Antifungal Susceptibilities of Cryptococcus neoformans

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Susceptibility profiles of medically important fungi in less-developed countries remain uncharacterized. We measured the MICs of amphotericin B, 5-flucytosine, fluconazole, itraconazole, and ketoconazole for Cryptococcus neoformans clinical isolates from Thailand, Malawi, and the United States and found no evidence of resistance or MIC profile differences among the countries.

Prompt identification of agents associated with emerging infectious diseases and documentation of resistance among these agents to available antimicrobial drugs depend on existing surveillance activities for emerging pathogens and antimicrobial resistance. Although the World Health Organization has undertaken initiatives (1) in these areas, surveillance of antimicrobial resistance in developing countries is lacking or has been generally ignored (2). Natural selective pressures exerted on microorganisms by routine, inappropriate, or excessive use of antimicrobial drugs are factors in the development of antimicrobial resistance. In tropical developing countries, unrestricted availability of antimicrobial drugs without prescriptions, suboptimal therapeutic regimens, blind empiric prescribing practices that are not epidemiologically directed, and lack of laboratory capacity or skilled personnel for susceptibility testing contribute to the spread of antimicrobial resistance (2). Although numerous studies have examined bacterial and mycobacterial resistance in the tropics, less is known about the susceptibility profiles of medically important fungi to antifungal agents (3–5). Given that only a few antimicrobial drugs may be available in developing countries because of limited resources or cost restrictions, the surveillance for resistance among common pathogens to available drug treatment is essential for appropriate patient care and improved patient outcome.

Cryptococcus neoformans, an opportunistic fungal pathogen that causes disease predominantly in immunocompromised patients, is a frequent cause of fatal mycotic infections among patients with AIDS (6). In sub-Saharan Africa, cryptococcal meningitis occurs in 30% of AIDS patients and is likely to remain a substantial cause of death in these patients unless highly active antiretroviral therapy becomes available (6–8). Until such a time, treatment with antifungal agents, including long-term, suppressive antifungal regimens, remains the only recourse.

The Study

We sought to determine if substantial differences in susceptibility profiles to common antifungal agents existed among clinical isolates of C. neoformans from three geographically diverse areas. Sixty-five clinical isolates of C. neoformans from Malawi, Thailand, and the United States were available for study. The 16 isolates from Malawi and 29 isolates from Thailand were recovered from the bloodstream of febrile, adult inpatients during previous bloodstream infection studies in these regions (9,10). The 20 isolates from the United States were recovered from the bloodstream, lung tissue, cerebrospinal fluid, and other sterile sites in routine clinical practice in the clinical microbiology laboratories of the Cleveland Clinic Foundation and Duke University Medical Center. The yeast isolates from all of the countries were shipped to Duke University Medical Center for testing and maintained in frozen stock vials at −70°C. Sixty-five yeast isolates were recovered from the frozen stock vials on potato dextrose agar and incubated at 30°C for 48 hours. The antifungal susceptibilities of the isolates were determined by using the Sensititre YeastOne system (Trek Diagnostic Systems Ltd., West Sussex, England), which includes amphotericin B, 5-flucytosine, fluconazole, itraconazole, and ketoconazole. All isolates were incubated for 72 hours, according to the manufacturer’s instructions. Inoculum assessments were performed on all trays and were within acceptable limits. The trays were visually inspected, and the MICs were determined according to the manufacturer’s guidelines. Interpretive guidelines and breakpoints for susceptibility testing of C. neoformans are not yet available from the National Committee for Clinical Laboratory Standards (NCCLS); therefore, only MIC comparisons were performed (11). For isolates from each country, we recorded the MIC at

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which 50% of the isolates were inhibited (MIC\(_{50}\)) and the
MIC at which 90% of the isolates were inhibited (MIC\(_{90}\))
and determined the MIC geometric mean for each thera-
petic agent. We compared the MIC geometric means for
the three countries with a one-way analysis of variance
(ANOVA) to determine if significant differences existed.
Additional comparisons between the MIC\(_{50}\) and MIC\(_{90}\)
were not undertaken, since these were within one dilution
of one another.

The \textit{C. neoformans} isolates from the United States,
Thailand, and Malawi demonstrated similar susceptibility
profiles to the common antifungal agents against which
they were tested (Table 1). The percentage of isolates
inhibited at each concentration of antifungal agent over
the full dilution series is summarized in Table 2. The isolates
from the three countries did not differ significantly in their
susceptibility to fluconazole (p = 0.198), itraconazole (p =
0.163), 5-flucytosine (p = 0.713), or ketoconazole (p =
0.531). The geometric mean of the MIC values for amphotericin
B in Thailand, the United States, and Malawi was
1.2 \(\mu\)g/mL, 1.4 \(\mu\)g/mL, and 1.6 \(\mu\)g/mL, respectively. These
mean values were significantly (p = 0.019) different.

\section*{Conclusions}

Resistance to antifungal drugs is rare among clinical
isolates of \textit{C. neoformans} but has been reported (4,12). The
use of antifungal agents, particularly in long-term suppressive
regimens, has raised concern about the development
of drug resistance in \textit{C. neoformans}. However, an extensive
survey of the susceptibility profiles of clinical isolates
of \textit{C. neoformans} at a university hospital during 1987 to
1994 helped to allay these fears by indicating no emergence
of resistance (13).

This study also demonstrates no evidence of resistance
among clinical isolates of \textit{C. neoformans} from Thailand,
Malawi, and the United States. For each country, the MIC\(_{50}\)
and MIC\(_{90}\) of isolates to commonly used antifungal agents
were within one dilution from each other. In addition, the
MIC ranges were similar. Statistical comparison of the MIC
geometric means confirmed that no significant differences
existed between the three regions for fluconazole, itracona-
zole, 5-flucytosine, or ketoconazole. The only statistically
significant differences were observed for amphotericin B
susceptibilities; however, this difference was believed to be
clinically irrelevant since the MIC geometric means for
amphotericin B were 1–1 \(\mu\)g/mL, or within one dilution.
Our documentation of the absence of resistance among \textit{C. neoformans}
isolates from the United States is consistent
with data published by the Centers for Disease Control and
Prevention, which showed in vitro resistance to antifungal
agents to be uncommon and unchanged among \textit{C. neoformans}

The similarity between the MICs of \textit{C. neoformans} isolates
from Malawi and the United States concurs with data
from a previous study of 164 African and 402 North
American clinical isolates of \textit{C. neoformans} isolates that
were tested and found to be susceptible to fluconazole and
other triazoles, with over 99% inhibited by concentrations
of fluconazole \(\leq 32\) \(\mu\)g/mL (5). The MIC\(_{50}\) and MIC\(_{90}\) in
that study were lower than those in this study, although the
YeastOne trays have been found to agree well with the
NCCLS reference method for itraconazole and the other
azoles (15). Also, the MICs of fluconazole documented in
our study are similar to those previously reported for isolates
of \textit{C. neoformans} from the United Kingdom and
Uganda (3,4); the MICs of 5-flucytosine in our study also
were similar to those previously reported for \textit{C. neoformans}
isolates from Uganda (4). The itraconazole MICs documented in
our study were lower than those reported for isolates from the United Kingdom, Africa, and the
United States (4,5). The differences between the susceptibility profiles of \textit{C. neoformans} to itraconazole reported in

\begin{table}[h]
\centering
\caption{Cryptococcus neoformans susceptibility results}
\begin{tabular}{|c|c|c|c|c|}
\hline
Antifungal agent & MIC range (\(\mu\)g/mL) & MIC\(_{50}\) (\(\mu\)g/mL) & MIC\(_{90}\) (\(\mu\)g/mL) & MIC geometric mean (\(\mu\)g/mL) \\
\hline
\textbf{U.S. isolates (N = 20)}
\hline
Amphotericin B & 1–2 & 1 & 2 & 1.4 \\
Flucytosine & 1–16 & 8 & 8 & 5.1 \\
Itraconazole & 0.016–0.125 & 0.06 & 0.125 & 0.06 \\
5-Flucytosine & 2–8 & 4 & 8 & 5.1 \\
Ketoconazole & \textless 0.008–0.250 & 0.06 & 0.06 & 0.05 \\
\hline
\textbf{Thailand isolates (N = 29)}
\hline
Amphotericin B & 0.5–2 & 1 & 2 & 1.2 \\
Flucytosine & 4–160 & 8 & 16 & 7.7 \\
Itraconazole & 0.030–0.125 & 0.06 & 0.06 & 0.06 \\
5-Flucytosine & 2–8 & 4 & 8 & 4.6 \\
Ketoconazole & 0.030–0.250 & 0.06 & 0.125 & 0.07 \\
\hline
\textbf{Malawi isolates (N = 16)}
\hline
Amphotericin B & 1–2 & 2 & 2 & 1.6 \\
Flucytosine & 4–32 & 8 & 16 & 7.6 \\
Itraconazole & 0.030–0.125 & 0.03 & 0.125 & 0.05 \\
5-Flucytosine & 1–16 & 4 & 8 & 4.5 \\
Ketoconazole & 0.016–0.250 & 0.03 & 0.25 & 0.03 \\
\hline
\end{tabular}
\end{table}
our study and those reported previously may be due in part to the poor solubility of this antimicrobial agent in an aqueous solution.

Using a standardized testing method, we found no significant or clinically meaningful differences between the antifungal susceptibility profiles of clinical isolates of *C. neoformans* from the United States, Thailand, and Malawi. Although rare strains of *C. neoformans* with elevated MICs to some antifungal agents may exist, they were not detected in this sampling of clinically significant *C. neoformans* isolates and, therefore, do not appear to be prominent in Cleveland, Ohio; Durham, North Carolina; Bangkok, Thailand; or Lilongwe, Malawi.

**Acknowledgment**

We thank Trek Diagnostic Systems Ltd., West Sussex, England, for providing the Sensititre YeastOne trays that made this study possible.

Dr. Archibald is the medical director of Regeneration Technologies, Inc., a biotechnology company in Florida. Previously, he was the acting medical director of the Epidemic Information Exchange at the Centers for Disease Control and Prevention (CDC) and a medical epidemiologist in the National Center for Infectious Diseases, CDC. His research interests include the study of bloodstream infections in less-developed countries and healthcare-associated infections.

**Table 2. Percentage of Cryptococcus neoformans isolates susceptible at each MIC dilution**

<table>
<thead>
<tr>
<th>MICs (µg/mL)</th>
<th>% Susceptible</th>
<th>U.S. isolates</th>
<th>Thailand isolates</th>
<th>Malawi isolates</th>
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</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td></td>
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<tr>
<td>0.5</td>
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<td>100</td>
<td>100</td>
<td></td>
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<tr>
<td>1</td>
<td>90</td>
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<tr>
<td>Fluconazole</td>
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<tr>
<td>0.016</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>0.030</td>
<td>75</td>
<td>95</td>
<td>87</td>
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<td>100</td>
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<td>0.125</td>
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<tr>
<td>5-Flucytosine</td>
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**References**


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