Changes in Karyotype and Azole Susceptibility of Sequential Bloodstream Isolates from Patients with *Candida glabrata* Candidemia[▽]

Jong Hee Shin,^{1*} Myung Jong Chae,¹ Jeong Won Song,¹ Sook-In Jung,² Duck Cho,¹ Seung Jung Kee,¹ Soo Hyun Kim,¹ Myung Geun Shin,¹ Soon Pal Suh,¹ and Dong Wook Ryang¹

Departments of Laboratory Medicine¹ and Internal Medicine,² Chonnam National University Medical School, Gwangju, South Korea

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We examined the changes in genotypes and azole susceptibilities among sequential bloodstream isolates of *Candida glabrata* during the course of fungemia and the relationship of these changes to antifungal therapy. Forty-one isolates were obtained from 15 patients (9 patients who received antifungal therapy and 6 patients who did not) over periods of up to 36 days. The isolates were analyzed using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) and tested for antifungal susceptibility to fluconazole, itraconazole, and voriconazole. PFGE typing consisted of electrophoretic karyotyping and restriction endonuclease analysis of genomic DNA by use of NotI (REAG-N). The 41 isolates yielded 23 different karyotypes and 11 different REAG-N patterns but only 3 MLST types. The sequential strains from each patient had identical or similar REAG-N patterns. However, they had two or three different karyotypes in 6 (40%) of 15 patients. The isolates from these six patients exhibited the same or similar azole susceptibilities, and five patients did not receive antifungal therapy. Development of acquired azole resistance in sequential isolates was detected for only one patient. For this patient, an isolate of the same genotype obtained after azole therapy showed three- or fourfold increases in the MICs of all three azole antifungals and exhibited increased expression of the *CgCDR1* efflux pump. This study shows that karyotypic changes can develop rapidly among sequential bloodstream strains of *C. glabrata* from the same patient without antifungal therapy. In addition, we confirmed that *C. glabrata* could acquire azole resistance during the course of fungemia in association with azole therapy.

Candida glabrata has recently emerged as one of the most common causes of *Candida* bloodstream infections (BSIs) worldwide (18, 19). This species is innately less susceptible to azoles than most other *Candida* species and mutates readily in vitro or in vivo and acquires secondary azole resistance rapidly following short-term exposure to triazole-derived agents (4, 5, 21–23). While acquired azole resistance is reported to be uncommon in most *Candida* species recovered from blood cultures, the development of azole resistance was recently documented in sequential BSI isolates of *C. glabrata* (15, 23). The reason for the rapid development of secondary antifungal resistance in *C. glabrata* is unknown, but its haploid nature is thought to be a contributing factor (10). Generally, the induction of stable azole resistance in *Candida albicans* isolates occurs gradually, which presumably reflects its diploid genome (8).

An assessment of the genotype patterns and antifungal susceptibilities of sequential *Candida* isolates from the same patient has been used to investigate whether the development of antifungal resistance was due to the acquisition of a new isolate or a change in an existing isolate (2, 4, 5, 21–23). Although sequential clinical isolates of *C. glabrata* from the same patient usually yield the same genotype (2, 12), *C. glabrata* may undergo rapid genotypic change during human infection due to

its haploid nature, unlike diploid *Candida* species. To date, however, research is lacking on genotypic variation and its relationship to antifungal therapy among clonal isolates of *C. glabrata* obtained sequentially from blood cultures from the same patient.

This study assessed the genotype change and acquisition of antifungal resistance in clonal isolates of *C. glabrata* from the same patient during the course of fungemia and their relationship with antifungal therapy. Forty-one sequential BSI isolates of *C. glabrata* obtained from 15 patients (6 patients who received antifungal therapy and 9 patients who did not receive antifungal therapy) who were admitted to the same hospital over a 7-year period were analyzed. Genotyping was performed using multilocus sequence typing (MLST), electrophoretic karyotyping (EK), and restriction endonuclease analysis of genomic DNA using NotI (REAG-N) followed by pulsed-field gel electrophoresis (PFGE). In addition, we compared the expressions of *CgCDR1* and *CgCDR2* among the sequential BSI isolates of *C. glabrata*.

MATERIALS AND METHODS

Microorganisms. Forty-one blood isolates were obtained from 15 patients, each of whom had one or more blood cultures positive for *C. glabrata* on two or more separate days. The patients were admitted to the Chonnam University Hospital between 1997 and 2003. The charts of the 15 patients with *C. glabrata* fungemia were reviewed retrospectively. Patient data (age, sex, and admission diagnoses), the number of positive blood cultures, the presence of central venous catheter (CVC)-related candidemia, the dates and dosages of antifungal drug administration, and outcomes of the fungemia were recorded (24). *Candida glabrata* was identified through assimilation tests by using the API 20C and ATB

* Corresponding author. Mailing address: Department of Laboratory Medicine, Chonnam National University Medical School, 8 Hakdong Dongku, Gwangju 501-757, South Korea. Phone: 82 (62) 220-5342. Fax: 82 (62) 224-2518. E-mail: shinjh@chonnam.ac.kr.

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TABLE 1. Clinical summary for 15 patients with *C. glabrata* fungemia in this study

Patient no.	Age (yr)/sex	Admission diagnosis	No. of positive cultures (days of culture) ^a	Antifungal therapy (days of treatment) ^b	Outcome
1	44/F	Panperitonitis due to multiple leakage	2 (0, 28)	None	Improved
2	40/F	Panperitonitis due to duodenal rupture	3 (0, 1, 2)	None	Improved
3	85/F	Renal failure, <i>Escherichia coli</i> bacteremia	2 (0, 1)	None	Not improved
4	61/M	Liver cirrhosis, gastrointestinal bleeding	5 (0, 1, 4, 6, 7)	None	Not improved
5	3/F	Acute lymphocytic leukemia	2 (0, 1)	None	Not improved
6	51/F	Cerebral hemorrhage	2 (0, 1)	None	Not improved
7	33/F	Renal transplantation	3 (0, 2, 36)	Flu 100 mg (4–11), Itra 100 mg (12–15), Flu 300 mg (35), AmB 50 mg (45–54, 62)	Not improved
8	40/F	Peritoneal carcinomatosis	4 (0, 1, 8, 22)	Flu 400 mg (7–10), Flu 400 mg (24–38)	Improved
9	53/M	Panperitonitis due to ileal injury	3 (0, 1, 23)	AmB 50 mg (7–33), Flu 400 mg (24–25)	Improved
10	62/M	Squamous cell carcinoma of lung	4 (0, 4, 8)	Flu 400 mg (2–11)	Not improved
11	55/M	Diabetes, liver cirrhosis, gastrointestinal bleeding	2 (0, 1)	Flu 200 mg (–19 to 4) ^c	Not improved
12	71/M	Acute drug intoxication, pneumonia	3 (0, 4, 7)	AmB 50 mg (9–37)	Improved
13	69/M	Epidural abscess	2 (0, 1)	Flu 400 mg (3–10), AmB 100 mg (10–13)	Improved
14	46/F	Panperitonitis due to ileal perforation	3 (0, 12, 24)	AmB 50 mg (16–49)	Improved
15	16/F	Renal transplantation	2 (0, 11)	Flu 100 mg (8–21)	Improved

^a The numbers in parentheses indicate the days on which positive blood cultures were made; for each patient, day 0 represents the day that the first positive blood culture was made, and the day(s) on which subsequent positive cultures were made are represented by the subsequent numbers.

^b The numbers in parentheses indicate the day(s) after positive blood culture that the corresponding treatment was given. AmB, amphotericin B; Flu, fluconazole; Itra, itraconazole.

^c Fungemia occurred during antifungal therapy (for the treatment of *C. albicans* candidemia) in patient 11.

32C systems (BioMérieux, Marcy l'Etoile, France) and by assessing the isolates on CHROMagar *Candida* medium (Gemini BioProducts, Woodland, CA).

PFGE analysis. PFGE was performed using a procedure previously described (24, 25) for EK and REAG-N. For EK, isolates that differed by one or more bands were considered to have different karyotypes (24, 25). For REAG-N, strains with banding patterns with identical sizes and numbers of bands were assigned to the same type, strains with banding patterns that differed by three or fewer bands were considered closely related and described as subtypes (a or b) of a given clonal type, and strains with banding patterns that differed by four or more bands were considered different and assigned to separate types (25, 28). When serial isolates from the same patient show a minor genotypic change (subtype) in the REAG-N pattern, it represents the occurrence of microevolution (25). All isolates were analyzed at least twice (mean, three times; range, two to five times) by repeating the procedure.

MLST. Multilocus enzyme sequence typing was performed using a previously described procedure (9). *Candida* DNA was extracted by using a QIAGEN DNA tissue kit (QIAGEN, Crawley, United Kingdom) according to the manufacturer's instructions. The six genes selected for MLST were *FKS*, *LEU2*, *NMT1*, *TRP1*, *UGP1*, and *URA3*. All loci were sequenced in both the forward and reverse directions with primers the same as those used for the PCRs. Sequencing reactions were performed in a 20- μ l volume with 3 pmol of oligonucleotide primer, 25 ng of template, 4 μ l of BigDye Terminator cycle sequencing ready reaction mix (PE Applied Biosystems, Foster City, CA), and 2 μ l of 5 $\times$ sequencing buffer (80 mM Tris-Cl [pH 9.0], 2 mM MgCl₂). The reaction products were analyzed with an ABI Prism 377 DNA sequencer (PE Applied Biosystems). The allele profiles of the strains were defined using the six MLST loci. Each unique allele profile was designated as a sequence type (ST), which was determined by comparing the database at the MLST website (www.mlst.net).

Real-time RT-PCR. Total RNA extraction and real-time reverse transcription-PCR (RT-PCR) were performed using the modified method of Sanguinetti et al. (23). The expression of *CgCDR1* and *CgCDR2* was quantified using real-time RT-PCR with ROTOR Gene 3000 (Corbett Research, Sydney, Australia). Each set of primer pairs and fluorescent probes was used as previously reported (23). For the target genes and the *URA3* reference gene, a primer pair and a TaqMan probe, which hybridize to the region between the primer-specific sequences, were synthesized and labeled by Operon Biotechnologies (Huntsville, AL). Real-time PCR was performed with a Quantitect PCR probe kit (QIAGEN, Hilden, Germany) with a 25- μ l volume of PCR mixture containing 2 $\times$ Quantitect buffer, 0.25 μ M each primer pair, 0.1 μ M each probe, and 8 μ l of cDNA. After 15 min of denaturation at 95°C, 45 cycles were performed. Cycling conditions were 95°C for 15 s and 59°C for 60 s. The concentration of each gene was calculated with

reference to the respective standard curve by use of Corbett Research software and normalized as the ratio of the target (*CgCDR1* and *CgCDR2*) and house-keeping (*CgURA3*) gene concentrations from separate reactions. Each reaction was performed in triplicate. For all isolates, the relative gene expression was reported as the change (*n*-fold) determined from the mean normalized expression relative to the mean normalized expression of *C. glabrata* ATCC 90030.

Antifungal susceptibility testing. Fungal susceptibilities to amphotericin B, fluconazole, itraconazole, and voriconazole were tested using the standard methods of the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) as given in document M27-A2 (17). Two reference strains, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258, were tested as quality control isolates in each antifungal susceptibility test.

RESULTS

Clinical characteristics of the patients. The patients included six (patients 1 to 6) who did not receive antifungal therapy and nine (patients 7 to 15) given antifungal therapy (Table 1). Seven patients (patients 7 to 11, 14, and 15) had sequential isolates even after the initiation of antifungal therapy. The most common underlying conditions in the patients were gastrointestinal diseases (seven patients). Three patients (patients 9, 10, and 13) were diagnosed with CVC-related fungemia. Previous fluconazole use was noted for two patients (patients 5 and 11). Only one patient had neutropenia (patient 5). The fungemia resolved completely in two of the six patients who did not receive antifungal therapy and in six of the nine patients who received antifungal therapy.

Comparison of genotypes among isolates from different patients. Table 2 presents the sequences of the isolates, antifungal susceptibilities, genotyping results, and *CgCDR1* and *CgCDR2* gene expression for the isolates from each of the 15 patients. When analyzed using the PFGE methods, the 41 isolates from the 15 patients yielded 23 different karyotypes and 11 different REAG-N patterns. MLST identified only three distinct types. Isolates from nine patients, five patients,

TABLE 2. Antifungal susceptibilities, genotypes, and expression characteristics of *CgCDR1* and *CgCDR2* genes for sequential *C. glabrata* isolates from each of 15 patients

Patient no.	Isolate no.	Isolation day ^a	MIC (μg/ml) for ^b :				Genotype by:			Fold gene expression for ^c :	
			AmB	Flu	Itra	Vori	EK	REAG-N ^c	MLST ^d	<i>CgCDR1</i>	<i>CgCDR2</i>
1	1-1	0	1	16	1	0.25	K1	N1	ST7	2.3	0.4
	1-2	28	1	16	1	0.5	K2	N1	ST7	2.0	1.6
2	2-1	0	1	8	0.125	0.125	K3	N2a	ST3	4.0	0.8
	2-2	1	1	8	0.125	0.125	K4	N2a	ST3	2.9	1.9
	2-3	2	1	8	0.125	0.125	K5	N2a	ST3	3.2	1.2
3	3-1	0	1	8	0.25	0.06	K6	N3a	ST3	4.4	3.6
	3-2	1	1	8	0.125	0.03	K7	N3b	ST3	1.8	1.2
4	4-1	0	1	8	0.125	0.25	K8	N2b	ST3	5.1	4.2
	4-2	1	1	8	0.25	0.125	K8	N2c	ST3	4.3	2.4
	4-3	4	1	8	0.25	0.06	K9	N2d	ST3	7.3	6.8
	4-4	6	1	8	0.25	0.06	K10	N2d	ST3	5.7	4.0
	4-5	7	1	8	0.25	0.06	K8	N2d	ST3	4.4	4.1
5	5-1	0	0.5	32	1	0.5	K11	N4a	New type	3.5	0.6
	5-2	1	1	32	1	0.5	K11	N4b	New type	4.1	1.7
6	6-1	0	1	32	1	0.25	K12	N5a	ST7	7.8	4.3
	6-2	1	1	32	1	0.25	K13	N5b	ST7	12.4	6.7
7	7-1	0	0.5	16	1	0.25	K14	N6a	ST7	11.0	1.0
	7-2	2	0.5	16	1	0.25	K14	N6a	ST7	6.5	0.6
	7-3 ^f	36	0.5	128	4	2	K14	N6a	ST7	145.0	3.5
8	8-1	0	0.5	16	0.5	0.25	K15	N7a	ST7	5.6	2.8
	8-2	1	1	16	0.25	0.125	K15	N7a	ST7	3.3	2.6
	8-3 ^f	8	0.5	16	0.25	0.25	K15	N7b	ST7	3.6	3.2
	8-4 ^f	22	0.5	32	1	0.5	K15	N7c	ST7	19.9	4.1
9	9-1	0	1	32	1	0.5	K16	N8a	ST7	3.1	2.5
	9-2	1	1	32	2	0.5	K16	N8b	ST7	3.1	3.8
	9-3 ^f	23	1	32	2	0.5	K16	N8a	ST7	3.7	4.4
10	10-1	0	1	32	1	0.5	K16	N8a	ST7	2.6	3.3
	10-2 ^f	4	1	32	1	0.5	K16	N8a	ST7	3.1	4.4
	10-3 ^f	8	1	32	2	0.5	K16	N8b	ST7	3.1	1.6
11	11-1 ^f	0	0.25	16	0.5	0.25	K17	N9	ST3	2.2	3.2
	11-2 ^f	1	0.25	16	0.5	0.25	K17	N9	ST3	3.7	6.7
12	12-1	0	0.5	16	0.5	0.25	K18	N10a	ST7	1.1	2.0
	12-2	4	0.5	32	0.5	0.25	K18	N10a	ST7	0.4	2.0
	12-3	7	1	16	1	0.25	K18	N10b	ST7	0.3	0.9
13	13-1	0	0.5	16	1	0.25	K19	N1	ST7	2.3	3.8
	13-2	1	1	16	1	0.25	K19	N1	ST7	2.7	5.6
14	14-1	0	0.5	8	0.5	0.25	K20	N6b	ST7	3.7	1.0
	14-2	12	0.5	8	0.5	0.25	K21	N6b	ST7	5.1	3.5
	14-3 ^f	24	0.5	8	0.5	0.25	K22	N6b	ST7	5.9	1.4
15	15-1	0	1	16	0.5	0.06	K23	N11a	ST3	10.0	3.7
	15-2 ^f	11	1	16	0.5	0.06	K23	N11b	ST3	12.2	4.9

^a The numbers indicate the days after the first positive blood culture in each patient.^b AmB, amphotericin B; Flu, fluconazole; Itra, itraconazole; Vori, voriconazole.^c A minor change (subtypes, a, b, c, and d) in REAG-N patterns for serial isolates from the same patient suggests microevolution.^d STs were assigned by combining the data from each of the six loci (*FKS*, *LEU2*, *NMT1*, *TRP1*, *UGP1*, and *URA3*) sequenced per isolate, as described in reference 9. The allele profiles for the six loci were 578736 for ST3, 344334 for ST7, and 365239 for the new ST.^e Quantification was performed using real-time RT-PCR. The values are averages of three independent experiments and represent increases in the levels of gene expression relative to that of *C. glabrata* ATCC 90030 (set as 1.0).^f Isolate was recovered after the initiation of antifungal therapy.

FIG. 1. Representative genotyping patterns of *C. glabrata* obtained by EK and REAG-N. Sequential BSI isolates of *C. glabrata* were obtained from five patients who did not receive antifungal therapy (patients 1 to 5) and from four patients who received antifungal therapy (patients 7 to 10). Table 2 shows detailed information on each isolate. The sequential isolates from each of patients 1 to 4 showed two or three different karyotypes, which demonstrates that karyotype change can occur without antifungal therapy. The sequential isolates from each of patients 3 to 5 and 8 to 10 showed one- or two-band differences in the REAG-N patterns, suggesting that microevolution occurs frequently among bloodstream isolates. Stars indicate the positions of added or deleted bands for clonal strains from the same patient. Two DNA types were shared by isolates from two patients (patients 9 and 10), which suggests nosocomial transmission. A, *C. glabrata* ATCC 90030. M, *Saccharomyces cerevisiae* DNA concatemers as a molecular size marker.

and one patient belonged to ST7, to ST3, and to a new ST type, respectively. The allele profiles for the six loci (*FKS*, *LEU2*, *NMT1*, *TRP1*, *UGP1*, and *URA3*) were 578736 for ST3, 344334 for ST7, and 365239 for the new ST type. Isolates from two patients (patients 9 and 10) shared identical EK, MLST, and REAG-N types (Fig. 1). These two patients had been admitted to the same surgical intensive care unit; their hospital stays overlapped, and both patients were diagnosed with CVC-related fungemia.

Comparison of genotypes among sequential isolates from the same patient. The sequential strains from each patient had identical karyotypes in nine (60%) patients, while they had two or three different karyotypes in six (40%) patients (patients 1 to 4, 6, and 14). For the last six patients, the isolates from the same patient differed from each other by only one or two chromosome bands in the molecular size range of >1,600 kb in EK, although they were the same in the low-molecular-weight chromosome bands (<1,600 kb). By REAG-N typing, the sequential isolates from each patient had identical patterns for six patients, while they exhibited minor genetic differences (one or two bands) for nine (60%) patients, suggesting that microevolution had occurred (Table 2 and Fig. 1). Overall, the sequential isolates from 11 of 15 patients (73%) exhibited karyotypic changes or microevolution. The isolation intervals

among these isolates for each patient ranged from 1 to 24 days. Five of the six patients whose isolates had two or more different karyotypes and four of the nine patients whose isolates showed microevolution had no association with previous antifungal therapy. For all patients, the sequential isolates from the same patient had the same MLST type.

Development of azole resistance in sequential isolates. With the exception of those from one patient, the sequential strains from each patient had the same or similar antifungal susceptibilities. The exception was patient 7, who had three positive blood cultures for *C. glabrata*; the second and third positive cultures were made 2 and 36 days, respectively, after the first. The patient received antifungal therapy after the second positive blood culture was obtained. The MICs of the first two isolates for fluconazole, itraconazole, and voriconazole were 16, 1, and 0.25 $\mu\text{g/ml}$, respectively. In contrast, the third isolate (isolate 7-3), obtained 36 days after the first positive culture, showed two- or threefold increases in the MICs of all three azole antifungals (fluconazole, 128 $\mu\text{g/ml}$; itraconazole, 4 $\mu\text{g/ml}$; and voriconazole, 2 $\mu\text{g/ml}$). By this time, the patient had received a cumulative dose of 1.1 g of fluconazole and 0.4 g of itraconazole. The three isolates from this patient retained the same MLST, karyotype, and REAG-N patterns. However, the resistant isolate showed an increase in the ethidium bromide

staining intensity of the karyotypic bands compared to that for the susceptible isolate, which was the consistent finding in repeated trials (Fig. 1).

Expression of *CgCDR1* and *CgCDR2* in sequential isolates. Levels of *CgCDR1* and *CgCDR2* expression relative to that of *C. glabrata* ATCC 90030 (fluconazole MIC, 8 µg/ml) for all isolates are shown in Table 2. The fluconazole MICs of the isolates from 14 patients (all except patient 7) were clustered between 8 and 32 µg. No significant differences were observed for the levels of expression of *CgCDR1* and *CgCDR2* between fluconazole-susceptible isolates (MIC, ≤8 µg/ml) and dose-dependently susceptible isolates (MIC, 16 to 32 µg/ml) (Table 2). In contrast, the strain with acquired azole resistance from patient 7 (isolate 7-3) revealed a high abundance of *CgCDR1* (145 times that of *C. glabrata* ATCC 90030) compared to the other BSI isolates (up to 19.9-fold). The level of *CgCDR2* (3.5 times that of *C. glabrata* ATCC 90030) for this isolate was not significantly higher than those for the other isolates (up to 6.8-fold). Compared to the first BSI isolate (isolate 7-1) from patient 7, the resistant isolate exhibited 13.2- and 3.5-fold upregulation of *CgCDR1* and *CgCDR2*, respectively. In contrast, for the other 14 patients, the sequential same-patient isolates differed somewhat in the levels of upregulation (0.3- to 3.6-fold for *CgCDR1* and 0.3- to 4.2-fold for *CgCDR2*, relative to that of the corresponding first blood isolate from each patient).

DISCUSSION

EK using PFGE can discriminate heterogeneous strains within the same *Candida* species, including *C. albicans*, *C. parapsilosis*, and *C. glabrata*, by use of chromosome number or size (2, 12, 14, 24, 25). Previously, we reported that same-patient sequential BSI isolates of *C. albicans* had a stable karyotype (25). In addition, the serial BSI isolates of *C. parapsilosis* from one episode of CVC-related fungemia showed the same stable karyotype (24). Sequential *C. glabrata* same-patient isolates are usually reported to yield the same karyotype (2, 12), and karyotyping has been used to study linking changes in the antifungal resistance of *C. glabrata* isolates to treatment in some studies (4, 21). Unlike other *Candida* species, however, we found that the sequential BSI isolates of *C. glabrata* from blood cultures from the same patient had two or three different karyotypes in 6 (40%) of 15 patients. Since the sequential BSI *Candida* isolates from the same patient are usually reported to yield the same clonal isolates (24, 25), our data strongly suggest that for epidemiological purposes, karyotypic differences should not always be interpreted as a new strain of *C. glabrata*.

In a previous study of *C. albicans*, different karyotypes of *C. albicans* isolates could occur only with a difference in REAG patterns, which represents the occurrence of a new strain (25). In contrast, in this study of *C. glabrata*, we found that same-patient sequential isolates of *C. glabrata* which had different karyotypes had the same or similar REAG patterns. This difference might have been due to the critical genetic characteristics distinguishing between *C. albicans* and *C. glabrata*: *C. glabrata* has a haploid genome, in contrast to the diploid genome of *C. albicans*. *C. glabrata* is more closely related to *Saccharomyces cerevisiae* than to other *Candida* species, and many studies have shown increased chromosomal recombination or rearrangements in the yeast *S. cerevisiae* (13).

Klemp-Selb et al. (14) reported that seven isolates from a patient with *C. glabrata* fungemia had five different karyotype patterns, although the differences were only minor. They suggested that these karyotypic differences were due to chromosome rearrangement within a single strain. Note that the changes observed in the EK patterns of isolates from a single patient in our study were usually restricted to one or two chromosome bands at molecular sizes of >1,600 kb. Therefore, one additional consideration is that the variation in the high-molecular-weight portion of the gel might indicate in vitro chromosomal instability, including artifactual breakage of large chromosomes.

In our study, the 41 BSI isolates from the 15 patients yielded 23 different karyotypes and 11 different REAG-N patterns but only 3 different MLST patterns, indicating that MLST is of limited value for differentiating the clinical isolates of *C. glabrata* compared to the two PFGE methods. One PFGE type was shared by isolates obtained from two patients with CVC-related fungemia who had been hospitalized in the same intensive care unit on overlapping dates, suggesting the intrahospital spread of this *C. glabrata* isolate. In addition, the sequential isolates of nine patients (60%) showed minor genetic differences (one or two bands) by REAG-N, suggesting that microevolution had occurred. In *C. albicans*, microevolution frequently occurred at colonization sites but occurred only rarely among consecutive blood isolates from the same patient (25). Our study shows that microevolution occurred frequently among sequential BSI isolates of *C. glabrata*.

Cormican et al. (7) described both microevolution and karyotype variation in serial clinical isolates of *C. glabrata*. They frequently noted pattern variation of a single band among isolates from a single patient on consecutive days and on occasion from isolates from the same body site on the same day. In this study, the BSI isolates from 11 patients exhibited karyotypic changes or microevolution, and 6 of these patients did not receive antifungal therapy. Our data show that prior antifungal exposure is not necessary for the development of karyotypic changes or microevolution in BSI isolates of *C. glabrata*. In 11 cases of candidemia in which the sequential isolates showed karyotypic changes or microevolution, the isolation intervals among the isolates in each patient ranged from 1 to 24 days, which indicates that these genotype changes can occur rapidly.

Our study shows that for one of seven candidemic patients who had sequential isolates after fluconazole therapy, the strain developed increased MICs to all triazole agents. Although the three sequential strains from this patient had the same genotype, the last strain with acquired azole resistance showed increased ethidium bromide staining of the karyotype bands, which suggests that the resistant isolate underwent mutations involving chromosome duplication (16). The resistant strain produced a 13.2-fold increase in *CgCDR1* transcripts, while *CgCDR2* was upregulated only 3.5-fold, compared to the first BSI isolate from the same patient. Sanguinetti et al. (23) documented the development of azole resistance in four pairs of sequential isolates obtained from separate patients with *C. glabrata* BSIs. They noticed that *CgCDR1* upregulation was always clearly manifested in fluconazole-resistant isolates, while *CgCDR2* was expressed at moderate levels, which supports the idea that *CgCDR1* is more closely associated with

azole resistance than CgCDR2 (22). Our patient had received cumulative doses of 1.5 g of triazole antifungals, which is a dose much smaller than those (6.0 to 12.2 g) reported by Sanguinetti et al. (23). Bennett et al. (4) reported that exposure of *C. glabrata* to subtherapeutic concentrations of fluconazole may result in resistance. Recently, another case of invasive candidiasis and candidemia due to a *C. glabrata* isolate that developed resistance to all triazole antifungals after a course of fluconazole treatment was reported (15). Our study, together with others (15, 23), confirms that *C. glabrata* can acquire azole resistance during BSI in association with azole therapy.

Although the specific reasons for the recent emergence of *C. glabrata* as a major cause of BSI are unknown, fluconazole use is most likely involved in the emergence of *C. glabrata* infections (11). However, of 15 patients with *C. glabrata* fungemia analyzed in this study, only 2 patients had a history of fluconazole use. The most common underlying conditions in our patients were gastrointestinal diseases. Since systemic *C. glabrata* infections frequently arise from the host's endogenous microflora, mainly that in the orointestinal and genitourinary tracts (5), this suggests the possible entrance of *C. glabrata* stains into the bloodstream from disruption of the gastrointestinal mucosal surfaces in our patients.

The molecular mechanism for the genotypic change in serial *C. glabrata* isolates is completely unknown. Although serial strains of *C. albicans* from patients usually have stable karyotypes (3, 25), chromosome translocations can contribute to karyotypic variability in vitro (26). Several studies have demonstrated that karyotypic variation in *C. albicans* is associated with high-frequency phenotypic switching, which occurs primarily in the chromosome harboring ribosomal DNA cistrons (20, 26). In our study on serial isolates of *C. glabrata*, one or two chromosomal bands larger than 1,600 bp are particularly variable, suggesting that this variation is due to a variable number of ribosomal DNA repeats (1). Brockert et al. (6) reported that the majority of *C. glabrata* strains switch spontaneously at high frequency between core phenotypes and the irregular wrinkle phenotype, and switching occurs at sites of infection. It has been suggested that the switching of *C. glabrata* represents a supervirulence factor regulating several genes, the combined expression of which facilitates pathogenesis (27). Our study showed that in addition to karyotype changes, microevolution occurs frequently in *C. glabrata* during the course of fungemia. However, the relevance of this genotypic change is unknown, and future investigation is needed to determine the mechanisms and phenotypic consequences of the genotypic changes in *C. glabrata* both in vivo and in vitro.

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