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Biofilm Production by Isolates of *Candida* Species Recovered from Nonneutropenic Patients: Comparison of Bloodstream Isolates with Isolates from Other Sources

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Biofilm production has been implicated as a potential virulence factor of some *Candida* species responsible for catheter-related fungemia in patients receiving parenteral nutrition. We therefore compared clinical bloodstream isolates representing seven different *Candida* species to each other and to those from other anatomical sites for the capacity to form biofilms in glucose-containing medium. Potential associations between the capacity to form biofilms and the clinical characteristics of fungemia were also analyzed. Isolates included the following from nonneutropenic patients: 101 bloodstream isolates (35 *C. parapsilosis*, 30 *C. albicans*, 18 *C. tropicalis*, 8 *C. glabrata*, and 10 other *Candida* species isolates) and 259 clinical isolates from other body sites (116 *C. albicans*, 53 *C. glabrata*, 43 *C. tropicalis*, 17 *C. parapsilosis*, and 30 other *Candida* species isolates). Organisms were grown in Sabouraud dextrose broth (SDB) containing a final concentration of 8% glucose to induce biofilm formation, as published previously. Biofilm production was determined by both visual and spectrophotometric methods. In this medium, biofilm production by *C. albicans* isolates was significantly less frequent (8%) than that by non-*C. albicans Candida* species (61%; $P < 0.0001$). The overall proportion of non-*C. albicans Candida* species isolates from the blood that produced biofilms was significantly higher than that of non-*C. albicans Candida* isolates obtained from other sites (79% versus 52%; $P = 0.0001$). Bloodstream isolates of *C. parapsilosis* alone were significantly more likely to be biofilm positive than were *C. parapsilosis* isolates from other sites (86% versus 47%; $P = 0.0032$). Non-*C. albicans Candida* species, including *C. parapsilosis*, were more likely to be biofilm positive if isolates were derived from patients whose candidemia was central venous catheter (CVC) related (95%; $P < 0.0001$) and was associated with the use of total parenteral nutrition (TPN) (94%; $P < 0.005$). These data suggest that the capacity of *Candida* species isolates to produce biofilms in vitro in glucose-containing SDB may be a reflection of the pathogenic potential of these isolates to cause CVC-related fungemia in patients receiving TPN.

The incidence of nosocomial candidemia has increased dramatically over the last few decades (1, 17, 18). Although *Candida albicans* remains the most common fungal isolate recovered from blood, recent reports indicate a trend toward an increasing prevalence of infections caused by species of *Candida* other than *C. albicans* (1, 15, 17, 18, 22). In particular, non-*C. albicans Candida* species, such as *C. parapsilosis*, *C. tropicalis*, and *C. glabrata*, are now approaching *C. albicans* as the most frequent cause of candidemia in some institutions (10, 15, 17, 18). The frequency of non-*C. albicans Candida* species recovery is influenced by the patient population studied, the therapeutic regimens employed, and the antibiotics or other supportive care measures used in specific institutions (1, 18). Thus, considerable attention has been paid to the importance of non-*C. albicans Candida* species as etiologic agents of bloodstream infections and to the potential nosocomial reservoirs from which these organisms may emerge.

Factors that predispose patients to disseminated candidiasis include increased colonization of the gastrointestinal tract by *Candida* species resulting from prolonged use of broad-spectrum antibacterial agents, disruption of the gastrointestinal mucosal surfaces by cytotoxic agents or hypotension, and neutropenia (6). Central venous catheters (CVCs), however, appear to be the most common risk factor for the development of candidemia in patients without neutropenia or major immunodeficiencies (20; J. H. Rex, Editorial Response, Clin. Infect. Dis. **22:**467–470, 1996). In addition, biofilm formation has been implicated as a potential virulence factor for at least one *Candida* species: *C. parapsilosis. C. parapsilosis* can proliferate in high concentrations of glucose and form biofilms on prosthetic materials. Biofilm formation has been associated with the enhanced capacity of *C. parapsilosis* to colonize indwelling CVCs (thus providing a reservoir from which the organism may enter the bloodstream) in individuals receiving intravenous hyperalimentation (3). Pfaller et al. (19) studied biofilm production by clinical isolates of *C. parapsilosis* grown in glucose-containing media. However, biofilm production by bloodstream isolates of *Candida* species other than *C. parapsilosis* and its potential relationship to CVC-related candidemia, as well as to other clinical characteristics, have rarely been assessed.

Therefore, we studied clinical bloodstream isolates of *C. albicans* and non-*C. albicans Candida* species recovered from nonneutropenic patients at Chonnam National University Hospital and compared *Candida* species to each other and to isolates from other anatomical sites for the capacity to produce

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biofilms. We also assessed the association between biofilm production and the clinical characteristics of candidemia, including the number of positive blood cultures, the presence of a CVC-related candidemia, the use of total parenteral nutrition (TPN), the clinical significance of candidemia, and the outcome of candidemia. The results of these investigations are presented here.

MATERIALS AND METHODS

Microorganisms. A total of 360 *Candida* species isolates recovered from clinical specimens as part of routine diagnostic procedures were tested for biofilm production. The isolates were obtained at Chonnam National University Hospital from 1994 to 1998. Isolates were obtained from nonneutropenic patients who had no antifungal drug exposure during hospitalization prior to the collection of the isolate. All bloodstream isolates studied were the first isolates recovered from a given patient. Bloodstream isolates from 101 nonneutropenic patients were tested, including 35 *C. parapsilosis*, 30 *C. albicans*, 18 *C. tropicalis*, 8 *C. glabrata*, 5 *C. guilliermondii*, 3 *C. pelliculosa*, and 2 *C. lipolytica* isolates. Bloodstream isolates collected from patients with hematologic malignancy or neutrophil counts of $<1{,}000/mm^3$ were excluded from this study. *Candida* species isolates from clinical specimens other than blood were obtained from 259 nonneutropenic patients. The isolates were cultured from urine ($n = 97$), respiratory specimens ($n = 89$), pus or wounds ($n = 41$), body fluids ($n = 12$), or other sites ($n = 20$) excluding catheter tip cultures. Non-bloodstream-derived *Candida* species isolates included 116 *C. albicans*, 53 *C. glabrata*, 43 *C. tropicalis*, and 17 *C. parapsilosis* isolates and isolates from 30 other species belonging to the genus *Candida*. The identification of *Candida* species was conducted by assessing germ tube and chlamydospore formation and API 20C (bioMerieux, Marcy l'Etoile, France) or ATB 32C (bioMerieux) sugar assimilation patterns.

Determination of biofilm production. Biofilm production was assessed by using a modification of the method established by others (3, 10, 19). Briefly, biofilm formation was determined by both visual and spectrophotometric methods. Sabouraud dextrose broth (SDB) was prepared from powdered Sabouraud broth-modified antibiotic medium 13 (BBL, Cockeysville, Md.) according to the manufacturer's instructions, except for supplementation with 60 g of glucose per liter (final glucose concentration, 80 g/liter or 8%). Organisms were grown for 24 h at 35°C on Sabouraud dextrose agar plates (BBL), and saline-washed suspensions of each strain of *Candida* species were prepared. We used two strains (*C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 96142) as controls in each experiment. The turbidity of each suspension was adjusted to the equivalent of 3×10^7 CFU/ml with SDB as determined by comparative plate counts and spectrophotometric readings. Next, 1 ml of suspension was inoculated into a polystyrene tube (Falcon #2095 17-by-120-mm conical tube with a screw cap [Becton Dickinson]) containing 9 ml of SDB. In addition, each well of microtitration plates (Nunclon; Nalge Nunc International, Roskilde, Denmark) was inoculated with aliquots of 20 μl of yeast cell suspension and 180 μl of SDB. Tubes and plates were then incubated at 35°C for 24 h without agitation. We observed that all *Candida* isolates tested grew well in this medium. In the microtiter plates, *Candida* isolates grown in this medium had A_{405} values of >1.0 at 24 h for all species with an initial inoculum of 10^6 CFU/ml.

After 24 h of incubation, the culture broth in the tube was aspirated gently, and tubes were washed once with distilled water. The walls of the tubes were stained with safranin after media and yeast cells were discarded. The adherent biofilm layer was scored as either negative or weakly (1+), moderately (2+ or 3+), or strongly (4+) positive as described by Pfaller et al. (19). Each isolate was tested at least three times, and two observers scored each tube independently in a blinded fashion. The intra- and interobserver reproducibility for deciding the presence or absence of biofilm by this method was 100% for all *Candida* species isolates. Since there was some disagreement (mean, 10%) regarding the intensity of positive results among samples, all positive results, including weak, moderate, or strong, were regarded as positive in this study.

After 24 h of incubation, the microtiter plate was also washed once with distilled water by using a microplate washer (ETL Testing Laboratories, Cortland, N.Y.). Spectrophotometric readings were performed at 405 nm with a microtiter plate reader (VERSAmax Tunable Microplate Reader; Molecular Devices Corp., Sunnyvale, Calif.) after 200 μl of distilled water was added to each well. As the percent transmittance (%T) was being measured, a wavelength of 405 nm was selected for reading the plates to minimize absorbance (7). The %T value for each test sample was subtracted from the %T value for the reagent blank to obtain a measure of the amount of light blocked when passing through the wells ($\%T_{bloc}$). Biofilm production by each isolate was scored as either negative ($\%T_{bloc}$, < 5), 1+ ($\%T_{bloc}$, 5 to 20), 2+ ($\%T_{bloc}$, 20 to 35), 3+ ($\%T_{bloc}$, 35 to 50), or 4+ ($\%T_{bloc}$, ≥50). Each isolate was tested at least twice. No major discrepancies occurred between results obtained by the visual reading method and those obtained by the spectrophotometric method.

TABLE 1. Comparison of biofilm production by *Candida* species isolates obtained from the bloodstream and from other sites in nonneutropenic patients

Candida species	No. biofilm positive/total no. (%)		
	Total	Bloodstream	Other sites
C. albicans	11/146 (8)	2/30 (7)	9/116 (8)
All non-*C. albicans* species	130/214 (61)	56/71 (79)[a]	74/143 (52)
C. tropicalis	49/61 (80)	16/18 (89)	33/43 (77)
C. parapsilosis	38/52 (73)	30/35 (86)[b]	8/17 (47)
C. glabrata	17/61 (28)	2/8 (25)	15/53 (28)
Other *Candida* species	26/40 (65)	8/10 (80)	18/30 (60)
Total	141/360 (39)	58/101 (57)[a]	83/259 (32)

[a] $P < 0.05$, bloodstream versus other sites.
[b] $P < 0.005$, bloodstream versus other sites.

Clinical correlation studies. The hospital records for the 101 nonneutropenic patients from whom the *Candida* species bloodstream isolates were recovered were reviewed. Biofilm positivity of the isolates was compared with the clinical characteristics of the candidemic episode, including the number of positive blood cultures, the presence of CVC-related candidemia, the use of TPN, the presence of clinically significant infection, and the outcome of the fungemia (cleared or uncleared). 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 *Candida* species (9). A *Candida* species' isolate was regarded as clinically insignificant and not associated with disease (i) if the patient had only one positive blood culture; (ii) if the candidemia cleared without any specific interventions, such as catheter removal or antifungal therapy; and (iii) the patients did not show any definitive signs and symptoms associated with candidemia. Candidemia was judged as having a "cleared outcome" if blood cultures became negative at any time during a 3-month follow-up period and if signs and symptoms of bloodstream infection (fever, hypotension, or presence of a *Candida* infection) were resolved (20).

A *Candida* species isolate obtained from clinical specimens other than blood was defined as clinically significant if it was (i) isolated from closed, normally sterile sites without evidence of contamination; (ii) if multiple specimens of the same patient were positive for the same *Candida* species; or (iii) if *Candida* colonies were grown only from confirmed infection sites.

Statistical analysis. Chi-square analysis was used to compare biofilm positivity between different *Candida* species or between isolates recovered from blood cultures and those recovered from all other sites. Fisher's exact test or the chi-square test was used to compare the biofilm positivity of *Candida* species isolates with clinical characteristics and outcome. Differences between groups were considered to be significant when P was < 0.05.

RESULTS

Comparison of biofilm production by different *Candida* species. A total of 141 (39%) of 360 *Candida* species isolates obtained from the bloodstream and from other anatomical sites were biofilm positive. Only 8% (11 of 146) of *C. albicans* isolates produced biofilms, which was significantly lower than the percentage of all non-*C. albicans Candida* species isolates producing biofilms (61%, 130 of 214; $P < 0.0001$; Table 1). The rank order of biofilm production for the non-*C. albicans Candida* species isolates tested is shown in Table 1. Among the four *Candida* species which were most commonly isolated from clinical specimens (*C. albicans*, 41%, 146 of 360; *C. tropicalis*, 17%, 61 of 360; *C. glabrata*, 17%, 61 of 360; and *C. parapsilosis*, 14%, 52 of 360), biofilm production was most frequently ob-

TABLE 2. Comparison of biofilm production by bloodstream isolates and clinical characteristics of candidemia

Candida species	No. biofilm positive/total no. for given category (%)										
	Total	No. of positive blood cultures		CVC-related candidemia		Total parenteral nutrition		Clinically significant disease		Outcome of candidemia	
		1	≥2	No	Yes	No	Yes	No	Yes	Cleared	Uncleared
C. albicans	2/30 (7)	2/15 (13)	0/15 (0)	1/13 (8)	1/17 (6)	1/18 (6)	1/12 (8)	1/2 (50)	1/28 (4)	1/19 (5)	1/11 (9)
All non-*C. albicans*	56/71 (79)	14/22 (64)	42/49 (86)[a]	16/29 (55)	40/42 (95)[a]	22/35 (63)	34/36 (94)[a]	5/12 (42)	51/59 (86)[a]	45/57 (79)	11/14 (79)
C. parapsilosis	30/35 (86)	8/13 (62)	22/22 (100)[a]	7/12 (58)	23/23 (100)[a]	11/16 (69)	19/19 (100)[a]	3/8 (38)	27/27 (100)[a]	27/32 (84)	3/3 (100)
All others[b]	26/36 (72)	6/9 (67)	20/27 (74)	9/17 (53)	17/19 (89)[a]	11/19 (58)	15/17 (88)[a]	2/3 (67)	24/32 (75)	18/25 (72)	8/11 (72)

[a] $P < 0.05$, significant difference between a *Candida* species and all other *Candida* species within a given category (i.e., number of positive blood cultures, 1 versus ≥2; CVC-related candidemia, yes versus no; clinically significant disease, yes versus no; outcome of candidemia, cleared versus uncleared).
[b] All *Candida* species other than *C. albicans* or *C. parapsilosis* included 18 *C. tropicalis*, 8 *C. glabrata*, 5 *C. guilliermondii*, 3 *C. pelliculosa*, and 2 *C. lipolytica* isolates.

served for isolates of *C. tropicalis* (80%, 49 of 61), followed by *C. parapsilosis* (73%, 38 of 52), *C. glabrata* (28%, 17 of 61), and *C. albicans* (8%, 11 of 146). Among biofilm-positive strains, the highest relative intensity of biofilm formation ($\%T_{bloc} > 35$ by the spectrophotometric method) was observed for *C. tropicalis* isolates (59%, 29 of 49), followed by *C. parapsilosis* (39%, 15 of 38).

Comparison of biofilm production by *Candida* species isolates obtained from the bloodstream versus those obtained from other anatomical sites. Biofilm production was detected in a total of 58 (57%) of 101 bloodstream isolates, whereas it was detected in 83 (32%) of 259 isolates from other sites (Table 1; $P < 0.0001$). Of the isolates of *Candida* species from clinical specimens other than blood, 24% (61 of 259) were associated with clinical disease; of these, 66% (40 of 61) were *C. albicans*. Almost all of the isolates of non-*C. albicans Candida* species obtained from respiratory specimens or urine were associated only with colonization. No significant difference between biofilm production by bloodstream isolates of *C. albicans* (7%, 2 of 30) and those obtained from other sites (8%, 9 of 116; $P > 0.05$) was observed. In contrast, biofilm positivity for non-*C. albicans Candida* species obtained from the bloodstream (79%, 56 of 71) was significantly higher than that for isolates from other sites (52%, 74 of 143; $P = 0.0001$). Bloodstream isolates of *C. parapsilosis* alone were significantly more likely to be biofilm positive than *C. parapsilosis* isolates from other sites (86% [30 of 35] versus 47% [8 of 17]; $P = 0.0032$). The combined biofilm positivity of bloodstream isolates of all other non-*C. albicans Candida* species, excluding *C. parapsilosis*, was also significantly higher than those from other sites (72% [26 of 36] versus 52% [66 of 126]; $P = 0.0341$).

Comparison of biofilm production by bloodstream isolates and clinical characteristics of candidemia. Table 2 depicts the relationship between biofilm positivity of *Candida* species isolates and the presence or absence of clinical characteristics of candidemia. Of the *Candida* species recovered from the blood of nonneutropenic patients, *C. parapsilosis* was most frequently isolated (35%, 35 of 101), followed by *C. albicans* (30%, 30 of 101), *C. tropicalis* (18%, 18 of 101), *C. glabrata* (8%, 8 of 101), *C. guilliermondii* (5%, 5 of 101), *C. pelliculosa* (3%, 3 of 101), and *C. lipolytica* (2%, 2 of 101). Of 101 patients with candidemia, 59 (58%) were diagnosed as having CVC-related candidemia. The underlying risk factors for candidemia for 59 patients with CVC-related fungemia were recent operation ($n = 30$), neurological disease ($n = 10$), gastrointestinal disease ($n = 5$), dialysis treatment for chronic renal failure ($n = 4$), cardiovascular disease ($n = 4$), prematurity ($n = 2$), pulmonary disease ($n = 2$), and acute drug intoxication ($n = 2$). Of these 59 patients, 46 (78%) were receiving TPN via CVC. When individual *Candida* species were compared with all other *Candida* species within a given category, *C. parapsilosis* was the only species associated with a statistically significant cleared outcome (91% [32 of 35]; $P = 0.0061$).

No significant association of biofilm production with clinical characteristics was found for *C. albicans* isolates, perhaps because only 2 of 30 bloodstream isolates were biofilm positive. Non-*C. albicans Candida* species were more likely to be biofilm positive if they were recovered multiple times from the bloodstream (86%; $P = 0.0351$), if candidemia was CVC related (95%; $P < 0.0001$), if the candidemia was associated with the use of TPN (94%; $P < 0.005$), and if the candidemia was associated with clinically significant disease (86%; $P = 0.0005$). No statistically significant differences in cleared (79%, 45 of 57) versus uncleared (79%; 11 of 14) candidemia were noted among non-*C. albicans Candida* species isolates ($P > 0.05$; Table 2).

Of 35 bloodstream isolates of *C. parapsilosis*, 22 were isolated from patients with multiple positive blood cultures, and all 22 of these isolates (100%) were biofilm positive. In contrast, only 8 (62%) of 13 *C. parapsilosis* isolates that were recovered from blood only once were biofilm-positive ($P = 0.0017$). The average number of positive blood cultures per patient was 3.0 for *C. parapsilosis*, 2.7 for *C. tropicalis*, 2.6 for *C. glabrata*, 1.7 for *C. albicans*, and 1.6 for other *Candida* species. All 23 isolates (100%) of *C. parapsilosis* recovered from CVC-related candidemia were biofilm positive, but only 7 (53%) of 12 isolates recovered from non-CVC-related candidemia were biofilm positive ($P = 0.0008$). All 19 isolates (100%) of *C. parapsilosis* recovered from patients receiving TPN were biofilm positive, but only 11 (69%) of 16 isolates recovered from patients without use of TPN were biofilm positive ($P < 0.01$). All 27 *C. parapsilosis* isolates associated with clinically significant disease were biofilm positive, whereas only 3 (38%) of 8 isolates without clinical significance were biofilm positive ($P < 0.0001$). The biofilm positivity of non-*C. albicans Candida* species, excluding *C. parapsilosis*, associated with different clinical characteristics of candidemia was also analyzed. Biofilm positivity was higher for non-*C. albicans Candida* isolates, other than *C. parapsilosis*, from CVC-related candidemia (89%, 17 of 19) than from non-CVC-related candidemia (53% [9 of 17]; $P < 0.05$). In addition, biofilm positivity was higher for non-*C. albicans Candida* isolates, other than *C. parapsilosis*, from

patients receiving TPN (88%; 15 of 17), than from patients not receiving TPN (58% [11 of 19]; $P < 0.05$). However, there was no significant association between biofilm positivity and the other three clinical characteristics of candidemia: number of positive blood cultures, clinical significance, and outcome (Table 2).

DISCUSSION

Despite sporadic reports implicating various *Candida* species as the cause of candidemia associated with the use of CVC or TPN (2, 15, 23; Rex, Editorial Response), few studies have examined biofilm production among *Candida* species isolates derived from the blood and compared these results to those obtained for isolates from other anatomical sites. Also, few studies have examined the relationship between biofilm production by bloodstream isolates and clinically significant disease. To our knowledge, we are the first to examine and compare such associations.

In this study, we used SDB medium that contained high glucose (8%) and protein (1%), which has been used to induce biofilm formation by *C. parapsilosis* isolates in several studies (3, 10, 19). Although SDB is not a defined medium, it is more similar to the milieu found in vivo (especially within the CVC lumen) of patients receiving TPN via CVC. TPN solutions usually contain high glucose (10 to 70%) and amino acid (up to 50%) concentrations, as well as other nutrients (16). These components result in an acidic pH which varies according to the content and concentration of the amino acids present. In glucose-containing SDB medium, biofilm production by *C. albicans* isolates was significantly less frequent than that by other *Candida* isolates, regardless of whether the isolates were derived from blood or other sites. It is not known why *C. albicans* isolates recovered from blood, even in cases of CVC-related candidemia, demonstrated a lower percentage of biofilm positivity than other *Candida* species isolates in this study. However, our study suggests that high protein and glucose conditions, mimicking those found in TPN solutions, do not promote biofilm formation by *C. albicans* isolates. These results suggested that *C. albicans* isolates possess mechanisms other than biofilm production to establish bloodstream infections. *C. albicans* is a highly pathogenic *Candida* species and adhesion may be facilitated by a number of protein receptors on epithelial, endothelial, and foreign body surfaces, including fibronectin (14), fibrinogen (5), and vitronectin (13). Perhaps other virulence factors are more important for the pathogenicity of *C. albicans*.

We found that considerable differences in biofilm production existed among *Candida* species grown in high-glucose medium. Biofilm positivity occurred most frequently in isolates of *C. tropicalis*, followed by *C. parapsilosis*, *C. glabrata*, and *C. albicans.* In contrast, Hawser and Douglas (11) reported that isolates of *C. parapsilosis* and *C. glabrata* were significantly less likely to produce biofilms than the more pathogenic *C. albicans*. However, these studies examined only a few selected strains of different *Candida* species and used yeast nitrogen base medium containing little glucose (50 nM). Therefore, this medium would not mimic the high glucose content of TPN and may explain the difference in the results obtained by these authors compared to our work.

Our data provide evidence that the majority of non-*C. albicans Candida* species recovered from the blood of nonneutropenic patients have the capacity to produce significant amounts of biofilm when grown in high-glucose medium. The proportion of biofilm producers was much higher among isolates of non-*C. albicans Candida* species recovered from blood than it was among isolates recovered from other sites. Of non-*C. albicans Candida* species, blood isolates of *C. parapsilosis* were significantly more likely to be biofilm positive than isolates from other sites. Pfaller et al. (19), also by using SDB with 8% glucose, reported that 83% of *C. parapsilosis* isolates recovered from blood or cultured from catheters were biofilm positive versus 53% of isolates from all other sites, a result similar to our findings. The combined biofilm positivity of bloodstream isolates of all other non-*C. albicans Candida* species, excluding *C. parapsilosis*, was also significantly higher than for isolates from other sites. Therefore, the enhanced capacity of isolates of non-*C. albicans Candida* species from the blood to produce biofilms relative to isolates from other sites suggests that the ability to produce a biofilm may be important in allowing non-*C. albicans Candida* species to cause candidemia in patients receiving TPN.

We did not find any significant association between biofilm production and the clinical characteristics of candidemia due to *C. albicans*, since only 2 of 30 blood isolates of *C. albicans* were biofilm positive. Studies by Hawser and Douglas (11) also failed to reveal any correlation between biofilm formation by different isolates of *C. albicans* and pathogenicity, even though all of the *C. albicans* isolates in their study were biofilm producers.

In contrast to *C. albicans* candidemia, there was a significant association between biofilm positivity of non-*C. albicans Candida* species and clinical characteristics of candidemia, including the number of positive blood cultures, the presence of a CVC-related candidemia, the use of TPN, and clinical significance of candidemia. The associations between biofilm positivity with two clinical features (i.e., the presence of clinically significant disease and multiple positive blood cultures) appear to be derived from the high proportion of *C. parapsilosis* isolates in our data set since there were no significant associations between these two clinical features and other non-*C. albicans Candida* species if the *C. parapsilosis* isolates were removed from the analysis. The lack of significant association with clinically significant disease and multiple positive blood cultures for individual non-*C. albicans Candida* species, except *C. parapsilosis*, may be a reflection of the smaller numbers of bloodstream isolates obtained for each of these *Candida* species. It may be that significant relationships between clinical disease and biofilm production by non-*C. albicans*, non-*C. parapsilosis Candida* species may become apparent in a larger clinical study of isolates. However, despite the small numbers of non-*C. albicans*, non-*C. parapsilosis Candida* species isolates present, there was nonetheless a significant association between biofilm production and CVC or TPN use for candidemias caused by non-*C. albicans Candida* species other than *C. parapsilosis*, thus underscoring the relationship between biofilm production and CVC or TPN use.

The lack of a correlation between biofilm formation and clinical outcome of fungemia by *C. albicans* or non-*C. albicans Candida* species observed in our study suggests that factors

other than biofilm formation are involved in the clearance of *Candida* species from the bloodstream. One factor affecting the outcome of candidemia is catheter removal, which has a powerful effect on the outcome of candidemia (Rex, Editorial Response). Since 1997, our hospital guidelines recommended removing a CVC at the first occurrence of candidemia. Of the 14 patients who died with catheters in place, 11 were admitted between 1994 and 1996. No deaths occurred among patients with CVC-related fungemia whose CVCs were removed except for three patients who died within 48 h of CVC removal (unpublished data). This suggests that early catheter removal has a powerful effect on the outcome of candidemia. Second, most cases of candidemia (80%) in our study had a favorable outcome, which was probably due to the high frequency of CVC-related candidemia in our patients (4; Rex, Editorial Response) or the high incidence of *C. parapsilosis* candidemia, which clears more easily than bloodstream infections by other *Candida* species (8, 12, 15, 21).

The spectrum of *Candida* species causing candidemia may vary by region and hospital (1, 10, 15, 17, 18). At Chonnam National University Hospital (an 850-bed tertiary care hospital), non-*C. albicans Candida* infections have increased during the past 5 years and account for approximately 70% of all *Candida* bloodstream infections. The *Candida* species recovered most frequently from blood was *C. parapsilosis.* The reason for the rising incidence of candidemia due to non-*C. albicans Candida* species is not completely understood. However, careful epidemiological studies of candidemia conducted at Chonnam National University Hospital have identified antibiotic exposure (100%), placement of CVCs (72%), and use of TPN (61%) as significant risk factors for the development of candidemia. Almost all (101 of 120) of the patients with candidemia were nonneutropenic (unpublished data). These results suggest that the increased incidence of candidemia due to non-*C. albicans Candida* species, including *C. parapsilosis*, in our hospital was mainly from CVC-related candidemia occurring in nonneutropenic patients receiving TPN. In addition, since no antifungal drug exposure occurred during patient hospitalization prior to the collection of bloodstream isolates, selective pressure favoring the growth of *Candida* species, such as *C. glabrata* or *C. krusei*, that are less susceptible to azole was not present.

Of the 59 patients with CVC-related fungemia described in this study, 46 (78%) were receiving TPN via CVC. The non-*C. albicans Candida* species recovered most frequently from blood at our hospital was *C. parapsilosis*, which also has a high frequency of biofilm production. Our study demonstrated that CVC-related candidemia due to non-*C. albicans Candida* species in nonneutropenic patients is much more likely to be associated with biofilm-producing strains than with biofilm-negative organisms. Furthermore, this study suggests that non-*C. albicans Candida* species, especially *C. parapsilosis*, have selective advantages for growth and biofilm formation if hyperalimentation fluid is present. These selective advantages appear to be independent of antifungal drug use. Future efforts directed at developing catheter materials that can resist biofilm formation may help to reduce the recent increase in candidemia caused by non-*C. albicans Candida* species.

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