Caspofungin-Resistant Strains of *Candida glabrata* Are Most Commonly Colonized in *Clostridioides difficile* Infection (CDI) Patient Guts in Houston, Texas

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ABSTRACT

Background: *Candida glabrata* is the second most common cause of invasive candidiasis in the United States. The echinocandin class of antifungals, including caspofungin has become the preferred therapy for invasive candidiasis due to *C. glabrata* and other species demonstrating decreased azole susceptibility. Caspofungin resistance has been uncommon, but reports suggest that the incidence is increasing, particularly among *C. glabrata* isolates. The dysbiosis associated with *Clostridioides difficile* allows for overgrowth of *Candida* spp. However, the prevalence of *C. glabrata* in stool of *C. difficile* infection (CDI) patients is not well studied. Therefore, our objectives were to investigate the incidence of potentially pathogenic species of *Candida* in stool samples of CDI patients.

Methods: We collected 1,241 CDI patient stool samples from two large hospitals in Houston, Texas and enrich the samples in brain heart infusion (BHI) broth at 37^{0}C for 48-72 hours. After that sub-cultured onto selective HardyChrom *Candida* agar and incubated at 37^{0}C for 48 to 72 hours. Characteristic *Candida* colonies were stocked in cryovials and kept at -80^{0}C for further analyses. Isolates were then identified by multiplex PCR. *C. glabrata* isolates were screened for caspofungin resistance on Muller-Hinton agar (with 8.0 ug/ml)

Results: Overall, 14.8% (184/1241) samples were culture positive for *Candida* spp. The predominant species was *C. glabrata* (9.2 %) followed by *C. albicans* (2.3%), *C. tropicalis* (1.6%), *C. parapsilosis* (1.2%), *C. krusei* (0.6%) or not speciated (6.9%). The majority of *C. glabrata* isolates (70.2%; 80/114) were caspofungin resistant.

Conclusion: The results of this study showed that colonization of *C. glabrata* is common in patients with CDI and could be a source of antifungal-resistant pathogens.

Objectives

The objectives of this study were to investigate the incidence of potentially pathogenic species of *Candida* in stool samples of CDI patients.

METHODS

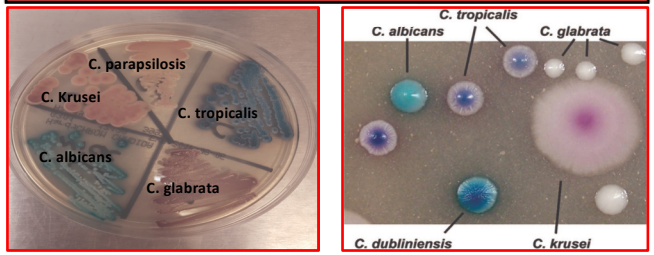

Figure 1. Growth of *Candida* spp on HardyChrom™ Candida Agar

1) Identifying *Candida* spp by Selective HardyChrom ™ Candida Agar Plates;

- 1,241 CDI patient stool samples were cultured onto HardyChrom Candida agar plates. Colony color and morphology were evaluated after 48 hours of incubation at 37^0 C.

2) Identifying *Candida* spp by Multiplex PCR;

- Colonies were collected from HardyChrom Candida agar plate then sub-cultured onto BHI agar. Single *Candida* colonies were stocked in cryovials and kept at -80C.
- The fungus- specific primers ITS1 and ITS2 were used for the identification of *C. albicans, C. galbrata, C. parapsilosis, C. tropicalis* and *C. Krusei*. ITS1 and ITS2 amplify a small conserved portion of the 18s rDNA and 28S rDNA regions.

- In addition, *C. albicans*-specific primers CA3 and CA4 were also included in PCR mixture to amplify a portion of ITS2 region of *C. albicans*.
- For the identification of other species (*C. kefyr, C. famata and C.dubliniensis*), ITS1F, ITS1K, and ITS2D primers were used.
- PCR was carried out under the following condition: initial denaturation, 92^{0}C, 2 min; 35 cycles of denaturation (95^{0}C,1 min), annealing (50^{0}C, 1 min), and extension (72^{0}C, 1 min); and final extension, 72^{0}C, 10 min. A negative control and also positive controls of each strain were run with unknown samples.
- Gel electrophoresis was conducted and then the results were compared with positive controls.

3) **Identifying Caspofungin- Resistant strains of *Candida glabrata* by Muller Hinton Agar plating with 8 microgram/mL caspofungin**

- *C. glabrata* ATCC 90030 was included for quality control .

RESULTS

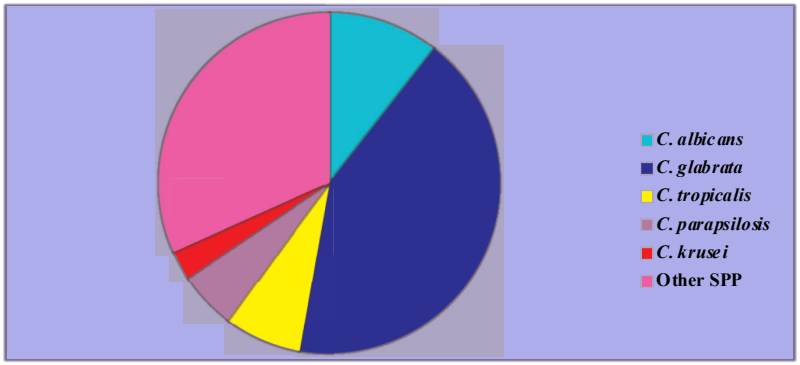

Figure 2. Distribution of *Candida* spp

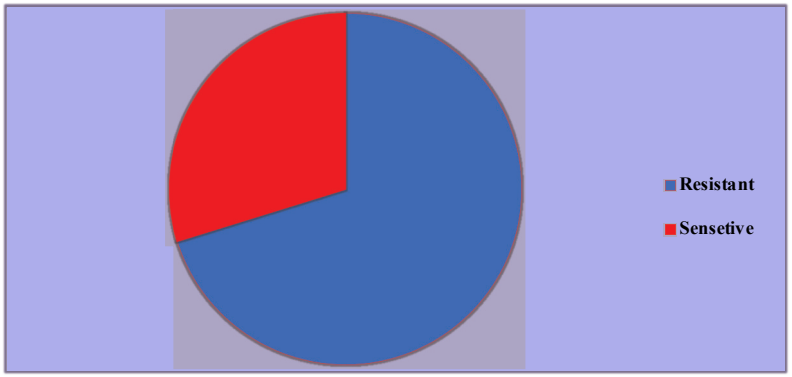

Figure 3. Distribution of *C. glabrata* Strains Resistant And Sensitive To Caspofungin

Conclusion

Occurrence of potentially pathogenic species of *Candida* spp in stool samples of CDI patients from Houston area hospitals has been investigated for the first time

- **Dominant species are *C. glabrata* (9.2%), *C. albicans* (2.3%), and *C. tropicalis* (1.6%) in stool samples of CDI patients**
- **CDI patient stools can be a potential source of caspofungin-resistant strains of *C. glabrata* and other *Candida* spp**

References

- **Liguori G, et al. Rapid identification of *Candida* species in oral rinse solutions by PCR. J Clin Pathol. 2007 60(9):1035-9.**
- **Shields RK, et al. Caspofungin MICs correlate with treatment outcomes among patients with *Candida glabrata* invasive candidiasis and prior echinocandin exposure. Antimicrob Agents Chemother. 2013 ;57(8):3528-35.**