Azole Resistance of Candida glabrata in a Case of Recurrent Fungemia

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We describe a case of recurrent Candida glabrata fungemia that became unresponsive to fluconazole treatment. Posttreatment isolates from blood and vaginal cultures of the immunocompetent patient were azole resistant and exhibited upregulated expression of CgCDR1/CgCDR2 efflux pumps compared to the original isolates. Amphotericin B therapy eradicated the infection.

CASE REPORT

In July 2002, a 35-year-old woman at 5 weeks’ gestation following intrauterine insemination was referred to our University Medical Center. She presented with fever (39°C), tachycardia (116 beats/min), and hypotension (90/60 mm Hg). The white blood cell count was 23.5 x 109/liter with 78% neutrophils, and the C-reactive protein level was elevated (40 mg/liter; normal range, <3 mg/liter). Liver and renal function tests were normal. She experienced spontaneous abortion. Severe chorioamnionitis was clinically diagnosed, placental and fetal tissues were cultured, and serial blood cultures and vaginal swabs were obtained. The patient was treated with a combination of ciprofloxacin and imipenem. Five days later, no clinical improvement was seen. The cultured blood and placental samples grew the yeast Candida glabrata, which was also isolated from the patient’s vaginal cultures. Because the C. glabrata isolates showed fluconazole susceptibility, the patient began to receive fluconazole at a dosage of 400 mg/day. Four weeks later, she experienced complete resolution of symptoms with eradication of the fungus from the bloodstream. Therefore, antifungal treatment was discontinued and she returned home. Clinically, the patient remained well, except for vaginal candidiasis and sporadic episodes of low-grade fever (<38°C) of 2 days’ duration. For each vaginitis episode (a total of four), she was given oral therapy with fluconazole (1-week courses of 100 mg/day), as prescribed by a family practitioner. Six months later, the patient was readmitted to the hospital with a new severe fever attack with temperatures of up to 40°C. She complained of chills and fatigue. Physical examination revealed diffuse attenuation of heart sounds, moderate hepatosplenomegaly, and white plaques on the vaginal mucosa. The white blood cell count had risen to 21.5 x 109/liter with 73% neutrophils. No abnormalities in liver or renal function were detected, and the chest X-ray was normal. An echocardiogram revealed no valvular lesions. Treatment with ceftriaxone (2 g/day) and fluconazole (400 mg/day) was initiated. Consecutive cultures of blood samples were again positive for C. glabrata, which was also found in cultures of vaginal exudates. All isolates were found to be resistant to fluconazole. On the basis of these microbiological findings, the patient was treated with 1- mg/kg intravenous amphotericin B deoxycholate daily. Within 1 week of the initiation of antifungal treatment, the patient’s clinical condition improved and her renal function remained normal. After 1 month of amphotericin B treatment, blood specimens were sterile and the patient was discharged from the hospital. Three years later, she remained free of any evidence of infection.

In vitro testing of C. glabrata isolates. Isolates from serial blood and vaginal cultures obtained from the patient at the onset of disease (T0 isolates) and at the subsequent episode of fungemia (T1 isolates) were identified by standard procedures (14) and compared for antifungal susceptibility (8, 10), genotyping (5, 13), and expression of the ABC transporter pump genes CgCDR1 and CgCDR2 (13). All isolates had the same genotype, as demonstrated by their Cg6-Cg12 DNA fingerprints and multilocus sequence typing (MLST) profiles obtained with an MLST system recently developed for C. glabrata (5) (data not shown). MLST profiles showed that isolates had the allelic profile 10-5-12-11-3-7 for the analyzed loci (FKS, LEU2, NMT1, TRP1, UGP1, and URA3). T0 isolates had developed resistance to fluconazole, itraconazole, and voriconazole and also exhibited increased MICs of ketoconazole (Table 1); no changes in amphotericin B susceptibility were noted. When the levels of CgCDR1 and CgCDR2 transcripts in T0 and T1 isolates were normalized to the amounts of URA3 transcript, theazole-resistant isolates (T1) had higher levels of CgCDR1 and CgCDR2 expression than the susceptible isolates (T0) (Table 1). This suggests that increased drug efflux activity was an important component of the observed resistance.

Discussion. In C. glabrata, upregulation of the ABC transporter genes CgCDR1 and CgCDR2 is primarily responsible for acquiredazole resistance that remains stable over time (7, 12). Increased expression of CgCDR1 and CgCDR2 upon fluconazole exposure has been observed in resistant C. glabrata clinical isolates (2, 11, 13) and in vitro (3). Treatment failures due to C. glabrata resistance to azoles have previously been described and occur mainly in AIDS patients with oropharyngeal candidiasis (2, 4, 6, 11). However, in only a few of
these cases was genotyping performed to determine whether resistance was acquired and selected for in vivo. To assess this, both the original azole-susceptible isolates and the subsequent azole-resistant isolates must be genotyped to determine whether all isolates from the same patient were of the same strain (2, 11).

Here we report a case of C. glabrata fungemia in which azole resistance appeared after fluconazole treatment. The patient’s initial, naive isolates showed minimal expression of the CgCDR1 and CgCDR2 genes, which are closely associated with acquired azole resistance in C. glabrata. However, subsequent isolates showed overexpression of both genes, suggesting acquisition of resistance, as was observed by an increase in fluconazole MIC from 4 μg/ml to 256 μg/ml. Not surprisingly, the fluconazole-induced upregulation of drug efflux pumps allowed later isolates to also become cross-resistant to itraconazole and voriconazole.

On the basis of the MICs for the initial isolates, the patient was promptly treated with fluconazole at 400 mg/day, a dosage well suited for the treatment of a generally susceptible organism such as Candida albicans. However, the infection recurred 6 months later, necessitating treatment with amphotericin B. In an immunocompetent individual such as our patient, it is difficult to establish whether vaginal colonization might have served as a portal of entry for the development of systemic candidiasis. It is also possible that if our patient harbored C. glabrata in the vagina, a uterine infection by the yeast might have accidentally occurred during the intrauterine insemination procedure, allowing the infected uterus to serve as a reservoir of C. glabrata for the onset and relapse of fungemia. In an incident similar to the scenario above, intra-amiotic C. tropicalis candidiasis complicated by severe maternal septicemia was described in a woman with a retained intrauterine contraceptive device, indicating that hematogenous spread of the infecting yeast can occur (1).

In conclusion, although fungemia due to C. glabrata has been treated successfully with fluconazole (at a dosage of 400 mg/day) (15), our results support the current opinion that amphotericin B is a good choice for the treatment of candidemia, especially when the species and/or antifungal susceptibility of the infecting isolate is known (9). According to the Infectious Diseases Society of America’s treatment guidelines for candidiasis, higher-dosage fluconazole (≥400 mg/day) is currently recommended as an option only for clinically stable patients who have not recently received azole therapy (9).

### TABLE 1. Expression levels of the CgCDR1 and CgCDR2 genes and azole susceptibilities for the C. glabrata isolates studied

<table>
<thead>
<tr>
<th>Isolate source (time of collection) or control strain</th>
<th>Gene expressiona</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CgCDR1</td>
<td>CgCDR2</td>
</tr>
<tr>
<td>Blood (T0)</td>
<td>0.54</td>
<td>1.21</td>
</tr>
<tr>
<td>Vagina (T0)</td>
<td>0.67</td>
<td>1.34</td>
</tr>
<tr>
<td>Blood (T1)</td>
<td>473.32</td>
<td>75.21</td>
</tr>
<tr>
<td>Vagina (T1)</td>
<td>471.23</td>
<td>76.98</td>
</tr>
<tr>
<td>ATCC 36909</td>
<td>0.69</td>
<td>1.05</td>
</tr>
</tbody>
</table>

a Times of collection are defined in the text. Reference strain C. glabrata ATCC 36909 was used as a control.

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### REFERENCES