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In Vitro Activities of Anidulafungin against More than 2,500 Clinical Isolates of *Candida* spp., Including 315 Isolates Resistant to Fluconazole

M. A. Pfaller,[1,2]* L. Boyken,[1] R. J. Hollis,[1] S. A. Messer,[1] S. Tendolkar,[1] and D. J. Diekema[1,3]

Departments of Pathology,[1] Epidemiology,[2] and Medicine,[3] University of Iowa College of Medicine and College of Public Health, Iowa City, Iowa 52242

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Anidulafungin is an echinocandin antifungal agent with potent activity against *Candida* spp. We assessed the in vitro activity of anidulafungin against 2,235 clinical isolates of *Candida* spp. using the CLSI broth microdilution method. Anidulafungin was very active against *Candida* spp. (the MIC at which 90% of strains are inhibited [MIC_{90}] was 2 μg/ml when MIC endpoint criteria of partial inhibition [MIC-2] were used). *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. kefyr* were the most susceptible species of *Candida* (MIC_{90}, 0.06 to 0.12 μg/ml), and *C. parapsilosis*, *C. lusitaniae*, and *C. guilliermondii* were the least susceptible (MIC_{90}, 0.5 to 2 μg/ml). In addition, 315 fluconazole-resistant isolates were tested, and 99% were inhibited by ≤1 μg/ml of anidulafungin. These results provide further evidence for the spectrum and potency of anidulafungin activity against a large and geographically diverse collection of clinically important isolates of *Candida* spp.

Anidulafungin is an investigational echinocandin with potent fungicidal activity against many species of *Candida* (1, 4, 5, 6, 11, 12, 13, 14, 17, 18). Anidulafungin is now in phase III clinical trials and has been shown to be safe and efficacious in treating invasive candidiasis (8). Although several studies documenting the in vitro activity of anidulafungin against *Candida* spp. have been published, these studies employed test methods that utilize either a more conservative MIC endpoint criterion (100% inhibition or MIC-0), an extended incubation time (48 h), or both and are either limited in the number of isolates of the various species of *Candida* tested or are restricted in the geographical distribution of the tested strains (1, 4, 11, 14, 17, 18).

In the present study, we determined the in vitro activity of anidulafungin against an international collection of more than 2,000 clinical isolates of *Candida* representing predominately bloodstream infection and other invasive forms of candidiasis. We also provide an evaluation of anidulafungin activity against 315 isolates with resistance (MIC, ≥64 μg/ml) to fluconazole. Because there is some controversy over the optimal method for performing in vitro susceptibility testing of the echinocandins (3), there is currently no standardized method for testing these agents against *Candida.* We have elected to use the method recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS), which employs RPMI 1640 broth, 24 h of incubation, and a partial inhibition (MIC-2, or ≥50% inhibition relative to control) MIC endpoint (3, 9, 10, 15, 16). The results are presented as the cumulative percentage of isolates inhibited at each concentration throughout the dilution series (full-range MICs) to facilitate comparison with other studies using the CLSI method.

MATERIALS AND METHODS

Organisms. A total of 2,235 clinical isolates of *Candida* spp. obtained from 91 different medical centers internationally were tested. These isolates were contributed as part of an ongoing prospective surveillance program and represent the incident isolate obtained from a given infectious episode. The collection included the following numbers of isolates: *C. albicans*, 1,181; *C. glabrata*, 265; *C. parapsilosis*, 328; *C. tropicalis*, 278; *C. krusei*, 59; *C. lusitaniae*, 34; *C. guilliermondii*, 57; *C. kefyr*, 15; and miscellaneous *Candida* spp., 18. The isolates represented the Asia-Pacific region (354 isolates from 16 study sites), Latin America (542 isolates from 15 study sites), Europe (668 isolates from 32 study sites), and North America (671 isolates from 28 study sites) (Table 1). In addition, a collection of 315 isolates of *Candida* spp. previously characterized as resistant to fluconazole (MIC, ≥64 μg/ml) (15) was tested in order to determine the activity of anidulafungin against these clinically important strains: *C. albicans* (41 isolates), *C. glabrata* (110 isolates), *C. krusei* (146 isolates), and *Candida* spp. (18 isolates). The isolates were all recent clinical isolates (2001 to 2004) and were from blood or normally sterile body fluids (cerebrospinal fluid, pleural fluid, or peritoneal fluid) and tissue. The isolates were identified by standard methods (7) and were stored as water suspensions until they were used in the study.

Antifungal agents. Standard antifungal powder of anidulafungin (Vicuron, Inc., King of Prussia, PA) was obtained from the manufacturer. Stock solutions were prepared in dimethyl sulfoxide. Serial twofold dilutions were prepared exactly as outlined in CLSI document M27-A2 (9). Final dilutions were made in RPMI 1640 medium (Sigma, St. Louis, MO) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma). Aliquots (0.1 ml) of the antifungal agent at twice the final concentration were dispensed into wells of plastic microdilution trays. The trays were sealed and frozen at −70°C until they were used.

Antifungal susceptibility studies. Broth microdilution testing of all 2,550 isolates was performed as described previously (16) in accordance with the guidelines of CLSI document M27-A2 (9), using a final inoculum concentration of (1.5 ± 1.0) × 10^3 cells/ml, RPMI 1640 medium, and incubation at 35°C for 24 h. MIC endpoints for anidulafungin were defined as the lowest concentration that produced a prominent decrease in turbidity (≥50% or MIC-2) relative to that of the drug-free control well (2, 9, 16).

RESULTS AND DISCUSSION

The species distribution of the isolates tested, stratified by the geographic region of origin, is shown in Table 1. All of the major species were represented, including less common and "emerging" species, such as *C. guilliermondii.* It is notable that

* Corresponding author. Mailing address: Medical Microbiology Division, C606 GH, Department of Pathology, University of Iowa College of Medicine, Iowa City, IA 52242. Phone: (319) 384-9566. Fax: (319) 356-4916. E-mail: michael-pfaller@uiowa.edu.

TABLE 1. Species distribution of *Candida* isolates by geographic region

Species	% by region[a]				
	APAC (354 isolates)	LAM (542 isolates)	EU (668 isolates)	NAM (671 isolates)	Total (2,235 isolates)
C. albicans	56.2	44.3	61.7	49.2	52.8
C. tropicalis	16.1	20.8	8.4	7.7	12.4
C. parapsilosis	15.3	18.1	10.9	15.4	14.7
C. glabrata	8.0	4.8	10.2	21.3	11.9
C. krusei	1.1	1.6	5.2	1.6	2.6
C. guilliermondii	1.4	7.6	0.9	0.8	2.6
C. lusitaniae	1.1	0.6	1.1	3.0	1.5
C. kefyr		0.6	1.3	0.4	0.7
Candida spp.[b]	0.8	1.6	0.3	0.6	0.8

[a] Regions: APAC, Asia-Pacific (16 study sites); LAM, Latin America (15 study sites); EU, Europe (32 study sites); NAM, North America (28 study sites).
[b] Includes *C. famata* (8 isolates), *C. dubliniensis* (4 isolates), *C. lipolytica* (2 isolates), and *C. rugosa* (2 isolates).

the species distributions of *Candida* bloodstream infection isolates contributed by study sites in the Asia-Pacific and Latin American regions were considerably different than that seen in North America.

Whereas *C. glabrata* was much more frequently isolated than either *C. parapsilosis* or *C. tropicalis* in North America, it was less common than either of these two species in the Asia-Pacific region and in Latin America. Similarly, *C. parapsilosis* was more common than *C. glabrata* among isolates contributed from Europe. Among the less frequently isolated species of *Candida*, *C. krusei* was more common among European isolates and *C. guilliermondii* was more common in Latin America, where it ranked above both *C. glabrata* and *C. krusei* among all bloodstream infection isolates. The species diversity, the number of contributing study sites, and the broad (worldwide) geographic representation are strengths of this database.

Table 2 summarizes the in vitro susceptibilities of 2,235 isolates of *Candida* spp. to anidulafungin using the MIC-2 endpoint method described above. Overall, anidulafungin was quite active against this broad range of *Candida* species (MIC at which 50% of the strains are inhibited [MIC_{50}], 0.06 μg/ml; MIC_{90}, 2 μg/ml). *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. kefyr* were the species most susceptible to anidulafungin (MIC_{90}, 0.06 to 0.12 μg/ml), and *C. parapsilosis* (MIC_{90}, 2 μg/ml), *C. lusitaniae* (MIC_{90}, 0.5 μg/ml), and *C. guilliermondii* (MIC_{50}, 2 μg/ml) were the least susceptible. Notably, 100% of *C. glabrata* and *C. krusei* isolates were inhibited by ≤0.25 μg/ml of anidulafungin. Despite differences in the species distribution across the four geographic regions, there was no difference in the activity of anidulafungin, overall or by species, when stratified by region.

The activity of anidulafungin against the 315 fluconazole-resistant isolates (Table 3) was equal to or better than its activity against the larger group of more azole-susceptible strains (Table 2). Notably, 100% of fluconazole-resistant isolates of *C. glabrata* and *C. krusei* were inhibited by ≤0.5 μg/ml of anidulafungin (Table 3).

These findings confirm and extend those reported previously regarding the anticandidal activity of anidulafungin (1, 4, 11, 15, 17, 18). Anidulafungin exhibited potent activity against virtually all species of *Candida*, including those with resistance to fluconazole. As seen with caspofungin (15, 16), there appear to be two broad groups of *Candida* species that can be differentiated by the degree of susceptibility to anidulafungin. One group includes the common species *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei* (as well as the less common *C. kefyr*) and is highly susceptible to anidulafungin (MIC_{90}, ≤0.12 μg/ml), whereas the second group includes *C. parapsilosis* and less common species, such as *C. lusitaniae* and *C. guilliermondii*, and is 4- to 16-fold less susceptible to anidulafungin (MIC_{90}, 0.5 to 2 μg/ml) (Table 2). Preliminary data suggest that all of these species may respond clinically in a similar fashion to anidulafungin treatment (8). The MICs for 99% of isolates in both groups are ≤2 μg/ml when tested using the partial inhibition endpoint criteria, a concentration that is exceeded

TABLE 2. In vitro susceptibilities of 2,235 clinical isolates of *Candida* spp. to anidulafungin[a]

Organism	No. tested	Cumulative % susceptible at:										
		0.007 μg/ml	0.015 μg/ml	0.03 μg/ml	0.06 μg/ml	0.12 μg/ml	0.25 μg/ml	0.5 μg/ml	1 μg/ml	2 μg/ml	4 μg/ml	8 μg/ml
C. albicans	1,181	4	28	61	88	99	99	99	99	100		
C. glabrata	265		1	19	70	97	100					
C. tropicalis	278	1	25	72	94	98	99	99	99	100		
C. krusei	59		5	64	95	100						
C. kefyr	15			7	67	100						
C. parapsilosis	328		1	1	1	1	2	6	39	97	100	
C. guilliermondii	57					2	5	9	60	93	100	
C. lusitaniae	34					12	59	97	100			
Candida spp.	18			6	13	13	25	31	56	94	94	100
Total	2,235	2	18	45	70	80	82	83	89	99	99	100

[a] Broth microdilution testing according to CLSI M27-A2 (10), using 24-h incubation and MIC-2 endpoint.

TABLE 3. In vitro activity of anidulafungin against 315 fluconazole-resistant clinical isolates of *Candida* spp.[a]

Organism	No. tested	Cumulative % susceptible to anidulafungin at:									
		0.007 μg/ml	0.015 μg/ml	0.03 μg/ml	0.06 μg/ml	0.12 μg/ml	0.25 μg/ml	0.5 μg/ml	1 μg/ml	2 μg/ml	4 μg/ml	8 μg/ml
C. albicans	41	15	42	66	95	95	95	98	100			
C. glabrata	110	1	3	36	81	98	100					
C. krusei	146	1	3	40	82	97	99	100				
Candida spp.	18	6	17	39	39	39	44	67	83	100		
Total	315	3	9	42	81	94	96	98	99	100		

[a] Broth microdilution testing was done according to CLSI M27-A2 (10), using 24-h incubation and MIC-2 endpoint. Fluconazole resistance is defined as a MIC of ≥64 μg/ml.

throughout the dosing interval following the administration of anidulafungin at standard doses of 100 mg/day (8, 13).

In summary, we have demonstrated that anidulafungin has potent in vitro activity against a broad range of *Candida* species from throughout the world. The emerging in vivo data from animal models and from clinical trials support the efficacy of anidulafungin in the treatment of invasive candidiasis. The fungicidal nature of anidulafungin coupled with sustained serum concentrations that exceed the MIC_{90} of virtually all *Candida* species makes it a very promising systemic antifungal agent.

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