Molecular Epidemiology of Candida Colonizing Critically Ill Patients in Intensive Care Units

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Background and Purpose: Nosocomial Candida infections are an important cause of morbidity and mortality in critically ill patients. Although there is growing evidence that candidemia develops primarily as a consequence of endogenous colonization, hospital outbreaks of Candida infection are not uncommon. To examine the prevalence and consequence of Candida colonization in critically ill patients and to elucidate the contribution of cross-transmission to the high frequency of nosocomial fungal infection in intensive care units (ICUs), a 6-month prospective surveillance study was conducted.

Patients and Methods: A total of 342 adult patients with an expected ICU stay of 48 hours or more were enrolled in the study. Surveillance cultures were taken from the rectal region, oropharynx, and urine on ICU entry and weekly thereafter. The electrophoretic karyotypes (EKs) of all isolates were characterized using pulsed-field gel electrophoresis.

Results: A total of 873 Candida strains were isolated from 208 of the 342 patients (60.8%) during ICU stay. A comparison of the EK patterns generated from Candida strains isolated from different patients demonstrated a variability of karyotypes, and failed to identify predominant clones colonizing or infecting ICU patients. For 62 of 102 patients colonized with multiple isolates of the same Candida spp., the EK patterns of the Candida strains isolated from individual patients were identical or similar, even when isolated from different anatomical sites, and the patterns remained the same for up to 62 days. A total of 57 episodes of Candida infection occurred in 53 (25.5%) of these 208 patients. Thirty-six episodes (63.1%) of Candida infection were preceded by colonization with the same Candida spp. All infecting strains had identical or similar EK patterns to prior colonizing strains.

Conclusions: While Candida colonization was common in ICU patients, karyotyping did not identify cross-transmission among these patients. Further, only 25.5% of patients with Candida colonization subsequently developed Candida infection. These findings suggest that universal prophylaxis is not warranted in critically ill patients with Candida colonization.

Nosocomial fungal infections are an important cause of morbidity and mortality in critically ill patients [1–5]. The frequency of nosocomial fungal infection has increased dramatically over the past decade [6–8], and Candida spp. have become important pathogens, particularly in the intensive care unit (ICU) [9–11]. Further, candidemia is an independent predictor of mortality [2–4, 12]. There is growing evidence that candidemia develops as a consequence of endogenous colonization [13–15]; however, hospital outbreaks of Candida infection have been reported [16–18]. In critical care settings such as ICUs, cross-infection is more likely and...
has been confirmed by various researchers [19–22]. Since 1993, fungi have become the leading pathogens for nosocomial bloodstream infection and nosocomial urinary tract infection at National Taiwan University Hospital (NTUH) [8]. Although severity of illness, use of invasive devices or procedures, and prior antimicrobial use might account for the high frequency of nosocomial fungal infection in our patients [23], the possibility of cross-transmission cannot be excluded. Nosocomial fungal infection was found to be an independent predictor of mortality in critically ill patients in ICUs [23], and it represents a factor that might be modified to improve the quality of care and outcome of patients. It is therefore of paramount importance to elucidate the pathogenesis of nosocomial fungal infection in order to establish an effective health-care policy for prevention and management. Because four *Candida* spp. (*Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida parapsilosis*) are the predominant isolates [5–8], intra-species delineation of *Candida* spp. is essential to determining the pathogenesis. A variety of DNA fingerprinting methods have been investigated, including restriction fragment length polymorphism analysis, electrophoretic karyotyping, randomly amplified polymorphic DNA (RAPD) analysis, and Southern blot hybridization with discriminating probes [24]. Among these methods, RAPD is attractive and easy to handle, but limited by reproducibility [24]. On the other hand, pulsed-field gel electrophoresis (PFGE) allows good resolution and reproducible separation of chromosomal-size DNA in yeast [14], and has high inter-laboratory agreement [25].

This 6-month, prospective surveillance study was conducted among adult patients with an estimated stay of 48 hours or more in medical and surgical ICUs. The electrophoretic karyotypes (EKs) for all colonizing or infecting isolates of *Candida* spp. were characterized using PFGE. Dendrograms were generated and used as quantitative measures of similarity.

### Materials and Methods

NTUH is an 1,800-bed teaching hospital, providing both primary and tertiary care. There were 86 beds designated as medical and surgical ICUs at the time of this study. Patients treated in coronary care and neuro-surgical care units were not included due to the low rate of nosocomial infection in these patient populations (authors’ unpublished data).

From September 1, 1996, through February 28, 1997, all adult patients admitted to medical and surgical ICUs, where the expected stay was 48 hours or more, were enrolled in the study and were followed for 10 weeks, or until discharge from the ICU. These patients represented a subset of patients treated in these ICUs during the study period. The inclusion criterion of an expected extended duration of stay of at least 48 hours was intended to identify patients who, because they were critically ill and expected to require prolonged physiologic support, would be at increased risk for infection.

### Microbiologic evaluation

Bacteria and fungi were identified and characterized by standard culture methods routinely used in our clinical microbiology laboratory [26]. For specimens that contained numerous species of bacteria or fungi, only the four dominant organisms were selected for analysis. Viruses and *Mycoplasma* were not cultured.

### Surveillance

Specimens for surveillance cultures were collected on admission to the ICU, and then weekly until discharge from the ICU. Specimens from three anatomical sites, including oropharyngeal swabs or endotracheal aspirates, rectal swabs, and urine samples, were collected from all patients. Specimens from other sites such as wounds, vagina, skin, and blood were also collected when clinically indicated. Isolates were labeled according to patient, *Candida* spp., temporal sequence of collection, anatomic location, and date of specimen collection. For example, the first isolate of *C. albicans* obtained from the rectal region of patient 35 on September 24 was labeled 35a1R-24/09.

### Definitions

Nosocomial infection was defined according to the criteria outlined by the Centers for Disease Control and Prevention [27]. The primary source of infection, causative pathogen, and adequacy of antimicrobial therapy were determined according to prospectively defined criteria [27, 28]. Colonization was defined when *Candida* spp. were isolated from surveillance cultures and patients did not have compatible signs or symptoms of infection. A patient was considered infected if there was documentation of either a candidemia or a severe candidal infection requiring the use of systemic antifungal therapy. Patients with *Candida* colonization and persistent signs and symptoms of infection despite apparently appropriate antibacterial therapy were considered to have *Candida* infection.

### Determination of electrophoretic karyotypes

After isolation on Sabouraud agar, two to three colonies of each *Candida* isolate were inoculated into YPD media (1% yeast extract, 2% peptone, 2% glucose) and
incubated at 30°C for 36 hours. Cells were harvested by centrifugation at 1,400 g for 5 minutes and washed twice in 50 mM ethylenediaminetetraacetic acid (EDTA; pH 8.0). The cells were then suspended in 0.5 mg/mL of lyticase (4,000 U/mg; Sigma, St. Louis, MO, USA) and incubated for 30 minutes at 37°C. The spheroplasts were washed and resuspended in 1 M sorbitol and 250 mM EDTA (pH 8.0) at 37°C. The cell suspensions were added to 1% low-melting-temperature chromosome grade agarose (Bio-Rad, Richmond, CA, USA) in 125 mM EDTA (pH 7.5) to prepare agarose plugs. The agarose plugs were then incubated overnight at 37°C in a buffer containing 0.45 M EDTA (pH 8.0), 0.01 M Tris (pH 7.5), and 7.5% β-mercaptoethanol. After washing three times in 50 mM EDTA (pH 8.0) for 15 minutes, the agarose plugs were incubated overnight at 50°C in a buffer containing 0.01 M Tris (pH 7.5), 0.45 M EDTA (pH 8.0), 1% lauroylsarcosine, and 1 mg/mL of proteinase K (Sigma). Sample agarose plugs were washed three times with 50 mM EDTA (pH 8.0), and then loaded into wells for electrophoresis. Extra plugs were stored at 4°C in 50 mM EDTA (pH 8.0).

Pulse-field electrophoresis in agarose containing *C. tropicalis*, *C. glabrata*, or *C. parapsilosis* was performed in a CHEF-DRII (BioRad) apparatus at 4°C for 27 hours at 150 V with pulse intervals ranging from 120 to 240 seconds. *C. albicans* karyotypes were determined in a BIO-RAD (BioRad) apparatus at 14°C for 48 hours at 4 V/cm with pulse intervals ranging from 90 to 325 seconds, at an angle of 120°. These conditions were chosen for best separation of *C. albicans* chromosome 1. *Saccharomyces cerevisiae* YNN 295 chromosomal DNA size standards (BioRad) were used as reference markers to determine the molecular sizes of the *Candida* DNA and were run in the outer two lanes of each gel in order to normalize the gel image.

Electrokaryotypic pattern analysis

Preliminary qualitative analysis of EK patterns obtained from the same gel was performed through visual inspection of photographs of ethidium bromide-stained gels. Isolates were considered different if banding patterns varied by one or more readily detectable band [29]. A one-band difference was used to categorize strain, since a one-band EK difference in yeast isolates signifies a large and detectable size variation in the chromosomal bands. To compare the EK patterns of colonizing and infecting isolates, together with EK patterns for isolates from different patients, the GelCompar software package (version 4.0; Applied Maths, Kortrijk, Belgium) was used. Autoradiogram images were digitized and processed for normalization using the Alpha Innotech IS1000 (Alpha Innotech Co., San Leandro, CA, USA). Dendrograms were generated using the unweighted pair-group method with arithmetic averages (UPGMA) [30]. Because a system for standardizing the interpretation of EK pattern in relation to fungal strain relatedness is not currently available, strains sharing at least 85% similarity of banding patterns were considered similar or identical, with others considered unrelated. Since the order of data input in UPGMA can affect branching and, thus, stability of clusters in a dendrogram, EK patterns from patients exhibiting temporal and/or spatial correlation, or from different patients where pattern similarity was marked, were run in the same gel and input in an orderly fashion, in order to ascertain the probability of persistence of strains from individual patients and of cross-transmission between patients.

**Results**

*Candida colonization and infection*

A total of 873 strains of *Candida* spp. were isolated from 208 of 342 adult patients (60.8%) who stayed in ICUs for 48 hours or more. A total of 525 strains of *C. albicans* were isolated from 160 patients (46.8%), 178 strains of *C. glabrata* from 69 (20.2%), 144 strains of *C. tropicalis* from 50 (14.6%), 22 strains of *C. parapsilosis* from 13 (3.8%), three strains of *Candida guilliermondii* from three (0.9%), and seven strains of *Candida krusei* from five patients (1.5%) (Fig. 1). *Candida* spp. were cultured most frequently from the rectal region (389 of 807 specimens, 48.2%), followed by the oropharynx (258 specimens, 32.0%), and urine (128 specimens, 15.9%). Of these 208 patients, 110 (52.9%) with *Candida* colonization carried the organisms simultaneously in more than one of the three areas of carriage.

A total of 57 episodes of *Candida* infection were diagnosed in 53 patients, including 17 episodes of candidemia, 27 episodes of urinary tract infection,
nine episodes of lower respiratory tract infection, and four episodes of surgical site infection or infection of other sites. *C. albicans* was the most common pathogen (25 episodes), followed by *C. tropicalis* (13), *C. glabrata* (12), *C. parapsilosis* (2), and *C. guilliermondii* (2). In addition, polymicrobial infection was found in five episodes. Among the 208 patients with *Candida* colonization, only 53 (25.5%) developed infection (Table 1). The interval between positive surveillance culture and the onset of nosocomial fungal infection varied from 1 to 26 days, with a median of 2 days. Thirty-six of the 57 episodes (63.1%) of *Candida* infection were preceded by colonization with the same *Candida* spp. in the rectal region and oropharynx (15 episodes), rectal region alone (15), oropharynx (4), or urine (2).

**Dissimilarity of strains isolated from different patients**

The EK patterns for the first *C. albicans* strain isolated from 16 patients are shown in Figure 2. Dendrograms of individual *Candida* spp. were generated for all isolates, and provide a measure of clustering among unrelated isolates at a selected threshold. The EK patterns for the majority of *Candida* isolates from different patients were unrelated (similarity < 85%). A minority of patients, however, from whom isolates with identical or similar EK patterns were collected, were not treated in the ICU at the same time, nor were they housed in the same ward after admission to NTUH (data not shown). There were no predominant clones colonizing ICU patients or showing preference for specific anatomical sites. The dendrogram for *C. albicans* generated from 25 infecting isolates showed that only two isolates from the same patient had a similarity of 85%, while others were unrelated.

![Fig. 2. Representative electrophoretic karyotype patterns for the first Candida albicans isolate from several patients. Reference markers, Saccharomyces cerevisiae YNN 295 chromosomal DNA size standard, were run in the outer two lanes of each gel. Isolates were labeled according to patient number, Candida spp., temporal sequence of collection, anatomic location, and date of collecting specimens. a = C. albicans; g = C. glabrata; t = C. tropicalis; p = C. parapsilosis; T = throat swab, sputum, endotracheal aspirate, or bronchial washing obtained by protective sheath; R = rectal swab; U = urine; B = blood; CVP = central venous catheter; O = other specimens obtained from infectious foci.](image)

**Genetic similarity and maintenance of strains at different anatomical locations in the same patient**

All strains from 102 patients with sequential isolates or multiple strains isolated from different anatomical sites were assessed for genetic relatedness. For 62 pa-

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**Table 1. Number of patients (%) treated in intensive care units with Candida colonization and/or Candida infection**

<table>
<thead>
<tr>
<th></th>
<th><em>C. albicans</em></th>
<th><em>C. glabrata</em></th>
<th><em>C. tropicalis</em></th>
<th><em>C. parapsilosis</em></th>
<th>Total*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with colonization</td>
<td>160</td>
<td>69</td>
<td>50</td>
<td>13</td>
<td>208</td>
</tr>
<tr>
<td>Patients with infection</td>
<td>25 (15.6)</td>
<td>12 (17.4)</td>
<td>13 (26.0)</td>
<td>2 (15.4)</td>
<td>53 (25.5)</td>
</tr>
<tr>
<td>Colonization with the same <em>Candida</em> spp. prior to development of infection</td>
<td>16 (64.0)</td>
<td>8 (66.7)</td>
<td>9 (69.2)</td>
<td>0 (0)</td>
<td>33 (62.3)</td>
</tr>
</tbody>
</table>

*Includes *Candida* spp. other than *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis.*

**Table 2. Number of patients (%) with multiple *Candida* isolates that had identical or similar electrophoretic karyotypic (EK) patterns**

<table>
<thead>
<tr>
<th></th>
<th><em>C. albicans</em></th>
<th><em>C. glabrata</em></th>
<th><em>C. tropicalis</em></th>
<th><em>C. parapsilosis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with multiple isolates of the same <em>Candida</em> spp.</td>
<td>54</td>
<td>32</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Patients with multiple <em>Candida</em> isolates with identical or similar EK patterns</td>
<td>34 (63.0)</td>
<td>12 (37.5)</td>
<td>14 (100)</td>
<td>2 (100)</td>
</tr>
</tbody>
</table>
Candida Colonizing Intensive Care Unit Patients

Fig. 3. Representative electrophoretic karyotype patterns for sequential Candida isolates from several patients. A) Candida albicans; B) Candida glabrata; C) Candida parapsilosis. Isolate labels as described in Fig. 2. Reference markers, Saccharomyces cerevisiae YNN295 chromosomal DNA size standard, were run in the outer two lanes of each gel.

patients (60.8%), multiple Candida isolates obtained from an individual patient had an identical or similar EK pattern, even when isolated from different anatomical sites, and the EK pattern remained the same over a prolonged period (up to 62 days). The proportion of patients with multiple Candida isolates with identical or similar EK patterns is shown in Table 2. The EK patterns of C. glabrata from the same patient were more diverse than those of C. albicans, C. tropicalis, or C. parapsilosis (Table 2, Fig. 3B). All infecting strains of Candida spp. revealed similar or identical EK patterns to prior colonizing strains.

Discussion

Significant increases in nosocomial fungal infections have been noted for all types of hospitals and at all sites of infection [6–8]. Four Candida spp. (C. albicans, C. glabrata, C. tropicalis, and C. parapsilosis) account for the predominant isolates [5–8, 29]. Because Candida spp. can be carried as commensal organisms, endogenous and exogenous origins for nosocomial infections are considered [31]. The present study confirmed the usefulness of PFGE in the delineation of a set of prospectively collected, epidemiologically well-defined isolates. Moreover, this study demonstrated the variability of karyotyping for Candida strains from different patients, as all patients were infected by unique (patient-specific) Candida strains. These findings are in accordance with previous studies [13–15] and suggest that, for the majority of patients, commensal organisms are the source of subsequent infection. These data do not support the hypothesis that cross-transmission is responsible for the high frequency of nosocomial fungal infection in these critically ill patients.

Thirty-six of 57 episodes of Candida infection were preceded by colonization with the same Candida spp. The remaining 21 episodes of Candida infection revealed either no positive surveillance cultures prior to the development of Candida infection, or a history of colonization with different Candida spp. This finding might reflect the inadequacy of our once-weekly surveillance system, commencing after entry to the ICU. On the other hand, a history of colonization with different Candida spp. would indicate very dynamic yeast colonization, with a single host harboring multiple Candida spp. or multiple genotypes of the same species. Further, since all isolates were obtained from each patient after entry into the ICU, the possibility of hospital acquisition remains.

In our study, a proportion of the isolates, particularly C. tropicalis strains, were indistinguishable by PFGE. This finding suggests that either patient-to-patient transfer occurred or that the typing methods used failed to distinguish these isolates. Moreover, cross-transmission and colonization by a specific Candida strain may
have occurred before surveillance isolates were obtained. Among several molecular typing methods available, PFGE was less discriminatory than restriction fragment length polymorphism analysis, Southern blot analysis, or RAPD analysis [32, 33]. For the present study, however, there was no evidence of temporal or geographic clustering of patients with identical or similar EK patterns.

Although there is growing evidence that nosocomial Candida infection may develop as a result of cross-transmission, particularly in ICUs [1], our results suggest that it was not likely to have contributed to the high prevalence of nosocomial Candida infection found in our ICUs during the study period. Up to 60.8% of patients who stayed in the ICU for 48 hours or more were colonized with Candida spp. during their stay in the ICU, and Candida colonization with the same strain preceded infection in 63.1% of Candida infection. These findings offer hope that efforts to eliminate colonization may reduce the incidence of nosocomial fungal infection. Nevertheless, only 25.5% of patients exhibiting Candida colonization in the present study subsequently developed Candida infection, raising doubts regarding the need for universal antifungal prophylaxis for critically ill patients with Candida colonization.

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References


