Candida species distribution and antifungal susceptibility testing according to European Committee on Antimicrobial Susceptibility Testing and new vs. old Clinical and Laboratory Standards Institute clinical breakpoints: a 6-year prospective candidaemia survey from the fungal infection network of Switzerland

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Abstract

We analyzed the species distribution of *Candida* blood isolates (CBIs), prospectively collected between 2004 and 2009 within FUNGINOS, and compared their antifungal susceptibility according to clinical breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in 2013, and the Clinical and Laboratory Standards Institute (CLSI) in 2008 (old CLSI breakpoints) and 2012 (new CLSI breakpoints). CBIs were tested for susceptiblity to fluconazole, voriconazole and caspofungin by microtitre broth dilution (Sensititre® YeastOne™ test panel). Of 1090 CBIs, 675 (61.9%) were *C. albicans,* 191 (17.5%) *C. glabrata*, 64 (5.9%) *C. tropicalis*, 59 (5.4%) *C. parapsilosis*, 33 (3%) *C. dubliniensis*, 22 (2%) *C. krusei* and 46 (4.2%) rare *Candida* species. Independently of the breakpoints applied, *C. albicans* was almost uniformly (>98%) susceptible to all three antifungal agents. In contrast, the proportions of fluconazole- and voriconazole-susceptible *C. tropicalis* and F-susceptible *C. parapsilosis* were lower according to EUCAST/new CLSI breakpoints than to the old CLSI breakpoints. For caspofungin, non-susceptibility occurred mainly in *C. krusei* (63.3%) and *C. glabrata* (9.4%). Nine isolates (five *C. tropicalis*, three *C. albicans* and one *C. parapsilosis*) were cross-resistant to azoles according to EUCAST breakpoints, compared with three isolates (two *C. albicans* and one *C. tropicalis*) according to new and two (2 *C. albicans*) according to old CLSI breakpoints. Four species (*C. albicans, C. glabrata, C. tropicalis* and *C. parapsilosis*) represented >90% of all CBIs. *In vitro* resistance to fluconazole, voriconazole and caspofungin was rare among *C. albicans*, but an increase of non-susceptibile isolates was observed among *C. tropicalis*/*C. parapsilosis* for the azoles and *C. glabrata*/*C. krusei* for caspofungin according to EUCAST and new CLSI breakpoints compared with old CLSI breakpoints.

Keywords: Breakpoint, *Candida*, candidaemia, Clinical and Laboratory Standards Institute, European Committee on Antimicrobial Susceptibility Testing, resistance, species
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Introduction

Candida species are among the top ten pathogens causing bloodstream infections [1]. Candidaemia is an invasive fungal infection associated with substantial morbidity, mortality and

healthcare costs [2]. Changes in species distribution and a shift to more resistant isolates are increasingly described [3–5]. There have been significant differences in clinical breakpoint values defined by the Antifungal Susceptibility Testing Subcommittee of the Clinical and Laboratory Standards Institute (CLSI) in the USA and by the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST) in Europe. In recent years a harmonization of these breakpoints as well as the definition of species-specific breakpoints has been achieved and the breakpoints have been lowered in order to better detect low level resistance [6–8].

The goal of our study was to analyse the species distribution of *Candida* blood isolates (CBIs) prospectively collected in the hospitals of the Fungal Infection Network of Switzerland (FUNGINOS) and to determine antifungal susceptibility to fluconazole, voriconazole and caspofungin according to the new species-specific clinical breakpoints defined by the EUCAST in Europe (in 2013) as well as by the CLSI (in 2008 [old CLSI breakpoints] and 2012 [new CLSI breakpoints]) in the USA. We also aimed to evaluate the frequency of cross- and multiresistant isolates.

Material and Methods

Participating hospitals and microbiology laboratories

All Swiss university hospitals (n = 7) and a representative sample of university-affiliated tertiary care centres (n = 10) of FUNGINOS prospectively collected CBIs between 1 January 2004 and 31 December 2009.

Sixteen microbiology laboratories were affiliated with the 17 participating hospitals. All laboratories used automated blood culture systems [11 Bactec (Becton Dickinson, Sparks, MD, USA) and five BacT/Alert (bioMérieux, Durham, NC, USA)]. The CBIs of each participating centre were sent to the FUNGINOS mycology reference laboratory in Lausanne.

Species identification, antifungal susceptibility testing and interpretation

In the mycology reference laboratory, the CBI were identified by recognized standard laboratory techniques [9] and antifungal susceptibility testing for fluconazole, voriconazole and caspofungin was performed using the microtitre broth dilution method with the Sensititre® YeastOne™ test panel (version 4.0 from 2004 to 2007; version 7.0 from 2007 to 2009).

Interpretation of susceptibility was performed by applying the clinical interpretive breakpoints defined by the CLSI in 2008 (≪old CLSI breakpoints≫) [10,11] and 2012 (≪new CLSI breakpoints≫) [12] as well as EUCAST in March 2013 (≪EUCAST breakpoints≫; http://www.eucast.org/clinical_breakpoints/; version 6.1). EUCAST has not yet defined clinical breakpoints for caspofungin.

The proportions of susceptible vs. non-susceptible or resistant CBIs were calculated and compared according to EUCAST and CLSI breakpoints.

Definitions

Susceptibility and non-susceptibility. A CBI was considered susceptible when the minimal inhibitory concentration (MIC) was at or below the breakpoint defined by EUCAST or CLSI. Non-susceptibility of a CBI was defined when its MIC was higher than the breakpoints defined by EUCAST/CLSI and includes both dose-dependent susceptible, intermediate and resistant isolates.

Cross-resistance. Cross- resistance was defined as resistance to two antifungals of the same drug class. We evaluated cross-resistance to azoles, defined as resistance to the two azoles tested (fluconazole and voriconazole).

Multi-resistance. Multi-resistance was defined as resistance to two antifungal drug classes, namely the azoles (fluconazole and voriconazole) and echinocandin tested (caspofungin).

Data collection and analysis

For data entry and analysis Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA) and its tools were used.

Results

Species distribution

Within the 6 years of the study, a total of 1090 CBIs underwent central re-identification and susceptibility testing. The most frequently isolated species were *C. albicans* (675; 61.9%) followed by *C. glabrata* (191; 17.5%), *C. tropicalis* (64; 5.9%) and *C. parapsilosis* (59; 5.4%), accounting for 90.7% of the total number of isolates. The remaining 9.3% of the species consisted of *C. dubliniensis* (33; 3%), *C. krusei* (22; 2%), *C. lusitaniae* C (12; 1.1%), *C. guilliermondii* (9; 0.8%), *C. kefyr* (8; 0.7%), *C. pelliculosa* (6; 0.6%), *C. famata* (4; 0.4%), *C. norvegensis* (3; 0.3%), *C. inconspicua* (2; 0.2%) and *C. rugosa* (2; 0.2%).

Antifungal susceptibility

We applied interpretive breakpoints defined by EUCAST and CLSI [6–8] [http://www.eucast.org/clinical_breakpoints/; version 6.1], summarized in Table 1. The percentage of susceptibility of the different *Candida* species to fluconazole, voriconazole and caspofungin is shown in Fig. 1(a–c).