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Original Article

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Impact of COVID-19 on the antifungal susceptibility profiles of isolates collected in a global surveillance program that monitors invasive fungal infections

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Abstract

Studies demonstrated the impact of the COVID-19 pandemic in the prevalence and susceptibility profiles of bacterial and fungal organisms. We analyzed 4821 invasive fungal isolates collected during 2018, 2019, and 2020 in 48 hospitals worldwide to evaluate the impact of this event in the occurrence and susceptibility rates of common fungal species. Isolates were tested using the CLSI broth microdilution method. While the percentage of total isolates that were *C. glabrata* ($n = 710$ isolates) or *C. krusei* ($n = 112$) slightly increased in 2020, the percentage for *C. parapsilosis* ($n = 542$), *A. fumigatus* ($n = 416$), and *C. lusitaniae* ($n = 84$) significantly decreased ($P < .05$). Fluconazole resistance in *C. glabrata* decreased from 5.8% in 2018–2019 to 2.0% in 2020, mainly due to fewer hospitals in the US having these isolates (5 vs. 1 hospital). Conversely, higher fluconazole-resistance rates were noted for *C. parapsilosis* (13.9 vs. 9.8%) and *C. tropicalis* (3.5 vs. 0.7%; $P < .05$) during 2020. Voriconazole resistance also increased for these species. Echinocandin resistance was unchanged among *Candida* spp. Voriconazole susceptibility rates in *A. fumigatus* were similar in these two periods (91.7% in 2018 and 2019 vs. 93.0% in 2020). Changes were also noticed in the organisms with smaller numbers of collected isolates. We observed variations in the occurrence of organisms submitted to a global surveillance and the susceptibility patterns for some organism-antifungal combinations. As the COVID-19 pandemic is still ongoing, the impact of this event must continue to be monitored to guide treatment of patients affected by bacterial and fungal infections.

Lay Summary

Secondary infections were documented in COVID-19 patients. We compared the prevalence of invasive fungal isolates consecutively collected in 48 worldwide hospitals and their susceptibility patterns between 2020, the year of the global COVID-19 pandemic, and the two prior years.

Key words: COVID-19, invasive fungal infections, antifungal susceptibility, azoles, echinocandins.

Background

The adverse impact of the COVID-19 pandemic on the health and welfare of the general population is without question.[1] Among the many consequences of this pandemic, secondary bacterial and fungal infections may impact antimicrobial resistance (AMR) through changes in antimicrobial use and healthcare-seeking behavior as well as infection prevention and control practices.[2–14] The complications of secondary infections are major risk factors for adverse COVID-19 outcomes.[10,11,14]

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Although most secondary infections in COVID-19 patients are bacterial,[5,10,11,15,16] many COVID-19 patients are at risk for invasive fungal infection (IFI) as well.[17–29] Early in the COVID-19 pandemic, secondary IFI were rarely reported.[30] But, as the pandemic spread globally, COVID-19 patients hospitalized in intensive care units (ICU) with acute respiratory distress syndrome (ARDS) and mechanical ventilation, COVID-associated pulmonary aspergillosis (CAPA;[17,20,21,31,32]), COVID-associated mucormycosis (CAM;[26,33,34]), and COVID-associated invasive candidiasis (CAIC;[11,18,22,24,25,29,35,36]) became important factors complicating the clinical course of the disease. Altered infection prevention practices in the pandemic setting may also increase the risk of CAIC in these seriously ill individuals.[18,22,35]

There is a paucity of data regarding the frequency of occurrence, species distribution, and antifungal resistance profiles of the infecting pathogens. Most reports of IFI complicating COVID-19 are case reports, case series, or literature reviews and contain few details of the infecting species and their antifungal resistance profiles.[11,17,18,22,25,26,31–33,35,36] Presently, no surveys describe the emerging species and resistant phenotypes of fungi causing invasive disease before and during the COVID-19 pandemic.

To address some of the shortcomings regarding investigations of IFI during the COVID-19 pandemic, we used the SENTRY Antifungal Surveillance Program database to examine isolates of yeasts and molds encountered pre-COVID-19 (2018–2019) and from the COVID-19 (2020) period. The primary objectives of this survey were first, to describe the frequency of isolated fungal species from significant infections in the pre-COVID and COVID periods, and second, to determine the phenotypic and genotypic resistance profiles for the pre-COVID and COVID fungal isolates against systemically active antifungal agents.

Methods

Fungal identification

All isolates were submitted to matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI–TOF MS) using the MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA) after a purity check. Yeasts that were not identified by these methods were identified using sequencing-based methods for the internal transcribed spacer (ITS) region, 28S ribosomal subunit, or IGS1 for *Trichosporon* spp.[37–40] Mold isolates that did not achieve an acceptable identification by MALDI–TOF MS were sequenced for the 28S and 1 of the following genes was analyzed: β-tubulin for *Aspergillus* spp., translation elongation factor (TEF) for *Fusarium* spp., or ITS for all other species of filamentous fungi.[37–40]

Susceptibility testing

All isolates were tested by broth microdilution methods as described in the M27[41] and M38[42] documents. Interpretive criteria, which included clinical breakpoints (CBPs) and epidemiological cut-off values (ECVs) where available, were published in the M59,[43] M60,[44] and M61.[45] Quality control (QC) was performed as recommended by in the M27[41] and M38[42] using *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, *Aspergillus flavus* ATCC 204304, and *Aspergillus fumigatus* ATCC MYA-3626.

Resistance mechanisms

Candida spp. isolates that exhibited echinocandin MIC values above the ECV and *A. fumigatus* isolates that displayed azole MIC values above the ECV were subjected to whole genome sequencing.[46] These isolates were selected to ensure that mutations were detected even if present in the tail-end of the wild-type population. DNA regions encoding the *FKS* hot spots in *Candida* spp. and *CYP* regions in *A. fumigatus* were compared to available sequences in the literature.[46,47]

Statistical analysis

Differences in percentages were further investigated using the Chi-square test to determine statistical significance ($P < .05$).

Results

Frequency of occurrence of fungal pathogens in each age group

Of the 4821 total non-duplicate fungal isolates collected from medical centers worldwide, 3272 were from the pre-COVID survey years 2018–2019 and 1549 were from the COVID survey year 2020 (Table 1). The isolates were collected from 48 medical centers located in Europe (2305 isolates from 21 medical centers), North America (1237 isolates from 13 medical centers), the Asia-Pacific region (824 isolates from 9 medical centers), and Latin America (455 isolates from 5 medical centers). All participating centers contributed isolates in each of the three survey years. These isolates were recovered from patients with bloodstream infection (BSI; 2460 isolates), pneumonia in hospitalized patients (PIHP; 757 isolates), skin and skin structure infection (SSSI; 316 isolates), urinary tract infection (UTI; 117 isolates), intraabdominal infection (IAI; 113 isolates), and other infection sites (1058 isolates).

Most isolates (80.0%) were *Candida* spp. (78.4%, pre-COVID; 83.4%, COVID), 1.7% were *Cryptococcus* spp. (2.4%, pre-COVID; 1.4%, COVID), 1.8% were other non-*Candida* yeasts (1.3%, pre-COVID; 0.5%, COVID), 13.4% were *Aspergillus* spp. (14.5%, pre-COVID; 11.0%, COVID), 0.8% were species within the Mucorales (0.9%, pre-COVID; 0.6%, COVID), 0.5% were *Fusarium* spp. (0.5%, pre-COVID; 0.6%, COVID), 0.7% were *Scedosporium* spp. (0.8%, pre-COVID; 0.6%, COVID), and 1.2% were other molds (1.2%, pre-COVID; 1.3%, COVID) (Table 1).

Table 1. Species distribution of opportunistic fungal pathogens from pre-COVID (2018–2019) and COVID (2020).

	Number (%) of isolates/total by category		
Organism	All isolates	2018–2019	2020
Overall	4821 (100.0%)	3272/4821 (67.9%)	1549/4821 (32.9%)
Candida spp.	3857/4821(80.0%)	2565/3272 (78.4%)	1292/1549 (83.4%)
Candida albicans	1724/3857 (44.7%)	1145/2565 (44.6%)	579/1292 (44.8%)
Candida glabrata	710/3857 (18.4%)	462/2565 (18.0%)	248/1292 (19.2%)
Candida parapsilosis	542/3857 (14.1%)	377/2565 (14.7%)	165/1292 (12.8%)
Candida tropicalis	419/3857 (10.9%)	277/2565 (10.8%)	142/1292 (11.0%)
Candida dubliniensis	107/3857 (2.8%)	68/2565 (2.7%)	39/1292 (3.0%)
Candida krusei	112/3857 (2.9%)	66/2565 (2.6%)	46/1292 (3.6%)
Candida lusitaniae	84/3857 (2.2%)	66/2565 (2.6%)	18/1292 (1.4%)
Candida orthopsilosis	43/3857 (1.1%)	27/2565 (1.1%)	16/1292 (1.2%)
Candida guilliermondii	30/3857 (0.8%)	18/2565 (0.7%)	12/1292 (0.9%)
Candida kefyr	28/3857 (0.7%)	18/2565 (0.7%)	10/1292 (0.8%)
Candida metapsilosis	14/3857 (3.6%)	13/2565 (0.5%)	1/1292 (<0.1%)
Candida pelliculosa	9/3857 (0.2%)	6/2565 (0.2%)	3/1292 (0.2%)
Candida fabianii	6/3857 (0.2%)	4/2565 (0.2%)	2/1292 (0.2%)
Candida inconspicua	6/3857 (0.2%)	3/2565 (0.1%)	3/1292 (0.2%)
Candida fermentati	5/3857 (0.1%)	4/2565 (0.2%)	1/1292 (<0.1%)
Candida nivariensis	3/3857 (<0.1%)	3/2565 (0.1%)	
Candida utilis	3/3857 (<0.1%)	1/2565 (<0.1%)	2/1292 (0.2%)
Candida bracarensis	2/3857 (<0.1%)		2/1292 (0.2%)
Candida haemulonii	2/3857 (<0.1%)	2/2565 (<0.1%)	
Candida rugosa	2/3857 (<0.1%)		2/1292 (0.2%)
Candida sphaerica	1/3857 (<0.1%)	1/2565 (<0.1%)	
Candida duobushaemulonii	1/3857 (<0.1%)	1/2565 (<0.1%)	
Candida pseudohaemulonii	1/3,857 (<0.1%)	1/2,565 (<0.1%)	
Candida quercitrusa	1/3,857 (<0.1%)	1/2,565 (<0.1%)	
Candida spencermartinsiae	1/3,857 (<0.1%)		1/1,292 (<0.1%)
Other yeasts			
Cryptococcus neoformans var. *grubii*	63/4,821 (1.3%)	51/3,272 (2.0%)	12/1,549 (1.2%)
Cryptococcus neoformans var. *neoformans*	8/4,821 (0.2%)	6/3,272 (0.2%)	3/1,549 (0.1%)
Cryptococcus gattii SC[b]	5/4,821 (0.1%)	4/3,272 (0.1%)	1/1,549 (<0.1%)
Cryptococcus laurentii	1/4,821 (<0.1%)	1/3,272 (<0.1%)	
Saccharomyces cerevisiae	20/4,821 (0.4%)	17/3,272 (0.5%)	3/1,549 (0.2%)
Trichosporon asahii	22/4,821 (0.5%)	15/3,272 (0.5%)	7/1,549 (0.5%)
Trichosporon louberi	1/4,821 (<0.1%)	1/3,272 (<0.1%)	
Trichosporon montevideense	1/4,821 (<0.1%)	1/3,272 (<0.1%)	
Trichosporon mycotoxinivorans	2/4,821 (<0.1%)	1/3,272 (<0.1%)	1/1,549 (<0.1%)
Trichosporon ovoides	1/4,821 (<0.1%)	1/3,272 (0.1%)	
Saprochaete clavata	12/4,821 (0.2%)	8/3,272 (0.2%)	4/1,549 (0.3%)
Geotrichum silvicola	1/4,821 (<0.1%)	1/3,272 (<0.1%)	
Yarrowia lipolytica	3/4,821 (<0.1%)	3/3,272 (<0.1%)	
Rhodotorula mucilaginosa	11/4,821 (0.2%)	5/3,272 (0.2%)	6/1,549 (0.4%)
Pichia norvegensis	4/4,821 (<0.1%)	4/3,272 (0.1%)	
Pichia sp. NOS[a]	1/4,821 (<0.1%)	1/3,272 (<0.1%)	
Debaromyces fabryi	1/4,821 (<0.1%)	1/3,272 (<0.1%)	
Loderomyces elongisporus	1/4,821 (<0.1%)	1/3,272 (<0.1%)	
Saprochaete capitatum	2/4,821 (<0.1%)	1/3,272 (<0.1%)	1/1,549 (<0.1%)
Kodamaea ohmeri	1/4821 (<0.1%)		1/1,549 (<0.1%)
Aspergillus spp.	645/4,821 (13.4%)	474/3,272 (14.5%)	171/1,549 (11.0%)
Aspergillus fumigatus	416/645 (64.5%)	302/474 (63.7%)	114/171 (66.7%)
Aspergillus section *Nigri*	81/645 (12.6%)	61/474 (12.9%)	20/171 (11.7%)
Aspergillus section *Flavi*	81/645 (12.6%)	61/474 (12.9%)	20/171 (11.7%)
Aspergillus section *Terrei*	30/645 (4.7%)	19/474 (4.0%)	11/171 (6.4%)

Table 1. Continued.

Organism	Number (%) of isolates/total by category		
	All isolates	2018–2019	2020
Aspergillus section *Nidulantes*	22/645 (3.4)	17/474 (3.6%)	5/171 (2.9%)
Aspergillus section *Usti*	7/645 (1.1%)	7/474 (1.5%)	
Aspergillus lentulus	2/645 (0.3%)	2/474 (0.4%)	
Aspergillus section *Versicolores*	3/645 (0.5%)	2/474 (0.4%)	1/171 (0.6%)
Aspergillus sclerotiorum	1/645 (0.2%)	1/474 (0.2%)	
Aspergillus sydowii	1/645 (0.2%)	1/474 (0.2%)	
Aspergillus sp. NOS[a]	1/645 (0.2%)	1/474 (0.2%)	
Mucorales group	38/4,821 (0.8%)	28/3,272 (0.9%)	10/1,549 (0.6%)
Lichtheimia corymbifera	6/38 (15.8%)	5/28 (17.9%)	1/10 (10.0%)
Lichtheimia ramosa	1/38 (2.6%)	1/28 (3.6%)	
Mucor circinelloides/M. ramosissimus	4/38 (10.5%)	2/28 (7.1%)	2/10 (20.0%)
Rhizomucor pusillus	3/38 (7.8%)	3/28 (10.7%)	
Rhizopus microsporus group	10/38 (26.3%)	9/28 (32.1%)	1/10 (10.0%)
Rhizopus arrhizus (syn. *R. oryzae)*	5/38 (13.2%)	3/28 (10.7%)	2/10 18 (20.0%)
Lichtheimia sp. NOS[a]	2/38 (5.3%)	2/28 (7.1%)	1/10 (10.0%)
Mucor sp. NOS[a]	2/38 (5.3%)	2/28 (7.1%)	
M. indicus	1/38 (2.6%)		1/10 (10.0%)
Rhizopus sp. NOS[a]	1/38 (2.6%)	1/28 (3.6%)	
Syncephalastrum sp. NOS[a]	1/38 (2.6%)	1/28 (3.6%)	
Cunninghamella sp. *NOS*	2/38 (5.3%)		2/10 (20.0%)
Fusarium spp.	25/4,821 (0.5%)	15/3,272 (0.5%)	10/1,549 (0.6%)
Fusarium incarnatum-equisete SC[b]	1/25 (4.0%)		1/10 (10.0%)
Fusarium oxysporum SC[b]	4/25 (16.0%)	4/15 (26.7%)	
Fusarium solani SC[b]	12/25 (48.0%)	7/15 (46.7%)	5/10 (50.0%)
Gibberella fujikuroi SC[b]	8/25 (32.0%)	4/15 (26.7%)	4/10 (36.4%)
Scedosporium spp.	35/4,821 (0.7%)	26/3,272 (0.8%)	9/1,549 (0.6%)
Scedosporium apiospermum/boydii	28/35 (80.0%)	22/26 (84.6%)	6/9 (66.7%)
Scedosporium aurantiacum	6/35 (17.1%)	3/26 (11.5%)	3/9 (33.3%)
Scedosporium sp. NOS[a]	1/35 (2.9%)	1/26 (3.8%)	
Other molds	59/4,821 (1.2%)	39/3,272 (1.2%)	20/1,549 (1.3%)
Alternaria alternata	2/59 (3.4%)	2/39 (5.1%)	
Aureobasidium pullulans	1/59 (1.7%)	1/39 (2.6%)	
Exophiala dermatitidis	4/59 (6.8%)	3/39 (7.7%)	1/20 (5.0%)
Lomentospora prolificans	8/59 (13.6%)	5/39 (12.8%)	3/20 (15.0%)
Medicopsis romeroi	1/59 (1.7%)	1/39 (2.6%)	
Microascus cirrosus	2/59 (3.4%)	2/39 (5.1%)	
Paecilomyces variotii	7/59 (11.9%)	1/39 (2.6%)	6/20 (30.0%)
Purpureocillium lilacinum	6/59 (10.2%)	4/39 (10.3%)	2/20 (10.0%)
Pleurostoma richardsiae	1/59 (1.7%)	1/39 (2.6%)	
Rasamsonia argillacea SC[b]	9/59 (15.3%)	5/39 (12.8%)	4/20 (20.0%)
Scopulariopsis brevicaulis/S. brumptii	6/59 (10.2%)	4/39 (10.3%)	2/20 (15.4%)
Bipolaris sp. NOS[a]	1/59 (1.7%)	1/39 (2.6%)	
Curvularia sp. NOS[a]	5/59 (8.5%)	5/39 (12.8%)	
Coprinellus sp. NOS	1/59 (1.7%)		1/20 (5.0%)
Paecilomyces sp. NOS[a]	2/59 (3.4%)	1/39 (2.6%)	1/20 (5.0%)
Phialemoniopsis sp. NOS[a]	1/59 (1.7%)	1/39 (2.6%)	
Phaeoacremonium sp. NOS	1/59 (1.7%)	1/39 (2.6%)	
Verruconis gallopava	1/59 (1.7%)	1/39 (2.6%)	

[a]NOS, not otherwise speciated.
[b]SC, species complex.

The rank order of the four most common species of *Candida* (*C. albicans* > *C. glabrata* > *C. parapsilosis* > *C. tropicalis*) was the same in both sets of isolates. Among the remaining 23 species of *Candida,* 14 species were common to both groups. *C. tropicalis*, *C. dubliniensis*, and *C. krusei* were more frequently isolated from the COVID set (11.0%, 3.0%, 3.6%, COVID; 10.8%, 2.7%, 2.6%, pre-COVID, respectively) whereas *C. parapsilosis* and *C. lusitaniae* were more frequent in the pre-COVID set (14.7%, 2.6%, pre-COVID set; 12.8% [*P* value = 0.172], 1.4% [*P* value = 0.028], COVID set, respectively). The frequencies of the remaining species of *Candida* were generally less than 1.0% and were comparable across both pre-COVID and COVID sets.

Aspergillus fumigatus was the most common species of *Aspergillus* in both sets (63.7%, pre-COVID; 66.7%, COVID, respectively). Of the remaining 10 *Aspergillus* species or species complexes, *Aspergillus* section *Flavi* and section *Nigri* were more frequently recovered from patients of the pre-COVID set (12.9%, pre-COVID; 11.7%, COVID [*P* value = 0.823]) while *Aspergillus* section *Terrei* was more frequently isolated from the COVID set (6.4%, COVID; 4.0%, pre-COVID [*P* value = 0.301]). The remaining species were similar in frequency in both groups (Table 1).

Differences in susceptibilities among isolates from pre-COVID and COVID periods to licensed antifungal agents

The isolates of yeasts and molds with at least 10 isolates collected in the pre-COVID and COVID surveillance years were tested and results are presented in Table 2. MIC results for fungal species, species complexes, or groups with <10 isolates are shown in Supplementary Tables 1 to 4.

The vast majority (>80%) of all tested species of *Candida* were susceptible (S) or wild-type (WT) to all echinocandins, triazoles, and amphotericin B, regardless of pre-COVID or COVID set (Table 2 and Supplementary Tables 1 and 2). Differences in the resistance or NWT rates to fluconazole between the COVID and pre-COVID sets was observed for *C. glabrata* (2.0% resistant, COVID; 5.8% resistant, pre-COVID [*P* value = 0.04]), *C. parapsilosis* (13.9% resistant, COVID; 9.8% resistant, pre-COVID [*P* value = 0.272]), *C. tropicalis* (3.5% resistant, COVID; 0.7% resistant, pre-COVID [*P* value = 0.09]), *C. dubliniensis* (0.0% NWT, COVID; 2.9% NWT, pre-COVID), *C. lusitaniae* (5.6% NWT, COVID; 7.5% NWT, pre-COVID), and *C. guilliermondii* (8.3% NWT, COVID; 22.2% NWT, pre-COVID) (Table 2 and Supplementary Tables 1 and 2). Elevated MIC values for fluconazole were observed in isolates of *C. orthopsilosis* from both sets (12.5% NWT, COVID; 14.8% NWT, pre-COVID). Resistance or NWT MIC values for voriconazole were noted among *C. glabrata* (4.8% NWT, COVID; 9.3% NWT, pre-COVID [*P* value = 0.065]), *C. parapsilosis* (4.2% resistant, COVID; 0.8% resistant, pre-COVID [*P* value = 0.021]), *C. tropicalis* (2.1% resistant, COVID; 0.0% resistant, pre-COVID [*P* value = 0.081]), and *C. orthopsilosis* (12.5% NWT, COVID; 3.7% NWT, pre-COVID; Table 2).

Among fluconazole-resistant isolates of *C. glabrata*, the decreased resistance to fluconazole and the other azoles during COVID was apparent in North America (7.3% resistant, pre-COVID; 1.4% resistant, COVID), Europe (6.3% resistant, pre-COVID; 3.7% resistant, COVID), Asia-Pacific (3.0% resistant, pre-COVID; 0.0% resistant, COVID), but not in Latin America (0.0% resistant, pre-COVID; 0.0% resistant, COVID). In contrast, the frequency of fluconazole resistance was greater during COVID for both *C. parapsilosis* and *C. tropicalis* in North America (4.5% resistant, pre-COVID; 10.0% resistant, COVID), Europe (16.1% resistant, pre-COVID; 19.8% resistant, COVID) and Asia-Pacific (2.3% resistant, pre-COVID; 8.0% resistant, COVID). Similar results were observed for fluconazole resistance in *C. tropicalis*, as there was an increase in resistance in most regions during COVID: North America (0.0% resistant, pre-COVID; 6.7% resistant, COVID), Europe (0.0% resistant, pre-COVID; 2.3% resistant, COVID), Asia-Pacific (1.4% resistant, pre-COVID; 4.7% resistant, COVID) and Latin America (1.8% resistant, pre-COVID; 0.0% resistant, COVID).

All *C. krusei* isolates were susceptible to voriconazole (Table 2). MIC_{90} values for isavuconazole were ≤1 mg/l for all species in both groups, except for *C. guilliermondii* isolates from the pre-COVID set (MIC_{90}, 4 mg/l). All isolates from the pre-COVID and COVID sets, save one isolate of *C. dubliniensis* (MIC, 1 mg/l; NWT), exhibited a WT phenotype to amphotericin B. The less common species of *Candida* identified in both groups included *C. fabianii*, *C. fermentati*, *C. inconspicua*, *C. metapsilosis*, *C. pelliculosa*, and *C. utilis*. Comparable activity was observed for both the echinocandins and triazoles in the COVID and pre-COVID sets (Supplementary Table 1).

Among the isolates of *Cryptococcus neoformans* var. *grubii*, the MIC_{90} values for each of the nine antifungal agents tested were identical or within a single dilution step for the COVID and pre-COVID sets (Table 2). The ECV for amphotericin B and *Cryptococcus neoformans* var. *grubii* was 0.5 mg/l and bisected the MIC distribution in both COVID and pre-COVID sets, resulting in 66.7% NWT for the COVID set and 47.1% NWT for the pre-COVID set (Table 2).

The non-*Candida* yeasts included five isolates of *Cryptococcus gattii* SC, four of which were from patients in the pre-COVID period and were WT to fluconazole and voriconazole (Supplementary Tables 3 and 4). Isolates of *Cryptococcus neoformans* var. *neoformans* were detected in both groups and were all WT to fluconazole, posaconazole, and voriconazole (Supplementary Tables 3 and 4). *Saprochaetae clavata* isolates were found in both groups and exhibited similar MIC values for fluconazole (MIC range, 4–16 mg/l), isavuconazole (MIC range, 0.12–1 mg/l), itraconazole (MIC range, 0.12–0.5 mg/l), posaconazole (MIC range, 0.25–0.5 mg/l), and voriconazole (MIC range, 0.06–0.5 mg/l) (Supplementary Tables 3 and 4).

Table 2. Activity of nine systemically active antifungal agents against fungal isolates from pre-COVID (2018–2019) and COVID (2020).

Organism/ antifungal agent	2018–2019						2020					
	MIC (mg/l; number of isolates)		CLSI[a,b]		ECV[a,b,c]		MIC (mg/l; number of isolates)		CLSI[a,b]		ECV[a,b,c]	
	50%	90%	% S	% R	% WT	% NWT	50%	90%	% S	% R	% WT	% NWT
C. albicans	(1,145)						(579)					
Anidulafungin	0.008	0.03	99.9	0.1	99.9	0.1	0.03	0.06	100.0	0.0	99.8	0.2
Caspofungin	0.015	0.03	99.9	0.1			0.015	0.03	100.0	0.0		
Micafungin	0.015	0.03	99.9	0.1	99.8	0.2	0.015	0.015	100.0	0.0	99.5	0.5
Fluconazole	0.12	0.25	99.7	0.1	97.8	2.2	0.12	0.25	99.5	0.3	99.0	1.0
Itraconazole	0.06	0.12					0.03	0.06				
Isavuconazole	0.004	0.008					0.004	0.008				
Posaconazole	0.03	0.06			98.1	1.9	0.03	0.06			98.8	1.2
Voriconazole	0.004	0.015	100.0	0.0	99.1	0.9	0.004	0.008	99.8	0.2	98.8	1.2
Amphotericin B	0.5	1			100.0	0.0	0.5	0.5			100.0	0.0
C. glabrata	(462)						(248)					
Anidulafungin	0.06	0.12	96.8	1.7	98.3	1.7	0.12	0.12	97.2	1.6	98.4	1.6
Caspofungin	0.015	0.03	97.4	1.7			0.03	0.06	98.4	0.8		
Micafungin	0.015	0.03	98.3	1.7	96.5	3.5	0.015	0.03	98.0	2.0	96.4	3.6
Fluconazole	4	16	94.2	5.8	89.6	10.4	4	8	98.0	2.0	94.8	5.2
Itraconazole	0.5	1			99.8	0.2	0.5	1			99.6	0.4
Isavuconazole	0.06	0.25					0.06	0.25				
Posaconazole	0.25	1			95.9	4.1	0.5	0.5			98.4	1.6
Voriconazole	0.06	0.25			90.7	9.3	0.12	0.25			95.2	4.8
Amphotericin B	1	1			100.0	0.0	1	1			100.0	0.0
C. parapsilosis	(377)						(165)					
Anidulafungin	2	2	94.4	0.0	100.0	0.0	2	2	93.9	0.0	100.0	0.0
Caspofungin	0.25	0.5	100.0	0.0	100.0	0.0	0.25	0.5	100.0	0.0	100.0	0.0
Micafungin	1	1	100.0	0.0	100.0	0.0	1	1	100.0	0.0	100.0	0.0
Fluconazole	0.5	4	88.3	9.8	88.3	11.7	0.5	16	86.1	13.9	86.1	13.9
Itraconazole	0.06	0.25			100.0	0.0	0.12	0.25			98.2	1.8
Isavuconazole	0.008	0.03					0.004	0.03				
Posaconazole	0.06	0.12			100.0	0.0	0.06	0.12			100.0	0.0
Voriconazole	0.008	0.06	90.7	0.8	87.0	13.0	0.008	0.25	89.7	4.2	86.1	13.9
Amphotericin B	0.5	1			100.0	0.0	0.5	1			100.0	0.0
C. tropicalis	(277)						(142)					
Anidulafungin	0.015	0.06	100.0	0.0	99.6	0.4	0.03	0.06	100.0	0.0	100.0	0.0
Caspofungin	0.03	0.06	100.0	0.0			0.015	0.03	100.0	0.0		
Micafungin	0.03	0.06	100.0	0.0	98.2	1.8	0.03	0.03	100.0	0.0	100.0	0.0
Fluconazole	0.25	0.5	98.9	0.7	96.8	3.2	0.5	1	94.4	3.5	93.0	7.0
Itraconazole	0.12	0.25			100.0	0.0	0.06	0.25			97.2	2.8
Isavuconazole	0.015	0.06					0.015	0.06				
Posaconazole	0.06	0.12			96.0	4.0	0.06	0.12			94.4	5.6
Voriconazole	0.015	0.03	99.3	0.0	99.3	0.7	0.03	0.06	94.4	2.1	94.4	5.6
Amphotericin B	0.5	1			100.0	0.0	0.5	1			100.0	0.0
C. krusei	(66)						(46)					
Anidulafungin	0.03	0.06	100.0	0.0	100.0	0.0	0.06	0.06	100.0	0.0	100.0	0.0
Caspofungin	0.06	0.12	100.0	0.0			0.06	0.25	100.0	0.0		
Micafungin	0.06	0.12	100.0	0.0	100.0	0.0	0.12	0.12	100.0	0.0	100.0	0.0
Fluconazole	32	32					32	64				
Itraconazole	0.25	0.5			100.0	0.0	0.5	0.5			100.0	0.0
Isavuconazole	0.25	0.5					0.12	0.25				
Posaconazole	0.25	0.5			100.0	0.0	0.25	0.5			100.0	0.0
Voriconazole	0.25	0.25	100.0	0.0	100.0	0.0	0.25	0.25	97.8	0.0	97.8	2.2
Amphotericin B	1	2			100.0	0.0	1	1			100.0	0.0

Table 2. Continued.

Organism/ antifungal agent	2018–2019 MIC (mg/l; number of isolates)		CLSI[a,b]		ECV[a,b,c]		2020 MIC (mg/l; number of isolates)		CLSI[a,b]		ECV[a,b,c]	
C. lusitaniae	(66)						(18)					
Anidulafungin	0.25	0.5			100.0	0.0	0.5	1			100.0	0.0
Caspofungin	0.25	0.25			100.0	0.0	0.12	0.25			100.0	0.0
Micafungin	0.12	0.25			100.0	0.0	0.12	0.25			100.0	0.0
Fluconazole	0.25	1			92.4	7.5	0.5	1			94.4	5.6
Itraconazole	0.12	0.25			100.0	0.0	0.25	0.25			100.0	0.0
Isavuconazole	0.008	0.015					0.008	0.03				
Posaconazole	0.06	0.06			90.9	9.1	0.06	0.12			88.9	11.1
Voriconazole	0.008	0.015					0.008	0.015				
Amphotericin B	0.5	1			100.0	0.0	0.5	0.5			100.0	0.0
C. dubliniensis	(68)						(39)					
Anidulafungin	0.03	0.12			100.0	0.0	0.06	0.12			97.4	2.6
Caspofungin	0.03	0.06					0.015	0.06				
Micafungin	0.015	0.03			100.0	0.0	0.015	0.03			97.4	2.6
Fluconazole	0.12	0.25			97.1	2.9	0.12	0.25			100.0	0.0
Itraconazole	0.06	0.12			97.1	2.9	0.06	0.06			100.0	0.0
Isavuconazole	≤0.002	0.008					≤0.002	0.004				
Posaconazole	0.03	0.06			97.1	2.9	0.03	0.06			100.0	0.0
Voriconazole	0.008	0.015					0.008	0.008				
Amphotericin B	0.25	0.5			98.5	1.5	0.25	0.5			100.0	0.0
C. orthopsilosis	(27)						(16)					
Anidulafungin	0.5	1			100.0	0.0	0.5	1			100.0	0.0
Caspofungin	0.12	0.25			100.0	0.0	0.12	0.25			100.0	0.0
Micafungin	0.5	1			100.0	0.0	0.25	0.5			100.0	0.0
Fluconazole	0.5	4			85.2	14.8	0.5	32			87.5	12.5
Itraconazole	0.12	0.25			100.0	0.0	0.12	0.5			93.8	6.2
Isavuconazole	0.015	0.06					0.015	0.12				
Posaconazole	0.06	0.12			100.0	0.0	0.12	0.25			93.8	6.2
Voriconazole	0.015	0.12			96.3	3.7	0.015	1			87.5	12.5
Amphotericin B	0.5	0.5			100.0	0.0	0.5	1			100.0	0.0
C. guilliermondii	(18)						(12)					
Anidulafungin	2	2			94.4	0.0	2	4	83.3	0.0	100.0	0.0
Caspofungin	0.5	1			100.0	0.0	0.25	0.5	100.0	0.0	100.0	0.0
Micafungin	0.5	1			100.0	0.0	0.5	1	100.0	0.0	100.0	0.0
Fluconazole	4	>128			77.8	22.2	2	8			91.7	8.3
Itraconazole	0.5	2			94.4	5.6	0.5	1			100.0	0.0
Isavuconazole	0.25	4					0.12	0.25				
Posaconazole	0.25	1			77.8	22.2	0.25	0.5			91.7	8.3
Voriconazole	0.06	>4					0.06	0.12				
Amphotericin B	0.5	0.5			100.0	0.0	0.5	1			100.0	0.0
C. kefyr	(18)						(10)					
Anidulafungin	0.03	0.06			100.0	0.0	0.12	0.25			100.0	0.0
Caspofungin	0.008	0.015					0.015	0.015				
Micafungin	0.03	0.06			100.0	0.0	0.06	0.12			100.0	0.0
Itraconazole	0.12	0.25			100.0	0.0	0.12	0.25			100.0	0.0
Isavuconazole	≤0.002	0.015					≤0.002	0.008				
Posaconazole	0.06	0.25			100.0	0.0	0.12	0.12			100.0	0.0
Voriconazole	0.008	0.015					0.008	0.015				
Amphotericin B	1	2			100.0	0.0	1	1			100.0	0.0

Table 2. Continued.

Organism/ antifungal agent	2018–2019 MIC (mg/l; number of isolates)		CLSI[a,b]		ECV[a,b,c]		2020 MIC (mg/l; number of isolates)		CLSI[a,b]		ECV[a,b,c]	
C. metapsilosis	(13)						(1)					
Anidulafungin	0.12	0.5			100.0	0.0	0.25				100.0	0.0
Caspofungin	0.06	0.25			100.0	0.0	0.06				100.0	0.0
Micafungin	0.25	0.5			100.0	0.0	0.25				100.0	0.0
Fluconazole	1	4			100.0	0.0	1				100.0	0.0
Itraconazole	0.12	0.25			100.0	0.0	0.12				100.0	0.0
Isavuconazole	0.015	0.03					0.015					
Posaconazole	0.06	0.12			100.0	0.0	0.12				100.0	0.0
Voriconazole	0.015	0.06			100.0	0.0	0.015				100.0	0.0
Amphotericin B	0.5	1			100.0	0.0	0.5				100.0	0.0
Cryptococcus neoformans var. *grubii*	(51)						(12)					
Anidulafungin	>4	>4					>4	>4				
Caspofungin	>4	>4					>4	>4				
Micafungin	>4	>4					>4	>4				
Fluconazole	4	4			100.0	0.0	4	8			100.0	0.0
Itraconazole	0.12	0.25			94.1	5.9	0.12	0.25			100.0	0.0
Isavuconazole	0.03	0.12					0.03	0.03				
Posaconazole	0.12	0.25			96.1	3.9	0.12	0.25			100.0	0.0
Voriconazole	0.03	0.12			100.0	0.0	0.06	0.06			100.0	0.0
Amphotericin B	0.5	1			52.9	47.1	1	1			33.3	66.7
Aspergillus fumigatus	(302)						(114)					
Anidulafungin	0.008	0.015					0.015	0.06				
Caspofungin	0.03	0.03			100.0	0.0	0.015	0.03			100.0	0.0
Micafungin	0.008	0.015					0.008	0.015				
Itraconazole	0.5	1			93.0	7.0	1	1			91.2	8.8
Isavuconazole	0.5	1			92.7	7.3	0.5	1			96.0	4.0
Posaconazole	0.25	0.5					0.25	0.5				
Voriconazole	0.5	0.5	91.7	3.6	96.4	3.6	0.5	0.5	93.0	2.6	97.4	2.6
Amphotericin B	1	2			99.7	0.3	1	2			99.1	0.9
Aspergillus section Nigri	(61)						(20)					
Anidulafungin	0.004	0.008					0.008	0.008				
Caspofungin	0.015	0.03			100.0	0.0	0.015	0.03			100.0	0.0
Micafungin	0.015	0.015					0.008	0.015				
Itraconazole	2	4			95.0	5.0	2	4			95.0	5.0
Isavuconazole	2	4			98.4	1.6	1	2			100.0	0.0
Posaconazole	0.5	1			100.0	0.0	0.5	1			100.0	0.0
Voriconazole	1	2			98.4	1.6	0.5	2			100.0	0.0
Amphotericin B	0.5	1			100.0	0.0	0.5	1			100.0	0.0
Aspergillus section Flavi	(61)						(20)					
Anidulafungin	0.004	0.008					0.008	0.015				
Caspofungin	0.015	0.03			100.0	0.0	0.015	0.015			100.0	0.0
Micafungin	0.015	0.015					0.008	0.015				
Itraconazole	0.5	1			100.0	0.0	1	1			100.0	0.0
Isavuconazole	0.5	1			100.0	0.0	0.5	1			100.0	0.0
Posaconazole	0.5	0.5			98.4	1.6	0.5	0.5			100.0	0.0
Voriconazole	0.5	1			100.0	0.0	0.5	1			100.0	0.0
Amphotericin B	2	2			100.0	0.0	2	2			95.0	5.0

Table 2. Continued.

Organism/ antifungal agent	2018–2019 MIC (mg/l; number of isolates)		2018–2019 CLSI[a,b]		2018–2019 ECV[a,b,c]		2020 MIC (mg/l; number of isolates)		2020 CLSI[a,b]		2020 ECV[a,b,c]	
Aspergillus section *Terrei*	(19)						(16)					
Anidulafungin	0.008	0.015					0.015	0.03				
Caspofungin	0.015	0.03			100.0	0.0	0.015	0.03			100.0	0.0
Micafungin	0.008	0.015					0.004	0.015				
Itraconazole	0.5	0.5			100.0	0.0	0.5	1			100.0	0.0
Isavuconazole	0.25	0.5			100.0	0.0	0.5	2			87.5	12.5
Posaconazole	0.25	0.25			100.0	0.0	0.25	0.5			100.0	0.0
Voriconazole	0.25	0.5			100.0	0.0	0.5	0.5			100.0	0.0
Amphotericin B	2	4			100.0	0.0	2	2			100.0	0.0
Aspergillus section *Nidulantes*	(17)						(5)					
Anidulafungin	0.008	0.015					0.015					
Caspofungin	0.03	2					0.015					
Micafungin	0.008	0.03					0.004					
Itraconazole	0.5	1					0.5					
Isavuconazole	0.12	0.25					0.12					
Posaconazole	0.25	0.5					0.25					
Voriconazole	0.12	0.25					0.12					
Amphotericin B	2	4					2					
Mucorales group	(28)						(10)					
Anidulafungin	>4	>4					>4	>4				
Caspofungin	>4	>4					>4	>4				
Micafungin	>4	>4					>4	>4				
Itraconazole	1	8					2	8				
Isavuconazole	2	8					4	>8				
Posaconazole	0.5	8					1	>8				
Voriconazole	>8	>8					>8	>8				
Amphotericin B	0.5	1					0.5	2				
Fusarium spp.	(15)						(10)					
Anidulafungin	>4	>4					>4	>4				
Caspofungin	>4	>4					>4	>4				
Micafungin	>4	>4					4	>4				
Itraconazole	>8	>8					>8	>8				
Isavuconazole	>8	>8					>8	>8				
Posaconazole	>8	>8					>8	>8				
Voriconazole	8	>8					8	>8				
Amphotericin B	2	2					2	2				
Scedosporium spp.	(26)						(9)					
Anidulafungin	4	>4					4					
Caspofungin	1	>4					>4					
Micafungin	0.5	>4					4					
Itraconazole	8	>8					>8					
Isavuconazole	8	8					>8					
Posaconazole	2	>8					>8					
Voriconazole	0.5	2					1					
Amphotericin B	>4	>4					>4					

[a] Abbreviations: CLSI, Clinical and Laboratory Standards Institute; ECV, epidemiological cut-off values; %S, percent susceptible; %R, percent resistant; %WT, percent wild-type; %NWT, percent non-wild-type.

[b] Criteria published by CLSI M60 (2020) and M61 (2020). ECV criteria published in CLSI M59 (2020).

[c] Isavuconazole ECVs were published by Espinell-Ingrof.[68]

Aspergillus fumigatus was the most common species of filamentous fungi isolated from both groups. The vast majority of *A. fumigatus* (>91.0%) were WT to the echinocandins, triazoles, and amphotericin B (Table 2). There were 31 isolates that were NWT to one or more of the triazoles, 10 in the COVID set and 21 in the pre-COVID set (*P* value = 0.708; Table 2). Most of the isolates were from Europe (17/31; 54.8%), followed by North America (9/31; 29.0%) and the Asia-Pacific region (5/31; 16.1%). Most of the NWT isolates of *A. fumigatus* in the pre-COVID set were from Europe (13/21; 61.9%) compared to only 40% (4/10) in the COVID set. Most of the NWT isolates (20/31; 64.5%) were NWT to more than one triazole, including 5/10 (50.0%) in the COVID set and 15/21 (71.4%) in the pre-COVID set.

Aspergillus section *Nigri* and section *Flavi* were tied for second in rank order among the *Aspergillus* species in both the COVID and pre-COVID sets (12.6%, overall; 12.9%, pre-COVID; 11.7%, COVID). All isolates (100.0%) of *Aspergillus* section *Nigri* from both groups were WT to caspofungin, posaconazole, and amphotericin B (Table 2). One isolate from Australia in the COVID set (5.0%) was NWT to itraconazole. Aside from itraconazole, isolates of *Aspergillus* section *Nigri* from the COVID set were 100.0% WT to the other antifungal agents tested (Table 2). There were three isolates in the pre-COVID set that were NWT to itraconazole (5.0%), one each from Europe, North America, and the Asia-Pacific region. The isolate from Europe was also NWT to voriconazole.

The majority (87.5–100.0%) of *Aspergillus* section *Flavii* and *Aspergillus* section *Terrei* isolates from both COVID and pre-COVID sets exhibited a WT phenotype for the echinocandins, triazoles, and amphotericin B (Table 2 and Supplementary Tables 5 and 6). Among the section *Flavi*, single isolates were NWT to posaconazole in the pre-COVID set and to amphotericin B in the COVID set. *Aspergillus* section *Terrei* isolates were all WT to the agents tested, except for the isavuconazole NWT isolates (MIC, 2 mg/L) from the COVID year. *Aspergillus* section *Nidulantes* isolates were found in both groups and exhibited similarly low MIC values for the triazoles and echinocandins (Table 2 and Supplementary Tables 5 and 6). Among the less common species of *Aspergillus*, isolates of *Aspergillus lentulus* and *Aspergillus* section *Usti* were only detected in the pre-COVID set and showed comparably elevated MIC values for all the triazoles (Supplementary Table 6).

Members of the Mucorales group were found in both COVID and pre-COVID sets, although only 5 of 10 species (Table 1) represented were common to both groups (Table 2 and Supplementary Tables 7 and 8). All the Mucorales group isolates were resistant to the echinocandins ($MIC_{50/90}$, >4/>4 mg/l) and voriconazole ($MIC_{50/90}$, >8/>8 mg/l) (Table 2 and Supplementary Tables 7 and 8). Based on MIC_{50} values, posaconazole was the most active agent (MIC_{50}, 0.5 mg/l, pre-COVID; 1 mg/l, COVID), followed by itraconazole (MIC_{50}, 1 mg/l, pre-COVID; 2 mg/l, COVID) and isavuconazole (MIC_{50}, 2 mg/l for pre-COVID; 4 mg/l, COVID). Among the species common to both groups, MIC values ranged from 0.5 to >8 mg/l for posaconazole, 0.5 to >8 mg/l for itraconazole, and 1 to 4 mg/l for isavuconazole (Supplementary Tables 7 and 8). Elevated MIC values (MICs, ≥8 mg/l) for posaconazole were observed for two isolates of *M. circinelloides/ramosissimus* (one each pre-COVID and COVID), two isolates of *Rhizopus* (pre-COVID), and one of *Mucor* sp. (pre-COVID). All of these isolates, save one isolate of *M. circinelloides/ramosissimus,* also exhibited elevated MIC values (MIC, >8 mg/l) to both isavuconazole and itraconazole.

None of the tested agents, except amphotericin B, showed useful *in vitro* activity against species of *Fusarium* (Table 2). The *Scedosporium* species in both groups showed decreased susceptibility to all agents tested except micafungin (MEC_{50}, 0.5 mg/l, pre-COVID), posaconazole (MIC_{50}, 2 mg/l, pre-COVID), and voriconazole (MIC_{50}, 0.5 mg/l, pre-COVID; 1 mg/l, COVID; Table 2).

The other molds in this survey represented a wide range of species, most of which were isolated only from the pre-COVID set (Table 2 and Supplementary Tables 7 and 8).

Resistance mechanisms

A total of 48 isolates of *Candida* spp. were NWT to one or more echinocandin (13, COVID; 35, pre-COVID). The COVID set NWT isolates consisted of 9 isolates of *C. glabrata*, 3 *C. albicans*, and 1 *C. dubliniensis*. Among these isolates, seven (1 *C. albicans,* 1 *C. dubliniensis*, and 5 *C. glabrata*) were found to harbor a mutation in *FKS1* or *FKS2* (Table 3). The NWT isolates in the pre-COVID set consisted of 16 isolates of *C. glabrata*, 5 *C. tropicalis*, 2 *C. albicans*, and 12 *C. parapsilosis*. Mutations in *FKS* were detected in 12 of 16 isolates of *C. glabrata* and 1 of 2 isolates of *C. albicans* (Table 3). There were no mutations detected in the NWT isolates of *C. tropicalis* and *C. parapsilosis*.

Among echinocandin-NWT *C. glabrata*, the most frequent mutation in the pre-COVID group of isolates was S663P in *FKS2* HS1 (6 isolates), followed by a deletion or alterations of F659 in *FKS2* HS1 (6 isolates). Notably, these mutations were not observed in the COVID group. Most of those isolates exhibited diverse mutations in FKS1 HS1, while only one isolate from the pre-COVID group had mutations in this region. All *C. albicans* and *C. dubliniensis* isolates displayed a S645P mutation in *FKS1* HS1 regardless of the year (Table 3). Among the *FKS* mutants, the majority (55.0%) were from North America (53.8%, pre-COVID; 57.1%, COVID).

There were a total of 31 isolates of *Aspergillus fumigatus* that were NWT to one or more of the triazoles (10, COVID; 21, pre-COVID). All but two (29/31, 93.5%) *A. fumigatus* isolates were NWT to itraconazole, 17/31 (54.8%) were NWT to isavuconazole, 13/31 (41.9%) were NWT to voriconazole, and 8/31 (25.8%) were NWT to posaconazole. Among the isolates from the COVID set, 3 of 10 (30%) isolates were found to have

Table 3. Summary of FKS alterations detected among non-wild-type *Candida spp.* from pre-COVID (2018–2019) and COVID (2020).[a]

Year	State and/or country	Organism	MIC (mg/l)[b,c]			1,3-β-D-glucan synthase mutations			
			Anidulafungin	Caspofungin	Micafungin	*FKS1* HS1	*FKS1* HS2	*FKS2* HS1	*FKS2* HS2
2018–2019	Hungary	*C. glabrata*	1 (R)	1 (R)	0.5 (R)	WT	WT	F659 deletion	WT
	Slovenia	*C. glabrata*	1 (R)	0.25 (I)	0.06 (S)	WT	WT	F659Y	WT
	NY, USA	*C. glabrata*	0.06 (S)	0.06 (S)	0.06 (S)	WT	WT	F659 deletion	WT
	NY, USA	*C. glabrata*	2 (R)	1 (R)	1 (R)	WT	WT	S663P	WT
	VA, USA	*C. glabrata*	4 (R)	4 (R)	4 (R)	Frame shift[d]	Frame shift[d]	S663P	WT
	Italy	*C. albicans*	1 (R)	1 (R)	1 (R)	S645P	WT	NT	NT
	Spain	*C. glabrata*	2 (R)	>4 (R)	1 (R)	WT	WT	S663P	WT
	CA, USA	*C. glabrata*	1 (R)	1 (R)	0.5 (R)	WT	WT	S663P	WT
	CO, USA	*C. glabrata*	0.25 (I)	0.25 (I)	0.25 (R)	WT	WT	F659 deletion	WT
	CO, USA	*C. glabrata*	0.25 (I)	0.12 (S)	0.06 (S)	WT	WT	F659 deletion	WT
	CO, USA	*C. glabrata*	1 (R)	1 (R)	0.25 (R)	WT	WT	S663P	WT
	Australia	*C. glabrata*	2 (R)	0.5 (R)	0.5 (R)	WT	WT	S663P	WT
	Australia	*C. glabrata*	0.25 (I)	0.25 (I)	0.06 (S)	WT	WT	F659S	WT
2020	CO, USA	*C. glabrata*	0.5 (R)	0.25 (I)	0.25 (R)	WT	WT	R665G	WT
	NY, USA	*C. glabrata*	2 (R)	4 (R)	1 (R)	S629P	WT	WT	WT
	Slovenia	*C. dubliniensis*	0.5 (NWT)	2	1 (NWT)	S645P	WT	NT	NT
	Slovenia	*C. albicans*	0.25 (S)	0.25 (S)	0.25 (S)	S645P	WT	NT	NT
	WA, USA	*C. glabrata*	0.25 (I)	0.12 (S)	0.06 (S)	L630Q	WT	WT	WT
	WA, USA	*C. glabrata*	4 (R)	>4 (R)	4 (R)	S629P	WT	WT	WT
	Chile	*C. glabrata*	1 (R)	0.25 (I)	0.25 (R)	WT	WT	Y657 deletion, F658Y	WT

[a]Abbreviations: WT, wild-type; NT, not tested.
[b]Categorical interpretations of susceptible (S), intermediate (I), and resistant (R) follow CLSI breakpoints.[44]
[c]NWT, non-wild-type based on ECV criteria (CLSI M59).
[d]The *FKS1* had 8 nucleotides insertion leading to frameshift beyond amino acid A597.

alterations in *CYP51A* (Table 4). Each of the 3 *A. fumigatus* isolates possessed a different set of alterations and a different geographical origin. The isolate from the US harbored multiple alterations in *CYP51A*, F46Y, M172V, N248T, D255E, and E427K. The isolate from France carried a Q42L alteration in *CYP51B*. The isolate from New Zealand carried a single alteration G138C in *CYP51A*.

The NWT isolates from the pre-COVID set included 21 isolates of *A. fumigatus*, 17 of which (81.0%) carried alterations in *CYP51* (Table 4). In contrast to the isolates from the COVID set, the most frequent alteration was *CYP51A* TR34/L98H in the pre-COVID set. This alteration was carried by nine isolates, seven from Italy, one from Belgium, and one from Slovenia. Three isolates from North America, two from the USA and one from Canada, carried the alteration I242V in *CYP51A*. One North American (USA) isolate carried the *CYP51A* alteration G448S (NWT to isavuconazole,[47] voriconazole, and itraconazole). A single isolate from Australia carried a *CYP51B* Q42L alteration and was only NWT to voriconazole. Multiple alterations in *CYP51A* were detected in isolates from the USA, Czech Republic, and Belgium.

Aside from these *A. fumigatus* isolates, there was 1 isolate of *Aspergillus* section *Nigri* from Europe that was NWT to itraconazole and voriconazole. This isolate was subjected to whole genome sequencing and was found to harbor a K77Q alteration in *CYP51A*. An isolate of *Aspergillus* section *Flavi* from Thailand was found to be NWT to voriconazole and posaconazole and carried alterations in *CYP51A* (R5H) and *CYP51B* (K165E).

Conclusion

COVID-19 presents with a spectrum of disease manifestations ranging from asymptomatic or non-specific flu-like symptoms to pneumonia, sepsis, and life-threatening complications such as ARDS and multiple organ failure. In addition, secondary bacterial and fungal infections have proven to be major risk factors for adverse COVID-19 outcomes.[10,11,14] Concern for these secondary infectious complications of COVID-19 have resulted in the increased use of empiric antimicrobial therapy with concomitant fears of increased AMR due to drug pressure.[13,48,49] Whereas resistance to antibacterial agents has garnered most of the attention in the literature,[5,10,11,15,16] emergence of antifungal-resistant fungi has largely been confined to reports of single cases of azole-resistant *A. fumigatus*[32] or azole- and echinocandin-resistant species of *Candida* following extended exposure to antifungal therapy.[25,36,50] More recently,

Table 4. Summary of CYP alterations detected among non-wild-type *Aspergillus* spp. isolates from pre-COVID (2018–2019) and COVID (2020).

Year	State and/or country	Organism	MIC (mg/l)[a]				CYP alterations	
			Isavuconazole[b]	Voriconazole	Itraconazole	Posaconazole[c]	CYP51A	CYP51B
2018–2019	Germany	*A. niger*	4 (WT)	4 (NWT)	8 (NWT)	1 (WT)	K77Q	WT
	IN, USA	*A. fumigatus*	1 (WT)	1 (WT)	2 (NWT)	1 (NWT)	I242V	WT
	Canada	*A. fumigatus*	1 (WT)	0.5 (WT)	2 (NWT)	0.5 (WT)	I242V	WT
	Australia	*A. fumigatus*	1 (WT)	2 (NWT)	1 (WT)	0.25 (WT)	WT	Q42L
	VT, USA	*A. fumigatus*	2 (NWT)	1 (WT)	2 (NWT)	0.5 (WT)	F46Y, M172V, E427K	WT
	Czech Republic	*A. fumigatus*	2 (NWT)	1 (WT)	2 (NWT)	0.5 (WT)	F46Y, M172V, N248T, D255E, E427K	WT
	Belgium	*A. fumigatus*	4 (NWT)	2 (NWT)	4 (NWT)	1 (NWT)	TR34/L98H	WT
	Italy	*A. fumigatus*	8 (NWT)	2 (NWT)	8 (NWT)	1 (NWT)	TR34/L98H	WT
	Italy	*A. fumigatus*	>8 (NWT)	>8 (NWT)	>8 (NWT)	4 (NWT)	TR34/L98H	WT
	Italy	*A. fumigatus*	4 (NWT)	2 (NWT)	4 (NWT)	1 (NWT)	TR34/L98H	WT
	Italy	*A. fumigatus*	4 (NWT)	1 (NWT)	4 (NWT)	0.5 (NWT)	TR34/L98H	WT
	Italy	*A. fumigatus*	4 (NWT)	2 (NWT)	4 (NWT)	1 (NWT)	TR34/L98H	WT
	Thailand	*A. flavus*	0.5 (WT)	2 (NWT)	1 (WT)	1 (NWT)	R5H	K165E
	VA, USA	*A. fumigatus*	1 (WT)	0.5 (WT)	2 (NWT)	0.5 (WT)	I242V	WT
	Slovenia	*A. fumigatus*	4 (NWT)	2 (NWT)	>8 (NWT)	0.5 (WT)	TR34/L98H	WT
	Italy	*A. fumigatus*	4 (NWT)	2 (NWT)	2 (NWT)	0.5 (WT)	TR34/L98H	WT
	Italy	*A. fumigatus*	2 (NWT)	2 (NWT)	2 (NWT)	0.5 (WT)	TR34/L98H	WT
	VA, USA	*A. fumigatus*	>8 (NWT)	4 (NWT)	>8 (NWT)	0.5 (WT)	G448S	WT
	Belgium	*A. fumigatus*	>8 (NWT)	>8 (NWT)	8 (NWT)	0.5 (WT)	Y121F, M172I, T289A, G448S, TR46	WT
2020	VT, USA	*A. fumigatus*	1 (WT)	0.5 (WT)	2 (NWT)	0.5 (WT)	F46Y, M172V, N248T, D255E, E427K	WT
	France	*A. fumigatus*	4 (NWT)	1 (WT)	4 (NWT)	1 (NWT)	WT	Q42L
	New Zealand	*A. fumigatus*	>8 (NWT)	8 (NWT)	>8 (NWT)	8 (NWT)	G138C	WT

[a]Categorical interpretations of non-wild-type (NWT) and wild-type (WT) are according to CLSI ECVs from document M59.[43]
[b]Isavuconazole ECVs were published by Espinell-Ingrof.[68]
[c]The ECV for posaconazole was 0.5 mg/l.[47]

invasive infections due to the Mucorales have been documented as COVID-19 complications in select areas of the world.[26,33,34]

Several reports raised concern regarding invasive candidiasis as a complication of severe COVID-19 infection.[18,22,24,25,29,35,36,50] These reports describe either clusters of infection (e.g., *Candida auris*) or variations in species distribution with the emergence of less common species.[18,22,24,25,29,35,36,50] When reported, resistance to antifungal agents during the COVID period is generally described as dependent upon species identification rather than assessment of *in vitro* resistance.[36] Investigations are required to determine if there are broad shifts in the pathogen distribution and emergence of both acquired and intrinsic antifungal resistance associated with the COVID pandemic.

Although the rank order of the most prominent species of *Candida* (*C. albicans* > *C. glabrata* > *C. parapsilosis* > *C. tropicalis*) is the same for both pre-COVID and COVID time periods, some differences should be noted. The occurrence of

C. glabrata, C. tropicalis, C. dubliniensis, and *C. krusei* increased during COVID compared to the pre-COVID years. Only *C. parapsilosis* and *C. lusitaniae* were less common during COVID than before COVID.

Changes to the *in vitro* susceptibility of these species are perhaps more interesting than subtle shifts in species. *C. glabrata* is possibly the one species that appeared to increase in both frequency and antifungal resistance in the past two decades.[51,52] This species is well-known for resistance to fluconazole and the other azoles. Most recently, *C. glabrata* has emerged as resistant to both the azoles and the echinocandin class of agents.[50,53] As such, it is notable that although the frequency of *C. glabrata* as a cause of infection slightly increased during COVID (19.2% of *Candida*, COVID; 18.0%, pre-COVID), resistance to fluconazole decreased from 5.8 to 2.0% over this time (Table 2). We previously noticed this trend[52] and found that it may be influenced by patient age, as resistance to the azoles decreased with patient age. However, there was no relationship between azole resistance and age in the present survey (data not shown).

C. parapsilosis is the one species of *Candida* that is most closely associated with central venous catheter bloodstream infection and breaks in infection prevention protocols.[54] As such, *C. parapsilosis* might be a prominent pathogen in the chaos of a COVID ICU. Indeed, fluconazole-resistant isolates of *C. parapsilosis* originated from the ICU more in the COVID set (52.2%) than pre-COVID (37.8%) set. This species was less frequent in the COVID set than in the pre-COVID set; however, increased resistance to fluconazole was prominent in the COVID set isolates (9.8%, pre-COVID; 13.9%, COVID). Notably, this level of resistance to fluconazole surpasses *C. glabrata*, a species notorious for decreased susceptibility to the azoles in general.[52] Whereas previously clinicians were fearful of using fluconazole empirically due to the threat of fluconazole-resistant *C. glabrata*,[52,55–57] now it is apparent that other species, namely *C. parapsilosis* and *C. tropicalis*, may be becoming more resistant to the azole class of agents than *C. glabrata*.[52,58] These observations underscore the importance identifying *Candida* to the species level and determining the antifungal susceptibility profile to optimize the care of patients with invasive candidiasis.

C. tropicalis has long been considered one of the most virulent species of *Candida*, causing severe infections in neutropenic cancer patients.[59] *C. tropicalis* was the fourth most frequent species of *Candida* in both pre-COVID and COVID sets. This species, while virulent, has not been especially resistant to any of the antifungal agents.[52] As with *C. parapsilosis*, resistance to the azole class of agents was more prominent in the COVID set (3.5% resistant to fluconazole) than in the pre-COVID set (0.7% resistant to fluconazole) and exceeded that of *C. glabrata* (2.0% resistant to fluconazole; Table 2).

The finding of emerging resistance to fluconazole among common species of *Candida* and very low rates of resistance to the echinocandins among species other than *C. glabrata* provides support for current recommendations regarding the use of echinocandins as an initial empirical therapy for invasive candidiasis pending the results of species identification and antifungal susceptibility testing.[56]

Perhaps one of the greatest concerns to treating IFI has been the emergence of resistance to the mold-active triazoles in *Aspergillus fumigatus*.[60–62] Triazole-resistant *A. fumigatus* has been detected worldwide, but it is most prevalent in Europe.[60] Characterization of the mechanisms of resistance to the azoles in *A. fumigatus* revealed important patterns relative to the epidemiology of infections.[60,62–64] Prolonged drug pressure, such as that seen in the management of chronic bronchopulmonary aspergillosis,[60,63,64] has been shown to result in several different point mutations in the *CYP51* genes. We detected more *CYP51* mutant strains of *A. fumigatus* in the pre-COVID set, likely due to the longer pre-COVID time period despite the comparable frequency of NWT strains in both groups (Table 2).

Although considerably less common than the *Candida* or *Aspergillus* species, the non-*Candida* yeasts and the non-*Aspergillus* molds present a diverse number of species and a diverse set of antifungal resistance profiles marked by either an intrinsic resistance to the azoles, the echinocandins, or both.[65–67] A greater diversity of species of both yeasts and molds were present in the pre-COVID than the COVID set, likely due to the longer pre-COVID survey time. The non-*Candida* yeasts that were common to both groups include several species with intrinsic resistance to one or more antifungal agent: *T. asahi*, which is resistant to echinocandins; *S. clavata*, which is resistant to fluconazole and the echinocandins; and *R. mucilaginosa*, which is resistant to the azoles and the echinocandins.

Among the non-*Aspergillus* molds, the Mucorales, *Fusarium*, *Scedosporium*, *L. prolificans*, and *Scopulariopsis brevicaulis*/*S. brumptii* represent fungi with intrinsic resistance to one or more antifungal agents.[65–67] These pathogens were detected in both pre-COVID and COVID sets, but the individual species were more frequent in the pre-COVID set. Two additional species with resistance to the triazoles were only detected in the pre-COVID set: *Microascus cirrosus* (MIC, >8 mg/l for all 4 triazoles) and *Rasamsonia argillacea* SC (MIC_{50}, >8 mg/l for isavuconazole and voriconazole).

These diverse species of yeasts and molds are frequently difficult to identify with routine methods and exhibit highly variable resistance profiles requiring the use of state-of-the-art species identification methods, such as MALDI–TOF MS or nucleic acid sequencing, as well as antifungal susceptibility testing to optimise infection management.[38,65]

During the COVID-19 pandemic, invasive infection with Mucorales species has emerged as an infrequent infectious complication relative to CAPA or CAIC.[26,33,34] Whereas cases of CAM have been reported in several areas of the world, most cases have been reported from India, where diabetes and glucocorticoid exposure in COVID are primary risk factors.[26] Unfortunately,

India was not among the participating countries in the SENTRY Program. There were few isolates of Mucorales from either group (0.8% of all isolates; 0.9%, pre-COVID; 0.6%, COVID). Posaconazole was the most active of the mold-active triazoles in both groups. Several isolates of Mucorales, including *M. circinelloides/ramosissimus*, *Rhizopus* spp., and *Mucor* sp., were resistant/NWT to posaconazole, itraconazole, and isavuconazole.

There are some limitations to this work that must be acknowledged. First, patient-level data is not collected in SENTRY. Second, patients were not identified as infected with COVID-19 in 2020; rather, we compared isolates from patients hospitalized in the pre-COVID and COVID periods. Third, we did not link the isolation of fungal species and associated resistance profiles with patient presentation, treatment, or outcome. Finally, the SENTRY program is not a population-based survey. Due to the prevalence-based study design of SENTRY (see ref 52), each medical center only sends a limited number of isolates each year. As such, the numbers of isolates may reflect what was seen in one part of the year but not necessarily the rest of the year.

In summary, we did not detect any major shift of antifungal resistance among clinical isolates of yeasts and molds from the pre-COVID and COVID periods. Most *Candida* and *Aspergillus* species from both eras were susceptible or WT to the azoles, echinocandins, and amphotericin B. The most notable changes in resistance profiles were seen with *Candida* and the azoles. When pre-COVID and COVID sets were compared, fluconazole resistance and resistance to other the azoles decreased among *C. glabrata* isolates while it increased among *C. parapsilosis* and *C. tropicalis* isolates. Importantly, azole resistance in both *C. parapsilosis* and *C. tropicalis* now exceeds that of *C. glabrata*, precluding the use of simple species identification to guide the use of fluconazole in treating *Candida* infections. These findings underscore the necessity for accurate species identification and determination of *in vitro* susceptibility of fungal isolates to optimize the treatment of IFI during the COVID pandemic.

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Transparency statement

JMI Laboratories contracted to perform services in 2018–2020 for Achaogen, Inc., Albany College of Pharmacy and Health Sciences, Allecra Therapeutics, Allergan, AmpliPhi Biosciences Corp., Amicrobe Advanced Biomaterials, Amplyx, Antabio, American Proficiency Institute, Arietis Corp., Arixa Pharmaceuticals, Inc., Astellas Pharma Inc., Athelas, Basilea Pharmaceutica Ltd., Bayer AG, Becton, Dickinson and Company, bioMerieux SA, Boston Pharmaceuticals, Bugworks Research Inc., Cepheid, Cidara Therapeutics, Inc., CorMedix Inc., DePuy Synthes, Destiny Pharma, Discuva Ltd., Dr. Falk Pharma GmbH, Emery Pharma, Entasis Therapeutics, Eurofarma Laboratorios SA, US Food and Drug Administration, Fox Chase Chemical Diversity Center, Inc., Gateway Pharmaceutical LLC, GenePOC Inc., Geom Therapeutics, Inc., GlaxoSmithKline plc, Harvard University, Helperby, HiMedia Laboratories, F. Hoffmann-La Roche Ltd., ICON plc, Idorsia Pharmaceuticals Ltd., Iterum Therapeutics plc, Laboratory Specialists, Inc., Melinta Therapeutics, Inc., Merck & Co., Inc., Microchem Laboratory, Micromyx, MicuRx Pharmaceuticals, Inc., Mutabilis Co., Nabriva Therapeutics plc, NAEJA-RGM, Novartis AG, Oxoid Ltd., Paratek Pharmaceuticals, Inc., Pfizer, Inc., Polyphor Ltd., Pharmaceutical Product Development, LLC, Prokaryotics Inc., Qpex Biopharma, Inc., Roivant Sciences, Ltd., Safeguard Biosystems, Scynexis, Inc.,SeLux Diagnostics, Inc., Shionogi and Co., Ltd., SinSa Labs, Spero Therapeutics, Summit Pharmaceuticals International Corp., Synlogic, T2 Biosystems, Inc., Taisho Pharmaceutical Co., Ltd., TenNor Therapeutics Ltd., Tetraphase Pharmaceuticals, Theravance Biopharma, University of Colorado, University of Southern California-San Diego, University of North Texas Health Science Center, VenatoRx Pharmaceuticals, Inc., Viosera Therapeutics, Vyome Therapeutics Inc., Wockhardt, Yukon Pharmaceuticals, Inc., Zai Lab, Zavante Therapeutics, Inc. There are no speakers' bureaus or stock options to declare.

Declaration of interest

The authors have declared no conflict of interest.

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