Susceptibility Testing of *Candida glabrata* Isolates Collected in a 7-year Study: Continued Need for Antifungal Susceptibility Monitoring of Bloodstream Isolates

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#1641
IDWeek 2016

Abstract

Background: Azoles and echinocandins are commonly used for treatment of invasive fungal infections. Resistance by *Candida glabrata* to echinocandins is emerging. Availability of antifungal susceptibility testing of bloodstream isolates (especially *C. glabrata*) is necessary for appropriate therapy. The aim of this study was to determine antifungal susceptibilities for *C. glabrata* and compare results from two testing methods.

Methods: A total of 429 *Candida* blood culture isolates were collected from unique New Orleans patients during 2009-2015. Of these, 151 (35%) were *C. glabrata* (146 viable for testing). Caspofungin and fluconazole MICs were determined by two FDA-approved antifungal susceptibility testing methods, the Vitek® 2 system and the Etest® method. Vitek MICs were finalized in an average time of 13h; Etest MICs were read at 24h.

Results: *C. glabrata* Vitek 2 and Etest determined caspofungin resistance ranged from 6% to 7%, respectively; fluconazole resistance, 12% to 23%, respectively. MICs determined by Etest were frequently higher than Vitek MICs. Perhaps this was due to subjectivity when reading an endpoint at 80% inhibition when diffuse growth or trailing endpoints were present. The Vitek 2 occasionally terminated the fluconazole MIC, due to insufficient growth. Current (2012) CLSI interpretive MIC (µg/mL) guidelines for *C. glabrata* are: caspofungin (≤0.12 S, 0.25 I, ≥0.5 R) and fluconazole (≤32 SDD, ≥64 R). Voriconazole was not evaluated in this study since CLSI breakpoints are not available for *C. glabrata*. Caspofungin MICs (≤0.25) reported by the Vitek could not be differentiated as S (≤0.12) or I (0.25). Essential agreement of MICs (within 2 two-fold dilutions) between Vitek and Etest was 144/146 (99%) for caspofungin and 129/144 (90%) for fluconazole.

Conclusion:
The agreement between the Vitek 2 and Etest methods was high, with the Vitek 2 being easier and more rapidly performed than Etest. Antifungal susceptibility testing should be performed on *Candida glabrata* isolates from bloodstream infections to detect *in vitro* resistance and optimize antifungal therapy.

Introduction

Candida glabrata has been a rising cause of candidemia in the last decade, second only to *Candida albicans*. During the last 7 years at our institution, *C. glabrata* was the most frequently *Candida* spp. isolated from blood cultures (35%) (Fig.1). Two large U.S. surveillance studies (2006-2010) found that approximately 10% of *C. glabrata* bloodstream infection (BSI) isolates were resistant to fluconazole, and resistance to one of the echinocandins (anidulafungin, caspofungin, or micafungin) was also present in 11% of these isolates (Pfaller, 2012, *Am J Med* 125:No. 1A).

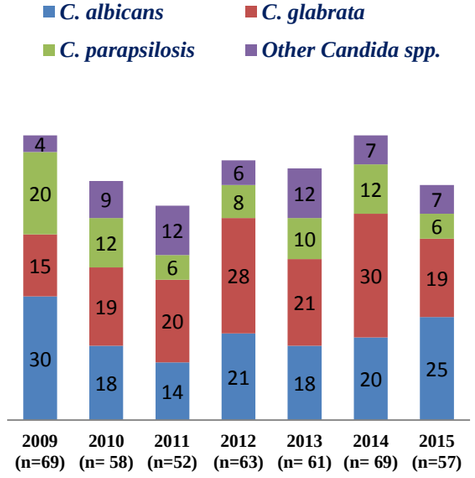
Emerging antifungal resistance by *Candida* species, primarily *Candida glabrata*, is making clinically relevant antifungal susceptibility testing necessary to help determine appropriate therapy. However, antifungal susceptibility testing is currently not *routinely* performed by many clinical laboratories. The delay to get MIC results is often impractical when fungal isolates are shipped to a reference lab for susceptibility testing. The aim of this study was to collect *Candida* spp. isolated from patients with bloodstream infections and compare antifungal susceptibilities using 2 different methods, Etest and Vitek 2, available options in many clinical microbiology laboratories. Both methods are FDA-approved and have been validated as acceptable alternatives when compared to the CLSI reference method, broth microdilution. The caspofungin Etest strip is for Research Use only in the U.S.

Methods

CANDIDA, MEDIA, AND ANTIFUNGAL AGENTS

- A total of 429 *Candida* isolates were collected from Ochsner Health System patients with bloodstream infections during 2009–2015 (Fig. 1).
- Of these, 151 (35%) were *C. glabrata* (*146* were viable for testing).
- Isolates were identified using the API® 20C yeast identification system (bioMérieux).
- Media included: Sabouraud dextrose agar plates for subcultures and RPMI 1640 agar with MOPS and 2% glucose plates for Etest MICs.
- MICs were determined by Etest and Vitek 2 (bioMérieux) for caspofungin (CAS), fluconazole (FLU), and voriconazole (VOR) (the only antifungals on the Vitek 2 card).
- Quality control testing was performed with *C. parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 (CLSI 2012); *C. albicans* ATCC 90028 (for Etest only).

Figure 1. Number of *Candida* Bloodstream Isolates by Year



- **Vitek 2 compact system.** The Vitek test cards (AST-YS05), containing CAS, FLU, and VOR were set up according to the manufacturer's instructions. The MICs were available from 10.5 h – 27.5 h, with an average time of 13 h. Voriconazole was not evaluated in this study since CLSI breakpoints are not available for *C. glabrata*.

- **Etest method.** The Etest was performed following manufacturer's guidelines for *Candida* species. Plates were incubated at 35° C and read at 24 h (occasionally plates were reincubated for confirmatory readings at 48 h). MICs were read at the first point of significant inhibition of growth or 80% inhibition of visual growth. The CAS Etest strip is for Research Use only in the U.S.

Results

- **Essential agreement** (MICs within two 2-fold dilutions) between Etest and Vitek 2 (Tables 1, 2):

  CAS- *C. glabrata* 99%;
  Note: CAS MICs (≤0.25) for *C. glabrata* reported by Vitek 2 could not be differentiated as S (≤0.12) or I (0.25).
  FLU- *C. glabrata* 90%

- **Categorical agreement** (MICs within the same interpretive category) between Etest and Vitek 2 (Tables 1, 2):

  CAS- *C. glabrata*, not determined-
  MICs ≤ 0.25 reported by Vitek 2 could not be differentiated as S (≤0.12) or I (0.25).
  FLU- *C. glabrata* 90%

Results

Table 1. Comparison of antifungal susceptibility testing of caspofungin and fluconazole by Etest and Vitek 2 for *C. glabrata* (*n*=146) collected from patients with bloodstream infections during 2009- 2015.

C. glabrata (n = 146)	Etest No. (%) of isolates in susceptibility category			Vitek 2 No. (%) of isolates in susceptibility category		Essential Agreement (MICs within two 2-fold dilutions)	Categorical Agreement (MICs within the same interpretive category)
	S	I	R	S/I	R		
Caspofungin	101 (69%)	35 (24%)	10 (7%)	137 (94%) S/I	9 (6%)	144/146 (99%)	--------
	S	SDD	R	S	SDD	R		
Fluconazole		113(77%)	33(23%)		126(87.5%)	18(12.5%)	129/144*(90%)	129/144 (90%)

S, susceptible; I, intermediate; SDD, susceptible-dose dependent; R, resistant.
S/I, MICs reported as ≤0.25 could not be differentiated as S (≤0.12) or I (0.25).
*MICs for 2 isolates not available due to termination of Vitek 2 card.

CLSI (2012) interpretive guidelines for *C. glabrata:* caspofungin ≤0.12 S, 0.25 I, ≥0.5 R; fluconazole ≤32 SDD, ≥64 R

Conclusions

- **The agreement between Vitek 2 and Etest methods was high for caspofungin and fluconazole for *C. glabrata*.**
- **Overall, the automated Vitek 2 was simpler and more rapidly performed than Etest, but caspofungin MICs could not be differentiated as susceptible or intermediate for *C. glabrata*. Additional antifungals on the Vitek 2 card are needed.**
- **Sufficient data are needed to demonstrate a correlation between *in vitro* susceptibility testing and clinical outcome for *C. glabrata* and voriconazole to establish interpretive breakpoints.**
- **Antifungal susceptibility testing should be performed on all *Candida* isolates from bloodstream infections to detect emerging *in vitro* resistance and optimize antifungal therapy.**