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Molecular Investigation of Two Consecutive Nosocomial Clusters of *Candida tropicalis* Candiduria Using Pulsed-Field Gel Electrophoresis

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Pulsed-field gel electrophoresis (PFGE) typing was applied to the epidemiological investigation of 21 *Candida tropicalis* isolates collected from urine specimens of 11 patients and one healthcare worker, in an intensive care unit (ICU) over a 4-month period. Seventeen epidemiologically unrelated strains from 14 patients were also tested to determine the discriminatory power of PFGE. PFGE typing consisted of electrophoretic karyotyping (EK) and restriction endonuclease analysis of genomic DNA (REAG), using two restriction enzymes (*Bss*HII and *Sfi*I). The EK pattern was the same in all 38 isolates, while REAG using *Sfi*I separated the isolates into nine types. However, 16 different PFGE types were identified by REAG with *Bss*HII, and the same results were obtained when the results of both REAG tests were combined. In serial urinary isolates from 10 patients, all strains from each patient had the same PFGE pattern. While the epidemiologically unrelated strains from 14 patients consisted of 13 different PFGE types, the 20 isolates from the 11 ICU patients fell into only two PFGE types (types C1 and C2), and these apparently originated from the two different outbreaks. All strains of type C1 (n = 12) were isolated from six patients, between November 1999 and January 2000, and all of the type C2 strains (n=8) were isolated from five patients, during January and February 2000. This study shows two consecutive clusters of *C. tropicalis* candiduria in an ICU, defined by PFGE typing, and also demonstrates that a PFGE typing method using *Bss*HII is perhaps the most useful method for investigating *C. tropicalis* candiduria.

Key words: *Candida tropicalis,* Candiduria, Pulsed-field gel electrophoresis (PFGE)

Candiduria, although rare in healthy people, is common in hospitalized patients (Rivett *et al.*, 1986). In tertiary care facilities, as many as 10% of positive urine cultures yield a candidal pathogen (Weber *et al.*, 1992). Candiduria incidence has increased in recent years, particularly in patients admitted to intensive care units (ICUs), most especially among patients requiring prolonged urinary catheterization, or receiving broad-spectrum antibiotics (Richards *et al.*, 1999; Kauffman *et al.*, 2000; Alvarez-Lerma *et al.*, 2003). Although most *Candida* urinary tract infections are nosocomial, and occur in patients with urinary bladder catheters, little is known about its molecular epidemiology.

Candida tropicalis has been reported to be one of the *Candida* species which is most likely to cause bloodstream and urinary tract infections in the hospital (Kauffman *et al.*, 2000; Roilides *et al.*, 2003). *C. tropicalis* is the *Candida* species which is third most frequently isolated from urine cultures, and it accounts for about 8% of urinary *Candida* isolation in most series (Kauffman *et al.*, 2000; Alvarez-Lerma *et al.* 2003). Although a few studies have documented the potential for *C. tropicalis* cross-infection in the hospital (Doebbeling *et al.*, 1991), rarely has actual transmission of urinary *C. tropicalis* strains among hospitalized patients been documented. We recently observed an increase in the number of urinary *C. tropicalis* isolates in an ICU, which prompted an investigation into the possibility of an outbreak of the organism. Although several molecular typing techniques have been used to investigate *C. tropicalis* infections, there is currently no "gold standard" method by which to determine the relatedness of *C. tropicalis* strains (Joly *et al.*, 1996). In this study, we used the pulsed-field gel electrophoresis (PFGE) method to investigate the increased isolation of urinary *C. tropicalis* from a number of patients in an ICU within a 4-month period, and found that the PFGE typing, using restriction enzyme *Bss*HII, served as a highly discriminatory and reproducible method for the investigation of the two consecutive outbreaks of *C. tropicalis* candiduria addressed by this study.

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Materials and Methods

Outbreak description

Chonnam National University Hospital is an 850-bed teaching hospital and tertiary care referral center. The surgical ICU is an open room with 20 patient beds. Between November 1999 and February 2000, 11 cases of *C. tropicalis* candiduria were documented in patients with adjacent beds in the surgical ICU. Not only were these patients kept close together, but they were also often cared for by the same healthcare workers (HCWs). A total of 20 isolates of *C. tropicalis* were recovered from the urinary specimens of 11 ICU patients during this period. The number of patients with *C. tropicalis* candiduria was higher than that identified in the previous 6 months (three patients) in this ICU. Since this cluster, however, these fungi have seldom been isolated from ICU patients, and only two patients were found to harbor *C. tropicalis* between March and June 2000. In order to determine whether the event was a true outbreak, we retrospectively reviewed the medical records of patients who tested positive for *C. tropicalis* during that time. We conducted a case-control study of the epidemic, comparing the clinical variables associated with *C. tropicalis* candiduria. The controls were 20 randomly selected, age- and sex-matched patients who were admitted to the same ICU, but did not have *C. tropicalis* candiduria. Fisher's exact test and the Chi-square test were used to compare clinical characteristics between the two groups. In all cases, statistical significance required a P value of <0.05.

Surveillance cultures

After the increased urinary isolation of *C. tropicalis* was identified, surveillance cultures were performed in the ICU on December 20, 1999, and then again on February 5, 2000. We obtained samples from environmental sources (urinals, floors, soap, disinfectant solution, mattresses, tap-water-supply system, fluid in humidifier jars, infusion pumps, and other hospital equipment). The hands and clothes of all 30 HCWs in the ICU were sampled twice, using the broth-bag technique (hands) and sterile premoistened swabs (clothes) (Shin *et al.*, 2000). The medical records of the 11 ICU patients with *C. tropicalis* candiduria were reviewed retrospectively. Prospective surveillance cultures (hands, throat, nares, urine, and stool) were also taken twice in an attempt to further isolate *C. tropicalis* in the 11 affected patients and the other patients who shared the ICU. The importance of hand washing and compliance with guidelines for the prevention of nosocomial infections was re-emphasized at the time the cluster was investigated.

C. tropicalis *isolates and identification*

Thirty-eight isolates of *C. tropicalis* were analyzed, including 20 urinary isolates from 11 ICU patients obtained during the outbreak periods, one isolate from one HCW obtained during an epidemiological survey, and 17 epidemiologically unrelated urinary strains from 14 patients. In 10 patients, serial urinary isolates (2-4) were obtained. The epidemiologically unrelated strains were from six patients admitted to Chonnam National University Hospital, into wards other than the surgical ICU, and from eight patients at Chosun University Hospital between 1999 and 2000. All *C. tropicalis* isolates were identified by assimilation tests, including conventional methods, using an API 20C and an ATB 32C (bioMerieux, France), and by assessing the isolates on cornmeal agar and CHROMagar Candida (Becton-Dickinson, USA).

Pulsed-field gel electrophoresis analysis

A total of 38 *C. tropicalis* isolates were analyzed by the PFGE methods as described previously (Doebbeling *et al.*, 1993; Zhang *et al.*, 1997; Shin *et al.*, 2001). PFGE typing consisted of electrophoretic karyotyping (EK) and restriction endonuclease analysis of genomic DNA (REAG), using two restriction enzymes (*Bss*HII and *Sfi*I). 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 mixed evenly with 30 U lyticase (Sigma, USA) and 150 μl of 1.6% low-melting temperature agarose (FMC BioProducts, USA), which was previously melted, and kept liquid at 50-55°C. Aliquots placed in plug molds were incubated at room temperature for 20 min. The agarose plugs were removed from the plug molds and placed in 500 μl of a lyticase buffer, containing 50 mM EDTA and 100 U/ml lyticase (Sigma, USA) for 2 h, and then washed once in 2 ml of distilled water. The plugs were incubated in Proteinase K solution (50 mM EDTA, and 100 μg Proteinase K [Gibco BRL, Life Technologies, USA]) at 50°C for 16-18 h and then washed five times in 50 mM sodium EDTA (pH 8.0).

Candida chromosomal DNA was separated by PFGE, using the GenePath system (Bio-Rad, USA). Electrophoresis was performed for 48 h in 0.7% 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 and final switch times of 90 and 325 sec, respectively.

For REAG, digestion was carried out with *Sfi*I at 37°C for 16 h and at 50°C for 16 h with *Bss*HII. Electrophoresis for REAG with *Sfi*I was performed using the EK mthod, except that 1% agarose gel (SeaKem GTG agarose; FMC BioProducts, USA) was used. Electrophoresis for REAG with *Bss*HII was performed for 20 h in 1% agarose gel 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 and final switch times of 5 and 50 sec, respectively. Isolates were considered to be different if banding patterns differed by more

than one readily detectable band (Zhang *et al.*, 1997). All isolates were analyzed at least twice (mean 3 times; range, 2 to 5 times) using a completely new procedure, which included sub-culturing of isolates from the original stock culture to SDA, preparation of DNA, and separation of the DNA by PFGE, to ascertain pattern relatedness and to ensure reproducibility.

Results

Although *C. tropicalis* was isolated from the urine of the 11 ICU patients with *C. tropicalis* candiduria, surveillance cultures from all other patients admitted to the same surgical ICU during the cluster period were negative for *C. tropicalis*. The examination of urinals, intravenous injection samples, and other hospital equipment failed to document fungal colonization by *C. tropicalis*. However, *C. tropicalis* was isolated from the hand of one HCW in the ICU.

A total of 38 isolates, including 21 isolates from 11 ICU patients and one HCW, and 17 epidemiologically unrelated isolates from 14 patients, were typed by three PFGE methods. All 38 *C. tropicalis* isolates had an identical EK pattern that did not differentiate ICU strains from epidemiologically unrelated strains (Figs. 1 and 2). While REAG using *Sfi*I separated the 38 isolates into nine types, 16 different types were identified by REAG with *Bss*HII. Overall, the combination of the three methods (composite PFGE types) resulted in 16 different profiles, which was the same result as that obtained by REAG with *Bss*HII alone. Among the 10 patients with two or more isolates, all strains from each patient had the same PFGE pattern (Tables 1 and 2).

Among the 12 ICU isolates, two types (C1 and C2)

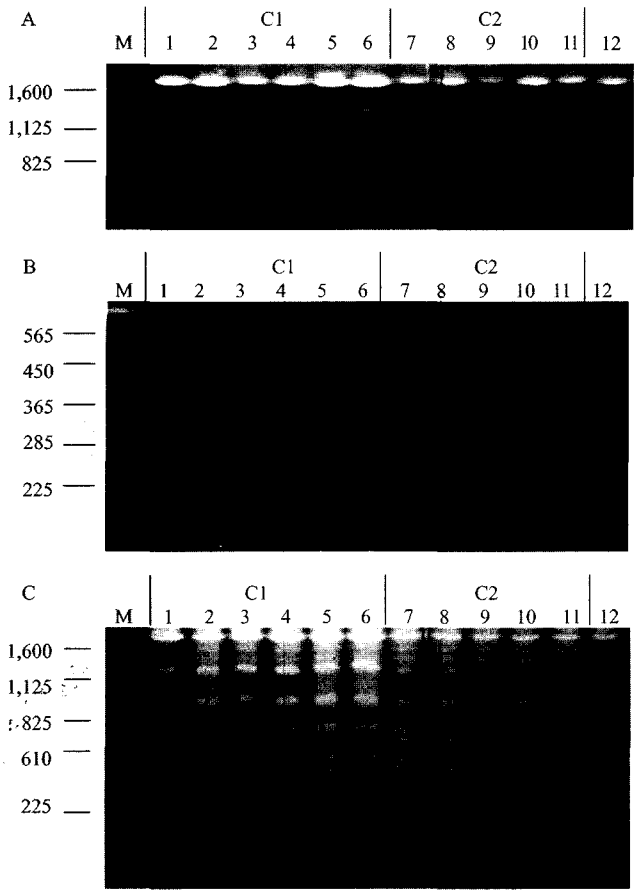

Fig. 1. Electrophoretic karyotyping (A) and restriction endonuclease analysis of genomic DNA (REAG) using *Bss*HII (B) and *Sfi*I (C) followed by PFGE for *C. tropicalis* urinary isolates from an ICU. Lanes 1 to 11 contain DNA digests of isolates from patients 1 to 11 (See Table 1 for the details of the isolates). Two PFGE types (types C1 and C2) were shared by isolates from 11 patients (type C1, lanes 1 to 6; type C2, lanes 7 to 11). The PFGE pattern of one isolate, from the HCW (lane 12) was different from the two outbreak isolates. M: DNA size standards or *Saccharomyces cerevisiae* chromosomal DNA standards. Sizes are shown in kilobases on the left.

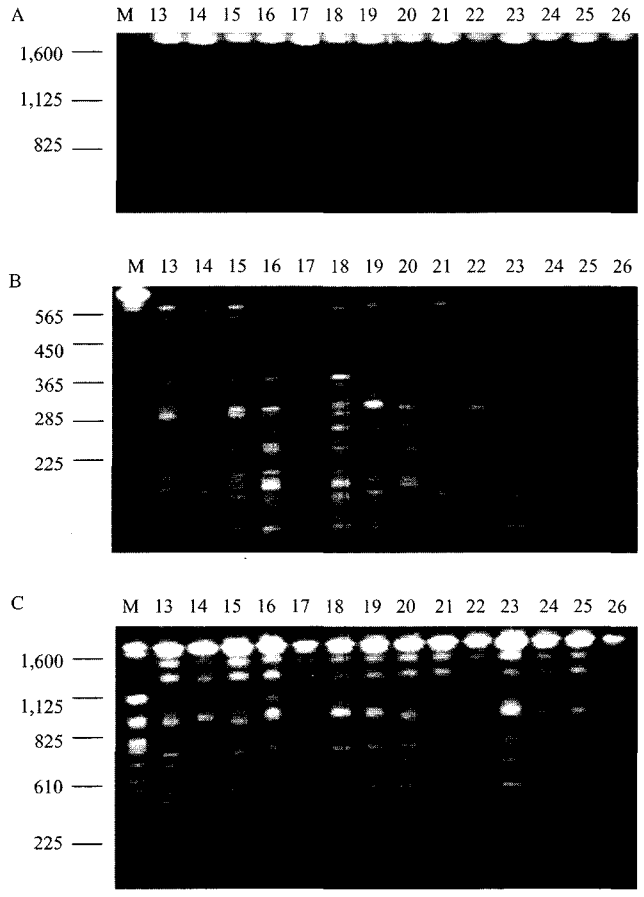

Fig. 2. PFGE types by Electrophoretic karyotyping (A) and REAG using *Bss*HII (B) and *Sfi*I (C) in epidemiologically unrelated isolates of *C. tropicalis* from 14 patients. Lanes 13 to 26 contain DNA digests of isolates from patients 13 to 26. Most of the patient isolates showed unique REAG patterns with *Bss*HII, except for two patients (patients 15 and 17). However, only 7 types were identified by REAG with *Sfi*I. Thus, REAG using *Bss*HII appears to be more discriminating than REAG using *Sfi*I in typing *C. tropicalis* isolates. M: DNA size standards or *S. cerevisiae* chromosomal DNA standards. Sizes are shown in kilobases on the left.

Table 1. Characteristics of outbreak-related *Candida tropicalis* isolates in this study[a]

Patient /HCW[b]	Isolate No.	Date of isolation (mo/day/yr)	Source	Hospital	Ward	PFGE type		
						REAG-S	REAG-B	Combined
1	1-1	11/12/1999	Urine	CNUH	SICU	S1	B1	C1
	1-2	11/15/1999	Urine	CNUH	SICU	S1	B1	C1
2	2-1	11/19/1999	Urine	CNUH	SICU	S1	B1	C1
	2-2	11/22/1999	Urine	CNUH	SICU	S1	B1	C1
3	3-1	11/20/1999	Urine	CNUH	SICU	S1	B1	C1
4	4-1	11/22/1999	Urine	CNUH	SICU	S1	B1	C1
	4-2	12/20/1999	Urine	CNUH	SICU	S1	B1	C1
5	5-1	12/27/1999	Urine	CNUH	SICU	S1	B1	C1
	5-2	12/29/1999	Urine	CNUH	SICU	S1	B1	C1
	5-3	01/03/2000	Urine	CNUH	SICU	S1	B1	C1
	5-4	01/05/2000	Urine	CNUH	SICU	S1	B1	C1
6	6-1	12/24/1999	Urine	CNUH	SICU	S1	B1	C1
7	7-1	01/25/2000	Urine	CNUH	SICU	S2	B2	C2
	7-2	01/27/2000	Urine	CNUH	SICU	S2	B2	C2
8	8-1	01/25/2000	Urine	CNUH	SICU	S2	B2	C2
9	9-1	01/28/2000	Urine	CNUH	SICU	S2	B2	C2
	9-2	01/31/2000	Urine	CNUH	SICU	S2	B2	C2
10	10-1	02/03/2000	Urine	CNUH	SICU	S2	B2	C2
11	11-1	02/10/2000	Urine	CNUH	SICU	S2	B2	C2
	11-2	02/15/2000	Urine	CNUH	SICU	S2	B2	C2
12[b]	12-1	02/05/2000	Hand	CNUH	SICU	S2	B3	C3

[a]CNUH, Chonnam National University Hospital; SICU, surgical intensive care unit; PFGE, pulsed-field gel electrophoresis; REAG-S, restriction endonuclease analysis of genomic DNA using *Sfi*I; REAG-B, restriction endonuclease analysis of genomic DNA using *Bss*HII.
[b]*C. tropicalis* was isolated from the hand of a healthcare worker (HCW) in the SICU.

were shared by 20 isolates from 11 ICU patients. These types came from the two different outbreaks (Table 1 and Fig. 1). All of the type C1 isolates (n=12) were recovered from six ICU patients (patients 1 to 6) between November 1999 and January 2000, and all of the type C2 strains (n=8) were isolated from five ICU patients (patients 7 to 11) in January and February 2000. REAG with *Sfi*I identified one isolate from an ICU HCW with the same pattern as those found after outbreak C2. However, REAG with *Bss*HII revealed a type that was different from those found in outbreak C2 isolates (Fig. 1). For epidemiologically unrelated isolates from 14 patients, seven types were identified by REAG with *Sfi*I, but 13 distinct types were revealed by REAG with *Bss*HII (Table 2 and Fig. 2). With the exception of two isolates from two patients (patients 15 and 17) from the same medical ICU, epidemiologically unrelated strains from each patient showed unique PFGE type.

During each outbreak of *C. tropicalis* candiduria, patient hospitalizations overlapped and the same HCWs cared for the patients. The most common factors associated with *C. tropicalis* candiduria among the 11 patients were the presence of urinary catheters (11/11, 100%), use of broad-spectrum antibiotic therapy (11/11, 100%), prior surgery (9/11, 82%), old age (mean age, 62 years), and diabetes (4/11, 36%). The prevalence of all three factors among the 11 patients with *C. tropicalis* candiduria was similar to that among 20 age- and sex-matched ICU patients without *C. tropicalis* candiduria [presence of urinary catheters (20/20, 100%), use of broad-spectrum antibiotic therapy (19/20, 95%), prior surgery (17/20, 85%), and diabetes (6/20, 30%)]. However, all 11 patients with *C. tropicalis* candiduria had had a history of urinary catheter manipulation (catheter insertion, exchange, or irrigation) within 2 weeks of each outbreak, compared to 8 of 20 patients without *C. tropicalis* candiduria ($P<0.005$). Of

Table 2. Characteristics of epidemiologically unrelated *Candida tropicalis* isolates in this study[a]

Patient No.	Isolate No.	Date of isolation (mo/day/yr)	Source	Hospital	Ward	PFGE type		
						REAG-S	REAG-B	Combined
13	13-1	05/15/00	Urine	CNUH	9W	S3	B4	C4
14	14-1	06/24/00	Urine	CNUH	6W	S4	B5	C5
	14-2	06/25/00	Urine	CNUH	6W	S4	B5	C5
15	15-1	08/06/00	Urine	CNUH	MICU	S3	B6	C6
	15-2	08/17/00	Urine	CNUH	MICU	S3	B6	C6
16	16-1	08/16/00	Urine	CNUH	6W	S5	B7	C7
17	17-1	08/15/00	Urine	CNUH	MICU	S3	B6	C6
	17-2	08/26/00	Urine	CNUH	MICU	S3	B6	C6
18	18-1	12/14/00	Urine	CNUH	8W	S6	B8	C8
19	19-1	12/03/99	Urine	CSU	1W	S7	B9	C9
20	20-1	12/21/99	Urine	CSU	2W	S8	B10	C10
21	21-1	01/02/00	Urine	CSU	OPD	S5	B11	C11
22	22-1	01/18/00	Urine	CSU	OPD	S2	B12	C12
23	23-1	02/19/00	Urine	CSU	ICU	S9	B13	C13
24	24-1	02/21/00	Urine	CSU	3W	S4	B14	C14
25	25-1	03/15/00	Urine	CSU	4W	S7	B15	C15
26	26-1	03/21/00	Urine	CSU	1W	S6	B16	C16

[a]CSU, Chosun University Hospital; MICU, medical intensive care unit; OPD, outpatient.

the 11 patients, nine exhibited concurrent pyuria, and two had concurrent bacterial urinary infections. None of the 11 patients with *C. tropicalis* candiduria developed systemic infections. All candiduria infections improved without antifungal therapy after urinary catheter removal.

Discussion

To date, several nosocomial outbreaks of candidiasis have been reported, but rarely has transmission of urinary *Candida* strains among hospitalized patients been documented. Schwab *et al.* (1997) reported that, at one hospital, urinary *C. glabrata* strains were genetically diverse, and that the association between *C. glabrata* and increases in urinary tract isolation did not appear to be due to horizontal transmission of a single or small number of strains. However, our PFGE study demonstrates two consecutive clusters of *C. tropicalis* candiduria occurring in an ICU. While epidemiologically unrelated strains had unique PFGE types, 20 isolates of the 11 ICU patients belonged to two only PFGE types (C1 and C2), and these came from the two different outbreaks. All patients from the two outbreaks had urinary catheters and were cared for by the same HCWs. All candiduria cases associated with these outbreaks occurred over a period of four months, in the same ICU. In this study, PFGE typing using restriction enzyme *Bss*HII allowed us to determine that there were two separate consecutive *C. tropicalis* outbreaks, rather than a single protracted outbreak.

To identify and control the transmission of epidemic infections caused by *C. tropicalis*, a suitable epidemiologic typing method is essential. Several genetic-based typing systems have been applied to *C. tropicalis*, including DNA restriction fragment length polymorphism patterns (Doebbeling *et al.*, 1991; Roilides *et al.*, 2003), PCR-based methods (Ferra *et al.*, 1994; Walsh *et al.*, 1995; Roilides *et al.*, 2003), PFGE (Doebbeling *et al.*, 1993; Vazquez *et al.*, 1993), isoenzyme profiles (Doebbeling *et al.*, 1993), and Southern blot hybridization with repetitive DNA probes (Walsh *et al.*, 1995; Joly *et al.*, 1996). However, there is currently no "gold standard" method for the epidemiological investigation of *C. tropicalis* infections. PFGE typing of *Candida* isolates, including *C. tropicalis*, has been accomplished by Pfaller *et al.* (1995) using EK analysis and by Doebbeling *et al.* (1993) and Khatib *et al.* (1998), using REAG with *Sfi*I. These authors concluded that the combination of EK and REAG with *Sfi*I typing methods was useful for investigating strain variations in clinical *Candida* strains. In our study, the EK pattern was the same in all 38 isolates tested,

while the REAG method with *Sfi*I identified only 9 patterns. However, 16 unique patterns were obtained after digestion with *Bss*HII. This shows that EK or REAG with *Sfi*I is of limited value, compared to REAG with *Bss*HII, for the differentiation of *C. tropicalis* urinary isolates. Zhang et al. (1997) analyzed a series of 89 clinical isolates of *C. tropicalis* from 56 patients by combining REAG with *Sfi*I and *Bss*HII, followed by PFGE. They reported that the combination of REAG with *Sfi*I and *Bss*HII was an excellent means of identifying individual strains of *C. tropicalis*. However, they did not describe or compare the REAG result with each restriction enzyme individually. In this study, REAG with *Bss*HII produced the same results as REAG with both *Sfi*I and *Bss*HII. We found that REAG with *Bss*HII alone identified epidemiologically unrelated strains as different, but was still able to identify multiple isolates from the same patient, or epidemiologically related isolates from a nosocomial cluster, as the same strain. These results suggest that REAG with *Bss*HII is the most suitable method for investigating *C. tropicalis* candiduria.

In this study, serial urinary isolates from 10 ICU patients were examined, and strains from each of all patients had the same PFGE pattern. Khatib *et al.* (1998) reported that strain persistence is exceedingly frequent in candiduria. They assessed *Candida* isolates (mostly *C. albicans*) from persistent and recurrent candiduria cases, including 4 cases with *C. tropicalis* candiduria, using REAG with *Sfi*I. We confirmed that recurrently positive urinary cultures usually yield the same strain of *C. tropicalis* using EK, or REAG with *Sfi*I and *Bss*HII.

Candida is now one of the most frequently isolated organisms from the urine of ICU patients. The presence of candiduria may signal diverse pathological states, including invasive renal parenchymal disease, fungal balls in obstructed ureters, superficial lower urinary tract infection, and lower urinary tract candidal colonization associated with urinary catheterization (Sobel *et al.*, 2000). A minority of patients with candiduria exhibit systemic infections with renal involvement, acquired by the hematogenous route (Sobel *et al.*, 2000). The most common risk factors for candiduria include urinary instrumentation, recent antibiotic therapy, and advanced age (Kauffman *et al.*, 2000; Alvarez-Lerma *et al.*, 2003). Urinary tract infections caused by *Candida* species are usually acquired via the ascending route, and most of these infections are due to the local spread of fungi from an indwelling urethral catheter, or from the genital or gastrointestinal tracts (Wise and Silver, 1993; Khatib *et al.*, 1998). Pfaller *et al* (1987) documented hematogenous dissemination of *C. tropicalis* in all patients who were persistently colonized with *C. tropicalis*. The importance of *C. tropicalis* infections is underscored by the observation, in several studies, of a high mortality rate associated with hematogenous *C. tropicalis* infection (Pfaller, 1996). In addition, *C. tropicalis* candiduria is associated with a significantly higher treatment failure rate than candiduria associated with other *Candida* species (Sobel *et al.*, 2000).

Since these outbreaks were identified and investigated only retrospectively, we have no evidence with respect to the mode of intrahospital transmission. Thus, it is difficult to say whether these patients became infected from a common source within the hospital environment, or from an individual, from whom the infection subsequently spread into the hospital environment. All environmental surveillance cultures were negative for *C. tropicalis,* but an *C. tropicalis* isolate was found on the hands of a HCW. Although the strain from the HCW evidenced a different PFGE type, which matched none of the patient isolates, the presence of *C. tropicalis* on the hands of a HCW suggests that this may be an important mode of nosocomial transmission. In addition, we found that the catheters of all the patients involved in the outbreaks were inserted or exchanged within 2 weeks of each outbreak. Therefore, we speculate that the *C. tropicalis* strain was transmitted to patients from the hands of the HCW who manipulated the urinary catheters. Local factors, such as biofilm formation of the *C. tropicalis* strain on the catheter surface (Shin *et al.*, 2002) may contribute to the transmission of isolates among patients with urinary catheters. No further clusters of *C. tropicalis* candiduria were detected in the ICU, after the importance of hand washing and compliance with the guidelines for preventing nosocomial infections were re-emphasized during the cluster investigation.

In summary, this study described the use of PFGE to investigate two consecutive outbreaks of *C. tropicalis* candiduria. The discriminating power of EK and REAG with *Sfi*I was found to be inadequate for the epidemiological investigation of *C. tropicalis* candiduria. However, the PFGE technique with *Bss*HII was found to be a highly discriminatory and reproducible method. Therefore, a PFGE typing method using *Bss*HII can facilitate the reliable evaluation of the clonal relationship of *C. tropicalis* isolates, and the identification of common sources of outbreaks.

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