Real-time antifungal susceptibility screening aids management of invasive yeast infections in immunocompromised patients

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We examined the utility of a semi-solid agar antifungal susceptibility screening (SAAS) test in real-time management of eight immunocompromised patients with invasive yeast infections. Tests of amphotericin B and fluconazole concentrations of 0.5 and 2 mg/L and 1, 8 and 40 mg/L, respectively, were performed on Candida albicans (two), Candida tropicalis (two), Candida krusei (one), Candida glabrata (one) and Trichosporon species (spp.) (two). All but the Trichosporon spp. and C. glabrata isolates were resistant to fluconazole at ≥40 mg/L, and patients were successfully managed accordingly. Real-time antifungal susceptibility screening can assist in clinical management of invasive yeast infections.

Introduction

A rise in invasive fungal infections (IFI) caused by less common Candida spp. associated with significant morbidity and mortality in immunocompromised and critically ill hosts is well documented over the last decade.1–4 Early appropriate therapy may alter the course of these infections. Treatment options, however, remain limited to a few licensed agents that are associated with toxicities, drug interactions or drug resistance. Accordingly, early knowledge of an organism’s drug susceptibility may optimize treatment and permit use of less toxic agents.

We have developed a semi-solid agar antifungal susceptibility screening (SAAS) test that has performed well for laboratory yeast strains.5 The aim of this study was to determine whether preliminary screening of a yeast isolate from a clinical specimen using this test, sometimes even before species identification, could assist in real-time management of IFIs.

Materials and methods

Patients

Patients were hospitalized at New England Medical Center over a 2 month period and diagnosed with IFI by blood cultures taken for evaluation of fever (seven patients), or by vitreal aspiration for evaluation of acute vision loss (one patient).

Definitions

Definitions used were: renal failure = estimated creatinine clearance <50 mL/min; neutropenia = absolute neutrophil count <500 cells/mm³ within 1 month of IFI; successful clinical outcome = resolution of symptoms (fever, eye pain and loss of visual acuity) on therapy; successful microbiological outcome = clearance of fungaemia on therapy without evidence of microbiological relapse.

Microbiology

The yeasts were subcultured onto corn meal and molybdate agar for rapid identification of Candida albicans by morphology and colour, respectively. Simultaneous subculture of the isolate onto a Sabouraud’s dextrose agar plate was performed for SAAS testing. If necessary, final identification was performed with the Vitek and API 20C methods (bioMérieux, Marcy l’Étoile, France) in the clinical microbiology laboratory.

Antifungal susceptibility screening

The semi-solid heart infusion medium was prepared as described previously.5 In brief, heart infusion broth (Difco Laboratories, Detroit, MI, USA) was mixed with agar (0.5%) (Bacto Agar; Difco Laboratories) and pharmacy stock amphotericin B and fluconazole in concentrations reflecting achievable serum levels and/or the MIC of the
drug. Concentrations of amphotericin B tested were 0.5 and 2 mg/L, and of fluconazole were 1, 8 and 40 mg/L. The drug mixtures and drug-free controls were stored in small lots in sterile, capped tubes. A just-turbid suspension (c. 0.5 McFarland standard) of bits of three to five single colonies from an overnight culture on Sabouraud’s dextrose agar was prepared and a standard loopful was inoculated vertically into the semi-solid agar media containing drugs and drug-free control. The tubes were incubated at 35°C for 48 h. Susceptibility was visually assessed by complete inhibition of growth in amphotericin B-containing tubes and at least 75% reduction in growth in fluconazole tubes when compared with controls. Candida parapsilosis American Type Culture Collection (ATCC) 22019 was used as a quality control (QC) for each test by determining MICs within 1 log₂ dilution of the NCCLS proposed QC range. No test results fell outside 1 log₂ of the proposed range (1–4 mg/L).

The yeast isolates were each tested singly by the SAAS method after isolation in the clinical microbiology laboratory, usually within 8 days of isolation. Later, comparison of MICs obtained by two-fold dilutions of amphotericin B (0.03–16 mg/L) and fluconazole (0.125–64 mg/L, including 40 mg/L as in the SAAS test) was made between the NCCLS M27-A broth microdilution and SAAS methods.

Antifungal treatment
All patients were treated in accordance with recommendations made by the infectious disease service and the primary team. When results of the SAAS test showed resistance to a drug, antifungal treatment was altered toward the active agent in accordance with the patient’s clinical course.

Results
The clinical characteristics of the eight immunocompromised patients with IFI are presented in Table 1. The mean age was 61 years. Three patients had renal failure at the time of IFI diagnosis. All seven patients with haematological malignancies had an indwelling central venous catheter (CVC), and six of these were neutropenic prior to IFI (range of neutropenia duration 4–15 days). Four patients received systemic antifungal therapy prior to the development of IFI (three amphotericin B, one fluconazole). Infecting organisms included: C. albicans (two); Candida tropicalis (two), Candida glabrata (one), Candida krusei (one) and Trichosporon spp. (two).

The antifungal susceptibility screening results, effects on systemic antifungal treatment and outcomes are shown in Table 1. The Candida isolates were either resistant (five isolates) or dose-dependently susceptible (one isolate) to fluconazole by the SAAS test. All organisms were susceptible to amphotericin B by the SAAS test. All CVCs were removed. Lipid formulation amphotericin B was initiated in all patients owing to receipt of concomitant nephrotoxic agents and/or baseline renal failure, and was continued in all patients when SAAS testing predicted fluconazole resistance, despite renal failure in three patients. As expected, the Trichosporon spp. isolates were susceptible to fluconazole by the SAAS test, and therapy for the two patients infected with this organism was changed to fluconazole, resulting in successful clinical and microbiological outcomes. Thus, therapy was altered for three of eight immunocompromised patients based on timely results of the SAAS test and the patient’s clinical status. All patients survived for 30 days after diagnosis of the IFI episode, with successful clinical and microbiological response. Two patients died 39 and 47 days after IFI without evidence of recurrent infection, although no autopsies were performed.

Antifungal susceptibility results comparing the NCCLS broth microdilution and the SAAS methods are presented in Table 2. The SAAS test results compared favourably to those of the NCCLS reference method. In all instances when the SAAS test predicted fluconazole resistance (MIC > 40 mg/L), the NCCLS MIC result was >64 mg/L. Fluconazole MICs for the C. glabrata isolate were 32 and 40 mg/L by the NCCLS and SAAS test methods, respectively.

Discussion
To our knowledge, this is the first study describing the real-time antifungal treatment management of patients with IFIs aided by results of an antifungal screening test. Other screening tests have shown promise in accurately predicting fluconazole resistance in the laboratory, but clinical correlation data are lacking. The accuracy of a new method of susceptibility testing may be assessed by comparing results with a standardized reference method. In this study, the MIC results achieved by the SAAS test compared favourably to those obtained by the NCCLS reference method. The high number of Candida spp. (five of six) resistant to fluconazole by both methods contrasts with recent reports of decreasing fluconazole resistance in blood isolates. Our results cannot be explained by prior fluconazole exposure in these patients and may reflect either the fact that most of the infections were non-albicans yeasts or the possibility of prior colonization with resistant organisms before the invasive illness. Nevertheless, all fluconazole-resistant organisms were accurately predicted by the screening test and later confirmed by the full SAAS and NCCLS MIC testing.

Ideally, susceptibility test results should correlate with clinical success of treatment with the active antifungal agent. Most experts agree that MICs achieved by the reference or other methods that predict antifungal resistance correlate best with clinical outcomes. To prove that susceptibility findings successfully influence patient manage-
<table>
<thead>
<tr>
<th>Pt</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Renal failure</th>
<th>CVC</th>
<th>Duration neutropenia (days)*</th>
<th>Prior systemic antifungals</th>
<th>Infecting isolate</th>
<th>SAAS results</th>
<th>Initial antifungal</th>
<th>Change in drug</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>s/p OLT</td>
<td>yes</td>
<td>no</td>
<td>–</td>
<td>no</td>
<td><em>C. albicans</em></td>
<td>S</td>
<td>R</td>
<td>AmB</td>
<td>no, despite RF</td>
</tr>
<tr>
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<td>69</td>
<td>AML</td>
<td>no</td>
<td>yes</td>
<td>10</td>
<td>no</td>
<td><em>C. tropicalis</em></td>
<td>S</td>
<td>R</td>
<td>AmB</td>
<td>survived; restored vision; required haemodialysis</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>CML, s/p re-BMT</td>
<td>yes</td>
<td>yes</td>
<td>6</td>
<td>yes</td>
<td><em>C. tropicalis</em></td>
<td>S</td>
<td>R</td>
<td>AmB, then FL</td>
<td>survived</td>
</tr>
<tr>
<td>4</td>
<td>78</td>
<td>AML</td>
<td>no</td>
<td>yes</td>
<td>0</td>
<td>no</td>
<td><em>Trichosporon sp.</em></td>
<td>S*</td>
<td>S</td>
<td>AmB</td>
<td>yes to FL</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>AML</td>
<td>no</td>
<td>yes</td>
<td>15</td>
<td>yes</td>
<td><em>C. krusei</em></td>
<td>S*</td>
<td>S</td>
<td>AmB</td>
<td>no dissemination to skin; survived</td>
</tr>
<tr>
<td>6</td>
<td>78</td>
<td>AML</td>
<td>no</td>
<td>yes</td>
<td>4</td>
<td>no</td>
<td><em>Trichosporon sp.</em></td>
<td>S*</td>
<td>S</td>
<td>AmB</td>
<td>yes to FL</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
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<td>yes</td>
<td>yes</td>
<td>13</td>
<td>yes</td>
<td><em>C. glabrata</em></td>
<td>S</td>
<td>S</td>
<td>AmB</td>
<td>no, expired 47 days after infection of unrelated cause</td>
</tr>
<tr>
<td>8</td>
<td>47</td>
<td>AML s/p BMT</td>
<td>yes</td>
<td>yes</td>
<td>6</td>
<td>yes</td>
<td><em>C. albicans</em></td>
<td>S</td>
<td>R</td>
<td>AmB</td>
<td>expired 39 days after infection of unrelated causes</td>
</tr>
</tbody>
</table>

CVC, central venous catheter; OLT, orthotopic liver transplantation; AML, acute myelogenous leukaemia; CML, chronic myelogenous leukaemia; BMT, bone marrow transplantation; s/p, status post; AB, amphotericin B; FL, fluconazole; S, sensitive; S*, sensitive to amphotericin B at 2 mg/L; SDD, sensitive to fluconazole >8 and <40 mg/L; R, resistant to fluconazole at 40 mg/L; AmB, AmBisome; RF, renal failure.

*Days preceding IFI.
ment would require a much larger study of clinical failures successfully treated by guidance of screening susceptibility test results. In this small study of mostly Candida non-albicans infections in severely immunocompromised hosts, only one patient demonstrated clinical failure that was predicted by the screening test results. However, in the two patients with C. albicans infections, fluconazole would have been an optimal choice to avoid toxicities and increased costs, yet resistance was documented by both the screening test and NCCLS results. Early susceptibility screening may have contributed to their successful clinical and microbiological outcomes.

Our study is limited by the small number of patients managed in accordance with their clinical course and SAAS test results. Thus, generalization of the utility of the SAAS test to a larger population of patients with IFIs should be carried out with caution. Our patients’ response to treatment may have simply reflected our use of amphotericin as the primary treatment. Additional limitations include that, while easy to perform, and reproducible in our hands, the SAAS test has not been validated in a multi-laboratory study and is subject to the inherent problems of subjective reading of results. Its use of a chemically undefined media and pharmacy stock antifungal agents, while rendering the procedure amenable to a busy clinical microbiology laboratory, precludes this test substituting for formal MIC determinations by the reference standard.

The simplicity of the SAAS test, the reproducibility of results demonstrated in laboratory yeast strains and the correlation with NCCLS MIC results shown here are exciting qualities of the test. For the eight patients studied here, we believe the test provided important early information that aided in their successful management. Further studies to assess the SAAS test’s accuracy with currently available and new antifungal agents and its reproducibility in multiple laboratories are warranted.

Acknowledgements

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References


Table 2. Antifungal susceptibilities of eight clinical isolates by NCCLS reference broth microdilution and SAAS methods

<table>
<thead>
<tr>
<th>Patient</th>
<th>Organism</th>
<th>NCCLS fluconazole</th>
<th>SAAS fluconazole</th>
<th>NCCLS amphotericin B</th>
<th>SAAS amphotericin B</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>C. albicans</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>C. tropicalis</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>C. tropicalis</td>
<td>&gt;64</td>
<td>64</td>
<td>0.5</td>
<td>1</td>
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<tr>
<td>4</td>
<td>Trichosporon sp.</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>C. krusei</td>
<td>64</td>
<td>&gt;64</td>
<td>0.5</td>
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</tr>
<tr>
<td>6</td>
<td>Trichosporon sp.</td>
<td>1</td>
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<td>2</td>
<td>0.125</td>
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<tr>
<td>7</td>
<td>C. glabrata</td>
<td>32</td>
<td>40</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>C. albicans</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>1</td>
<td>0.25</td>
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Antifungal susceptibility screening in real time


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