In vitro susceptibilities of bloodstream isolates of *Candida* spp.: results from a multicenter active surveillance program in Andalusia

Carmen Florez a,*, Estrella Martín-Mazuelos a, Maite Ruiz b, José Miguel Cisneros c, Marta Herrero c, Mª Victoria García d, Manuel Márquez e, José Porras f, Patricia Martín g, Carmen Gamero h, Juan José Castón i and Grupo de Estudio de las Candidemias en Andalucía (Andalusian Study Group for Candidemia)

b Servicio de Microbiología, Hospital Virgen del Rocío, Seville, Spain
c Servicio de Enfermedades Infecciosas, Hospital Virgen del Rocío, Seville, Spain
a Servicio de Microbiología, Hospital de Valme, Seville, Spain
d Servicio de Microbiología, Hospital Virgen de la Victoria, Malaga, Spain
e Servicio de Enfermedades Infecciosas, Hospital Carlos Haya, Malaga, Spain
f Servicio de Microbiología, Hospital Carlos Haya, Malaga, Spain
g Servicio de Enfermedades Infecciosas, Hospital Reina Sofía, Cordoba, Spain
h Servicio de Microbiología, Hospital Reina Sofía, Cordoba, Spain
i Servicio de Enfermedades Infecciosas, Hospital Reina Sofía, Cordoba, Spain

ABSTRACT

Objectives: The aim of this study was to determine the antifungal drug susceptibilities of *Candida* bloodstream isolates in Andalusia, obtained through a multicenter active laboratory-based surveillance between October 2005 and September 2006.

Methods: One hundred and ninety-seven *Candida* isolates were collected. The MICs of amphotericin B, fluconazole, itraconazole and voriconazole were established using the Sensititre YeastOne panel. The MICs of posaconazole and caspofungin were determined by Etest.

Results: *C. albicans* was the most frequently isolated species (49.2%), followed by *C. parapsilosis* (17.3%), *C. tropicalis* (15.2%), *C. glabrata* (13.7%) and *C. krusei* (3.6%). All strains were inhibited at MICs of \( \leq 1 \) mg/L of amphotericin B and 98.5% of isolates were inhibited at MICs of \( \leq 1 \) mg/L of posaconazole. A total of 8 isolates (4.1%) were classified as resistant to fluconazole (MIC \( \geq 64 \) mg/L) and 7 (3.6%) were considered resistant to itraconazole (MIC \( \geq 1 \) mg/L). All the isolates were susceptible to voriconazole and caspofungin.

Conclusion: In our study *C. krusei* and *C. glabrata* were identified in over 18% of cases of candidemia. Most clinical isolates of these species are resistant or susceptible-dose-dependent to fluconazole but susceptible to voriconazole and caspofungin. These agents must be used in the empiric treatment of candidemia rather than fluconazole.

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Palabras clave:
Candidemia
*Candida* spp.
Sensibilidad antifúngica
Introduction

Candidemia is the fourth most common hospital-acquired bloodstream infection in the USA\(^1\) and is associated with high morbidity and mortality.\(^2\) Within the rising incidence of bloodstream infections caused by yeasts, there has been a sharp increase in the percentage caused by Candida species other than Candida albicans, such as Candida glabrata and Candida krusei.\(^3\) C. krusei is innately resistant to fluconazole,\(^4\) and C. glabrata often develops acquired resistance to this agent.\(^5\) Previous investigations have suggested that prior exposure to fluconazole may be a risk factor for subsequent infection with C. glabrata and C. krusei.\(^6,7\) The increase in infections caused by these Candida species presents a particular challenge for the clinical efficacy of triazole antifungal agents. Among several new triazole and echinocandin agents, voriconazole, posaconazole, and caspofungin appear to be highly active against all Candida species, including those that are less susceptible or resistant to fluconazole. There are certain differences in the distribution of species and antifungal drug susceptibilities between countries, and this fact underscores the need for continuing surveillance to monitor the trends related to these factors.\(^8–10\)

To date, there is little data on Candida bloodstream infection in Andalusia. Thus, we conducted a prospective multicenter surveillance study from 2005 to 2006 for Candida bloodstream infection in Andalusian adults to determine the distribution of the species involved in these infections and the percentages of antifungal drug resistance among the isolated strains. Herein, we report the antifungal drug susceptibility profiles of the strains found to amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, and caspofungin.

Materials and methods

Isolates

Between 1 October 2005 and 30 September 2006, 197 Candida isolates from adults with bloodstream infection were collected as part of a multicenter active surveillance program conducted in the region of Andalusia (Spain) by SAEI-SAMPAC (Andalusian Infectious Diseases Society-Andalusian Society of Clinical Microbiology and Parasitology). Seventeen hospitals participated (6 university tertiary care centers and 11 secondary care hospitals). Among the total of isolates collected, 115 were from tertiary hospitals and 82 from secondary care hospitals. Candidemia detection and identification of the species involved were performed at the participating laboratories and confirmed by the Valme laboratory, using the Vitek 2 ID-YST card (bioMerieux Vitek, Inc., Hazelwood, MO).\(^11\) Candida parapsilosis ATCC 22019 and C. krusei ATCC 6258 were used as quality control organisms for antifungal drug susceptibility testing.

Susceptibility testing

The minimum inhibitory concentrations (MICs) of amphotericin B, fluconazole, itraconazole, and voriconazole were determined at Valme Laboratory using the Sensititre YeastOne panel (Trek Diagnostic Systems, East Grinstead, UK).\(^12\) which incorporates alamarBlue as the oxidation-reduction colorimetric indicator (fungal growth changes the indicator from blue to pink). Susceptibility testing, reading, and interpretation of the results were performed in accordance with the manufacturer’s instructions, as follows: 20 μL of inoculum suspension was added to 11 mL of RPMI 1640 broth to obtain a working suspension (approximately 1.5–5 × 10\(^3\) cells/mL), and 100 μL of this suspension was added to each well. The panels were sealed and incubated in air at 37 °C. The azoles were read after 24 h, and amphotericin B was read after 48 h; The MIC was the first well that did not show a color change from blue to pink.

The MICs of posaconazole and caspofungin were determined at the Valme Laboratory by Etest (AB BIODISK, Solna, Sweden)\(^12\) on RPMI 1640 agar plates with 2% glucose according to the manufacturer’s instructions. Etest strips containing concentrations ranging from 0.002 mg/L to 32 mg/L were used. MICs were read where the edge of the inhibition ellipse intersected the MIC scale on the Etest strip after 24 h of incubation in air at 37 °C.

The interpretive breakpoints were those proposed in the Clinical and Laboratory Standards Institute (CLSI) M44-S1 reference method for fluconazole, itraconazole and voriconazole\(^13\) and in the CLSI M27-A3 reference method for caspofungin.\(^14\) Isolates showing fluconazole MICs of ≤8.0 mg/L were considered susceptible (S), those with MICs of 16 to 32 mg/L were considered susceptible dose dependent (S-DD), and isolates with fluconazole MICs of ≥64 mg/L were considered resistant (R). These breakpoints applied to all Candida spp. with the exception of C. krusei, which is considered inherently resistant to fluconazole, regardless of the MIC value.\(^4\) The interpretive breakpoints defined for itraconazole were the following: S, ≤0.12 mg/L; S-DD, 0.25 to 0.5 mg/L; and R, >1.0 mg/L. Isolates showing voriconazole MICs of ≤1 mg/L were classified as susceptible, those with MICs of 2 mg/L as S-DD, and those with MICs of ≥4 mg/L as resistant. The interpretive breakpoints defined for caspofungin were S, ≤2 mg/L; and R, >4 mg/L. Although interpretive breakpoints for amphotericin B have not been established, Candida isolates showing MICs of >1 mg/L are likely to be resistant to amphotericin B.\(^4\) Interpretive criteria have not been established for posaconazole.

Results

During the 12-month study period, a total of 197 Candida bloodstream infections in adults were reported by the 17 SAEI-SAMPAC Candidemia Program participants. The species distribution and in vitro susceptibilities of the 197 Candida strains to amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole and caspofungin are shown in Table 1. The results are reported as MIC ranges, and the MICs at which 50% (MIC\(_{50}\)) and 90% (MIC\(_{90}\)) of the isolates tested were inhibited by each drug. C. albicans was the most common isolate (49.2%), followed by C. parapsilosis (17.3%), Candida tropicalis (15.2%), C. glabrata (13.7%), and C. krusei (3.6%).

Amphotericin B MICs were in the range 0.01–1 mg/L, with C. krusei isolates demonstrating the highest MIC\(_{90}\) values (1 mg/L).
C. krusei and C. glabrata showed the highest MICs of fluconazole, itraconazole, voriconazole, and posaconazole. A total of 8 isolates (4.1%) were classified as resistant to fluconazole (MIC ≥64 mg/L) and 13 (6.6%) were S-DD (16–32 mg/L). Seven isolates (3.6%) were classified as resistant to itraconazole (MIC ≥1 mg/L) and 37 (18.8%) were S-DD (MIC 0.25–0.5 mg/L). All isolates were classified as susceptible to voriconazole (<0.1 mg/L). Posaconazole MICs were in the range 0.002–8 mg/L, with a MIC 50 and MIC 90 of 0.04 and 0.19 mg/L, respectively; C. glabrata showed the highest MICs. Posaconazole MICs of ≥1 mg/L were demonstrated for only 3 C. glabrata isolates (1.5%), 1 of which was resistant and 2 S-DD to fluconazole. All isolates were susceptible to caspofungin, with C. parapsilosis and Candida guilliermondii showing the highest MICs. C. albicans was the most susceptible species to all the antifungal drugs studied.

Discussion

We present the species distribution and in vitro susceptibility data obtained in a multicenter active surveillance program for Candida spp. isolates causing bloodstream infection in adults in the south of Spain between 1 October 2005 and 30 September 2006. Although C. albicans remains the predominant species in these infections, the frequency with which it occurs varies worldwide from as low as 37% in the USA to a high of 70% in Norway. C. albicans was the predominant species in our geographic area, accounting for 49% of all Candida bloodstream infections, a value similar to reported rates in Spain and other countries in Europe. C. parapsilosis and Candida guilliermondii showed the highest MICs. C. albicans was the most susceptible species to all the antifungal drugs studied.
been reported in other studies from Spain, although at a lower incidence than the 29% and 23% described in these studies. It is, however, higher than the 1% to 6% reported for C. parapsilosis from Switzerland, Denmark and Norway. These differences might be attributable to differences in the population studied and healthcare practices. In other reports, C. parapsilosis was isolated in 45% to 67% of neonatal candidemias, and was associated with nosocomial spread.

In the present study, C. glabrata was the fourth most common species isolated, whereas in the United States and Europe it was the second most common. The low percentage of species isolated, whereas in the United States and Europe it was similar to other reported rates, but lower than the 15% seen in Germany, and the USA.

Our susceptibility results are in keeping with those of other studies: resistance is uncommon among C. albicans and C. parapsilosis, and there is a high level of reducedazole susceptibility among C. glabrata. Overall resistance to fluconazole was documented in 4.1% of the tested strains, a percentage similar to other reported rates, but lower than the 15% seen in Portugal. Complete resistance to itraconazole was recorded in 3.6% of isolates. This finding is consistent with a report from the USA, but is lower than the 12.6% to 19.4% described in Belgium, Germany, and Spain.

In this study, amphotericin B MICs were in the range of 0.01–1 mg/L, with a MIC of 1 mg/L for C. krusei. The decreased susceptibility of C. krusei to amphotericin B is consistent with previous reports for this microorganism. In other studies, 0.4% to 5% of isolates demonstrated potential resistance, with amphotericin B MICs of ≥2 mg/L.

Fluconazole resistance was not always associated with resistance to the other azoles studied; in some cases, only an increase in the MIC endpoint was observed. Moreover, intrinsically fluconazole-resistant C. krusei showed no cross resistance with other azoles. The new azoles, posaconazole and voriconazole, displayed potent antifungal activity against all Candida spp. including C. glabrata and C. krusei. In this study, all the isolates were susceptible to voriconazole and 98.5% of the strains were inhibited at MICs of ≤1 mg/mL of posaconazole. Our results are consistent with those of other studies.

All the isolates studied were susceptible to caspofungin. Of note are the relatively high MICs that were seen for C. parapsilosis and C. guilliermondii. These results are consistent with other reports.

In conclusion, this study identified C. krusei and C. glabrata in 17.3% of candidemia cases. Most clinical isolates of these species were resistant or susceptible dose dependent to fluconazole, but were susceptible to voriconazole and caspofungin. Hence, these latter agents should be used in empirical treatment for candidemia rather than fluconazole.

Acknowledgments

The other members of the Andalusian Candidaemia Study Group are Consuelo Miranda and Elisa Vidal (Hospital Virgen de las Nieves, Granada), Ana García and Francisca María Guerrero (Hospital Puerta del Mar, Cadiz), María Dolores López and Carolina García (Hospital de Jerez, Jerez), Natalia Montiel and Alfonso del Arco (Hospital Costa del Sol, Marbella), Petra Navas and Antonio Collado (Hospital de Torrecardenas, Almeria), José María Saavedra and Francisco Martínez (Hospital Juan Ramón Jiménez, Huelva), Trinidad Escobar and Alejandro Peña (Hospital San Cecilio, Granada), María Jesús Pérez and Fernando Salgado (Hospital de Ronda, Ronda), Marina Cueto and Jesús Rodríguez (Hospital Virgen Macarena, Sevilla), Juan Corzo (Hospital de Valme, Seville), Iria Jesús de la Calle and Antonio Vergara (Hospital de Puerto Real, Puerto Real), Antonio Sánchez and Montserrat Pérez (Hospital de La Linea, La Linea de La Concepción).

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