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Trends in Antifungal Susceptibility of *Candida* Species – one Year Observation

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Abstract

In the past years opportunistic fungal infections have seriously increased, mainly in immunocompromised patients. The aim of the study was to determine the prevalence of yeast-like fungi in invasive candidiasis and to estimate its susceptibility to chosen antifungal agents. One hundred and sixty strains of yeast-like fungi were cultured from various clinical material: samples from lower respiratory tract, blood, the peritoneal cavity and others. The susceptibility tests were established according to the quantitative E-test method. The *Candida* genus represented the main etiological factor of invasive candidiasis. The predominant species were: *C. glabrata* (71/160), *C. albicans* (34/160), *C. krusei* (17/160), *C. tropicalis* (14/160). All tested strains were the most resistant to itraconazole. *Candida glabrata* presented the 100% susceptibility to amphotericin B and caspofungin and was the least susceptible to itraconazole, posaconazole and voriconazole. *Candida albicans* was the most susceptible species to all antymicotics.

Key words: *Candida glabrata*, antifungal susceptibility, Candidiasis, E-test

Introduction

In the past years, opportunistic fungal infections have seriously increased, mainly in immunocompromised patients. Systemic mycoses are most often caused by *Candida* genus yeasts and moulds, especially the *Aspergillus* genus. The increasing number of infections often results from advanced and more aggressive medical treatment, such as chemotherapy, abdominal or cardiothoracic surgery complications, organs and hematopoietic cells transplantation, prolonged broad spectrum antibiotic therapy, biopolymer devices such as indwelling catheters and prolonged hospitalization (Passos *et al.*, 2007).

According to data published by many authors, *C. albicans* isolated from clinical materials remains to be the predominant pathogen. The percentage of infections caused by this microorganism varied from 48.5% (Passos *et al.*, 2007) to 72.7% (Kubisiak-Rzepczyk *et al.*, 2008) depending on localisation of the infection and sample origin. In spite of a broad antifungal prophylaxis, these infections still represented a severe therapeutic problem (Cuenca-Estrella *et al.*, 2002; Nucci *et al.*, 2005; Laupland *et al.*, 2005; Passos *et al.*, 2007; Batura-Gabryel 2007; Kubisiak-Rzepczyk *et al.*, 2008).

Candida glabrata is one of the most frequently isolated non-*albicans Candida* species (Lockhart *et al.*, 1999; Fadda *et al.*, 2008; Costa de Oliveira *et al.*, 2011). Especially it was a frequent cause of candidaemia in patients from high risk group (Lockhart *et al.*, 1999). In the p ast years, *C. glabrata* infections have increased possibly as a result of wide use of azoles that promote rapid selection of resistance (Fadda *et al.*, 2008; Costa de Oliveira *et al.*, 2011). Lockhart et al. showed that *C. glabrata* was a second most common pathogen of candidaemia and its prevalence was increasing (Lockhart *et al.*, 1999).

The percentage of infections caused by non-*albicans Candida* species (*NAC* spp.*)* constantly increase, but their number does not exceed the number of infections caused by *C. albicans*. Different species of non-albicans *Candida* spp. predominated in infections depending on the study and geographical location. *C. parapsilosis* caused 36.4% of infections in Latin America, and the percentage of infections other than *C. albicans* represented approx. 65% (Cuenca-Estrella *et al.*, 2002; Berg von Zepelin *et al.*, 2007; Saracli *et al.*, 2009). *C. glabrata* and *C. parapsilosis* are indicated as the second etiological factor in yeast infections. A higher number of *C. glabrata* infections could result from

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the widespread use of fluconazole (Nucci *et al.*, 2005; Laupland *et al.*, 2005).

Among non-albicans *Candida* species causing infections, *C. parapsilosis, C. tropicalis* and *C. glabrata* were most often isolated, and less often were isolated: *C. krusei, C. guilliermondii*, *C. kefyr, C. lusitaniae* and *C. inconspicua* (Cuenca-Estrella *et al.*, 2002; Laupland *et al.*, 2005; Nucci *et al.*, 2005; Passos *et al.*, 2007; Kubisiak-Rzepczyk *et al.*, 2008; Pfaller *et al.*, 2008).

This study was performed to evaluate species distribution, antifungal susceptibility and also contemporary epidemiology in Polish hospital.

Experimental

Materials and Methods

The study was conducted from September 1, 2009 to August 31, 2010 at the Central Clinical Hospital in Warsaw. Strains were collected from hospitalized patients with diagnosed fungal infections (fungal infection was identified when strain was isolated from sterile body sites, or when the same species was isolated at least from 3 different body sites) on15 wards.

Clinical samples originated from: lower respiratory tracts: tracheal aspirates (56), bronchovesicular lavage (BAL) (15) and sputum (11), blood (25), peritoneal cavity samples (16), bile tracts samples (9) and other different clinical materials (28).

The samples were cultured on standard Sabouraud Dextrose Agar with gentamicin and chloramphenicol (bioMeriéux, Marcy l'Etoile, France). Blood was cultured on enriched fluid medium within the Bact/Alert automatic system (bioMeriéux, Marcy l'Etoile, France). Species identification was carried out using commercial biochemical tests ID32C (bioMeriéux, Marcy l'Etoile, France).

The quality control strains used in every batch of susceptibility test were *Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 90028 (CLSI, 2000).

Inoculum preparation for susceptibility test: The inocula were prepared following the guidelines of Clinical Laboratory Standards Institute (CLSI, 2000) Document M27-A2. After preparing 24 h cultures of the isolates at 37°C, 5 colonies were suspended in a sterile test tube containing 1 mL of 0.85% NaCl for each isolate. The mixture was vortexed at low speed to obtain homogeneity. The cell density was equivalent to 0.5 McFarland standards. Antifungal agents used in this test: amphotericin B, fluconazole, posaconazole, voriconazole, itraconazole, caspofungin (bioMeriéux, Marcy l'Etoile, France). All strips were stored at –20°C and thawed at room temperature before use.

All isolates were tested *in vitro* against 6 antifungal agents standard used in mycological diagnostic and recommended for treatment invasive candidiasis: amphotericin B, fluconazole, posaconazole, voriconazole, itraconazole and caspofungin. Lawn cultures of the *Candida* isolates were prepared on RPMI-1640 agar medium (Biomed, Warsaw, Poland) supplemented with 2% glucose (pH 7.0) in Petri dishes. E-test (bioMeriéux, Marcy l'Etoile, France) strips were gently placed on the lawn cultures with the MIC scale facing upwards using a sterile forcep. The Petri dishes were incubated at 37°C for 24 h. The MIC's value were read where the inhibition ellipse intersected the strip which was interpreted at the lowest concentration at which 80% of the growth was inhibited for the azoles group and 100% of the growth was inhibited for the amphotericin B and echinocandins. The result interpretation was performed by MIC determination within clinical categories (S, I or R) according to CLSI recommendations.

Results

From September 1, 2009 to August 31, 2010, a total of 17 387 clinical materials were tested for yeast presence. One thousand six hundred ninety one positive culture samples were analyzed, among them 160 isolates were cultured from patients with invasive candidiasis. Ninety six percent of this infection were caused by *Candida* spp.

There were 71 isolates (71/160, 44.4%) of *C. glabrata*, 34 of *C. albicans*, 17 of *C. krusei*, 14 of *C. tropicalis* and seven of both *C. inconspicua* and *C. parapsilosis*. Figure 1 presents the distribution of the eight most common species.

Other than *Candida* spp. isolates cultured during the study period were: *Saccharomyces cerevisiae* (5/160, 3%), *Trichosporon asahii* (1/160, 0.6%), and *Geotrichum capitatum* (1/160, 0.6%).

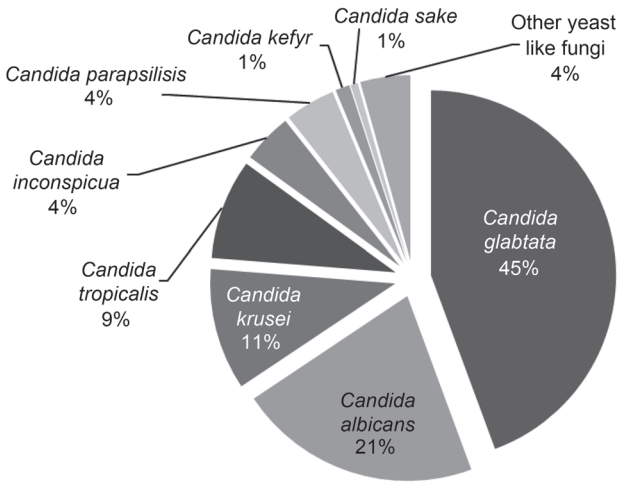
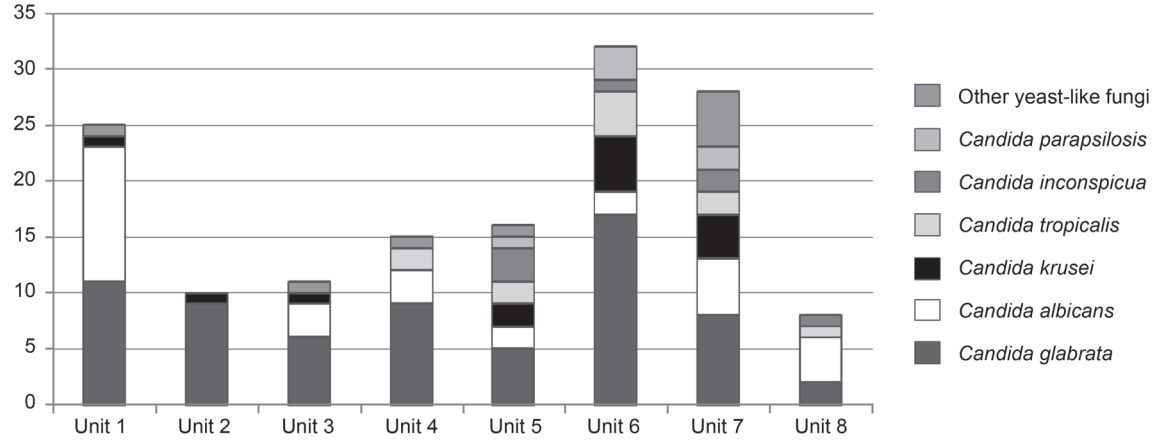


Fig. 1. Distribution of *Candida* species causing invasive fungal infection during the study period.



Unit 1. Department of General, Gastroenterological and Oncological Surgery
Unit 2. Department of General, Vascular and Transplant Surgery
Unit 3. Department of Hematology
Unit 4. Department of Internal Medicine, Pneumonology, and Allergology
Unit 5. Department of Immunology, Transplantology and Internal Medicine, Transplantation Institute
Unit 6. Department of Cardiac Surgery
Unit 7. Intensive Care Unit
Unit 8. Intensive Care Unit A

Fig. 2. Distribution of the yeast like fungi according to medical unit

The distribution of isolated *Candida species* in predominated wards is presented in Figure 2. *C. glabrata* dominated in almost all Surgery Units with range, 31.25–81.8% and 60% in Department of Internal Medicine, Pneumonology, and Allergology. Regarding *C. albicans* with 48% of the species isolated from Department of General, Gastroenterological and Oncological Surgery. Also frequently cultured in ICUs with range, 17.8–50%.

C. glabrata strains were mainly isolated from tracheal aspirates (26/71), material from peritoneal cavity (9/71) and – bronchovesicular washing samples (8). *C. albicans* was mostly isolated from blood – 18 strains. Also from tracheal aspirate 8 strains of *C. krusei* were isolated.

The susceptibility to antifungal agents of the isolated *Candida* species are presented in Table I. Data are reported as MIC ranges, MIC_{90}, number of susceptible isolates and number of resistant isolates. Best activity against all species tested exhibited amphotericin B (100% susceptible strains) and caspofungin (100% susceptible strains). Caspofungin were potent against all species with the MIC_{90} range: 0.016 mg/l for *C. kefyr* to 0.5 mg/l for *C. parapsilosis*. Sixty six (93%) of the 71 *C. glabrata* showed resistance to itraconazole. Nineteen (12%) of the 160 isolates were intermediate resistant to itraconazole, including six *C. inconspicua*, three *C. krusei*, three *C. tropicalis* and one of each species: *C. glabrata, C. albicans*, *C. parapsilosis, C. kefyr and C. sake*. All isolates were susceptible to voriconazole except for three resistant and four intermediate resistant isolates of *C. glabrata*. Overall, fluconazole exhibited good activity against most species (98%), with MIC_{90} range, 1 mg/l–16 mg/l. In particular, *C. albicans*, *C. tropicalis*, *C. parapsilosis, C. kefyr* and *C. sake*. In contrast, *C. inconspicua* was less susceptible to fluconazole (MIC_{90}, 16 mg/l). All isolates were susceptible to posaconazole except for 15 resistant strains of *C. glabrata*.

All triazoles demonstrated potent activity against *C. albicans* (susceptible 99.25%), *C. parapsilosis* (susceptible 96.43%) and *C. sake* (susceptible 100%). Regarding *C. glabrata*, 4/71 isolates resulted susceptible to itraconazole, and 56/71 isolates resulted susceptible to voriconazole. *C. glabrata* was the least susceptible species to itraconazole (MIC_{90}, > 32 mg/l), posaconazole (MIC_{90}, 1.5 mg/l) and voriconazole (MIC_{90}, 1.5 mg/l).

Discussion

Resistance to microbial agents emerges and spreads as a consequence of the use antimicrobial drugs. Similarly to other candidaemia studies, (Martin *et al.*, 2005; Tortorano *et al.*, 2006; Borg von Zepelin *et al.*, 2007; Chen *et al.*, 2009; Swinne *et al.*, 2009; Tortorano *et al.*, 2009; Falagas *et al.*, 2010; Nishikaku *et al.*, 2010; Arendrup *et al.*, 2011; Arendrup *et al.*, 2011) the highest number of infections was reported from Surgery, ICU and Hematology units. Yeast like fungi appeared as the predominant factor in invasive fungal infection at the Central Clinical Hospital. Over the past ten years, studies reported a shift in the etiology of candidaemia. While *Candida albicans* is still considered

Table I
MICs and antifungal susceptibility of *Candida* species

Species (isolates)	Amphotericin B			Fluconazole			Itraconazole		
	Range [mg/l]	MIC_{90} [mg/l]	S/R S%	Range [mg/l]	MIC_{90} [mg/l]	S/R S%	Range [mg/l]	MIC_{90} [mg/l]	S/R S%
C. glabrata (71)	0.032–0,38	0.25	71/0 100	not tested	–	not tested	0.023–32	>32	4/1/66 5.6
C. albicans (34)	0.004–0,19	0.094	34/0 100	0,016–2	1	26/0 100	0.012–0.25	0.125	33/1/0 97
C. krusei (17)	0.047–0,5	0.38	17/0 100	not tested	–	not tested	0,. 16–32	0.5	12/3/2 70.6
C. tropicalis (14)	0.047–0,5	0.38	14/0 100	0.25–3.0	3	3/0 100	0.064–1.5	1	9/3/2 64.3
C. parapsilosis (7)	0.016–0.25	0.125	7/0 100	1.5–6.0	6	2/1 100	0.023–0.25	0.125	6/1/0 857
C. inconspicua (7)	0.006–0.094	0.094	7/0 100	6.0–16.0	16	5/1 83	0.125–0.5	0.38	1/6/0 14.3
C. kefyr (2)	0.125–0.19	0.125	2/0 100	1	1	2/0 100	0.5–1.0	1	0/1/1 0
C. sake (1)	0094	–	1/0 100	8	–	1/0 100	0.38	–	0/1/0 0

Species (isolates)	Voriconazole			Posaconazole			Caspofungin		
	Range [mg/l]	MIC_{90} [mg/l]	S/R S%	Range [mg/l]	MIC_{90} [mg/l]	S/R S%	Range [mg/l]	MIC_{90} [mg/l]	S/R S%
C. glabrata (71)	0.012–32	1.5	64/4/3 90,2	0.008–32	1.5	56/15 78.9	0,032–0,19	0,125	71/0 100
C. albicans (34)	0.008–0.064	0.032	34/0/0 100	0.016–0.19	0.19	34/0 100	0,006–0,125	0,125	33/0 100
C. krusei (17)	0.016–0.38	0.25	17/0/0 100	0.006–1.5	0.19	17/0 100	0,094–0,5	0,38	17/0 100
C. tropicalis (14)	0.002–0.19	0.125	14/0/0 100	0.032–0.19	0.19	13/0 100	0,023–0,25	0,125	14/0 100
C. parapsilosis (7)	0.023–0.25	0.125	7/0/0 100	0.023–0.125	0.125	7/0 100	0,064–0,5	0,5	7/0 100
C. inconspicua (7)	0.064–0.19	0.19	7/0/0 100	0.064–0.19	0.125	6/0 100	0,047–0,125	0,125	7/0 100
C. kefyr (2)	0.032–0.047	0.047	2/0/0 100	0.25–0.38	0. 38	2/0 100	0,012–0,016	0,016	2/0 100
C. sake (1)	not tested	–	not tested	0.064	–	1/0 100	0,064	–	1/0 100

the most common etiological factor of candidaemia (Laupland *et al.*, 2005; Passos *et al.*, 2007; Kubisiak-Rzepczyk *et al.*, 2008; Macura *et al.*, 2009; Saracli *et al.*, 2009; Swinne *et al.*, 2009), recent epidemiologic studies have demonstrated an increasing incidence of non *albicans* species candidaemia, with *C. glabarata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis* (Nguyen *et al.*, 1996; Rocco *et al.*, 2000; Richet *et al.*, 2002; Wisplinghoff *et al.*, 2004; Basetti *et al.*, 2006) . The reason for change in the pattern of *Candida* spp distribution has not been understood yet, but there are some predisposing factors identified, such as indwelling catheters and parenteral nutrition for *C. parapsilosis* (Horasan *et al.*, 2010), cancer and neutropenia for *C. tropicalis* (Negri *et al.*, 2012) and previous exposure to azoles for *C. krusei* and *C. glabrata* (Bassetti *et al.*, 2009) The results presented here demonstrate that non-*C. albicans* species caused the majority of cases of ICU. In our study *C. glabrata* was found to cause 44,4% of the cases of IFI. This is similar to the proportion of cases of infections caused by *C. glabrata* that the Ruan et al. found in its latest study (Ruan *et al.*, 2009). *C. albicans* was ranked as second etiological factor of candidaemia, causing 21,25% of invasive infections. Third and fourth came *C. krusei* and *C. tropicalis* causing respectively 10,6% and 8,75% of infections. These study results are similar to those in North America (Trick *et al.*, 2002) and Ireland (McMullan *et al.*, 2002) in which the prevalence of non-*albicans Candida* is higher than that of *C. albicans.*

The emergence of *C. glabrata* as the major non-albicans *Candida* species was not suprising given its ability to develop resistance to azole drugs and expand under the selection pressure provided by the common use of fluconazole and itraconazole for prophylaxis, preemptive therapy and empirical therapy, discriminately or indiscriminately in high risk settings (Hitchcock *et al.*, 1993; Rex *et al.*, 1995; Gumbo *et al.*, 1999; Sobel 2000; Safdar *et al.*, 2001; Uzun *et al.*, 2001; Vazques *et al.*, 2001; Cuenca-Estrella *et al.*, 2002; Passos *et al.*, 2007; Kubisiak-Rzepczyk *et al.*, 2008; Saracli *et al.*, 2009). Antifungal resistance in our study was a rare finding and was restricted to azoles. As with a Spanish study (Cisterna *et al.*, 2010), none of our *Candida* bloodstream isolates and other isolates from invasive fungal infections had MICs of >2 mg/l for amphotericin B. According to E-test interpretative criteria, all of the *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. kefyr* and *C. sake* isolates included here were susceptible to fluconazole (MIC ≤ 8 mg/l), voriconazole (MIC ≤ 1 mg/l) and posaconazole (MIC ≤ 0.5 mg/l). Voriconazole was fully-active against all *C. krusei* isolates but only in 90.2% of isolates of *C. glabrata*, while only 4/71

C. glabrata isolates were susceptible to itraconazole, and 66/71 and 2/17 *C. krusei* isolates were resistant to this drug. Our proportion of fluconazole-resistant isolates (2.2%) was lower than the rates observed with European (6.3%) and North America (6.6%) isolates (Messer et al. 2009; Cisterna *et al.*, 2010). Caspofungin demonstrated excellent activity as reported by others (Pffaler *et al.*, 2011). The azole which exhibited the best in vitro activity was voriconazole. Also other authors shows similar results (Berg von Zepelin *et al.*, 2007; Fleck *et al.*, 2007; González *et al.*, 2008; Swinne *et al.*, 2009).

Macura et al. observed *C. albicans* high susceptibility to amphotericin B and fluconazole, strains tested in our study were also highly susceptible to this agents. (Macura *et al.*, 2009). The data presented in our paper demonstrated a high biological activity of posaconazole (89.7% of the strains).

In conclusion yeast like fungal strains belonging to non-albicans *Candida* species represented the main cause of invasive fungal infections with predominant azole resistant species such as: *Candida glabrata* (71/160) and *C. krusei* (17/160). We reported that all isolated *Candida* species strains were susceptible to amphotericin B and caspofungin. Analysed strains were most resistant to itraconazole. A similar observation was described by Swinne *et al.* (Swinne *et al.*, 2009) and other authors (Berg von Zepelin *et al.*, 2007; Szymankiewicz *et al.*, 2007; González *et al.*, 2008; Saracli *et al.*, 2009; Sipsas *et al.*, 2009). Due to increasing MICs (intermediate-resistant and resistant strains), itraconazole must be used with caution for the treatment of invasive candidiasis due to *C. glabrata* and *C. krusei*. Periodic analysis is critical in determining the rapidly evolving susceptibility trends among *Candida* species, especially at centers caring for patients at risk.

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Conflict of interest

No conflict of interest.

Literature

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