

Evaluation of Disk Diffusion Susceptibility Testing for CD101, a Novel Echinocandin, Against *Candida* spp.

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Abstract

Background: CD101, a novel echinocandin with an extended half-life, is undergoing development to treat candidemia and other forms of invasive candidiasis. As part of this development, it is important to evaluate susceptibility testing methodology. In this study, the feasibility of disk diffusion susceptibility testing for CD101 at various disk masses was evaluated alongside caspofungin against *Candida* spp., including echinocandin-resistant isolates.

Methods: CD101 5, 10, 15, 20, and 25 µg disks were made in house. Caspofungin 5 µg manufactured disks were tested as comparators. Broth microdilution and disk diffusion susceptibility testing of yeast was conducted in parallel by standard methods (CLSI M27-A3 and CLSI M44-A2). A total of 60 *Candida* spp. (10 each of *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* along with 10 *Candida* spp. selected for resistance to echinocandins) were tested. Correlation between broth MIC and disk zones was determined.

Results: MIC values and disk zone diameters for caspofungin were within quality control ranges during testing, and a linear correlation ($r=0.6760$) was observed with increasing disk zones correlating to decreasing MICs. Disk zones for CD101 for all disk masses were smaller than those observed for caspofungin indicating some difference in diffusion of CD101 into agar relative to caspofungin. Overall, CD101 disk zones increased only slightly with increasing disk mass, with typically less than 3 mm separating the disk zones observed with the 5 µg mass from the 25 µg mass. Nonetheless, for all evaluated disk masses, disk zones observed with CD101 showed a linear correlation to broth MICs with r -values ranging from 0.7374 (25 µg disks) to 0.7812 (5 µg disks). Regardless of disk mass, zone sizes typically ranged from 13 – 19 mm for isolates with CD101 MICs ≤ 0.06 µg/mL and from 6 – 14 mm for isolates with CD101 MICs ≥ 0.25 µg/mL.

Conclusions: It is feasible to conduct disk diffusion susceptibility testing with CD101 across a variety of disk masses. For all disk masses there appears to be the capability to distinguish potential “susceptible” isolates from “resistant” isolates, though the clinically predictive value of such a test will not be realized until later in clinical development.

Objective

To evaluate whether disk diffusion susceptibility testing of CD101 against *Candida* spp. is feasible and to investigate the performance of various CD101 disk masses.

Methods

• Test isolates for MIC testing included clinical isolates from the Micromyx Repository (Kalamazoo, MI) and isolates with reduced echinocandin-susceptibility supplied by Cidara Therapeutics, Inc. (San Diego, CA).

• A total of 10 each of *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*, alongside 10 *Candida* spp. selected for reduced susceptibility to echinocandins based on prior results, were tested.

• Tested isolates included standard quality control isolates from the American Type Culture Collection (ATCC; Manassas, VA).

• The susceptibility of test isolates to CD101 was determined by broth microdilution in accordance with CLSI guideline M27-A3¹ and M27-S3² in Roswell Park Memorial Institute (RPMI) media.

• The susceptibility of test isolates to CD101 was determined by disk diffusion in accordance with CLSI guideline M44-A2³ and M44-S3⁴ using Mueller-Hinton Agar supplemented with 2% glucose and 0.5 µg/mL methylene blue.

• Disks used for the testing of CD101 were made in house at Micromyx by spotting 5, 10, 15, 20, and 25 µg aliquots from DMSO stock onto sterile Whatman paper disks. Disks containing DMSO only were evaluated as controls.

• Caspofungin was tested in parallel as a comparator by broth microdilution and disk diffusion using 5 µg commercially acquired disks (Liofilchem, Italy).

Results

Table 1. Summary of Activity by MIC

Organism (N)	Drug	Minimum Inhibitory Concentration (µg/mL)		
		Range	MIC ₅₀	MIC ₉₀
<i>C. albicans</i> (10)	CD101	≤0.001 - 0.015	0.008	0.03
	caspofungin	0.008 - 0.06	0.015	0.06
<i>C. krusei</i> (10)	CD101	0.004 - 0.06	0.03	0.03
	caspofungin	0.12 - 0.25	0.25	0.25
<i>C. parapsilosis</i> (10)	CD101	0.004 - 1	0.5	1
	caspofungin	0.06 - 1	0.5	0.5
<i>C. tropicalis</i> (10)	CD101	≤0.001 - 0.03	0.008	0.015
	caspofungin	0.002 - 0.12	0.06	0.12
<i>C. glabrata</i> (10)	CD101	0.03 - 0.5	0.06	0.25
	caspofungin	0.06 - 0.5	0.12	0.5
<i>Candida</i> spp. (10 - reduced susceptibility ¹)	CD101	0.12 - 4	1	2
	caspofungin	0.5 - 8	1	4

¹isolates consist of 6 *C. glabrata*, 2 *C. tropicalis*, 1 *C. krusei*, and 1 *C. parapsilosis*

Results

• By broth microdilution (**Table 1**), CD101 was most potent by MIC₅₀/MIC₉₀ (µg/mL) against *C. albicans* (0.008/0.03) and *C. tropicalis* (0.008/0.015) followed by *C. krusei* (0.03/0.03), *C. glabrata* (0.06/0.25), and *C. parapsilosis* (0.5/1). CD101 was typically more potent than caspofungin.

• Against *Candida* spp. preferentially selected for reduced echinocandin susceptibility, the MIC₅₀/MIC₉₀ of CD101 and caspofungin was elevated (1/2 and 1/4 µg/mL, respectively) relative to representative susceptible clinical isolates.

• By disk diffusion (**Table 2**), the largest mean zone diameter for CD101 at each disk mass by species were observed with *C. albicans* (16.2 – 17.5 mm) followed by *C. glabrata*, *C. krusei*, and *C. tropicalis* (14.5 – 16.7 mm) with comparatively smaller mean zone diameters for *C. parapsilosis* (10.6 – 12.7 mm).

• Caspofungin at standard disk mass had comparatively larger mean zone sizes by species relative to CD101 (19.6 – 21.4 mm for *C. albicans*, *C. krusei*, *C. tropicalis*, and *C. glabrata*; 17.7 mm for *C. parapsilosis*).

Table 2. Summary of Activity by Disk Diffusion

Organism (N)	Disk/mass (µg)	Zone Diameter (mm)			
		Range	Median	Mode	Mean
<i>C. albicans</i> (10)	CD101/5	15 - 19	16	16	16.2
	CD101/10	16 - 18	16	16	16.5
	CD101/15 ¹	16 - 19	17	16	16.9
	CD101/20	16 - 19	17	17	17.2
	CD101/25	16 - 19	17	17	17.5
	caspofungin/5	20 - 23	21	20,22	21.4
<i>C. krusei</i> (10)	CD101/5	13 - 16	15	15	14.9
	CD101/10	14 - 16	15	15	15.0
	CD101/15	15 - 16	16	16	15.6
	CD101/20	15 - 17	16	15,16	15.8
	CD101/25	15 - 17	16	16	15.9
	caspofungin/5	17 - 22	20	20	20.1
<i>C. parapsilosis</i> (10)	CD101/5	8 - 16	9	9	10.6
	CD101/10	10 - 17	12	10	12.3
	CD101/15	10 - 17	12	10	12.3
	CD101/20	10 - 18	11	10	12.3
	CD101/25	10 - 18	12	10	12.7
	caspofungin/5	11 - 21	18	NA	17.7
<i>C. tropicalis</i> (10)	CD101/5	14 - 16	14	14	14.5
	CD101/10	14 - 17	15	14	15.3
	CD101/15	14 - 18	15	15	15.7
	CD101/20	14 - 17	16	16	15.7
	CD101/25	14 - 18	16	16	15.7
	caspofungin/5	19 - 22	20	19,20	19.9
<i>C. glabrata</i> (10)	CD101/5	12 - 17	15	14,16	14.9
	CD101/10	13 - 17	16	17	15.9
	CD101/15	13 - 18	17	17	16.4
	CD101/20	12 - 18	16	18	16.3
	CD101/25	14 - 18	17	18	16.7
	caspofungin/5	14 - 23	21	23	19.6
<i>Candida</i> spp. (10 - reduced susceptibility ²)	CD101/5	6 - 13	9	9	9.5
	CD101/10	6 - 14	11	NA	10.9
	CD101/15	6 - 14	12	12	11.2
	CD101/20	6 - 14	11	11	11.1
	CD101/25	7 - 14	12	12	11.7
	caspofungin/5	6 - 19	12	10	12.9

¹ data only available for 9 *C. albicans* for the CD101 15 µg disks

² isolates consist of 6 *C. glabrata*, 2 *C. tropicalis*, 1 *C. krusei*, and 1 *C. parapsilosis*

NA: not applicable, no clear mode

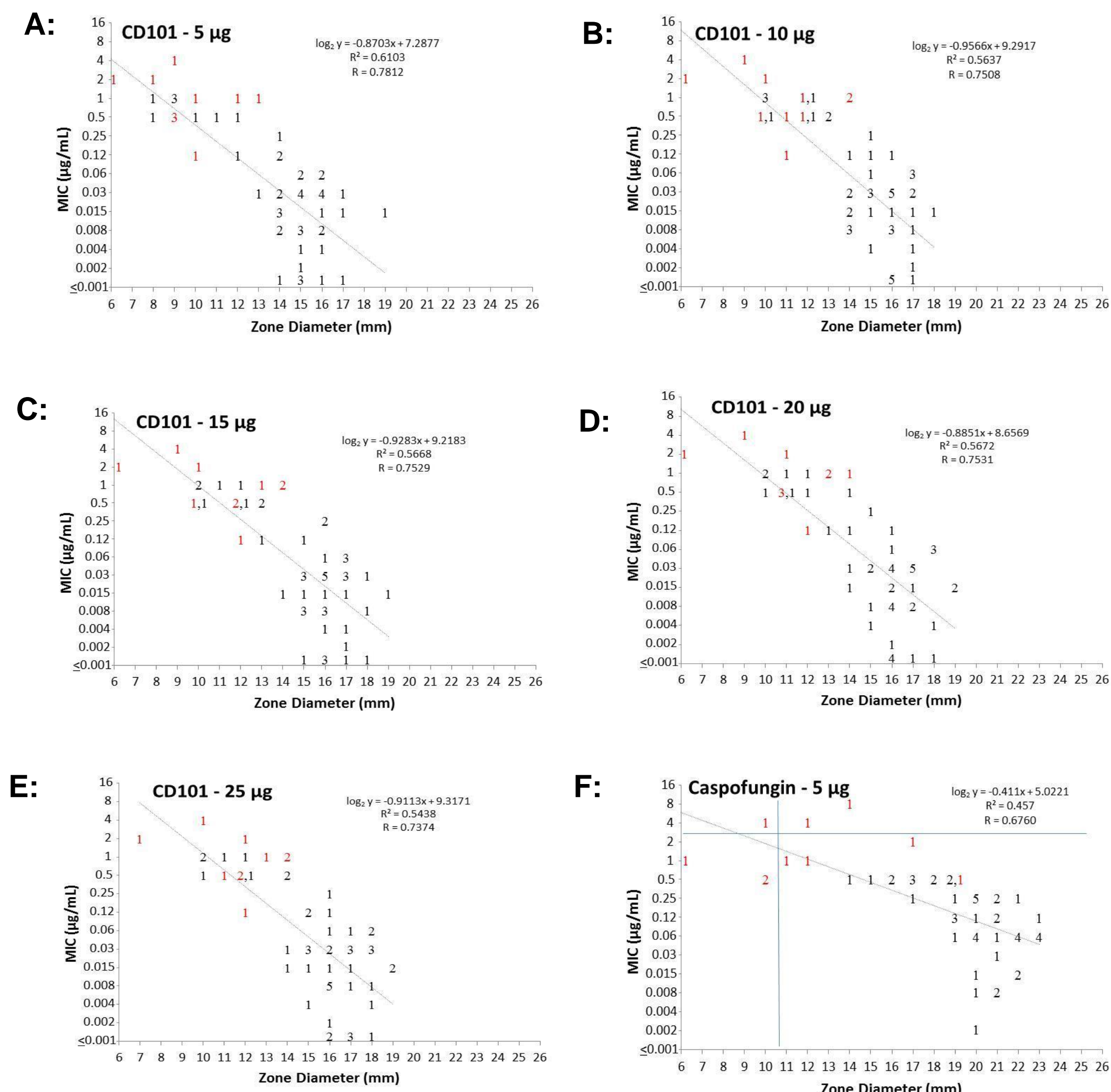
• Against *Candida* spp. preferentially selected for reduced echinocandin susceptibility, smaller mean zone sizes were observed for CD101 across disk masses (9.5 – 11.7 mm) and caspofungin at standard disk mass (12.9 mm) relative to representative susceptible clinical isolates (**Table 2**).

• Overall, there was little increase in CD101 zone diameter with increasing disk mass, with typically a <2 mm difference in mean zone diameter between the smallest and largest disk mass evaluated across species. (**Table 2**).

• The broth versus disk correlation for CD101 at each evaluated disk mass is shown in **Figure 1A – Figure 1E**. Regardless of mass, CD101 zone diameters for isolates with MICs ≤ 0.06 µg/mL were typically 13 – 19 mm and were typically 6 – 14 mm for isolates with MICs ≥ 0.25 µg/mL. Correlation coefficients ranged from 0.7374 at the 25 µg disk mass to 0.7812 at the 5 µg disk mass.

• The broth versus disk correlation observed with caspofungin in this study ($R = 0.6760$) is shown in **Figure 1F**.

Figure 1. Broth vs. Disk Correlation for CD101 at Varied Disk Masses (A-E) and for Caspofungin (F) Against *Candida* spp. (n=60)



red numbers indicate isolates preferentially selected for reduced susceptibility to echinocandins based on prior test results

Conclusions

• Zones of inhibition were apparent with *Candida* spp. using CD101 loaded disks, demonstrating that disk diffusion testing is feasible.

• There was little difference in zone diameter with increasing disk mass of CD101 across the evaluated *Candida* spp. Despite enhanced potency by MIC relative to caspofungin, CD101 disk zones were smaller than caspofungin, which may reflect decreased diffusion of CD101 into agar relative to caspofungin.

• Despite the small zone diameters observed with CD101 overall (<19 mm), zone diameters for isolates with low CD101 MIC values could still generally be distinguished from those with higher CD101 MIC values across the evaluated disk masses.

• The ability to distinguish putative “susceptible” from “resistant” isolates and the linear correlation between MIC and zone diameter observed across disk masses indicates potential utility of the disk diffusion test for evaluating the susceptibility of *Candida* spp. to CD101.

Acknowledgements

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References

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⁴ CLSI. Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints, and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Third Informational Supplement. CLSI document M44-S3. CLSI, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2009.

Introduction

• CD101 is a novel echinocandin with a long half-life currently under development by Cidara Therapeutics, Inc. for the treatment of candidemia and other forms of invasive candidiasis.

• As part of the ongoing development of CD101, it is important to evaluate susceptibility testing methodology, including whether susceptibility testing by disk diffusion is feasible and correlates well with susceptibility observed by standard broth microdilution testing.

• In this study, CD101 was tested by disk diffusion at various disk masses and by broth microdilution in parallel against clinical isolates of *Candida* spp., including isolates with reduced susceptibility to echinocandins.