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Molecular Epidemiological Analysis of Bloodstream Isolates of *Candida albicans* from a University Hospital over a Five-Year Period

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We assessed the genetic relations and epidemiological links among bloodstream isolates of *Candida albicans,* which were obtained from a university hospital over a period of five years. The 54 bloodstream isolates from the 38 patients yielded 14 different karyotypes, 29 different patterns after digestion with *Sfi*I (REAG-S), and 31 different patterns after digestion with *Bss*HII (REAG-B) when analyzed using three different pulsed-field gel electrophoresis (PFGE) typing methods. In 11 patients with serial bloodstream isolates, all strains from each patient had the same PFGE pattern. The dendrograms for all of the strains revealed that the distribution of similarity values ranged from 0.70 to 1.0 in the REAG-S patterns, and from 0.35 to 1.0 in the REAG-B patterns. Overall, the combination of the three different PFGE methods identified 31 distinct types, reflecting the results obtained using the REAG-B alone different. different Five PFGE types were shared among 22 isolates from 12 patients. These types of strains were more frequently associated with central venous catheter-related fungemia than the other 26 type strains (92% versus 31%; $P < 0.005$). Of five PFGE types, four isolates were determined to be epidemiologically related: each of these types was primarily from two or three patients who had been hospitalized concurrently within the same intensive care unit. Our results suggest that the REAG-B constitutes perhaps the most useful PFGE method for investigating *C. albicans* candidemia and also shows that a relatively high proportion of *C. albicans* candidemia may be associated with exogenous acquisition of clonal strains.

Key words: bloodstream infections, *Candida albicans,* candidemia, pulsed-field gel electrophoresis (PFGE).

Candida bloodstream infections (BSIs) are associated with the relatively high rates of both morbidity and attributable mortality (38%) (Wenzel *et al.*, 1995; Edmond *et al.*, 1999). A marked rise in the occurrence of *Candida* BSIs has occurred over the past two decades, and *Candida albicans* has been shown to account for 50 to 70% of all nosocomial BSIs attributed to the *Candida* species (Banerjee *et al.*, 1991; Pfaller *et al.*, 1996; Abi-Said *et al.*, 1997; Ellepola and Morrison, 2005). The development of *C. albicans* candidemia has been classically considered to be more or less exclusively attributable to autoinfection by endogenous *C. albicans* strains initially colonizing the patient. However, exogenous infection originating from hospital staff or the environment has also been determined as occurring (Ruiz-Diez *et al.*, 1997).

The genotyping of the *Candida* BSI isolates, which were obtained from a hospital, can provide important information for understanding and controlling the nosocomial spread of *Candida* BSI within a hospital (Pfaller *et al.*, 1995; Marco *et al.*, 1999). Different types of candidemia pathogenesis will clearly require different, although not exclusive, prophylactic approaches. With regard to cases which are related to endogenous flora, it is appropriate to attempt to reduce the risk of the translocation of isolates from the gut, via the administration of prophylactic antifungal agents, or by at least surveying the digestive flora. However, in cases in which an exogenous source is involved, the aggressive reinforcement of sanitary measures is mandatory (Pfaller *et al.*, 1994).

Due to the ever increasing incidence and constant possibility of exogenous nosocomial acquisition of *Candida* BSIs, efforts to detect and prevent the cross-transmission of *C. albicans* BSI isolates are clearly warranted. In this study, we attempted to characterize the genetic relations and epidemiological links occurring among *C. albicans* BSI isolates. They were recovered from the same hospital over a five-year period, employing profiles generated via three pulsed-field gel electrophoresis (PFGE) methods.

Materials and Methods

Patients and microorganisms

Fifty-four *C. albicans* isolates were obtained from the

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blood cultures of 38 patients. These patients had been admitted to Chonnam National University Hospital (CNUH) over a five-year period between January 1996 and December 2000. During the study period, we identified 47 patients whose blood was positive for *C. albicans*. Among these, the *C. albicans* isolates of 38 of these patients were retained by a microbiological laboratory, and subsequently enrolled in this study. We were able to obtain serial BSI isolates (2-3 isolates) in 11 patients. We used the *C. albicans* strain ATCC 20098 as a control in this study. *C. albicans* was identified via the evaluation of germ tubes and chlamydospore formation, and an assessment of API 20C (bioMerieux, France) or ATB 32C (bioMerieux, France) sugar assimilation patterns.

Pulsed-field gel electrophoresis (PFGE) analysis

The PFGE analyses were conducted according to a previously described procedure (Rho *et al.*, 2004; Shin *et al.*, 2004). This procedure involved electrophoretic karyotyping (EK) and the restriction endonuclease analysis of genomic DNA, using *Bss*HII (REAG-B) or *Sfi*I (REAG-S) restriction enzymes. In brief, one colony of each *Candida* isolate from the 48-h Sabouraud dextrose agar (SDA) cultures was incubated overnight at 37°C in 10 ml of YPD broth (glucose, 2%; yeast extract, 1%; Bacto-peptone, 2%; Difco, USA). A 150-µl aliquot of the cell suspension was then mixed with 30 U lyticase (Sigma, USA) and 150 µl of 1.6% agarose (FMC BioProducts, USA), which was melted and maintained at a temperature of 50-55°C. Then, aliquots of the agarose/cells mixture were poured into individual molds, in order to allow the formation of agarose plugs, which were permitted to harden for 20 min at room temperature. These agarose plugs were then removed from the molds, immersed for 2 h in 500 µl of a lyticase buffer containing 50 mM EDTA and 100 U/ml lyticase, then washed once in 2 ml of distilled water. The plugs were then incubated in proteinase K solution (50 mM EDTA, and 100 µg proteinase K; Invitrogen, USA) for 16-18 h at 50°C, and finally washed five times in 50 mM sodium EDTA (pH 8.0).

During the EK, the *Candida* chromosomal DNA was separated via PFGE, using a GenePath system (Bio-Rad, USA). The electrophoresis was conducted for 48 h in 0.7% agarose gel (SeaKem GTG agarose; FMC BioProduct, USA) in 0.5× TBE buffer (0.1 M Tris, 0.09 M boric acid, 0.01 M EDTA, pH 8.0), at 4 V/cm, with an initial switch time of 90 sec, and a final switch time of 325 sec. Immediately following the electrophoresis, the gels were stained with 0.5 µg/ml ethidium bromide solution, then photographed under UV illumination. Isolates which were determined to differ by one or more bands were designated as possessing different karyotypes (Rho *et al.*, 2004; Shin *et al.*, 2004).

In the REAG procedure, digestion was conducted using *Sfi*I at 37°C for 16 h, or *Bss*HII at 50°C for 16 h. Electrophoresis for the REAG-S was performed in a manner identical to the EK phase of the experiment, except that the 1% agarose gel was used. The electrophoresis for the REAG-B was conducted for 20 h in 1.0% agarose gel (SeaKem GTG agarose; FMC BioProducts, USA) in 0.5× TBE buffer (0.1 M Tris, 0.09 M boric acid, 0.01 M EDTA, pH 8.0) at 4 V/cm, with an initial switch time of 5 sec and a final switch time of 50 sec. Isolates were considered identical when all of the bands matched. For the REAG, banding patterns with $\geq$ 95%, but less than 100% of the bands matching, were termed "similar" and grouped into subtypes (a or b) of a given clonal types. Isolates with less than 95% of bands matching were considered different (Voss, 1995; Shin *et al.*, 2004). Overall, isolates were considered the same when all of the EK bands matched and $\geq$95% of the REAG bands matched.

All of the isolates in this study were analyzed at least twice (mean: 3 times, range: 2 to 5 times), which involved the subculturing of isolates from the original stock culture to SDA, DNA preparation, endonuclease digestion, the separation of the DNA via PFGE in order to discern pattern relations and ensure reproducibility.

In order to compare the two REAG patterns of the isolates, we assessed the similarity coefficients via the unweighted pair-group method using arithmetic means (UPGMA), in the PAUP ver. 4.0b10 program (D.L. Swofford, Smithsonian Institution, USA). A genetic similarity score of 1.00 indicates that all of the bands of the strains were identical. Similarity values ranged between 0.01 and 0.99, indicating increasing degrees of similarity. We clustered the similarity values via the unweighted pair group method, and generated dendrograms in order to visualize the relationships occurring among the isolates. In addition, we assessed the discriminatory power inherent to each of the PFGE methods by applying Simpson's index of diversity, as was previously described (Hunter and Gaston, 1988).

Antifungal susceptibility testing

Antifungal susceptibility to fluconazole was determined via the standard methods established by the National Committee for Clinical Laboratory Standards (1997). Two reference strains, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258, were employed as quality control isolates in each of the antifungal susceptibility tests. The final fluconazole (Pfizer, UK) concentrations ranged between 0.125-64 µg/ml. The fluconazole MICs were defined as the lowest drug concentrations required to induce a prominent decrease in turbidity (score 2).

Clinical data and definitions

We reviewed the hospital records for the 38 patients for whom the *C. albicans* BSI isolates were recovered. The demographic and clinical data included the gender, age, date of hospitalization, admission wards, underlying con-

ditions, predisposing factors, central venous catheter (CVC) withdrawal and culturing, and outcome of the fungemia (cleared or uncleared). Neutropenia was defined as a neutrophil count of <1,000/mm^3 at the time of infection onset. Candidemia was defined as CVC-related if no other source of infection was found, and if the semiquantitative catheter-tip culture yielded more than 15 colonies of the same *C. albicans* type (Richard *et al.*, 2003). Candidemia was judged to have a "cleared outcome" if the blood cultures returned negative at any time during a 3-

Table 1. Results of karyotyping, restriction endonuclease analysis of genomic DNA using *Bss*HII (REAG-B) and *Sfi*I (REAG-S) for 54 bloodstream isolates of *C. albicans* from 38 patients

Patient	Isolate No.	Isolation date (mo/day/yr)	Fluconazole MIC (ug/mL)	PFGE type			
				EK	REAG-S	REAG –B	Combined
1	1a	07/14/2000	0.12	K1	S1a	B1	
	1b	07/26/2000	0.12	K1	S1a	B1	
	1c	08/19/2000	0.12	K1	S1a	B1	
2	2	06/13/1999	0.25	K2	S2	B2	
3	3	08/09/1999	0.25	K1	S3	B3	
4	4	05/03/1999	0.12	K3	S4	B4	
5	5a	01/09/1999	0.25	K4	S5	B5	
	5b	02/28/1999	0.12	K4	S5	B5	
6	6	11/18/1998	0.12	K3	S6a	B6	C1
7	7	10/08/1998	0.12	K1	S7	B7	
8	8	10/26/1998	0.12	K5	S8	B8	
9	9	06/10/1998	0.12	K6	S9	B9	
10	10	10/29/1998	0.12	K3	S6b	B6	C1
11	11a	01/27/2000	0.25	K7	S10	B10	C2
	11b	01/31/2000	0.12	K7	S10	B10	C2
12	12	06/28/1999	0.25	K7	S10	B10	C2
13	13	01/04/1999	0.25	K7	S10	B10	C2
14	14	03/02/1998	0.25	K7	S11	B11	
15	15	05/03/1997	0.50	K7	S12	B12	
16	16a	11/28/1996	0.12	K7	S13	B13	C3
	16b	12/02/1996	0.12	K7	S13	B13	C3
	16c	12/11/1996	0.12	K7	S13	B13	C3
17	17	05/04/1996	0.50	K7	S13	B13	C3
18	18a	03/11/1998	0.25	K8	S14a	B14	C4
	18b	03/13/1998	0.25	K8	S14a	B14	C4
	18c	03/15/1998	0.25	K8	S14a	B14	C4
19	19a	04/28/1998	0.25	K8	S14b	B14	C4
	19b	05/04/1998	0.25	K8	S14b	B14	C4
	19c	05/09/1998	0.25	K8	S14b	B14	C4
20	20a	05/09/1998	0.25	K8	S14b	B14	C4
	20b	05/23/1998	0.25	K8	S14b	B14	C4
21	21	01/12/1996	0.25	K9	S15	B15	
22	22	06/07/1996	0.25	K6	S16	B16	
23	23	04/14/1997	0.50	K10	S17	B17	
24	24	08/09/1997	0.25	K11	S18	B18	
25	25	08/30/1997	0.50	K7	S19	B19	
26	26	09/26/1997	0.50	K7	S20	B20	C5
27	27	11/20/1997	0.12	K6	S1b	B21	
28	28	02/01/1998	0.12	K1	S21	B22	
29	29	09/01/1998	0.12	K7	S22	B23	
30	30	11/06/1998	0.25	K1	S23	B24	
31	31	09/13/1999	0.12	K7	S24	B25	
32	32	08/21/2000	0.12	K1	S25	B26	

Table 1. Continued.

Patient	Isolate No.	Isolation date (mo/day/yr)	Fluconazole MIC (ug/mL)	PFGE type			
				EK	REAG-S	REAG-B	Combined
33	33	10/02/2000	0.12	K12	S26	B27	
34	34	11/06/2000	0.12	K8	S27	B28	
35	35a	09/12/1996	0.25	K7	S13	B29	
	35b	09/16/1996	0.25	K7	S13	B29	
36	36a	09/15/1997	0.25	K7	S20	B20	C5
	36b	09/15/1997	0.50	K7	S20	B20	C5
	36c	10/26/1997	0.25	K7	S20	B20	C5
37	37a	08/01/2000	0.12	K13	S28	B30	
	37b	08/02/2000	0.12	K13	S28	B30	
38	38a	02/08/1997	0.12	K14	S29	B31	
	38b	02/12/1997	0.12	K14	S29	B31	

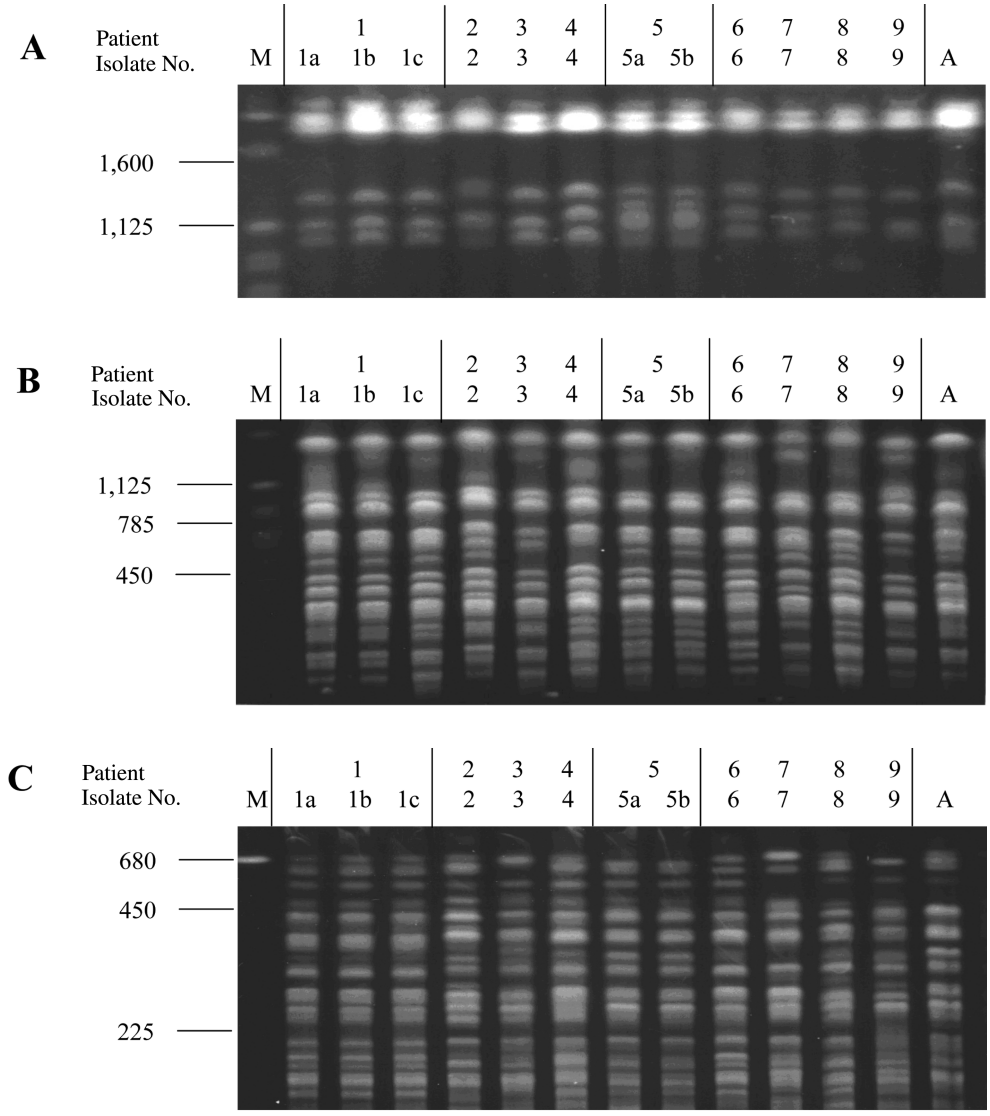

Fig. 1. Electrophoretic karyotyping (A) and restriction endonuclease analysis of genomic DNA using *Sfi*I (REAG-S) (B) and *Bss*HII (REAG-B) (C) followed by PFGE for 12 *C. albicans* isolates obtained from the blood cultures of 9 patients. See Table 1 for the details of the isolates. Of the 12 isolates obtained from nine patients, nine different types were identified by the REAG using *Sfi*I or *Bss*HII, but only 6 types were identified by karyotyping. The strains which were sequentially isolated from each of two patients (patients 1 and 5) had identical karyotypes, and evidenced the same REAG patterns. Lane M, *Saccharomyces cerevisiae* DNA concatamers as molecular size markers. Lane A, *C. albicans* ATCC 90028.

month follow-up period, and if the signs and symptoms of bloodstream infection (fever, hypotension, presence of *Candida* infection) resolved (Shin *et al.*, 2004). Fisher's exact test and the Chi-square test were used to compare the clinical characteristics associated with *C. albicans* BSI caused by epidemic or endemic type strains, with those of *C. albicans* BSI by other strains. In all cases, statistical significance required a P value of <0.05.

Results

A total of 54 isolates, obtained from 38 patients, were typed using three different PFGE methods. In Table 1, the results of PFGE typing, the date of isolation, and the fluconazole MICs are presented for the isolates from each of the 38 patients. EK separated the 54 isolates into 14 types. The REAG-S method identified 29 unique patterns and the REAG-B identified 31 patterns. Among the 11 patients, in which two or more isolates were obtained, all of the strains from each patient manifested identical EK, REAG-S, and REAG-B patterns. The representative PFGE patterns of 12 isolates, obtained from 9 patients, are shown in Fig. 1. The combination of the three methods resulted in the final identification of 31 different profiles, consistent with the results obtained using just the REAG-B. Among the 31 identified PFGE types, 22 isolates from 12 patients were determined to belong to only five (C1, C2, C3, C4, and C5) of these types.

All of the *C. albicans* isolates were determined to be susceptible to fluconazole, and the fluconazole MICs ranged from 0.25-1.0 μg/ml among the isolates. There was no significant increase in the MICs of fluconazole among the sequential BSI isolates obtained from each of 11 patients over the progression of the candidemia. The comparative discriminatory indixes were as follows: 0.835 for EK, 0.953 for the REAG-S, and 0.962 for the REAG-B.

We also prepared dendrograms from the REAG-S and REAG-B results determined from the 55 isolates, including a dendrogram for the ATCC 20098 *C. albicans* strain (Fig. 2). The dendrogram generated from the REAG-S patterns of all of the *C. albicans* strains evidenced similarity values which ranged from 0.70 to 1.0, whereas the dendrogram generated as the result of the REAG-B patterns ranged from 0.35 to 1.0 similarity. The dendrograms generated for each REAG show that 22 of 54 isolates were allocated to five identical PFGE types, which evidenced similarity values of ≥ 0.95, according to both the

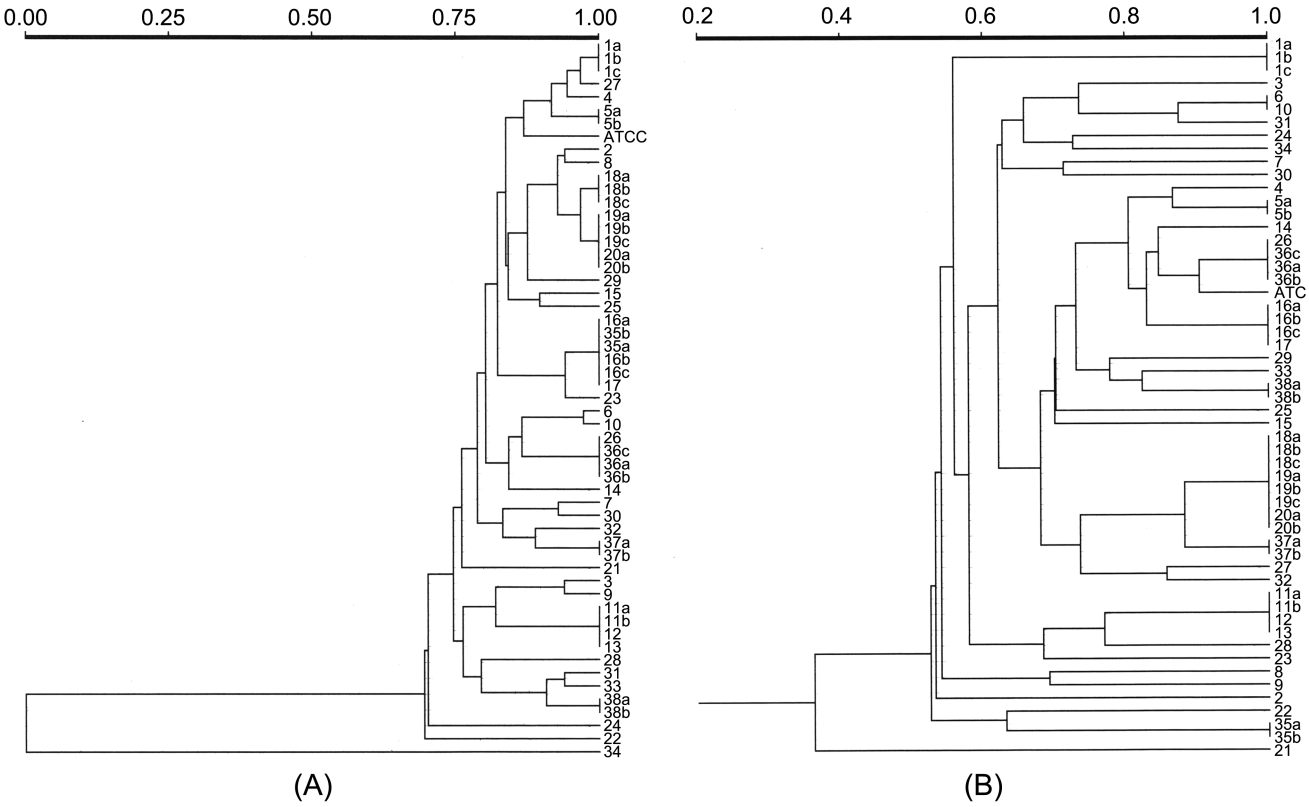

Fig. 2. Dendrogram showing the estimates of similarity values among 55 strains including 54 bloodstream isolates and a ATCC 20098 *C. albicans* strain, as determined by the REAG-S (A) and the REAG-B (B). The dendrogram generated by the REAG-S patterns of all *C. albicans* strains was found to have a Dice similarity coefficient ranging from 0.70 to 1.0, whereas the dendrogram generated by the REAG-B patterns ranged from 0.35 to 1.0 similarity. Thus, the REAG-B appears to have more discriminatory ability than is associated with the REAG-S, with regard to the typing of *C. albicans* isolates.

Table 2. Data summary of 12 patients with *C. albicans* candidemia caused by putatively epidemic or endemic clones (five PFGE type strains)

Pt. No.	Age /sex	Date of hospitalization	Ward	Admisssion diagnosis	CVC-related candidemia	Isolate PFGE type
P6	59y / M	1998/10/16 – 1998/11/25	SICU	Esophageal cancer	Yes	C1
P10	36y / M	1998/09/23 – 1998/11/07	SICU	Congenital cardiac anomaly	No	C1
P11	16d / M	2000/01/10 – 2000/04/26	NICU	Low birth weight	Yes	C2
P12	33d / M	1999/05/25 – 1999/07/17	NICU	Low birth weight	Yes	C2
P13	3m / F	1998/12/15 – 1999/03/23	NICU	Pierre-Robin syndrome	Yes	C2
P16	1y / F	1996/11/18 – 1997/01/20	5W	Hypoxic encephalopathy	Yes	C3
P17	37y / F	1996/04/19 – 1996/05/13	78W	Chronic renal failure	Yes	C3
P18	10d / F	1998/03/01 – 1998/04/28	NICU	Low birth weight	Yes	C4
P19	2.5m / M	1998/02/10 – 1998/07/21	NICU	Duodenal atresia	Yes	C4
P20	3.5m / F	1998/04/20 – 1998/06/13	5W	Hepatoblastoma	Yes	C4
P26	43y / M	1997/07/19 – 1997/10/26	SICU	Traffic accident	Yes	C5
P36	22y / M	1997/08/02 – 1998/07/06	SICU	Traffic accident	Yes	C5

Abbreviations: M, male; F, female; d, day; m, month; y, year; CVC, central venous catheter; NICU, neonatal intensive care unit; SICU, surgical intensive care unit.

REAG-S and REAG-B analyses.

Table 2 summarizes the data obtained from 12 patients, whose isolates shared five (C1 to C5) PFGE types (Fig. 3). This set of patients consisted of five adult and seven pediatric patients. Four of the five adult patients had been admitted to a surgical intensive care unit (SICU), while five out of the seven pediatric patients were admitted to neonatal intensive care units (NICU). The clinical characteristics of these 12 patients were as follows: the presence of CVC (11/12, 92%), use of broad-spectrum antibiotic therapy (12/12, 100%), prior surgery (7/12, 58%), neutropenia (1/12, 8%), ICU stay (9/12, 75%) and cleared outcome (7/12, 58%). The prevalence of all these characteristics among the 12 patients was similar to those among other 26 patients with *C. albicans* candidemia enrolled in this study [the presence of CVC (23/26, 88%), use of broad-spectrum antibiotic therapy (26/26, 100%), prior surgery (16/26, 62%), neutropenia (2/26, 8%), ICU stay (15/26, 58%) and cleared outcome (18/26, 69%)] ($P > 0.05$). However, the prevalence of CVC-related candidemia was different between two groups: CVC-related candidemia had been diagnosed in 92% (11/12) of patients with *C. albicans* candidemia caused by five type (C1 to C5) strains, but in only 31% (8/26) of other patients ($P < 0.005$).

Epidemiological analysis of clinical data indicated that the isolates in each of four PFGE types (C1, C2, C4 and C5) were epidemiologically related. The C1 isolates were proven to have come from two patients who were hospitalized concurrently, and had occupied the same SICU. The C2 cluster type was shown to have occurred in three patients who had been admitted in the same NICU. The C2 isolates were recovered sporadically between January 1999 to January 2000, and the hospitalization of these three patients was not concurrent and did not overlap, thereby suggesting the persistence of endemic clones in the NICU. The cluster C4 isolates were obtained from three pediatric patients with overlapping hospital stays. Although one of these patients (patient 20) had not occupied the same NICU, the same members of the medical staff from the Pediatric department had cared for this patient as the ones who had treated the other two. The cluster C5 isolates were obtained from two patients, who had been concurrently hospitalized, and had occupied the same SICU. However, we were not able to identify epidemiological links among the C3 genotype from two patients (patient 16 and 17), who had been hospitalized in the Pediatric Ward and in the Nephrology Ward, respectively. These two patients did not share the same hospital floor, and the dates of the hospitalization of these two patients did not overlap.

Discussion

Although nosocomial candidemia (due to *C. albicans*) constitutes a growing issue, it has proven quite difficult to achieve a precise understanding of its epidemiology. This is largely because of the absence of a reliable system for the evaluation of strain differences or relations (Soll *et al.*, 2003). Here, we report the findings of a comparison among three PFGE methods, all of which were utilized in the assessment of genetic diversity and relations among isolates of *C. albicans* BSI obtained from a university hospital over a period of five years.

PFGE typing of *Candida* isolates including *C. albicans* has been accomplished by Voss *et al.* (1994), Pfaller *et al.* (1995) and Waggoner-Fountain *et al.* (1996). Voss *et al.* (1994) analyzed a series of 29 clinical *C. albicans* isolates, using three different PFGE methods. They reported that their 29 isolates yielded 9 different EK patterns, 11 differ-

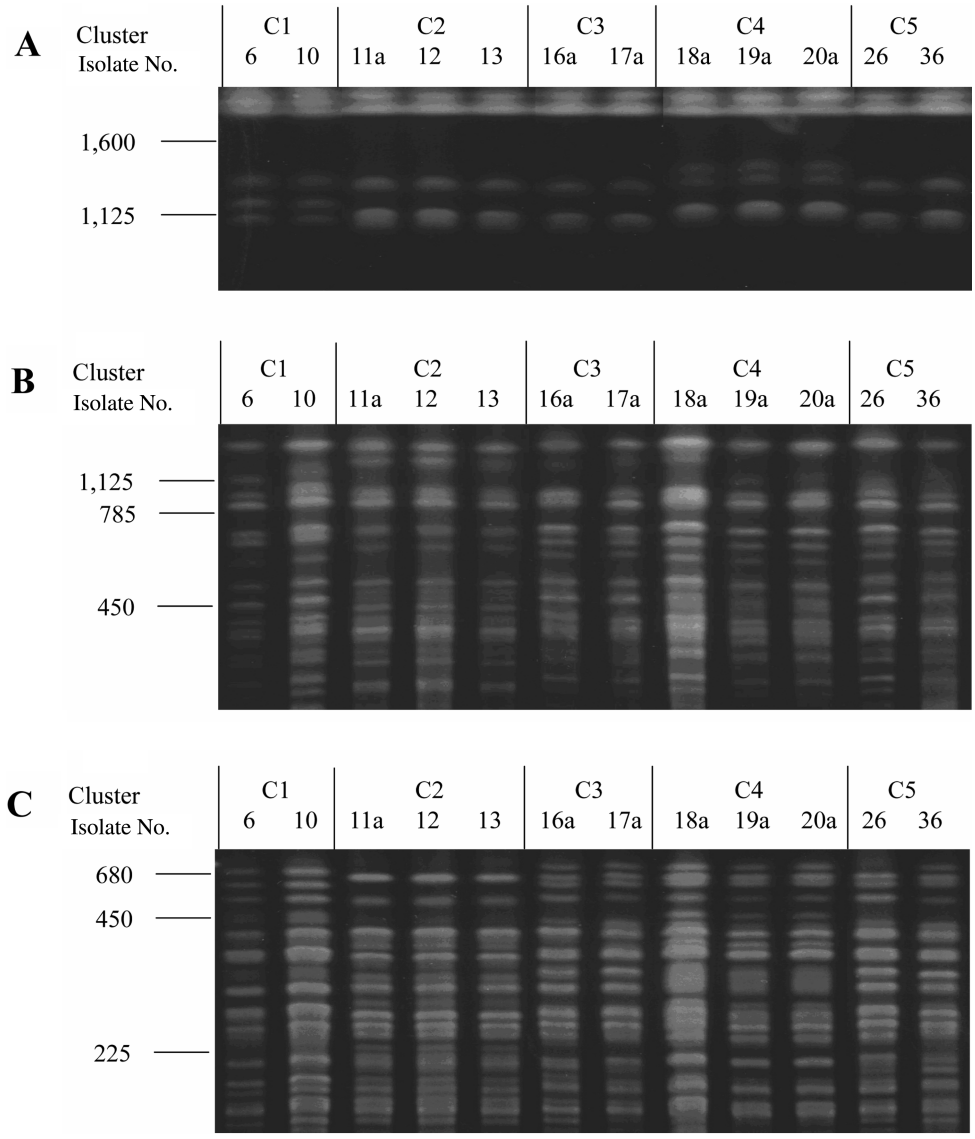

Fig 3. Electrophoretic karyotyping (A) and REAG-S (B) and REAG-B (C) followed by PFGE for 12 *C. albicans* isolates obtained from 12 patients. Five PFGE types (clusters C1 to C5) were shared in the isolates from 12 patients (type C1, patients 6 and 10; type C2, patients 11-13; C3, patients 16 and 17; C4, patients 18-20, C5, patients 26 and 36), suggesting the clonal spread of *C. albicans* bloodstream isolates.

ent REAG-S patterns, and 12 distinct REAG-B patterns. They also asserted that the combination of the REAG with *Sfi*I and *BssH*II constituted an excellent technique for the identification of individual *C. albicans* strains. Pfaller *et al.* (1995) and Waggoner-Fountain *et al.* (1996) conducted PFGE analyses of *Candida* isolates, including *C. albicans*. They concluded that the combination of the EK and REAG-S typing methods could be useful in the identification of strain variations among clinical *Candida* strains. However, in these three studies, the patterns generated by the PFGE methods were not characterized with regard to their level of discrimination. The methods used were not verified with regard to their capacity to measure genetic distance between independent isolates.

In this study, we determined the discriminatory indices to be 0.835 for EK, 0.953 for the REAG-S, and 0.962 for the REAG-B. In addition, the 54 isolates from our 38 patients yielded 14 different EK patterns, 29 different REAG-S patterns, and 31 different REAG-B patterns. This demonstrates that EK is a method of rather limited value in terms of its ability to differentiate between *C. albicans* isolates, especially as compared to the REAG. When the two REAG methods were compared, the REAG-B was found to yield results identical to those yielded by the REAG with both *Sfi*I and *Bss*HII. In addition, the dendrogram generated on the basis of the REAG patterns of all of the *C. albicans* strains demonstrated that the distribution of similarity values ranged from 0.70 to 1.0 for the REAG-S patterns, but from 0.35 to 1.0 for the REAG-B patterns. These results clearly indicate that the REAG-B is the most

discriminatory and suitable PFGE method for the characterization of *C. albicans* BSI isolates.

Karyotype stability has been reported in the serial BSI isolates of *Candida* isolates, including isolates of *C. albicans* (Shin *et al.*, 2004) or *C. parapsilosis* (Shin *et al.*, 2001) obtained from the same patient. These results also show that the karyotypes in the serial BSI isolates of *C. albicans* from each of the 11 patients were identical. In addition, serial isolates from the same patient yielded the same REAG type. Antifungal susceptibility testing revealed no significant increases in the MICs of fluconazole among the serial isolates of each of the 11 patients for whom serial isolates could be obtained over the course of therapy.

In this study, we found that PFGE typing was capable of identifying some clusters of epidemiologially related isolates, which suggests the possibility that *C. albicans* candidemia has been nosocomially transmitted. Among 31 PFGE patterns, 5 PFGE types were shared by 22 isolates obtained from 12 patients, and the isolates in each of four PFGE types (C1, C2, C4 and C5) were obtained from two or three patients who had been hospitalized on overlapping dates, or patients who had occupied the same SICU or NICU. Although the isolates of four PFGE types were determined to be related epidemiologically, the C3 type isolates were obtained from epidemiologically unrelated patients, who had occupied different wards. We are currently unable to explain this observation. However, Shin *et al.* (2004) reported the existence of potentially endemic clones of *Candida tropicalis* isolates within a hospital, the isolates of which were obtained from the blood cultures of patients from different wards, and had been collected sporadically, for periods ranging from four months to three years.

Marco *et al.* (1999) reported that multiple endemic strains might be responsible for a significant amount of the cases of nosocomial BSIs attributable to *C. albicans* occurring in NICUs and SICUs. Our results also indicated that four epidemic or endemic strains were responsible for nosocomial candidemia cases occurring in the NICU or SICU in our hospital during the study period. In addition, almost all of our isolates were recovered from patients who had used CVCs, and 11 of 12 patients had been diagnosed with CVC-related fungemia. In this study, other clinical characteristics associated with *C. albicans* BSI caused by these type strains, except CVC-related fungemia, do not seem to be different from those of the other *C. albicans* BSI -associated strains. As CVC has recently become the most frequently-observed risk factor for candidemia development in patients without neutropenia (Rex *et al.*, 1996; Raad 1998; Shin *et al.*, 2002), our data strongly indicates that *C. albicans* BSI can be acquired exogenously at a high rate via CVC.

In cases of nosocomial infections occurring in newborns, most of the infections originate within a hospital setting, because the fetus is sterile *in utero* (Marco *et al.*, 1999). In our study, two clusters of the C2 and C4 genotypes were found to occur in patients who had occupied the same NICU. As our cases of nosocomial clusters were only retrospectively identified and investigated, we were not able to gather any definite evidence regarding the mode of intrahospital transmission. However, one *C. albicans* strain obtained from the health care workers (HCWs) in NICU, which had been taken as surveillance cultures after the discovery of C2 clusters, evidenced PFGE types identical to those seen in the type C2 isolates (data not shown). This finding suggests that *C. albicans* strains might have been transmitted to the patients from the hands of the HCW who manipulated the CVC.

In addition, our follow-up PFGE study on *C. albicans* BSI isolates from ICU patients showed that two REAG-B patterns were common in isolates from the seven NICU patients between January 2001 and August 2002 (Shin *et al.*, 2004). All these seven patients were using CVC, which indicates a high rate of exogenous origin of CVC-related BSI caused by *C. albicans* in NICU. Therefore, the infection control strategies employed in our hospital have become more sharply focused on high-risk ICU patients who require CVCs. We have also emphasized the importance of hand washing by HCWs. The hospital infection control team regularly carried out inspections to ensure compliance with guidelines for the prevention of nosocomial BSI. After these control measures were instituted, no further clusters of *C. albicans* candidemia were noted in the NICU.

In summary, the results of our study indicated that a relatively high proportion of *C. albicans* candidemia might be exogenously and nosocomially acquired, and that such infections occur quite frequently in conjunction with the use of a CVC. In addition, our results indicate that the REAG-B by PFGE is a reliable method for the tracking of endemic or epidemic *C. albicans* clones, thereby enhancing the effectiveness of strategies for the control of infection.

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