Antifungal use influences Candida species distribution and susceptibility in the intensive care unit

Pierre Fournier1*, Carole Schwebel2, Danièle Maubon1, Aurélien Vesin3, Bernadette Lebeau1, Luc Foroni4, Rebecca Hamidfar-Roy2, Muriel Cornet1, Jean-François Timsit2,3 and Hervé Pelloux1

1Parasitology-Mycology Laboratory, Infectious Agent Department, Albert Michallon University Hospital and Joseph Fourier University, Grenoble cedex 9, France; 2Medical ICU, Albert Michallon University Hospital, Grenoble cedex 9, France; 3Inserm U823 (Outcome of cancer and critically ill patients), Albert Bonniot Institute, University Grenoble 1, Grenoble cedex 9, France; 4Pharmacy Department, Albert Michallon University Hospital and Joseph Fourier University, Grenoble cedex 9, France

*Corresponding author. Tel: +33-476-765-490; Fax: +33-476-765-228; E-mail: pfournier@chu-grenoble.fr

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Objectives: Antifungal prescription practices have changed over the last decade, and the impact of these changes is unclear. Our objective here was to evaluate the effect of antifungal drug use on the distribution and drug susceptibility of Candida spp. in a French intensive care unit (ICU).

Methods: Antifungal drug use was measured as the number of defined daily doses per 1000 hospital days (DDDs/1000HD). The distribution of Candida spp. over a 6 year period (2004–09) and the MICs of antifungal drugs over 2007–09 were determined. Statistical analyses were performed to assess relationships between antifungal drug use, Candida spp. distribution and MIC changes over time.

Results: Of 26450 samples from 3391 patients, 1511 were positive for Candida spp. Candida albicans predominated (52.5%), followed by Candida glabrata (16.6%) and Candida parapsilosis (7.5%). C. parapsilosis increased significantly, from 5.7% in 2004 to 12.5% in 2009 (P=0.0005). Caspofungin use increased significantly between 2004 (17.9 DDDs/1000HD) and 2009 (69.9 DDDs/1000HD) (P<0.0001). Between 2007 and 2009, the increase in caspofungin use correlated significantly with the increase in caspofungin MICs displayed by C. parapsilosis (P<0.0001) and C. glabrata (P=0.03). Amphotericin B consumption changed over time and correlated with an increase in amphotericin B MICs for C. albicans (P=0.0002) and C. glabrata (P=0.0005). Significant declines occurred in both fluconazole use (P<0.0001) and fluconazole MICs of C. albicans (P<0.001).

Conclusions: Antifungal drug use in the ICU is associated with major changes in the distribution and drug susceptibility of Candida spp.

Keywords: antifungal agents, drug utilization, fungal susceptibility tests

Introduction

Candida spp. are the most common cause of fungal infections in humans.1 The incidence of Candida infections has risen over the last two decades,2 in parallel with the increasing use of immuno-suppressive treatments and transplantation procedures. Critically ill patients are at high risk of opportunistic invasive fungal infections (IFIs),3 which are associated with high attributable mortality rates (10%–49%).3 These high mortality rates can be ascribed in part to the difficulty of diagnosing IFI at an early stage. Because the diagnosis is difficult, antifungal drugs are widely used as empirical or preemptive treatments in patients with suspected IFI.4

Since 2003, several new antifungals have been introduced. Among these, the echinocandins and new azoles are active against a broader spectrum of fungi, including Candida glabrata and Candida krusei. The echinocandin caspofungin and the new azole voriconazole are now used as first-line antifungal agents in many clinical situations.5 Several studies on changes in Candida spp. epidemiology identified prophylactic fluconazole therapy as a contributing factor.6/7 The emergence of non-albicans Candida species correlated with fluconazole use in some studies,8,9 although this finding was not replicated in other studies.10–12 The potential impact of extensive use of the new antifungal drugs on the epidemiology and susceptibility of Candida spp. is not systematically monitored. In two studies, increasing caspofungin use was associated with an increased incidence of Candida parapsilosis candidaemia.13,14 Antibiotic use has been proven to influence...
the epidemiology and drug susceptibility of bacteria responsible for human disease.\textsuperscript{15,16} The influence of antifungal drug use on species distribution or drug susceptibility has been assessed in studies focusing on candidaemia.\textsuperscript{6,8,13} Here, we hypothesized that antifungal drug use impacts the overall species distribution and drug susceptibility of Candida spp. Identifying effects of the use of a specific antifungal agent on Candida spp. distribution and susceptibility might inform treatment decisions. We evaluated antifungal drug use, and we determined the distribution and susceptibility of Candida spp. over a 6-year period in our intensive care unit (ICU). Correlations between observed changes were assessed.

**Methods**

The study was carried out in the adult ICU of the Michallon University Hospital in Grenoble, France, between 1 January 2004 and 31 December 2009. The ICU has 18 beds and serves medical and surgical patients, including transplant recipients and patients with haematological and solid malignancies.

**Antifungal drug use**

Data on antifungal drug use were extracted from the electronic database of the hospital pharmacy. In our study, we selected the most commonly used antifungal drugs: polyenes (including amphotericin B and liposomal amphotericin B), caspofungin, voriconazole and flucytosine. For itraconazole, posaconazole, 5-fluorocytosine, and the other echinocandins (micafungin and anidulafungin), the levels of use in our ICU was considered too low during the study period to exert major effects.

We converted antifungal drug doses from milligrams to defined daily doses per 1000 hospital days (DDDs/1000HD), in accordance with the Guidelines for ATC classification and DDD assignment (WHO Collaborating Centre for Drug Statistics Methodology; www.whocc.no). The DDDs were 70 mg for amphotericin B, 210 mg for liposomal amphotericin B, 50 mg for caspofungin, 400 mg for voriconazole and 400 mg for flucytosine. In a second step, we pooled the data for amphotericin B and liposomal amphotericin B to reflect overall polyene use.

**Sampling and Candida spp. identification**

All Candida-positive specimens from all sampling sources were considered, except those from superficial skin samples. When multiple isolates were obtained from the same patient, all species were included in the study but only the first isolate of a given species was considered in the analysis. Specimens were inoculated onto CAN2 chromogenic isolation plates and/or Sabouraud chloramphenicol tubes (bioMérieux, Lyon, France) and incubated for 3–6 days at 35°C or 27°C depending on the sampling source. The following rapid tests were used for identification: rapid assimilation or agglutination tests (Glabrata RTT, Bichro-Latex Albicans and Krusei-Color; Fumouze Diagnostics, Levallois-Perret, France) and api-ID32C (bioMérieux, Lyon, France). Blood samples were cultured on Mycosis IC/F and Bactec 9240 media (Becton Dickinson Inc., Sparks, Krusei-Color; Fumouze Diagnostics, Levallois-Perret, France) and use of a specific antifungal agent on changes were assessed.

**Antifungal drug susceptibility**

Yeast isolated from blood cultures, deep sites and normally sterile sites were tested routinely. For non-sterile sites (e.g. the lower respiratory tract), the decision to perform antifungal drug susceptibility testing was based on the underlying disease or at the physician's request. The Etest\textsuperscript{17} method (AB bioMérieux, Solna, Sweden) was used, and spanned the period from the beginning of 2007 to the end of 2009. Fluconazole, amphotericin B, caspofungin and voriconazole Etest strips were placed on RPMI 1640 agar (AES, Reuz, France) and incubated at 35°C for 24 h, as recommended by the manufacturer.

Antifungal breakpoints and MIC interpretation were in accordance with the CLSI.\textsuperscript{18} Many studies have shown good correlations between broth microdilution and Etest methods.\textsuperscript{19–23} To ensure reliable detection of MIC fluctuations, we created subcategories within the clinical categories defining susceptibility and resistance, for the three main species (C. albicans, C. glabrata and C. parapsilosis), as follows: fluconazole, 0–0.5 mg/L, 0.5–2 mg/L and ≥2 mg/L; amphotericin B, 0–0.5 mg/L, 0.5–1 mg/L and ≥1 to 4 mg/L; voriconazole, 0–1 mg/L and ≥1 to 32 mg/L; and caspofungin, 0–0.25 mg/L and ≥0.25 to 1 mg/L.

**Statistical analysis**

For the six most commonly isolated Candida spp., the monthly incidences and their changes over time were assessed using linear regression with correction for autocorrelation (AUTOREG procedure, SAS Inc., Cary, NC, USA). The Durbin–Watson statistic was used to identify significant autocorrelation terms. The same method was used to test for linear trends in antifungal drug use over time. Fisher’s test was performed to evaluate relationships between MIC categories and time periods; the χ² test for trend was used when only two MIC categories were available.

The relationship between monthly antifungal drug consumption and monthly median MIC of the same drug for a specific Candida spp. was assessed using a dynamic regression model.\textsuperscript{24} In our study, this method consisted of modelling MIC using the ARIMA model\textsuperscript{25} and adding drug consumption as an explanatory variable through a specific function (called ‘transfer function’). ARIMA was designed to model a quantitative series over time by identifying the correlation with the past values of the same variable (AR stands for autoregressive) and abrupt changes in the recent past (MA stand for moving average). This method allowed us to determine the most plausible time to occurrence of the potential effect of antifungal drug use on the MIC for each particular drug. The model-building process for each antifungal drug–Candida pair involved three steps: (i) an ARIMA model was fitted to the MIC series and to the drug-use series; (ii) the cross-correlations of the series were estimated to identify any significant and relevant delayed association over time; and (iii) the drug-use series was entered in the MIC ARIMA model using the transfer function in ARIMA (0,0,0) according to the lag found in step 2.

Series stationarity was tested (Dickey and Fuller test) and ensured by data differencing or transformation. The model yielding the lowest Akaike Information Criterion (AIC) value was chosen as the best model. Goodness-of-fit was assessed throughout model fitting using a white noise test of residuals and cross-correlation check of residuals.

**Results**

**Patient population**

During the 6-year period, we included 3391 patients, among whom 1061 had at least one positive specimen. The mean number of ICU admissions per year was 951 (SD 117; median 954), and the mean number of ICU hospitalization days per year was 5488 (SD 397; median 5530).

**Antifungal drug use**

Table 1 reports the overall antifungal drug use during the study period. In 2009 caspofungin was the most heavily used...
antifungal drug (69.9 DDDS/1000HD), followed by amphotericin B (45.3 DDDS/1000HD), voriconazole (37.5 DDDS/1000HD) and fluconazole (17.9 DDDS/1000HD). Caspofungin use increased steadily and significantly \((P<0.0001)\) during the study period. Fluconazole use showed a significant decrease \((P<0.0001)\). The use of voriconazole and amphotericin B remained unchanged over the study period \((P=0.22\) and \(P=0.5\), respectively).

### Distribution of Candida spp.

Table 2 reports the sources of the samples and the Candida culture results. Of the 26450 samples collected during the study period, 4886 (18.5%) were positive. Each strain was counted only once per patient, which yielded a total of 1511 Candida spp. isolates. Table 3 shows the distribution of Candida spp. C. albicans predominated (52.5%), followed by C. glabrata (16.6%), C. parapsilosis (7.5%), C. tropicalis (7.3%), Candida kefyr (5.4%), C. krusei (4.6%) and other Candida spp. (6.1%). The proportion of C. parapsilosis strains increased significantly, from 5.7% (10/174) in 2004 to 12.5% (32/256) in 2009 \((P<0.0001)\). No changes occurred over time for the other Candida spp., most notably C. glabrata.

### Antifungal susceptibility profiles

Table 4 reports the antifungal susceptibility data. The statistical analysis of drug susceptibility data for 2007–09 was confined to the three most common species, namely, C. albicans \((n=177)\), C. glabrata \((n=51)\) and C. parapsilosis \((n=34)\), resulting in 262 Etest determinations. The proportion of C. albicans strains with fluconazole MICs between 0 and 0.5 mg/L increased significantly, from 52% in 2007 to 90% in 2009 \((P<0.0001)\). All C. albicans, C. glabrata and C. parapsilosis strains had caspofungin MICs within the susceptible range. However, we found an increase in the proportion of C. parapsilosis strains displaying caspofungin MICs between 2.5 and 1 mg/L, from 71% in 2007 to 82% in 2009 \((P=0.86)\). All Candida spp. displayed stable and low voriconazole MICs over time. C. glabrata susceptibility against amphotericin B declined non-significantly from 2007 to 2009 \((P=0.59)\).

### Correlations with antifungal drug use

Increased caspofungin use correlated significantly with increased caspofungin MIC values of C. parapsilosis strains \((P<0.0001)\) 3 months later, and with increased caspofungin MIC values of C. glabrata strains \((P=0.03)\) 2 months later (Table 5). As an example, Figure 1 shows the influence of high caspofungin use in period 61 on predicted and actual caspofungin MICs of C. parapsilosis in period 64 (3 months later). Greater amphotericin B use correlated with increased amphotericin B MICs for C. albicans \((P=0.0002)\) and C. glabrata \((P=0.0005)\) 3–4 months later.

No other significant correlations were found; in particular, fluconazole use at any given point in time did not correlate significantly with the fluconazole MIC decrease.

### Discussion

The number of critically ill patients at high risk of IFIs is growing, and consequently antifungal drugs are used on an everyday basis in the ICU. The pharmacoeconomic impact of these drugs was assessed recently. Here, we assessed the impact of antifungal drug use on the epidemiology and susceptibility of Candida spp. in ICU patients.

The emergence of Candida strains with increased virulence and/or decreased drug susceptibility is of considerable concern, not only in the ICU but also in oncology and surgical wards. Close monitoring is needed, and if the results show an adverse impact of antifungal drug use, then new prescription strategies would need to be considered. Whereas many earlier studies focused on candidaemia,12–14,27 we investigated the impact of antifungal drug use on the distribution and susceptibility of Candida spp. in the ICU. Over a 6-year period, antifungal drug use significantly affected both of our study parameters.
Of all the Candida spp., the rise in C. parapsilosis incidence from 5.7% to 12.5% is one of the main findings of our study. Recent epidemiological studies have shown an increase in the incidence of C. parapsilosis infections; in some regions C. parapsilosis is now the second most frequently isolated Candida sp. from blood culture and from normally sterile body-sites of hospitalized patients. This rise has been attributed to a variety of risk factors, such as the high ability of C. parapsilosis to colonize intravascular material and prosthetic devices. Patients requiring prolonged use of a central venous catheter, such as ICU patients, are at increased risk of infection with C. parapsilosis. Colonization of the skin or gastrointestinal tract is frequently the first step in the pathogenesis of invasive candidiasis. In our study, we did not assess possible links between the rise in C. parapsilosis and other well-known risk factors, such as the extensive use of intravascular catheters, broad-spectrum antibiotics and surgical procedures. Nevertheless, during the study period, we noted a 4-fold increase in caspofungin use, which directly and significantly affected C. parapsilosis MIC values. Breakthrough C. parapsilosis bloodstream infections have been reported after prolonged caspofungin therapy. However, there are only limited data linking the increasing incidence of C. parapsilosis candidaemia to the growing use of caspofungin. According to the 2009 Infectious Diseases Society of America (IDSA) update of the clinical guidelines for managing candidiasis, echinocandins are now the recommended first-line drugs in many clinical situations, most notably for patients previously exposed to fluconazole and in those with C. glabrata infections. The potential effect of this practice on Candida spp. epidemiology and susceptibility is unclear. Here, using subcategories within those recommended by the CLSI, we documented significant changes in MICs over time. According to the 2 mg/L CLSI Table 4.

### Table 3. Global Candida spp. distribution from 2004 to 2009

<table>
<thead>
<tr>
<th>Organism</th>
<th>2004 (n = 177)</th>
<th>2005 (n = 147)</th>
<th>2006 (n = 144)</th>
<th>2007 (n = 136)</th>
<th>2008 (n = 126)</th>
<th>2009 (n = 141)</th>
<th>Total (n = 794)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>100 (57.5%)</td>
<td>147 (54.0%)</td>
<td>144 (50.9%)</td>
<td>136 (49.5%)</td>
<td>126 (50.2%)</td>
<td>141 (55.1%)</td>
<td>794 (52.5%)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>26 (14.9%)</td>
<td>57 (20.9%)</td>
<td>56 (19.8%)</td>
<td>42 (15.3%)</td>
<td>35 (13.9%)</td>
<td>35 (13.7%)</td>
<td>251 (16.6%)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>10 (5.7%)</td>
<td>10 (3.7%)</td>
<td>16 (5.6%)</td>
<td>24 (8.7%)</td>
<td>22 (8.8%)</td>
<td>32 (12.5%)</td>
<td>114 (7.5%)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>13 (7.8%)</td>
<td>16 (5.9%)</td>
<td>23 (8.1%)</td>
<td>22 (8.8%)</td>
<td>15 (6.0%)</td>
<td>15 (6.0%)</td>
<td>111 (7.3%)</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>6 (3.4%)</td>
<td>13 (4.8%)</td>
<td>14 (4.9%)</td>
<td>13 (4.7%)</td>
<td>19 (7.7%)</td>
<td>10 (4.0%)</td>
<td>111 (7.3%)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>6 (3.4%)</td>
<td>13 (4.8%)</td>
<td>14 (4.9%)</td>
<td>13 (4.7%)</td>
<td>19 (7.7%)</td>
<td>10 (4.0%)</td>
<td>111 (7.3%)</td>
</tr>
<tr>
<td>Other Candida spp.</td>
<td>9 (5%)</td>
<td>15 (5.6%)</td>
<td>15 (5.4%)</td>
<td>24 (8.7%)</td>
<td>14 (5.4%)</td>
<td>12 (4.7%)</td>
<td>89 (6.1%)</td>
</tr>
<tr>
<td>All Candida spp.</td>
<td>174 (100%)</td>
<td>272 (100%)</td>
<td>283 (100%)</td>
<td>275 (100%)</td>
<td>251 (100%)</td>
<td>256 (100%)</td>
<td>1511 (100%)</td>
</tr>
</tbody>
</table>

*P value of the autocorrelated error model.

### Table 4. MICs of fluconazole, amphotericin B, caspofungin and voriconazole for C. albicans, C. glabrata and C. parapsilosis from 2007 to 2009

<table>
<thead>
<tr>
<th>Drug and MIC range (mg/L)</th>
<th>C. albicans (n = 177)</th>
<th>C. glabrata (n = 51)</th>
<th>C. parapsilosis (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2007 (n = 58)</td>
<td>2008 (n = 54)</td>
<td>2009 (n = 65)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5-2</td>
<td>30 (52)</td>
<td>43 (80)</td>
<td>59 (90)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>7 (12)</td>
<td>1 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5-1</td>
<td>56 (97)</td>
<td>53 (98)</td>
<td>65 (100)</td>
</tr>
<tr>
<td>1-4</td>
<td>2 (3)</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Voriconazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1-1</td>
<td>58 (100)</td>
<td>53 (98)</td>
<td>65 (100)</td>
</tr>
<tr>
<td>1-32</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Caspofungin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25-1</td>
<td>56 (97)</td>
<td>54 (100)</td>
<td>63 (97)</td>
</tr>
<tr>
<td>0.25-2</td>
<td>2 (3)</td>
<td>0 (0)</td>
<td>2 (3)</td>
</tr>
</tbody>
</table>
Candida of fungin MICs between 0.25 and 1 mg/L. A recent 7-year study of correlation between these increasing MICs and increased caspofungin Drug C. albicans C. glabrata C. parapsilosis based on ARIMA models with transfer function caspofungin MIC values for MIC changes. We found not only a trend toward increasing categories used in our study allowed a more accurate analysis of echinocandins and amphotericin B as first- or second-line antifungals in C. glabrata infections may lead to the selection of resistant strains. C. glabrata and C. krusei occasionally show decreased susceptibility to amphotericin B, but resistance to amphotericin B during treatment is common with species such as Candida guilliermondii and Candida lusitaniae. For C. glabrata strains resistant to amphotericin B, mutations in the erg gene and modifications in the ergosterol composition of the plasma membrane have been described, suggesting adaptation or selection of these strains in response to amphotericin B exposure. For C. albicans, this phenomenon is less often reported, and further studies would be of interest. Overall, the use of caspofungin and amphotericin B seems to impact the antifungal susceptibility of Candida, an effect that requires close monitoring.

C. albicans fluconazole MICs diminished significantly over time in our study. There was no significant correlation with the decrease in fluconazole use, but improved fluconazole dosages and durations in patients with suspected or proven IFI may have contributed to these changes. In contrast to the C. glabrata increase reported in ICUs in the United States, this species did not appear as an emerging pathogen in our unit. Voriconazole use affected neither Candida spp. distribution nor antifungal drug susceptibility.

Alternative explanations for our findings include changes in the endogenous flora of patients with long hospital stays, and transmission among patients of a modified Candida population in the ICU. Previous data suggest an impact of both factors on the colonizing of Candida flora in ICU patients. In our study we took into account only the first available MIC value for a given strain, to avoid bias due to autocorrelation among MIC values of multiple isolates of the same strain. We were unable to determine whether this first isolate was endogenous or exogenous in origin. Antifungal drugs have a direct impact on the flora of patients receiving these drugs. Our results suggest that they may also have an indirect impact on patients who are not receiving antifungal drugs but who are exposed to these more-resistant strains. C. parapsilosis is a common skin commensal, and can therefore be readily transmitted. Clearly, the impact of antifungal drug use must be monitored closely, particularly for species that are easily spread via the hands of healthcare workers.

In conclusion, in our ICU the epidemiology and drug susceptibility of Candida spp. changed over a 6-year period. Increased caspofungin use was associated with increases in the incidence and caspofungin MICs of C. parapsilosis and C. glabrata, while amphotericin B consumption affected C. glabrata and C. albicans. On the other hand, decreased fluconazole use was accompanied by increased overall susceptibility of C. albicans to fluconazole. Significant MIC changes occurred during our brief study period, suggesting a rapid adaptation mechanism. Given the rapid increase in antifungal drug use, close monitoring is recommended. Strict rules for antifungal drug use should be devised to minimize the emergence of strains characterized by decreased susceptibility, especially in the ICU, where factors such as the cumulative length of hospital stays enhance antifungal resistance. Furthermore, MIC thresholds should be assessed.

### Table 5. Relationship between monthly antifungal consumption and Candida spp. distribution nor antifungal drug susceptibility.

<table>
<thead>
<tr>
<th>Drug</th>
<th>C. albicans</th>
<th>C. glabrata</th>
<th>C. parapsilosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspofungin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$ (SE)$^a$</td>
<td>0.0007 (0.0003)</td>
<td>0.003 (0.0003)</td>
<td></td>
</tr>
<tr>
<td>time lag 2 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NSCC</td>
<td>NSCC</td>
<td>NSCC</td>
</tr>
<tr>
<td>Fluconazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$ (SE)$^a$</td>
<td>0.01 (0.008)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>time lag 6 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value 0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NSCC</td>
<td>NSCC</td>
<td>NSCC</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$ (SE)$^a$</td>
<td>0.0017 (0.0004)</td>
<td>0.0066 (0.0017)</td>
<td>NSCC</td>
</tr>
<tr>
<td>time lag 3 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value 0.0002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NSCC</td>
<td>NSCC</td>
<td>NSCC</td>
</tr>
<tr>
<td>Voriconazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$ (SE)$^a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>time lag</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NSCC, no significant cross-correlation.

$^a$ $\beta$ (SE) is the estimate of the effect of antifungal use in previous months (time lag) on Candida MICs for an antifungal, after inclusion in an ARIMA model designed to predict the MIC time series.

breakpoint, all Candida spp. tested in this study remained susceptible in vitro to caspofungin, as reported previously. However, recent results indicate that the CLSI breakpoints lack sensitivity for detecting echinocandin resistance. The MIC subcategories used in our study allowed a more accurate analysis of MIC changes. We found not only a trend toward increasing caspofungin MIC values for C. parapsilosis, but also a significant correlation between these increasing MICs and increased caspofungin use at our institution. We documented a surprisingly rapid increase in the incidence of C. parapsilosis isolates having caspofungin MICs between 0.25 and 1 mg/L. A recent 7-year study of patients with candidaemia in France showed that previous caspofungin exposure was associated with increased proportions of Candida spp. having high caspofungin MICs, including C. parapsilosis and C. glabrata. Similarly, we have demonstrated that a high level of caspofungin use in the ICU was associated with an increase in C. parapsilosis and C. glabrata strains exhibiting high caspofungin MICs after a few months. Our study confirms the presence of a selective pressure exerted by echinocandins on Candida spp. The high in vitro echinocandin MICs usually found for C. parapsilosis are generally attributed to the natural polymorphism in the fks1 gene sequence of this organism. For C. glabrata, hot-spot mutations in fks2 contribute significantly to the decreased susceptibility to echinocandins. Our study not only confirms the relationship between C. parapsilosis and caspofungin, but also corroborates recent data about the impact of caspofungin on C. glabrata.

Although the antifungal activity of amphotericin B was excellent, increased use of amphotericin B was followed by increased amphotericin B MICs for C. albicans and C. glabrata. These new results are unsurprising, as the recommendation to use echinocandins and amphotericin B as first- or second-line antifungals in C. glabrata infections may lead to the selection of resistant strains. C. glabrata and C. krusei occasionally show decreased susceptibility to amphotericin B, but resistance to amphotericin B during treatment is common with species such as Candida guilliermondii and Candida lusitaniae. For C. glabrata strains resistant to amphotericin B, mutations in the erg gene and modifications in the ergosterol composition of the plasma membrane have been described, suggesting adaptation or selection of these strains in response to amphotericin B exposure. For C. albicans, this phenomenon is less often reported, and further studies would be of interest. Overall, the use of caspofungin and amphotericin B seems to impact the antifungal susceptibility of Candida, an effect that requires close monitoring.

Amphotericin B fluconazole MICs diminished significantly over time in our study. There was no significant correlation with the decrease in fluconazole use, but improved fluconazole dosages and durations in patients with suspected or proven IFI may have contributed to these changes. In contrast to the C. glabrata increase reported in ICUs in the United States, this species did not appear as an emerging pathogen in our unit. Voriconazole use affected neither Candida spp. distribution nor antifungal drug susceptibility.

Alternative explanations for our findings include changes in the endogenous flora of patients with long hospital stays, and transmission among patients of a modified Candida population in the ICU. Previous data suggest an impact of both factors on the colonizing of Candida flora in ICU patients. In our study we took into account only the first available MIC value for a given strain, to avoid bias due to autocorrelation among MIC values of multiple isolates of the same strain. We were unable to determine whether this first isolate was endogenous or exogenous in origin. Antifungal drugs have a direct impact on the flora of patients receiving these drugs. Our results suggest that they may also have an indirect impact on patients who are not receiving antifungal drugs but who are exposed to these more-resistant strains. C. parapsilosis is a common skin commensal, and can therefore be readily transmitted. Clearly, the impact of antifungal drug use must be monitored closely, particularly for species that are easily spread via the hands of healthcare workers.

In conclusion, in our ICU the epidemiology and drug susceptibility of Candida spp. changed over a 6-year period. Increased caspofungin use was associated with increases in the incidence and caspofungin MICs of C. parapsilosis and C. glabrata, while amphotericin B consumption affected C. glabrata and C. albicans. On the other hand, decreased fluconazole use was accompanied by increased overall susceptibility of C. albicans to fluconazole. Significant MIC changes occurred during our brief study period, suggesting a rapid adaptation mechanism. Given the rapid increase in antifungal drug use, close monitoring is recommended. Strict rules for antifungal drug use should be devised to minimize the emergence of strains characterized by decreased susceptibility, especially in the ICU, where factors such as the cumulative length of hospital stays enhance antifungal resistance. Furthermore, MIC thresholds should be assessed.

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as tools for improving the detection of changes in susceptibility to antifungal drugs.

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