

CANDIDA BLOODSTREAM INFECTION: A CLINICAL MICROBIOLOGY LABORATORY PERSPECTIVE

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The incidence of *Candida* bloodstream infection (BSI) has been on the rise in several countries worldwide. Species distribution is changing; an increase in the percentage of non-*albicans* species, mainly fluconazole non-susceptible *C. glabrata* was reported. Existing microbiology diagnostic methods lack sensitivity, and new methods need to be developed or further evaluation for routine application is necessary. Although reliable, standardized methods for antifungal susceptibility testing are available, the determination of clinical breakpoints remains challenging. Correct species identification is important and provides information on the intrinsic susceptibility profile of the isolate. Currently, acquired resistance in clinical *Candida* isolates is rare, but reports indicate that it could be an issue in the future. The role of the clinical microbiology laboratory is to isolate and correctly identify the infective agent and provide relevant and reliable susceptibility data as soon as possible to guide antifungal therapy.

Keywords: *Candida* spp., bloodstream infection, clinical breakpoints

Introduction

Candida BSI has become a major healthcare issue in the past couple of decades due to a growing patient population at risk. *Candida* spp. are the fourth most common cause of nosocomial BSI in the US, accounting for 4.6 BSI per 10,000 hospital admissions [1]. The annual incidence in Europe ranges between 3.0 to

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8.6/100,000 inhabitants according to nationwide studies performed in Norway [2], Spain [3], and Denmark [4]. *Candida* BSI is most prevalent in the ICU following abdominal surgery: in a survey of 47,707 cases, Kuhns et al. identified *Candida* spp. as the cause of 20.3% of BSIs [5].

The most common isolated species is *C. albicans*, but reports show a growing proportion of non-albicans species [6–8]. Increasing resistance rates have also become an issue in some countries [9]. The clinical microbiology laboratory has a vital role in the timely identification and antifungal susceptibility testing of isolates in order to aid the clinician in selecting the most appropriate treatment regime.

Microbiological diagnosis

The gold standard of diagnosing *Candida* BSI is blood culture. European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines for the diagnosis of candidaemia recommend the culture of 3 pairs (aerobic and anaerobic bottles) of blood cultures every day if candidaemia is suspected [10]. An additional special mycosis blood culture bottle may increase sensitivity and decrease time to detection, especially in the case of *C. glabrata* and the BACTEC system [2]. The incubation time to positivity varies by species: Arendrup found that 75% of cases of *C. tropicalis* and *C. krusei* BSI were detected in 2 days, *C. albicans* and *C. parapsilosis* in 3 days and *C. glabrata* in 4 days [11].

The sensitivity of blood culture in detecting *Candida* spp. is only 50–75%, with even lower rates among haematologic patients and patients receiving antifungal treatment [4, 12]. Furthermore, mortality is increased greatly by the delay of antifungal therapy (from 10–15% if treatment was started on the day of blood culture to 35–40% if treatment was started 3 days later) [13, 14]. Consequently, faster and more sensitive diagnostic methods are needed to detect *Candida* BSI.

Since colonization by *Candida* spp. in non-sterile sites have been shown to precede an invasive infection, different score systems, such as the Candida Colonization Index (CCI) can be used successfully to identify patients at risk of invasive *Candida* infection with a positive predictive value of 66% and a negative predictive value of 100% if the CCI > 0.5 [12, 15, 16].

Non-culture based diagnostic methods have also become available. The combined detection of mannan and anti-mannan antibodies from serum has been recommended by ESCMID to rule out infection. The test has a high negative pre-

dictive value (>85%), a sensitivity of 80% and specificity of 85%; it can also be positive 6 days on average before the blood culture. The detection of β -1,3-D-glucan, with a similar high negative predictive value, was also recommended to rule out invasive fungal disease [10, 17].

PCR-based methods may be valuable tools in the future, but currently there is no recommendation for their use due to the lack of standardized technique and furthermore there is only scarce data available regarding their clinical relevance [10, 18, 19].

***Candida* spp.: Species characteristics and epidemiology**

C. albicans remains the most frequently isolated species in BSI in most countries worldwide, although numerous reports have detected a shift toward non-*albicans* species [3, 20, 21]. The rise in the number of BSI caused by *C. glabrata* has been shown to correlate with increased fluconazole exposure [22].

Correct species identification is vital in guiding antifungal therapy, since different species possess different intrinsic susceptibility patterns.

Besides conventional identification methods based on biochemical profile analysis and the morphology of pseudohyphae on malt agar, new techniques have become available in the diagnostic laboratory such as Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) and molecular-based techniques making possible the identification of rare, emerging and cryptic species.

C. nivariensis was identified in 2005 and was found to be genetically closely related to *C. glabrata*, thus they are difficult to separate from each other by conventional methods. However, *C. nivariensis* frequently exhibits multidrug resistance against several azoles and flucytosine [23].

C. palmioleophila has been found to be more prevalent in Denmark than previously thought, since the species is frequently misidentified as *C. famata* or *C. guilliermondii* by conventional methods. *C. famata* displays low MIC values against fluconazole and echinocandins, while fluconazole MIC values for *C. palmioleophila* and *C. guilliermondii* are higher ($\geq 16 \mu\text{g/ml}$ and $\geq 2 \mu\text{g/ml}$); *C. guilliermondii* has reduced echinocandin susceptibility, while *C. palmioleophila* is susceptible *in vitro* [24].

The different species differ in virulence and can be divided into three groups: the most virulent species are *C. albicans* and *C. tropicalis*, followed by *C. glabrata*, *C. lusitanae* and *C. kefyr*, and the least virulent are *C. parapsilosis*,

C. krusei and *C. guilliermondii* [11]. These differences do not only translate into epidemiologic differences and mortality rates that vary by species, but must be taken into consideration when antifungal breakpoints are determined.

Biofilm production may be an important virulence factor of *Candida* spp., and has been associated with higher mortality in patients with *Candida* BSI. Biofilms provide a highly sheltered environment and demonstrate *in vitro* resistance to azoles, while amphotericin B lipid formulations and echinocandins remain effective [25–28].

Antifungal drugs: intrinsic and acquired resistance

A number of antifungal compounds with different modes of action are available in the treatment of *Candida* infections. Amphotericin B and azoles interact with ergosterol in the fungal cell membrane; amphotericin B is fungicidal by creating pores in the membrane leading to leakage and cell death. Azoles are fungistatic and inhibit ergosterol synthesis through the cytochrome P450 enzyme 14- α -sterol-demethylase. The newest drug group, the echinocandins inhibit the β -D-glucan synthase enzyme essential to cell wall synthesis and are fungicidal.

Candida spp. have diverse *in vitro* intrinsic susceptibility patterns, as shown in Table I.

Table I

Intrinsic susceptibility pattern of the most frequently isolated *Candida* spp.

	Fluconazole	Voriconazole	Amphotericin B	Echinocandins
<i>C. albicans</i>	S	S	S	S
<i>C. glabrata</i>	I–R	S–I–R	S	S
<i>C. tropicalis</i>	S	S	S	S
<i>C. parapsilosis</i>	S	S	S	S–I
<i>C. krusei</i>	R	S–I–R	S	S
<i>C. lusitanae</i>	S	S	R	S

Acquired resistance is rare in most countries and since the source of candidaemia is the patient's own microbial flora, usually occurs in patients who had received previous antifungal treatment. The underlying mechanisms for acquired resistance to azoles include mutation of the target gene, upregulation of the target gene and efflux pumps. Echinocandin resistant isolates harbor target gene mutations in the FKS1 and FKS2 genes [11, 29].

In countries where echinocandin use is widespread, a growing incidence of echinocandin-resistant *C. glabrata* isolates are reported (from 4.9% in 2001 to 12.3% in 2010) [9]. Recently, *Candida* isolates resistant to amphotericin B and echinocandins or azoles have been detected [30, 31].

***In vitro* susceptibility and clinical breakpoints**

Two reference methods have been described for *in vitro* susceptibility testing by the Clinical and Laboratory Standards Institute (CLSI) in the United States and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in Europe [32, 33]. Reliable, reproducible *in vitro* susceptibility data can be obtained with either method, providing useful data to determine the epidemiological cutoff to differentiate wild type organisms from those with acquired resistance mechanisms.

However, *in vitro* susceptibility data is not easily translated into clinical breakpoints. *In vitro* MIC values do not always correlate with clinical outcome: therapeutic failure occurs with low MIC, and treatment may be successful despite *in vitro* resistance to any given agent [34].

There has been ongoing discussion on the therapeutic efficacy of echinocandins against *C. parapsilosis*. *C. parapsilosis* exhibits slightly elevated echinocandin MIC levels, due to an alteration in the target gene of β -D-glucan synthase. Nonetheless, clinical studies evaluating echinocandin therapy did not demonstrate a statistically different outcome in the case of *C. parapsilosis* compared to other species with lower MICs, which is probably in connection with the low virulence of the species [35, 36]. On the other hand, a larger number of persistent fungemia and breakthrough infections caused by *C. parapsilosis* were reported during echinocandin treatment compared to other antifungal agents [37–39]. Further data is necessary, but current ESCMID guidelines consider fluconazole a slightly better treatment alternative to echinocandins [40].

No correlation was found between *C. glabrata* voriconazole MIC values and clinical outcome, therefore clinical breakpoints could not be established. CLSI recommended the use of ≤ 0.5 $\mu\text{g/ml}$ cutoff to differentiate between wild-type and non-wild type strains for resistance surveillance purposes [41].

There was no relation between amphotericin B MIC values of *Candida* bloodstream isolates and clinical success or failure [42].

New methods may prove to be a sensitive and quicker way to detect antifungal resistance. FKS mutations connected with echinocandin resistance can be

detected by molecular methods. After a 3-hour incubation in different concentrations of caspofungin, and analysis of the spectra measured by MALDI-TOF MS, an isolate can be categorized as susceptible or resistant [43]. Flow cytometry can detect cell viability by fluorescence following exposure to an antifungal agent [44].

Antifungal susceptibility testing in the routine laboratory is recommended on isolates from sterile sites, such as the bloodstream, especially in persistent or breakthrough infections, when emerging resistance may occur. If no clinical breakpoints are available, the epidemiological cutoff values (ECV) may be used to predict the presence of acquired resistance in an isolate [45].

Conclusions

The microbiological diagnosis of *Candida* BSI and the determination of antifungal susceptibility are among the most challenging tasks of the clinical microbiology laboratory.

Blood culture lacks sensitivity, and in up to half of the patients, *Candida* BSI remains an empirical diagnosis. New diagnostic methods, such as serum markers and PCR are promising, but require further evaluation.

The correct identification of an isolate is essential in determining antifungal susceptibility, since *Candida* spp. possess diverse intrinsic resistance patterns. Acquired antifungal resistance is rare, but reports of multiresistant isolates are alarming for future prospects. Clinical breakpoints are not available for a number of agents due to the lack of data and no correlation between *in vitro* and clinical data. The ECV is a useful tool in detecting isolates with possible acquired resistance mechanisms. New tools may be useful to detect resistance earlier.

In the routine laboratory, susceptibility testing of isolates from the bloodstream (and other sterile sites) with standard methods and of relevant antifungal agents following proper species identification is recommended to guide antifungal therapy.

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