

## Azole Resistance of *Candida glabrata* in a Case of Recurrent Fungemia

Brunella Posteraro,<sup>1</sup> Mario Tumbarello,<sup>2</sup> Marilena La Sorda,<sup>1</sup> Teresa Spanu,<sup>1</sup>  
Enrico Maria Trearichi,<sup>2</sup> Flavia De Bernardis,<sup>3</sup> Giancarlo Scoppettuolo,<sup>2</sup>  
Maurizio Sanguinetti,<sup>1\*</sup> and Giovanni Fadda<sup>1</sup>

Departments of Microbiology<sup>1</sup> and Infectious Diseases,<sup>2</sup> Università Cattolica del Sacro Cuore, and Department of Infectious,  
Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità,<sup>3</sup> Rome, Italy

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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  $23.5 \times 10^9$ /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  $21.5 \times 10^9$ /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 ( $T_0$  isolates) and at the subsequent episode of fungemia ( $T_1$  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*).  $T_1$  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  $T_0$  and  $T_1$  isolates were normalized to the amounts of *URA3* transcript, the azole-resistant isolates ( $T_1$ ) had higher levels of *CgCDR1* and *CgCDR2* expression than the susceptible isolates ( $T_0$ ) (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 acquired azole 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

\* Corresponding author. Mailing address: Department of Microbiology, Università Cattolica del Sacro Cuore, Largo F. Vito 1, Rome 00168, Italy. Phone: 39 6 30154964. Fax: 39 6 3051152. E-mail: msanguinetti@rm.unicatt.it.

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

Isolate source (time of collection) or control strain <sup>a</sup>	Gene expression <sup>b</sup>		MIC ( $\mu\text{g/ml}$ )			
	<i>CgCDR1</i>	<i>CgCDR2</i>	Fluconazole	Ketoconazole	Itraconazole	Voriconazole
Blood (T <sub>0</sub> )	0.54	1.21	4	0.25	0.25	0.125
Vagina (T <sub>0</sub> )	0.67	1.34	4	0.125	0.125	0.125
Blood (T <sub>1</sub> )	473.32	75.21	256	>16	4	8
Vagina (T <sub>1</sub> )	471.23	76.98	256	>16	8	8
ATCC 36909	0.69	1.05	2	0.125	0.125	0.06

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

<sup>b</sup> Quantification was performed by real-time reverse transcription-PCR. Values are averages of four independent experiments and represent increases in gene expression relative to azole-susceptible *C. glabrata* strain DSY562 (which was arbitrarily set at a value 1.00) (13).

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  $\mu\text{g/ml}$  to 256  $\mu\text{g/ml}$ . 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-amniotic *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 ( $\geq 400$  mg/day) is currently recommended as an option only for clinically stable patients who have not recently received azole therapy (9).

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