Activity of a Long-Acting Echinocandin, Rezafungin, and Comparator Antifungal Agents Tested against Contemporary Invasive Fungal Isolates: SENTRY Program 2016-2018

Running title: In vitro activity of rezafungin

Michael A. Pfaller¹, Cecilia Carvalhaes¹, Shawn A. Messer¹, Paul R. Rhomberg¹, Mariana Castanheira¹

¹JMI Laboratories, North Liberty, Iowa, USA
²University of Iowa, Iowa City, Iowa, USA

Contact Information: Cecilia Carvalhaes, MD, PhD, D(ABMM)
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, IA 52317
Phone: (319) 665-3370
Fax: (319) 665-3371
Email: cecilia-carvalhaes@jmilabs.com
We evaluated the activity of rezafungin and comparators using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods against worldwide collection of 2,205 invasive fungal isolates recovered from 2016-2018. Candida spp. (1,904 isolates; 6 species), Cryptococcus neoformans (73), Aspergillus fumigatus (183) and Aspergillus flavus (45) isolates were susceptibility (S) tested for rezafungin as well as the comparators caspofungin, anidulafungin, micafungin, and azoles. Interpretive criteria were applied following CLSI published clinical breakpoint (CBP) and epidemiological cutoff values (ECV). Isolates displaying non-WT echinocandin MIC values were sequenced for fks hot spot (HS) mutations. Rezafungin inhibited 99.8% of Candida albicans isolates (MIC\textsubscript{50/90}, 0.03/0.06 \(\mu\text{g/mL}\)), 95.7% of Candida glabrata (MIC\textsubscript{50/90}, 0.06/0.12 \(\mu\text{g/mL}\)), 97.4% of Candida tropicalis (MIC\textsubscript{50/90}, 0.03/0.06 \(\mu\text{g/mL}\)), 100.0% of Candida krusei (MIC\textsubscript{50/90}, 0.03/0.06 \(\mu\text{g/mL}\)), and 100.0% of Candida dubliniensis (MIC\textsubscript{50/90}, 0.06/0.12 \(\mu\text{g/mL}\)) at \(\leq 0.12 \mu\text{g/mL}\). All (329/329 [100.0%]) Candida parapsilosis isolates (MIC\textsubscript{50/90}, 1/2 \(\mu\text{g/mL}\)) were inhibited by rezafungin at \(\leq 4 \mu\text{g/mL}\). Fluconazole resistance was detected among 8.6% of C. glabrata, 12.5% of C. parapsilosis, 3.2% of C. dubliniensis, and 2.6% of C. tropicalis. Rezafungin activity against these 6 Candida spp. was similar to the activity of other echinocandins. Detection of fks HS mutation was performed by sequencing echinocandin resistant or non-WT Candida spp. isolates. Good activity was observed by fluconazole and other azoles against Cr. neoformans, whereas echinocandins, including rezafungin, displayed limited activity. Rezafungin displayed similar activity as other echinocandins against A. fumigatus and A. flavus. These in vitro data contribute to accumulating research demonstrating rezafungin potential for preventing and treating invasive fungal infections.
Among the available systemically active antifungal agents, the echinocandins, including caspofungin, anidulafungin, and micafungin, and the azoles, such as fluconazole, voriconazole, isavuconazole, and posaconazole, are all employed empirically as directed therapy and for prophylaxis in patients with suspected or documented invasive fungal infection (1-7). Whereas fluconazole remains the most frequently employed antifungal globally, the echinocandin class has steadily increased in use in academic and community hospital settings (2, 4, 7-11).

The documented potency, spectrum, and safety of the echinocandins has led many experts in infectious diseases to consider echinocandins as initial therapy for treating candidemia (5, 7, 9, 12). A meta-analysis of randomized clinical trials comparing treatment for candidemia and invasive candidiasis (IC) showed that initial therapy with an echinocandin was a significant predictor of survival (13). Once clinically stable, de-escalation to an oral azole (usually fluconazole) is suggested for all patients (1, 5, 7, 12, 14).

Echinocandins have some important limitations, despite proven safety and efficacy (9, 15). Most notably, the daily parenteral dosing requirement complicates administration post discharge in patients requiring extended therapy. Indeed, much of the growth in outpatient antifungal expenditure, as documented in a recent survey of antifungal use in US hospitals, was for echinocandins. This survey suggests that outpatient antifungal use may be increasing (7). Although step-down therapy from an echinocandin to fluconazole may partially address the outpatient antifungal expenditure, it is complicated by increasing resistance to fluconazole among common species of Candida (1, 7, 9, 14, 16). Other potential drawbacks of the available echinocandins for clinical application are use of a fixed dose irrespective of body size or species susceptibility and emerging resistance mediated by mutations in the FKS genes (15, 17). It has been suggested that underdosing echinocandins coupled with poor penetration to certain body sites may partially account for emerging echinocandin resistance (15, 18, 19). An echinocandin that could be safely administered at higher doses to ensure optimal pharmacokinetic (PK) and pharmacodynamic (PD) features and target attainment may facilitate outpatient therapy, reduce hospital stay, and possibly delay or prevent the development of echinocandin resistance, thus becoming an important step toward improving the ability to effectively manage candidemia and IC (15, 20).
Rezafungin (Cidara Therapeutics, Inc.) is a novel echinocandin that exhibits a prolonged half-life and displays chemical stability in plasma, aqueous solution, and at elevated temperature (15, 21-27). The in vitro activity of rezafungin against Candida spp. has been shown to be comparable to other clinically available echinocandins (2, 28-36). Rezafungin is being developed for treating of candidemia and other forms of IC on a once-weekly IV administration (27). A phase 3, randomized, double-blind, multicenter clinical trial of the efficacy and safety of rezafungin for injection compared with intravenous caspofungin followed by oral fluconazole step down in the treatment of subjects with candidemia and/or IC (NCT03667690; ReSTORE) is underway.

In the present study, we examined the in vitro activity of rezafungin compared with the other systemically active antifungal agents by testing a global collection of 2,205 clinical isolates of yeasts (Candida and Cryptococcus spp.) and molds (Aspergillus spp.) obtained during the 2016-2018 SENTRY Surveillance Program. All isolates were submitted to broth microdilution (BMD) susceptibility testing following Clinical and Laboratory Standards Institute (CLSI) methods (37, 38). Some results have been presented in part, for the individual years included in the study period, at the following scientific conferences: ASM Microbe 2018, IDWeek 2018, and IDWeek 2019 (34-36).

RESULTS

Geographic distribution of Candida species. Among the 1,904 Candida isolates submitted for testing from 2016 through 2018, 43.9% were Candida albicans, 19.6% were Candida glabrata, 17.3% were Candida parapsilosis, 10.3% were Candida tropicalis, 4.9% were Candida dubliniensis, and 4.0% were Candida krusei (Table 1). Table 1 lists the frequencies of the most common species of Candida in each geographic region included in the SENTRY Program. C. albicans was most common in the Asia-Pacific (APAC) region (49.8%) and Europe (EUR) (49.6%) and least common in North America (NA [USA and Canada]; 34.1%), whereas C. glabrata was most common in NA (27.7%) and least common in Latin America (LATAM) (8.7%). C. parapsilosis and C. tropicalis were more common than C. glabrata in LATAM (20.2% and 20.2% versus 8.7%). C. tropicalis also was a frequent cause of IC in the APAC region (16.9%). C. krusei was more common in LATAM (6.2%), whereas C. dubliniensis was more common in NA (9.0%).
Rezafungin activity against Candida spp., Cr. neoformans var. grubii, and Aspergillus spp.
isolates. Among the 6 species of Candida shown in Table 2, rezafungin was most active against C.
albicans (MIC$_{90}$, 0.06 µg/mL), C. tropicalis (MIC$_{90}$, 0.06 µg/mL), and C. krusei (MIC$_{90}$, 0.06 µg/mL) andleast active against C. parapsilosis (MIC$_{90}$, 2 µg/mL). With minimal variation over the 3-year time period,theclassical MIC values were 0.03 µg/mL for C. albicans, C. tropicalis, and C. krusei, 0.06 µg/mL for C.
glabrata and C. dubliniensis, and 1 µg/mL for C. parapsilosis. The MIC distribution data was employed todevelop tentative ECVs using the iterative statistical method recommended by the CLSI (41) to establishthe WT distribution for rezafungin and each of the tested species. The ECV of rezafungin for eachspecies was 0.12 µg/mL for C. albicans (99.8% WT), C. glabrata (95.7% WT), C. tropicalis (97.4% WT), and C. krusei (100.0% WT), 0.25 µg/mL for C. dubliniensis (100.0% WT), and 4 µg/mL for C. parapsilosis in Table 2). Overall, 98.5% of the Candida spp. tested, aside from C. parapsilosis, wereinhibited by ≤0.12 µg/mL and 99.2% were inhibited by ≤0.25 µg/mL of rezafungin (Table 2). Rezafunginshowed limited activity against Cr. neoformans (MIC$_{90}$, >4 µg/mL) and was highly active againstAspergillus species (MEC$_{100}$, ≤0.03 µg/mL). The ECV calculated for A. fumigatus was 0.03 µg/mL.

Rezafungin and comparators in vitro activity against Candida spp., Cr. neoformans var. grubii andAspergillus spp. isolates. Rezafungin (MIC$_{50/90}$, 0.03/0.06 µg/mL; 99.8% WT) displayed comparableactivity against C. albicans to that of anidulafungin, micafungin, and caspofungin (MIC$_{50/90}$, 0.015/0.03 µg/mL [anidulafungin, caspofungin and micafungin]; Table 3). One C. albicans isolate was resistant (MIC,1 µg/mL) to both caspofungin and micafungin and non-wild type (NWT) (MIC > ECV, 0.25 µg/mL) torezafungin while harboring a mutation in fks1 HS1 (S645P; Table 4). Three fluconazole-resistant strainswere detected, one from LATAM and two from NA (Table 5).

Among 374 C. glabrata isolates tested, 95.7% were inhibited by rezafungin (MIC$_{50/90}$, 0.06/0.12 µg/mL) atthe ECV cutoff value of ≤0.12 µg/mL (Tables 2 and 3). Micafungin (MIC$_{50/90}$, 0.015/0.03 µg/mL),caspofungin (MIC$_{50/90}$, 0.03/0.06 µg/mL), and anidulafungin (MIC$_{50/90}$, 0.06/0.12 µg/mL) respectivelyinhibited 96.0%, 97.1%, and 94.4% of these isolates at the current CLSI breakpoints (39). Mutationswithin fks HS leading to amino acid alterations were found in 17 (68.0%) out of 25 C. glabrata isolates.
displaying echinocandin MIC values greater than the ECV (Table 4). The most common substitutions were fks2 HS1 S663P (7 isolates), fks2 HS1 F659_del (2 isolates), fks2 HS1 Y657_del/F658Y (2 isolates), and fks1 HS1 S629P (2 isolates). The corresponding rezafungin MIC values ranged from 0.06 to 2 µg/mL (82.4% > ECV [0.12 µg/ml]) for all 17 isolates with an fks mutation (Table 4). Among all C. glabrata isolates from 2016-2018, 8.6% displayed a fluconazole-resistant phenotype. Based on the ECV cutoff published by CLSI, 7.0% and 12.8% of these isolates were categorized as NWT to posaconazole and voriconazole, respectively (39,40) (Table 3). High rates of resistance to fluconazole were seen in C. glabrata isolates from EUR (6.0%) and NA (13.2%) (Table 5). Not only was C. glabrata a rare cause of IC in LATAM (Table 1), it was also less resistant to fluconazole (0.0%) compared to the other monitored regions (Table 5).

Rezafungin inhibited all C. parapsilosis isolates (n = 329) at the ECV of ≤4 µg/mL (Table 2). Rezafungin activity (MIC₉₀, 2 µg/mL) was similar to that observed for micafungin (MIC₉₀, 1 µg/mL; 100.0% S) and anidulafungin (MIC₉₀, 2 µg/mL; 93.9% S) and was 4-fold lower than caspofungin (MIC₉₀, 0.5 µg/mL; 100.0% S) (Tables 2 and 3). Among C. parapsilosis, a total of 41 isolates (12.5%) were categorized as fluconazole resistant, and 36 of these strains (87.8%) were from European medical centers (24.8% fluconazole resistant) (Table 5). Although C. parapsilosis was common in LATAM (20.2% of Candida isolates, second in rank order; Table 1), no fluconazole-resistant strains were detected among 49 isolates tested (Table 5).

C. tropicalis (n = 196) isolates were largely susceptible to anidulafungin, caspofungin, and micafungin (99.0% S; Table 3). Rezafungin (MIC₉₀₉₀, 0.03/0.06 µg/mL) inhibited 97.4% of isolates at the proposed ECV of ≤0.12 µg/mL (Tables 2 and 3). Among 7 C. tropicalis isolates categorized as NWT to echinocandin and submitted to fks sequencing, 2 harbored fks1 HS1 mutations leading to amino acid alterations (S645P and F650S; Table 4). Both isolates were resistant to anidulafungin (MIC values of 1 µg/mL for both), caspofungin (MIC values of >8 and 2 µg/mL), and micafungin (MIC values of 2 and 1 µg/mL) and NWT (MIC > ECV, 2 and 1 µg/mL) to rezafungin. The remaining 5 isolates did not contain fks1 mutations and 4 were WT to rezafungin (MIC values ≤0.12 µg/mL). Fluconazole resistance was observed in 5 C. tropicalis isolates (2.6% of total; Table 5). No fluconazole-resistant strains were among 45 isolates from NA and 5.0% of isolates from APAC were resistant to fluconazole (Table 5).
Rezafungin (MIC$_{50/90}$, 0.03/0.06 µg/mL) was active against 77 C. krusei; 100.0% of isolates were inhibited at ≤0.12 µg/mL, the ECV for this species (100.0% WT; Tables 2 and 3). These isolates were susceptible to anidulafungin (MIC$_{50/90}$, 0.06/0.12 µg/mL; 100.0% S), micafungin (MIC$_{50/90}$, 0.06/0.12 µg/mL; 100.0% S), and caspofungin (MIC$_{50/90}$, 0.12/0.25 µg/mL; 98.7% S) (Table 3) according to CLSI breakpoint criteria. Four C. krusei isolates were NWT to one or more echinocandin, none of which were shown to possess an fks mutation: all were WT to rezafungin (Table 4). Voriconazole was active against 96.1% of C. krusei isolates and all isolates displayed posaconazole WT phenotype (Table 3).

Fluconazole (MIC$_{50/90}$, 2/4 µg/mL) and other azoles (MIC$_{50/90}$ values were 0.12/0.25, and 0.03/0.12 µg/mL for posaconazole, and voriconazole, respectively) displayed good activity against Cr. neoformans, whereas echinocandins, including rezafungin, displayed limited activity.

The activity of rezafungin against 183 A. fumigatus isolates tested (MEC$_{50/90}$, 0.015/0.03 µg/mL; all inhibited at ECV of ≤0.03 µg/mL [100.0% WT]) was comparable to that of caspofungin (MEC$_{50/90}$, 0.015/0.03 µg/mL, 100% WT), and micafungin (MEC$_{50/90}$, ≤0.008/0.015 µg/mL). Voriconazole and itraconazole showed WT MIC values against over 98% of A. fumigatus isolates (Table 3).

Against A. flavus species complex isolates (n = 45), comparable activity was observed for rezafungin (MEC$_{50/90}$, ≤0.008/0.015 µg/mL) and other echinocandins such as caspofungin (MEC$_{50/90}$, 0.015/0.03 µg/mL, 100% WT), anidulafungin (MEC$_{50/90}$, 0.015/0.03 µg/mL, 100% WT), and micafungin (0.015/0.03 µg/mL). A WT phenotype was observed for itraconazole, posaconazole, and voriconazole against all A. flavus species complex isolates (Table 3).

DISCUSSION
This study provides a robust estimate of the WT MIC/MEC distributions of rezafungin for 6 species of *Candida* as well as *A. fumigatus* and *A. flavus* and expands upon our earlier rezafungin activity observations (31-33). Although establishing definitive ECVs and CBPs for rezafungin requires multicenter studies involving larger numbers of isolates of each species (41), we suggest that the ECV determined using CLSI BMD methods in the present study is ≤0.12 μg/mL for *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei* (98.5% of 1,482 isolates; Table 2), ≤0.25 μg/mL for *C. dubliniensis* (100.0% of 93 isolates), ≤4 μg/mL for *C. parapsilosis* (100.0% of 329 isolates), and ≤0.03 μg/mL for *A. fumigatus* (100.0% of 183 isolates) (Table 2). Notably, these values are far below the peak achievable plasma concentrations of 22-30 μg/mL at the 400 mg dose (15, 26, 27) and are equivalent to the ECVs established for these species/species groups and the clinically available echinocandins (40, 42, 43).

Additional support for these ECVs is found in a recent multicenter study of rezafungin activity against *Candida* spp. determined using the EUCAST BMD method and both visual and statistical means of determining possible wild type-upper limit (WT-UL) values (28). In the four-laboratory study, WT-UL cutoffs were proposed for *C. glabrata* (0.125 μg/mL), *C. krusei* (0.125 μg/mL), and *C. parapsilosis* (4 μg/mL). Although interlaboratory variation precluded proposing cutoffs for *C. albicans* and *C. tropicalis*, the WT-UL statistical 97.5% endpoint was 0.063 μg/mL for *C. albicans* and 0.25 μg/mL for *C. tropicalis* (28). These values compare favorably with the ECVs generated by the CLSI BMD method in the present study. Although an essential agreement rate (+/− 2 dilution steps) of 92.0% for *C. albicans* and 100.0% for *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* between CLSI and EUCAST methods for rezafungin was observed previously (31), alignment between CLSI and EUCAST susceptibility profiles and breakpoints is yet to be determined, as significant interlaboratory EUCAST MIC variability (likely attributed to nonspecific binding of the drug to plastics) has been identified for rezafungin against a more susceptible collection of *Candida* spp. (28, 44).

As seen in Table 4, the highest rezafungin MIC values for fks mutant strains of *C. albicans* and *C. glabrata* were 0.25 μg/mL and 2 μg/mL, respectively. Both of these mutant MIC values are within the range of concentrations that Bader et al (20) estimated would achieve percent probabilities of PK-PD.
target attainment of 100% through week 6, suggesting that weekly regimens of rezafungin can achieve exposures associated with efficacy against some fks mutant Candida isolates (20). In addition, the same study showed that the mutant prevention concentration, the concentration of drug that would inhibit emerging resistant mutants, for both rezafungin and micafungin was 16 µg/mL (27). Given that the high plasma drug exposure of rezafungin easily exceeds the mutant prevention concentration for Candida, a possible advantage of rezafungin may be to prevent resistance development in the echinocandin class of antifungal agents (20, 22, 24, 27).

Expert panel guidelines from both NA (5) and EUR (12) favor step-down therapy to fluconazole or voriconazole for patients with Candidiasis in specific clinical situations, that is when clinical improvement and clearance of Candida from the bloodstream was achieved by initial echinocandin therapy. In addition, the organism must be susceptible to fluconazole (e.g., C. albicans, C. parapsilosis, and C. tropicalis) or voriconazole (e.g., C. krusei). Unfortunately, antifungal susceptibility testing is still not routinely available in many patient care settings. In these circumstances, clinicians are forced to rely on simple species identification of Candida as a predictor of fluconazole susceptibility (5, 12). In most instances, isolates of C. albicans, C. parapsilosis, and C. tropicalis are considered to be reliably susceptible to fluconazole (16), whereas C. glabrata and C. krusei are considered to be intrinsically less susceptible or resistant and are suboptimal targets for using fluconazole (5, 12). This approach may be seriously flawed if fluconazole resistance emerges among the traditionally susceptible species. Concern regarding this approach has been raised by Oxman et al (45) who found that despite the small proportion of C. albicans, C. parapsilosis, and C. tropicalis with resistance/decreased susceptibility to fluconazole, these species comprised 36% of the reduced susceptibility group (including C. glabrata and C. krusei), potentially compromising therapy with resultant clinical failure. These concerns are supported by data from the current survey showing that resistance to fluconazole was 0.4% for C. albicans, 12.5% for C. parapsilosis, and 2.6% for C. tropicalis (Tables 3 and 5). In aggregate, these three normally susceptible species accounts for 31% of all fluconazole-resistant isolates. Species identification should be used cautiously as the sole criterion for anti-Candida agent selection (5, 45).
The increased rate of fluconazole resistance among *C. parapsilosis* (12.5% overall) and *C. tropicalis* (2.6% overall) in the present study is important as these species are the most commonly isolated non-*C. albicans* species in LATAM (Table 1). Although less common than *C. glabrata* in EUR, the finding of fluconazole resistance in 24.8% of *C. parapsilosis* isolates exceeds that observed in *C. glabrata* (6.0%) isolates and is cause for alarm (Table 5).

This survey has some limitations as noted elsewhere (16): the SENTRY Surveillance Program is a sentinel surveillance and not population-based; therefore, we may over/underestimate the activity of the tested agents. In addition, we do not collect data concerning antifungal use or outcomes of therapy. The purpose of SENTRY is to identify trends in antifungal resistance and to document the emergence of new species as well as the activity of new and established agents against key fungal pathogens. The broad geographic distribution, longitudinal nature of the surveillance, and the use of molecular and proteomic identification methods and determination of resistance mechanisms is a strength of the SENTRY Program.

In conclusion, we have provided additional *in vitro* data demonstrating the activity of rezafungin against a collection of largely echinocandin-WT isolates of *Candida* spp., *C. neoformans*, *A. fumigatus*, and *A. flavus* species complex. Given these findings, we suggest that MIC values of ≤0.12 µg/mL (*C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei*), ≤0.25 µg/mL (*C. dubliniensis*), ≤4 µg/mL (*C. parapsilosis*), and MEC ≤0.03 µg/mL (*A. fumigatus*) approximate the ECV/WT-UL MIC/MEC distributions for rezafungin and the common species of *Candida* and *Aspergillus*. Further evaluations, including at least 100 MIC values per species tested by 3 different laboratories, should be performed to define the ECVs for rezafungin, a fundamental step in establishing clinical breakpoints.

This survey provides new information regarding emerging fluconazole resistance among *C. parapsilosis* and *C. tropicalis* clinical isolates from geographic regions beyond NA in addition to demonstrating evidence of the sustained activity of rezafungin and the other echinocandins against *Candida* and *Aspergillus* species. Whereas the highest rates of fluconazole resistance in NA isolates were seen in *C. glabrata* (13.2%), fluconazole-resistant *C. parapsilosis* (24.8%) was most prominent in EUR and fluconazole-resistant *C. tropicalis* was most prominent in APAC (5.0%) and LATAM (4.1%). In all three...
instances, fluconazole resistance was highest in species of Candida other than C. glabrata. Species identification should be used cautiously as the sole criterion for selecting antifungal therapy.

**MATERIALS AND METHODS**

**Organisms.** During 2016-2018, 2,205 non-duplicate fungal isolates were prospectively collected from 57 medical centers located in North America (723 isolates; 18 sites, 17 USA and 1 Canada), EUR (927 isolates; 22 sites, 14 countries), the APAC region (279 isolates; 11 sites, 5 countries) and LATAM (276 isolates; 6 sites, 4 countries). Isolates were recovered from the following sources: bloodstream infections (1,460 isolates), pneumonia in hospitalized patients (306), intra-abdominal infections (32), skin and skin structure infections (106), urinary tract infections (35), and other or non-specified body sites (266).

**Fungal identification methods.** Yeast isolates were subcultured and screened using CHROMagar Candida (Becton Dickinson, Sparks, MD) to ensure purity. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was applied for identification of all yeast isolates using the MALDI Biotyper according to the manufacturer’s instructions (Bruker Daltonics, Billerica, MA). Isolates that were not identified by proteomic methods were submitted to the described sequencing-based methods (43, 46, 47).

Moulds were cultured and identified by MALDI–TOF MS or DNA sequencing analysis when an acceptable identification was not achieved by MALDI–TOF MS. Sequencing of 28S rDNA and β-tubulin genes for Aspergillus spp. were analyzed (47-50).

Nucleotide sequences were analyzed using Lasergene® software (DNAStar, Madison, Wisconsin, USA) and compared to available sequences using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Antifungal susceptibility testing.** All isolates were tested by CLSI BMD methods as described in documents M27 and M38 (37, 38). Only systemically active antifungal agents were tested, including rezafungin, anidulafungin, micafungin, caspofungin, itraconazole, fluconazole, voriconazole, posaconazole, and amphotericin B. The range of antifungal agent concentrations tested were 0.008 – 4 µg/mL for itraconazole, posaconazole, and voriconazole, 0.12 – 2 µg/mL for amphotericin B, and 0.12 – 128 µg/mL for fluconazole. Echinocandins concentration range tested during 2016 and 2017 was 0.008 – 4 µg/mL whereas this range was expanded to 0.002 – 4 µg/mL in 2018. MIC results were determined...
visually after 24 (Candida spp.), 48 (Aspergillus spp.), or 72 (Cr. neoformans) hours of incubation at 35°C.

Azoles and echinocandins’ MIC values against yeasts were read as the lowest concentration of drug that resulted in ≥50% inhibition of growth relative to the growth control. Complete (100%) inhibition was used to determine itraconazole, posaconazole, and voriconazole MIC values against Aspergillus spp. and amphotericin B against yeasts and moulds. Echinocandins minimum effective concentration (MEC) values, including rezafungin, were read against Aspergillus spp. as described in CLSI document M38 (38).

Echinocandins, fluconazole, and voriconazole susceptibility categories were applied for the five most common species of Candida (C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, and C. krusei) following CLSI clinical breakpoints (CBPs) (39). Epidemiological cutoff values (ECVs/ECOFFs) were used to differentiate wild-type (WT) from non-wild-type (NWT) isolates of the species for which there are no CLSI CBPs (40, 41). Neither CBPs nor ECVs/ECOFFs have been determined by CLSI methods for rezafungin against Candida, Aspergillus, or Cryptococcus spp. For comparison, we established tentative ECVs for rezafungin and each species using the iterative statistical method recommended by CLSI (28, 32, 39-41). These ECVs must be considered tentative given the CLSI requirement that ECVs be determined using MIC/MEC data acquired from a minimum of three different laboratories including at least 100 MIC/MEC values from 100 individual isolates, all determined by CLSI reference methods (41).

Quality control. To ensure proper test conditions and procedures, concurrent quality control (QC) testing was performed. QC strains recommended by CLSI included C. krusei ATCC 6258, C. parapsilosis ATCC 22019, A. flavus ATCC 204304, and A. fumigatus ATCC MYA-3626.

Screening for 1,3-β-D-glucan synthase mutations. All Candida spp. isolates that were echinocandin-resistant or showed MIC values higher than the ECV for any echinocandin were submitted to whole genome sequencing for detecting mutations in the HS regions of fks1 and fks2 (C. glabrata only) as described previously (43, 48, 50).
ACKNOWLEDGEMENTS

The authors wish to thank the following staff members at JMI Laboratories (North Liberty, Iowa, USA): R. D. Dietrich, L. N. Woosley, and S. E. Costello for technical support during susceptibility testing and characterization of the fks mutant isolates.

Funding

This study was performed by JMI Laboratories and sponsored by research grants from Cidara Therapeutics, Inc.

Transparency declaration

JMI Laboratories contracted to perform services in 2018 for Achaogen, Inc., Albany College of Pharmacy and Health Sciences, Allecra Therapeutics, Allergan, AmpliPhi Biosciences Corp., Amplyx, Antabio, American Proficiency Institute, Arietis Corp., Arixa Pharmaceuticals, Inc., Astellas Pharma Inc., Athelas, Basilea Pharmaceutica Ltd., Bayer AG, Becton, Dickinson and Company, bioMerieux SA, Boston Pharmaceuticals, Bugworks Research Inc., CEM-102 Pharmaceuticals, Cepheid, Cidara Therapeutics, Inc., CorMedix Inc., DePuy Synthes, Destiny Pharma, Discuva Ltd., Dr. Falk Pharma GmbH, Emery Pharma, Entasis Therapeutics, Eurofarma Laboratorios SA, US Food and Drug Administration, Fox Chase Chemical Diversity Center, Inc., Gateway Pharmaceutical LLC, GenePOC Inc., Geom Therapeutics, Inc., GlaxoSmithKline plc, Harvard University, Helperby, HiMedia Laboratories, F. Hoffmann-La Roche Ltd., ICON plc, Idorsia Pharmaceuticals Ltd., Iterum Therapeutics plc, Laboratory Specialists, Inc., Melinta Therapeutics, Inc., Merck & Co., Inc., Microchem Laboratory, Micromyx, MicuRx Pharmaceuticals, Inc., Mutabilis Co., Nabriiva Therapeutics plc, NAEJA-RGM, Novartis AG, Oxoid Ltd., Paratek Pharmaceuticals, Inc., Pfizer, Inc., Polyphor Ltd., Pharmaceutical Product Development, LLC, Prokaryotics Inc., Qpex Biopharma, Inc., Ra Pharmaceuticals, Inc., Reivant Sciences, Ltd., Safeguard Biosystems, Scynexis, Inc., SeLux Diagnostics, Inc., Shionogi and Co., Ltd., SinSa Labs, Spero Therapeutics, Summit Pharmaceuticals International Corp., Synlogic, T2 Biosystems, Inc., Taisho Pharmaceutical Co., Ltd., TenNor Therapeutics Ltd., Tetraphase Pharmaceuticals, The Medicines Company, Theravance Biopharma, University of Colorado, University of Southern California-San Diego,
University of North Texas Health Science Center, VenatoRx Pharmaceuticals, Inc., Vyome Therapeutics Inc., Wockhardt, Yukon Pharmaceuticals, Inc., Zai Lab, Zavante Therapeutics, Inc. There are no speakers' bureaus or stock options to declare.
REFERENCES

1. Bassetti M, Righi E, Montravers P, Cornely OA. 2018. What has changed in the treatment of invasive candidiasis? A look at the past 10 years and ahead. J Antimicrob Chemother 73:i14-i25.

2. Berkow EL, Lockhart SR. 2017. Fluconazole resistance in Candida species: a current perspective. Infect Drug Resist 10:237-245.

3. Lockhart SR, Berkow EL. 2019. Antifungal agents, p 2319-2333. In Carroll KC, Pfaller MA, Landry ML, McAdam AJ, Patel R, Richter SS, Warnock DW (ed), Manual of Clinical Microbiology 12th ed, vol 2. ASM Press, Washington, DC, USA.

4. Pakyz AL, Gurgle HE, Oinonen MJ. 2011. Antifungal use in hospitalized adults in U.S. academic health centers. Am J Health Syst Pharm 68:415-418.

5. Pappas PG, Kauffman CA, Andes D, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. 2016. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 62:409-417.

6. Patterson TF, Thompson GR, 3rd, Denning DW, Fishman JA, Hadley S, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Nguyen MH, Segal BH, Steinbach WJ, Stevens DA, Walsh TJ, Wingard JR, Young JA, Bennett JE. 2016. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 63:e1-e60.

7. Vallabhaneni S, Baggs J, Tsay S, Srinivasan AR, Jernigan JA, Jackson BR. 2018. Trends in antifungal use in US hospitals, 2006-12. J Antimicrob Chemother 73:2867-2875.

8. Fondevilla E, Grau S, Mojal S, Palomar M, Matas L, Gudiol F, Group VI. 2016. Consumption of systemic antifungal agents among acute care hospitals in Catalonia (Spain), 2008-2013. Expert Rev Anti Infect Ther 14:137-144.

9. Garey KW, Aitken SL, Dima-Ala A, Beyda ND, Kuper K, Xie Y, Koo HL. 2015. Echinocandin use in hospitalized patients: a multi-institutional study. Am J Med Sci 349:316-320.
10. Goemaere B, Lagrou K, Spriet I, Hendrickx M, Vandael E, Becker P, Catry B. 2019. Systemic antifungal drug use in Belgium—One of the biggest antifungal consumers in Europe. Mycoses 62:542-550.

11. Stultz JS, Kohinke R, Pakyz AL. 2018. Variability in antifungal utilization among neonatal, pediatric, and adult inpatients in academic medical centers throughout the United States of America. BMC Infect Dis 18:501.

12. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, Meersseman W, Akova M, Arendrup MC, Arikani-Akdagli S, Bille J, Castagnola E, Cuenca-Estrella M, Donnelly JP, Groll AH, Herbrecht R, Hope WW, Jensen HE, Lass-Florl C, Petrikos G, Richardson MD, Rolides E, Verweij PE, Viscoli C, Ullmann AJ, Group EFIS. 2012. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect 18 Suppl 7:19-37.

13. Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH, Sobel JD, Pappas PG, Kullberg BJ, Mycoses Study G. 2012. 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 54:1110-1122.

14. Lamoth F, Lockhart SR, Berkow EL, Calandra T. 2018. Changes in the epidemiological landscape of invasive candidiasis. J Antimicrob Chemother 73:i4-i13.

15. Sofjan AK, Mitchell A, Shah DN, Nguyen T, Sim M, Trojnak A, Beyda ND, Garey KW. 2018. Rezafungin (CD101), a next-generation echinocandin: A systematic literature review and assessment of possible place in therapy. J Glob Antimicrob Resist 14:58-64.

16. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. 2019. Twenty years of the SENTRY Antifungal Surveillance Program: Results for Candida species From 1997-2016. Open Forum Infect Dis 6:S79-S94.

17. Beyda ND, Lewis RE, Garey KW. 2012. Echinocandin resistance in Candida species: mechanisms of reduced susceptibility and therapeutic approaches. Ann Pharmacother 46:1086-1096.
18. Yamaguchi M, Kurokawa T, Ishiyama K, Aoki G, Ueda M, Matano S, Takami A, Yamazaki H, Sawazaki A, Yamauchi H, Yoshida T, Nakao S. 2011. Efficacy and safety of micafungin as an empirical therapy for invasive fungal infections in patients with hematologic disorders: a multicenter, prospective study. Ann Hematol 90:1209-1217.

19. Yamazaki S, Nakamura F, Yoshi A, Ichikawa M, Nannya Y, Kurokawa M. 2014. Safety of high-dose micafungin for patients with hematological diseases. Leuk Lymphoma 55:2572-2576.

20. Bader JC, Lakota EA, Flanagan S, Ong V, Sandison T, Rubino CM, Bhavnani SM, Ambrose PG. 2018. Overcoming the Resistance hurdle: Pharmacokinetic-pharmacodynamic target attainment analyses for rezafungin (CD101) against Candida albicans and Candida glabrata. Antimicrob Agents Chemother 62:e02614.

21. James KD, Laudeman CP, Malkar NB, Krishnan R, Polowy K. 2017. Structure-activity relationships of a series of echinocandins and the discovery of CD101, a highly stable and soluble echinocandin with distinctive pharmacokinetic properties. Antimicrob Agents Chemother 61:e01541.

22. Krishnan BR, James KD, Polowy K, Bryant BJ, Vaidya A, Smith S, Laudeman CP. 2017. CD101, a novel echinocandin with exceptional stability properties and enhanced aqueous solubility. J Antibiot (Tokyo) 70:130-135.

23. Lepak AJ, Zhao M, VanScoy B, Ambrose PG, Andes DR. 2018. Pharmacodynamics of a long-acting echinocandin, CD101, in a neutropenic invasive-candidiasis murine model using an extended-interval dosing design. Antimicrob Agents Chemother 62:e02154.

24. Locke JB, Almaguer AL, Zuill DE, Bartzal K. 2016. Characterization of in vitro resistance development to the novel echinocandin, CD101, in Candida species. Antimicrob Agents Chemother 60:6100-6107.

25. Ong V, Hough G, Schlosser M, Bartzal K, Balkovec JM, James KD, Krishnan BR. 2016. Preclinical evaluation of the stability, safety and efficacy of CD101, a novel echinocandin. Antimicrob Agents Chemother doi:10.1128/AAC.00701-16:in press.

26. Sandison T, Ong V, Lee J, Thye D. 2017. Safety and pharmacokinetics of CD101 IV, a novel Echinocandin, in healthy adults. Antimicrob Agents Chemother 61:e01627.
Zhao Y, Perez WB, Jimenez-Ortigosa C, Hough G, Locke JB, Ong V, Bartizal K, Perlin DS. 2016. CD101: a novel long-acting echinocandin. Cell Microbiol 18:1308-1316.

Arendrup MC, Meletiadis J, Zaragoza O, Jorgensen KM, Marcos-Zambrano LJ, Kanioura L, Cuenca-Estrella M, Mouton JW, Guinea J. 2018. Multicentre determination of rezafungin (CD101) susceptibility of Candida species by the EUCAST method. Clin Microbiol Infect 24:1200-1204.

Chandra J, Ghannoum MA. 2018. CD101, a novel echinocandin, possesses potent antibiofilm activity against early and mature Candida albicans biofilms. Antimicrob Agents Chemother 62:e01750.

Hall D, Bonifas R, Stapert L, Thwaites M, Shinabarger DL, Pillar CM. 2017. In vitro potency and fungicidal activity of CD101, a novel echinocandin, against recent clinical isolates of Candida spp. Diagn Microbiol Infect Dis 89:205-211.

Pfaller MA, Messer SA, Rhomberg PR, Jones RN, Castanheira M. 2016. Activity of a long-acting echinocandin, CD101, determined using CLSI and EUCAST reference methods, against Candida and Aspergillus spp., including echinocandin- and azole-resistant isolates. J Antimicrob Chemother 71:e02045.

Castanheira M, Messer SA, Rhomberg PR, Dietrich RR, Pfaller MA. 2018. Activity of a long-acting echinocandin rezafungin (CD101) and comparator antifungal agents tested against contemporary invasive fungal isolates: SENTRY 2016, Poster #Friday-485. ASM Microbe, June 7-11, Atlanta, GA, USA.
Pfaller MA, Messer SA, Rhomberg PR, Schaefer BA, Castanheira M. 2018. Activity of a long-acting echinocandin rezafungin and comparator antifungal agents tested against contemporary invasive fungal isolates: SENTRY 2018, Poster #2400. IDWeek, October 3-7, San Francisco, CA, USA.

Pfaller MA, Carvalhaes CG, Messer SA, Rhomberg PR, Castanheira M. 2019. Activity of a long-acting echinocandin, rezafungin, tested against invasive fungal isolates collected worldwide, Poster #2115. IDWeek, October 2-6, Washington, DC, USA.

CLSI. 2017. M27Ed4. Reference method for broth dilution antifungal susceptibility testing of yeasts. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI. 2018. M38Ed3. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, third edition. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI. 2018. M60Ed1. Performance standards for antifungal susceptibility of testing yeasts. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI. 2018. M59Ed2. Epidemiological cutoff values for antifungal susceptibility testing, second edition. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI. 2016. M57. Principles and procedures for the development of epidemiological cutoff values for antifungal susceptibility testing, 1st edition. Clinical and Laboratory Standards Institute, Wayne, PA.

Pfaller MA, Diekema DJ. 2012. Progress in antifungal susceptibility testing of Candida spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. J Clin Microbiol 50:2846-2856.

Pfaller MA, Messer SA, Woosley LN, Jones RN, Castanheira M. 2013. Echinocandin and triazole antifungal susceptibility profiles of opportunistic yeast and mould clinical isolates (2010-2011): Application of new CLSI clinical breakpoints and epidemiological cutoff values to characterize geographic and temporal trends of antifungal resistance. J Clin Microbiol 51:2571-2581.
44. Arendrup MC, Jorgensen KM, Hare RK, Cuenca-Estrella M, Zaragoza O. 2019. EUCAST reference testing of rezafungin susceptibility and impact of choice of plastic plates. Antimicrob Agents Chemother 63:e00659.

45. Oxman DA, Chow JK, Frendl G, Hadley S, Hershkovitz S, Ireland P, McDermott LA, Tsai K, Marty FM, Kontoyiannis DP, Golan Y. 2010. Candidaemia associated with decreased in vitro fluconazole susceptibility: Is Candida speciation predictive of the susceptibility pattern? J Antimicrob Chemother 65:1460-1465.

46. Castanheira M, Woosley LN, Diekema DJ, Jones RN, Pfaller MA. 2013. Candida guilliermondii and other species of Candida misidentified as Candida famata: Assessment by Vitek2, ITS sequence analysis and Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry in two global surveillance programs. J Clin Microbiol 51:117-124.

47. Pfaller MA, Woosley LN, Messer SA, Jones RN, Castanheira M. 2012. Significance of molecular identification and antifungal susceptibility of clinically significant yeasts and moulds in a global antifungal surveillance program. Mycopathologia 174:259-271.

48. Castanheira M, Messer SA, Jones RN, Farrell DJ, Pfaller MA. 2014. Activity of echinocandins and triazoles against a contemporary (2012) worldwide collection of yeast and moulds collected from invasive infections. Int J Antimicrob Agents 44:320-326.

49. Pfaller MA. 2015. Invasive fungal infections and approaches to their diagnosis, p 219-287. In Sails A, Tang Y (ed), Methods in Microbiology, vol 72. Oxford: Academic Press.

50. Pfaller MA, Rhomberg PR, Wiederhold NP, Gibas C, Sanders C, Fan H, Mele J, Kovanda LL, Castanheira M. 2018. In vitro activity of isavuconazole versus opportunistic fungal pathogens from two mycology reference laboratories. Antimicrob Agents Chemother 62:e01230.
Table 1. Species distribution of *Candida* isolates by geographic region: SENTRY Program, 2016-2018

| Region     | No. tested | CA  | CG  | CP  | CT  | CD  | CK  |
|------------|------------|-----|-----|-----|-----|-----|-----|
| APAC       | 237        | 49.8| 15.2| 12.2| 16.9| 2.1 | 3.8 |
| EUR        | 823        | 49.6| 18.2| 17.6| 7.5 | 3.6 | 3.4 |
| LATAM      | 242        | 43.0| 8.7 | 20.2| 20.2| 1.7 | 6.2 |
| NA         | 602        | 34.1| 27.7| 17.6| 7.5 | 9.0 | 4.2 |
| Total      | 1,904      | 43.9| 19.6| 17.3| 10.3| 4.9 | 4.0 |

Abbreviations: CA, *C. albicans*; CG, *C. glabrata*; CP, *C. parapsilosis*; CT, *C. tropicalis*; CD, *C. dubliniensis*; CK, *C. krusei*; APAC, Asia-Pacific; EUR, Europe; LATAM, Latin America; NA, North America.
Table 2. Antimicrobial activity of rezafungin tested against the main organisms and organism groups using the CLSI method from all years

| Organism/organism group (no. of isolates) | No. and cumulative % of isolates inhibited at MIC (µg/mL) of: | MIC<sub>50</sub> | MIC<sub>90</sub> |
|------------------------------------------|---------------------------------------------------------------|----------------|----------------|
| **Candida albicans** (835)               |                                                               |                |                |
| 2016 (276)                               |                                                               |                |                |
| 11                                       | 6                                                             | 270            | 39 28 2        |
|                                          | 3.8                                                            | 10.4           | 42.8 79.8 96.4 |
|                                          | 1.0                                                            | 13             | 113 38 11      |
|                                          | 0.07                                                           | 4.7            | 40.9 81.9 95.7 |
|                                          | 0.03                                                           | 0.06           | 0.06 0.66 10   |
|                                          | 0.015                                                          | 0.12           | 0.12 0.12      |
| 2017 (267)                               |                                                               |                |                |
| 11                                       | 6                                                             | 83             | 66 10          |
|                                          | 3.8                                                            | 21.2           | 510 85.3 97.3 |
|                                          | 0.07                                                           | 4.5            | 35.6 71.5 96.3 |
|                                          | 0.03                                                           | 0.06           | 0.06 0.12      |
| 2018 (292)                               |                                                               |                |                |
| 11                                       | 6                                                             | 45             | 87 100 35 7    |
|                                          | 3.8                                                            | 21.2           | 510 85.3 97.3 |
|                                          | 0.07                                                           | 4.5            | 35.6 71.5 96.3 |
|                                          | 0.03                                                           | 0.06           | 0.06 0.12      |
| **Candida glabrata** (374)               |                                                               |                |                |
| 2016 (135)                               |                                                               |                |                |
| 1                                        | 0                                                             | 1              | 38 65 27 3     |
|                                          | 0.3                                                           | 0.3            | 38 65 27 3     |
|                                          | 0.07                                                          | 0.07           | 0.07 28.9 77.0 |
|                                          | 0.03                                                          | 0.12           | 0.12 0.12      |
| 2017 (121)                               |                                                               |                |                |
| 1                                        | 0                                                             | 0              | 33 60 20 1     |
|                                          | 0.8                                                           | 0.8            | 4.2 59.3 90.7 98.6 |
|                                          | 0.07                                                          | 0.07           | 0.07 27.3 76.9 |
|                                          | 0.03                                                          | 0.12           | 0.12 0.12      |
| 2018 (118)                               |                                                               |                |                |
| 0.8                                      | 0                                                             | 0              | 65 37 7 1      |
|                                          | 0.8                                                           | 0.8            | 4.2 59.3 90.7 98.6 |
|                                          | 0.07                                                          | 0.07           | 0.07 27.3 76.9 |
|                                          | 0.03                                                          | 0.12           | 0.12 0.12      |
| **Candida parapsilosis** (329)           |                                                               |                |                |
| 2016 (94)                                |                                                               |                |                |
| 0                                        | 1                                                             | 1              | 10 124 124 2   |
|                                          | 0.0                                                           | 0.0            | 0.0 1.6 38.0 81.3 |
|                                          | 0.07                                                          | 0.07           | 0.07 0.7 28.9 |
|                                          | 0.03                                                          | 0.06           | 0.06 0.12      |
| 2017 (118)                               |                                                               |                |                |
| 0                                        | 1                                                             | 0              | 14 48 51 2     |
|                                          | 0.0                                                           | 0.0            | 0.0 0.8 0.8    |
|                                          | 0.07                                                          | 0.07           | 0.07 27.3 76.9 |
|                                          | 0.03                                                          | 0.12           | 0.12 0.12      |
| 2018 (117)                               |                                                               |                |                |
| 0.8                                      | 0                                                             | 0              | 35 49 31       |
|                                          | 0.8                                                           | 0.8            | 4.2 59.3 90.7 98.6 |
|                                          | 0.07                                                          | 0.07           | 0.07 27.3 76.9 |
|                                          | 0.03                                                          | 0.12           | 0.12 0.12      |
| **Candida tropicalis** (196)             |                                                               |                |                |
| 2016 (64)                                |                                                               |                |                |
| 0                                        | 1                                                             | 2              | 13 37 42       |
|                                          | 0.0                                                           | 0.0            | 0.0 2.1 28.9 77.0 |
|                                          | 0.07                                                          | 0.07           | 0.07 0.7 28.9 |
|                                          | 0.03                                                          | 0.12           | 0.12 0.12      |
| 2017 (54)                                |                                                               |                |                |
| 0                                        | 1                                                             | 1              | 19 17 5 2      |
|                                          | 0.0                                                           | 0.0            | 0.0 20.4 55.8 87.0 |
|                                          | 0.07                                                          | 0.07           | 0.07 27.3 76.9 |
|                                          | 0.03                                                          | 0.12           | 0.12 0.12      |
| 2018 (76)                                |                                                               |                |                |
| 0                                        | 9                                                             | 21             | 32 12 3 1      |
|                                          | 0.0                                                           | 1.1            | 11.5 38.5 79.5 94.9 |
|                                          | 0.07                                                          | 0.07           | 0.07 27.3 76.9 |
|                                          | 0.03                                                          | 0.06           | 0.06 0.12      |
| **Candida krusei** (77)                  |                                                               |                |                |
| 2016 (33)                                |                                                               |                |                |
| 0                                        | 2                                                             | 22             | 31 17 7       |
|                                          | 0.0                                                           | 0.0            | 0.0 28.6 88.8 90.9 |
|                                          | 0.07                                                          | 0.07           | 0.07 0.7 28.6 |
|                                          | 0.03                                                          | 0.06           | 0.06 0.12      |
| 2017 (28)                                |                                                               |                |                |
| 0                                        | 3                                                             | 12             | 10 3         |
|                                          | 0.06                                                          | 0.12           | 0.12 0.12      |
| 2018 (16)                                |                                                               |                |                |
| 0                                        | 1                                                             | 2              | 0 3          |
|                                          | 0.06                                                          | 0.12           | 0.12 0.12      |
| **Candida dubliniensis** (93)            |                                                               |                |                |
| 1                                        | 1                                                             | 4              | 30 39 18      |
|                                          | 1.1                                                           | 1.1            | 2.2 6.5 38.7 89.7 |
|                                          | 0.07                                                          | 0.07           | 0.07 0.7 28.6 |
|                                          | 0.03                                                          | 0.12           | 0.12 0.12      |
| 2016 (30)                                |                                                               |                |                |
| 0                                        | 1                                                             | 8              | 15 7         |
|                                          | 0.0                                                           | 0.0            | 0.0 26.7 76.7 89.7 |
|                                          | 0.07                                                          | 0.07           | 0.07 0.7 28.6 |
|                                          | 0.03                                                          | 0.12           | 0.12 0.12      |
| 2017 (28)                                |                                                               |                |                |
| 0                                        | 1                                                             | 9              | 11 7         |
|                                          | 0.06                                                          | 0.12           | 0.12 0.12      |
| Organism/organism group (no. of isolates) | No. and cumulative % of isolates inhibited at MIC (µg/mL) of: | MIC<sub>50</sub> | MIC<sub>90</sub> |
|----------------------------------------|----------------------------------------------------------|----------------|----------------|
| ±0.002 <sup>a</sup> | 0.004 <sup>a</sup> | 0.008 | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | > b |
| 2018 (35) | 1 | 2.9 | 0 | 2.9 | 1 | 5.7 | 3 | 14.3 | 3 | 13 | 13 | 4 | 0.03 | 0.12 |
| Cryptococcus neoformans var. grubii (73) | 0 | 0.0 | 9 | 12.3 | 64 | >4 | >4 | |
| 2016 (27) | 0 | 0.0 | 27 | 0.0 | 100.0 | >4 | >4 | |
| 2017 (25) | 0 | 0.0 | 7 | 28.0 | 18 | >4 | >4 | |
| 2018 (21) | 0 | 0.0 | 2 | 5.5 | 19 | >4 | >4 | |
| Aspergillus fumigatus (183) | 3 | 64 | 88 | 28 | 0.015 | 0.03 |
| 2016 (48) | 26 | 54.2 | 20 | 96.8 | 2 | <=0.008 | 0.015 |
| 2017 (60) | 25 | 41.7 | 29 | 90.0 | 6 | 0.015 | 0.015 |
| 2018 (75) | 0 | 13 | 39 | 100.0 | 20 | 0.015 | 0.03 |
| Aspergillus section Flavi (45) | 5 | 33.3 | 20 | 18 | 2 | <=0.008 | 0.015 |
| 2016 (12) | 3 | 25.0 | 7 | 83.3 | 2 | 0.015 | 0.03 |
| 2017 (18) | 8 | 55.6 | 10 | 95.6 | 94.4 | 0.015 | 0.015 |
| 2018 (15) | 0 | 33.3 | 9 | 93.3 | 1 | 0.008 | 0.008 |

<sup>a</sup> During 2016 and 2017 study years, the lowest echinocandins concentration tested was 0.008 µg/mL. The range was expanded to 0.002 µg/mL in 2018.

<sup>b</sup> Greater than the last concentration tested.
Table 3. Antimicrobial activity of rezafungin and comparators agents tested against fungal isolates from the worldwide 2016-2018 Rezafungin Surveillance Program

| Antimicrobial agent | MIC50 | MIC90 | CLSI% | ECV% |
|---------------------|-------|-------|-------|-------|
|                     | %S    | %R    | %WT   | %NWT  |
| **Candida albicans (n = 835)** |       |       |       |       |
| Rezafungin          | 0.03  | 0.06  | 99.8  | 0.2   |
| Anidulafungin       | 0.015 | 0.03  | 100.0 | 0.0   |
| Caspofungin         | 0.015 | 0.03  | 99.9  | 0.1   |
| Micafungin          | 0.015 | 0.03  | 99.9  | 0.1   |
| Fluconazole         | ≤0.12 | 0.25  | 99.5  | 0.4   |
| Itraconazole        | ≤0.06 | 0.12  |       |       |
| Posaconazole        | 0.03  | 0.06  | 96.5  | 3.5   |
| Voriconazole        | ≤0.008| 0.015 | 99.9  | 0.0   |
| Amphotericin B      | 0.5   | 1     | 100.0 | 0.0   |
| **Candida glabrata (n = 374)** |       |       |       |       |
| Rezafungin          | 0.06  | 0.12  | 95.7  | 4.3   |
| Anidulafungin       | 0.06  | 0.12  | 94.4  | 3.2   |
| Caspofungin         | 0.03  | 0.06  | 97.1  | 2.1   |
| Micafungin          | 0.015 | 0.03  | 96.0  | 2.4   |
| Fluconazole         | 2     | 32    | 91.4  | 8.6   |
| Itraconazole        | 0.5   | 2     | 98.7  | 1.3   |
| Posaconazole        | 0.25  | 1     | 93.0  | 7.0   |
| Voriconazole        | 0.06  | 1     | 87.2  | 12.8  |
| Amphotericin B      | 1     | 1     | 100.0 | 0.0   |
| **Candida parapsilosis (n = 329)** |       |       |       |       |
| Rezafungin          | 1     | 2     | 100.0 | 0.0   |
| Anidulafungin       | 2     | 2     | 93.9  | 0.0   |
| Caspofungin         | 0.25  | 0.5   | 100.0 | 0.0   |
| Micafungin          | 1     | 1     | 100.0 | 0.0   |
| Fluconazole         | 0.5   | 32    | 86.0  | 12.5  |
| Itraconazole        | 0.12  | 0.25  |       |       |
| Posaconazole        | 0.06  | 0.12  | 100.0 | 0.0   |
| Voriconazole        | ≤0.008| 0.25  | 88.4  | 15.5  |
| Amphotericin B      | 0.5   | 1     | 100.0 | 0.0   |
| **Candida tropicalis (n = 196)** |       |       |       |       |
| Rezafungin          | 0.03  | 0.06  | 100.0 | 0.0   |
| Anidulafungin       | 0.03  | 0.06  | 99.0  | 1.0   |
| Caspofungin         | 0.015 | 0.06  | 99.0  | 1.0   |
| Micafungin          | 0.03  | 0.06  | 99.0  | 1.0   |
| Fluconazole         | 0.25  | 1     | 96.9  | 3.1   |
| Itraconazole        | 0.12  | 0.5   | 100.0 | 0.0   |
| Posaconazole        | 0.06  | 0.12  | 92.9  | 7.1   |
| Voriconazole        | 0.015 | 0.06  | 96.9  | 3.1   |
## Table

| Antimicrobial agent | MIC<sub>50</sub> | MIC<sub>90</sub> | CLSI<sup>a</sup> | ECV<sup>a</sup> |
|---------------------|-----------------|-----------------|-----------------|-----------------|
|                     | %S   | %R   | %WT | %NWT |
| **Amphotericin B**  | 0.5  | 1    | 100.0 | 0.0   |
| **Candida krusei (n = 77)** | | | | |
| Rezafungin          | 0.03 | 0.06 | 100.0 | 0.0   |
| Anidulafungin       | 0.06 | 0.12 | 100.0 | 0.0   |
| Caspofungin         | 0.12 | 0.25 | 98.7  | 0.0   |
| Micafungin          | 0.06 | 0.12 | 100.0 | 0.0   |
| Fluconazole         | 32   | 64   |      |      |
| Itraconazole        | 0.5  | 1    | 100.0 | 0.0   |
| Posaconazole        | 0.5  | 0.5  | 100.0 | 0.0   |
| Voriconazole        | 0.25 | 0.5  | 96.1  | 1.3   |
| **Amphotericin B**  | 1    | 2    | 100.0 | 0.0   |
| **Candida dubliniensis (n = 93)** | | | | |
| Rezafungin          | 0.06 | 0.12 | 100.0 | 0.0   |
| Anidulafungin       | 0.03 | 0.12 | 100.0 | 0.0   |
| Caspofungin         | 0.03 | 0.03 |      |      |
| Micafungin          | 0.03 | 0.03 | 100.0 | 0.0   |
| Fluconazole         | ≤0.12| 0.25 | 96.8  | 3.2   |
| Itraconazole        | ≤0.06| 0.25 |      |      |
| Posaconazole        | 0.03 | 0.06 | 100.0 | 0.0   |
| Voriconazole        | ≤0.008| 0.015|      |      |
| **Amphotericin B**  | 0.5  | 0.5  |      |      |
| **Cryptococcus neoformans var. grubii (n = 73)** | | | | |
| Rezafungin          | >4   | >4   | 100.0 | 0.0   |
| Anidulafungin       | >4   | >4   | 100.0 | 0.0   |
| Caspofungin         | >4   | >4   | 100.0 | 0.0   |
| Micafungin          | >4   | >4   | 100.0 | 0.0   |
| Fluconazole         | 2    | 4    | 93.5  | 6.5   |
| Itraconazole        | 0.25 | 0.25 | 97.3  | 2.7   |
| Posaconazole        | 0.12 | 0.25 | 100.0 | 0.0   |
| Voriconazole        | 0.03 | 0.12 |      |      |
| **Amphotericin B**  | 0.5  | 1    | 52.1  | 47.9  |
| **Aspergillus fumigatus (n = 183)** | | | | |
| Rezafungin          | 0.015| 0.03 | 100.0 | 0.0   |
| Anidulafungin       | 0.015| 0.03 | 100.0 | 0.0   |
| Caspofungin         | 0.015| 0.03 | 100.0 | 0.0   |
| Micafungin          | ≤0.008| 0.015|      |      |
| Itraconazole        | 0.5  | 1    | 98.4  | 1.6   |
| Posaconazole        | 0.25 | 0.5  |      |      |
| Voriconazole        | 0.25 | 0.5  | 98.9  | 1.1   |
| **Amphotericin B**  | 1    | 2    | 100.0 | 0.0   |
| **Aspergillus section Flavi (n = 45)** | | | | |
| Rezafungin          | ≤0.008| 0.015|      |      |

---

<sup>a</sup> CLSI = Clinical and Laboratory Standards Institute, ECV = EUCAST.
| Antimicrobial agent | MIC<sub>50</sub> | MIC<sub>90</sub> | CLSI<sup>a</sup> | ECV<sup>a</sup> |
|--------------------|----------------|----------------|----------------|--------------|
|                    | %S  | %R  | %WT | %NWT  |
| Anidulafungin      | ≤0.008 | 0.015 | 100.0 | 0.0 |
| Caspofungin        | 0.015 | 0.03 | 100.0 | 0.0 |
| Micafungin         | 0.015 | 0.03 | 100.0 | 0.0 |
| Itraconazole       | 0.5 | 1 | 100.0 | 0.0 |
| Posaconazole       | 0.25 | 0.5 | 100.0 | 0.0 |
| Voriconazole       | 0.5 | 1 | 100.0 | 0.0 |
| Amphotericin B     | 2   | 2   | 100.0 | 0.0 |

<sup>a</sup> Criteria published by CLSI M60 (39). Epidemiological cutoff value (ECV) criteria published in CLSI M59 (40). ECV for rezafungin and each species determined from data in the present study.

<sup>b</sup> Non-resistant interpreted as susceptible-dose dependent.
### Table 4. Summary of fks alterations detected in Candida spp. strains as part of the 2016-2018 Rezafungin surveillance program

| Isolate  | Country | Year | Organism     | MIC according to CLSI method (μg/mL): | 1,3-β-D-glucan synthase mutations: |
|----------|---------|------|--------------|--------------------------------------|-----------------------------------|
|          |         |      |              | RFG | AFG | CAS | MFG | fks1 | fks1 | fks2 | fks2 |
| 1051621  | Hungary | 2018 | C. tropicalis| 0.25 | 0.25 | 0.12 | 0.12 | WT   | WT   | NT   | NT   |
| 1051641  | Hungary | 2018 | C. glabrata  | 1    | 1    | 1    | 0.5  | WT   | WT   | F659_del | WT   |
| 1053234  | Canada  | 2018 | C. glabrata  | 0.12 | 0.12 | 0.06 | 0.06 | WT   | WT   | WT   | WT   |
| 1075570  | Belgium | 2018 | C. glabrata  | 0.06 | 0.12 | 0.06 | 0.06 | WT   | WT   | WT   | WT   |
| 1078854  | USA     | 2018 | C. glabrata  | 0.06 | 0.06 | 0.06 | 0.06 | WT   | WT   | F659_del | WT   |
| 1078861  | USA     | 2018 | C. glabrata  | 2    | 2    | 1    | 1    | WT   | WT   | S663P | WT   |
| 1085740  | Spain   | 2018 | C. tropicalis| 0.06 | 0.06 | 0.06 | 0.12 | WT   | WT   | NT   | NT   |
| 1087598  | USA     | 2018 | C. glabrata  | 2    | 4    | 4    | 4    | WT   | WT   | S663P | WT   |
| 997524   | Mexico  | 2017 | C. glabrata  | 0.5  | 0.5  | 0.25 | 0.06 | F625S | WT   | WT   | WT   |
| 999721   | Italy   | 2017 | C. glabrata  | 0.06 | 0.06 | 0.06 | 0.06 | WT   | WT   | WT   | WT   |
| 1015009  | Spain   | 2017 | C. glabrata  | 0.5  | 1    | 0.5  | 0.25 | WT   | WT   | Y657 deletion, F658Y | WT   |
| 1020535  | USA     | 2017 | C. glabrata  | 0.25 | 0.25 | 0.12 | 0.06 | WT   | WT   | WT   | WT   |
| 1021070  | France  | 2017 | C. glabrata  | 1    | 2    | 0.5  | 0.5  | WT   | WT   | S663P | WT   |
| 1025460  | USA     | 2017 | C. glabrata  | 0.5  | 1    | 0.5  | 1    | S629P | WT   | R665G | WT   |
| 1026179  | Spain   | 2017 | C. glabrata  | 1    | 1    | 0.25 | 0.25 | WT   | WT   | Y657 deletion, F658Y | WT   |
| 1034513  | Ireland | 2017 | C. glabrata  | 2    | 4    | 2    | 0.5  | WT   | WT   | S663P | WT   |
| 1034514  | Ireland | 2017 | C. glabrata  | 0.25 | 0.5  | 0.12 | 0.12 | WT   | WT   | S663P | WT   |
| 1034803  | USA     | 2017 | C. glabrata  | 0.12 | 0.12 | 0.06 | 0.06 | WT   | WT   | WT   | WT   |
| 1034767  | Turkey  | 2017 | C. tropicalis| 0.06 | 0.06 | 0.25 | 0.12 | WT   | NT   | NT   | NT   |
| 1034766  | Turkey  | 2017 | C. tropicalis| 0.12 | 0.25 | 0.03 | 0.06 | WT   | NT   | NT   | NT   |
| 1041544  | Greece  | 2017 | C. tropicalis| 0.06 | 0.06 | 0.03 | 0.12 | WT   | NT   | NT   | NT   |
| 984357   | Ireland | 2016 | C. albicans  | 0.25 | 0.12 | 1    | 1    | S645P | WT   | NT   | NT   |
| 978825   | Turkey  | 2016 | C. albicans  | 0.12 | 0.12 | 0.12 | 0.06 | WT   | WT   | NT   | NT   |
| 949247   | USA     | 2016 | C. glabrata  | 0.06 | 0.12 | 0.03 | 0.03 | WT   | WT   | WT   | WT   |
| 949151   | USA     | 2016 | C. glabrata  | 0.03 | 0.06 | 0.06 | 0.12 | WT   | WT   | WT   | WT   |
| 970382   | USA     | 2016 | C. glabrata  | 0.25 | 0.25 | 0.12 | 0.12 | S629P | WT   | WT   | WT   |
| 970397   | USA     | 2016 | C. glabrata  | 0.12 | 0.25 | 0.25 | 0.12 | WT   | WT   | P667H | WT   |
| 974239   | USA     | 2016 | C. glabrata  | 0.25 | 0.25 | 0.06 | 0.12 | S629P | WT   | WT   | WT   |
| 974249   | USA     | 2016 | C. glabrata  | 2    | 2    | 1    | 1    | WT   | S663P | WT   |
| 978819   | Turkey  | 2016 | C. glabrata  | 0.25 | 0.25 | 0.06 | 0.06 | WT   | WT   | WT   | WT   |
| 983007   | USA     | 2016 | C. glabrata  | 0.12 | 0.5  | 0.06 | 0.12 | WT   | WT   | F658_del | WT   |
| 985673   | USA     | 2016 | C. glabrata  | 0.06 | 0.12 | 0.06 | 0.06 | WT   | WT   | S663P | WT   |
| 936285   | Germany | 2016 | C. krusei  | 0.12 | 0.12 | 0.25 | 0.12 | WT   | WT   | NT   | NT   |
| 954660   | Italy   | 2016 | C. krusei  | 0.015 | 0.03 | 0.06 | 0.06 | WT   | WT   | NT   | NT   |
| 975669   | USA     | 2016 | C. krusei  | 0.015 | 0.06 | 0.12 | 0.06 | WT   | WT   | NT   | NT   |
| 977046   | Brazil  | 2016 | C. krusei  | 0.015 | 0.015 | 0.06 | 0.06 | WT   | WT   | NT   | NT   |

\( \text{a}: \)
| Isolate  | Country | Year | Organism     | MIC according to CLSI method (µg/mL): | 1,3-β-D-glucan synthase mutations<sup>a</sup> |
|---------|---------|------|--------------|--------------------------------------|---------------------------------------------|
|         |         |      |              | RFG | AFG | CAS | MFG | fks1 | fks1 | fks2 | fks2 |
| 970388  | USA     | 2016 | *C. tropicalis* | 2   | 1   | >8  | 2   | S654P | WT   | NT   | NT   |
| 977041  | Brazil  | 2016 | *C. tropicalis* | 1   | 1   | 2   | 1   | F650S | WT   | NT   | NT   |

<sup>a</sup> RFG, rezafungin; AFG, anidulafungin; CAS, caspofungin; MFG, micafungin; WT, wild-type; NT, not tested.
| Species       | Region | No. tested | % resistant (n) |
|---------------|--------|------------|-----------------|
| *C. albicans* | APAC   | 118        | 0.0 (0)         |
|               | EUR    | 408        | 0.0 (0)         |
|               | LATAM  | 104        | 1.0 (1)         |
|               | NA     | 205        | 1.0 (2)         |
|               | Total  | 835        | 0.4 (3)         |
| *C. glabrata* | APAC   | 36         | 2.8 (1)         |
|               | EUR    | 150        | 6.0 (9)         |
|               | LATAM  | 21         | 0.0 (0)         |
|               | NA     | 167        | 13.2 (22)       |
|               | Total  | 374        | 8.6 (32)        |
| *C. parapsilosis* | APAC   | 29         | 3.4 (1)         |
|               | EUR    | 145        | 24.8 (36)       |
|               | LATAM  | 49         | 0.0 (0)         |
|               | NA     | 106        | 3.8 (4)         |
|               | Total  | 329        | 12.5 (41)       |
| *C. tropicalis* | APAC   | 40         | 5.0 (2)         |
|                | EUR    | 62         | 1.6 (1)         |
|                | LATAM  | 49         | 4.1 (2)         |
|                | NA     | 45         | 0.0 (0)         |
|                | Total  | 196        | 2.6 (5)         |
| *C. dubliniensis* | APAC   | 5          | 0.0 (0)         |
|                | EUR    | 30         | 0.0 (0)         |
|                | LATAM  | 4          | 0.0 (0)         |
|                | NA     | 54         | 5.6 (3)         |
|                | Total  | 93         | 3.2 (3)         |

APAC, Asia-Pacific region; EUR, Europe; LATAM, Latin America; NA, North America

% of wide-type isolates based on Epidemiological cutoff value (ECV) criteria published in CLSI M59 (40).