In Vitro Activity of Isavuconazole against Opportunistic Fungal Pathogens from Two Mycology Reference Laboratories

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ABSTRACT Monitoring antifungal susceptibility patterns for new and established antifungal agents seems prudent given the increasing prevalence of uncommon species associated with higher antifungal resistance. We evaluated the activity of isavuconazole against 4,856 invasive yeasts and molds collected worldwide. The 4,856 clinical fungal isolates, including 2,351 Candida species isolates, 97 non-Candida yeasts, 1,972 Aspergillus species isolates, and 361 non-Aspergillus molds, including 292 Mucorales isolates collected in 2015 to 2016, were tested using CLSI methods. The MIC values for isavuconazole versus Aspergillus ranged from 0.06 to ≥16 μg/ml. The modal MIC for isavuconazole was 0.5 μg/ml (range, 0.25 [A. nidulans and A. terreus species complex] to 4 μg/ml [A. calidoustus and A. tubingensis]). Eight A. fumigatus isolates had elevated isavuconazole MIC values at ≥8 μg/ml (non-wild type). Isavuconazole showed comparable activity to itraconazole against the Mucorales. The lowest modal isavuconazole MIC values were seen for Rhizopus spp., R. arrhizus var. arrhizus, and R. microsporus (all 1 μg/ml). Candida species isolates were inhibited by ≤0.25 μg/ml of isavuconazole (range, 96.1% [C. lusitaniae] to 100.0% [C. albicans, C. dublinensis, C. kefyr, and C. orthopsilosis]). MIC values were ≤1 μg/ml for 95.5% of C. glabrata isolates and 100.0% of C. krusei isolates. Isavuconazole was active against the non-Candida yeasts, including Cryptococcus neoformans (100.0% at ≤0.5 μg/ml). Isavuconazole exhibited excellent activity against most species of Candida and Aspergillus. Isavuconazole was comparable to posaconazole and voriconazole against the less common yeasts and molds. Isavuconazole was generally less active than posaconazole and more active than voriconazole against the 292 Mucorales isolates. We confirm the potentially useful activity of isavuconazole against species of Rhizopus as determined by CLSI methods.

KEYWORDS azoles, isavuconazole, molds, yeasts

The burden of invasive fungal infections (IFIs) for patients and health care systems is difficult to measure (1, 2); however, it is well recognized that IFIs are associated with high morbidity and mortality rates and elevated health care costs. A higher prevalence of IFIs has been observed over the last 3 decades due to the increasing immunocompromised population, which includes individuals living with human immunodeficiency virus, transplant recipients, and cancer patients (1, 3–6). Additionally, increases in the elderly population, neonates, and patients requiring invasive therapies also contribute to the higher IFI rates (4, 7, 8).

The most common fungal pathogens associated with IFIs in humans include Candida spp., Aspergillus spp., and members of the order Mucorales (1). Notably, though the incidences of candidemia and invasive candidiasis (including infections of normally
sterile body fluids, deep tissues, and organs) have declined in recent U.S. surveys (9, 10),
they are increasing in many other regions of the world (4, 11–19). Although much less
common than candidiasis, invasive infections due to Aspergillus and the mucormycetes
are increasing in the U.S. and elsewhere (12, 20–22). Infections due to members of each
of these organism groups carry high rates of mortality and cost (1, 10, 20, 23–26).
Isolates displaying resistance to clinically available antifungal agents are increasingly
reported worldwide, but they are still uncommon (12, 25, 27–31). Emerging multidrug-
resistant (MDR [resistant to 2 or more classes of agents]) species of Candida (7, 25, 32,
33) and azole-resistant Aspergillus fumigatus (30, 34, 35) are now reported globally and
are associated with excess health care costs in addition to considerable morbidity and
mortality (23, 36, 37). The increase in invasive mucormycosis is especially notable as
these organisms are intrinsically resistant to many antifungal agents. Thus, the increasing
number of breakthrough infections reported in patients receiving mold-active
agents (e.g., voriconazole and echinocandins) is of great concern (20–22, 26, 38). For
this reason, continuous monitoring of the antifungal susceptibility patterns and resis-
tance mechanisms to clinically used antifungal agents is of increased importance.

The systemically active antifungal armamentarium currently includes the polyenes,
flucytosine, fluconazole, the extended-spectrum (mold-active) triazoles (isavuconazole,
itracnazone, posaconazole, and voriconazole), and the echinocandins. Despite the fact
that these agents cover the vast majority of opportunistic fungal pathogens and are
increasingly employed in either a prophylactic or preemptive treatment strategy,
breakthrough invasive fungal infections continue to be reported and increasingly
involve yeasts and/or molds that are relatively uncommon and tend to exhibit de-
creased susceptibility to the available antifungal agents (27, 29, 31).

Isavuconazole, a mold-active triazole, may be administered orally or parenterally and
offers advantages in terms of predictable pharmacokinetics and safety over the other
mold-active triazoles, including itraconazole, posaconazole, and voriconazole (39–42).
Specifically, isavuconazonium sulfate (the prodrug formulation of isavuconazole) may
be administered intravenously to patients with decreased renal function without the
need for dose adjustment, due to the lack of cyclodextrin and minimal renal excretion
(42).

Previous studies have documented activity of isavuconazole against common spe-
cies of both Candida and Aspergillus (41, 43). Isavuconazole is also active against many
of the less common yeasts and molds, including members of the order Mucorales
(44–47), and has been approved by the U.S. Food and Drug Administration for the
treatment of invasive aspergillosis and invasive mucormycosis (38–40, 42, 48, 49).
Studies to assess the clinical activity of isavuconazole against Candida and uncommon
yeasts and molds have been completed (42).

In the present study, we examined the in vitro activities of isavuconazole and
comparator antifungal agents against 4,856 clinical fungal isolates (2,351 of Candida
spp., 1,972 of Aspergillus spp., 97 of non-Candida yeasts, and 361 of non-Aspergillus
molds, including 292 Mucorales isolates) collected in 2015 to 2016 from clinically
significant infections as part of two fungal surveillance efforts: the global SENTRY
Antimicrobial Surveillance Program (JMI Laboratories, North Liberty, IA [Candida spp.,
non-Candida yeasts, and rare molds]) and the Fungus Testing Laboratory (San Antonio,
TX [Aspergillus spp. and Mucorales]). All isolates were tested using Clinical and Labora-
tory Standards Institute (CLSI) broth microdilution (BMD) methods, species-specific
clinical breakpoints (CBPs), and proposed epidemiological cutoff values (ECVs), where
available, for each agent to detect emerging resistance among Candida spp., Aspergillus
spp., and selected mucormycetes. Molecular and proteomic methods were used to
confirm the identification of the less common species of Candida, non-Candida yeasts,
and all filamentous fungi.

**RESULTS**

All fungal clinical isolates (species with 10 or more isolates) collected and tested in
surveillance years 2015 and 2016 are presented in Table 1. Of the 4,856 fungal clinical
isolates tested, 40.6% (1,972 isolates) consisted of *Aspergillus* spp., the majority of which (78.6%; 1,550 isolates) were from the U.S. Species of the *Mucorales* order comprised 6.0% (292 isolates) of the tested isolates, including *Lichtheimia*, *Mucor*, *Rhizomucor*, *Rhizopus*, and *Syncephalastrum* species (Table 1). Most (94.5% or 276 isolates) of the *Mucorales* isolates were from the United States. Among the other fungal species tested, the majority were *Candida* spp. (48.4% overall [2,351 isolates]), most of which (58.8% [1,382 isolates]) were non-U.S. isolates (Table 1).

| Organism* | No. (%) of isolates/total |
|-----------|---------------------------|
| Overall   | United States | Non-United States | Total |
| 2,937/4,856 (60.48) | 1,919/4,856 (39.52) | 4,856 |

| *Aspergillus* | |
|--------------|--------------------------------------------------|
| *Aspergillus* spp. | 1,550/1,972 (78.60) | 422/1,972 (21.40) | 1,972/4,856 (40.61) |
| A. calidoustus | 34/1,972 (1.72) | 2/1,972 (0.10) | 36/4,856 (0.74) |
| A. flavus | 108/1,972 (5.48) | 0/1,972 | 108/4,856 (2.22) |
| A. flavus species complex | 20/1,972 (1.01) | 42/1,972 (2.13) | 62/4,856 (1.28) |
| A. fumigatus | 884/1,972 (44.83) | 310/1,972 (15.72) | 1,194/4,856 (24.59) |
| A. lentulus | 9/1,972 (0.46) | 2/1,972 (0.10) | 11/4,856 (0.23) |
| A. nidulans | 22/1,972 (1.12) | 7/1,972 (0.35) | 29/4,856 (0.60) |
| A. niger | 48/1,972 (2.43) | 14/1,972 (0.71) | 62/4,856 (1.28) |
| A. niger species complex | 87/1,972 (4.41) | 16/1,972 (0.81) | 103/4,856 (2.12) |
| A. sydowii | 9/1,972 (0.46) | 2/1,972 (0.10) | 11/4,856 (0.23) |

| *Mucorales* | |
|--------------|--------------------------------------------------|
| Mucorales spp. | 276/292 (94.52) | 16/292 (5.48) | 292/4,856 (6.01) |
| Lichtheimia spp. | 20/23 (86.96) | 3/23 (13.04) | 23/4,856 (0.47) |
| Mucor spp. | 67/69 (97.10) | 2/69 (2.90) | 69/4,856 (1.42) |
| M. circinelloides f. circinelloides | 17/69 (24.64) | 0/69 | 17/4,856 (0.35) |
| M. circinelloides f. janssenii | 67/69 (97.10) | 2/69 (2.90) | 69/4,856 (1.42) |
| Rhizomucor spp. | 12/14 (85.71) | 2/14 (14.29) | 14/4,856 (0.29) |
| Rhizopus spp. | 153/162 (94.44) | 9/162 (5.56) | 162/4,856 (3.34) |
| R. arrhizus var. arrhizus | 61/162 (37.65) | 1/162 (0.62) | 62/4,856 (1.28) |
| R. arrhizus var. delemar | 41/162 (25.31) | 0/162 | 41/4,856 (0.84) |
| R. microsporus | 41/162 (25.31) | 1/162 (0.62) | 42/4,856 (0.86) |
| Syncephalastrum spp. | 11/11 (100) | 0/11 | 11/4,856 (0.23) |

| *Candida* and other fungal species | |
|-----------------|----------------------|----------------------|
| *Candida* spp. | 969/2,351 (41.22) | 1382/2,351 (58.78) | 2,351/4,856 (48.41) |
| C. albicans | 382/2,351 (16.25) | 674/2,351 (28.67) | 1,056/4,856 (21.75) |
| C. dubliniensis | 43/2,351 (1.83) | 19/2,351 (0.81) | 62/4,856 (1.28) |
| C. glabrata | 244/2,351 (10.38) | 245/2,351 (10.42) | 489/4,856 (10.07) |
| C. guilliermondii | 3/2,351 (0.13) | 10/2,351 (0.43) | 13/4,856 (0.27) |
| C. kefyr | 6/2,351 (0.26) | 9/2,351 (0.38) | 15/4,856 (0.31) |
| C. krusei | 28/2,351 (1.19) | 40/2,351 (1.70) | 68/4,856 (1.40) |
| C. lusitaniae | 29/2,351 (1.23) | 22/2,351 (0.94) | 51/4,856 (1.05) |
| C. orthosporus | 9/2,351 (0.38) | 13/2,351 (0.55) | 22/4,856 (0.45) |
| C. parapsilosis | 132/2,351 (5.61) | 217/2,351 (9.23) | 349/4,856 (7.19) |
| C. tropicalis | 76/2,351 (3.23) | 111/2,351 (4.72) | 187/4,856 (3.85) |
| Cryptococcus spp. | 46/84 (54.76) | 38/84 (45.24) | 84/4,856 (1.73) |
| C. neoformans var. grubii | 41/84 (48.81) | 35/84 (41.67) | 76/4,856 (1.57) |
| Fusarium spp. | 15/24 (62.50) | 9/24 (37.50) | 24/4,856 (0.49) |
| F. solani species complex | 11/24 (45.83) | 7/24 (29.17) | 18/4,856 (0.37) |
| Saccharomyces spp. | 3/13 (23.08) | 10/13 (76.92) | 13/4,856 (0.27) |
| S. cerevisiae | 3/13 (23.08) | 10/13 (76.92) | 13/4,856 (0.27) |
| Scedosporium spp. | 30/45 (66.67) | 15/45 (33.33) | 45/4,856 (0.93) |
| S. apiospermum/S. boydii | 22/45 (48.89) | 4/45 (8.89) | 26/4,856 (0.54) |

*Species with 10 or more isolates overall are included.*
TABLE 2 MIC distributions for isavuconazole against Aspergillus spp. and species of the Mucorales order using CLSI broth microdilution methods.

| Species (no. tested) | No. of isolates with MIC (µg/ml) of isavuconazole | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | ≥16 |
|----------------------|--------------------------------------------------|------|------|------|------|-----|---|---|---|---|-----|
| Aspergillus spp. (1,964) | 0                                                | 8    | 37   | 165  | 751  | 676 | 190 | 113 | 18 | 6  |
| A. cladosporioides (36) | 0                                                | 0    | 0    | 0    | 0    | 0   | 2  | 20 | 14 | 0  |
| A. flavus (107)        | 0                                                | 0    | 0    | 0    | 1    | 23  | 65 | 17 | 1  | 0  |
| A. flavus species complex (62) | 0                                              | 0    | 0    | 2    | 20   | 40  | 0  | 0  | 0  | 0  |
| A. fumigatus (1189)    | 0                                                | 0    | 1    | 81   | 611  | 451 | 24 | 13 | 2  | 6  |
| A. lentulus (11)       | 0                                                | 0    | 0    | 0    | 1    | 2   | 6  | 1  | 1  | 0  |
| A. nidulans (29)       | 0                                                | 1    | 13   | 14   | 1    | 0   | 0  | 0  | 0  | 0  |
| A. niger (62)          | 0                                                | 0    | 0    | 2    | 2    | 13  | 39 | 5  | 1  | 0  |
| A. niger species complex (103) | 0                                | 1    | 2    | 1    | 3    | 27  | 41 | 25 | 3  | 0  |
| A. sydowi (22)         | 0                                                | 0    | 0    | 5    | 6    | 11  | 0  | 0  | 0  | 0  |
| A. terreus (96)        | 0                                                | 0    | 1    | 15   | 49   | 28  | 2  | 0  | 1  | 0  |
| A. terreus species complex (14) | 0                              | 0    | 1    | 8    | 5    | 0   | 0  | 0  | 0  | 0  |
| A. tubingensis (66)    | 0                                                | 0    | 0    | 0    | 0    | 3   | 10 | 44 | 9  | 0  |
| A. welwitschiae (25)   | 0                                                | 0    | 0    | 0    | 0    | 10  | 14 | 1  | 0  | 0  |
| Lichtheimia spp. (22) | 0                                                | 0    | 0    | 0    | 0    | 3   | 6  | 7  | 6  | 0  |
| Mucor spp. (69)        | 0                                                | 0    | 0    | 0    | 0    | 1   | 0  | 7  | 17 | 26 | 18 |
| M. circinelloides f. circinelloides (34) | 0                  | 0    | 0    | 0    | 0    | 0   | 2  | 4  | 19 | 9  |
| M. circinelloides f. janssennii (17) | 0                  | 0    | 0    | 0    | 0    | 0   | 5  | 10 | 2  | 0  |
| Rhizomucor pusillus (14) | 0                                             | 0    | 0    | 0    | 1    | 2   | 9  | 0  | 2  | 0  |
| Rhizopus spp. (161)    | 0                                                | 0    | 0    | 0    | 22   | 57  | 37 | 20 | 9  | 15 |
| R. arrhizus var. arrhizus (62) | 0                  | 0    | 0    | 1    | 13   | 29  | 17 | 2  | 0  | 0  |
| R. arrhizus var. delemar (41) | 0                  | 0    | 0    | 0    | 0    | 1   | 8  | 13 | 7  | 12 |
| R. microsporus (41)    | 0                                                | 0    | 0    | 0    | 6    | 25  | 5  | 3  | 1  | 1  |
| Syncphalastrum spp. (11) | 0                                             | 0    | 0    | 0    | 1    | 2   | 0  | 0  | 1  | 7  |

Numbers in boldface are modal MIC values.

Isavuconazole activity against Aspergillus and Mucorales isolates. The most common Aspergillus species (with 10 or more isolates overall) in the 2015 and 2016 cumulative isolate collection that were tested against isavuconazole included the following 13 Aspergillus species, in order of frequency: A. fumigatus, A. flavus, A. niger species complex (SC), A. terreus, A. tubingensis, A. flavus SC, A. niger, A. cladosporioides, A. nidulans, A. welwitschiae, A. sydowi, A. terreus SC, and A. lentulus (Table 2). The cumulative frequencies of MIC distributions for isavuconazole are presented for Aspergillus species in Table 2.

Among the tested species of Aspergillus, the MIC values for isavuconazole ranged from 0.06 to ≥16 µg/ml. The modal MIC for isavuconazole among all Aspergillus spp. was 0.5 µg/ml, with a low modal MIC of 0.25 µg/ml for A. nidulans and A. terreus SC and a high modal MIC of 4 µg/ml for A. cladosporioides and A. tubingensis. Isavuconazole ECVs have been defined for A. flavus, A. fumigatus, A. niger, and A. terreus (50). According to the species-specific ECVs, the vast majority of isolates represented wild-type (WT) strains of Aspergillus spp. (MIC ≤ ECV; range, 83.2 to 100.0%) (Tables 2 and 3). The isavuconazole MIC values were elevated at ≥8 µg/ml for 8 A. fumigatus isolates, which suggests resistance mediated by mutations in cyp51A.

The activity of isavuconazole against Mucorales isolates was generally lower than that seen with Aspergillus spp., with a MIC range of 0.25 to ≥16 µg/ml (Table 2). Modal MIC values of 4 to ≥16 µg/ml were seen with Lichtheimia spp., Mucor spp., Rhizopus arrhizus var. delemar, and Syncphalastrum spp. The lowest modal MIC values were seen for Rhizopus spp., R. arrhizus var. arrhizus, and R. microsporus (all 1 µg/ml [Table 2]). ECV values have not been established for isavuconazole and the Mucorales.

Activity of isavuconazole and comparators against Aspergillus and Mucorales isolates. Isavuconazole and itraconazole (MIC₉₀, 1 µg/ml for both compounds [Table 3]) had similar activities against 1,189 A. fumigatus isolates that were one 2-fold dilution
| Species (no. of isolates collected) | Antifungal agent (no. of isolates tested) | MIC (µg/ml) | % ECV by category:<br>Range 50% 90% WT NWT |
|-----------------------------------|------------------------------------------|-------------|----------------------------------|
| **Aspergillus spp. (1,964)**      | Isavuconazole (1,964)                     | 0.015–32    | 1 2                              |
|                                  | Itraconazole (1,413)                     | 0.12–32     | 1 2                              |
|                                  | Posaconazole (1,246)                     | 0.008–16    | 0.25 0.5                          |
|                                  | Voriconazole (1,834)                     | 0.03–32     | 0.5 1                            |
| A. calidoustus (36)              | Isavuconazole (36)                       | 1–4         | 2 4                              |
|                                  | Itraconazole (14)                        | 1–4         | 2 4                              |
|                                  | Posaconazole (28)                        | 2–8         | 4 4                              |
|                                  | Voriconazole (31)                        | 2–8         | 4 8                              |
| A. flavus (107)                  | Isavuconazole (107)                      | 0.25–4      | 1 2 83.2 16.8                    |
|                                  | Itraconazole (57)                        | 0.25–1      | 1 1 100.0 0.0                    |
|                                  | Posaconazole (44)                        | 0.12–0.5    | 0.25 0.5 63.6 36.4               |
|                                  | Voriconazole (95)                        | 0.25–16     | 0.5 1 95.8 4.2                   |
| A. flavus SC (62)                | Isavuconazole (62)                       | 0.25–1      | 1 1 100.0 0.0                    |
|                                  | Itraconazole (62)                        | 0.25–1      | 0.5 1 100.0 0.0                  |
|                                  | Posaconazole (62)                        | 0.12–0.5    | 0.25 0.5 59.7 40.3               |
|                                  | Voriconazole (62)                        | 0.12–1      | 1 1 100.0 0.0                    |
| A. fumigatus (1,189)             | Isavuconazole (1,189)                    | 0.12–32     | 0.5 1 96.2 3.8                   |
|                                  | Itraconazole (876)                       | 0.12–32     | 1 1 95.8 4.2                     |
|                                  | Posaconazole (817)                       | 0.008–4     | 0.25 0.5 79.4 20.6               |
|                                  | Voriconazole (1,122)                     | 0.12–32     | 0.5 0.5 98.1 1.9                 |
| A. lentulus (11)                 | Isavuconazole (11)                       | 0.5–8       | 2 4                              |
|                                  | Itraconazole (8)                         | 0.25–4      |                                  |
|                                  | Posaconazole (7)                         | 0.25–1      |                                  |
|                                  | Voriconazole (11)                        | 0.5–8       | 2 4                              |
| A. nidulans (29)                 | Isavuconazole (29)                       | 0.06–0.5    | 0.25 0.25 96.6 3.4               |
|                                  | Itraconazole (17)                        | 0.25–1      | 0.5 1 100.0 0.0                  |
|                                  | Posaconazole (19)                        | 0.12–0.5    | 0.25 0.5 100.0 0.0               |
|                                  | Voriconazole (25)                        | 0.06–0.5    | 0.12 0.25 100.0 0.0              |
| A. niger (62)                    | Isavuconazole (62)                       | 0.25–8      | 2 2 98.4 1.6                     |
|                                  | Itraconazole (50)                        | 0.25–4      | 2 2 96.0 4.0                     |
|                                  | Posaconazole (44)                        | 0.06–1      | 0.5 0.5 95.5 4.5                 |
|                                  | Voriconazole (59)                        | 0.12–16     | 1 2 98.3 1.7                     |
| A. niger species complex (103)   | Isavuconazole (103)                      | 0.06–8      | 2 4 97.1 2.9                     |
|                                  | Itraconazole (85)                        | 0.25–4      | 2 2 92.9 7.1                     |
|                                  | Posaconazole (47)                        | 0.06–1      | 0.5 0.5 91.5 8.5                 |
|                                  | Voriconazole (99)                        | 0.03–2      | 1 2 100.0 0.0                    |
| A. sydowii (22)                  | Isavuconazole (22)                       | 0.25–1      | 0.5 1                            |
|                                  | Itraconazole (13)                        | 0.5–2       | 1 2                              |
|                                  | Posaconazole (10)                        | 0.12–0.5    | 0.5 0.5                          |
|                                  | Voriconazole (21)                        | 0.12–2      | 0.5 1                            |
| A. terreus (96)                  | Isavuconazole (96)                       | 0.12–8      | 0.5 1 96.9 3.1                   |
|                                  | Itraconazole (63)                        | 0.12–1      | 0.5 1 100.0 0.0                  |
|                                  | Posaconazole (61)                        | 0.12–1      | 0.25 0.25 98.4 1.6               |
|                                  | Voriconazole (89)                        | 0.12–8      | 0.5 1 97.8 2.2                   |
| A. terreus species complex (14)  | Isavuconazole (14)                       | 0.12–0.5    | 0.25 0.5 100.0 0.0               |
|                                  | Itraconazole (14)                        | 0.25–0.5    | 0.5 0.5 100.0 0.0                |
|                                  | Posaconazole (12)                        | 0.12–0.25   | 0.25 0.25 100.0 0.0              |
|                                  | Voriconazole (14)                        | 0.25–0.5    | 0.25 0.5 100.0 0.0               |
| A. tubingensis (66)              | Isavuconazole (66)                       | 1–8         | 4 8                              |
|                                  | Itraconazole (48)                        | 1–4         | 2 4                              |
|                                  | Posaconazole (13)                        | 0.25–1      | 0.5 1                            |
|                                  | Voriconazole (56)                        | 0.5–2       | 2 2                              |
| A. welwitschiae (25)             | Isavuconazole (25)                       | 1–4         | 2 2                              |
|                                  | Itraconazole (17)                        | 2–2         | 2 2                              |
|                                  | Posaconazole (3)                         | 0.12–0.5    |                                  |
|                                  | Voriconazole (21)                        | 0.25–1      | 0.5 0.5                          |
| Lichtheimia spp. (22)            | Isavuconazole (22)                       | 1–16        | 4 8                              |
|                                  | Itraconazole (9)                         | 1–16        |                                  |
|                                  | Posaconazole (20)                        | 0.25–2      | 0.5 1                            |
|                                  | Voriconazole (12)                        | 16–32       | 16 32                            |

(Continued on next page)
higher than those of posaconazole and voriconazole (MIC\textsubscript{90} 0.5 \textmu{g/ml for both). More than 95% of the \textit{A. fumigatus} isolates tested were WT to isavuconazole (96.2%), itraconazole (95.8%), and voriconazole (98.1%), whereas only 79.4% were WT to posaconazole. Regarding the posaconazole data, note that there has been discussion whether the ECV should be 0.25 or 0.5 \textmu{g/ml} (51). If the ECV for posaconazole were to be set at 0.5 \textmu{g/ml}, the percentage of WT would be 97.9% for this collection, comparable to that of the other triazoles (data not shown). The recently revised ECV for posaconazole and \textit{A. fumigatus} of 0.25 \textmu{g/ml was determined as the optimal cutoff for the separation of WT strains from mutants harboring \textit{cyp51A} mutations (51).}

The isavuconazole MIC\textsubscript{90} values were 2 \textmu{g/ml for \textit{A. flavus and 1 \textmu{g/ml for \textit{A. flavus SC}, resulting in 83.2% and 100.0% wild type, respectively (Table 3). There were 17 \textit{A. flavus} isolates for which the isavuconazole MIC value was 2 \textmu{g/ml, and if the ECV was increased from 1 \textmu{g/ml to 2 \textmu{g/ml, the percentage of WT would increase to 99.1%, comparable to that seen for the \textit{A. flavus SC, itraconazole (100.0% WT), and voriconazole (95.8% WT). Whereas the isavuconazole ECV for this species was determined using MIC values from 7 different laboratories (50), the reproducibility of the CLSI method for a single laboratory (± one 2-fold dilution) should be kept in mind when evaluating such data. Given the potential for dose escalation with isavuconazole, it may be possible to...
treat *Aspergillus* infections for which the isavuconazole MIC is 2 μg/ml (52). Although dose escalation is less feasible with posaconazole, similar considerations may apply where an ECV of 0.5 μg/ml applied to *A. flavus* and *A. flavus* SC would increase the percentage of WT from 63.6% and 59.7%, respectively (determined at the CLSI ECV of 0.25 μg/ml), to 100.0% for both organism groups (data not shown).

The respective isavuconazole MIC₉₀ values of 2 and 4 μg/ml for *A. niger* and *A. niger* SC (Table 3) were comparable to that of itraconazole (2 μg/ml) and voriconazole (2 μg/ml) and higher than that of posaconazole (0.5 μg/ml). The wild-type percentages against *A. niger* and *A. niger* SC were 97.1 to 98.4% for isavuconazole, 92.9 to 96.0% for itraconazole, 91.5 to 95.5% for posaconazole, and 98.3 to 100.0% for voriconazole.

Greater than 95% of *A. nidulans*, *A. terreus*, and *A. terreus* SC isolates were WT to all four triazoles, and these species were among the most susceptible to these agents, with MIC₉₀ values of 0.25 to 1 μg/ml. The highest MIC₉₀ values (4 to 8 μg/ml) for the tested triazoles were seen with *A. calidoustus*, *A. lentulus*, and *A. tubingensis* (MIC₉₀: 8 μg/ml [isavuconazole]) (Table 3).

All triazole antifungal agents showed variable activity across the *Mucorales* tested (0.06 to 32 μg/ml), with the lowest MIC₉₀ values observed for *Rhizomucor pusillus* (MIC₉₀: 0.5 to 8 μg/ml), *Rhizopus arhizus* var. *arhizus* (MIC₉₀: 0.5 to 8 μg/ml), and *R. microsporus* (MIC₉₀: 1 to 8 μg/ml) and the highest MIC values observed for *Mucor* spp. (MIC₉₀: 2 to 32 μg/ml), *Mucor circinelloides* f. *circinelloides* (MIC₉₀: 4 to 32 μg/ml), and *Syncephalastrum* spp. (MIC₉₀: ≥16 μg/ml [isavuconazole]) (Table 3). Whereas voriconazole lacked any useful activity against the *Mucorales* (MIC₉₀: 8 to 32 μg/ml across all species), the lowest MIC₉₀ values were observed with posaconazole (MIC₉₀ range, 0.5 to 4 μg/ml). Among the species for which an ECV has been proposed for posaconazole (53), 100.0% of *M. circinelloides* f. *circinelloides*, *M. circinelloides* f. *janssenii*, and *Rhizopus arhizus* var. *arhizus* isolates, 90.6% of *Rhizopus arhizus* var. *delemar* isolates, and 93.3% of *Rhizopus microsporus* isolates expressed a WT phenotype: 100.0% of *Rhizopus arhizus* var. *arhizus* isolates and 90.0% of *Rhizopus arhizus* var. *delemar* isolates were WT to itraconazole. The activity of isavuconazole against the *Mucorales* most closely mirrored that of itraconazole (Table 3).

**Isavuconazole activity against Candida species isolates.** Among the 10 species of *Candida* shown in Tables 4 and 5, isavuconazole was most active against *Candida dubliniensis* (MIC₉₀: 0.008 μg/ml) and *Candida albicans* (MIC₉₀: 0.008 μg/ml) and least active against *Candida krusei* (MIC₉₀: 0.5 μg/ml), *Candida glabrata* (MIC₉₀: 1 μg/ml), and *Candida guilliermondii* (MIC₉₀: 4 μg/ml). The vast majority of each species, except for *C. glabrata*, *C. krusei*, and *C. guilliermondii*, were inhibited by ≤0.25 μg/ml of isavuconazole (range, 96.1% [*Candida lusitaniae*] to 100.0% [*C. albicans*, *C. dubliniensis*, *C. krusei*, and *C. orthopsilosis*]). *C. glabrata* and *C. krusei* were susceptible to isavuconazole at MIC values of ≤1 μg/ml (95.5 and 100.0%, respectively).

| Species (no. of isolates tested) | No. of isolates with MIC (μg/ml) a of: |
|----------------------------------|----------------------------------------|
|                                  | 0.008 | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | ≥4 |
| *Candida* spp. (2,351) | 1,494 | 202 | 165 | 222 | 77 | 71 | 50 | 42 | 19 | 9 |
| *C. albicans* (1,056) | 1,034 | 20 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. dubliniensis* (62) | 61 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. glabrata* (489) | 10 | 33 | 108 | 179 | 51 | 24 | 26 | 36 | 16 | 6 |
| *C. guilliermondii* (13) | 0 | 0 | 1 | 0 | 1 | 2 | 5 | 0 | 2 | 2 |
| *C. kefyr* (15) | 13 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. krusei* (68) | 0 | 0 | 0 | 4 | 12 | 38 | 13 | 1 | 0 | 0 |
| *C. lusitaniae* (51) | 35 | 8 | 4 | 1 | 0 | 1 | 2 | 0 | 0 | 0 |
| *C. orthopsilosis* (22) | 9 | 4 | 5 | 3 | 1 | 0 | 0 | 0 | 0 | 0 |
| *C. parapsilosis* (349) | 224 | 86 | 19 | 15 | 3 | 1 | 1 | 0 | 0 | 0 |
| *C. tropicalis* (187) | 104 | 47 | 20 | 11 | 3 | 1 | 0 | 0 | 1 | 0 |

aNumbers in boldface are modal MIC values.
Activity of isavuconazole and comparators against *Candida* species isolates.

The antifungal activities of isavuconazole, fluconazole, posaconazole, and voriconazole against 2,351 *Candida* isolates (10 species) as determined by CLSI BMD methods are shown in Table 5. Results are categorized using CLSI CBPs and/or ECVs, as appropriate. The majority of these isolates represented WT strains, as determined by the respective ECVs, and few (*C. glabrata* and *C. parapsilosis*) were resistant to triazoles, based on CBPs. Neither CBPs nor ECV values have been established for isavuconazole and *Candida* spp.

| Species (no. of isolates collected) | Antifungal agent (no. of isolates tested) | MIC (µg/ml) | % by category* | CLSI | ECV |
|-----------------------------------|-------------------------------------------|------------|----------------|------|-----|
|                                   |                                           | Range      | 50% | 90%   | S | R | WT | NWT |
| *C. albicans* (1,056)             | Isavuconazole (1,056)                      | 0.008–0.03 | 0.008 | 0.008 |   |   |   |     |
|                                   | Posaconazole (1,056)                       | 0.008–0.12 | 0.03 | 0.03 | 99.3 | 0.7 |
|                                   | Voriconazole (1,056)                       | 0.008–0.06 | 0.008 | 0.015 | 100.0 | 0.0 | 99.5 | 0.5 |
|                                   | Fluconazole (1,056)                        | 0.12–1 | 0.12 | 0.25 | 100.0 | 0.0 | 99.4 | 0.6 |
| *C. glabrata* (489)               | Isavuconazole (489)                        | 0.008–8 | 0.06 | 1 |   |   |   |     |
|                                   | Posaconazole (489)                         | 0.03–16 | 0.25 | 1 | 99.2 | 0.8 |
|                                   | Voriconazole (489)                         | 0.008–8 | 0.06 | 0.5 | 88.8 | 11.2 |
|                                   | Fluconazole (489)                          | 0.25–256 | 2 | 16 | 93.7 | 6.3 | 87.3 | 12.7 |
| *C. parapsilosis* (349)           | Isavuconazole (349)                        | 0.008–0.5 | 0.008 | 0.03 |   |   |   |     |
|                                   | Posaconazole (349)                         | 0.008–0.5 | 0.06 | 0.12 |   |   |   |     |
|                                   | Voriconazole (349)                         | 0.008–1 | 0.015 | 0.03 | 96.3 | 0.3 | 91.1 | 8.9 |
|                                   | Fluconazole (349)                          | 0.12–128 | 0.5 | 2 | 94.8 | 4.3 | 89.4 | 10.6 |
| *C. tropicalis* (187)             | Isavuconazole (187)                        | 0.008–2 | 0.008 | 0.03 |   |   |   |     |
|                                   | Posaconazole (187)                         | 0.015–1 | 0.03 | 0.06 |   |   | 98.4 | 1.6 |
|                                   | Voriconazole (187)                         | 0.008–16 | 0.015 | 0.06 | 97.9 | 0.5 | 97.9 | 2.1 |
|                                   | Fluconazole (187)                          | 0.12–256 | 0.25 | 0.5 | 97.9 | 1.6 | 97.3 | 2.7 |
| *C. krusei* (68)                  | Isavuconazole (68)                         | 0.06–1 | 0.25 | 0.5 |   |   |   |     |
|                                   | Posaconazole (68)                          | 0.12–0.5 | 0.25 | 0.5 |   |   |   |     |
|                                   | Voriconazole (68)                          | 0.12–4 | 0.25 | 0.5 | 94.1 | 1.5 | 94.1 | 5.9 |
|                                   | Fluconazole (68)                           | 16–128 | 32 | 64 | 85.3 | 14.7 |
| *C. lusitaniae* (51)              | Isavuconazole (51)                         | 0.008–0.5 | 0.008 | 0.03 |   |   |   |     |
|                                   | Posaconazole (51)                          | 0.03–0.5 | 0.06 | 0.12 |   |   | 76.5 | 23.5 |
|                                   | Voriconazole (51)                          | 0.008–0.5 | 0.008 | 0.015 |   |   | 94.1 | 5.9 |
|                                   | Fluconazole (51)                           | 0.12–64 | 0.5 | 1 |   |   | 90.2 | 9.8 |
| *C. dubliniensis* (62)            | Isavuconazole (62)                         | 0.008–0.06 | 0.008 | 0.008 |   |   |   |     |
|                                   | Posaconazole (62)                          | 0.008–0.12 | 0.03 | 0.03 |   |   | 100.0 | 0.0 |
|                                   | Voriconazole (62)                          | 0.008–0.06 | 0.008 | 0.015 |   |   | 98.4 | 1.6 |
|                                   | Fluconazole (62)                           | 0.12–16 | 0.12 | 0.25 |   |   | 98.4 | 1.6 |
| *C. guillermondii* (13)           | Isavuconazole (13)                         | 0.03–4 | 0.5 | 4 |   |   |   |     |
|                                   | Posaconazole (13)                          | 0.25–1 | 0.5 | 1 | 76.9 | 23.1 |
|                                   | Voriconazole (13)                          | 0.015–2 | 0.25 | 2 | 46.2 | 53.8 |
|                                   | Fluconazole (13)                           | 1–128 | 8 | 128 | 61.5 | 38.5 |
| *C. orthopsilosis* (22)           | Isavuconazole (22)                         | 0.008–0.12 | 0.015 | 0.06 |   |   |   |     |
|                                   | Posaconazole (22)                          | 0.03–0.12 | 0.06 | 0.12 |   |   | 100.0 | 0.0 |
|                                   | Voriconazole (22)                          | 0.008–0.25 | 0.015 | 0.03 |   |   | 90.9 | 9.1 |
|                                   | Fluconazole (22)                           | 0.25–4 | 0.5 | 1 | 90.9 | 9.1 |
| *C. kefyr* (15)                   | Isavuconazole (15)                         | 0.008–0.03 | 0.008 | 0.015 |   |   |   |     |
|                                   | Posaconazole (15)                          | 0.03–0.25 | 0.12 | 0.25 |   |   | 100.0 | 0.0 |
|                                   | Voriconazole (15)                          | 0.008–0.03 | 0.008 | 0.015 |   |   | 100.0 | 0.0 |
|                                   | Fluconazole (15)                           | 0.12–1 | 0.25 | 0.5 |   |   | 100.0 | 0.0 |

*Interpretive categories as recommended by CLSI (70) and the use of ECVs (71, 73, 76). ECV, epidemiological cutoff value; S, susceptible; R, resistant; WT, wild type; NWT, non-wild type.

*Category designation is susceptible dose dependent.*
Using species-specific breakpoints, 100.0% of *C. albicans* isolates were susceptible to fluconazole and voriconazole. Fluconazole and voriconazole were also active against *C. parapsilosis* (94.8 and 96.3% susceptible, respectively, at the CLSI CBP) and *Candida tropicalis* (97.9 and 97.9% susceptible, respectively, at the CLSI CBP). Voriconazole was also active against *C. krusei* (94.1% susceptible). Among the 10 species of *Candida* tested against posaconazole, 98.7% showed a WT phenotype based on the established ECVs (54). Only *C. lusitaniae* (76.5% WT) and *C. guilliermondii* (76.9% WT) exhibited greater than 3% strains non-WT to posaconazole (Table 5).

The *in vitro* potency of isavuconazole against *Candida* spp. was most comparable to that of voriconazole. Based on MIC\(_{90}\) values, isavuconazole was 2- to 16-fold more active than posaconazole against all species, although *C. guilliermondii* displayed much higher MIC\(_{90}\) values for all agents (Table 5). *C. guilliermondii* is known to exhibit decreased susceptibility to fluconazole, posaconazole, and voriconazole (55–57), and this phenotype was apparent in isolates from the present study as well (23.1 to 53.8% non-WT [Table 5]).

**Isavuconazole activity against non-*Candida* yeasts and rare molds.** Isavuconazole MIC ranges were 0.008 to 0.5 \(\mu g/ml\) across *Cryptococcus* spp. (modal MIC, 0.03 \(\mu g/ml\)), *Cryptococcus neoforms* var. *grubii* (modal MIC, 0.015 \(\mu g/ml\)), and *Saccharomyces cerevisiae* (modal MIC, 0.015 \(\mu g/ml\)). In contrast, the modal MICs were all \(\geq 4\) \(\mu g/ml\) for *Fusarium* spp. and *Scedosporium* spp. (Table 6).

**DISCUSSION**

Several important observations can be made from this global survey. First, we have used molecular methods of species identification to further document the broad array of fungi implicated as causes of IFI in U.S. and non-U.S. medical centers. We have tested all fungi for susceptibility to isavuconazole and the other systemically active triazoles using reference CLSI BMD methods and have applied the most recent CBPs and ECVs to assess the relative activity of these important antifungal agents. In general, the more common species of *Candida* and *Aspergillus* remain susceptible to all the mold-active triazole antifungal agents. Resistance to multiple azoles is apparent in both *C. glabrata* and *C. guilliermondii*, and both species must be monitored closely for the emergence of multidrug resistance. Likewise, the azole-resistant non-*fumigatus* species of *Aspergillus*, such as *A. calidoustus*, *A. lentulus*, and *A. tubingensis*, along with emerging MDR strains of *A. fumigatus*, must be actively sought in clinical material and undergo accurate species identification as well as antifungal susceptibility testing to ensure optimal patient management (29, 30, 34, 35). Whereas isavuconazole has been approved for the treatment of invasive mucormycosis (49), the available clinical and *in vitro* data to support this application have been limited to date (44–49). In the present study, we have documented the variable activities of isavuconazole, itraconazole, and posaconazole across all of the *Mucorales* isolates tested and have confirmed the potentially useful activity of isavuconazole against select species of *Rhizopus* as determined by CLSI methods (44–47). Given the modal MIC value of 1 \(\mu g/ml\) for isavuconazole and species of *Rhizopus*, it is important to note that an analysis of real-world usage, along with an analysis of clinical trial samples, showed that drug concentrations of \(> 1\) \(\mu g/ml\) are achieved with standard doses of isavuconazole (58).

| Species (no. of isolates tested) | No. of isolates with MIC (\(\mu g/ml\)) of: |
|---------------------------------|-------------------------------------|
|                                 | 0.008 | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | \(\geq 4\) |
| *Cryptococcus* spp. (84)        | 12    | 27    | 28   | 9    | 3    | 1    | 4    | 0  | 0  | 0  |
| *C. neoforms* var. *grubii* (76)| 11    | 26    | 24   | 9    | 2    | 1    | 3    | 0  | 0  | 0  |
| *Saccharomyces cerevisiae* (13) | 2     | 5     | 5    | 3    | 2    | 1    | 0    | 0  | 0  | 0  |
| *Fusarium* spp. (24)            | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0  | 0  | 0  |
| *F. solani* species complex (18)| 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0  | 0  | 18 |
| *Scedosporium* spp. (45)        | 0     | 0     | 1    | 0    | 0    | 0    | 1    | 5  | 6  | 32 |
| *S. apiospermum/S. boydii* (26) | 0     | 0     | 0    | 0    | 0    | 0    | 3    | 5  | 18 | 18 |

*Numbers in boldface are modal MIC values.*
Isavuconazole MIC distributions examined for *Candida* spp., *Aspergillus* spp., and the *Mucorales* from the most recent 2-year surveillance period (2015 to 2016) demonstrated little to no change in the distributions compared to reports from previous years (43, 46, 59, 60), with activity comparable to those of itraconazole, posaconazole, and voriconazole. Isavuconazole and the other triazoles continue to be highly active against *Aspergillus* spp., but are less potent against the non-*Aspergillus* molds, including the *Mucorales*. The triazoles, including isavuconazole, appear to be more reliably active against the non-*Candida* yeasts than against rare molds, such as *Fusarium* spp.

In summary, the increasing application of molecular and proteomic methods of identification reveals a broad spectrum of opportunistic fungal pathogens. Isavuconazole exhibited excellent activity against most species of *Candida* and *Aspergillus* and is comparable to posaconazole and voriconazole against the less common yeasts and molds. Whereas most *Candida* and *Aspergillus* spp. remain susceptible to isavuconazole and the other triazoles, emergence of resistance during therapy, especially in patients with previous antifungal exposure, must be kept in mind. Given the extensive use of voriconazole in prevention and treatment of invasive aspergillosis, emergence of the *Mucorales* as breakthrough infections is a clear threat and underscores the importance of new agents, such as isavuconazole, in patients with invasive mucormycosis who are unable to tolerate amphotericin B therapy (42, 49).

**MATERIALS AND METHODS**

Organisms. A total of 4,856 nonduplicate clinical isolates from patients with IFI were collected during 2015 to 2016 from U.S. (2,937 isolates) and non-U.S. (1,919 isolates) medical centers (Table 1). There were 75 isolates (1.5% of total) from species with <10 representatives (data not shown). The isolates were received from patients with bloodstream infections, from normally sterile body fluids (e.g., cerebrospinal, pleural, and peritoneal fluids), tissues, or abscesses, from respiratory tract specimens, or from unspecified infection sites. Molds included 1,194 isolates of *A. fumigatus sensu stricto* and 108 *A. flavus*, 62 *A. flavus* SC, 608 other *Aspergillus* species (36 *A. caldostus*, 11 *A. lentulus*, 29 *A. nidulans*, 62 *A. niger*, 103 *A. niger* SC, 22 *Aspergillus* sydowi, 98 *A. terreus*, 14 *A. terreus* SC, 66 *A. tubingensis*, and 25 *A. welwitschiae* isolates), 24 *Fusarium* species, and 45 *Scedosporium* species isolates (Table 1). There were 292 isolates of the *Mucorales* order, including 23 *Litchheimia* species, 69 *Mucor* species, 14 *R. pusillus* species, 162 *Rhizopus* species, and 11 *Syccephalum* species isolates. Among the 2,351 isolates of *Candida* spp. were 1,056 *C. albicans*, 489 *C. glabrata*, 349 *C. parapsilosis*, 187 *C. tropicalis*, 68 *C. krusei*, 51 *C. lusitaniae*, 62 *C. dubliniensis*, 13 *C. guilliermondii*, 22 *C. orthopsilosis*, and 15 *C. kefyr* isolates. The collection also included 84 Cryptococcus species and 13 *S. cerevisiae* isolates.

Isolates were identified at participating institutions using methods routinely employed at the submitting laboratory, including the use of Vitek, MicroScan, API strips, and AuxaColor systems supplemented by conventional methods for yeast and mold identification (61–63). Isolates were submitted to JMI Laboratories (North Liberty, IA) or the Fungus Testing Laboratory (San Antonio, TX), where species identification was confirmed using morphological, biochemical, molecular, and proteomic tests (64–66). Yeast isolates were subcultured and screened using CHROMagar *Candida* (Becton, Dickinson, Sparks, MD) to ensure purity and to differentiate *C. albicans/C. dubliniensis*, *C. tropicalis*, and *C. krusei*. Additionally, biochemical tests, including Vitek 2 (bioMérieux, Hazelwood, MO), trehalose assimilation (for *C. glabrata*), or growth at 45°C (for *C. albicans/C. dubliniensis*), were used to identify common *Candida* species. Identity of isolates was confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS [Bruker, Billerica, MA]). Isolates that were not identified by either phenotypic or proteomic methods, including all rare and sibling species, were identified using sequence-based methods as previously described (64).

Identification of *Aspergillus* spp. and the *Mucorales* spp. was performed by combined morphology/phenotypic assessment and DNA sequence analysis. All rare and sibling species were identified by DNA sequencing. For morphological/phenotypic assessment, macroscopic and microscopic features were evaluated and temperature studies performed. For DNA sequence analysis, regions of the β-tubulin and calmodulin genes were amplified and sequenced. For *Mucorales* isolates, the internal transcribed spacer and D1/D2 regions were amplified and sequenced. *Scedosporium* spp. were also identified by amplifying and sequencing regions of the β-tubulin and calmodulin genes. Nucleotide sequences were examined using Lasergene software (DNASTar, Madison, WI) or Sequencer software (Gene Codes, Ann Arbor, MI) and then compared to database sequences using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). *Fusarium* species isolates were analyzed for TEF sequence using the *Fusarium*-ID database through 2016 and the *Fusarium* multilocus sequence typing database (http://www.westerdijkinstitute.nl/fusarium/) (64). Results were considered acceptable if homology was >99.5% with other entries in the databases used for comparison. Sequences that were considerably different from the majority of entries for a species were considered outliers and were excluded from the analysis. The DNA sequence results were combined with the morphological/phenotypic assessment to assign a species identity to each isolate (67).
Antifungal susceptibility testing. All yeast isolates were tested for in vitro susceptibility to fluconazole, isavuconazole, posaconazole, and voriconazole using CLSI (68) BMD methods. MIC results for all agents were read after 24 h of incubation, when the agents were tested against Candida spp., whereas MIC results were read after 48 h, when the agents were tested against non-Candida yeasts. MIC values were determined visually as the lowest concentration of drug that caused significant (≥50%) growth diminution levels relative to the growth control (69, 70).

In vitro susceptibility testing of Aspergillus spp., members of the Mucorales order, and other molds against the triazoles (isavuconazole, itraconazole, posaconazole, and voriconazole) was performed by BMD as described in CLSI document M38-A2 (69). For Aspergillus spp., the MICs for isavuconazole and comparators were read as 100% inhibition of growth after 48 h of incubation at 35°C. Against the Mucorales isolates, MICs for isavuconazole and comparators were also read at 100% inhibition of growth, but after 24 h of incubation.

We used the revised species-specific CLSI CBPs to identify strains of the 6 most common species of Candida (C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, and C. guilliermondii) that were susceptible and resistant to fluconazole and voriconazole (70, 71). All C. krusei isolates were defined as resistant to fluconazole. CLSI has not assigned CBPs for voriconazole and C. glabrata and recommends the ECV of 0.5 μg/ml to be used to differentiate WT (MIC ≤ ECV) from non-WT (MIC > ECV) strains of this species (54, 71).

CBPs have not been established for isavuconazole or posaconazole and the common species of Candida or for any antifungal agent and the less common species of Candida, non-Candida yeasts, Aspergillus spp., or the non-Aspergillus molds; however, ECVs have been proposed for the triazoles (fluconazole, posaconazole, and voriconazole) and 6 Candida species that are encountered less frequently (C. lusitaniae, C. guilliermondii, C. dubliniensis, C. kefyr, C. orthopsilosis, and Candida pelliculosa) (54, 71, 72). ECVs have also been developed for A. fumigatus, A. flavus, A. terreus, A. nidulans, and A. niger and isavuconazole, itraconazole, posaconazole, and voriconazole (50, 54, 73): isavuconazole, itraconazole, and voriconazole MIC values of >1 μg/ml were considered non-WT for A. fumigatus, A. flavus, and A. terreus, and isavuconazole and posaconazole MIC values of >1 μg/ml and voriconazole MIC values of >2 μg/ml were considered non-WT for A. nidulans. Posaconazole MIC values of >0.25 μg/ml were considered non-WT for A. fumigatus and A. flavus, and MIC results of >0.5 μg/ml were non-WT for A. niger and A. terreus; isavuconazole MIC values of >1 μg/ml were non-WT for A. nidulans, and MIC values of >4 μg/ml were non-WT for A. niger. Isolates of these Aspergillus spp. for which triazole MIC results exceed the ECV are considered to be non-WT and may harbor acquired mutations in the cyp51A gene (74, 75).

Among the Mucorales, there are no CBPs, and ECVs have only been proposed for posaconazole and L. corymbifera (2 μg/ml), M. circinelloides (4 μg/ml), R. arrhizus (2 μg/ml), and R. microsporus (2 μg/ml) and for itraconazole and R. arrhizus (2 μg/ml) (53).

Quality control was performed as recommended in CLSI documents M27-A3 (68) and M38-A2 (69) using strains C. krusei ATCC 6258, C. parapsilosis ATCC 22019, A. flavus ATCC 204304, and A. fumigatus MYA-3626.

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