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Evaluation of Rezafungin Provisional CLSI Clinical Breakpoints and Epidemiological Cutoff Values Tested against a Worldwide Collection of Contemporaneous Invasive Fungal Isolates (2019 to 2020)

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ABSTRACT Rezafungin is a new echinocandin under development for the treatment of candidemia and invasive candidiasis. CLSI recently approved provisional susceptible-only breakpoints and epidemiological cutoff values for *Candida* spp. and rezafungin. The activities of rezafungin and comparators against 2019 to 2020 invasive fungal isolates was evaluated by applying the new CLSI breakpoints. Rezafungin demonstrated potent activity against *Candida albicans* (MIC_{50}/MIC_{90}, 0.03/0.06 mg/L; 100.0% susceptible), *Candida tropicalis* (MIC_{50}/MIC_{90}, 0.03/0.06 mg/L; 100% susceptible), *Candida glabrata* (MIC_{50}/MIC_{90}, 0.06/0.06 mg/L; 98.3% susceptible), *Candida krusei* (MIC_{50}/MIC_{90}, 0.03/0.03 mg/L; 100% susceptible), and *Candida dubliniensis* (MIC_{50}/MIC_{90}, 0.06/0.12 mg/L; 100% susceptible) when tested by the CLSI broth microdilution method. Rezafungin inhibited 99.6% of *Candida parapsilosis* isolates (MIC_{50}/MIC_{90}, 1/2 mg/L) at the susceptible breakpoint of ≤2 mg/L. All *C. albicans*, *C. tropicalis*, and *C. krusei* isolates, as well as most *C. glabrata* (96.2% to 97.9%) and *C. parapsilosis* (86.2% to 100%) isolates, were susceptible to comparator echinocandins. Fluconazole resistance was detected among 0.5%, 4.5%, 10.5%, and 1.2% of *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* isolates, respectively. All echinocandins displayed limited activity against *Cryptococcus neoformans*. Rezafungin and other echinocandins were active against *Aspergillus fumigatus* (minimum effective concentration for 90% of isolates tested [MEC_{90}] range, 0.015 to 0.06 mg/L) and *Aspergillus* section *Flavi* (MEC_{90} range, 0.015 to 0.03 mg/L). All but 16 (8.6%) *A. fumigatus* isolates were susceptible to voriconazole, and 100% of *Aspergillus* section *Flavi* isolates were WT to mold-active azoles. When applying the CLSI clinical breakpoints, rezafungin displayed high susceptibility rates (>98.0%) against *Candida* isolates from invasive fungal infections and showed potent activity against *Aspergillus* isolates.

KEYWORDS echinocandin, *Candida*, *Aspergillus*, *C. albicans*, *A. fumigatus*, antifungal

Invasive fungal infections (IFIs) are associated with elevated morbidity and mortality in immunocompromised and critically ill patients (1, 2). These infections are most frequently caused by species of *Candida* and *Aspergillus*, and among these genera, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, and *Aspergillus fumigatus* are the most prevalent species (3, 4). It is important to note that although an update on the taxonomic status of many fungi of medical importance was recently published (5), which reclassified *Candida krusei* and *Candida glabrata* as *Pichia kudriavzevii* and *Nakaseomyces glabrata*, respectively, the former names of these fungi will be retained in this article.

The therapeutic armamentarium to treat IFIs is limited to a few classes that include the azoles, polyenes, pyrimidine inhibitors, and the echinocandins. The latter are the

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mainstay treatment for invasive candidiasis in both neutropenic and nonneutropenic patients, as well as critically ill patients, and are recommended as salvage therapy in patients with invasive aspergillosis (IA) (1, 2).

Rezafungin is a new echinocandin and a structural analog of anidulafungin. This echinocandin differs from the previous U.S. FDA-approved echinocandins due to its long half-life and front-loaded drug exposure, which allows for once-weekly intravenous (i.v.) administration instead of the once-daily administration required for caspofungin, micafungin, and anidulafungin (6). Rezafungin is currently in phase 3 clinical development for the treatment of candidemia and invasive candidiasis (ReSTORE study, ClinicalTrials registration no. NCT03667690) and the prevention of invasive fungal disease caused by *Candida*, *Aspergillus*, and *Pneumocystis* spp. in allogeneic blood and marrow transplant recipients (ReSPECT study, ClinicalTrials registration no. NCT04368559).

In June 2021, the CLSI Subcommittee on Antifungal Susceptibility Tests (AFSC) approved rezafungin provisional susceptible-only clinical breakpoint and epidemiological cutoff value (ECV) criteria for multiple *Candida* species (7). These breakpoints were approved after review of microbiological data, pharmacokinetics/pharmacodynamics (PK/PD), and results from phase 2 clinical trials, including patient outcomes by MIC (8). ECVs differ from clinical breakpoints since they do not classify isolates as susceptible or resistant. An ECV is the MIC or minimum effective concentration (MEC) value that defines the upper limit of the wild-type (WT) distribution, and it is helpful to distinguish between WT isolates without acquired resistance mechanisms and non-WT (NWT) isolates that harbor acquired resistance mechanisms. If breakpoints are not available, clinicians may use ECVs alone when deciding whether to treat a patient with a certain agent. However, ECVs do not predict a therapeutic response (9). In this scenario, the evaluation of the species-specific ECV or further investigation of the presence of resistance mechanisms may be useful to drive treatment decisions. Accordingly, the activities of rezafungin and comparator agents against invasive fungal isolates from the SENTRY Antimicrobial Surveillance Program (2019 to 2020) were evaluated using the recently approved CLSI clinical breakpoints and ECVs in this study.

MATERIALS AND METHODS

Fungal isolates. A total of 1,679 nonduplicate fungal isolates causing invasive infections were collected from 48 medical centers located in North America (573 isolates from 16 medical centers in the USA and Canada), Europe (647 isolates from 18 medical centers in 13 countries), the Asia-Pacific region (242 isolates from 8 medical centers in 4 countries), and Latin America (217 isolates from 6 medical centers in 5 countries). Participant medical centers submitted consecutively collected fungal isolates deemed by local criteria to cause invasive infections to a central monitoring laboratory (JMI Laboratories, North Liberty, IA, USA) as part of the 2019 to 2020 SENTRY Antimicrobial Surveillance Program. Only a single isolate per patient was included. Fungal isolates were collected from bloodstream infections (939 isolates), pneumonia in hospitalized patients (226), skin and skin structure infections (120), intra-abdominal infections (49), and other nonspecified sites (345). Fungal isolates were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) using MALDI MBT Compass and JMI proprietary libraries. Isolates not scoring $\geq$2.0 by spectrometry were submitted to confirmatory identification by sequencing and analysis of the 28S ribosomal subunit for all isolates and β-tubulin for *Aspergillus* spp. (10, 11). Notably, the ability of the MALDI MBT Compass library to discriminate among species within the *A. fumigatus* complex, *Aspergillus* section Flavi, and variants of *Cryptococcus neoformans* may be limited. The JMI proprietary library and β-tubulin gene sequencing enhanced our ability to discriminate among *Cryptococcus neoformans* variants and *Aspergillus fumigatus* species complex members, including *A. fumigatus* and *A. lentulus*. In this collection, no *A. lentulus* or *C. neoformans* var. *grubii* isolates were detected. Due to the similarity among species of the section *Flavi*, these isolates were only identified to the section level. Invasive aspergillosis caused by species of the section *Flavi* may involve several taxa, including *A. flavus*, *A. oryzae*, *A. tamarii*, *A. parasiticus*, *Petromyces alliaceus*, *A. nomius*, *A. qizutongi*, *A. beijingensis*, and *A. novoparasiticus* (12). Nucleotide sequences were analyzed using Lasergene software (DNAStar, Madison, WI, USA) and compared to available sequences through the Internet by using BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Results were considered acceptable if homology was $\geq$99.5% compared to other entries in the databases.

Antifungal susceptibility testing. Isolates were tested for susceptibility by broth microdilution following the guidelines in the CLSI M27 and M38 documents, with the exception that panels were made by dispensing 10 μL of a 20$\times$ drug stock solution into wells that contained 90 μL of RPMI and mixing (13, 14). The following antifungal agents were included in this study: rezafungin, caspofungin, micafungin, anidulafungin, fluconazole, voriconazole, posaconazole, itraconazole, and amphotericin B. Quality control was performed and interpreted as recommended in the CLSI documents M60 and M61 by using

C. krusei ATCC 6258, *C. parapsilosis* ATCC 22019, *Aspergillus flavus* ATCC 204304, *A. fumigatus* MYA-3626, and *Hamigera insecticola* ATCC MYA-3630 (15, 16). CLSI species-specific clinical breakpoints and ECVs were applied where available (9, 15, 16), including recently approved breakpoints and ECVs for rezafungin against *Candida* spp. (7).

Screening for *FKS* mutations. Rezafungin activity was also evaluated against *Candida* species isolates displaying NWT MIC values to micafungin or anidulafungin (9). These isolates were submitted to whole-genome sequencing. Total genomic DNA was used as input material for the library, which was sequenced using a MiSeq or NextSeq 1000 Sequencer (Illumina). Reads were trimmed with Sickle v.1.33 and error corrected using BayesHammer from SPAdes 3.11.1. Each sample was assembled using a reference-guided assembly in DNASTAR SeqMan Ngen v.16.0 (Madison, WI, USA). DNA regions containing *FKS* genes were compared to the sequences available in the literature (17).

RESULTS

Activity of rezafungin and comparator agents against yeasts. Rezafungin was active against 651 *C. albicans* isolates (MIC_{50}/MIC_{90}, 0.03/0.06 mg/L) (Table 1) and inhibited all of these isolates at MIC values of ≤0.25 mg/L (100% susceptible), which is the recently approved CLSI provisional breakpoint for this agent against this species. In addition, 97.8% of these isolates were wild type (WT) to rezafungin when the ECV criteria were applied (7). All *C. albicans* isolates were susceptible to the previously approved echinocandins (Table 2). Rezafungin activity against *C. albicans* was equivalent to the activity displayed by anidulafungin (MIC_{50}/MIC_{90}, 0.03/0.06 mg/L) and within ±1 dilution of those displayed by micafungin (MIC_{50}/MIC_{90}, 0.015/0.03 mg/L) and caspofungin (MIC_{50}/MIC_{90}, 0.015/0.03 mg/L). Two micafungin non-wild-type (NWT) *C. albicans* isolates were detected in this collection (Table 3). These isolates were from the USA (Massachusetts) and South Korea (1 each) and carried no mutations in *FKS1*. The micafungin NWT isolates were also NWT to rezafungin (MIC, 0.12 mg/L) when the recently approved CLSI ECV criteria were applied (≤0.06 mg/L), but were anidulafungin WT (MIC, 0.12 mg/L).

Fluconazole and voriconazole inhibited 99.2% and 99.8% of *C. albicans* isolates, respectively. Of the 5 fluconazole-nonsusceptible *C. albicans* isolates, 3 were from the USA (Washington, Kansas, and Iowa) and 1 each were from Argentina and Colombia (Table 4). Rezafungin (MIC values, ≤0.03 mg/L) and the other echinocandins (MIC values, ≤0.03 mg/L) remained active against fluconazole-nonsusceptible isolates.

The echinocandins displayed susceptibility rates of 96.2%, 97.2%, 97.9%, and 98.3% for anidulafungin, caspofungin, micafungin, and rezafungin, respectively, against the 289 *C. glabrata* isolates tested (Table 2). Rezafungin (MIC_{50}/MIC_{90}, 0.06/0.06 mg/L) exhibited similar activity against this *Candida* species compared to other echinocandins (MIC_{50}/MIC_{90} range, 0.015 to 0.06/0.03 to 0.12 mg/L) (Table 2).

Nine (3.1%) *C. glabrata* isolates were NWT to micafungin, and 8 of them displayed mutations on *FKS1* HS1 (3 isolates) and/or *FKS2* HS1 (6 isolates) gene (Table 3). The most frequent 1,3-β-D-glucan synthase mutation was S663P in Fks2 HS1 (3 occurrences), followed by S629P in Fks1 HS1 (2 occurrences). Alterations L630Q in Fks1 HS1, F659S, and a deletion of F659 Fks2 HS1 (1 occurrence each) were also observed. One isolate had an S629P alteration in Fks1 HS1 plus a stop codon at position Q1704 of the Fks2 HS1. This isolate displayed elevated MIC values for all echinocandins: ≥4 mg/L for anidulafungin, caspofungin, and micafungin and 2 mg/L for rezafungin. Seven micafungin NWT *C. glabrata* isolates were from the USA, including Colorado (3 isolates), Washington (2 isolates), California (1 isolate), and New York (1 isolate). *FKS* mutant *C. glabrata* isolates were also detected in Spain and Australia (1 isolate each). Rezafungin MIC values varied from 0.12 to 2 mg/L among micafungin NWT *C. glabrata* isolates.

The fluconazole resistance rate among *C. glabrata* was 4.5% and, although no breakpoints are available for the other azoles, NWT rates were 10.0% to voriconazole and 3.5% to posaconazole (Table 2). All fluconazole-resistant *C. glabrata* isolates were inhibited by rezafungin at the susceptible CLSI breakpoint (≤0.5 mg/L), and all but 1 isolate were also susceptible to other echinocandins (Table 4).

Notably, 10.5% of *C. parapsilosis* isolates were resistant to fluconazole and 8.4% were nonsusceptible to voriconazole. Rezafungin displayed a 99.6% susceptibility rate, and its activities (MIC_{50}/MIC_{90}, 1/2 mg/L) against this challenging *Candida* species were similar to those of micafungin (MIC_{50}/MIC_{90}, 1/1 mg/L; 100% susceptible)

TABLE 1 Antimicrobial activity of rezafungin tested against invasive fungal isolates from 2019 to 2020

Organism/group (no. of isolates)	No. (cumulative %) of isolates inhibited at MIC (mg/L) of:												MIC mg/L)[a]		CLSI provisional criterion[b]	
	≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4 >4	50%	90%	S (mg/L)[c]	ECV (mg/L)[d]
Candida albicans (651)	1 (0.2)	2 (0.5)	41 (6.8)	168 (32.6)	264 (73.1)	161 (97.8)	13 (99.8)	1 (100.0)					0.03	0.06	≤0.25	0.06
Candida glabrata (289)		0 (0.0)	1 (0.3)	4 (1.7)	105 (38.1)	163 (94.5)	8 (97.2)	3 (98.3)	0 (98.3)	1 (98.6)	4 (100.0)		0.06	0.06	≤0.5	0.12
Candida parapsilosis (239)				0 (0.0)	1 (0.4)	0 (0.4)	1 (0.8)	11 (5.4)	56 (28.9)	135 (85.4)	34 (99.6)	1 (100.0)	1	2	≤2	4
Candida tropicalis (166)		0 (0.0)	12 (7.2)	28 (24.1)	65 (63.3)	51 (94.0)	9 (99.4)	1 (100.0)					0.03	0.06	≤0.25	0.12
Candida krusei (40)		0 (0.0)	1 (2.5)	11 (30.0)	24 (90.0)	4 (100.0)							0.03	0.03	≤0.25	0.12
Candida dubliniensis (42)		0 (0.0)	2 (4.8)	0 (4.8)	16 (42.9)	17 (83.3)	7 (100.0)						0.06	0.12	≤0.12	0.12
Cryptococcus neoformans var. *grubii* (38)											0 (0.0)	38 (100.0)	>2	>2	–	–
Aspergillus fumigatus (186)	0 (0.0)	4 (2.2)	16 (10.8)	103 (66.1)	53 (94.6)	10 (100.0)							0.015	0.03	–	–
Aspergillus section *Flavi* (28)		0 (0.0)	13 (46.4)	12 (89.3)	3 (100.0)								0.015	0.03	–	–

[a]50%, MIC_{50}; 90%, MIC_{90}.

[b]S, susceptible; ECV, epidemiological cutoff value; –, not available.

[c]Rezafungin provisional susceptible-only breakpoint criteria approved in the CLSI June 2021 meeting are not yet official and should not be implemented by laboratories until they are published in the upcoming CLSI M27M44S and M57S documents.

[d]Rezafungin provisional epidemiological cutoff value criteria approved in the CLSI June 2021 meeting are not yet official and should not be implemented by laboratories until they are published in the upcoming CLSI M27M44S and M57S documents.

TABLE 2 Antimicrobial activities of rezafungin and comparator agents tested against *Candida* species isolated worldwide during 2019 to 2020

Species and antimicrobial agent (no. of isolates)	MIC (mg/L)[a]			CLSI[b]			ECV[c]	
	50%	90%	Range	% S	% I	% R	% WT	% NWT
C. albicans (651)								
Rezafungin[d]	0.03	0.06	≤0.002 to 0.25	100.0	–	–	97.8	2.2
Anidulafungin	0.03	0.06	≤0.002 to 0.12	100.0	0.0	0.0	100.0	0.0
Caspofungin	0.015	0.03	≤0.002 to 0.06	100.0	0.0	0.0	–	–
Micafungin	0.015	0.03	≤0.002 to 0.06	100.0	0.0	0.0	99.7	0.3
Fluconazole	0.12	0.25	≤0.008 to 16	99.2	0.3[e]	0.5	98.3	1.7
Posaconazole	0.03	0.06	≤0.002 to 0.25	–	–	–	98.0	2.0
Voriconazole	0.004	0.015	≤0.002 to 0.25	99.8	0.2	0.0	98.5	1.5
Amphotericin B	0.5	1	0.06 to 1	–	–	–	100.0	0.0
C. glabrata (289)								
Rezafungin[d]	0.06	0.06	0.008 to 2	98.3	–	–	97.2	2.8
Anidulafungin	0.06	0.12	0.015 to 4	96.2	1.4	2.4	97.6	2.4
Caspofungin	0.03	0.06	0.004 to >4	97.2	1.0	1.7	–	–
Micafungin	0.015	0.03	0.008 to 4	97.9	0.0	2.1	96.9	3.1
Fluconazole	4	16	0.06 to 128	–	95.5[e]	4.5	89.3	10.7
Posaconazole	0.5	1	0.03 to >8	–	–	–	96.5	3.5
Voriconazole	0.06	0.5	0.004 to 4	–	–	–	90.0	10.0
Amphotericin B	1	1	0.25 to 2	–	–	–	100.0	0.0
C. parapsilosis (239)								
Rezafungin[d]	1	2	0.03 to >2	99.6	–	–	99.6	0.4
Anidulafungin	2	4	0.03 to >4	86.2	13.4	0.4	99.6	0.4
Caspofungin	0.25	0.5	0.03 to 0.5	100.0	0.0	0.0	100.0	0.0
Micafungin	1	1	0.25 to 2	100.0	0.0	0.0	100.0	0.0
Fluconazole	0.5	8	0.06 to 128	87.9	1.7[e]	10.5	87.9	12.1
Posaconazole	0.06	0.12	0.008 to 0.25	–	–	–	100.0	0.0
Voriconazole	0.008	0.12	≤0.002 to 2	91.6	6.3	2.1	87.0	13.0
Amphotericin B	0.5	1	0.25 to 1	–	–	–	100.0	0.0
C. tropicalis (166)								
Rezafungin[d]	0.03	0.06	0.008 to 0.25	100.0	–	–	99.4	0.6
Anidulafungin	0.03	0.06	0.008 to 0.25	100.0	0.0	0.0	98.8	1.2
Caspofungin	0.03	0.03	≤0.002 to 0.12	100.0	0.0	0.0	–	–
Micafungin	0.03	0.06	0.008 to 0.06	100.0	0.0	0.0	100.0	0.0
Fluconazole	0.25	1	0.12 to >128	98.2	0.6[e]	1.2	97.0	3.0
Posaconazole	0.06	0.12	0.015 to 0.25	–	–	–	97.0	3.0
Voriconazole	0.03	0.06	0.004 to >8	98.8	0.6	0.6	98.8	1.2
Amphotericin B	0.5	1	0.25 to 1	–	–	–	100.0	0.0
C. krusei (40)								
Rezafungin[d]	0.03	0.03	0.008 to 0.06	100.0	–	–	100.0	0.0
Anidulafungin	0.06	0.06	0.03 to 0.12	100.0	0.0	0.0	100.0	0.0
Caspofungin	0.06	0.12	0.015 to 0.25	100.0	0.0	0.0	–	–
Micafungin	0.06	0.12	0.06 to 0.12	100.0	0.0	0.0	100.0	0.0
Fluconazole	32	32	16 to 64	–	–	–	–	–
Posaconazole	0.25	0.5	0.06 to 0.5	–	–	–	100.0	0.0
Voriconazole	0.25	0.25	0.06 to 0.5	100.0	0.0	0.0	100.0	0.0
Amphotericin B	1	2	1 to 2	–	–	–	100.0	0.0
C. dubliniensis (42)								
Rezafungin[d]	0.06	0.12	0.008 to 0.12	100.0	–	–	100.0	0.0
Anidulafungin	0.06	0.12	0.015 to 0.12	–	–	–	100.0	0.0
Caspofungin	0.03	0.06	0.015 to 0.06	–	–	–	–	–
Micafungin	0.015	0.03	0.008 to 0.06	–	–	–	100.0	0.0
Fluconazole	0.12	0.25	0.03 to 0.25	–	–	–	100.0	0.0
Posaconazole	0.03	0.06	0.015 to 0.06	–	–	–	100.0	0.0
Voriconazole	0.004	0.008	≤0.002 to 0.015	–	–	–	–	–
Amphotericin B	0.25	0.5	0.12 to 0.5	–	–	–	100.0	0.0

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TABLE 2 (Continued)

Species and antimicrobial agent (no. of isolates)	MIC (mg/L)[a]			CLSI[b]			ECV[c]	
	50%	90%	Range	% S	% I	% R	% WT	% NWT
C. neoformans var. *grubii* (38)								
Rezafungin	>2	>2	>2 to >2	–	–	–	–	–
Anidulafungin	>4	>4	4 to >4	–	–	–	–	–
Caspofungin	>4	>4	4 to >4	–	–	–	–	–
Micafungin	>4	>4	4 to >4	–	–	–	–	–
Fluconazole	4	8	0.5 to 16	–	–	–	97.4	2.6
Itraconazole	0.12	0.25	0.03 to 0.5	–	–	–	94.7	5.3
Posaconazole	0.12	0.25	0.015 to 0.5	–	–	–	97.4	2.6
Voriconazole	0.06	0.12	0.008 to 0.25	–	–	–	100.0	0.0
Amphotericin B	1	1	0.5 to 1	–	–	–	26.3	73.7

[a]50%, MIC_{50}; 90%, MIC_{90}.
[b]Criteria published in CLSI M60 (15). S, susceptible; I, intermediate; R, resistant; –, not available.
[c]Epidemiological cutoff value (ECV) criteria published in CLSI M59 (9). WT, wild type; NWT, not wild type; –, not available.
[d]Rezafungin provisional susceptible-only breakpoints and ECV criteria were approved in the CLSI June 2021 meeting but are not yet published in the upcoming CLSI M27M44S and M57 documents.
[e]Intermediate is interpreted as susceptible dose dependent.

and anidulafungin (MIC_{50}/MIC_{90}, 2/4 mg/L; 86.2% susceptible). Caspofungin showed greater activity (MIC_{50}/MIC_{90}, 0.25/0.5 mg/L; 100% susceptible) than other echinocandins against *C. parapsilosis* isolates from this collection. Rezafungin and other echinocandins inhibited all fluconazole-nonsusceptible *C. parapsilosis* isolates (29 isolates; 12.1% of all *C. parapsilosis*) from this collection (Table 4).

All *C. krusei* (40 isolates) and *C. tropicalis* (166 isolates) isolates displayed 100% susceptibility to echinocandins. Fks alterations were not detected in the single *C. tropicalis* isolate displaying an anidulafungin NWT MIC value (0.25 mg/L) (Tables 2 and 3). Rezafungin also inhibited all *C. dubliniensis* isolates (100% susceptible) at the proposed CLSI breakpoint of ≤0.12 mg/L. CLSI susceptibility breakpoints are not available for *C. dubliniensis* for the other echinocandins, but all isolates were WT to micafungin, anidulafungin, and rezafungin (Table 2).

Echinocandins, including rezafungin, exhibited limited activity against *Cryptococcus neoformans* var. *grubii* (MIC_{50}/MIC_{90}, >2/>4 mg/L). Fluconazole (MIC_{50}/MIC_{90}, 4/8 mg/L; 97.4% WT) and other systemic triazoles (MIC_{50}/MIC_{90} range, 0.06 to 0.12/0.12 to 0.25 mg/L; 94.7% to 100.0% WT) were active against these isolates.

Activities of rezafungin and comparator agents against *Aspergillus* spp. Rezafungin was active (MEC_{50}/minimal effective concentration for 90% of isolates

TABLE 3 *FKS* alterations detected in strains of *Candida* spp.

Organism	State and/or country	MIC (mg/L) according to CLSI method[a]				1,3-β-D-Glucan synthase mutation[b]:			
		RZF	ANF	CSF	MCF	Fks1 HS1	Fks1 HS2	Fks2 HS1	Fks2 HS2
Candida albicans	Korea	0.12	0.12	0.06	0.06	WT	WT	NT	NT
	MA, USA	0.12	0.12	0.06	0.06	WT	WT	NT	NT
Candida glabrata	Australia	0.25	1	0.25	0.06	WT	WT	**F659S**	WT
	CA, USA	2	4	1	0.5	WT	WT	**S663P**	WT
	CO, USA	0.25	0.5	0.25	0.25	WT	WT	**F659 deletion**	WT
	CO, USA	2	4	1	0.25	WT	WT	**S663P**	WT
	CO, USA	0.12	0.12	0.12	0.06	WT	WT	WT	WT
	NY, USA	1	2	4	1	**S629P**	WT	WT	WT
	Spain	2	2	>4	1	WT	WT	**S663P**	WT
	WA, USA	0.25	0.25	0.12	0.06	**L630Q**	WT	WT	WT
	WA, USA	2	4	>4	4	**S629P**	WT	**Disrupted (Q1704X[c])**	**Disrupted (Q1704X[c])**
Candida tropicalis	NY, USA	0.12	0.25	0.03	0.03	WT	WT	NT	NT

[a]RZF, rezafungin; ANF, anidulafungin; CSF, caspofungin; MCF, micafungin.
[b]Mutations are in boldface. WT, wild type; NT, not tested.
[c]Stop codon.

TABLE 4 Echinocandin activity against fluconazole-nonsusceptible *Candida* species isolates[a]

Organism (no. of isolates)	Country (no. of isolates [state])	MIC range (mg/L) according to CLSI method[b]			
		RZF	ANF	CSF	MCF
Candida albicans (5)	Argentina (1), Colombia (1), USA (3 [1 each in WA, KS, and IA])	0.015–0.03	0.008–0.03	0.015	0.008–0.015
Candida glabrata (13)	Hungary (1), Israel (1), Spain (5), UK (1), USA (5 [1 each in CA, CO, IN, NY, and WA])	0.03–0.25	0.06–0.5	0.03–0.25	0.015–0.25
Candida parapsilosis (29)	Australia (1), Germany (1), Greece (3), Italy (14), Israel (1), South Korea (1), Spain (1), Turkey (3), USA (4 [2 in TX and 1 each in CA and NY])	0.5–2	1–2	0.12–0.5	0.25–1
Candida tropicalis (3)	Philippines (1), USA (2 [WA])	0.015–0.03	0.015–0.06	0.015	0.008–0.06

[a]Criteria published in CLSI M60 (15).
[b]RZF, rezafungin; ANF, anidulafungin; CSF, caspofungin; MCF, micafungin.

tested [MEC_{90}], 0.015/0.03 mg/L) (Table 5) against 186 *A. fumigatus* isolates collected from invasive infections. All *A. fumigatus* isolates were inhibited by rezafungin at an MEC value of 0.06 mg/L. Similar activity was observed for anidulafungin (MEC_{50}/MEC_{90}, 0.015/0.06 mg/L), caspofungin (MEC_{50}/MEC_{90}, 0.015/0.03 mg/L; 100% WT), and micafungin (MEC_{50}/MEC_{90}, 0.008/0.015 mg/L).

The rezafungin activity (MEC_{50}/MEC_{90}, 0.015/0.03 mg/L) against *Aspergillus* section *Flavi* (28 isolates) was equivalent to the activity of caspofungin (MEC_{50}/MEC_{90}, 0.015/0.03 mg/L; 100% WT) and similar to the activities of anidulafungin (MEC_{50}/MEC_{90}, 0.008/0.015 mg/L) and micafungin (MEC_{50}/MEC_{90}, 0.008/0.015 mg/L). *Aspergillus* section *Flavi* isolates were all inhibited by the four echinocandins at an MEC of 0.03 mg/L. The mold-active azoles were also active against *A. fumigatus* (MIC_{50}/MIC_{90} range, 0.25 to 1/0.5 to 1 mg/L; 91.4% to 96.8% WT) and *Aspergillus* section *Flavi* (MIC_{50}/MIC_{90}, 0.25 to 0.5/0.5 to 1 mg/L; 100% WT). A total of 16 (8.6%) *A. fumigatus* isolates were nonsusceptible to voriconazole (MIC, $\geq$1 mg/L): 7 isolates from Europe, 3 from Asia-Pacific, and 6 from North America. Rezafungin MEC values ranged from 0.004 mg/L to 0.03 mg/L against voriconazole-nonsusceptible *A. fumigatus* isolates.

DISCUSSION

A change in the epidemiology of IFIs has occurred in recent decades. IFIs shifted from being most frequently associated with HIV-positive patients to aggravating the clinical course of patients undergoing drug-induced immunosuppression and critically ill patients in ICUs (18). Nevertheless, *Candida*, *Aspergillus*, *Pneumocystis*, and endemic mycoses can cause disease in both immunocompetent and immunocompromised hosts, with a state of impaired immunity resulting in more severe disease (19). In addition to the increased frequency of IFIs among non-HIV patients, Rayens and colleagues showed that the mortality rate of patients in the USA with a IFI diagnosis has increased in the past decade, mostly due to the expansion of the at-risk patient populations with risk factors other than HIV (18).

The rising incidence of fungal infection and antifungal resistance has prompted the need for novel antifungal agents. Existing systemic antifungal agents available to treat the burden of invasive fungal diseases have a variety of limitations, from toxicity and drug interactions to increasing resistance rates in common fungal pathogens, such as *Candida* and *Aspergillus* species. Currently, echinocandins are the recommended first-line treatment for candidemia and invasive candidiasis, as amphotericin B is associated with higher rates of toxicity and the azoles displayed higher rates of antifungal resistance, drug-drug interactions, and decreased efficacy (20, 21).

Rezafungin is a next-generation echinocandin with a modified choline moiety at the cyclic echinocandin core. This structure confers greater chemical and metabolic stability and solubility, which potentially contributes to lower toxicity (22, 23). Similar

TABLE 5 Antimicrobial activity of rezafungin and comparator agents tested against *Aspergillus* spp. and *Cryptococcus* species isolated worldwide during 2019 to 2020

Species and antimicrobial agent (no. of isolates)	MIC/MEC (mg/L)[a]			ECV[b]	
	50%	90%	MIC range (mg/L)	% WT	% NWT
Aspergillus fumigatus (186)					
Rezafungin	0.015	0.03	0.004 to 0.06	–	–
Anidulafungin	0.015	0.06	0.004 to 0.12	–	–
Caspofungin	0.015	0.03	≤0.002 to 0.06	100.0	0.0
Micafungin	0.008	0.015	≤0.002 to 0.015	–	–
Itraconazole	1	1	0.25 to >8	91.4	8.6
Posaconazole	0.25	0.5	0.06 to 8	–	–
Voriconazole	0.5	0.5	0.12 to >8	96.8	3.2
Amphotericin B	2	2	0.5 to 4	98.9	1.1
Aspergillus section *Flavi* (28)					
Rezafungin	0.015	0.03	0.008 to 0.03	–	–
Anidulafungin	0.008	0.015	0.004 to 0.03	–	–
Caspofungin	0.015	0.03	0.004 to 0.03	100.0	0.0
Micafungin	0.008	0.015	≤0.002 to 0.03	–	–
Itraconazole	0.5	1	0.25 to 1	100.0	0.0
Posaconazole	0.25	0.5	0.25 to 0.5	100.0	0.0
Voriconazole	0.5	1	0.25 to 1	100.0	0.0
Amphotericin B	2	4	1 to >4	96.4	3.6

[a]50%, MIC_{50}/MEC_{50}; 90%, MIC_{90}/MEC_{90}.
[b]Epidemiological cutoff value (ECV) criteria published in CLSI M59 (9). WT, wild type; NWT, not wild type; –, not available.

to other echinocandins, rezafungin inhibits the synthesis of β-1,3-glucan and compromises the integrity of the fungal cell wall. PK/PD studies have shown that rezafungin provides advantages over other echinocandins, including a prolonged half-life (~133 h) and high plasma drug concentrations (24, 25). Rezafungin has concentration-dependent fungicidal activity and is highly bound to plasma proteins, with extensive tissue distribution and minimal urinary concentration (22, 26). Rubino and Flanagan demonstrated that rezafungin achieved a plasma maximum concentration of drug in serum (C_{max}) of 16.4 ± 2.17 μg/mL after the first 400-mg dose and prior to the second dose in patients with candidemia from the phase 2 STRIVE study, which is >4, >64, >64, >130, and >260 times higher than the rezafungin MIC values displayed in this collection by *C. glabrata*, *C. albicans*, *C. tropicalis*, *C. dubliniensis*, and *C. krusei*, respectively (24, 27). In addition, 99.6% of *C. parapsilosis* isolates were inhibited by rezafungin at an MIC of 2 mg/L, which is 8 times lower than the plasma C_{max} demonstrated by the first 400-mg dose.

Rezafungin PK/PD studies using models of neutropenic mouse invasive candidiasis against *C. tropicalis* and *C. dubliniensis* demonstrated potent activity and area under the concentration-time curve (AUC)/MIC targets likely to be exceeded for >99% of these isolates with i.v. doses of 400 mg once weekly (28). Moreover, Lepak and colleagues also demonstrated that if a patient were to receive 400 mg of rezafungin on day 1, followed by 200 mg on day 8 to complete 2 weeks of therapy, the stasis target would be expected to be achieved against all *C. albicans* and *C. parapsilosis* isolates with MICs of ≤1 mg/L and against all *C. glabrata* isolates with MICs of ≤16 mg/L. This result would correspond to the rezafungin concentration that inhibited 100%, 85.4%, and 100% of *C. albicans*, *C. parapsilosis*, and *C. glabrata* isolates from this collection, respectively (29).

Results from the STRIVE trial, a phase 2, randomized, double-blind study comparing rezafungin at either 400 mg weekly or 400 mg in the first week and then 200 mg weekly to caspofungin at 70 mg for day 1 followed by 50 mg daily, showed overall cure rates of rezafungin at 400 mg/200 mg of 76.1% compared to 60.5% for the rezafungin 400-mg arm and 67.2% for the caspofungin in 207 patients with candidemia (30). The 400-mg/

200-mg regimen was selected to continue the clinical development of rezafungin in two phase 3 trials. The ReSTORE trial enrolled patients to determine the noninferiority of rezafungin compared to caspofungin for the treatment of candidemia and/or invasive candidiasis. In addition, the ReSPECT trial is currently recruiting patients to evaluate the efficacy of rezafungin in a 90-day prophylactic regimen for the prevention of *Candida*, *Aspergillus*, and *Pneumocystis* infections in allogeneic blood and marrow transplant patients.

Based on microbiological data, PK/PD, and phase 2 trial results, the CLSI Subcommittee on Antifungal Susceptibility Tests (AFSC) approved rezafungin susceptible-only provisional breakpoints against key prevalent *Candida* species (7). Notably, rezafungin susceptible-only provisional breakpoints were established due to the absence or rare occurrence of resistant strains. Therefore, if an MIC value above the susceptible-only provisional breakpoint is obtained, that does not necessarily mean that the isolate has a resistance mechanism. Isolates displaying MIC values above the susceptible breakpoint should be reported as nonsusceptible (31). In this case, the evaluation of the species-specific ECV or further investigation of the presence of resistance mechanisms may be useful to drive treatment decisions. Applying these provisional breakpoints, our results demonstrate that rezafungin displayed comparable rates of susceptibility to other echinocandins against the same species. Susceptibility rates to rezafungin varied from 98.3% in *C. glabrata* to 100% in *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. dubliniensis*. Additionally, we showed that the *FKS* HS mutations confer some level of cross-resistance among echinocandins, including rezafungin, and were mainly observed in *C. glabrata*. These results are in accordance with previous studies where similar rezafungin activity was observed (32). In this study, fluconazole resistance was detected among 0.5%, 4.5%, 10.5%, and 1.2% of *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* isolates, respectively, causing invasive infections worldwide. Rezafungin and other echinocandins were active against 100% and 98.0% of the fluconazole-resistant isolates, respectively.

Although azoles are the first-line therapy recommended for aspergillosis by the Infectious Diseases Society of America, there is rising concern about the emergence and dissemination of azole-resistant *A. fumigatus* (2). Resistance to the azoles may be due to prolonged exposure to these agents in patients with invasive or chronic aspergillosis or environmental exposure in agriculture (33). In a retrospective cohort study conducted by Lestrade and colleagues, IA caused by voriconazole-resistant *A. fumigatus* was associated with an increased overall mortality rate compared to voriconazole-susceptible IA (27). Furthermore, inappropriate empirical therapy with voriconazole was also associated with increased mortality rates, despite switching to appropriate antifungal therapy. For these reasons, echinocandins are recommended as salvage therapy for aspergillosis. However, as appropriate initial antifungal therapy was found to be critical, up-front combination antifungal therapy may be required to increase the probability of survival for patients at risk for IA in geographic regions with high azole resistance rates (2, 34). Combination therapy includes voriconazole or isavuconazole combined with an echinocandin or liposomal amphotericin B, but clinical evidence supporting these treatment options is lacking (33, 34). Here, 8.6% of *A. fumigatus* isolates were nonsusceptible to voriconazole, but rezafungin showed potent *in vitro* activity against these isolates, with MIC values ranging from 0.004 mg/L to 0.03 mg/L.

In summary, rezafungin and other echinocandins displayed similar activities against *Candida* and *Aspergillus* species isolates from IFIs. While the rezafungin breakpoints and epidemiological cutoff values approved by the AFSC are not yet official and should not be implemented by laboratories until they are published in the upcoming CLSI M27M44S and M57S documents, the *in vitro* results of rezafungin against the evaluated 2019 to 2020 isolates support the continued development of rezafungin for the treatment and prevention of invasive fungal disease.

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