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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. Pfaller[1,2], Cecilia Carvalhaes[1], Shawn A. Messer[1], Paul R. Rhomberg[1], Mariana Castanheira[1]

[1]JMI Laboratories, North Liberty, Iowa, USA

[2]University 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

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ABSTRACT

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 breakpoints (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 ($MIC_{50/90}$, 0.03/0.06 µg/mL), 95.7% of *Candida glabrata* ($MIC_{50/90}$, 0.06/0.12 µg/mL), 97.4% of *Candida tropicalis* ($MIC_{50/90}$, 0.03/0.06 µg/mL), 100.0% of *Candida krusei* ($MIC_{50/90}$, 0.03/0.06 µg/mL), and 100.0% of *Candida dubliniensis* ($MIC_{50/90}$, 0.06/0.12 µg/mL) at ≤0.12 µg/m. All (329/329 [100.0%]) *Candida parapsilosis* isolates ($MIC_{50/90}$,1/2 µg/mL) were inhibited by rezafungin at ≤4 µg/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.

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INTRODUCTION

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).

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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%).

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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) and least active against *C. parapsilosis* (MIC_{90}, 2 µg/mL). With minimal variation over the 3-year time period, the modal 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 to develop tentative ECVs using the iterative statistical method recommended by the CLSI (41) to establish the WT distribution for rezafungin and each of the tested species. The ECV of rezafungin for each species 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* (100.0% WT) (Table 2). Overall, 98.5% of the *Candida* spp. tested, aside from *C. parapsilosis*, were inhibited by ≤0.12 µg/mL and 99.2% were inhibited by ≤0.25 µg/mL of rezafungin (Table 2). Rezafungin showed limited activity against *Cr. neoformans* (MIC_{90}, >4 µg/mL) and was highly active against *Aspergillus* 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* and *Aspergillus* spp. isolates. Rezafungin ($MIC_{50/90}$, 0.03/0.06 µg/mL;99.8% WT) displayed comparable activity 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) to rezafungin while harboring a mutation in *fks1* HS1 (S645P; Table 4). Three fluconazole-resistant strains were 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) at the 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) respectively inhibited 96.0%, 97.1%, and 94.4% of these isolates at the current CLSI breakpoints (39). Mutations within *fks* HS leading to amino acid alterations were found in 17 (68.0%) out of 25 *C. glabrata* isolates

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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 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 (MIC_{90}, 2 µg/mL) was similar to that observed for micafungin (MIC_{90}, 1 µg/mL; 100.0% S) and anidulafungin (MIC_{90}, 2 µg/mL; 93.9% S) and was 4-fold lower than caspofungin (MIC_{90}, 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 ($MIC_{50/90}$, 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).

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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).

All echinocandins (anidulafungin [$MIC_{50/90}$, 0.03/0.12 µg/mL; 100.0% WT], caspofungin [$MIC_{50/90}$, 0.03/0.03 µg/mL], and micafungin [$MIC_{50/90}$, 0.03/0.03 µg/mL; 100.0% WT]) displayed similar activity to rezafungin ($MIC_{50/90}$, 0.06/0.12 µg/mL, 100.0% WT; Tables 2 and 3) against 93 *C. dubliniensis* isolates. Three isolates were resistant/NWT to fluconazole, all from patients hospitalized in NA (5.6% resistant) (Table 5).

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), anidulafungin ($MEC_{50/90}$, 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 ($MEC_{50/90}$, ≤0.008/0.015 µg/mL), 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

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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

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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).

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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., *Cr. 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

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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

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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).

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353 Prokaryotics Inc., Qpex Biopharma, Inc., Ra Pharmaceuticals, Inc., Roivant Sciences, Ltd., Safeguard
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520 Table 1. Species distribution of *Candida* isolates by geographic region: SENTRY Program, 2016-2018

		% by species					
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**

521 Abbreviations: CA, *C. albicans*; CG, *C. glabrata*; CP, *C. parapsilosis*; CT, *C. tropicalis*; CD, *C. dubliniensis;* CK, *C. krusei;* APAC,
522 Asia-Pacific; EUR, Europe; LATAM, Latin America; NA, North America.

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524 Table 2. Antimicrobial activity of rezafungin tested against the main organisms and organism groups

525 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$_{50}$	MIC$_{90}$
	≤0.002 [a]	0.004 [a]	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	> [b]		
Candida albicans (835)	11 3.8	6 5.8	87 10.4	270 42.8	309 79.8	139 96.4	28 99.8	2 100.0						0.03	0.06
2016 (276)			13 4.7	100 40.9	113 81.9	38 95.7	11 99.6	1 100.0						0.03	0.06
2017 (267)			12 4.5	83 35.6	96 71.5	66 96.3	10 100.0							0.03	0.06
2018 (292)	11 3.8	6 5.8	45 21.2	87 51.0	100 85.3	35 97.3	7 99.7	1 100.0						0.015	0.06
Candida glabrata (374)	1 0.3	0 0.3	0 0.3	5 1.6	136 38.0	162 81.3	54 95.7	6 97.3	3 98.1	3 98.9	4 100.0			0.06	0.12
2016 (135)			0 0.0	1 0.7	38 28.9	65 77.0	27 97.0	3 99.3	0 99.3	0 99.3	1 100.0			0.06	0.12
2017 (121)				0 0.0	33 27.3	60 76.9	20 93.4	2 95.0	3 97.5	2 99.2	1 100.0			0.06	0.12
2018 (118)	1 0.8	0 0.8	0 0.8	4 4.2	65 59.3	37 90.7	7 96.6	1 97.5	0 97.5	1 98.3	2 100.0			0.03	0.06
Candida parapsilosis (329)			0 0.0	1 0.3	0 0.3	0 0.3	1 0.6	5 2.1	62 21.0	134 61.7	124 99.4	2 100.0		1	2
2016 (94)							0 0.0	2 2.1	13 16.0	37 55.3	42 100.0			1	2
2017 (118)			0 0.0	1 0.8	0 0.8	0 0.8	0 0.8	2 2.5	14 14.4	48 55.1	51 98.3	2 100.0		1	2
2018 (117)						0 0.0	1 0.9	1 1.7	35 31.6	49 73.5	31 100.0			1	2
Candida tropicalis (196)			12 6.1	52 32.7	75 70.9	41 91.8	11 97.4	3 99.0	0 99.0	1 99.5	1 100.0			0.03	0.06
2016 (64)			3 4.7	20 35.9	24 73.4	12 92.2	3 96.9	0 96.9	0 96.9	1 98.4	1 100.0			0.03	0.06
2017 (54)			0 0.0	11 20.4	19 55.6	17 87.0	5 96.3	2 100.0						0.03	0.12
2018 (78)		0 0.0	9 11.5	21 38.5	32 79.5	12 94.9	3 98.7	1 100.0						0.03	0.06
Candida krusei (77)			0 0.0	22 28.6	31 68.8	17 90.9	7 100.0							0.03	0.06
2016 (33)			0 0.0	8 24.2	17 75.8	7 97.0	1 100.0							0.03	0.06
2017 (28)			0 0.0	3 10.7	12 53.6	10 89.3	3 100.0							0.03	0.12
2018 (16)			0 0.0	11 68.8	2 81.2	0 81.2	3 100.0							0.015	0.12
Candida dubliniensis (93)	1 1.1	0 1.1	1 2.2	4 6.5	30 38.7	39 80.6	18 100.0							0.06	0.12
2016 (30)				0 0.0	8 26.7	15 76.7	7 100.0							0.06	0.12
2017 (28)			0 0.0	1 3.6	9 35.7	11 75.0	7 100.0							0.06	0.12

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Organism/organism group (no. of isolates)	No. and cumulative % of isolates inhibited at MIC (µg/mL) of:													MIC$_{50}$	MIC$_{90}$
	≤0.002 [a]	0.004 [a]	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	13 51.4	13 88.6	4 100.0							0.03	0.12
Cryptococcus neoformans var. *grubii* (73)											0 0.0	9 12.3	64 100.0	>4	>4
2016 (27)												0 0.0	27 100.0	>4	>4
2017 (25)											0 0.0	7 28.0	18 100.0	>4	>4
2018 (21)											0 0.0	2 9.5	19 100.0	>4	>4
Aspergillus fumigatus (183)		3 4.0	64 36.6	88 84.7	28 100.0									0.015	0.03
2016 (48)			26 54.2	20 95.8	2 100.0									≤0.008	0.015
2017 (60)			25 41.7	29 90.0	6 100.0									0.015	0.015
2018 (75)	0 0.0	3 4.0	13 21.3	39 73.3	20 100.0									0.015	0.03
Aspergillus section *Flavi* (45)		5 33.3	20 55.6	18 95.6	2 100.0									≤0.008	0.015
2016 (12)			3 25.0	7 83.3	2 100.0									0.015	0.03
2017 (18)			8 44.4	10 100.0										0.015	0.015
2018 (15)	0 0.0	5 33.3	9 93.3	1 100.0										0.008	0.008

526 a. During 2016 and 2017 study years, the lowest echinocandins concentration tested was 0.008 µg/mL. The range was
527 expanded to 0.002 µg/mL in 2018.
528 b. Greater than the last concentration tested.
529
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532 Table 3. Antimicrobial activity of rezafungin and comparators agents tested against fungal isolates from

533 the worldwide 2016-2018 Rezafungin Surveillance Program

Antimicrobial agent	MIC_{50}	MIC_{90}	CLSI[a]		ECV[a]	
			%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	100.0	0.0
Caspofungin	0.015	0.03	99.9	0.1		
Micafungin	0.015	0.03	99.9	0.1	99.6	0.4
Fluconazole	≤0.12	0.25	99.5	0.4	98.1	1.9
Itraconazole	≤0.06	0.12				
Posaconazole	0.03	0.06			96.5	3.5
Voriconazole	≤0.008	0.015	99.9	0.0	99.0	1.0
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	96.8	3.2
Caspofungin	0.03	0.06	97.1	2.1		
Micafungin	0.015	0.03	96.0	2.4	93.3	6.7
Fluconazole	2	32	91.4[b]	8.6	85.6	14.4
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	100.0	0.0
Caspofungin	0.25	0.5	100.0	0.0		
Micafungin	1	1	100.0	0.0	100.0	0.0
Fluconazole	0.5	32	86.0	12.5	83.6	16.4
Itraconazole	0.12	0.25				
Posaconazole	0.06	0.12			100.0	0.0
Voriconazole	≤0.008	0.25	88.4	0.9	84.5	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	98.0	2.0
Caspofungin	0.015	0.06	99.0	1.0		
Micafungin	0.03	0.06	99.0	1.0	96.4	3.6
Fluconazole	0.25	1	96.9	2.6	94.9	5.1
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	0.0	96.9	3.1

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Antimicrobial agent	MIC_{50}	MIC_{90}	CLSI[a]		ECV[a]	
			%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	100.0	0.0
Caspofungin	0.12	0.25	98.7	0.0		
Micafungin	0.06	0.12	100.0	0.0	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	96.1	3.9
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				
Voriconazole	≤0.008	0.015				
Amphotericin B	0.5	0.5				
Cryptococcus neoformans var. *grubii* (*n* = 73)						
Rezafungin	>4	>4				
Anidulafungin	>4	>4				
Caspofungin	>4	>4				
Micafungin	>4	>4				
Fluconazole	2	4			100.0	0.0
Itraconazole	0.25	0.25			93.5	6.5
Posaconazole	0.12	0.25			97.3	2.7
Voriconazole	0.03	0.12			100.0	0.0
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				
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				

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Antimicrobial agent	MIC_{50}	MIC_{90}	CLSI[a]		ECV[a]	
			%S	%R	%WT	%NWT
Anidulafungin	≤0.008	0.015				
Caspofungin	0.015	0.03			100.0	0.0
Micafungin	0.015	0.03				
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

534 [a] Criteria published by CLSI M60 (39). Epidemiological cutoff value (ECV) criteria published in CLSI M59 (40). ECV for
535 rezafungin and each species determined from data in the present study.
536 [b] Non-resistant interpreted as susceptible-dose dependent.

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541 Table 4. Summary of *fks* alterations detected in *Candida spp.* strains as part of the 2016-2018

542 Rezafungin surveillance program

Isolate	Country	Year	Organism	MIC according to CLSI method (µg/mL):				1,3-β-D-glucan synthase mutations[a]:			
				RFG	AFG	CAS	MFG	*fks1* HS1	*fks1* HS2	*fks2* HS1	*fks2* HS2
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[b]	WT[b]	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
1034763	Turkey	2017	*C. tropicalis*	0.06	0.06	0.25	0.12	WT	WT	NT	NT
1034766	Turkey	2017	*C. tropicalis*	0.12	0.25	0.03	0.06	WT	WT	NT	NT
1041544	Greece	2017	*C. tropicalis*	0.06	0.06	0.03	0.12	WT	WT	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
948247	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	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
975699	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

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Isolate	Country	Year	Organism	MIC according to CLSI method (µg/mL):				1,3-β-D-glucan synthase mutations[a]:			
				RFG	AFG	CAS	MFG	*fks1* HS1	*fks1* HS2	*fks2* HS1	*fks2* HS2
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

543 [a] RFG, rezafungin; AFG, anidulafungin; CAS, caspofungin; MFG, micafungin; WT, wild-type; NT, not tested.

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545 Table 5. Fluconazole resistance by geographic region for *Candida species*: SENTRY, 2016-2018

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 [a]	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)

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

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

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