National surveillance of antifungal susceptibility of Candida species in South Korean hospitals

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We investigated the species distribution and antifungal susceptibility of Candida isolates from tertiary and non-tertiary hospitals in South Korea from 2002–2004. Of the 612 Candida isolates that were collected, Candida albicans, C. parapsilosis, C. tropicalis, and C. glabrata occurred most frequently, accounting for 97.3% and 96.8% of the isolates in tertiary and non-tertiary hospitals, respectively. C. albicans was the most common isolate, but the incidence of non-C. albicans Candida species was higher than that of C. albicans in tertiary hospitals. The Candida species had much lower MIC90 to voriconazole (tertiary hospitals: 0.5 µg/ml, non-tertiary hospitals: 0.25 µg/ml) than to fluconazole (tertiary hospitals: 8 µg/ml, non-tertiary hospitals: 4 µg/ml). The MIC90 of Candida isolates to 5-flucytosine in non-tertiary hospitals was two times higher than that observed in tertiary facilities. The C. glabrata isolates showed a tendency toward strong resistance to fluconazole, but C. parapsilosis isolates were susceptible to all of the evaluated antifungal agents. Voriconazole showed strong in vitro activity against Candida species, especially C. krusei, which is resistant to fluconazole and 5-flucytosine. To our knowledge, this is the first report of Candida antifungal susceptibility that includes non-tertiary hospitals in South Korea.

Keywords Candida species, antifungal susceptibility, tertiary hospitals, non-tertiary hospitals

Introduction

The number of life-threatening fungal infections observed worldwide has increased significantly [1–3] and Candida species are the major etiologic agents of these disease. The most recent surveys have shown Candida to be the third or fourth most commonly isolated bloodstream pathogen in US hospitals, currently surpassing Gram-negative bacilli in frequency [4,5].

Although C. albicans remains the most common cause of oropharyngeal and cutaneous candidiasis, non-C. albicans species of Candida are observed more frequently in both invasive and vaginal candidiasis [1,2,6–8]. The need for a variety of antifungal agents has increased due to the increasing frequency of fungal infections. In particular, fluconazole is a highly effective broad-spectrum antifungal agent. It has been widely used because it is non-toxic, active against several species of yeast, and can be administered via both the intravenous and oral routes. But widespread and prolonged use of azoles during recent years has led to the rapid development of drug resistance in Candida species [7,9]. Large-scale surveillance studies of Candida isolates has produced useful information regarding resistance trends, the distribution of species.
in various countries, and descriptions of infection types [10–12]. Despite this progress, there have not been any reported investigations of the incidence or susceptibility of Candida species in non-tertiary hospitals. Therefore, in this study, we assessed the distribution of Candida species recovered in tertiary and non-tertiary hospitals and in vitro susceptibility testing to amphotericin B, 5-flucytosine, fluconazole, and voriconazole Candida isolates collection between 2002 and 2004 from these types of hospitals.

Materials and methods

Clinical isolates and organism identification

In this study, all Candida isolates recovered from clinical specimens in tertiary and non-tertiary hospitals were consecutively collected during 2002–2004 and 2003–2004, respectively. All were identified at the participating institutions using routine laboratory methods. The isolates were stored in Microbank vials at −80°C until needed.

Antifungal susceptibility testing

Antifungal susceptibility testing was performed in exact accordance with the reference broth microdilution method described by the M27-A2 guidelines of the Clinical and Laboratory Standards Institute (formerly NCCLS) [13]. Preparations of amphotericin B (Sigma, St Louis, MO, USA), 5-flucytosine (Sigma), fluconazole (Pfizer, Inc., Korea), and voriconazole (Pfizer) were obtained from the respective manufacturers. The MICs of 5-flucytosine, fluconazole and voriconazole were read as the lowest concentrations that produced a prominent decrease in turbidity relative to the growth in drug free controls. The interpretive susceptibility criteria used for 5-flucytosine, amphotericin B (>1 μg/ml, likely resistance), voriconazole and fluconazole were those specified by the CLSI [13,14]. The MIC of amphotericin B was defined as the lowest concentration resulting in complete inhibition of growth. Testing of the control strains that were recommended by the CLSI (C. krusei ATCC 6258 and C. parapsilosis ATCC 22019) ensured the quality of each MIC50 and MIC90 measurement.

Results

Species distribution of Candida isolates

A total of 612 Candida isolates were collected from nine tertiary hospitals (333 isolates) and 95 non-tertiary hospitals (279 isolates) between 2002 and 2004. The overall species distributions are shown in Table 1. Most of our 612 Candida isolates, were c. albicans, C. parapsilosis, C. tropicalis, and C. glabrata were the most common, C. albicans was the most prevalent species in tertiary and non-tertiary hospitals. These four major species accounted for 97.3% and 96.8% of the isolates from tertiary and non-tertiary hospitals, respectively. C. tropicalis (29%) was isolated more often than C. parapsilosis (3.6%) in non-tertiary hospitals. In contrast, C. parapsilosis (22.2%) was recovered more often than C. tropicalis (13.8%) in tertiary hospitals. The other Candida species showed frequencies below 2%, with C. guilliermondii, C. krusei, and C. lusitaniae collected in both tertiary and non-tertiary hospitals, but C. famata, C. intermedia, C. utilis, C. holmii, C. humicola, and C. rugosa were only isolated in tertiary hospitals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tertiary hospitals</th>
<th>Non-tertiary hospitals</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>333 (%)</td>
<td>279 (%)</td>
<td>612</td>
</tr>
<tr>
<td>C. albicans</td>
<td>155 (46.6)</td>
<td>160 (57.4)</td>
<td>315</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>74 (22.2)</td>
<td>10 (3.6)</td>
<td>184</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>46 (13.8)</td>
<td>81 (29.0)</td>
<td>127</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>37 (11.1)</td>
<td>19 (6.8)</td>
<td>56</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>5 (1.5)</td>
<td>2 (0.7)</td>
<td>7</td>
</tr>
<tr>
<td>C. famata</td>
<td>5 (1.5)</td>
<td>5 (0.8)</td>
<td>10</td>
</tr>
<tr>
<td>C. intermedia</td>
<td>1 (0.3)</td>
<td>1 (0.2)</td>
<td>2</td>
</tr>
<tr>
<td>C. krusei</td>
<td>3 (0.9)</td>
<td>5 (1.8)</td>
<td>8</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>3 (0.9)</td>
<td>2 (0.7)</td>
<td>5</td>
</tr>
<tr>
<td>C. utilis</td>
<td>1 (0.3)</td>
<td>1 (0.2)</td>
<td>2</td>
</tr>
<tr>
<td>C. holmii</td>
<td>1 (0.3)</td>
<td>1 (0.2)</td>
<td>2</td>
</tr>
<tr>
<td>C. humicola</td>
<td>1 (0.3)</td>
<td>1 (0.2)</td>
<td>2</td>
</tr>
<tr>
<td>C. rugosa</td>
<td>1 (0.3)</td>
<td>1 (0.2)</td>
<td>2</td>
</tr>
</tbody>
</table>

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Susceptibility to amphotericin B

The MIC ranges, including both MIC_{50} and MIC_{90}, are summarized in Table 2. The \textit{in vitro} amphotericin B MIC level for all isolates was never higher than 1 \( \mu \text{g/ml} \), with most \textit{Candida} isolates having MICs between 0.5–1 \( \mu \text{g/ml} \) against this antifungal agent. Among the \textit{Candida} species from all of the evaluated hospitals, seven out of eight \textit{C. krusei} isolates from tertiary and non-tertiary hospitals showed MICs of 1 \( \mu \text{g/ml} \). The MIC ranges of \textit{C. parapsilosis} and \textit{C. glabrata} isolates from non-tertiary hospitals were higher than those of isolates of the same species recovered in tertiary hospitals.

Susceptibility to 5-flucytosine

\textit{C. albicans} showed a higher MIC_{90} against 5-flucytosine than \textit{C. tropicalis}, \textit{C. parapsilosis}, or \textit{C. glabrata} collected in both types of hospitals (Table 2). Importantly, five of eight and three of eight \textit{C. krusei} isolates showed intermediate and full resistance to 5-flucytosine, respectively. A low level of 5-flucytosine antifungal activity was demonstrated \textit{in vitro} against \textit{C. krusei} isolates (tertiary hospital 16 \( \mu \text{g/ml} \), non-tertiary hospital 32 \( \mu \text{g/ml} \)), but this antifungal was highly active against \textit{C. glabrata} isolates (tertiary hospital 0.125 \( \mu \text{g/ml} \), non tertiary hospital 0.25 \( \mu \text{g/ml} \)).

\textit{Susceptibility to fluconazole and voriconazole}

Of the 56 \textit{C. glabrata} isolates, 24 (43\%) showed fluconazole dose-dependent susceptibility (SDD) and resistance patterns. The MIC_{90} values of fluconazole against \textit{C. glabrata} from both tertiary and non-tertiary hospitals were 64 \( \mu \text{g/ml} \). The other major \textit{Candida} species (\textit{C. albicans}, \textit{C. tropicalis}, and \textit{C. parapsilosis}) showed low MIC_{90} values ranging from 0.5–8 \( \mu \text{g/ml} \). Among the non-major \textit{Candida} species, five \textit{C. krusei} isolates were resistant to fluconazole. With the exception of six \textit{Candida} isolates, most had MICs \( \leq 2 \mu \text{g/ml} \) against voriconazole. One voriconazole-resistant \textit{C. albicans} isolate was identified from a tertiary hospital. The \textit{C. parapsilosis} isolates had the lowest MIC_{90} values for voriconazole, at 0.062 \( \mu \text{g/ml} \) and \( < 0.031 \mu \text{g/ml} \) in tertiary and non-tertiary hospitals, respectively. The MIC_{90} values of fluconazole against \textit{Candida} were 8 \( \mu \text{g/ml} \) and 4 \( \mu \text{g/ml} \) in tertiary and non-tertiary hospitals, respectively.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Species} & \textbf{Antifungal agent} & \multicolumn{3}{c|}{\textbf{Tertiary hospitals MIC (\( \mu \text{g/ml} \))}} & \multicolumn{3}{c|}{\textbf{Non tertiary hospitals MIC (\( \mu \text{g/ml} \))}} \\
\hline
\textbf{(no.of Tertiary isolates/ non tertiary isolates)} & & \textbf{Range} & \textbf{50\%} & \textbf{90\%} & \textbf{Range} & \textbf{50\%} & \textbf{90\%} \\
\hline
\hline
\textbf{C. albicans} & amphotericin B & 0.062–1 & 0.5 & 0.5 & 0.125–1 & 0.5 & 1 \\
(155/160) & flucytosine & \(< 0.125–64\) & \(< 0.125–64\) & \(< 0.125–64\) & \(< 0.125–64\) & 1 & 1 \\
& fluconazole & 0.031–8 & 0.25 & 2 & 0.125–8 & 0.25 & 0.5 \\
& voriconazole & 0.031–8 & 0.031 & 0.5 & 0.031 & 0.031 & 0.125 \\
& amphotericin B & 0.125–1 & 0.5 & 1 & 0.125–1 & 0.5 & 1 \\
\hline
\textbf{C. tropicalis} & flucytosine & \(< 0.125–64 \leq 0.125\) & \(< 0.125–64 \leq 0.125\) & \(< 0.125–64 \leq 0.125\) & \(< 0.125–64 \leq 0.125\) & 0.5 & 0.5 \\
(46/81) & fluconazole & 0.25–16 & 0.5 & 8 & 0.125–16 & 0.5 & 4 \\
& voriconazole & 0.031–4 & 0.031 & 1 & 0.031–4 & 0.031 & 0.25 \\
& amphotericin B & 0.125–1 & 0.5 & 1 & 0.5 & 1 & 1 \\
\hline
\textbf{C. parapsilosis} & fluconazole & 0.125–1 & 0.125 & 0.25 & \(< 0.125–0.5\) & \(< 0.125–0.5\) & 1 & 1 \\
(74/10) & fluconazole & 0.125–8 & 0.5 & 1 & 0.25–4 & 0.5 & 2 \\
& voriconazole & \(< 0.031–0.25\) & \(< 0.031–0.25\) & \(< 0.031–0.25\) & \(< 0.031–0.25\) & \(< 0.031–0.25\) & \(< 0.031–0.25\) \\
& amphotericin B & 0.25–1 & 0.5 & 1 & 0.25–1 & 1 & 1 \\
\hline
\textbf{C. glabrata} & fluconazole & 0.125–1 & \(< 0.125–125\) & \(< 0.125–125\) & \(< 0.125–125\) & \(< 0.125–125\) & 0.25 & 0.25 \\
(37/19) & fluconazole & 0.125–64 & 8 & 64 & 2–64 & 32 & 64 \\
& voriconazole & 0.031–4 & 0.25 & 1 & 0.062–1 & 0.5 & 1 \\
& amphotericin B & 0.031–1 & 0.25 & 1 & 0.125–1 & 1 & 1 \\
\hline
\textbf{Candida spp.} & fluconazole & 0.125–32 & 0.125 & 16 & 0.125–32 & 4 & 32 \\
(21/9) & fluconazole & 0.25–64 & 4 & 32 & 0.125–64 & 16 & 64 \\
& voriconazole & 0.031–4 & 0.125 & 0.25 & 0.031–0.41 & 0.25 & 1 \\
& amphotericin B & 0.031–1 & 0.5 & 1 & 0.125–1 & 0.5 & 1 \\
\hline
\textbf{All isolates} & flucytosine & \(< 0.125–64\) & \(< 0.125–64\) & \(< 0.125–64\) & \(< 0.125–64\) & 1 & 1 \\
(333/279) & fluconazole & 0.125–64 & 0.5 & 8 & \(< 0.125–64\) & \(< 0.125–64\) & 4 \\
& voriconazole & 0.031–8 & 0.031 & 0.5 & 0.031–4 & 0.031 & 0.25 \\
\hline
\end{tabular}
\caption{Antifungal susceptibilities of 612 \textit{Candida} isolates from tertiary and non-tertiary hospitals, as determined by the CLSI microdilution method after 48h of incubation.}
\end{table}
Discussion

We investigated Candida species distribution and in vitro antifungal susceptibility. Until now, the most prevalent Candida species in hospitals was reported to be C. albicans. In this study, the frequency of non-C. albicans Candida species was higher (53.4%) than that of C. albicans (46.6%) in tertiary hospitals of South Korea, indicating an increase in the frequency of non-C. albicans Candida species recovered from hospitals. The distribution of C. albicans and non-C. albicans Candida species in South Korea was similar to that reported for other countries [15,16]. In this study, most Candida isolates were susceptible to the antifungal agents tested. However, we identified a few Candida species that were resistant to 5-flucytosine, fluconazole, and voriconazole agents. The Candida species that showed intermediate or complete resistance to 5-flucytosine were C. albicans, C. tropicalis, C. krusei, and C. humicola. Fluconazole SDD and resistant isolates of C. tropicalis, C. glabrata, and C. krusei were detected in our study. C. glabrata and C. krusei are intrinsically resistant to fluconazole. Importantly, five (63%) out of the eight C. krusei isolates showed 5-flucytosine and fluconazole resistance. In contrast, C. krusei isolates showed low MIC values (1 µg/ml) to voriconazole. The MIC of C. glabrata isolates to fluconazole was higher than that of the other Candida species. The tendency of C. glabrata and C. krusei isolates to be resistant makes treatment of infections caused by these agents to be quite difficult.

In this study, antifungal susceptibility testing showed that the MIC values of fluconazole and voriconazole for isolates from tertiary hospitals were generally two times higher than those from non-tertiary hospitals. However, the MIC values of 5-flucytosine of isolates from non-tertiary hospitals were two times higher than those from tertiary hospitals. The two types of hospitals showed similar MIC values (1 µg/ml) for amphotericin B. It was previously presumed that there were no significant differences in the trends towards resistance found with Candida isolates between tertiary hospitals and non-tertiary hospitals. The results of this study suggest that fluconazole resistance among C. albicans, C. parapsilosis, and C. tropicalis is still extremely rare in South Korea. Although the frequency of fluconazole resistance in C. albicans is very low, a few fluconazole-resistant C. albicans isolates have been detected in other Asian countries, such as Japan, India, Thailand, and Taiwan [12,15–17]. Importantly, fluconazole-resistant C. albicans was not detected but voriconazole-resistant isolates were detected in tertiary hospitals. Although C. albicans is the most prevalent Candida species, it is unclear why fluconazole-resistant C. albicans isolates were not detected in South Korea. A few antifungal agents are available for the treatment of fungal infections, especially those due to Candida species. Therefore, rapid development of drug resistance in Candida seriously hinders the successful use of antifungals as therapeutics. There is little information regarding the prevalence of Candida drug resistance from national-level surveillance of non-tertiary hospitals in South Korea. To our knowledge, this is the first report of Candida antifungal susceptibility that includes non-tertiary hospitals in South Korea.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

12 Takakura S, Fujihara N, Saito T, et al. National surveillance of species distribution in blood isolates of Candida species in Japan and their susceptibility to six antifungal agents including...


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