Nosocomial candidiasis in Rio de Janeiro State: Distribution and fluconazole susceptibility profile

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Abstract

One hundred and forty-one Candida species isolated from clinical specimens of hospitalized patients in Rio de Janeiro, Brazil, during 2002 to 2007, were analyzed in order to evaluate the distribution and susceptibility of these species to fluconazole. Candida albicans was the most frequent species (45.4%), followed by C. parapsilosis sensu lato (28.4%), C. tropicalis (14.2%), C. guilliermondii (6.4%), C. famata (2.8%), C. glabrata (1.4%), C. krusei (0.7%) and C. lamberca (0.7%). The sources of fungal isolates were blood (47.5%), respiratory tract (17.7%), urinary tract (16.3%), skin and mucous membrane (7.1%), catheter (5.6%), feces (2.1%) and mitral valve tissue (0.7%). The susceptibility test was performed using the methodology of disk-diffusion in agar as recommended in the M44-A2 Document of the Clinical and Laboratory Standards Institute (CLSI). The majority of the clinical isolates (97.2%) was susceptible (S) to fluconazole, although three isolates (2.1%) were susceptible-dose dependent (S-DD) and one of them (0.7%) was resistant (R). The S-DD isolates were C. albicans, C. parapsilosis sensu lato and C. tropicalis. One isolate of C. krusei was resistant to fluconazole. This work documents the high susceptibility to fluconazole by Candida species isolated in Rio de Janeiro, Brazil.

Key words: Candida, fluconazole, antifungal susceptibility, disk diffusion method.

Introduction

Since the 1980 decade, invasive fungal infections have grown considerably and the focus of this process has been immunocompromised patients (Beck-Sagué and Jarvis, 1993; Martin et al., 2003). Among the factors that make the defense system fragile and the individuals susceptible to a variety of opportunistic fungi are the seriousness of the base disease, time of permanence in the intensive therapy unit, antibiotic therapy of large spectrum, chemotherapy, radiotherapy, immunosuppressive therapy, central venous catheter, total parenteral feeding, attended ventilation, burns, abdominal surgeries and organ as well as bone marrow transplantations (Colombo and Guimarães, 2003; Colombo et al., 2007; Pemán and Salavert, 2012). Although,
new fungal species are described each year as agents of nosocomial infection (Chakrabarti and Singh, 2011; Nucci and Marr, 2005). Candida spp. are still considered as the most current pathogen (Alangaden, 2011; Pfaffer et al., 2006a). Based on the literature, C. albicans is detected in the majority of the cases in Latin America followed by C. tropicalis, C. parapsilosis sensu lato, and C. glabrata (Pfaffer et al., 2010). Nevertheless, C. famata, C. kefyr, C. guilliermondii, C. lusitaniae, C. pelliculosa and C. rugosa have presented increasing isolation rates (Colombo et al., 2006; Matta et al., 2007; Pfaffer and Diekema, 2007).

Due to the long use for fluconazole to the invasive candidiasis treatment, strains of non-albicans species with low susceptibility have appeared in the hospital ambient (Chen et al., 2012). As the resistance to fluconazole can be the cause of therapeutic failure, the fast identification of the etiologic agent and the analysis of the susceptibility profile to the antifungal drugs can help to decide the most appropriate treatment (Montravers and Jabbour, 2006; Pfaffer and Diekema, 2007; Shah et al., 2011).

The method employed to evaluate antifungal susceptibility must present a good clinical correlation and has to be reproducible (Hospenthal et al., 2004; Lass-Flör et al., 2010). Within this context, an effort has been made by the Clinical and Laboratory Standards Institute - CLSI, which developed a reference test to evaluate the in vitro susceptibility of Candida spp. to fluconazole (CLSI, 2009), using the disk-diffusion in agar methodology (M44-A2). Due to its simplicity, facility of execution and low cost, this method can be easily incorporated in the routine of the public and private clinical laboratories, working as a predictor of clinical response as well as a tool of surveillance and control of the emergence of Candida spp. strains with low sensitivity to fluconazole (Pfaffer et al., 2004).

The present study describes the distribution of Candida species isolated from clinical specimens obtained from hospitalized adult and infant patients in Rio de Janeiro, Brazil, and their susceptibility profile to fluconazole employing the agar disk diffusion method described by CLSI (CLSI, 2009).

Material and Methods

Samples

One hundred and forty-one nosocomial isolates of Candida spp. (one isolate by patient) were obtained from 2002 to 2007 from five medical centers (a tertiary teaching hospital, a tertiary private hospital, a tertiary military hospital, a hematological and hemotherapeutic center and an university pediatric center), one public and one private laboratory of clinical analyses, all of them located in the City of Rio de Janeiro. Despite this localization, the nosocomial samples came from different regions of the State of Rio de Janeiro. In this investigation, the hospital-acquired infections were those that were diagnosed while the patient was hospitalized in the assistance unit. Candida species were isolated from blood, catheter, gastrointestinal tract, genitourinary tract, skin lesions and mitral valve tissue from adult and infant immunocompromised patients. The identification of Candida isolates and the fluconazole disk diffusion susceptibility testing were performed at the Clinical Mycology Laboratory of Pharmacy College of the Federal University of Rio de Janeiro and at the Mycology Sector of Parasitology Service of Adolfo Lutz Institute, São Paulo, Brazil. All information about the samples were obtained from the data records sent with requests for mycological analyses. However, many of them were incomplete. Thus, it was not possible to classify all samples studied.

Yeast Identification

All isolates of Candida species were identified based on their morphophysiological characteristics. The cultivation in CHROMagar-Candida medium (Company, France) was performed to confirm the viability and pureness, as well as for a preliminary identification of the isolates by the production of chromogen pigments (green: C. albicans, blue: C. tropicalis and rose: C. krusei) (Odds and Bernaerts, 1994). The observation of the formation of germinative tube in human serum and the production of chlamydospores in cornmeal agar (Oxoid, England) with tween 80 (Reagen, Brazil) was performed in order to identify C. albicans (Dalmau, 1929; Taschdjian et al., 1960). The biochemical identification was conducted through the Vitek commercial system (BioMerieux, France) according to the manufacturer recommendation. The isolates were maintained in sterile distilled water at room temperature up to the moment of the susceptibility tests.

Antifungal Susceptibility Testing

The agar disk-diffusion test was performed in accordance to the methodology described in M44-A2 document published by CLSI (CLSI, 2009). Paper disks containing 25 μg of fluconazole (CECON, Brazil) and Petri dishes (90 mm of diameter) containing Mueller-Hinton agar (Difco, England) supplemented with 2% of glycosed and 0.5 μg mL⁻¹ of methylene blue at a depth of 4.0 mm were used. In order to monitor the precision, accuracy and performance of the test, Candida albicans ATCC 90028 and Candida parapsilosis ATCC 22019 were used as control strains. Each isolate of Candida spp. was subcultivated into plates with Sabouraud dextrose agar (Difco, England), which were incubated at 35-37 °C for 24 h. Five colonies of the isolates were collected and suspended in 5 mL of sterile saline (0.85%).

The turbidity suspension was adjusted to the 0.5 McFarland scale (10⁶ cells/mL) in a spectrophotometer (Biospectro, Brazil) using 530 nm wavelength. The yeast suspension was inoculated using a sterile swab over the surface of agar Mueller-Hinton. The disk with fluconazole was aseptically deposited over the inoculated agar and the plate
was incubated aerobically at a 35-37 °C temperature for 24 h. The diameter of the inhibition area was measured for determining the susceptibility and calculating the minimal inhibitory concentration (MIC). The interpretative criteria of the fluconazole disk-diffusion test were those suggested by CLSI 7: susceptible (S): ≥ 19 mm; susceptible-dose dependent (S-DD): 15-18 mm; resistant (R): ≤ 14 mm. The values of the corresponding MIC to these diameters CLSI (CLSI, 2009) are the following: S: MIC ≤ 8 μg mL⁻¹; S-DD: MIC 16-32 μg mL⁻¹; R: MIC ≥ 64 μg mL⁻¹. The quality control test was conducted every day during the procedure according to CLSI (CLSI, 2009) and the results obtained were within expected limit for each control strain.

Results

The distribution of Candida species at the seven health institutions involved in this investigation is presented in Table 1. From the total of isolations, 43.3% occurred in the Hemotherapy and Hematology Center. The largest percentage of recovery of Candida spp. from bloodstream infections (46.3%) was also observed in this same institution. In the present study, Candida albicans (Table 1) was the yeast with the largest isolation rate (45.4%), followed by C. parapsilosis sensu lato (28.4%), C. tropicalis (14.2%), C. guilliermondii (6.4%) and C. famata (2.8%), besides C. glabrata (1.4%), C. krusei (0.7%) and C. lambica (0.7%). The isolates of Candida here evaluated were obtained from hospitalized patients with different risk factors, among them, onco-hematological diseases, solid tumors and AIDS.

Blood (47.5%), respiratory tract (17.7%), urinary tract (16.3%), skin and mucous membrane (7.1%), catheter (5.6%), feces (2.1%) and biopsy of the mitral valve (0.7%) were the sources of isolation. It was not possible to define anatomical localization for 3.0% of the isolates. The distribution of Candida isolates by clinical specimens is summarized in the Table 2. C. albicans and C. parapsilosis sensu lato were the most frequent species isolates from hemoculture, presenting rates of 41.8% and 37.3%, respectively. Other yeasts isolated from hemoculture were C. tropicalis (10.4%), C. guilliermondii (6.0%), C. famata (3.0%) and C. glabrata (1.5%). From samples of catheter only C. albicans (62.5%) and C. parapsilosis sensu lato (37.5%) were isolated. The single isolate of C. krusei was retrieved from respiratory specimens.

The diameters of inhibition zones produced by the agar disk-diffusion for all of the isolates tested varied from 10 to 50 mm with average value of 35.8 mm. Table 3 presents the intervals and the means of the diameters from the inhibition zone for Candida species regarding the relationship to different anatomical sites. Among the species, C. krusei (10 mm) presented the smallest inhibition diameter. C. albicans (38.6 mm), C. parapsilosis sensu lato (36.5 mm) and C. guilliermondii (33 mm) were the species

| Table 1 - Distribution of Candida clinical isolates by health centers. |
|--------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Center                        | C. albicans | C. parapsilosis | C. tropicalis | C. guilliermondii | C. famata | C. glabrata | C. krusei | C. lambica | Overall |
| Tertiary teaching hospital       | 03 (60)              | 01 (20)                | 01 (20)                | -                    | -                    | -                    | -                    | -                    | 05 (3.5)             |
| Tertiary private hospital       | 09 (81.8)           | 01 (9.1)               | -                    | -                    | -                    | -                    | -                    | -                    | 11 (7.8)             |
| Tertiary militar hospital       | 02 (100)            | -                    | -                    | -                    | -                    | -                    | -                    | -                    | 02 (1.4)             |
| Hematological and homothepathic center | 18 (29.5)         | -                    | -                    | -                    | -                    | -                    | -                    | -                    | 02 (1.4)             |
| Pediatric university laboratory  | 17 (37.7)           | -                    | -                    | -                    | -                    | -                    | -                    | -                    | 17 (12.1)            |
| Public clinical laboratory       | 07 (17.6)           | 03 (17.6)             | -                    | -                    | -                    | -                    | -                    | -                    | 16 (11.3)            |
| Private clinical laboratory      | 09 (58.2)           | 05 (10.6)             | 05 (10.6)             | -                    | -                    | -                    | -                    | -                    | 29 (20.6)            |
| Overall                        | 64 (44.4)           | 64 (44.4)             | 64 (44.4)             | 64 (44.4)            | 64 (44.4)            | 64 (44.4)            | 64 (44.4)            | 64 (44.4)            | 141 (100)            |
Table 2 - Distribution of *Candida* isolates by clinical specimens.

| Species                     | Blood (No.) | Catheter (No.) | Urinary tract (No.) | Respiratory tract (No.) | Skin/soft tissue (No.) | Stool (No.) | Mitral valve (No.) | NC (No.) | Total (No.) [No. (%)] |
|-----------------------------|-------------|----------------|---------------------|-------------------------|------------------------|-------------|-------------------|---------|---------------------|
| *C. albicans*               | 28 (41.8)   | 05 (62.5)      | 13 (56.6)           | 12 (48)                 | 03 (30)                | 02 (66.7)   | 01 (100)          | -       | 64 (45.4)          |
| *C. parapsilosis* [sensu lato] | 25 (37.3)   | 03 (37.5)      | 04 (17.4)           | 03 (12)                 | 05 (50)                | -           | -                 | -       | 40 (28.4)          |
| *C. tropicalis*             | 07 (10.4)   | -              | 02 (8.7)            | 07 (28)                 | 01 (10)                | -           | -                 | -       | 03 (75)            |
| *C. guilliermondii*         | 04 (6)      | -              | 03 (13)             | 01 (4)                  | -                      | -           | -                 | -       | 09 (6.4)           |
| *C. famata*                 | 02 (3)      | -              | 01 (4.3)            | -                       | -                      | -           | -                 | -       | 01 (25)            |
| *C. glabrata*               | 01 (1.5)    | -              | -                   | 01 (10)                 | -                      | -           | -                 | -       | 02 (1.4)           |
| *C. krusei*                 | -           | -              | -                   | 01 (4)                  | -                      | -           | -                 | -       | 01 (0.7)           |
| *C. lambica*                | -           | -              | -                   | 01 (4)                  | -                      | -           | -                 | -       | 01 (0.7)           |
| Total                       | 67 (47.5)   | 08 (5.6)       | 23 (16.3)           | 25 (17.7)               | 10 (7.1)               | 03 (2.1)    | 01 (0.7)          | 04 (3)  | 141 (100)          |

NC, not-classified specimens.

Table 3 - Range and average zone diameters (mm) by *Candida* isolates and specimen types.

| Specimens          | Range (average) [mm] | Total range (average) |
|--------------------|----------------------|-----------------------|
|                    | *C. albicans*        | *C. parapsilosis*     | *C. tropicalis*       | *C. guilliermondii*    | *C. famata*          | *C. glabrata*    | *C. krusei*       | *C. lambica*    |
| Blood              | 16-40 (40.2)         | 15-50 (37.2)          | 26-37 (30.5)          | 28-36 (32.5)           | 30-32 (31)           | 35                  | -                 | -                 | 15-50 (37.2) |
| Catheter           | 30-48 (40.8)         | 22-44 (36.6)          | -                    | -                      | -                    | -                   | -                 | -                 | 22-44 (39.2) |
| Urinary tract      | 30-44 (39.9)         | 30-38 (34.7)          | 30-33 (31.5)          | 26-38 (32)             | 34                   | -                   | -                 | -                 | 26-44 (34.7) |
| Respiratory tract  | 30-47 (37)           | 30-36 (33.2)          | 30-36 (33.6)          | 20                    | -                    | 10                  | 24                | -                 | 10-47 (33)  |
| Skin/soft tissue   | 32-50 (38.3)         | 30-48 (37.2)          | 38                   | -                     | -                    | 20                  | -                 | -                 | 20-50 (35.9) |
| Stool              | 32-46 (39)           | -                    | -                    | 33                    | -                    | -                   | -                 | -                 | 32-46 (37)  |
| Mitral valve       | 37                   | -                    | -                    | -                     | -                    | -                   | -                 | -                 | 37            |
| NC                 | -                    | -                    | 15-35 (26)            | -                     | 25                   | -                   | -                 | -                 | 15-35 (25.7) |
| Total              | 16-50 (38.6)         | 15-50 (36.5)          | 15-38 (31.2)          | 20-38 (33)            | 25-34 (30.2)         | 20-35 (27.5)       | 10                | 24                | 10-50 (35.8) |

NC, not-classified specimens.
Table 4 - Fluconazole susceptibility of 141 Candida clinical isolates.

| Species             | Clinical specimens | Fluconazole susceptibility category (%) |
|---------------------|--------------------|----------------------------------------|
|                     |                    | S  | S-DD | R  |
| C. albicans         | Blood              | 96.4 | 3.6 | -  |
|                     | Non-blood          | 100 | -    | -  |
|                     | All                | 98.4 | 1.6 | -  |
| C. parapsilosis     | Blood              | 96  | 4.0  | -  |
| [sensu lato]        | Non-blood          | 100 | -    | -  |
|                     | All                | 97.5 | 2.5 | -  |
| C. tropicalis       | Blood              | 100 | -    | -  |
|                     | Non-blood          | 100 | -    | -  |
|                     | NC                 | 66.7 | 33.3 | -  |
|                     | All                | 95  | 5    | -  |
| C. guilliermondii   | Blood              | 100 | -    | -  |
|                     | Non-blood          | 100 | -    | -  |
|                     | All                | 100 | -    | -  |
| C. famata           | Blood              | 100 | -    | -  |
|                     | Non-blood          | 100 | -    | -  |
|                     | NC                 | -   | -    | -  |
|                     | All                | 100 | -    | -  |
| C. glabrata         | Blood              | 100 | -    | -  |
|                     | Non-blood          | 100 | -    | -  |
|                     | All                | 100 | -    | -  |
| C. krusei           | Blood              | -   | -    | -  |
|                     | Non-blood          | -   | -    | 100|
|                     | All                | -   | -    | 100|
| C. lambica          | Blood              | -   | -    | -  |
|                     | Non-blood          | 100 | -    | -  |
|                     | All                | 100 | -    | -  |
| Overall             |                    | 97.2 | 2.1 | 0.7|

S, susceptible; S-DD, susceptible-dose dependent; R, resistant; NC, not-classified specimens.

Discussion

From the medical centers analyzed, the one specialized in the treatment of hematological patients was the center that presented the largest rate of fungal isolation, including isolates from hemocultures (46.3%). Because of its consumptive nature and its more aggressive treatment protocols, the hematological pathologies, in general, make the patients extremely susceptible to invasive fungal infections, therefore becoming one of the major risk factors (Wisplinghoff et al., 2003b). High percentages of fungal recovery from these institutions (or hematological unit) are to be expected (Martin et al., 2003). Within the group of tertiary hospitals and the Pediatric Care Center, the percent ages of positive hemoculture were 6.0% and 13.4%, respectively. Similar rates have been documented by different authors (Velasco and Bigni, 2008; Velasco et al., 2000; Wisplinghoff et al., 2003a). Based on these isolations, nowadays the genus Candida is considered the third most frequent pathogen in infections of stream blood, after Staphylococcus epidermidis and S. aureus (Wisplinghoff et al., 2003b, 2004).

The results here documented highlight the high incidence of C. albicans in hospitalized patients. In the present work, C. albicans represented 41.8% of all isolates from the hemoculture. This percentage agrees with the data recently pointed out regarding this species (48.7%) by the International Program of Epidemiologic Surveillance (SENTRY), which investigated the distribution of Candida species isolated from candidemias and their susceptibility profile to antifungics in North America, Latin America and Europe (Messer et al., 2006). In Brazil, C. albicans has been equally the most isolated yeast in candidemias in many regions of the country (Aquino et al., 2005; Barberino et al., 2006; Passos et al., 2007). The explanation for the fact that C. albicans presents the highest percentage of recovery may be related to its large adaptability and pathogenic versatility (Kumamoto and Vincea, 2005).

In spite of C. albicans being the most frequent agent in this investigation (41.8%), as a whole, the non-C. albicans Candida species represented the majority of the isolates (58.2%) with preponderance of C. parapsilosis sensu lato (37.3%) and C. tropicalis (10.4%). Currently, C. parapsilosis sensu lato and C. tropicalis correspond conjunctly to about 70% of the non-C. albicans Candida isolates from Brazilian candidemias (Colombo et al., 2006). The progressive increase in the rates of recovery of non-C. albicans Candida species has been widely related (Bassetti et al., 2011; Pfaffer et al., 2010). In Brazil, this tendency was equally confirmed (Nucci et al., 2010; Sampaio-Camargo et al., 2010) and isolation rates up to 75% have been reported to non-C. albicans Candida species (Pasqualotto et al., 2005). The emergence of non-C. albicans Candida isolates may be due to the selection of more resistant strains because of ostensive use of azoles derivatives (Mario et al., 2012).
In this investigation, blood was the main source of Candida species (47.5%). Nevertheless, the respiratory tract (17.7%), urinary tract (16.3%), skin and mucous (7.1%) also contributed as important sources. In accordance to the findings of Comert et al. (2006), C. parapsilosis sensu lato, C. tropicalis and C. guilliermondii in the present study were also the main non-C. albicans Candida species recovered from non-sterile specimens. In spite of the hemoculture being the main marker of invasive infection (Martin et al., 2003) and the non-sterile specimens being considered of relative importance to the infection diagnostic (Wang et al., 2004), the isolation of Candida spp. from these specimens may present certain predictive value for candidemias (Sandford et al., 1980), because many invasive processes are associated to the host’s microbiota (Agvald-Öhman et al., 2007).

The invasive infections represent high cost to human economy. In the United States the annual cost with these infections is about US$ 17 billions (Martin et al., 2003). For the candidemia treatment, a cost of about US$1 billion per year is estimated (Pfaller et al., 2006b). Among the different factors that contribute in a critical way to calculate economical and social costs of candidemias, it is the microbial resistance to the antifungics of clinical use (Pfaller et al., 2006b, 2007). As a result, the demand for susceptibility tests has been growing (Pfaller, 2012). The agar disk diffusion method may be useful for the selection of more effective, non-toxic and less expensive therapies (Pfaller et al., 2007; Shah et al., 2011). Its effectiveness in the confirmation of the susceptibility or in the detection of resistance to fluconazole has been evaluated in various multicentric international studies (Pfaller et al., 2005, 2007). Good levels of agreement (87.4 to 97%) between zone diameters by disk-diffusion method and MIC by CLSI reference microdilution method has been found (Barry et al., 2002; Rodero et al., 2006), suggesting an in vivo/in vitro correlation equivalent to the reference method (Meis et al., 2000).

In the present study it was demonstrated the low percentage (0.7%) of resistance to fluconazole, corroborating with what has been reported previously by other authors (Azvedo, 2011; Pfaller et al., 2003, 2004). The only resistant isolate was C. krusei that was recovered from the respiratory tract. The resistance of this yeast was expected, since it is considered intrinsically resistant to fluconazole (Comert et al., 2006). In spite of the occurrence of susceptibility-dose dependent (2.1% from the total of isolates), 98.4% of C. albicans isolates, 97.5% of C. parapsilosis sensu lato isolates and 95.0% of C. tropicalis isolates were susceptible to fluconazole. Pfaller et al. (2005, 2007) also reported similar rates from global studies, which included the Latin America and Brazil. To C. famata, C. glabrata, C. guilliermondii and C. lambica, the fluconazole was 100% effective. Nevertheless, acquired resistance within isolates of C. glabrata has been reported (Colombo et al., 2006; Matta et al., 2007). The high susceptibility of Candida isolates to fluconazole described here is also very relevant, since this antifungal medicine is the main availableazole drug to treat invasive fungal infections within Brazilian public hospitals (Brasil, 2007). The simplicity and validity of the use of the disk-diffusion method were also confirmed.

The study on resistance to fluconazole and other antifungals comparing CLSI disk-diffusion method to CLSI microdilution method and to EUCAST method was conducted and the results will be published soon, as well as the results of the study comparing the genotypic and phenotypic identification of these isolates.

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