Endemic Genotypes of *Candida albicans* Causing Fungemia Are Frequent in the Hospital

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Genotyping of *Candida albicans* strains causing candidemia can uncover the presence of endemic genotypes in the hospital. Using a highly reproducible and discriminatory microsatellite marker panel, we studied the genetic diversity of 217 *C. albicans* isolates from the blood cultures of 202 patients with candidemia (from January 2007 to December 2011). Each isolate represented 1 candidemia episode. Multiple episodes were defined as the isolation of *C. albicans* in further blood cultures taken ≥7 days after the last isolation in blood culture. Of the 202 patients, 188 had 1 episode, 13 had 2 episodes, and 1 had 3 episodes. Identical genotypes showed the same alleles for all 6 markers. The genotypes causing both episodes were identical in most patients with 2 episodes (11/13; 84.6%). In contrast, 2 different genotypes were found in the patient with 3 episodes, one causing the first and second episodes and the other causing the third episode (isolated 6 months later). We found marked genetic diversity in 174 different genotypes: 155 were unique, and 19 were endemic and formed 19 clusters (2 to 6 patients per cluster). Up to 25% of the patients were infected by endemic genotypes that infected 2 or more different patients. Some of these endemic genotypes were found in the same unit of the hospital, mainly neonatology, whereas others infected patients in different wards.

Candidemia is generally a nosocomial infection, and half of all cases are caused by *Candida albicans* (1–5). Studying the genetic relationship between *C. albicans* causing fungemia in the hospital can uncover the presence of endemic genotypes, which may suggest horizontal transmission and enable us to implement prevention measures.

However, in the absence of genotyping, the potential routes of infection and the presence of endemic genotypes of *C. albicans* in the hospital are unknown. Several procedures are used to genotype *C. albicans* (6–8), and microsatellites in particular have a high discriminatory power, the ability to detect heterozygote diploid organisms (codominance), and a high reproducibility (9–12).

Previous studies have shown the presence of endemic genotypes of *C. albicans* causing candidemia in specific hospital units, mostly adult and neonatal intensive care units (ICUs) (8, 13, 14). However, it is unknown whether endemic genotypes can be found in other parts of the hospital. Furthermore, the proportion of patients infected by endemic *C. albicans* genotypes has been poorly studied.

We investigated the genotypic diversity of *C. albicans* isolates from patients with candidemia who were admitted to a large tertiary hospital in order to determine the percentage of patients infected by endemic genotypes and the ward of hospitalization.

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**MATERIALS AND METHODS**

**Hospital description, definition of candidemia episodes, and patients studied.** This study was carried out at a large teaching hospital that serves a population of approximately 715,000 inhabitants in the city of Madrid, Spain. The institution cares for all types of patients at risk of acquiring candidemia, including patients admitted to medical and surgical ICUs, neonates, patients with hematological malignancies, solid organ transplant recipients, and patients with central venous catheters.

During the study period, blood samples for culture were obtained by standard procedures and incubated in the automated Bactec-NR system (Becton-Dickinson, Cockeysville, MD).

From January 2007 to December 2011, 202 patients admitted to the hospital had 217 episodes of candidemia caused by *C. albicans*. An episode of candidemia was defined as the isolation of *C. albicans* from a blood culture. In the absence of a consensus for the definition of additional episodes of candidemia, we arbitrarily defined additional episodes as the isolation of *C. albicans* in further blood cultures taken ≥7 days after the last isolation in the previous episode.

**Identification of the isolates.** Blood cultures with presumptive visualization of yeasts in the Gram stain were subcultured on CHROMagar Candida plates (CHROMagar, Paris, France) and incubated at 35°C. Isolates were identified by means of the ID 32C system (bioMérieux, Marcy l’Étoile, France). Identification of *C. albicans* was confirmed by amplification and sequencing of the ITS1-5.8S-ITS2 region (15).

**Genotyping procedure.** We genotyped 1 *C. albicans* strain representing one episode per patient using a panel of 6 short tandem repeats (STRs), as reported elsewhere (9, 11, 12). The sizes of the amplified fragments were determined by capillary electrophoresis with a 3130xl analyzer (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA) using the GeneScan ROX marker. Electropherograms were analyzed using GeneMapper v.4.0 software (Applied Biosystems-Life Technologies Corporation, CA). A *C. albicans* strain was used as a control in each run to ensure accuracy of the size and to minimize run-to-run variation.

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RESULTS

Distribution of episodes of candidemia. At the time of diagnosis, the 202 patients admitted to the medical oncology and oncohematology units, 188 had 1 episode, 13 had 2 episodes, and 1 had 3 episodes. In most of the patients with 2 episodes (11/13; 84.6%), the genotypes involved with both episodes were identical (mean, 10 days between episodes). In the remaining 2 patients, the second episode occurred 10 and 13 days after the first episode, respectively. Genotypes from the first and second episodes differed in 2 and 3 markers, respectively.

In contrast, 2 different genotypes were found in the patient with 3 episodes, one causing the first 2 episodes (9 days between the first and the second episodes) and the other causing the third episode (isolated 6 months later). The genotypes differed in 4 markers.

Genetic diversity and interpatient genotyping. The parameters of genetic diversity are shown in Table 1. We found high genetic diversity among the 217 C. albicans strains studied, as shown by the high number of alleles detected, the low frequency of null alleles, and the high heterozygosity. Despite the high diversity, we observed heterozygote deficiency, as shown by the positive values of Wright’s fixation index and the statistically significant (P < 0.001) departure from Hardy-Weinberg equilibrium in the allele frequencies of the 6 loci. The probability of identity index was 1.05 × 10^-8, which showed that the markers with the highest numbers of different alleles were the most informative.

A total of 174 genotypes were found in the 217 strains studied; the genotype distribution is shown in Fig. 2. Of the 174 genotypes, 155 were unique and infected 1 patient each; the remaining 19 were endemic and formed 19 clusters (named 1 to 19) that involved 51 patients (2 to 6 patients per cluster) (Fig. 2). Clusters were classified according to the ward of hospitalization at the time of blood sample collection.

The patients involved in 10 of the 19 clusters (53%) were geographically related. The first group accounted for 7 of the 19 clusters and involved patients admitted to the same ward at the time of blood sample collection, mostly in the neonatology unit (Table 2). The 5 clusters involving neonates were observed from 2008 to 2010; 3 out of the 5 clusters included patients diagnosed in 2010, when the highest number of cases of candidemia caused by C. albicans was found in the unit (Fig. 1). These findings suggest the

![Image](http://jcm.asm.org/Downloaded from http://jcm.asm.org)

**Fig 1** Distribution of episodes of candidemia diagnosed in each year of the study period. The distribution of patients is also shown grouped by unit of admission at the time of diagnosis.

**TABLE 1** Genetic diversity in the C. albicans isolates studied

<table>
<thead>
<tr>
<th>STRa</th>
<th>No. of different alleles</th>
<th>Frequency of null allelesb</th>
<th>Observed heterozygosityc</th>
<th>Expected heterozygosity</th>
<th>Wright’s indexd</th>
<th>Probability of identitye</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT</td>
<td>36</td>
<td>0.082</td>
<td>0.75</td>
<td>0.91</td>
<td>0.17</td>
<td>0.012</td>
</tr>
<tr>
<td>CAII</td>
<td>8</td>
<td>0.008</td>
<td>0.65</td>
<td>0.67</td>
<td>0.02</td>
<td>0.139</td>
</tr>
<tr>
<td>CAIV</td>
<td>36</td>
<td>0.113</td>
<td>0.65</td>
<td>0.86</td>
<td>0.24</td>
<td>0.028</td>
</tr>
<tr>
<td>CDC2</td>
<td>8</td>
<td>-0.067</td>
<td>0.77</td>
<td>0.66</td>
<td>-0.16</td>
<td>0.164</td>
</tr>
<tr>
<td>HIS3</td>
<td>33</td>
<td>0.149</td>
<td>0.57</td>
<td>0.85</td>
<td>0.32</td>
<td>0.035</td>
</tr>
<tr>
<td>EF3</td>
<td>20</td>
<td>0.143</td>
<td>0.59</td>
<td>0.85</td>
<td>0.31</td>
<td>0.035</td>
</tr>
<tr>
<td>Mean</td>
<td>23.5</td>
<td>0.071</td>
<td>0.67</td>
<td>0.80</td>
<td>0.15</td>
<td>0.07</td>
</tr>
</tbody>
</table>

a Short tandem repeat. Allele frequencies of the 6 loci differed significantly (P < 0.001) from those expected in a population in Hardy-Weinberg equilibrium.

b A frequency of null alleles of <0.07 was considered nonsignificant.

c Observed and expected heterozygosities ranged from 0 (no heterozygosity) to 1 (highest heterozygosity).

d Wright’s index indicates a deficiency of heterozygosity (positive values) or excess heterozygosity (negative values).

e Probability of identity values near zero indicate the highest discriminative power of the STR.
presence of outbreaks of candidemia, as most of the patients involved were in the unit at the same time (Fig. 3). The second group accounted for 3 of the 19 clusters that involved patients who were in different wards at the time of blood sample collection, although they had previously shared a hospital ward (Table 3).

The 27 patients in the remaining 9 clusters (47%) did not show a geographical relationship either at the time of blood sample collection or during the previous 2 years. The patients who were admitted were mainly adults (Table 4).

## DISCUSSION

Candidemia caused by *C. albicans* is generally nosocomial (5). Although *C. albicans* is part of the microbiota of patients with candidemia, the disease can also be caused by exogenous strains acquired during a hospital stay (10, 22, 23). Candidemia may be transmitted horizontally in hospitalized patients when it is caused by exogenous strains. Genotyping of isolates allows us to understand the role of nosocomial transmission of *C. albicans* strains in hospitalized patients (24, 25).

We observed that most patients (75%) were infected by differ-
ent genotypes, suggesting an endogenous origin, as reported by others (26, 27). However, we found that up to 25% of patients can be infected by endemic *C. albicans* genotypes. Consequently, the strains might have a common source, such as health care workers, biomedical devices, parenteral nutrition, and the hospital environment (13, 28, 29). Interestingly, only half of the patients infected by endemic genotypes were or had been admitted to the same ward at the time of blood sample collection; in these cases, the patients were usually in the ward at the same time. Genotyping of the strains from the patients admitted to the neonatology ward showed that endemic genotypes persisted in the unit for up to several months, as illustrated by the patients in clusters 1 and 18 (Fig. 3). However, several of the clusters were found in 2010 among patients who were in the unit at the same time, suggesting the presence of an outbreak of candidemia during that period.

Of note, 13% of the patients were infected by endemic genotypes, although we were unable to demonstrate any geographical relationship between them. The patients were mainly adults and had been admitted to the hospital at different times, as shown in Table 4. Some patients in these clusters (cluster codes 2, 3, 8, 11, and 12) were diagnosed with candidemia at similar times, thus suggesting a common source for the isolates. A potential explanation is the presence of persistent endemic genotypes adapted to surviving in common areas of the hospital. Patients may become infected when visiting these areas during their stay, after ingestion of contaminated food, or even after receiving contaminated medication. Another explanation might be that these genotypes occur more frequently than others (12, 30, 31) and can be actively transmitted from person to person, from the environment to patients, and from health care workers to patients.

The presence of clusters involving patients who are not geographically related may be a consequence of the limitation of the genotyping procedure. A lack of discrimination of the STR markers used was ruled out for different reasons. First, we found marked diversity, as shown by the total probability of identity of 1.05, which indicates that the probability of finding 2 strains with the same genotype was almost zero. Second, a clonal nature for the population structure is suggested by the statistically significant deviation from Hardy-Weinberg equilibrium, probably owing to heterozygote deficiency. Finally, heterozygote deficiency was not due to a lack of amplification of markers, as shown by the low frequency of null alleles. Heterozygote deficiency might be caused by the clonal nature of the *C. albicans* populations (31–34), by chromosomal rearrangements such as aneuploidy (a lack of chromosomes or presence of extra chromosomes), or by a loss of heterozygosity as a response to antifungal stress (35–37).

Our study has several limitations. We did not determine a potential source of infection or route of transmission in the hospital because we did not study isolates from the hospital environment, from health care workers, from other anatomical sites of the pa-

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**TABLE 3** Clusters involving patients who were admitted to different wards at the time of diagnosis of candidemia but who had a shared ward of admission in the previous 2 years

<table>
<thead>
<tr>
<th>Cluster code</th>
<th>No. of patients involved</th>
<th>Ward of admission at time of diagnosis</th>
<th>Date of blood culture collection (month/day/yr)</th>
<th>Ward of hospitalization in the previous month</th>
<th>Month of coincidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2</td>
<td>Pediatric ICU</td>
<td>04/13/2008</td>
<td>Pediatric hematology</td>
<td>March 2008</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>General surgery</td>
<td>05/09/2008</td>
<td>General surgery</td>
<td>May 2008</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
<td>Internal medicine</td>
<td>06/28/2007</td>
<td>Internal medicine</td>
<td>May 2008</td>
</tr>
</tbody>
</table>

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patients with candidemia, or even from mothers who could colonize and further infect neonates during delivery. Since strains may have been commensal fungi in the host, transmission between patients could be ruled out. Furthermore, we cannot exclude the possibility that endemic genotypes are a consequence of chromosomal rearrangements in the isolates or homoplasy (alleles with identical sizes but different sequences), so we must therefore accept them as an intrinsic limitation of microsatellite analysis.

In summary, we found marked genetic diversity among Candida albicans isolates causing candidemia. However, up to 25% of the patients were infected by endemic genotypes detected in 2 or more patients. Some of these endemic genotypes were found in the same units, whereas others infected patients in different wards. Future studies are necessary to clarify the sources and routes of transmission of endemic genotypes in hospitals.

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REFERENCES

TABLE 4 Clusters involving patients who were not admitted to the same ward at the time of blood sample collection or in the previous 2 years

<table>
<thead>
<tr>
<th>Cluster code</th>
<th>No. of patients involved</th>
<th>Date of blood culture collection (month/day/yr)</th>
<th>Ward of admission at time of diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
<td>7/17/2010</td>
<td>Angiology</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>4/26/2008</td>
<td>Digestive medicine</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>3/20/2008</td>
<td>Pediatric ICU</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>11/3/2008</td>
<td>General surgery</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>5/9/2008</td>
<td>Pneumology</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>5/10/2007</td>
<td>General surgery</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>12/10/2007</td>
<td>Digestive medicine</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>1/3/2008</td>
<td>Digestive medicine</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>7/7/2010</td>
<td>Angiology</td>
</tr>
</tbody>
</table>

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