The persistence of multifocal colonisation by a single ABC genotype of Candida albicans may predict the transition from commensalism to infection

Guilherme Maranhão Chaves¹, Fernanda Pahim Santos², Arnaldo Lopes Colombo²/+ ¹Laboratório de Micologia Médica e Molecular, Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Norte, Natal, RN, Brasil ²Disciplina de Infectologia, Laboratório Especial de Micologia, Universidade Federal de São Paulo, Rua Botucatu 740, 04023-062 São Paulo, SP, Brasil

Candida albicans is a common member of the human microbiota and may cause invasive disease in susceptible populations. Several risk factors have been proposed for candidaemia acquisition. Previous Candida multifocal colonisation among hospitalised patients may be crucial for the successful establishment of candidaemia. Nevertheless, it is still not clear whether the persistence or replacement of a single clone of C. albicans in multiple anatomical sites of the organism may represent an additional risk for candidaemia acquisition. Therefore, we prospectively evaluated the dynamics of the colonising strains of C. albicans for two groups of seven critically ill patients: group I included patients colonised by C. albicans in multiple sites who did not develop candidaemia and group II included patients who were colonised and who developed candidaemia. ABC and microsatellite genotyping of 51 strains of C. albicans revealed that patients who did not develop candidaemia were multiply colonised by at least two ABC genotypes of C. albicans, whereas candidaemic patients had highly related microsatellites and the same ABC genotype in colonising and bloodstream isolates that were probably present in different body sites before the onset of candidaemia.

Key words: Candida albicans - genotyping - colonisation - bloodstream infection - strain maintenance or replacement

Financial support: FAPESP, CNPq (475338/2006-5)
GMC received a postdoctoral fellowship from FAPESP (2005/04442-1) and FPS a Master’s degree studentship from FAPESP (2007/05880-8).
GMC and FPS contributed equally to this work.
+ Corresponding author: arnaldocolombo@gmail.com
Received 23 May 2011
Accepted 27 October 2011

Previous authors have suggested that the risk of candidaemia increases with the number of body sites colonised by Candida. The intensity of Candida colonisation in critically ill patients may predict subsequent infections with identical strains (Pittet et al. 1994, Vincent et al. 1998). Recently, Leon et al. (2006) developed a scoring system named the “Candida score” and proved that patients with Candida multifocal colonisation had higher overall mortality rates compared to those patients with unifocal colonisation by this yeast. The calculation of a Candida score was based on the variables generated from a logistic regression model that evaluated the risk factors independently associated with candidaemia. The authors rounded the score for patients with exposure to total parenteral nutrition, surgery or multifocal Candida species colonisation up to 1 and the weight for clinically severe sepsis up to 2 and established a cut-off value of 2.5. Therefore, clinically ill patients with candidaemia who had a score of 2.5 were considered to be 7.75 times more likely to have a proven infection than patients with a Candida score up to 2.5.

It is clear that the number of body sites colonised by the Candida species impacts the risk of acquiring candidaemia; however, whether the persistence or replacement of a single clone of C. albicans in multiple anatomical sites of the organism may represent an additional risk in the transition from commensal to invasive candidiasis has not been largely investigated.

By evaluating the genotypes of 165 C. albicans isolates using multilocus sequence typing (MLST) and ABC genotyping, Odds et al. (2006) have shown that in cases of candidaemia, strain maintenance, predominant clonality and microvariation occur in isolates obtained from...
non-sterile sites cultured before the onset of Candida bloodstream infection. These results strongly suggest that candidaemic patients are usually infected with their own commensal isolates that exhibit multiple colonisation and clonal dissemination as previously described (Reagan et al. 1990, Pittet et al. 1991, 1994, Caugant & Sandven 1993, Voss et al. 1994, Marr et al. 2001, Verma et al. 2003).

In contrast, few authors have suggested that multiple strain types may coexist in the same clinical sample from a unique patient (Takasuka et al. 1998, Kam & Xu 2002, Samaranyake et al. 2003). By investigating different C. albicans colonies obtained from healthy volunteers and patients with oral and vaginal candidiasis, Jacobsen et al. (2008) reported a higher prevalence of strain microevolution and substitution among healthy volunteers compared to infected patients. The authors inferred that superficial infection may result from the selective overgrowth of a single subtype or fewer subtypes before invasive infection.

By comparing the concordance among MLST and ABC genotyping, Odds et al. (2006) stated that the latest technique is reliable when strains differ in more than one single nucleotide polymorphism. However, ABC genotyping should not be used as a unique method for C. albicans typing. First described in 1999 by McCullough et al., ABC genotyping was developed on the basis of the presence or absence of an insert in the DNA that encodes for the 25S ribosomal RNA gene among different C. albicans genotypes (McCullough et al. 1999). Microsatellite typing using primer M13, which amplifies short tandem repeats, was first employed to type Cryptococcus strains in Brazil (Casali et al. 2003) and has been extensively used to type this encapsulated yeast (Loperena-Alvaréz et al. 2010, Pedroso et al. 2010, Ferreira-Paim 2011).

Nevertheless, only a few studies have employed the M13 primer to type C. albicans (Bartie et al. 2001).

It seems reasonable to hypothesise that the proliferation of a single C. albicans subtype may be a predictor for candidaemia. However, there is a lack of prospective studies in the medical literature that have determined whether the dissemination of a single clone of C. albicans to multiple anatomical sites occurs in patients who remain colonised by this yeast, but do not develop candidaemia during hospitalisation. In this study, we prospectively evaluated the dynamics of the persistence or replacement of C. albicans genotypes for two well established groups of critically ill patients: group I included three patients colonised by C. albicans in multiple sites who did not develop candidaemia during hospitalisation and group II included four patients who were colonised by C. albicans and who developed candidaemia.

SUBJECTS, MATERIALS AND METHODS

Strains - We selected a total of 51 isolates of C. albicans that were prospectively collected from the different anatomical sites of six patients admitted at a tertiary care hospital in São Paulo, Brazil. In addition, we included a patient (patient 5) who had two positive urine cultures for C. albicans collected at a seven-day interval and who developed candidaemia. These isolates were obtained from patients sequentially admitted to the ICU who agreed to participate in a surveillance confidential study. Only patients who required more than 72 h of ICU admission were enrolled in this study. Samples were systematically collected from urine, rectal swabs, skin surrounding the catheter entry site, surgery wounds, tracheal secretions and blood until the onset of candidaemia or discharge from the ICU (Table). The clinical isolates were collected during the study over a period of two years. The isolates were stored on YPD glycerol (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose and 3% glycerol) at -70°C. The C. albicans strains ATCC90028 and SC5314 were used as external control strains for the phenotypic and molecular identification and typing of our isolates.

Procedures for species identification - The isolates were plated on CHROMagar Candida® (CHROMagar Microbiology, Paris, France) to check for purity, viability and screening for green colonies. The production of chlamydoconidia on cornmeal agar and green colour on CHROMagar Candida® were the presumptive tests adopted for the initial identification of C. albicans. The final identification of the strains was confirmed by the sequencing of the ribosomal DNA (rDNA) internal transcribed spacer (ITS) region (White et al. 1990).

C. albicans genotyping methods - To establish the genetic relatedness of the colonising and bloodstream isolates of C. albicans, all of the strains were typed by microsatellite and ABC genotyping (based on the amplification of an intron present in the 26S rDNA gene).

DNA extraction - The isolates were grown in Falcon tubes that contained 2 mL YPD medium for 16 h at 30°C at 200 rpm in a gyratory shaker for 48 h. DNA was extracted using the fast small scale isolation protocol previously described (Melo et al. 1998).

Polymerase chain reaction (PCR) assay and ITS region sequencing - The primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATAT-3') were used to amplify the ITS region (White et al. 1990). The samples were amplified in a Thermocycler (model 9600) (Applied Biosystems, Foster City, CA, USA) using the following cycling parameters: one initial cycle of 94°C for 3 min followed by 30 cycles of 1 min at 94°C, 1 min at 57°C, 1 min at 72°C and a final cycle of 5 min at 72°C. The PCR products were size-separated by agarose gel electrophoresis and the gel was stained in a 0.5 μg/mL ethidium bromide buffer solution (1 x tris-acetate-ethylenediamine tetraacetic acid). The purified PCR products were sequenced using the dideoxynucleotide method in an ABI PRISM 3100 automated sequencer (Applied Biosystems, CA, USA). The sequencing reaction included each primer separately and the BigDyeTM Terminator reagent kit (Applied Biosystems) and it was performed according to the manufacturer’s instructions. The nucleotide sequences were submitted for BLAST analysis (blastn) at the National Center for Biotechnology Information (ncbi.nlm.nih.gov) website for species identification.

Microsatellite typing PCR and ABC genotyping PCR - Microsatellite typing was performed using the primer M13 (5'-GAGGGTGGCGGTTCT-3') (IDT) as
previously described (Casali et al. 2003). ABC genotyping was performed with the following primers: CA-INT-L (5′-ATAAGGGAGTCGGCACAATAGATCCGTAAG-3′) and CA-INT-R (5′-CCTGCGTGTGCTTGCCTAGATGATGAG-3′) (McCullough et al. 1999). Briefly, 1.0 µL of DNA 40 ng/µL, 2.5 µL of 10 x PCR buffer (100 mM tris-HCl, pH 8.3, 500 mM KCl, 3.5 mM MgCl2), 5 µL of dNTPmix (100 mM each dNTP), 1.0 µL of each primer (50 pmol/µL), 0.13 µL of Tween 20 and 1.0 unit of Taq DNA polymerase were added to a final volume of 25 µL. Forty-five cycles of amplification were performed using the same cycling parameters described previously, except the annealing temperature was 36°C for the microsatellite typing.

Computer-assisted microsatellite data analysis - Gel images were analysed with the GelCompar II software, version 4.5 and BioNumerics (Applied Maths, Kortrijk, Belgium). The similarities between the profiles were calculated using the Dice coefficient to generate the matrixes of similarity coefficients to dendrogram constructions. For profile clustering, the unweighted pair-group method with arithmetic averages was used with a tolerance of 2%.

RESULTS

All of the isolates that were phenotypically identified as C. albicans had their identification confirmed with rDNA ITS region sequencing. Notably, the patients included in this analysis were not on antifungal therapy or prophylaxis at the time of enrolment. Patient demographic and underlying conditions are described in Table.

The genomic DNA of all of the isolates was successfully amplified with the M13 primer, which targets genome repetitive sequences. The DNA amplification generated well-defined band patterns, ranging from 500 pb-2 KB and the microsatellite technique showed sufficient discriminatory power for recognising intraspecific variation (Figure). As expected, the external control strains of C. albicans, SC5314 and ATCC90028, were placed in completely different clusters by the dendrogram analysis performed using GelCompar II, proving the efficiency of the technique in separating strains isolated from different geographic areas (Figure).

C. albicans microsatellite typing results of colonised vs. candidaemic patients - The results obtained using microsatellite typing suggest that each patient was colonised by a group of specific strains. Isolates with some degree of genetic variability were obtained from group I patients (who did not develop candidaemia). This finding suggests that these isolates underwent microevolution or were completely replaced during the patient’s hospitalisation. The majority of the genotypic profiles of the sequential isolates from patient 1 were identical (100% similarity). Isolates 904 C1 (catheter tip), 904 A (rectal swab) and 904 B (urine) were placed within different clusters. Patient 2 was colonised in three different body sites. Strains 143 A (rectal swab) and 143 C (surgery wound) showed 100% similarity, whereas an isolate from tracheal secretion (138 C) had a very different genotypic profile compared to the two other strains and was placed in a different cluster after the dendrogram analysis (84% similarity). This trend was still more remarkable among the isolates from patient 3. Some of the isolates presented only 60% similarity and were placed in very distinct clusters, except for two strains that were considered identical (170 A and 184 A1 both from rectal swabs collected on different days) (Figure).

Regarding the patients who were colonised by C. albicans and who developed candidaemia (group II), bloodstream isolates were indistinguishable or highly related to other strains of the same patient, although some degree of genetic variability was observed among previously colonising strains. Patient 4 had colonising strains that exhibited genetic variability ranging from 85-100% similarity, suggesting that strain microevolution occurred during the patient’s ICU hospitalisation. Furthermore, isolate 731 D from blood was considered indistinguishable from isolate 704 B (100% similarity), a strain recovered from urine before the onset of candidaemia. Patient 5 was included in the analysis because of two positive urine cultures that were collected within a week of developing candidaemia. These strains (19 B and 15 B1) were considered highly related (above 97% similarity) to the 15 D bloodstream isolate. Patient 6 showed some degree of difference among the colonising strains of C. albicans (86-100% similarity), possibly due to microvariations and similarity of 93% among a C. albicans bloodstream isolate (757) and the previous colonising strains. Finally, patient 7 had a blood isolate (997, 5 G) that was 100% similar to the colonising isolates of different body sites, such as urine (1002 B) and surgery wounds (982 E) and 97% similarity among all of the isolates, proving that the strains were highly related.

C. albicans ABC genotyping results of colonised vs. candidaemic patients - When ABC genotyping was used, we observed that at least two different A, B or C genotypes were observed within the colonising strains of C. albicans from group I (patients who did not develop candidaemia). Nevertheless, patients who were colonised and developed candidaemia (group II) due to C. albicans had a single A or B genotype strain at the beginning of colonisation through the onset of candidaemia (Table).

Regarding group I, patient 1 was colonised by several genotype C strains, but a genotype A strain was also isolated. This isolate (904 C1; skin surrounding the catheter entry site) was placed in a different cluster from the majority of the isolates from the same patient when the microsatellite technique was used. Patient 2 had two genotype B strains that were also considered to be indistinguishable (143 A, rectal swab; 143 C, surgery wound) and a genotype C strain (138 C; tracheal secretion) that was again placed in a different cluster when the microsatellite method was used. Interestingly, patient 3 was colonised by a combination of A and B genotypes (Table).

In contrast, group II patients were solely colonised by a single A or B genotype of C. albicans in all anatomical sites. Patients 4 and 7 were only colonised by genotype A strains, whereas patients 5 and 6 were only colonised by genotype B strains (Table). None of the strains isolated from the candidaemic patients belonged to the C genotype.
| Patient | Isolate number | Site of isolation | ABC typing | Apache II score | Age (years) | Gender | Associated conditions | Clinical outcome |
|---------|----------------|------------------|------------|----------------|-------------|--------|----------------------|-----------------|
| 1 (group I) | 868 A1 | Rectal swab | C | 22 | 58 | Female | Cerebral vascular accident | Discharged |
|          | 881 A  | Rectal swab | C | 58 | 58 | Female | - | - |
|          | 893 A  | Rectal swab | C | 58 | 58 | Female | - | - |
|          | 904 A  | Rectal swab | C | 58 | 58 | Female | - | - |
|          | 904 B  | Urine         | C | 58 | 58 | Female | - | - |
|          | 904 C1 | Catheter      | A | 58 | 58 | Female | - | - |
|          | 914 A  | Rectal swab  | C | 58 | 58 | Female | - | - |
|          | 914 B  | Urine        | C | 58 | 58 | Female | - | - |
| 2 (group I) | 138 C | Tracheal secretion | C | 21 | 61 | Male | Cranioencephalic trauma | Discharged |
|          | 143 A  | Rectal swab | B | 61 | 61 | Male | - | - |
|          | 143 C  | Surgery wound | B | 61 | 61 | Male | - | - |
| 3 (group I) | 133 C1 | Tracheal secretion | B | 20 | 59 | Male | Cardiovascular disease | Discharged |
|          | 170 A  | Rectal swab | A | 59 | 59 | Male | - | - |
|          | 170 D1 | Skin surrounding catheter entry | B | 59 | 59 | Male | - | - |
|          | 176 A  | Rectal swab | B | 59 | 59 | Male | - | - |
|          | 184 A1 | Rectal swab | A | 59 | 59 | Male | - | - |
|          | 184 C  | Skin surrounding catheter entry | A | 59 | 59 | Male | - | - |
| 4 (group II) | 624 A  | Rectal swab | A | 25 | 47 | Female | Cardiovascular disease | Candidaemia (discharged) |
|          | 645 A  | Rectal swab | A | 47 | 47 | Female | - | - |
|          | 657 A  | Rectal swab | A | 47 | 47 | Female | - | - |
|          | 657 B  | Urine       | A | 47 | 47 | Female | - | - |
|          | 672 A  | Rectal swab | A | 47 | 47 | Female | - | - |
|          | 672 B  | Urine       | A | 47 | 47 | Female | - | - |
|          | 672 D  | Skin surrounding catheter entry | A | 47 | 47 | Female | - | - |
|          | 687 A1 | Rectal swab | A | 47 | 47 | Female | - | - |
|          | 687 B  | Urine       | A | 47 | 47 | Female | - | - |
|          | 687 C  | Skin surrounding catheter entry | A | 47 | 47 | Female | - | - |
|          | 704 A  | Rectal swab | A | 47 | 47 | Female | - | - |
|          | 704 B  | Urine       | A | 47 | 47 | Female | - | - |
|          | 731 A  | Rectal swab | A | 47 | 47 | Female | - | - |
|          | 731 D  | Blood       | A | 47 | 47 | Female | - | - |
| 5 (group II) | 15 B1  | Urine | B | 20 | 62 | Male | Cardiovascular disease, benign gastrointestinal disease | Candidaemia (discharged) |
|          | 15 D   | Blood       | B | 62 | 62 | Male | - | - |
|          | 19 B   | Urine       | B | 62 | 62 | Male | - | - |
### DISCUSSION

In this study, we prospectively evaluated the genotypes of the colonising and infecting strains of *C. albicans* from patients sequentially admitted for more than 72 h in the ICU. Overall, we found a great diversity among the different colonising strains of *C. albicans* obtained from various anatomical sites of patients who did not develop candidaemia despite previous multifocal colonisation, whereas the persistence of a single A or B genotype was documented in patients who developed candidaemia. These data support the hypothesis that the presence of a single, well-adapted dominant strain in non-sterile sites obtained from patients with candidaemia may increase the risk of transition from commensalism to infection.

It is important to emphasise that the patients who did not develop candidaemia were colonised by different *C. albicans* genotypes and strain replacement (as supported by 2 different techniques) occurred even among *Candida* strains isolated from the same anatomical site at two different time points of collection. Patient 3 was sequentially colonised by a combination of genotype A and B strains within the same anatomical site. According to Jacobsen et al. (2008), a combination of different genotypes is expected more among colonised individuals compared to patients exhibiting superficial infections with *C. albicans*. Therefore, it is reasonable to hypothesise that an adapted genotype succeeded during disease development, whereas strain replacement may have occurred in patients who did not develop candidaemia during hospitalisation.

It is very unlikely that commensal strain replacement occurs in patients who develop candidaemia (Reagan et al. 1990, Pittet et al. 1991, 1994, Caugant & Sandven 1993, Voss et al. 1994, Marr et al. 2001, Verma et al. 2003, Odds 2010). By evaluating 30 hospitalised patients with multifocal colonisation by *C. albicans* who developed candidaemia, Marco et al. (1999) found highly similar or identical genetic patterns between colonising and infecting strains typed with a Ca3 probe. Odds et al. (2006) demonstrated a high genetic relatedness within the colonising strains from multiple sites and the bloodstream isolates obtained from 11 different sets of strains of a patient admitted to the ICU.

Several authors have demonstrated that candidaemic patients are previously colonised in multiple non-sterile sites by *Candida* strains of clonal origin; however, we did not find any prospective study that evaluated whether this phenomenon occurs in critically ill patients with multifocal colonisation who do not develop candidaemia. Our findings suggest that *C. albicans* strain replacement may occur in colonised patients who do not progress to candidaemia.

Despite being a controversial issue, several authors have recommended serial fungal surveillance cultures in critically ill patients to better identify individuals who should be treated with empirical antifungal therapy when a substantial degree of fungal colonisation is observed (Eggimann et al. 2005, Leon et al. 2006). According to our data, ABC genotyping could help clinicians predict whether or not a critically ill patient who is multicolonised by *C. albicans* would develop candidaemia.

| Site of isolation                  | Clinical outcome       | Associated conditions | Clinical outcome       |
|-----------------------------------|------------------------|-----------------------|------------------------|
| ABC typing                        | Isolate number         | Age (years)           | Gender                 |
| ABC typing                        | Site of isolation      | Age (years)           | Gender                 |
| ABC typing                        | Site of isolation      | Age (years)           | Gender                 |
In conclusion, as demonstrated by ABC and microsatellite genotyping, we suggest that in addition to the number of body sites colonised by Candida, multifocal colonisation by a single genotype may increase the risk of developing candidaemia in colonised patients. We demonstrated that a patient who was colonised in a single anatomical site by a persistent C. albicans genotype B (isolated from urine) progressed to invasive infection (patient 5). The limitations of our study are represented by the small number of patients evaluated in the present series and by the fact that strain virulence factors were not included in our analysis. Further studies are necessary to confirm whether our findings may be extrapolated to larger populations.

REFERENCES

Almirante B, Rodriguez D, Park BJ, Cuenca-Estrella M, Planes AM, Almela M, Mensa J, Sanchez F, Ayats J, Gimenez M, Saballs P, Fridkin SK, Morgan J, Rodriguez-Tudela JL, Warnock DW, Pahissa A, The Barcelona Candidemia Project Study Group 2005. Epidemiology and predictors of mortality in cases of Candida bloodstream infection: results from population-based surveillance, Barcelona, Spain, from 2002 to 2003. J Clin Microbiol 43: 1829-1835.

Bartie KL, Williams DW, Wilson MJ, Potts AJ, Lewis MA 2001. PCR fingerprinting of Candida albicans associated with chronic hyperplastic candidosis and other oral conditions. J Clin Microbiol 39: 4066-4075.

Borzotta AP, Beardsley K 1999. Candida infections in critically ill trauma patients: a retrospective case-control study. Arch Surg 134: 657-664.

Casali AK, Goulart L, Rosa e Silva LK, Ribeiro AM, Amaral AA, Alves SH, Schrank A, Meyer W, Vainstein MH 2003. Molecular typing of clinical and environmental Cryptococcus neoformans isolates in the Brazilian state Rio Grande do Sul. FEMS Yeast Res 3: 405-415.

Caugant DA, Sandven P 1993. Epidemiological analysis of Candida albicans strains by multilocus enzyme electrophoresis. J Clin Microbiol 31: 215-220.

Colombo AL, Guimarães T 2003. Epidemiology of hematogenous infections due to Candida spp. Rev Soc Bras Med Trop 36: 599-607.

Colombo AL, Nucci M, Park BJ, Nouer SA, Arthington-Skaggs B, da Matta DA, Warnock D, Morgan J, Brazilian Network Candidemia Study 2006. Epidemiology of candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven medical centers. J Clin Microbiol 44: 2816-2823.
Cornwell EE 3rd, Belzberg H, Offne TV, Dougherty WR, Morales IR, Asensio J, Demetriades D 1995. The pattern of fungal infections in critically ill surgical patients. Am Surg 61: 847-850.

Eggimann P, Calandra T, Fluckiger U, Bille J, Garbino J, Glauser MP, Marchetti O, Ruet C, Täuber M, Pittet D 2005. Invasive candidiasis: comparison of management choices by infectious disease and critical care specialists. Intensive Care Med 31: 1514-1521.

Ferreira-Paim K, Andrade-Silva L, Mora DJ, Pedrosa AL, Rodrigues V, Silva-Vergara ML 2011. Genotyping of Cryptococcus neoformans isolated from captive birds in Uberaba, Minas Gerais, Brazil. Mycoses 54: 294-300.

Godoy P, Tiraboschi IN, Severo LC, Bustamante B, Calvo B, Almeida LP, da Matta DA, Colombo AL 2003. Species distribution and antifungal susceptibility profile of Candida spp bloodstream isolates from Latin American hospitals. Mem Inst Oswaldo Cruz 98: 401-405.

Gudlaugsson O, Gillespie S, Lee K, Vande Berg J, Hu J, Messer S, Herwaldt L, Pfaller M, Diekema D 2003. Attributable mortality of nosocomial candidemia, revisited. Clin Infect Dis 37: 1172-1177.

Jacobsen MD, Duncan AD, Bain J, Johnson EM, Naglik JR, Shaw DJ, Gow NA, Odds FC 2008. Mixed Candida albicans strain populations in colonized and infected mucosal tissues. FEMS Yeast Res 8: 1334-1338.

Kam AP, Xu J 2002. Diversity of commensal yeasts within and among healthy hosts. Diagn Microbiol Infect Dis 43: 19-28.

Leon C, Ruiz-Santana S, Saavedra P, Almirante B, Nolla-Salas J, Alvarez-Lerma F 2006. A bedside scoring system (“Candida score”) for early antifungal treatment in nonneutropenic critically ill patients with Candida colonization. Crit Care Med 34: 730-737.

Loperena-Alvarez Y, Ren P, Li X, Schoonmaker-Bopp DJ, Ruiz A, Chaturvedi V, Rios-Velazquez C 2010. Genotypic characterization of environmental isolates of Cryptococcus gattii from Puerto Rico. Mycopathologia 170: 279-285.

Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruet C, Garbino J, Calandra T, Glauser MP, Täuber MG, Pittet D 2004. Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991-2000. Clin Infect Dis 38: 311-320.

Marcos F, Lockhart SR, Pfäffler MA, Pujol C, Rangel-Frausto MS, Wiblin T, Blumberg HM, Edwards JE, Jarvis W, Saiman L, Patterson JE, Rinaldi MG, Wenzel RP, Soll DR 1999. Elucidating the origins of nosocomial infections with Candida albicans by DNA fingerprinting with the complex probe Ca3. J Clin Microbiol 37: 2817-2828.

Marr KA, Lyons CN, Ha K, Rastad TR, White TC 2001. Inducible azole resistance associated with a heterogeneous phenotype in Candida albicans. Antimicrob Agents Chemother 45: 52-59.

McCullough MJ, Clemens KV, Stevens DA 1999. Molecular and phenotypic characterization of genotype Candida albicans subgroups and comparison with Candida dubliniensis and Candida stellatoidea. J Clin Microbiol 37: 417-421.

Melo AS, de Almeida LP, Colombo AL, Briones MR 1998. Evolutionary distances and identification of Candida species in clinical isolates by randomly amplified polymorphic DNA (RAPD). Mycopathologia 42: 57-66.

Odds FC 2010. Molecular phylogenetics and epidemiology of Candida albicans. Future Microbiol 5: 67-79.

Odds FC, Davidson AD, Jacobsen MD, Tavanti A, Whyte JA, Kibbler CC, Ellis DH, Maiden MC, Shaw DJ, Gow NA 2006. Candida albicans strain maintenance, replacement and micro-variation demonstrated by multilocus sequence typing. J Clin Microbiol 44: 3647-3658.

Ostrosky-Zeichner L, Kullberg BJ, Bow EJ, Hadley S, Leon C, Nucci M, Patterson TF, Perfect JR 2011. Early treatment of candidemia in adults: a review. Med Mycol 9: 113-120.

Pedroso RS, Lavrador MA, Ferreira JC, Candido RC, Maffei CM 2010. Cryptococcus neoformans var. grubii - Pathogenicity of environmental isolates correlated to virulence factors, susceptibility to fluconazole and molecular profile. Mem Inst Oswaldo Cruz 105: 993-1000.

Pittet D, Monod M, Filthuth I, Frenk E, Suter PM, Auckenthaler R 1991. Contour-clamped homogeneous electric field gel electrophoresis as a powerful epidemiologic tool in yeast infections. Am J Med 91: 265S-268S.

Pittet D, Monod M, Suter PM, Frenk E, Auckenthaler R 1994. Candida colonization and subsequent infections in critically ill surgical patients. Ann Surg 220: 751-758.

Reagan DR, Pfäffler MA, Hollis RJ, Wenzel RP 1990. Characterization of the sequence of colonization and nosocomial candidemia using DNA fingerprinting and a DNA probe. J Clin Microbiol 28: 2733-2738.

Samaranayake YH, Samaranayake LP, Dassanayake RS, Yau JY, Tsang WK, Cheung BP, Yeung KW 2003. ‘Genotypic shuffling’ of sequential clones of Candida albicans in HIV-infected individuals with and without symptomatic oral candidiasis. J Med Microbiol 32: 349-359.

Takasuka T, Baily GG, Birch M, Anderson MJ, Law D, Denning DW 1998. Variation in morphotype, karyotype and DNA type of fluconazole resistant Candida albicans from an AIDS patient. J Infect 36: 57-62.

Verma AK, Prasad KN, Singh M, Dixit AK, Ayyagari A 2003. Candidaemia in patients of a tertiary health care hospital from north India. Indian J Med Res 117: 122-128.

Vincent JL, Anaissie E, Bruining H, Demajo W, el-Biary M, Haber J, Hiramatsu Y, Nitenberg G, Nyström PO, Pittet D, Rogers T, Sandven P, Sganga G, Schaller MD, Solonkin J 1998. Epidemiology, diagnosis and treatment of systemic Candida infection in surgical patients under intensive care. Intensive Care Med 24: 206-216.

Voss A, Hollis RJ, Pfäffler MA, Wenzel RP, Doebbeling BN 1994. Investigation of the sequence of colonization and candidemia in nonneutropenic patients. J Clin Microbiol 32: 975-980.

Wey SB, Mori M, Pfäffler MA, Woolson RF, Wenzel RP 1988. Hospital-acquired candidemia. The attributable mortality and excess length of stay. Arch Intern Med 148: 2642-2645.

White TJ, Bruns TD, Lee SB, Taylor JW 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In MA Innis, DH Gelfand, JE Sninsky, TJ White (eds.), PCR protocols: a guide to methods and applications, Academic Press, San Diego, p. 315-322.