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Investigation of *Candida albicans* Transmission in a Surgical Intensive Care Unit Cluster by Using Genomic DNA Typing Methods

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An apparent outbreak of serious *Candida albicans* infections (n = 6) occurred in a surgical intensive care unit over a 4-week period. Four patients developed *C. albicans* bloodstream infections. An additional patient developed catheter-related *C. albicans* infection; the sixth patient developed an infection of cerebrospinal fluid. *C. albicans* was isolated from the hands of five health care workers (17%) and the throat of one health care worker (3%) during the outbreak investigation. Karyotyping and restriction endonuclease analysis of genomic DNA with BsmIII of 23 *C. albicans* isolates from patients and the 6 health care worker isolates revealed 9 and 12 different patterns, respectively. Three of six patients appeared to be infected with the same *C. albicans* strain (two bloodstream infections and one cerebrospinal fluid infection). The hands of a health care worker were colonized with strain that appeared identical to an isolate from a patient prior to infection of the patient. However, restriction endonuclease analysis with SfiI found differences among the isolates determined to be identical by the other two methods. Karyotyping alone does not appear to be sufficient to differentiate between outbreak and control isolates. Restriction endonuclease analysis typing may be a more sensitive method than karyotyping alone in the investigation of a cluster of *C. albicans* infections. Furthermore, the use of more than one restriction enzyme may be necessary for optimal strain discrimination in restriction endonuclease analysis of genomic DNA.

In recent years, the *Candida* spp. have emerged as important nosocomial pathogens. Over the past decade, the incidence of *Candida* bloodstream infections increased by 219 to 487% in U.S. teaching hospitals and by 75 to 370% in nonteaching hospitals (1, 21). Candidemia occurs most frequently in immunocompromised patients with an underlying malignancy or hematological disorder (14, 15) and in severely ill burn patients, as well as surgical intensive care unit (SICU) and neonatal intensive care unit (ICU) patients (3–5, 18, 23, 26). Therefore, incidence rates of candidemia are highest in tertiary care referral hospitals. The incidence rate at the University of Iowa Hospitals and Clinics is 8.5 per 10,000 admissions, with 57% attributable mortalities (30).

Until recently, the development of invasive *Candida* infections was thought to be more or less exclusively due to autoinfection by endogenous *Candida* strains which initially colonize the patient. Using molecular typing methods, investigators have confirmed this hypothesis in neutropenic and nonneutropenic patients (20, 25, 27). However, exogenous sources responsible for outbreaks have been described increasingly, including cross-infection among ICU patients attributed to hand carriage by health care workers (HCWs) (3, 12), contaminated medications, pressure transducers, parenteral nutrition, and reused disposable devices (16, 22, 24, 28, 29, 31). In half of such outbreaks reviewed by Sherertz et al. (22), the source could not be identified, possibly because of the lack of reliable and readily available typing methods for *Candida* spp.

We report an outbreak of systemic *Candida albicans* infections in an SICU in which apparently identical isolates were recovered from three patients' sites of infection and from the hands of an HCW, prior to development of infection in one of the patients. The epidemiology of the carriage and transmission of *C. albicans* among patients in the SICU was further clarified by the application of molecular typing methods.

**MATERIALS AND METHODS**

**Hospital and patients.** The University of Iowa Hospitals and Clinics is a 901-bed teaching hospital and tertiary care referral center, with approximately 200 beds designated for intensive care. The SICU (Fig. 1) at the time of the outbreak was divided into four bays with a total of 24 beds. The nurse-to-patient ratio ranged from 1:2 to 1:3, depending on the patients' needs and availability of nursing staff. A mean of 164 patients per month (standard deviation, ±12) were admitted to the SICU during the 9 months prior to the outbreak. During the 4-week period of the outbreak, 163 patients had been admitted to the SICU. The unit is prospectively surveyed by trained infection control nurses each weekday. Additionally, the surveillance system has been prospectively validated recently (2). No significant changes in personnel, equipment, or infection control surveillance or definitions occurred during the outbreak period or in the previous 9 months.

**Outbreak description.** During the period of the cluster, the observed rate of *C. albicans* bloodstream infections exceeded the upper limit of the predicted 95% confidence interval, and an outbreak investigation was initiated. Microbiological data, infection control surveillance data, and clinical information for all patients were reviewed in search of a common source or vehicle. In order to determine current rates of carriage among SICU workers, the hands and throats of 30 HCWs were sampled prospectively at random intervals over the next 2 weeks by using the broth-bag technique (19) and sterile premoistened rayon-tipped swabs (Cultivette II; Marion Scientific, Kansas City, Mo.), respectively. Pressure transducers of patients receiving intra-arterial monitoring and the ward stock insulin in use were also cultured. Similarly, the throats and stools of patients in the SICU over the 5 weeks following the identification of the outbreak were also cultured at random intervals. All *Candida* isolates were banked and identified to the
species level by using the API 20C system (Analytab Products, Plainview, N.Y.).

Cases were identified by reviewing microbiology reports and patient data from charts and the hospital’s mainframe computer and data gathered by infection control nurses. The importance of hand washing and compliance with the guidelines for prevention of nosocomial infections were reemphasized at the time of the identification of the cluster.

**Molecular typing.** Molecular typing of all isolates was accomplished by electrophoretic karyotype (EK) analysis and by restriction endonuclease analysis of genomic DNA (REAG) using the restriction enzymes BsuHI (REAG-B) and SfiI (REAG-S) followed by pulsed-field gel electrophoresis. Electrophoretic karyotyping and REAG were performed as described previously (6, 8, 27).

Analysis. EK and REAG profiles were analyzed by visual inspection of photographs of ethidium bromide-stained gels. Photographs were analyzed to detect similarities and differences in banding patterns by three observers who were blinded to the origin of the isolate and the results of the other observers. Isolates were considered identical when all of the bands matched, similar when ≥95% but <100% of the bands matched, and different when <95% of the bands matched. Interobserver reliability was calculated by using the kappa coefficient (32).

**RESULTS**

**Patients.** Between 14 October and 22 November 1990, a total of six serious *C. albicans* infections occurred in an open SICU ward with adjacent beds, in which patients were close together and often cared for by the same HCW (Fig. 1). Four of the six patients had candidemia, one had a catheter-related infection, and one had a central nervous system infection. The incidence density ratio for candidemia during the outbreak period was significantly elevated: 4 infections per 577 patient days versus 4 per 6,430 patient days in the entire year (incidence density ratio, 11.1; 95% confidence interval, 2.79 to 44.56). Similarly, the incidence density rate difference was 0.0063 (95% confidence interval, 0.0005 to 0.01). The underlying diseases and risk factors for infection of the six patients involved in the cluster are shown in Table 1. Four patients had undergone abdominal surgery, and five had diabetes mellitus as their major underlying disease. Risk factors for infection included intravascular catheters for six patients, five or more antibiotics for five patients, and total parenteral nutrition for four patients (Table 1).

**Microbiological surveillance.** Following identification of the cluster, the hands and throats of 30 different HCWs were cultured. Seventeen percent of the hand cultures and 33% of the throat cultures of HCWs yielded *C. albicans*. At the same time, the throats of 33% of the patients and the stools of 15% of the patients were colonized with *C. albicans*. In the subsequent 3 weeks, rates of *C. albicans* colonization among patients were 30 and 12% for the throat and stool, respectively. This difference was not statistically significant (P > 0.20).

**Molecular typing.** In the analysis of the DNA profiles of the isolates, a high degree of interobserver reliability was observed: the generalized kappa statistics were 0.84 or greater for EK and REAG-B and REAG-S profiles (P < 0.001 for each). The results of the overall interpretation (consensus call of the three observers) are given in Table 2.

A total of 20 isolates from six outbreak patients (patients S, A, W, D, C, and K), the hand isolates from the five HCWs with positive cultures, and isolates from two additional SICU patients (patients 1 and 2) colonized with *C. albicans* following the outbreak were typed by all three methods. EK analysis and REAG-B and REAG-S identified 9, 10, and 10 different patterns, respectively (Table 2 and Fig. 2 to 4). The combination of 17 patterns identified by EK and REAG-B and REAG-S was used to type 15 isolates included in a previous study (32).

**FIG. 1.** Schematic diagram of bays 1 to 4 of the SICU, showing the initial localization and transfer of the cluster patients.

**TABLE 1.** Underlying diseases and risk factors of the SICU cluster patients

<table>
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<tr>
<th>Risk factor</th>
<th>S</th>
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<th>K</th>
<th>C</th>
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<th>D</th>
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* CRI, catheter-related infection; CSF, cerebrospinal fluid; BSI, bloodstream infection.

b TPN, total parenteral nutrition.
of all three methods (composite DNA type) identified 16 different profiles.

Identical EK patterns (EK A) were obtained for all isolates from patients S and A and from the hands and throat of HCW 1. Additionally, EK A was found in the blood and trachea of patients D and K, respectively (Table 2). In contrast, distinctly different EKs were observed for isolates from patients W and D and HCW 1. Lane numbers correspond to the isolate numbers in Table 2. Banding patterns of isolates from patients S and A and HCW 1 represent identical or closely related subtypes (≥95% of bands are shared). Lanes L, molecular size standards (lambda phage DNA concatemers; sizes are indicated on the right).

The relationship among isolates determined by REAG-B generally supported that determined by EK analysis (Table 2). All isolates from patients S and A and HCW 1 had the same basic REAG-B profile (type 1), allowing for minor banding differences (shared ≥95% of bands; Fig. 3). REAG-B also found the blood and tracheal isolates from patients D and K to be identical (type 3) but different from the isolates of patients S, A, and HCW 1 despite a common EK (Fig. 3). As with EK analysis, the isolates from patients W and C, SICU patients 1 and 2, and HCWs 2 to 5 were all different as determined by REAG-B. Likewise, REAG-B demonstrated that multiple isolates from the same patient generally had the same DNA banding pattern (Fig. 3).

In contrast to the findings with EK analysis and REAG-B, REAG-S identified differences among the outbreak isolates (Table 2 and Fig. 4). Specifically, isolates from patients S and A and HCW 1 each had a different REAG-S profile despite having the same EK and REAG-B types (Table 2 and Fig. 3).

![FIG. 2. Representative EK profiles of C. albicans. Lanes: S, Saccharomyces cerevisiae chromosome DNA size standards (sizes are indicated on the right); 1, C. albicans control strain; 2 and 3, EK E; 4, EK H; 5, EK F; 6, EK G; 7, EK D; 8 to 10, EK A.](image)

![FIG. 3. Types of C. albicans obtained by REAG-B followed by pulsed-field gel electrophoresis. Results are shown for blood culture and other-site isolates from patients S, A, W, and D and HCW 1. Lane numbers correspond to the isolate numbers in Table 2. Banding patterns of isolates from patients S and A and HCW 1 represent identical or closely related subtypes (≥95% of bands are shared). Lanes L, molecular size standards (lambda phage DNA concatemers; sizes are indicated on the right).](image)

![FIG. 4. Types of C. albicans obtained by REAG-S followed by pulsed-field gel electrophoresis. Isolates are the same as for Fig. 3. Differences in banding patterns are seen among isolates from patients S and A and HCW 1, as well as patients W and D. Lanes L, molecular size standards (lambda phage DNA concatemers; sizes are indicated on the right).](image)
and 4). Several patients had the same REAG-S profile: REAG-S type III was shared by patients W, D, and C; type IV was shared by patients D and K and HCW 2; type V was shared by HCW 1 and patient K; and type VI was shared by HCW 1 and patient 1 (Table 2). However, these isolates were identified as different strains on the basis of REAG-B or EK analysis (Table 2). As with the other typing methods, multiple isolates from the same patient generally had the same REAG-S profile.

When the results of all three typing methods were considered (composite DNA type), a total of 16 DNA types were identified among 27 isolates from 13 individuals (Table 2). None of the composite DNA types were shared by two or more individuals. Patients S, A, W, and C each maintained their own unique DNA type of C. albicans over time and from multiple anatomic sites, whereas patients D and K and HCW 1 each had two different DNA types colonizing or infecting different anatomic sites.

**DISCUSSION**

One of the important considerations regarding the prevention and control of nosocomial candidiasis is whether the infection is endogenous or exogenous to the patient (17). It is reasonable to assume that endogenous forms of infection may require strategies for prevention (e.g., antifungal prophylaxis) which are different from those for exogenous infections due to transmission of any organism from one infected patient to another (hand washing and other infection control measures). Our understanding of this area is still evolving; however, the use of molecular typing methods to fingerprint Candida species has been very useful in epidemiologic studies designed to address these issues (6, 20, 22, 24, 27).

The evidence for endogenous candidal infection in hospitalized patients includes the findings that unique strains of Candida are usually seen for each patient, that the same Candida DNA type is usually isolated from multiple anatomic sites over time, that colonizing and infecting strains are usually the same DNA type, and that the colonizing isolate usually precedes the infecting isolate (20, 27).

Despite the importance of endogenous infection, there are now several reports documenting transmission of Candida species from patient to patient, particularly in the ICU setting (3, 10, 12, 23, 26). The open nature of many ICUs and the level of activity make it difficult to prevent or control infections among healthcare workers, thus facilitating nosocomial transmission of pathogenic organisms such as Candida spp. Evidence for carriage of the infecting strain of Candida on the hands of HCWs is mounting, suggesting that simple hand washing may be an effective infection control measure (6, 7). Unfortunately, compliance with hand-washing policies among HCW personnel is less than optimal and underscores the problem of controlling nosocomial transmission of Candida and other pathogens (9).

In the present study, we investigated a cluster of Candida infections in a single ICU in which surveillance cultures indicated hand carriage by several HCWs of the same species of Candida (C. albicans) carried by the infected patients. The clustering of these infections in time and space coupled with hand carriage of the infecting species suggested the possibility of nosocomial transmission. The epidemiology was clarified by the use of DNA-based typing methods, including EK analysis and REAG-B and REAG-S.

Application of EK analysis alone suggested infection of four patients and colonization of one HCW with a single strain of C. albicans. Two additional patients shared a second EK of C. albicans, and the remaining patients and HCWs were each colonized with their own unique EK. This relationship among isolates and patients was corroborated by REAG-B. Importantly, apparently the same strain eventually causing infection in a previously uninfected patient was identified on the hands of an HCW prior to development of infection in that patient. Isolates from two patients and one HCW had the same EK and REAG-B typing, suggesting limited nosocomial transmission of a single strain of C. albicans and linking the HCW to the two infected patients.

One of the major tenets of epidemiologic typing is that although one or more typing methods may be used to demonstrate convincingly that two nosocomial pathogens are different from one another, it is very difficult to prove that they are the same strain. To this end, epidemiologic typing systems are used in combination to enhance discrimination among bacterial isolates. However, this combined approach has been used infrequently for Candida species. Previously, Hurter and Fraser (11) have demonstrated enhanced discriminatory power for typing isolates of C. albicans with the combination of restriction endonuclease analysis, which alone is not adequate, and other typing methods, such as Southern hybridization.

In the present study, several typing methods were used in combination, which suggests that although these isolates may have had a common origin, direct patient-to-patient or HCW-to-patient transmission is unlikely. Molecular typing methods may be used effectively to clarify the epidemiology of nosocomial infections; however, the various typing methods must be used with the
understanding that more than one approach may be necessary to achieve optimal strain discrimination.

REFERENCES


