Original Article

Antifungal Susceptibility of Candida Isolates at One Institution

Shinji Katsuragi1,2, Makoto Sata1, Yoshinari Kobayashi1, Takekazu Miyoshi1, Yasuki Yamashita1, Reiko Neki1, Chinami Horiuchi1, Kaoru Yamanaka1, Chizuko Kamiya1, Naoko Iwanaga1, Hiroaki Tanaka1, Tomoaki Ikeda1, Jun Yoshimatsu1

1National Cerebral and Cardiovascular Center
2Sakakibara Heart Institute

ABSTRACT

Species distribution and antifungal susceptibility of Candida isolates at one institution were evaluated. Detection rates of fungi were examined for 5 years between 2007 and 2011. Sensitivities of fungi to amphotericin B, flucytosine, fluconazole, micafungin, itraconazole, and voriconazole were evaluated in blood culture-positive patients. A total of 3,832 fungal isolates were detected, including Candida albicans 66.5%, Candida glabrata 20.3%, Candida parapsilosis 6.2%, Candida tropicalis 5.5%, and others 1.5%. Candidemia was diagnosed in 131 patients, and C. albicans, C. parapsilosis, C. glabrata, C. tropicalis, and others were present in 42.0%, 27.5%, 16.0%, 8.4%, and 6.1% of these patients, respectively. Voriconazole had the lowest MIC90s against C. albicans and C. parapsilosis (0.015 and 0.25). Micafungin had a low MIC90 against C. glabrata and C. tropicalis. C. albicans was the most common fungus in patients with candidemia. Voriconazole and micafungin were effective against C. albicans. Amphotericin B was effective for C. parapsilosis, and micafungin showed good efficacy against C. glabrata and C. tropicalis.

Key words: fungus, antifungal susceptibility, antifungal agents, Candida, candidemia

Introduction

Recent developments in medical technology have improved the survival of patients with severe tissue damage, those who undergo complex surgery, and those with severe circulatory failure. However, deep mycosis in these patients may induce opportunistic infections that may be difficult to diagnose and treat. The incidence of deep mycosis has increased in surgery, ambulatory care, and intensive care units, and is commonly treated with antimicrobial agents. Candida mycosis is treated with empirical therapy using azole antifungal drugs including fluconazole, and the prognosis is good. However, Candida albicans may show low sensitivity or resistance to these drugs, and non-albicans Candida species are also increasingly being isolated.

In this study, the detection rate of deep mycosis, the drug sensitivity of the causal species, and the use of antifungal drugs were examined at the National Cerebral and Cardiovascular Center (NCVC). The goals of the study were to establish the antifungal drug sensitivity of Candida strains isolated in the center and to identify appropriate agents for treatment of deep mycosis.

Materials and Methods

The detection rates of fungi at the NCVC were determined for 5 years between January 2007 and December 2011. Sensitivities of fungi to amphotericin B, flucytosine, fluconazole, micafungin, itraconazole, and voriconazole were examined in blood culture-positive patients. The NCVC is located in
an urban area in Japan, and specializes in surgical treatment of cardiovascular diseases, including cerebrovascular and internal injuries. The facility also manages pregnancy and delivery for women with maternal cardiac diseases. The NCVC has 612 beds and about 10,000 new hospital stays each year. The average hospital stay is 17 days, and 650 and 190 heart surgeries are performed annually for adults and infants, respectively. Ten heart transplantations are performed each year.

**Culture media**

CHROMagar Candida (CHROMagar, Paris, France) was purchased as a powder. CHROMagar is composed (per liter) of 10 g peptone, 20 g glucose, 15 g agar, 0.5 g chloramphenicol, and 2 g chromogenic mix. The medium was prepared according to the manufacturer’s instructions and dispensed in petri dishes (20 ml in a 90-mm diameter dish).

**Identification of fungus species**

Clinical specimens from cases with suspected mycogenic infections were inoculated onto CHROMagar and incubated at 37°C for 48 h. Macroscopic identification was performed based on the color and shape of the grown colonies. Strains without typical characteristics on the CHROMagar were identified with a ID 32°C Yeast Identification System (bioMerieux S. A.), using colonies on the CHROMagar prepared using the solution provided with this system.

**Determination of sensitivity to antifungal drugs**

The microdilution method was used to study drug sensitivity, using an Antifungal Susceptibility Test for Yeast (Kyokuto Pharmaceutical Industrial Co.) that complied with Clinical and Laboratory Standards Institute (CLSI) criteria. M27-A3 was used to determine the minimum inhibitory concentration (MIC) of amphotericin B (measurable concentration range 0.03-16 μg/ml), flucytosine (0.125-64 μg/ml), fluconazole (0.125-64 μg/ml), micafungin (0.03-16 μg/ml), itraconazole (0.015-8 μg/ml), and voriconazole (0.03-16 μg/ml). Sensitive (S), sensitive dose-dependent (S-DD), intermediate (I), and resistant (R) responses to flucytosine, fluconazole, and itraconazole were evaluated using CLSI M27-S3 criteria.

The study was exempted from Committee on Human Research approval (National Cerebral and Cardiovascular Center) because there no longer exists a key or code sheet relating the individuals’ identities to their private health information.

**Results**

A total of 3,832 patients had a detected fungal infection in the 5-year period from 2007 to 2011 in the NCVC, including 2,548 patients with *C. albicans* (66.5%), 776 with *C. glabrata* (20.3%), 239 with *C. parapsilosis* (6.2%), and 212 with *C. tropicalis* (5.5%) (Table 1). Non-albicans infections accounted for 33.5% of cases. The location and materials of isolated *Candida* species are shown in Table 2.

The number of blood culture performed were 2,819, 3,306, 2,900, 3,797, 4,239 in 2007, 2008, 2009, 2010, and 2011, respectively. The number and percentages of patients with fungemia caused by *C. albicans, C. parapsilosis, C. glabrata, C. tropicalis, Candida lusitaniae, Candida guilliermondii,* and *Candida krusei* were 55 (42.0%), 36 (27.5%), 21...
Drug sensitivity

Data for the sensitivity of fungi to amphotericin B, flucytosine, fluconazole, micafungin, itraconazole, and voriconazole are shown in Table 4. Amphotericin B was not classified into S, S-DD, I, and R categories in the CLSI 2009 criteria.

The MIC₉₀ of voriconazole against C. *albicans* (0.015) was the lowest among the 6 antifungal drugs, followed by micafungin (0.06), flucytosine (0.25), itraconazole (0.25), amphotericin B (0.5), and fluconazole (0.5). However, none of the 55 patients with candidemia caused by C. *albicans* showed resistance in the CLSI criteria (flucytosine ≥ 32, fluconazole ≥ 64, itraconazole ≥ 1, voriconazole ≥ 4). Of these 55 cases, 46 (83.6%) were S-DD to itraconazole and all 55 were sensitive to the other 5 antifungal drugs.

The MIC₉₀ of voriconazole and against C. *parapsilosis* (0.125) was also the lowest among the antifungal drugs, followed by flucytosine (0.25), amphotericin B (0.5), itraconazole (1), micafungin (2), and fluconazole (16). The resistance rates of C. *parapsilosis* to fluconazole and itraconazole were 5.6% and 25.0%, respectively. The percentages of patients with S (< 0.125), S-DD (0.25-0.5), and R (≥ 1) responses were 27.8%, 47.2%, and 25.0%, respectively, for itraconazole. The percentages of patients with S (< 8), S-DD (16-32), and R (≥ 64) responses were 83.3%, 11.1%, and 5.6%, respectively, for fluconazole.

The MIC₉₀ of micafungin against C. *glabrata*

### Table 2. Location and materials of isolated Candida species

<table>
<thead>
<tr>
<th>Species</th>
<th>Sputum</th>
<th>Urogenital</th>
<th>Stool</th>
<th>Intra-body material</th>
<th>Blood</th>
<th>Skin</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. <em>albicans</em></td>
<td>1,309</td>
<td>800</td>
<td>150</td>
<td>113</td>
<td>55</td>
<td>81</td>
<td>40</td>
<td>2,548</td>
</tr>
<tr>
<td>C. <em>glabrata</em></td>
<td>291</td>
<td>333</td>
<td>87</td>
<td>25</td>
<td>21</td>
<td>10</td>
<td>9</td>
<td>776</td>
</tr>
<tr>
<td>C. <em>parapsilosis</em></td>
<td>56</td>
<td>77</td>
<td>8</td>
<td>38</td>
<td>36</td>
<td>11</td>
<td>13</td>
<td>239</td>
</tr>
<tr>
<td>C. <em>tropicalis</em></td>
<td>123</td>
<td>49</td>
<td>17</td>
<td>11</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>212</td>
</tr>
<tr>
<td>C. <em>krusei</em></td>
<td>13</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>C. <em>luisitaniae</em></td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>C. <em>guiliemondii</em></td>
<td>2</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>15</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,798</td>
<td>1,276</td>
<td>268</td>
<td>192</td>
<td>131</td>
<td>102</td>
<td>65</td>
<td>3,832</td>
</tr>
</tbody>
</table>

All data are shown as a number with the percentage in parentheses. Sputum includes respiratory related materials. Intra-body materials include catheters and drainage tube. Aspiration fluid indicates ascites, pleural effusion, and pericardial effusion.

### Table 3. Yearly changes of species distribution of 131 Candida blood isolates detected at the National Cerebral and Cardiovascular Center from 2007 to 2011

<table>
<thead>
<tr>
<th>Species</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. <em>albicans</em></td>
<td>7 (41.1)</td>
<td>7 (43.8)</td>
<td>8 (44.4)</td>
<td>25 (59.5)</td>
<td>8 (25.0)</td>
<td>55 (42.0)</td>
</tr>
<tr>
<td>C. <em>parapsilosis</em></td>
<td>2 (11.8)</td>
<td>4 (25.0)</td>
<td>3 (16.7)</td>
<td>11 (25.0)</td>
<td>16 (44.4)</td>
<td>36 (27.5)</td>
</tr>
<tr>
<td>C. <em>glabrata</em></td>
<td>3 (17.7)</td>
<td>3 (18.8)</td>
<td>4 (22.2)</td>
<td>5 (11.4)</td>
<td>6 (16.7)</td>
<td>21 (16.0)</td>
</tr>
<tr>
<td>C. <em>tropicalis</em></td>
<td>4 (23.5)</td>
<td>1 (6.3)</td>
<td>2 (11.1)</td>
<td>2 (4.6)</td>
<td>2 (5.6)</td>
<td>11 (8.4)</td>
</tr>
<tr>
<td>C. <em>luisitaniae</em></td>
<td>1 (5.9)</td>
<td>1 (6.3)</td>
<td>1 (5.6)</td>
<td>1 (2.8)</td>
<td>1 (1.5)</td>
<td></td>
</tr>
<tr>
<td>C. <em>kruusei</em></td>
<td>1 (5.9)</td>
<td>1 (6.3)</td>
<td>1 (5.6)</td>
<td>1 (2.8)</td>
<td>1 (1.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>17</td>
<td>16</td>
<td>18</td>
<td>44</td>
<td>36</td>
<td>131</td>
</tr>
</tbody>
</table>

All data are shown as a number with the percentage for each year in parentheses.
was the lowest among the antifungal drugs, followed by flucytosine (0.13), amphotericin B (1), and voriconazole (1). The resistance rate to voriconazole was 14.3%. The drug resistance rates of itraconazole and fluconazole were 100% and 19.1%, respectively.

The MICs of micafungin against \textit{C. tropicalis} (0.13) was significantly lower than those for other drugs, followed by amphotericin B and voriconazole (both 0.5). The resistance rate of \textit{C. tropicalis} against voriconazole was 0%.

**Discussion**

131 \textit{Candida} strains isolated from blood at the NCVC from 2007 to 2011 showed species distribution, \textit{C. albicans} 42.0%, \textit{C. parapsilosis} 27.5%, \textit{C. glabrata} 16.0%, \textit{C. tropicalis} 8.4%, and \textit{C. krusei} 0.8%. Our data and the results of a national surveillance study indicate that \textit{C. albicans} is still the major causal fungus of candidemia in Japan. In \textit{C. albicans} no isolate with resistance to fluconazole $\geq 64 \mu g/ml$ was found in this study. The 90\%MIC was 0.5 $\mu g/ml$ and the isolate with
lowest susceptibility required 2μg/ml. These findings led us to use an antifungal susceptibility-based management strategy in NCVC for treatment for known C. albicans infection in which fluconazole is the first line antifungal drug.

The rates of resistance of C. glabrata to itraconazole and fluconazole (100% and 20%) were greater than those (56.3% and 5.2%) in Takakura et al[7]. High rates of resistance to itraconazole for C. glabrata detected in the bloodstream were also found by Myoken (100%, 8/8) and St-Germain et al. (83.3%, 65/78). In our study, none of the 25 Candida isolates with reduced susceptibility to fluconazole (MIC >16μg/ml) was susceptible to itraconazole (MIC < 0.12 μg/ml). However, Pfaffer et al. suggested that MICs of < 1 μg/ml may better reflect ‘susceptibility’ in invasive candidiasis, due to the higher serum concentrations achievable with the new nanocrystal intravenous formulation of itraconazole[10]. Given this new threshold, 40.0% of our isolates with reduced susceptibility to fluconazole would be considered susceptible to itraconazole. Furthermore, our observations are similar to those of Pfaffer et al., with all four of our C. glabrata isolates that were resistant to fluconazole also showing resistance to itraconazole.

The higher resistance rate of C. tropicalis to fluconazole (36.4%) compared to reports from the USA (6.2%) and Spain (16.6%) is another characteristic of non-albicans candidemia in this study[11,12]. We attribute this high resistance to the consistent and high frequency use of fluconazole in our facility. Interestingly, for each case of fluconazole-resistant non-albicans candidemia (4 isolates of C. glabrata, and 4 isolates of C. tropicalis), micafungin showed high sensitivity and can be regarded as the first choice for treatment of fluconazole-resistant C. glabrata and C. tropicalis. Voriconazole showed no resistance to C. tropicalis and may be used as the second choice for these isolates in our hospital; however, voriconazole showed a resistance rate of 43.5% in a national survey[7]. This discrepancy suggests that the susceptibility of each species of Candida differs from hospital to hospital, due to the different disease backgrounds and treatments at each center. This indicates that antifungal drug susceptibility at each facility should be considered in the selection of antifungal drugs.

In this study, the greatest number of fungi in the bloodstream was detected in 2011 and the incidence of the disease caused by C. parapsilosis (n = 16) was the highest in the same year. The incidence of candidemia caused by C. albicans gradually decreased in the study period. We attribute this increase of C. parapsilosis to the increase in operations for candidates for heart transplantation and for neonates with congenital heart diseases. These immunologically compromised patients underwent treatments including central line management, which is a known risk
factor for *C. parapsilosis*. This increase in *C. parapsilosis* caused a temporary increase in use of amphotericin B in 2011 (Fig. 1). The selection of this drug for *C. parapsilosis* has turned out to be appropriate because in this study fluconazole showed a resistance rate of 5.6% and a MIC90 with micafungin that was as high as 2.0 μg/ml. The MIC90 of voriconazole was 0.125, which makes this drug the second choice for *C. parapsilosis* in the NCVC.

We introduced micafungin for treatment of deep mycosis in 2004. By 2006, micafungin accounted for 27% of all antifungal drugs used in the NCVC and from 2009 to 2011 this rate reached 70%. This increased use has occurred because micafungin is an echinocandin that has a broad antifungal spectrum and exhibits good activity against azole antifungal drug-resistant strains. Micafungin is effective in fungal cell lines and several reports have shown excellent tissue penetration and clinical effects. Thus, micafungin has been most commonly used at the NCVC since 2007, including preservational use for immunocompromised patients, such as those undergoing cardiac transplantation or in extremely low-birthweight infants in the NICU. However, several clinical isolates of *Candida* with low resistance to echinocandin antifungal drugs have been described and care is taken regarding this issue at the NCVC. The mechanism of this reduced sensitivity involves a mutation in Fkslp, which is a 1,3β-D-glucan synthase subunit of the target enzyme of echinocandins. No strains with reduced sensitivity to micafungin were found in this study. However, as clinical use of the drug continues to increase, particular attention should be paid to the sensitivity of clinical isolates to micafungin.

*C. lusitaniae* is an infrequent cause of fungemia, but the rate obtained in this study (3.8%) was 6.8 times higher than that in Takakura and Minari et al. The reported underlying conditions for patients with deep seated *C. lusitaniae* infections are malignancy 53%, neurogenic 35%, receiving broad-spectrum antibiotics 27%, receiving long-term corticosteroid therapy 16%, and having a central venous catheter 27%. Although fungemia is the most common type of *C. lusitaniae* infection (80%), primary infection focuses were identified in 20% of cases. These included endocarditis, infection of a left ventricular device, meningitis, chorioamnionitis, peritonitis, abdominal abscess, and cutaneous infection, and most of these diseases are treated at our center. These facts may be related to the higher detected rate of *C. lusitaniae* fungemia at our institution. *C. tropicalis* and *C. krusei* are likely to cause deep mycosis in patients with hematologic tumors undergoing digestive tract surgery, but this type of surgery is not performed at the NCVC. This may explain the low incidence of fungemia at the NCVC due to these species.

In summary, it is important to comprehend the susceptibility for antifungal drug and distribution of each *Candida* isolate of each hospital in selection of antifungal drug, due to the different disease backgrounds and treatments at each center.

**Limitations**

The sample population was small in this study. In particular, only 11 patients had *C. tropicalis*, which is the minimum required to calculate MIC, and further validation of this result is required. Drug sensitivity may vary depending on the actual treatment in medical institutions, in particular regarding use of the antifungal drug. The NCVC is a specialized center for internal medicine and cardiovascular surgery in patients with cardiovascular disorders or cerebrovascular accident, in contrast to the roles of secondary or tertiary hospitals for general patients. Therefore, it is important to study the drug sensitivity of fungi and measures to be taken against infections in centers such as the NCVC, in which immunocompromised patients are treated, including those undergoing cardiac transplantation, even if the study population is small.

**Acknowledgments**

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**Conflict of Interest Statement**

None of the authors has a conflict of interest regarding the work in this study.

**References**
