Inc.), micafungin (Astellas Pharma, Japan), anidulafungin (Pfizer, Inc.) and the MXP-4509 experimental compound ("Petru Poni" Institute of Macromolecular Chemistry – Iasi, Romania), which is a triazole based nanoconjugate with β-cyclodextrin as a carrier molecule (Marangoci *et al.*, 2011). Two reference strains (*C. albicans* ATCC 90028 and *Candida krusei* ATCC 6258) were used for quality control. The interpretation of the MICs for the commercial antifungal agents was done according to the recent EUCAST document "Antifungal Agents. Breakpoint tables for interpretation of MICs", version 7.0 (Subcommittee on Antifungal Susceptibility Testing (AFST) of the European Committee for Antimicrobial Susceptibility Testing (EUCAST), 2014).

Specific statistical parameters (Mode, MIC_{50} and MFC_{50} – for $n \geq 5$, MIC_{90} and MFC_{90} – for $n \geq 10$ and Geometric Mean–for $n \geq 2$, where n = the number of isolates) were calculated for each tested drug using Microsoft® Excel® (Dannaoui *et al.*, 2008). Statistical analysis was performed using a fully functional trial version of GraphPad Prism version 6.04 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com. Two-tailed *P*-values were calculated and $P < 0.05$ was considered significant. The level of significance was signalled in the text with one superscript asterisk (*) for $P \leq 0.05$ and two superscript asterisks (**) for $P \leq 0.01$. To calculate the geometric means and run the statistical tests, right censored values (MIC > the maximum tested concentration) were treated as the next theoretical value *i.e.* "> 8 mg/l" was treated as "16 mg/l" (Dannaoui *et al.*, 2008).

The overall species distribution and some of the calculated statistical parameters of the MICs are shown in Table I. Nine species were identified. In both types of chronic condition *Candida albicans* was the dominant species. Although it was surpassed by non-*albicans Candida* in the T1DM isolates, the statistical analysis revealed no significant differences in the distribution of species (*C. albicans* vs. non-*albicans Candida*) between the two categories. Cumulative antifungal susceptibility data (MIC_{50}, MIC_{90}, MFC_{50}, MFC_{90}) along with susceptibility and resistance rates for *C. albicans* and the non-*albicans Candida* group are presented in Table II. All the T1DM isolates and all *C. albicans* HIV isolates were susceptible to FLC. Based mostly on the 2 mg/l non-specific BP for FLC, eight (30.77%) non-*albicans Candida* HIV isolates can be considered resistant, *i.e.* four *C. krusei* isolates, two of *Candida inconspicua* and two of *Candida norvegensis*. There was also two intermediately susceptible *Candida utilis* isolate. All isolates were susceptible to VOR. Two *C. albicans* and two *Candida tropicalis* T1DM isolates were resistant to all echinocandins, but they were susceptible to azoles. The *C. tropicalis* isolates also had high MFC values for CAS and ANI. There were also two *Candida lusitaniae* T1DM isolates resistant to CAS. Within the HIV isolates there were four of *C. albicans* that were resistant to MCA and ANI but were susceptible to azoles. All the non-*albicans Candida* HIV isolates were susceptible to echinocandins.

The antifungals MICs for the reference strains used for quality control were: *C. albicans* ATCC 90028 (0.125–0.25 mg/l for FLC, 0.0156 mg/l for VOR, 0.0156–0.0312 mg/l for MXP, 0.0625–0.125 mg/l for CAS, 0.0156–0.0312 for MCA, and 0.0156 mg/l for ANI); *C. krusei* ATCC 6258 (16–32 mg/l for FLC, 0.0625–0.125 mg/l for VOR, 0.0312–0.0625 mg/l for MXP, 0.0312–0.0625 mg/l for CAS, 0.0156–0.0312 for MCA, and 0.0312–0.0625 mg/l for ANI).

Articles regarding species distribution and antifungal susceptibility of oral isolates from patients with diabetes are relatively scarce and, unlike our study, they investigate isolates resulted from colonisation and not from OC. Even fewer go as far as testing antifungal susceptibility. Despite reports of increased presence of non-*albicans Candida* species, the most recent surveys from Brazil (Sanitá *et al.*, 2013; Bremenkamp *et al.*, 2011) or Western Europe (Manfredi *et al.*, 2002; 2006) document isolation rates of 70% and higher for *C. albicans*. The proportion in our study, approximately 50%, is more similar to reports from geographically closer areas such as Poland (Drozdowska and Drzewoski, 2008; Nawrot *et al.*, 2006), Slovakia (Dorko *et al.*, 2005) or Turkey (Kadir *et al.*, 2002). Regarding the non-*albicans* species, most studies report the isolation of *C. tropicalis*, but also *Candida glabrata* and *Candida parapsilosis*; the latter two did not occur in our investigation. The number of isolates and also the geographical gradient are important reasons for these differences. The Turkish survey reports *Candida kefyr*, while an older British survey reports *C. lusitaniae* (Manfredi *et al.*, 2002), species also reported by this study.

Our findings regarding FLC susceptibility are in agreement with the most recent Brazilian (Sanitá *et al.*, 2013) and British-Italian (Manfredi *et al.*, 2006) researches that found no FLC resistance. In contrast to the situation in Brazil, the Romanian isolates had a high rate of CAS resistance. Since there are no established BPs for CAS, we did use a non-specific BP of 0.25 mg/l which encompasses most of the already

Table I
Species distribution and *in vitro* antifungal susceptibility in oral candidosis isolates

Species (no. of isolates % of all isolates)	Compound	MIC (μg/ml)			MFC (μg/ml)		
		Range	Mode	GM[1]	Range	Mode	GM
T1DM isolates (n = 30)	FLC	≤ 0.125–0.25	≤ 0.125	0.1682			
C. albicans	VOR	NA[1]	≤ 0.0156	0.1566			
(14–46.67%,	MXP	≤ 0.0156–0.0625	≤ 0.0156	0.0210			
95% CI = 24.80–69.89%)	CAS	0.0312–0.5	0.0312	0.0464	0.125–2.0	0.25	0.4529
	MCA	≤ 0.0156–0.25	≤ 0.0156	0.0283	0.0625–2.0	0.125; 0.25	0.2500
	ANI	0.0312–0.25	0.0312	0.0420	0.125–1.0	0.125	0.2051
C. kefyr	FLC	0.25–0.5	0.25	0.3150			
(6–20.00%)	VOR	NA	≤ 0.0156	0.0156			
	MXP	≤ 0.0156–0.0312	≤ 0.0156	0.0197			
	CAS	NA	0.0312	0.0312	0.0625–0.25	0.25	0.1575
	MCA	0.0312–0.0625	0.0625	0.0496	0.125–0.5	NA	0.2500
	ANI	0.0625–0.25	NA	0.1250	0.125–0.5	0.5	0.3150
C. lusitaniae	FLC	≤ 0.125–0.5	≤ 0.125	0.1984			
(6–20.00%)	VOR	NA	≤ 0.0156	0.0156			
	MXP	≤ 0.0156–0.0312	≤ 0.0156	0.0197			
	CAS	0.0312–0.5	NA	0.1249	0.5–1.0	0.5	0.6300
	MCA	0.0312–0.25	NA	0.0787	0.125–0.5	0.5	0.3150
	ANI	0.0625–0.25	0.0625	0.0992	0.25–1.0	0.25	0.3969
C. tropicalis	FLC	≤ 0.125–1.0	NA	0.3536			
(4–13.33%)	VOR	≤ 0.0156–0.0312	NA	0.0221			
	MXP	≤ 0.0156–0.0625	NA	0.0884			
	CAS	0.0312–1.0	NA	0.1766	0.25–16.0	NA	2.0000
	MCA	0.0625–1.0	NA	0.2500	0.25–1.0	NA	0.5000
	ANI	0.0625–2.0	NA	0.3536	1.0–8.0	NA	2.8284
Non-albicans *Candida*	FLC	≤ 0.125–1.0	≤ 0.125	0.2726			
(16–53.33%,	VOR	≤ 0.0156–0.0312	≤ 0.0156	0.0170			
95% CI = 30.11%–75.20%)	MXP	≤ 0.0156–0.0625	≤ 0.0156	0.0286			
	CAS	0.0312–1.0	0.0312	0.0810	0.0625–16.0	0.25	0.5000
	MCA	0.0312–1.0	0.0625	0.0884	0.125–1.0	0.5	0.3242
	ANI	0.0625–2.0	0.0625	0.1487	0.125–8.0	0.25; 0.5; 1.0	0.5946
Overall	**FLC**	**≤ 0.125–1.0**	**≤ 0.125**	**0.2176**			
	VOR	**≤ 0.0156–0.0312**	**≤ 0.0156**	**0.0163**			
	MXP	**≤ 0.0156–0.0625**	**≤ 0.0156**	**0.0248**			
	CAS	**0.0312–1.0**	**0.0312**	**0.0624**	**0.0625–16.0**	**0.25**	**0.4774**
	MCA	**≤ 0.0156–1.0**	**≤ 0.0156; 0.0625**	**0.0519**	**0.0625–2.0**	**0.125; 0.25; 0.5**	**0.2872**
	ANI	**0.0312–2.0**	**0.0312**	**0.0824**	**0.0625–8.0**	**0.125**	**0.3618**
HIV isolates (n = 86)	FLC	≤ 0.125–0.5	0.5	0.2872			
C. albicans	VOR	NA	≤ 0.0156	0.0156			
(60–69.77%,	MXP	NA	≤ 0.0156	0.0156			
95% CI = 54.80%–81.49%)	CAS	≤ 0.0156–0.0312	0.0312	0.0291	0.0625–2.0	0.0625	0.1984
	MCA	≤ 0.0156–0.0312	≤ 0.0156	0.0163	0.0312–2.0	0.0625	0.1575
	ANI	≤ 0.0156–0.0625	0.0312	0.0312	0.0625–2.0	0.0625	0.1469
C. dubliniensis	FLC	0.25–0.5	0.25	0.2973			
(8–9.30%)	VOR	NA	≤ 0.0156	0.0156			
	MXP	NA	≤ 0.0156	0.0156			
	CAS	NA	0.0312	0.0312	0.5–1.0	1.0	0.8409
	MCA	0.0312–0.0625	0.0312; 0.0625	0.0442	1.0–4.0	1.0	1.4142
	ANI	0.0625–0.125	0.125	0.1051	0.125–1.0	0.5	0.4204
C. kefyr	FLC	NA	0.5	0.5000			
(4–4.65%)	VOR	NA	≤ 0.0156	0.0156			
	MXP	NA	≤ 0.0156	0.0156			
	CAS	NA	0.0312	0.0312	0.0312–0.0625	NA	0.0442
	MCA	NA	0.0625	0.0625	0.125–0.25	NA	0.1768
	ANI	NA	0.125	0.1250	0.25–0.5	NA	0.3536

Table I. Continued.

Species (no. of isolates % of all isolates)	Compound	MIC (μg/ml)			MFC (μg/ml)		
		Range	Mode	GM[1]	Range	Mode	GM
C. krusei (4–4.65%)	FLC	32.0–64.0	NA	45.2548			
	VOR	0.25–0.5	NA	0.3536			
	MXP	0.25–0.5	NA	0.3536			
	CAS	NA	0.125	0.1250	0.125–0.25	NA	0.1768
	MCA	0.0625–0.125	NA	0.0884	0.125–0.25	NA	0.1768
	ANI	NA	0.0625	0.0625	NA	0.125	0.1250
C. tropicalis (4–4.65%)	FLC	0.25–0.5	NA	0.3536			
	VOR	NA	0.0312	0.0312			
	MXP	0.0312–0.0625	NA	0.0442			
	CAS	NA	0.0312	0.0312	0.125–2.0	NA	0.5000
	MCA	NA	0.0312	0.0312	NA	1.0	1.0000
	ANI	NA	0.0625	0.0625	0.25–1.0	NA	0.5000
C. inconspicua (2–2.33%)	FLC	NA	32.0	32			
	VOR	NA	0.25	0.25			
	MXP	NA	0.125	0.125			
	CAS	NA	0.125	0.125	NA	0.125	NA
	MCA	NA	0.0312	0.0312	NA	0.0625	NA
	ANI	NA	0.0625	0.0625	NA	0.125	NA
C. norvegensis (2–2.33%)	FLC	NA	16.0	16			
	VOR	NA	0.0312	0.0312			
	MXP	NA	0.0312	0.0312			
	CAS	NA	0.0625	0.0625	NA	0.25	NA
	MCA	NA	0.0312	0.0312	NA	0.125	NA
	ANI	NA	0.0312	0.0312	NA	0.125	NA
C. utilis (2–2.33%)	FLC	NA	4.0	4			
	VOR	NA	0.125	0.125			
	MXP	NA	0.0625	0.0625			
	CAS	NA	0.0312	0.0312	NA	0.0312	NA
	MCA	NA	0.0312	0.0312	NA	0.0625	NA
	ANI	NA	0.0312	0.0312	NA	0.0312	NA
Non-*albicans Candida* (26–30.23%, 95% CI = 18.51%–45.20%)	FLC	0.25–64.0	0.25; 0.5	1.7044			
	VOR	≤ 0.0156–0.5	≤ 0.0156	0.0430			
	MXP	≤ 0.0156–0.5	≤ 0.0156	0.0408			
	CAS	0.0312–0.125	0.0312	0.0453	0.0312–2.0	0.125; 1.0	0.2370
	MCA	0.0312–0.125	0.0312	0.0453	0.0625–4.0	1.0	0.3631
	ANI	0.0312–0.125	0.0625	0.0733	0.0312–1.0	0.125	0.2370
Overall	**FLC**	**≤ 0.125–64.0**	**0.5**	**0.4920**			
	VOR	**≤ 0.0156–0.5**	**≤ 0.0156**	**0.0212**			
	MXP	**≤ 0.0156–0.5**	**≤ 0.0156**	**0.0209**			
	CAS	**≤ 0.0156–0.125**	**0.0312**	**0.0333**	**0.0312–2.0**	**0.125**	**0.2094**
	MCA	**≤ 0.0156–0.125**	**0.0156**	**0.0222**	**0.0312–4.0**	**0.0625**	**0.2027**
	ANI	**≤ 0.0156–0.125**	**0.0312**	**0.0404**	**0.0312–2.0**	**0.125**	**0.1698**

[1] GM–Geometric Mean; [2] NA–Not Applicable

established echinocandin BPs. This situation requires further research, especially considering the findings of a recent study that echinocandins would be a safer choice for diabetes patients since they do not seem to be affected by glucose, which appears to significantly lower the antifungal activity of azoles and polyenes (Mandal *et al.*, 2014).

Studies that investigate isolates from HIV patients are more abundant, but similarly to those targeting diabetes, more of them focus on the asymptomatic carriage of yeasts in the oral cavities. Nevertheless, oral colonisation of HIV-infected patients in conjunction to low counts of CD4 cells are strong premises for subsequent development of OPC (Fong *et al.*, 1997). The same increase of prevalence for the non-*albicans* species is documented for HIV patients and, equally, *C. albicans* remains the dominant species. *C. dubliniensis*, *C. glabrata*, and *C. tropicalis* are considered as

Table II
Cumulative antifungal susceptibility data and resistance (R) rates of oral candidosis isolates

Species	T1DM				HIV				
	Compound	MIC_{50} (μg/ml)	MFC_{50} (μg/ml)	R (n–%)	MIC (μg/ml)		MFC (μg/ml)		R (n–%)
					MIC_{50}	MIC_{90}	MFC_{50}	MFC_{90}	
Candida albicans	FLC	≤0.125		0–0.0%	0.25	0.5			0–0.0%
	VOR	≤0.0156		0–0.0%	≤0.0156	≤0.0156			0–0.0%
	MXP	≤0.0156		NA	≤0.0156	≤0.0156			NA
	CAS	0.0312	0.25	2–14.3%	0.0312	0.0312	0.125	1.0	0–0.0%
	MCA	≤0.0156	0.25	4–28.6%	≤0.0156	≤0.0156	0.0625	2.0	4–6.7%
	ANI	0.0312	0.125	2–14.3%	0.0312	0.0312	0.125	0.5	4–6.7%
Non-*albicans Candida*	FLC	0.25		0–0.0%	0.5	32.0			8–30.8%
	VOR	≤0.0156		0–0.0%	0.0312	0.25			0–0.0%
	MXP	≤0.0156		NA	0.0312	0.25			NA
	CAS	0.0312	0.25	4–25.0%	0.0312	0.125	0.25	1.0	0–0.0%
	MCA	0.0625	0.25	2–12.5%	0.0312	0.0625	0.25	1.0	0–0.0%
	ANI	0.0625	0.5	2–12.5%	0.0625	0.125	0.25	1.0	0–0.0%
Overall	FLC	0.25		0–0.0%	0.5	4.0			8–9.3%
	VOR	≤0.0156		0–0.0%	≤0.0156	0.0312			0–0.0%
	MXP	≤0.0156		NA	≤0.0156	0.0625			NA
	CAS	0.0312	0.25	6–20.0%	0.0312	0.0312	0.125	1.0	0.0
	MCA	0.0625	0.25	6–20.0%	≤0.0156	0.0625	0.125	2.0	4–4.7%
	ANI	0.0625	0.25	4–13.3%	0.0312	0.125	0.125	0.5	4–4.7%

emerging pathogens (Lin *et al.*, 2013; Drozdowska and Drzewoski, 2008; Binolfi *et al.*, 2005).

Regarding *C. albicans* proportion within the HIV isolates, values similar to the one in this study (70%) have been reported for Taiwan (Ho *et al.*, 2014), Cameroun (dos Santos Abrantes *et al.*, 2014), USA (Merenstein *et al.*, 2013), Spain (Ramírez *et al.*, 2006) or Turkey (Erköse and Erturan, 2007). Percentages can go as high as 90% in India (Maurya *et al.*, 2013), Italy (Giammanco *et al.*, 2002) or UK (Cartledge *et al.*, 1999), 83% in South Africa (dos Santos Abrantes *et al.*, 2014), 79% in Serbia (Mitrovic *et al.*, 1996), or can go as low as 62% in Turkey (Erköse and Erturan, 2007) or Brazil (Costa *et al.*, 2006). Again, *C. glabrata* is missing from the isolates in our non-*albicans Candida* group.

Reported levels of FLC resistance vary widely from 0.9% in Taiwan (Ho *et al.*, 2014) and 3.4% in China (Li *et al.*, 2013) to about 50% in South Africa and Cameroun (dos Santos Abrantes *et al.*, 2014) for *C. albicans*. The differences can have a few possible causes such as street availability of antifungals, without prescription (dos Santos Abrantes *et al.*, 2014), or the different susceptibility testing methods used in each investigation. In our study, FLC resistance was present only in the non-*albicans Candida* group, in agreement with the above mentioned sources which found higher resistance rates for this group by up to 13% (Li *et al.*, 2013).

All the Romanian isolates were susceptible to VOR, a situation similar to that in Taiwan (Ho *et al.*, 2014). Some resistance was found in China–3% for *C. albicans* and 14.5 % for non-*albicans Candida* (Li *et al.*, 2013) and very high values, were reported for *C. albicans* from South Africa and Cameroun (dos Santos Abrantes *et al.*, 2014). This study also signals the occurrence of resistance to echinocandins that other investigations did not report. Although these antifungals are not the first choice in treating patients with OC, they can be an effective alternative if topical or systemic azoles have definitely failed (Lortholary *et al.*, 2012).

Our study confirms a few worldwide reported tendencies such as the increasing prevalence and lower antifungal susceptibility of non-*albicans Candida* species, and *C. dubliniensis* as an emerging oral pathogen in HIV patients. It also supports the status of FLC as the first option for treatment, but not advisable for prophylaxis, and VOR as a viable second line of defence.

As a triazole based antifungal, MXP-4509 inhibits the ergosterol biosynthesis, similar to FLC and VOR. The experimental drug had a good antifungal activity with MIC values similar to those of VOR. Further, *in vivo* studies are warranted.

In conclusion, strict oral hygiene and adherence to specific treatment are the best prophylactic approaches to prevent OC in both chronic conditions, while FLC is recommended only as a first line of defense after the occurrence of the infection. As a second line of defense, in case of FLC therapeutic failure, echinocandins are a viable option for HIV patients. In the case

of diabetes patients, however, the risk of azole cross-resistance should be evaluated first; for patients without prior exposure to azoles, VOR may be a better option.

Acknowledgements

This publication benefited from the financial support of the project "Programme of excellency in the multidisciplinary doctoral and post-doctoral research of chronic diseases", contract no. POSDRU/159/1.5/S/133377, beneficiary "Gr. T. Popa" University of Medicine and Pharmacy of Iasi, project co-financed from the European Social Fund through the Sectoral Operational Programme Human Resources Development (SOP HRD) 2007–2013.

Literature

Binolfi A., M.S. Biasoli, A.G. Luque, M.E. Tosello and H.M. Magaró. 2005. High prevalence of oral colonization by *Candida dubliniensis* in HIV-positive patients in Argentina. *Med. Mycol.* 43(5): 431–437.

Bremenkamp R.M., A.R. Caris, A.O.C. Jorge, G.N. Back-Brito, A.J. Mota, I. Balducci, F.L. Brighenti and C.Y. Koga-Ito. 2011. Prevalence and antifungal resistance profile of *Candida* spp. oral isolates from patients with type 1 and 2 diabetes mellitus. *Arch. Oral Biol.* 56(6): 549–555.

Cartledge J.D., J. Midgley and B.G. Gazzard. 1999. Non-albicans oral candidosis in HIV-positive patients. *J. Antimicrob. Chemother.* 43(3): 419–422.

Costa C.R., J.A. de Lemos, X.S. Passos, C.R. de Araújo, A.J. Cohen, L.K.H.E. Souza and M. do R.R. Silva. 2006. Species distribution and antifungal susceptibility profile of oral *Candida* isolates from HIV-infected patients in the antiretroviral therapy era. *Mycopathologia* 162(1): 45–50.

Dannaoui E., O. Lortholary, D. Raoux, M.E. Bougnoux, G. Galeazzi, C. Lawrence, D. Moissenet, I. Poilane, D. Hoinard and F. Dromer. 2008. Comparative *in vitro* activities of caspofungin and micafungin, determined using the method of the European Committee on antimicrobial susceptibility testing, against yeast isolates obtained in France in 2005–2006. *Antimicrob. Agents Chemother.* 52 (2): 778–781.

Dorko E., Z. Baranová, A. Jenča, P. Kizek, E. Pilipčinec and L. Tkáčiková. 2005. Diabetes mellitus and candidiases. *Folia Microbiol. (Praha)* 50(3): 255–261.

Dos Santos Abrantes P.M., C.P. McArthur and C.W.J. Africa. 2014. Multi-drug resistant oral *Candida* species isolated from HIV-positive patients in South Africa and Cameroon. *Diagn. Microbiol. Infect. Dis.* 79(2): 222–227.

Drozdowska A. and J. Drzewoski. 2008. Mycoses in diabetes-difficult diagnostic and therapeutic problem. Review of literature. *Diabetol. Doświadczalna Klin.* 8(1): 1–11.

Erköse G. and Z. Erturan. 2007. Oral *Candida* colonization of human immunodeficiency virus infected subjects in Turkey and its relation with viral load and CD4+ T-lymphocyte count. *Mycoses* 50(6): 485–490.

Fong I.W., M. Laurel and A. Burford-Mason. 1997. Asymptomatic oral carriage of *Candida albicans* in patients with HIV infection. *Clin. Investig. Med. Médecine Clin. Exp.* 20(2): 85–93.

Giammanco G.M., G. Pizzo, S. Pecorella, S. Distefano, V. Pecoraro and M.E. Milici. 2002. Identification of *Candida dubliniensis* among oral yeast isolates from an Italian population of human immunodeficiency virus-infected (HIV+) subjects. *Oral Microbiol. Immunol.* 17(2): 89–94.

Ho M.-W., Y.-L. Yang, C.-C. Lin, C.-Y. Chi, H.-T. Chen, P.-C. Lin, L.-Y. Hsieh, C.-H. Chou, W.-L. Chu, C.-P. Wu and others. 2014. Yeast oropharyngeal colonization in human immunodeficiency virus-infected patients in central Taiwan. *Mycopathologia* 177(5–6): 309–317.

Kadir T., R. Pisiriciler, S. Akyüz, A. Yarat, N. Emekli and A. Ipbüker. 2002. Mycological and cytological examination of oral candidal carriage in diabetic patients and non-diabetic control subjects: thorough analysis of local aetiologic and systemic factors. *J. Oral Rehabil.* 29(5): 452–457.

Kolecka A., K. Khayhan, M. Groenewald, B. Theelen, M. Arabatzis, A. Velegraki, M. Kostrzewa, M. Mares, S.J. Taj-Aldeen and T. Boekhout. 2013. Identification of medically relevant species of arthroconidial yeasts by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J. Clin. Microbiol.* 51(8): 2491–2500.

Leigh J.E., K. Shetty and P.L. Fidel. 2004. Oral opportunistic infections in HIV-positive individuals: review and role of mucosal immunity. *AIDS Patient Care STDs* 18(8): 443–56.

Lin J.-N., C.-C. Lin, C.-H. Lai, Y.-L. Yang, H.-T. Chen, H.-C. Weng, L.-Y. Hsieh, Y.-C. Kuo, T.-L. Lauderdale, F.-C. Tseng and others. 2013. Predisposing factors for oropharyngeal colonization of yeasts in human immunodeficiency virus-infected patients: A prospective cross-sectional study. *J. Microbiol. Immunol. Infect.* 46(2): 129–135.

Li Y.-Y., W.-Y. Chen, X. Li, H.-B. Li, H.-Q. Li, L. Wang, L. He, X.-P. Yang, X.-C. Wang, Y.-L. Huang and others. 2013. Asymptomatic oral yeast carriage and antifungal susceptibility profile of HIV-infected patients in Kunming, Yunnan Province of China. *BMC Infect. Dis.* 13(1): 46.

Lortholary O., G. Petrikkos, M. Akova, M.C. Arendrup, S. Arikan-Akdagli, M. Bassetti, J. Bille, T. Calandra, E. Castagnola, O.A. Cornely and others. 2012. ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: patients with HIV infection or AIDS. *Clin. Microbiol. Infect.* 18(Suppl. 7): 68–77.

Mandal S.M., D. Mahata, L. Migliolo, A. Parekh, P.S. Addy, M. Mandal and A. Basak. 2014. Glucose directly promotes antifungal resistance in the fungal pathogen, *Candida* spp. *J. Biol. Chem.* 289(37): 25468–25473.

Manfredi M., M.J. McCullough, Z.M. Al-Karaawi, S.J. Hurel and S.R. Porter. 2002. The isolation, identification and molecular analysis of *Candida* spp. isolated from the oral cavities of patients with diabetes mellitus. *Oral Microbiol. Immunol.* 17(3): 181–185.

Manfredi M., M.J. McCullough, L. Polonelli, S. Conti, Z.M. Al-Karaawi, P. Vescovi and S.R. Porter. 2006. *In vitro* antifungal susceptibility to six antifungal agents of 229 *Candida* isolates from patients with diabetes mellitus. *Oral Microbiol. Immunol.* 21(3): 177–182.

Marangoci N., M. Mares, M. Silion, A. Fifere, C. Varganici, A. Nicolescu, C. Deleanu, A. Coroaba, M. Pinteala and B.C. Simionescu. 2011. Inclusion complex of a new propiconazole derivative with β-cyclodextrin: NMR, ESI-MS and preliminary pharmacological studies. *Results Pharma Sci.* 1(1): 27–37.

Maurya V., A. Srivastava, J. Mishra, R. Gaind, R.S.K. Marak, A.K. Tripathi, M. Singh and V. Venkatesh. 2013. Oropharyngeal candidiasis and *Candida* colonization in HIV positive patients in northern India. *J. Infect. Dev. Ctries.* 7(8): 608–613.

Merenstein D., H. Hu, C. Wang, P. Hamilton, M. Blackmon, H. Chen, R. Calderone and D. Li. 2013. Colonization by *Candida* species of the oral and vaginal mucosa in HIV-infected and non-infected women. *AIDS Res. Hum. Retroviruses* 29(1): 30–34.

Mitrovic S., V. Radovic, I. KranjcicZec, V. Arsic, A. Dzamic and D.J. Jevtovic. 1996. *Oral candidiasis in HIV infected patients caused by Candida albicans and associated Candida species*, Berkarda B. (eds). Monduzzi Editore, Bologna.

Nawrot U., M. Cisło, A. Noczyńska, K. Włodarczyk and E. Baran. 2006. Susceptibility to selected antifungal agents of yeast-like fungi

isolated from gastrointestinal tract of diabetes children type 1. *Mikol. Lek*. 13(1): 35–38.

Ramírez M., M.C. Serrano, C. Castro, E. López, C. Almeida, A. Fernández, A. Romero and E. Martín-Mazuelos. 2006. Comparative study of disc diffusion and microdilution methods in susceptibility testing of micafungin against *Candida* species. *J. Antimicrob. Chemother.* 58(4): 861–863.

Romeo O. and G. Criseo. 2011. *Candida africana* and its closest relatives. *Mycoses* 54(6):475–86.

Sangeorzan J.A., S.F. Bradley, X. He, L.T. Zarins, G.L. Ridenour, R.N. Tiballi and C.A. Kauffman. 1994. Epidemiology of oral candidiasis in HIV-infected patients: colonization, infection, treatment, and emergence of fluconazole resistance. *Am. J. Med.* 97(4): 339–46.

Sanitá P.V., E.G. de Oliveira Mima, A.C. Pavarina, J.H. Jorge, A.L. Machado and C.E. Vergani. 2013. Susceptibility profile of a Brazilian yeast stock collection of *Candida* species isolated from subjects with *Candida*-associated denture stomatitis with or without diabetes. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* 116(5): 562–569.

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST definitive document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* 14(4): 398–405.

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2014. Antifungal Agents. Breakpoint tables for interpretation of MICs. Version 7.0, valid from 2014-08-12. www.eucast.org.

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(2): 341–350.

Vergani C.E., P.V. Sanitá, E.G.O. Mima, A.C. Pavarina and A.L. Machado. 2013. Oral candidiasis: conventional and alternative treatment options. In: *Candidiasis Epidemiol. Symptoms Treat. Options*, pp. 85–115.