Published in final edited form as:

Diagn Microbiol Infect Dis. 2016 March ; 84(3): 223–226. doi:10.1016/j.diagmicrobio.2015.11.018.

Utility of in-house fluconazole disk diffusion susceptibility testing in the treatment of candidemia

David W. Kubiak^{a,b,*}, Dimitrios Farmakiotis^c, Viktoria Arons^a, Randy M. Hollins^a, Sara E. Rostas^a, Linda M. Weiser^d, Lindsey R. Baden^{b,e,f}, Francisco M. Marty^{b,e,f}, and Sophia Koo^{b,e,f,**}

David W. Kubiak: dwkubiak@partners.org, kubiak26@gmail.com; Sophia Koo: skoo@bwh.harvard.edu

^aDepartment of Pharmacy, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA

^bDivision of Infectious Diseases, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA

^cWarren Alpert Medical School of Brown University, Division of Infectious Diseases, Rhode Island Hospital, 593 Eddy Street, Providence, RI 02115, USA

^dClinical Microbiology Laboratory, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA

^eDivision of Infectious Diseases, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, USA

^fHarvard Medical School, Boston, MA 02115, USA

Abstract

Among 302 first candidemia episodes, 210 (69.6%) were initially treated with an echinocandin or polyene (E/P) antifungal drug. In 137 (72.5%) patients with fluconazole-susceptible isolates, treatment was changed to fluconazole based on disk diffusion susceptibility testing. Clinical outcomes were not compromised in patients receiving E/P who were de-escalated to fluconazole for treatment of candidemia based on disk diffusion results.

Keywords

Candidemia; Fluconazole; Disk diffusion susceptibility testing

1. Introduction

Candida is one of the most frequent causes of nosocomial bloodstream infections and the most common fungal pathogen (Pfaller and Diekema, 2007). Despite advances in the diagnosis and treatment of invasive candidiasis (IC), mortality rates close to 50% are still

*Corresponding author. Tel.: +1-617-525-2255. **Corresponding author. Tel.: +1-617-525-8448.

Results from this study were presented in part at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO USA 2013 (Abstract M-218).

reported (Farmakiotis et al., 2015; Gudlaugsson et al., 2003). There are many therapeutic options for treatment of IC. The prevalence of fluconazole resistance remains relatively low for bloodstream *Candida* isolates, with the exception of *Candida glabrata* and *Candida krusei* (Pfaller et al., 2010). The use of routine and reliable antifungal susceptibility testing is imperative for clinical care, resistance surveillance, and enhancement of antifungal stewardship programs (Ananda-Rajah et al., 2012; Pfaller et al., 2010).

The use of disk diffusion susceptibility testing is a simple, inexpensive, and accurate method of assessing susceptibility of *Candida* species to fluconazole (Pfaller et al., 2004). The Clinical and Laboratory Standards Institute (CLSI) has endorsed the use of antifungal disk diffusion susceptibility testing of yeasts as a reliable and validated method to guide antifungal therapy (CLSI, 2009). Moreover, current guidelines (Pappas et al., 2009) recommend switching antifungal therapy in patients with fluconazole-susceptible *Candida* strains from intravenous echinocandin or polyene (E/P) therapy to oral fluconazole when clinically appropriate, a practice of increasing clinical significance in view of polyene toxicity, high costs associated with E/P use relative to fluconazole, and emerging echinocandin resistance (Alexander et al., 2013; Beyda et al., 2014; Farmakiotis et al., 2014; Shields et al., 2012).

Despite its relative ease to perform and low cost, antifungal disk diffusion susceptibility testing is not performed in many clinical microbiology laboratories where *Candida* isolates are routinely tested for antifungal susceptibility (Pfaller et al., 2009). In 2006, we implemented fluconazole disk diffusion susceptibility testing for all *Candida* bloodstream isolates at our institution, in accordance with CLSI standards and susceptibility breakpoints (CLSI, 2009). Results are generally available within 24 hours after a positive blood culture result and have greatly enhanced our antifungal stewardship efforts. In this study, we sought to evaluate the safety of antifungal modification of treatment for candidemia following implementation of in-house fluconazole disk diffusion susceptibility testing.

2. Methods

We identified all patients hospitalized at our institution with a first candidemia episode between 12/1/2006 and 12/31/2012, who received at least 1 dose of systemic antifungal therapy. We collected data on patient demographics, risk factors for candidemia, *Candida* species, fluconazole disk diffusion zone diameters (CLSI, 2009), and antifungal therapy.

Disk diffusion testing of fluconazole and voriconazole was performed as recommended in CLSI (formerly NCCLS) document M44-A (Shields et al., 2012). Briefly, we used Mueller–Hinton agar plates (Hardy Diagnostics, Santa Maria, CA, USA) supplemented with 2% glucose and 0.5 g of methylene blue per milliliter at a depth of 4.0 mm. The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard. Fluconazole (25 g) and voriconazole (1 g) disks (Becton Dickinson, Franklin Lakes, NJ, USA) were placed onto the surfaces of the inoculated plates, and the plates were incubated in air at 35 to 37 °C and read at 18–24 hours. Zone diameter endpoints were read at 80% growth inhibition by the technologist using a standard ruler. We used CLSI interpretive criteria for the fluconazole and voriconazole disk diffusion tests: susceptible (S),

zone diameters of 19 mm (fluconazole) and 17 mm (voriconazole); susceptible dose dependent (SDD), zone diameters of 15–18 mm (fluconazole) and 14–16 mm (voriconazole); and resistant (R), zone diameters of 14 mm (fluconazole) and 13 mm (voriconazole).

We assessed recurrence of candidemia within 30 days and survival to hospital discharge. Categorical data were compared with the χ^2 or Fisher exact test, as appropriate. Continuous variables were compared with the Student *t* test or the Mann–Whitney *U* test, if they were not normally distributed. Because of lower in-hospital mortality but higher candidemia recurrence rates in patients transitioned to oral fluconazole, compared to those treated with E/P (Fig. 1), we used competing risks regression by the method of Fine and Gray (1999), using recurrent candidemia and in-hospital mortality as potential outcomes, to assess the relationship between switching from E/P to oral fluconazole and recurrent candidemia. Two-tailed *P* values <0.05 were considered significant. The Partners Healthcare Human Research Committee approved this study.

3. Results

We studied first candidemia episodes in 302 patients. Demographic, clinical, and laboratory characteristics are summarized in Table 1. There were no differences in length of hospitalization between fluconazole-susceptible versus nonsusceptible isolates or among patients treated with different antifungal drug regimens. Twenty-five episodes (8.3%) were caused by fluconazole-resistant strains.

Fluconazole was the initial therapy in 92 (30.5%) candidemia episodes; 4 (4.3%) of these bloodstream isolates were fluconazole resistant, and these patients were switched to E/P. E/P was initially prescribed in the remaining 210 (69.9%) candidemia episodes, and 189 (90%) of these isolates were fluconazole susceptible (Fig. 1). Within a median of 3 days after the positive blood culture result returned, 137 (72.5%) of 189 patients with fluconazole-susceptible isolates were switched from E/P to fluconazole. In-hospital mortality was lower in patients transitioned to oral fluconazole, compared to those treated with E/P (Fig. 1; *P* < 0.01). There was no statistically significant association between type of anti-fungal therapy and recurrent candidemia (subdistribution hazard ratio [SHR] 1.01, 95% confidence interval [CI] 0.35–2.90); only neutropenia was associated with recurrent candidemia (SHR 3.52, 95% CI 1.28–9.70).

4. Discussion

Fluconazole disk diffusion susceptibility testing is a rapid and inexpensive method of guiding hospital-wide and patient-level use of anti-fungal therapy and surveillance of antifungal resistance in *Candida* species. The correlation between disk diffusion and broth microdilution MIC testing has been validated, with >90% in vitro agreement (Pappas et al., 2009; Pfaller et al., 2004, 2009; 2010). Nevertheless, to our knowledge, there is limited evidence about the utility of disk diffusion antifungal susceptibility testing in clinical settings.

Fluconazole susceptibility testing led to treatment modification in 141 (46.7%) patients, with early transition to fluconazole in patients initially treated with E/P. A prompt switch to oral antifungal therapy has the potential to reduce complications associated with intravenous access devices, polyene nephrotoxicity, and infusion-related reactions.

De-escalation of antifungal treatment also has broader benefits on a population level: the increasing use of echinocandins has been associated with the development of resistance to this class of antifungals, which are considered fungicidal and the least toxic agents for the treatment of candidemia. Echinocandin resistance rates >10% (Alexander et al., 2013; Beyda et al., 2014; Farmakiotis et al., 2014, 2015; Shields et al., 2012) and multidrug resistance (MDR) (Farmakiotis et al., 2014) have been reported from different tertiary centers. The optimal treatment for MDR *Candida* isolates has not been defined, and many clinicians would consider using polyene-based regimens, which have been associated with nephrotoxicity and increased mortality rates (Alexander et al., 2013).

It should be noted that, in 27.5% of patients with fluconazole-susceptible isolates, clinicians chose to continue treatment with E/P, mostly due to concern for potential mold infections or to avoid drug–drug interactions. The higher mortality (65.3%) in these patients compared to those whose treatment was changed to fluconazole (15.3%) likely reflects greater underlying illness and comorbidities. For critically ill and neutropenic patients, national guidelines (Pappas et al., 2009) recommend E/P as first-line therapy for candidemia; therefore, disk susceptibility testing might not be as useful in guiding therapy as it is for noncritically ill, nonneutropenic patients. In our cohort, however, fluconazole disk susceptibility testing led to rapid and safe de-escalation of treatment in over 70% of patients initially treated with E/P therapy.

Our study has limitations that should be taken into consideration. First, it was a single-center retrospective study, and in-hospital mortality was 27.5%, which is lower than in other candidemia series (Andes et al., 2012; Gudlaugsson et al., 2003). Also, it is not yet determined how disk diameter cutoffs correlate with the amended species-specific MIC breakpoints by broth microdilution (CLSI, 2012), but the clinical implications of these definitions remain to be validated. Our institution does not perform in-house echinocandin susceptibility testing nor do we test for the presence of FKS mutations.

5. Conclusions

We found that fluconazole susceptibility testing of *Candida* bloodstream isolates by disk diffusion is a rapid and inexpensive method that can be safely utilized to de-escalate antifungal treatment and enhance antifungal stewardship efforts. Universal assessment and reporting of fluconazole susceptibility of *Candida* bloodstream isolates should be encouraged in the management of invasive candidiasis.

Acknowledgments

Funding: There was no financial support for this study. SK is supported by a grant from the National Institutes of Health (K23 AI097225).

References

- Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R, et al. Increasing echinocandin resistance in *Candida glabrata*: clinical failure correlates with presence of FKS mutations and elevated MIC. Clin Infect Dis. 2013; 56:1724–32. [PubMed: 23487382]
- Ananda-Rajah MR, Slavin MA, Thursky KT. The case for antifungal stewardship. Curr Opin Infect Dis. 2012; 25:107–15. [PubMed: 22123667]
- Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH, et al. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. Clin Infect Dis. 2012; 54:1110–22. [PubMed: 22412055]
- Beyda ND, John J, Kilic A, Alam MJ, Lasco TM, Garey KW. FKS mutant *Candida glabrata*: risk factors and outcomes in patients with candidemia. Clin Infect Dis. 2014; 59:819–25. [PubMed: 24879785]
- Clinical and Laboratory Standards Institute (CLSI). Method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline, M44-A2. 2. Wayne, PA: CLSI; 2009.
- Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts, M27-S4. 4. Wayne, PA: CLSI; 2012.
- Farmakiotis D, Tarrand JJ, Kontoyiannis DP. Drug-resistant *Candida glabrata* infection in cancer patients. Emerg Infect Dis. 2014; 20:1833–40. [PubMed: 25340258]
- Farmakiotis D, Kyvernitakis A, Tarrand JJ, Kontoyiannis DP. Early initiation of appropriate treatment is associated with increased survival in cancer patients with *Candida glabrata* fungaemia: a potential benefit from infectious disease consultation. Clin Microbiol Infect. 2015; 21:79–86. [PubMed: 25636931]
- Fine J, Gray R. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999; 94:496–509.
- Gudlaugsson O, Gillespie S, Lee K, Vande Berg J, Hu J, Messer S, et al. Attributable mortality of nosocomial candidemia, revisited. Clin Infect Dis. 2003; 37:1172–7. [PubMed: 14557960]
- Pappas PG, Kauffman CA, Andes D, Benjamin DK Jr, Calandra TF, Edwards JE Jr, et al. Clinical practice guidelines for the management of candidiasis: 2009 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2009; 48:503–35. [PubMed: 19191635]
- Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20:133–63. [PubMed: 17223626]
- Pfaller MA, Hazen KC, Messer SA, Boyken L, Tendolkar S, Hollis RJ, et al. Comparison of results of fluconazole disk diffusion testing for *Candida* species with results from a central reference laboratory in the ARTEMIS global antifungal surveillance program. J Clin Microbiol. 2004; 42:3607–12. [PubMed: 15297505]
- Pfaller MA, Boyken L, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, et al. Comparison of results of fluconazole and voriconazole disk diffusion testing for *Candida* spp. with results from a central reference laboratory in the ARTEMIS DISK Global Antifungal Surveillance Program. Diagn Microbiol Infect Dis. 2009; 65:27–34. [PubMed: 19679232]
- Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10. 5-year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J Clin Microbiol. 2010; 48:1366–77. [PubMed: 20164282]
- Shields RK, Nguyen MH, Press EG, Kwa AL, Cheng S, Du C, et al. The presence of an FKS mutation rather than MIC is an independent risk factor for failure of echinocandin therapy among patients with invasive candidiasis due to *Candida glabrata*. Antimicrob Agents Chemother. 2012; 56:4862–9. [PubMed: 22751546]

Fig. 1.
Initial treatment, changes after susceptibility testing, and outcomes.

Table 1

Cohort characteristics.

Patients (N)		302
Age (years), median (IQR; range)		57 (46–66; 15–90)
Female (%)		143 (47.4)
Risk factors for candidemia (%)		
Active malignancy		182 (60.3)
Hematologic malignancy		92 (30.5)
Intensive care unit stay		89 (29.5)
CVC		251 (83.1)
Diabetes mellitus		60 (19.9)
Total parental nutrition		122 (40.4)
Exposure to T-cell immunosuppressants ^a		104 (34.4)
Chronic corticosteroid exposure ^b		57 (18.9)
Prolonged neutropenia ^c		39 (12.9)
Hematopoietic stem cell transplantation		39 (12.9)
Solid organ transplantation		14 (4.6)
Prior antifungal exposure ^d		72 (23.8)
Duration of candidemia, median days (IQR; range)		1 (1–2; 1–15)
CVC replaced or removed (of 251 patients)		220 (87.6%)
Hospital length of stay, median (IQR; range)		
Entire cohort (n = 302)		26 (13–42; 1–175)
Fluconazole-susceptible isolates (n = 277)		26 (13–42; 1–175)
Fluconazole-nonsusceptible isolates (n = 25)		26 (15–41; 5–105)
Fluconazole treated (n = 225)		26 (13–42; 1–161)
E/P treated (n = 77)		25 (16–42; 5–175)
Fluconazole disk diameter (mm), median (IQR; range)		29 (25–32; 6–45)
<i>Candida</i> species	N (%)	FLUC-S ^e (%)
<i>C. albicans</i>	136 (45.0)	98.5
<i>C. parapsilosis</i>	77 (25.5)	97.4
<i>C. glabrata</i>	34 (11.3)	94.1
<i>C. tropicalis</i>	22 (7.3)	95.5
<i>C. krusei</i>	10 (3.3)	0
<i>C. guilliermondii</i>	5 (1.6)	60
Other <i>Candida</i> spp. ^f	18 (6.0)	

^aWithin 90 days prior to candidemia.^b0.3 mg/kg/day of prednisone equivalent >3 weeks.^cAbsolute neutrophil count <500 cells/mm³ lasting >10 days prior to candidemia.^dExposure to systemic antifungal therapy within 30 days prior to candidemia.^eBy fluconazole disk diffusion susceptibility testing; diffusion diameter 19 mm.

^fFive *C. dublinensis*, 4 *C. lusitaniae*, 3 *C. pelliculosa*, 2 *C. kefir*, 3 *C. albicans*/*C. glabrata*, 1 *C. albicans*/*C. krusei*.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript