A Retrospective Analysis of Antifungal Susceptibilities of Candida Bloodstream Isolates From Singapore Hospitals

Thean Yen Tan, MB.BCh, MRCPath, Ai Ling Tan, MBBS, Dip Bact, FRCPA, Nancy WS Tee, MBBS, FRCPA, Lily SY Ng, DQE (Bacteriology), SpDip (Microbiology)

Abstract

Introduction: Worldwide, Candida albicans is the most common Candida species implicated in bloodstream infections. However, the proportion of non-albicans bloodstream infections is increasing. Fluconazole resistance is known to be more common in non-albicans species, but is also reported in C. albicans. This retrospective study was performed to determine the species epidemiology of Candida bloodstream infections in Singapore hospitals, and to perform susceptibility testing to a range of antifungal drugs. Materials and Methods: Candida spp. isolated from bloodstream infections from October 2004 to December 2006 were collected from 3 participating hospitals: a tertiary referral hospital (Singapore General Hospital), a secondary referral hospital (Changi General Hospital) and an obstetrics/paediatric hospital (KK Women’s and Children’s Hospital (KKWCH)). Isolate collection was also retrospectively extended to January 2000 for KKWCH because of the limited number of cases from this hospital. Isolates were identified by a common protocol, and antifungal susceptibility testing was performed by microbroth dilution (Sensititre One, Trek Diagnostics, United Kingdom). Results: The most common isolates were C. albicans (37%), C. tropicalis (27%) and C. glabrata (16%). There were differences in species distribution between institutions, with C. parapsilosis and C. albicans predominant in KKWCH, and C. albicans and C. tropicalis predominant in the other 2 institutions. Fluconazole resistance was detected in 3.2% of all Candida spp., and 85.3% were classified as susceptible. All C. albicans and C. parapsilosis were susceptible to fluconazole and voriconazole, while susceptibility to fluconazole was much more variable for C. glabrata and C. krusei. Conclusion: This study shows that C. albicans remains the predominant Candida species isolated from bloodstream infections in the 3 participating hospitals. However, non-albicans species accounted for nearly two-thirds of all cases of candidaemia. Resistance to fluconazole was uncommon, and was generally confined to C. krusei and C. glabrata.

Key words: Antifungal agents, Antifungal drug resistance, Fungaemia

Introduction

Nosocomial infection with Candida species is increasing in significance worldwide. A recent review of positive blood cultures noted the relative increase in importance of fungal bloodstream infections (BSI), and Candida was reported as the fourth most common bloodstream pathogen in the United States. Candida BSI is associated with a very high crude mortality of over 60%, while the attributable mortality may be as high as 49%.

C. albicans remains the predominant pathogen associated with BSI caused by Candida spp. Population-based studies in Europe and the United States demonstrate that approximately 95% of candidaemia is caused by 4 species: C. albicans, C. glabrata, C. parapsilosis and C. tropicalis. There has been a documented increase in the proportion of infections caused by other non-albicans sp., particularly C. glabrata. In addition to this shift in species distribution, geographic variations are apparent in the distribution of Candida species implicated in BSI, emphasising the importance of knowing local epidemiological patterns.

1 Division of Laboratory Medicine, Changi General Hospital, Singapore
2 Department of Pathology, Singapore General Hospital, Singapore
3 Laboratory, KK Women and Children’s Hospital, Singapore
Address for Correspondence: Dr Thean Yen Tan, Division of Laboratory Medicine, Changi General Hospital, 2 Simei Street 3, Singapore 529889. Email: thean_yen_tan@cgh.com.sg


October 2008, Vol. 37 No. 10
Fluconazole has remained the mainstay of empiric antifungal therapy since its introduction in the early 1990’s, because of favourable pharmacokinetic and side effect profiles. However, C. glabrata and C. krusei exhibit reduced susceptibilities or frank resistance to fluconazole. In addition, there is documented increasing fluconazole resistance in all Candida spp., including C. albicans, C. lusitaniae, C. tropicalis and C. dubliniensis. The gold standard of antifungal therapy for fluconazole resistant species and for severe infections has remained Amphotericin B. Amphotericin resistance in Candida spp. has been documented, particularly in C. lusitaniae and therapeutic failures with this drug have been documented. The recent introduction of new azole derivatives and the echinocandins has offered increased choices for the treatment of resistant or refractory fungal infections.

Antifungal susceptibility is a rapidly changing area of knowledge. Current internationally accepted standards of testing require broth dilution methods which are labour intensive, time consuming and expensive. An alternative commercially available equivalent method (Sensititre YeastOne, Trek Diagnostics, UK) has been demonstrated to show good correlation and reproducibility with the reference CLSI M27-A method for the azoles, amphotericin B and the newer antifungal agents.

There is patchy information available on the spectrum of Candida BSI in Singapore. A 6-month study performed in 2001 was based on Candida species isolated from most body sites, including a limited number of BSI isolates, and documented a lower prevalence of C. albicans and a different distribution of non-albicans species. A recent study performed at a single institution reported that C. tropicalis was the predominant species isolated from BSI. This study was implemented as the first systematic multi-centre survey of Candida BSI infections in Singapore. The participating hospitals were chosen to include a pediatric, an acute care and a tertiary referral hospital in order to sample a representative patient population.

Materials and Methods

Collection of Isolates

All strains of Candida spp. isolated from BSI between October 2004 and December 2006 from 3 participating Singapore hospitals were included in the study. The 3 participating hospitals were Singapore General Hospital (SGH), Changi General Hospital (CGH) and KK Women’s and Children’s Hospital (KKWCH). Due to the low incidence of Candida BSI in paediatric patients, archived bloodstream isolates dating from 2000 from KKWCH were also included in the study. The 3 study hospitals represent 28% of all hospital beds available in Singapore, and 56% of acute-care beds available in the 6 public acute-care hospitals.

Identification of Yeast Isolates

Species determination was initially performed at each participating laboratory using a standardised protocol. Isolates were identified based on API 20C AUX (bioMérieux, France) and morphology expression on cornmeal agar. Isolates were then forwarded to CGH for further testing.

All isolates were sub-cultured on to CHROMagar Candida (Becton Dickinson, USA).

All isolates submitted as C. albicans with concordant colonial morphology on CHROMagar Candida were screened for growth at 42°C in order to differentiate possible C. dubliniensis isolates. The species identification of strains with poor growth at 42°C was confirmed using ID-YST panels (Vitek 2 Compact, bioMérieux, France).

For non-albican isolates, species identification was accepted as accurate if the submitted identification by participating laboratories was concordant with colonial morphology on CHROMagar Candida.

Isolates with discordant colonial morphologies were further tested using ID-YST panels and a repeat test to check morphology expression on cornmeal agar supplemented with Tween 80.

Minimum Inhibitory Concentration Testing

Antifungal susceptibility testing was performed at CGH using the Sensititre YeastOne YO-5/6 panels. MIC values were obtained for the following antifungals: fluconazole, itraconazole, ketoconazole, voriconazole, amphotericin and 5-flucytosine. Posaconazole and caspofungin MIC were available for a subset of tested isolates. C. krusei ATCC 6258 and C. parapsilosis ATCC 22019 were used as controls for each batch of testing. The accuracy of inoculum density was checked by quantitative plating.

Test panels were read following 24 hours incubation in ambient condition. Following the manufacturer’s guidelines, the MIC was interpreted as the lowest concentration of antifungal that produced a colour change from blue to red. For isolates with trailing colorimetric endpoints for fluconazole and theazole drugs, the MIC was interpreted as the first well showing a less intense colour change compared to the positive growth well.

Susceptibility breakpoints and categorical interpretations (sensitive, resistant and susceptible-dose-dependent) for fluconazole, itraconazole and fluocytosine were interpreted according to those set by the Clinical Laboratory Standards Institute (CLSI). Proposed susceptibility breakpoints were used for voriconazole. There is insufficient data at present for interpretive breakpoints for amphotericin, caspofungin and posaconazole.


Results

Two hundred seventy-nine isolates of Candida spp. were collected from the 3 participating institutions over the study period: 49 from KKWCH (February 2000 to December 2006), 53 from CGH (October 2004 to December 2006) and 165 from SGH (October 2004 to December 2006). The most common isolates were C. albicans (37%), C. tropicalis (27%), C. glabrata (16%) and C. parapsilosis (14%). If isolates collected prior to Oct 2004 were excluded from analysis, there was no change in the relative frequency ranking for each Candida spp. The distribution of Candida spp. isolated from each institution is shown in Table 1.

There were some differences in species distribution between the institutions. C. parapsilosis was significantly more frequently isolated in KKWCH as compared with SGH (relative risk RR 3.2; 95%CI 2.0-5.0) and CGH (RR 2.4, 95%CI 1.7-3.3), and was the most common species isolated from Candida BSI in this institution. C. albicans remained the most common species for BSI infection in both CGH and SGH (45.3% and 35.2% respectively). C. glabrata was significantly more common in CGH compared with KKWCH (RR 1.6; 95%CI 1.1-2.3), but there were no significant differences in isolation rate for this species between the other 2 institutions.

Antifungal Susceptibility

85.3% of Candida isolates were susceptible to fluconazole based on CLSI breakpoints, and a further 11.5% were classified as susceptible-dose-dependant (S-DD). Fluconazole resistance was detected in 3.2% of all isolates, predominantly in C. glabrata but also for C. krusei and C. tropicalis.

Susceptibilities to the other azoles varied: only 62.5% of isolates were susceptible to itraconazole, while 98.0% were considered susceptible to voriconazole, using suggested susceptible breakpoints of ≤1 μg/mL. As in other studies, susceptibilities to the azoles could be predicted according by species, as shown in Table 2. C. albicans and C. parapsilosis were uniformly susceptible to fluconazole and voriconazole, and resistance to fluconazole was low. 2.7% of C. tropicalis were resistant to fluconazole and voriconazole. Susceptibility to fluconazole was much more variable for C. glabrata, with 66.7% of isolates categorised as S-DD, and 20% categorised as resistant. Voriconazole retained in-vitro activity in C. glabrata, with 97.8% of isolates being classified as susceptible.

Amphotericin MIC for the study isolates ranged from 0.0625 μg/ml to 1 μg/mL, with most isolates clustering around 0.25 μg/ml to 1 μg/mL. The mean amphotericin MIC was significantly higher for C. tropicalis and C. glabrata when analysed by analysis of variance.

MIC distributions for the other tested antifungals showed a wider distribution of results, including caspofungin (0.008 -1 μg/mL), posaconazole (0.008-16 μg/mL) and ketoconazole (0.008-32 μg/mL). The MIC distributions for these drugs against the 4 commonest Candida species are shown in Table 3.

Discussion

This study provides the first 2 years of data from a 3-year surveillance program for Candida spp isolated from BSI in 3 Singapore hospitals. In line with other published studies, C. albicans was the most common species isolated from BSI. However, in contrast to other population-based studies where C. glabrata is ranked as the second most commonly reported species, C. tropicalis was the second most common species isolated, comprising 27% of bloodstream isolates. Although there is limited regional data available, C. tropicalis appears to predominate in Latin America, with a higher prevalence in South East Asia. Data available from 2 previous studies performed in Singapore confirmed the
relative higher incidence of *C. tropicalis* from BSI, while a more recent study by Chai and colleagues report that a predominant clonal strain of *C. tropicalis* accounted for 36% of Candida BSI in their institution.20

There were also other subtle inter-hospital differences: *C. parapsilosis* was the predominant species from BSI in KKWH, followed by *C. albicans*, whereas in SGH, *C. albicans* and *C. tropicalis* predominated. CGH showed the highest proportion of *C. albicans* and *C. glabrata* BSI when compared with the other 2 institutions. The predominance of *C. parapsilosis* in KKWH specimens is probably due to the fact that 64% of isolates from this hospital were isolated from patients <2 years of age. The predominance of *C. parapsilosis* infections in paediatric patients has previously been noted in other surveillance programs.24

Antifungal susceptibilities to fluconazole were consistent with previously reported data, and could be reliably predicted by speciation. *C. albicans* was uniformly susceptible to fluconazole. Fluconazole resistance was detected in only 3% of all Candida isolates, although 12% of isolates (mainly *C. glabrata*) were categorised as S-DD. MIC data for fluconazole showed a trend towards higher fluconazole MIC for non-albicans species, even when the data for *C. glabrata* and *C. krusei* were excluded. Voriconazole retained activity against Candida sp. (including those with reduced susceptibility to fluconazole), and in-vitro resistance was rare.

Similarly, resistance to flucytosine was uncommon across all Candida spp.

There are no accepted susceptibility breakpoints for amphoterin in *Candida* spp. A susceptibility breakpoint of ≤1 μg/ml has been suggested, based on predicted microbiologic failure. Based on this breakpoint, none of the study isolates were resistant to amphoterin. However, it has been apparent that amphoterin MIC testing, when performed by the current reference susceptibility methods from the CLSI, results in a narrow spread of MIC values, and may poorly differentiate resistant from susceptible isolates.25 As the Sensititre panels are based on CLSI methods, amphoterin MIC for our study isolates were similarly constrained. Alternative testing methods such as Etest or the use of other susceptibility testing media (e.g. antibiotic medium 3) may better elucidate amphoterin resistance within *Candida* spp.25, 27

Antifungal susceptibility testing remains a rapidly changing field. Standardised testing methods (such as those from the CLSI or EUCAST) are labour-intensive, but have improved the reproducibility of testing results. Existing issues such as the interpretation of trailing endpoints, poor discrimination of amphoterin-resistant yeasts and correlation with clinical endpoints remain to be resolved. The availability of commercial testing systems28-30 and disc diffusion guidelines 31 has enabled wider adoption of antifungal susceptibility testing, though it has to be emphasised that limitations in each method, when compared to the reference methods, should always be considered.

The Sensititre panel used in this study is based on the CLSI microbroth dilution methodology. Evaluations of this test method have shown good reproducibility of categorical interpretations when compared with the reference method, although some investigators report that the Sensititre panel tended to yield higher MIC for most tested drugs, with lower rates of categorical agreement for *C. glabrata* when tested against fluconazole.28

Antifungal susceptibility testing remains a rapidly changing field. Standardised testing methods (such as those from the CLSI or EUCAST) are labour-intensive, but have improved the reproducibility of testing results. Existing issues such as the interpretation of trailing endpoints, poor discrimination of amphoterin-resistant yeasts and correlation with clinical endpoints remain to be resolved. The availability of commercial testing systems28-30 and disc diffusion guidelines 31 has enabled wider adoption of antifungal susceptibility testing, though it has to be emphasised that limitations in each method, when compared to the reference methods, should always be considered.

The Sensititre panel used in this study is based on the CLSI microbroth dilution methodology. Evaluations of this test method have shown good reproducibility of categorical interpretations when compared with the reference method, although some investigators report that the Sensititre panel tended to yield higher MIC for most tested drugs, with lower rates of categorical agreement for *C. glabrata* when tested against fluconazole.28

This study demonstrates that *C. albicans* remains the predominant species isolated from Candida BSI, although inter-institutional differences were apparent. Non-albicans
Table 3. MIC Distributions for Amphotericin B, Caspofungin, Ketoconazole and Posaconazole of the Four Most Common Species From BSI

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>Candida spp.</th>
<th>n</th>
<th>MIC90</th>
<th>MIC range</th>
<th>% MIC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(μg/mL)</td>
<td></td>
<td>≤0.05 0.01 0.02 0.03 0.06 0.13 0.25 0.5 1 2 4 8 16 ≥32</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>C. albicans</td>
<td>107</td>
<td>0.016</td>
<td>0.008 - 0.12</td>
<td>47.3 45.5 3.6 1.8 1.8</td>
</tr>
<tr>
<td></td>
<td>C. glabrata</td>
<td>28</td>
<td>0.125</td>
<td>0.03 - 0.25</td>
<td>0.9 0.9 33.6 52.3 12.1</td>
</tr>
<tr>
<td></td>
<td>C. tropicalis</td>
<td>45</td>
<td>1</td>
<td>0.25 - 1</td>
<td>13.3 37.8 48.9</td>
</tr>
<tr>
<td></td>
<td>C. parapsilosis</td>
<td>18</td>
<td>1</td>
<td>0.12 - 1</td>
<td>2.5 2.5 35 35 25</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>C. albicans</td>
<td>56</td>
<td>0.125</td>
<td>0.03 - 0.25</td>
<td>1.8 69.6 26.8 1.8</td>
</tr>
<tr>
<td></td>
<td>C. glabrata</td>
<td>28</td>
<td>0.125</td>
<td>0.03 - 0.25</td>
<td>3.6 14.3 75 7.1</td>
</tr>
<tr>
<td></td>
<td>C. tropicalis</td>
<td>46</td>
<td>0.125</td>
<td>0.015 - 0.25</td>
<td>6.5 52.2 21.7 15.2 4.3</td>
</tr>
<tr>
<td></td>
<td>C. parapsilosis</td>
<td>18</td>
<td>1</td>
<td>0.12 - 1</td>
<td>11.1 22.2 44.4 22.2</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>C. albicans</td>
<td>55</td>
<td>0.016</td>
<td>0.008 - 0.12</td>
<td>79.4 17.8 0.9 0.9 0.9</td>
</tr>
<tr>
<td></td>
<td>C. glabrata</td>
<td>28</td>
<td>1</td>
<td>0.008 - 2</td>
<td>4.4 2.2 2.2 2.2 2.2</td>
</tr>
<tr>
<td></td>
<td>C. tropicalis</td>
<td>75</td>
<td>0.25</td>
<td>0.015 - 16</td>
<td>1.3 9.3 28 41.3 12 5.3</td>
</tr>
<tr>
<td></td>
<td>C. parapsilosis</td>
<td>40</td>
<td>0.125</td>
<td>0.008 - 0.5</td>
<td>12.5 20 35 20 10</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>C. albicans</td>
<td>107</td>
<td>0.016</td>
<td>0.008 - 0.12</td>
<td>79.4 17.8 0.9 0.9 0.9</td>
</tr>
<tr>
<td></td>
<td>C. glabrata</td>
<td>45</td>
<td>1</td>
<td>0.008 - 2</td>
<td>4.4 2.2 2.2 2.2 2.2</td>
</tr>
<tr>
<td></td>
<td>C. tropicalis</td>
<td>75</td>
<td>0.25</td>
<td>0.015 - 16</td>
<td>1.3 9.3 28 41.3 12 5.3</td>
</tr>
<tr>
<td></td>
<td>C. parapsilosis</td>
<td>40</td>
<td>0.125</td>
<td>0.008 - 0.5</td>
<td>12.5 20 35 20 10 2.5</td>
</tr>
</tbody>
</table>
species now account for nearly two-thirds of BSI. The main non-\textit{albicans} species isolated were \textit{C. tropicalis} in the hospitals serving mainly adult populations, and \textit{C. parapsilosis} in the hospital serving mainly a paediatric and obstetric population. Other than for \textit{C. krusei}, and to a lesser extent, \textit{C. glabrata}, resistance to fluconazole was uncommon.

Acknowledgements

We would like to thank Ms Chloe Wang Jun Chee for her unflagging technical assistance and to all staff members involved in each participating institution for making this study possible. This study was funded by a grant from the National Medical Research Council, Singapore.

REFERENCES


