Acta Dermatovenerol Croat 2006;14(4):219-224 ORIGINAL SCIENTIFIC ARTICLE

Biotypes of *Candida albicans* Isolated from Cardiovascular System and Skin Surveillance Cultures of Hospitalized Patients

Verica Važić-Babić[1], Emilija Mlinarić-Missoni[1], Smilja Kalenić[2]

[1]Mycology Department, Croatian National Institute of Public Health, Zagreb, Croatia
[2]Department of Clinical and Molecular Microbiology, Zagreb University Hospital Center, Zagreb, Croatia

Corresponding author:
Verica Važić-Babić, PhD
Croatian National Institute of Public Health
Rockefellerova 2
HR-10000 Zagreb,
Croatia
verica.vazic.babic@hzjz.hr

Received: July 14, 2006
Accepted: September 13, 2006

SUMMARY The aim of the study was to biotype 59 isolates of *Candida (C.) albicans* from cardiovascular system samples (blood and intravenous catheter) and 123 isolates of the same species from skin surveillance cultures (swabs of the armpit, groins and intravenous catheter insertion sites) of hospitalized patients using the Odds and Abbott biotyping method. Biotyping of 59 isolates of *C. albicans* taken from the cardiovascular system samples revealed the presence of 16 biotypes. Biotype 355 was the most common biotype, accounting for 35.6% of all biotype isolates from this system. Biotyping of 123 *C. albicans* isolates from skin surveillance cultures detected 21 biotypes. Biotype 355 was most common, accounting for 17.9% of all biotype isolates from these samples. The two systems had 10 biotypes in common: 355, 155, 257, 305, 105, 315, 300, 015, 157, and 345. These biotypes accounted for 88.3% and 81.4% of all *C. albicans* biotypes isolated from the cardiovascular system and skin surveillance cultures, respectively. Biotypes 355, 155, and 257 were the biotypes most frequently shared in isolates from the two systems. These biotypes accounted for 57.7% and 43.1% of all *C. albicans* biotypes isolated from the cardiovascular system and skin surveillance cultures, respectively.

KEY WORDS: biotype; *Candida albicans*; cardiovascular system; skin surveillance culture

INTRODUCTION

Candida (C.) albicans is ubiquitous commensal yeast in humans in whom it is part of the physiological flora of the skin and mucous membranes. In individuals with preserved immunity, it may cause disease exceptionally rarely. When body defenses fail for any reason, this yeast behaves opportunistically. As the number of immunocompromised patients across the world is steadily increasing, the incidence of fungal infections follows this trend (1,2). *Candida* spp. are the most common agents (78.3%) of fungal nosocomial infections, and *C. albicans* has been demonstrated to be the causative agent in more than 50% of these infections (3-5).

The ever greater use of intravenous catheters is the reason for this increase in the incidence of fungemia coupled with the use of catheter in immunocompromised patients. This is due to the

Acta Dermatovenerol Croat
2006;14(4):219-224

opportunistic species of the fungus from the patient's skin and his surrounding (6,7). In 50% to 80% of candidemia in patients with hematologic malignancies, intravenous catheter is the site of *Candida* introduction into the bloodstream (8). In these patients candidemia may result in the development of severe and often fatal invasive and disseminated candidiasis (endocarditis, endophthalmitis, microabscesses in the brain and kidney, etc.) (5,9). In some 50% of immunocompromised patients, *C. albicans* is isolated from the skin around the catheter insertion site. In addition to the source of yeast from the patient's own skin, yeasts from the patient's environment (e.g., contaminated objects and infusion solutions as well as the hands of hospital staff and secretions from their respiratory systems) may also lead to colonization of intravenous catheter. This may also occur secondarily by hematogenous dissemination of the yeast from a remote focus of inflammation with the same yeast (6,8,10,11).

A variety of phenotyping (biotyping, serotyping, morphotyping, enzyme typing, assessment of sensitivity to antimycotics and "killer" toxins, etc.) and genotyping methods have been employed to identify the types of *C. albicans* (12,13). Results reported by other workers have pointed to the biotyping method as being highly reproducible and able to differentiate particular types within the *C. albicans* species (13).

The aim of the present study was to identify the types of *C. albicans* isolates taken from samples of the cardiovascular system and skin surveillance cultures of inpatients using the Odds and Abbott biotyping method (14,15). This typing method allows for identification of 512 different biotypes of this species, which enables monitoring of the frequency of individual biotypes in different systems of the patient as well as detection of the potential sources of infection. The method is suitable to examine a large number of isolates and does not entail major material expenses or expensive equipment.

PATIENTS AND METHODS

Samples

Fifty-nine isolates of *C. albicans* from the cardiovascular system (blood and intravenous catheter) and 123 *C. albicans* isolates from skin surveillance cultures (swabs of armpit, groins and intravenous catheter insertion sites) were typed. The samples derived from patients hospitalized at Sterile Unit, Department of Hematology, University Department of Medicine, Zagreb University Hospital Center.

Isolation and identification methods

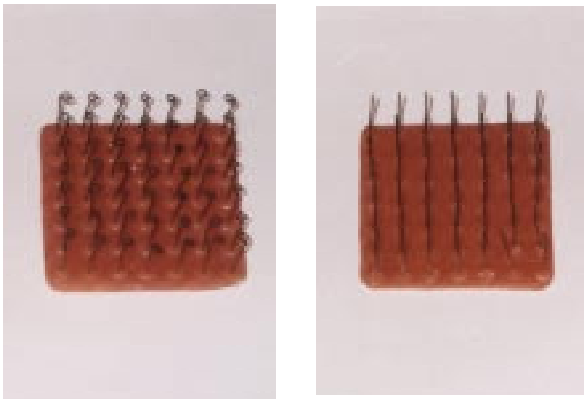
All clinical material samples were inoculated onto Sabouraud's glucose agar and incubated aerobically at 37 ºC for 3 to 7 days. Identification of *C. albicans* was made by standard tests (test of germination, assimilation and sugar fermentation tests, and development of chlamydospores on cornmeal agar) and commercial tests (ID 32 C and API 20 C AUX, BioMerieux) (3,16).

Biotyping method

The isolates were stored at -26 ºC in glycerin broth until analysis. Resuscitated isolates were typed by using the Odds and Abbott method (14,15). Each isolate underwent testing on nine different media (those to examine its tolerance of pH 1.4 and increased NaCl concentrations, thus testing its ability to excrete proteinase and assimilate urea, citrates and sorbose, and its resistance to 5-fluorocytosine, boric acid and safranin). The isolate growth on a given medium was considered as positive test outcome; the same person read all test media (Figs. 1-4). Test media were divided into three groups, the biotype of each isolate being marked with a 3-digit number accordingly. Each digit was calculated based on the sum of test results within each group.

RESULTS

Table 1 illustrates the frequency of *C. albicans* biotypes isolated from cardiovascular system. Sixteen biotypes were typed in 59 isolates. The



a) b)

Figure 1. (a) Multiple inoculator for preparing suspension of *Candida albicans* isolates; (b) multiple inoculator for inoculating *Candida albicans* isolates onto different media.

Acta Dermatovenerol Croat
2006;14(4):219-224

Table 1. Frequency of *Candida albicans* biotypes isolated from cardiovascular system

Biotype		No. of isolates (%)
355		21 (35.6)
155		8 (13.6)
257, 305		10 (17.0)
105		4 (6.8)
315, 300		6 (10.2)
017		2 (3.4)
015, 045, 100, 115, 157, 314, 345, 360		8 (13.6)
Total	16	59 (100)

four most common biotypes (355, 155, 257 and 305) accounted for 66.2% and the remaining 12 biotypes for 33.8% of all biotypes from the cardiovascular system samples. The prevalence of biotype 355 was highest with 35.6% of all biotypes. Biotype 155 had a prevalence of 13.6%, and biotypes 257 and 305 of 8.5% each (Fig. 5).

The frequency of *C. albicans* biotypes isolated from skin surveillance cultures (armpit, groin and catheter insertion site swabs) is shown in Table 2. Typing of 123 isolates yielded 21 biotypes. The four most common biotypes (355, 155, 257 and 300) accounted for 53.7% of all biotypes from skin surveillance cultures. Biotype 355 was most prevalent (17.9%), followed by biotypes 155 (13.0%), 257 (12.2%) and 300 (10.6%) (Fig. 6).

Table 3 presents biotypes of *C. albicans* isolates taken from samples of the cardiovascular system and skin surveillance cultures of 16 hematological patients. Isolates taken from these samples of each individual patient belonged to one to three biotypes. In 14 (87.5%) patients, the biotypes found in the isolates from cardiovascular system and skin surveillance cultures were the same.

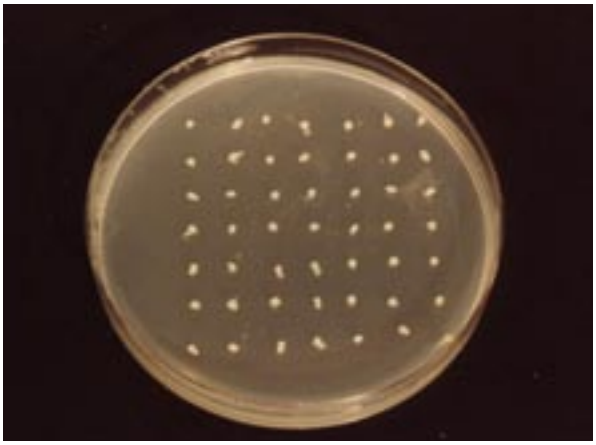


Figure 2. Growth of 49 *Candida albicans* isolates on control medium.

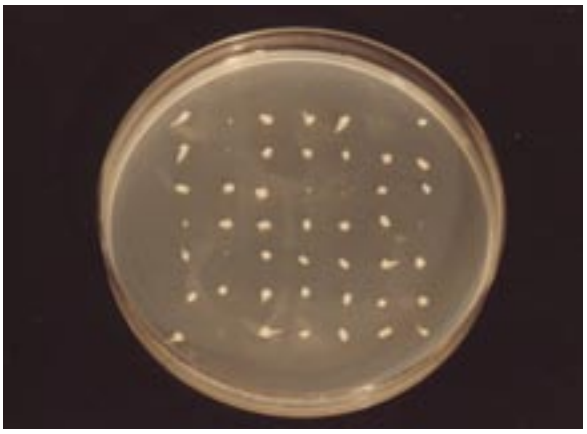


Figure 3. Growth of 40 *Candida albicans* isolates on glycine medium to demonstrate its ability to use glycine as the source of nitrogen and carbon.

DISCUSSION

C. albicans isolates typed in the present study originated from samples of the cardiovascular system (blood and intravenous catheter) and of the skin surveillance cultures (armpit, groin, and/or catheter insertion site swabs) from hospitalized hematological patients. Intravenous catheter was the site of *Candida* spp. introduction in the bloodstream in 50% to 80% of candidemia, and *C. albicans* was responsible for 55% of these infections (2,5,6,8,10). In about 50% of immunocompromised patients, *C. albicans* was isolated from the skin around the intravenous catheter insertion site (6,7,10,11).

These data have prompted us to attempt typing and comparing the detected types of *C. albicans* isolated from the cardiovascular system and surveillance skin cultures of hospitalized hemato-

Table 2. Frequency of *Candida albicans* biotypes isolated from skin surveillance cultures

Biotype		No. of isolates (%)
355		22 (17.9)
155		16 (13.0)
257		15 (12.2)
300		13 (10.6)
105		9 (7.3)
305, 345		16 (13.0)
144, 157, 241, 315, 357		20 (16.5)
041		3 (2.4)
147		2 (1.5)
005, 015, 055, 175, 205, 325, 755		7 (5.6)
Total	21	123 (100)

Acta Dermatovenerol Croat
2006;14(4):219-224

Table 3. Biotypes of *Candida albicans* isolated from cardiovascular system samples (blood and/or intravenous catheter) and skin surveillance cultures (swabs of armpit, groin and/or site of catheterization) in 16 hospitalized hematological patients

Patient	Cardiovascular system		Skin surveillance cultures	
	Blood	Intravenous catheter	Catheterization site	Armpit/groin
1	355		355	
2	155	155	155	
3	315	300		305
4	355	355	355	
5	357	355	357	
6	357			357
7	155			155
8	157	257		157,257
9	155	157		155, 157
10	355	315	315	
11	257		257	
12	300			300
13	345	345		345
14	305		315	
15	105	105		105
16	157			157

logical patients. The Odds and Abbott biotyping method was chosen for typing for its simple performance and allowing for examination of a large number of samples without entailing great material expenses or expensive equipment. Although the methods of genotyping are currently also available for microorganism (and yeast) typing, phenotyping methods are still in common use in epidemiological studies of nosocomial infections (17). Literature reports of research findings on the biotyping of *C. albicans* isolates with the Odds and Abbott method are very scarce. Most researchers typed isolates of this species originating from two systems, genitourinary and digestive systems (14,18). Although this method permits typing of 512 different *C. albicans* biotypes, the reported typing results have revealed much fewer (from 25 to 79) biotypes (14,18).

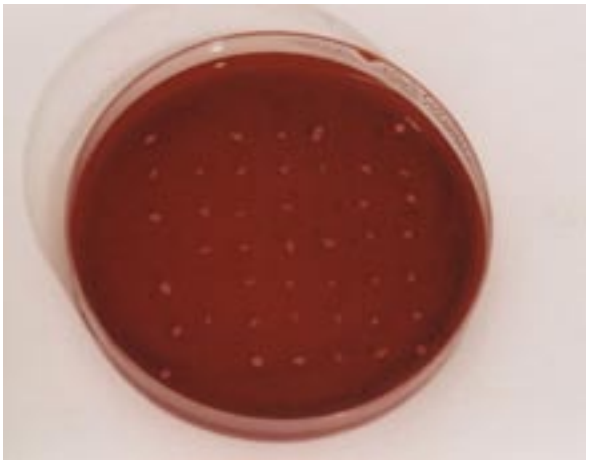


Figure 4. Growth of 43 *Candida albicans* isolates on safranin medium to demonstrate resistance to safranin stain.

On typing *C. albicans* isolates taken from skin surveillance cultures of hematological inpatients, the present study found a greater number of biotypes (n=21) in these than in the cardiovascular system samples from the same patients (n=16). In the isolates from these samples, 10 biotypes were the same: their frequency in the isolates from samples of the cardiovascular system and skin surveillance cultures was 88.3% and 81.4%, respectively. The present study demonstrated a high coincidence (87.5%) between the biotypes recorded in isolates from the cardiovascular system samples

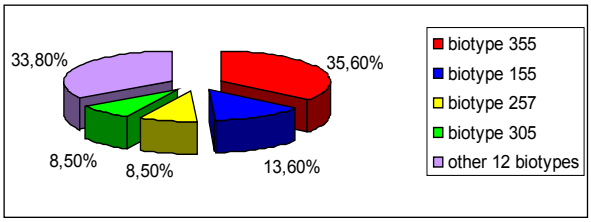


Figure 5. Prevalence of the four most common *Candida albicans* biotypes isolated from the cardiovascular system.

Acta Dermatovenerol Croat
2006;14(4):219-224

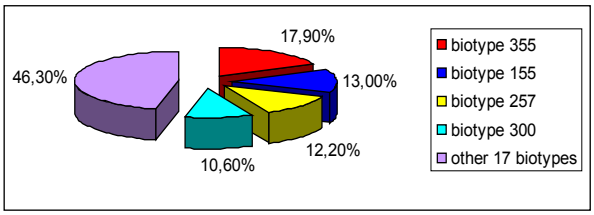


Figure 6. Prevalence of the four most common *Candida albicans* biotypes isolated from the skin surveillance cultures.

and skin surveillance cultures in 16 hematological inpatients. Klempp-Selb *et al.* obtained comparable results. They typed chromosomal DNA of 19 *C. albicans* isolates taken from blood samples and 36 *C. albicans* isolates taken from other samples (stool, urine, sputum, vaginal swabs, and swabs of the skin around the site of central venous catheter insertion) from six hospitalized patients. Karyotyping revealed the presence of 10 different karyotypes. In 5 (83.3%) patients, the karyotypes in the isolates from blood and other samples proved to be the same (19). Using the "killer-yeasts" typing method, Candido *et al.* typed 146 *C. albicans* isolates taken from various samples of hospitalized patients, i.e. blood, stool, bronchoalveolar lavage, urine, swabs of the oral cavity, vagina, skin lesions and intact skin). Typing detected 23 biotypes, the most common being 211, 111 and 811. Type 211 was most common among the isolates from blood samples (20). Betremieux *et al.* typed *C. albicans* isolates obtained from blood samples of seven premature infants admitted to intensive care unit (ICU), and *C. albicans* isolates from swabs of the skin of hospital staff hands. Five methods (serotyping, morphotyping, resistance typing, "killer-yeast" typing, and genotyping) were used on typing. All blood sample isolates were of the same type as the isolate from the skin of the hand of an ICU worker, indicating the role of yeasts from the patient's surrounding in intrahospital candidemia (21).

CONCLUSION

Biotyping of 59 *C. albicans* isolates obtained from the cardiovascular system of hospitalized hematological patients showed 16 biotypes. Biotyping of 123 *C. albicans* isolates from surveillance cultures of the skin of the same patients revealed 21 biotypes. Three most common biotypes (355, 155 and 257) accounted for 57.7% of all *C. albicans* biotypes isolated from the cardiovascular system and 43.1% of all biotypes of the *C. albicans* isolates from surveillance cultures of the skin. The results of our study showed *C. albicans* to be not only a major colonizer of the skin and intravenous catheters in hematological inpatients, but also an agent of candidemia in this population. Along with other phenotyping and genotyping methods, the Odds and Abbott biotyping helps in differentiating particular types within the *C. albicans* species, permitting detection of the epidemiologic sources of nosocomial infections and routes of spread of this yeast.

References

1. Raad II. Intravascular catheter-related infections. Lancet 1998;351:893-8.
2. Edwards JE. *Candida* species. In: Mandell GL, Bennett JE, Dolin RD, editors. Mandell, Douglas, Bennett's Principles and practice of infectious diseases. Philadelphia: Churchill Livingstone; 2000. p. 2656-74.
3. Warren NG, Hazen KC. *Candida, Cryptococcus* and other yeasts of medical importance. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RM, editors. Manual of clinical microbiology. Washington DC: ASM Press; 1999. p. 723-37.
4. Reef SE, Lasker BA, Butcher DS, McNeil MM, Pruitt R, Keyserling H, *et al*. Non-perinatal nosocomial transmission of *Candida albicans* in neonatal intensive care unit; prospective study. J Clin Microbiol 1998;36:1255-9.
5. Crump JA, Collignon PJ. Intravascular catheter-associated infections. Eur J Clin Microbiol Infect Dis 2000;19:1-8.
6. Diekema DJ, Messer SA, Brueggemann AB, Coffman SL, Doern GV, Herwaldt LA, *et al*. Epidemiology of candidemia: 3-year results from the emerging infections and the epidemiology of Iowa Organisms Study. J Clin Microbiol 2002;4:1298-302.
7. Raad II, Darouiche RO. Catheter-related septicemia: risk reduction. Infect Med 1996;13:807-23.
8. Raad II. Central venous catheter fungaemia in cancer patients. Cliniguide Fungal Infect 1995;6:5-8.
9. Odds FC. Candida endocarditis, myocarditis and other cardiovascular candida infections. In: Odds FC. Candida and candidosis. London: Bailliere Tindall; 1988. p. 175-81.
10. Shin JH, Park MR, Song JW, Shin DH, Jung SI, Cho D, *et al*. Microevolution of *Candida*

albicans strains during catheter-related candidemia. J Clin Microbiol 2004;9:4025-31.

11. Warren DK, Zack JE, Mayfield JL, Chen A, Prentice D, Fraser VJ, *et al*. The effect of an education program on the incidence of central venous catheter-associated bloodstream infection in a medical ICU. Chest 2004;126: 1612-8.
12. Merz WG. *Candida albicans* strain delineation. Clin Microbiol Rev 1990;4:321-34.
13. Otero L, Vazquez F, Palacio V, Vazquez S, Carreno F, Mendez FJ. Comparison of seven phenotyping methods for *Candida albicans*. Eur J Epidemiol 1995;2:221-4.
14. Odds FC, Abbott AB. A simple system for the presumptive identification of *Candida albicans* and differentiation of strains within the species. Sabouraudia 1980;18:301-17.
15. Odds FC, Abbott AB. Modification and extension of tests for differentiation of *Candida* species and strains. Sabouraudia 1983;21:79-81.
16. Larone DH. Yeasts and yeastlike organisms. In: Larone DH, ed. Medically important fungi. Washington DC: ASM Press; 2002; p. 113-32.
17. Martin C, Roberts D, van der Weide M, Rossau R, Jannes G, Smith T, *et al*. Development of a PCR-based lineprobe assay for identification of fungal pathogens. J Clin Microbiol 2000;38:3735-42.
18. O'Connor MI, Sobel JD. Epidemiology of recurrent vulvovaginal candidiasis identification and strain differentiation of *Candida albicans*. J Infect Dis 1986;2:358-62.
19. Klempp-Selb B, Rimek D, Kappe R. Karyotyping of *Candida albicans* and *Candida glabrata* from patients with *Candida* sepsis. Mycoses 2000;43:159-63.
20. Candido RC, Fischman O, Zaror L, Ito IY. The differentiation of *Candida albicans* strains by the killer system. Rev Soc Brasil Med Trop 1995;28:321-4.
21. Betremieux P, Chevrer S, Quindos G, Sullivan D, Polonelli L, Guiguen C. Use of DNA fingerprinting and biotyping methods to study a *Candida albicans* outbreak in a neonatal intensive care unit. Pediatr Infect Dis J 1994;13:899-905.

Elida cream, for the use every hour; year 1935.
(from the collection of Mr. Zlatko Puntijar)