International Surveillance of *Candida* spp. and *Aspergillus* spp.: Report from the SENTRY Antimicrobial Surveillance Program (2003)

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AMENDED ABSTRACT

Background: Standardized testing methodologies now allow us to generate meaningful information to track resistance to antifungal agents. Results from the fungal surveillance component of the SENTRY Program for 2003 are presented here.
Methods: A total of 1,397 *Candida* spp. (CSP), 73 *Aspergillus* spp. (ASP), 53 *C. neoformans* and 25 other isolates from infected sterile-site sources in patients from North America (NA), Europe (EU) and Latin America (LA) were submitted for testing (JMI Laboratories, IA). MICs for 5-fluorocytosine (FC), fluconazole (FLU), itraconazole (ITR), ketoconazole (KET), voriconazole (VOR), ravuconazole (RAV) and amphotericin B (AMP) were determined using NCCLS reference methods (M38-A, M27-A). A S breakpoint of ≤ 1 µg/ml was used with RAV, VOR and amphotericin B for comparative purposes only.
Results: Rank order of CSP occurrence was: *C. albicans* (CA; 48.7%), *C. parapsilosis* (CP; 17.3%) *C. glabrata* (CG; 17.2%), *C. tropicalis* (CT; 10.9%), *C. krusei* (CK; 1.9%) and CSP (4.0%). CA accounted for 51.5, 47.8 and 36.5% of candidal infections in NA, EU and LA, respectively. Among ASP, *A. fumigatus* (AF) was predominant (71.2%). CA, CP and CT were highly S to RAV, VOR and FLU (≥ 98.8% S) with the former two compounds being more potent (MIC_{90} ≤ 0.008 to 0.12 µg/ml) than FLU (MIC_{90}, 0.5 to 2 µg/ml). CG were less susceptible to RAV, VOR and FLU with MIC_{90}, in µg/ml (% S) of 1 (91.7), 1 (90.8) and 64 (52.1), respectively. CG from LA were less susceptible to RAV and AMB (86.7 and 73.3%, respectively) than from NA (92.8 and 86.8%). RAV and VOR were most active against CK, with an MIC_{90} of 0.5 µg/ml (92.6 to 96.3% S); most strains were R to FLU (74.1%). AF was R to some antifungals with VOR, RAV, and ITR being most active (MIC_{90}, % S, respectively: 1.0, 96.2; 1.0, 96.2 and 2.0, 84.6).
Conclusions: RAV and VOR displayed the greatest spectrum of activity against CSP and ASP, regardless of geographic origin. These results are congruent with previous SENTRY Program surveys, demonstrating that use of antifungals has had little impact on emergence of R.

INTRODUCTION

The development of standardized antifungal testing methodologies are allowing us to generate meaningful data to detect and track resistance to antifungal agents, and monitor the emergence of yeast species with innate resistance profiles as well as those of mould pathogens. The coupling of such technologies with global-scale antimicrobial surveillance programs provide a ready resource for monitoring and assessing prevalence of etiologic agents and detecting changes in efficacy of licensed antifungal agents through the emergence of resistance in recognized pathogens or selection of innately resistant species. In addition, these programs allow for detection of geographic differences in susceptibility as well as changing trends occurring in species distributions secondary to changing patient demographics.

In this report we summarize the results of the international (North America, Europe and Latin America) SENTRY Antimicrobial Surveillance Program comparing the activity of ravuconazole with currently marketed antifungal agents against contemporary, clinical isolates (2003). A total of 1,397 fungal (yeast and mould) strains were tested by reference NCCLS [2002] methods with susceptibilities to comparator agents interpreted by NCCLS breakpoint criteria.

MATERIALS AND METHODS

Specimen Collection: A total of 1,397 *Candida* spp. (predominantly from blood stream infections), 73 *Aspergillus* spp. (ASP; respiratory tract infections), and 53 *C. neoformans* and 25 other isolates (any infected sterile body site source) were submitted from participating medical centers in North America (882 strains), Europe (350 strains) and Latin America 316 strains) to the central monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA) for testing. Confirmation of identification was performed using standard biochemical methods and use of the Vitek identification system (Hazelwood, Missouri, USA).

Susceptibility Testing Methodologies: All strains were tested by the reference broth microdilution methods for yeasts and filamentous fungi as recommended by the NCCLS M27-A2 and M38-A approved standards [2002] using MOPS-buffered RPMI 1640 medium. Agents routinely tested included: 5-fluorocytosine, fluconazole, itraconazole, ketoconazole, voriconazole, ravuconazole and amphotericin B. Interpretive criteria used for yeasts when testing fluconazole, itraconazole and flucytosine are those of NCCLS [M27-A2]; interpretive breakpoints for moulds have not been established. A susceptible breakpoint of ≤ 1 µg/ml was used with ravuconazole, voriconazole and amphotericin B for comparative purposes (Diagn. Microbiol. Infect. Dis. 2004; 48:101). Quality control strains utilized included *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 [NCCLS, 2002].

RESULTS

- Rank order of *Candida* spp. recovered during the surveillance year from all sources was: *C. albicans* (48.7%), *C. parapsilosis* (17.3%), *C. glabrata* (17.2%), *C. tropicalis* (10.9%) and *C. krusei* (1.9%).
- *C. albicans* accounted for 51.5, 47.8 and 36.5% of candidal infections in North America, Europe and Latin America, respectively.
- *C. albicans*, *C. parapsilosis*, and *C. tropicalis* were highly susceptible to ravuconazole, voriconazole and fluconazole (≥ 98.0% susceptible) with the former two compounds being more potent (MIC_{90}, ≤ 0.008 to 0.12 µg/ml) than fluconazole (MIC_{90}, 0.5 to 2 µg/ml; Table 1).
- *C. glabrata* were less susceptible to ravuconazole, voriconazole and fluconazole with MIC_{90} values of 1 µg/ml (91.7% susceptible), 1 µg/ml (90.8% susceptible) and 64 µg/ml (52.1% susceptible), respectively.
- *C. glabrata* from Latin America were less susceptible to ravuconazole and amphotericin B (86.7 and 73.3% susceptible, respectively) than from North America (92.8 and 86.8% susceptible; Table 2).
- Ravuconazole and voriconazole were the most active agents tested against *C. krusei*, with a MIC_{90} of 0.5 µg/ml (92.6 to 96.3% susceptible); most strains were resistant to fluconazole (74.1%).
- Among *Aspergillus* spp., *A. fumigatus* was the predominant (71.2%) species.
- *A. fumigatus* was resistant to most antifungals with voriconazole (MIC_{90}, 1 µg/ml), ravuconazole (MIC_{90}, 1 µg/ml) and itraconazole (MIC_{90}, 2 µg/ml) being most active (96.2, 96.2 and 84.6% of isolates having MIC values ≤ 1 µg/ml, respectively; Table 3).

Table 1. In vitro susceptibilities of *Candida* spp. and *C. neoformans* isolates to seven antifungal agents (SENTRY Program, 2003).

Species (no. tested)	MIC (µg/ml) 50/90%	MIC (µg/ml) Range	% by category Susceptible	% by category Susceptible-dose dependent	% by category Resistant
All *Candida* spp. (1,397)					
Amphotericin B[a]	1/1	0.25-2	95.3	-	-
5-FC[b]	0.12/1	≤0.03->64	95.3	(1.4)[c]	3.3
Fluconazole	0.5/16	≤0.12-256	88.5	7.3	4.2
Ketoconazole	0.03/1	≤0.008-4	-	-	-
Itraconazole	0.12/1	≤0.008->16	55.6	28.9	15.5
Voriconazole[a]	0.016/0.25	≤0.008-8	98.2	-	-
Ravuconazole[a]	0.016/0.25	≤0.008-4	98.3	-	-
C. albicans (680)					
Amphotericin B	1/1	0.25-2	99.3	-	-
5-FC	0.12/2	≤0.03->64	97.8	(0.4)	1.8
Fluconazole	≤0.25/0.5	≤0.12-128	99.4	0.1	0.4
Ketoconazole	≤0.008/0.016	≤0.008-2	-	-	-
Itraconazole	0.06/0.12	≤0.008-2	92.8	6.9	0.3
Voriconazole	≤0.008/≤0.008	≤0.008-4	99.9	-	-
Ravuconazole	≤0.008/≤0.008	≤0.008-4	99.9	-	-
C. parapsilosis (242)					
Amphotericin B	1/1	0.5-2	97.9	-	-
5-FC	0.12/0.5	≤0.03-2	100.0	(0.0)	0.0
Fluconazole	1/2	0.25-64	98.8	0.8	0.4
Ketoconazole	0.12/0.25	0.016-1	-	-	-
Itraconazole	0.25/0.5	0.03-1	32.6	65.7	1.7
Voriconazole	0.03/0.12	≤0.008-1	100.0	-	-
Ravuconazole	0.03/0.12	≤0.008-0.5	100.0	-	-
C. glabrata (240)					
Amphotericin B	1/1	0.25-2	85.4	-	-
5-FC	0.06/0.12	≤0.03-2	100.0	(0.0)	0.0
Fluconazole	8/64	1-128	52.1	35.8	12.1
Ketoconazole	0.5/2	0.12-4	-	-	-
Itraconazole	1/2	0.25->16	0.0	26.3	73.7
Voriconazole	0.25/1	0.03-4	90.8	-	-
Ravuconazole	0.25/1	0.03-4	91.7	-	-
C. tropicalis (152)					
Amphotericin B	1/1	0.5-2	94.7	-	-
5-FC	0.25/1	≤0.03->64	90.1	(0.7)	9.2
Fluconazole	1/2	≤0.12-128	98.0	0.7	1.3
Ketoconazole	0.03/0.12	≤0.008-4	-	-	-
Itraconazole	0.25/0.5	0.016-2	32.2	64.5	3.3
Voriconazole	0.06/0.12	≤0.008-8	99.3	-	-
Ravuconazole	0.03/0.12	≤0.008-4	99.3	-	-
C. krusei (27)					
Amphotericin B	1/2	1-2	66.7	-	-
5-FC	32/64	8->64	0.0	(44.4)	55.6
Fluconazole	64/128	16-256	0.0	25.9	74.1
Ketoconazole	1/2	0.5-4	-	-	-
Itraconazole	1/1	0.25-2	0.0	40.7	59.3
Voriconazole	0.5/0.5	0.12-4	96.3	-	-
Ravuconazole	0.5/0.5	0.12-2	92.6	-	-
Candida spp. (56)					
Amphotericin B	1/1	0.25-2	92.9	-	-
5-FC	0.12/16	≤0.03->64	83.9	(7.2)	8.9
Fluconazole	0.12/16	≤0.12-128	83.9	10.7	5.4
Ketoconazole	0.12/0.5	≤0.008-2	-	-	-
Itraconazole	0.25/1	0.3-2	32.1	46.5	21.4
Voriconazole	0.03/0.25	≤0.008-1	100.0	-	-
Ravuconazole	0.03/0.5	≤0.008-1	100.0	-	-
C. neoformans (53)					
Amphotericin B	1/1	0.25-2	-	-	-
5-FC	8/16	2-16	-	-	-
Fluconazole	16/32	4-64	-	-	-
Ketoconazole	0.5/1	0.12-1	-	-	-
Itraconazole	0.5/1	0.25-1	-	-	-
Voriconazole	0.12/0.5	0.03-0.5	-	-	-
Ravuconazole	0.12/0.5	0.016-0.5	-	-	-

a. Breakpoint criteria have not been established by NCCLS; for comparative purposes a susceptible breakpoint of ≤ 1 µg/ml was used (Diagn. Microbiol. Infect. Dis. 2004; 48:101).
b. 5-FC = 5-Fluorocytosine.
c. Number in parenthesis = % intermediate [NCCLS, 2002].

Table 2. In vitro susceptibilities of *Candida* spp. and *C. neoformans* isolates from North America, Europe and Latin America to seven antifungal agents (SENTRY Program, 2003).

Species (no. tested)	$MIC_{50/90}$ µg/ml (% susceptible) North America	Europe	Latin America
All *Candida* spp.	(784)	(336)	(277)
Amphotericin B[a]	1/1(94.9)	1/1(96.7)	1/1(94.6)
5-FC[b]	0.12/2(95.9)	0.25/8(92.6)	0.12/1(96.8)
Fluconazole	0.5/16(86.4)	0.5/16(89.3)	0.5/8(93.5)
Ketoconazole	0.016/1(-)	0.016/0.5(-)	0.03/0.5(-)
Itraconazole	0.12/1(57.1)	0.12/1(59.5)	0.25/1(46.6)
Voriconazole[a]	0.016/0.25(97.8)	≤0.008/0.25(98.2)	0.03/0.25(99.3)
Ravuconazole[a]	≤0.008/0.5(98.2)	≤0.008/0.25(98.2)	0.03/0.25(98.6)
C. albicans	(404)	(175)	(101)
Amphotericin B	1/1(99.0)	1/1(100.0)	1/1(99.0)
5-FC	0.25/2(97.0)	0.12/0.5(98.9)	0.12/2(99.0)
Fluconazole	0.25/0.5(99.0)	0.25/0.5(100.0)	≤0.12/0.5(100.0)
Ketoconazole	≤0.008/0.016(-)	≤0.008/0.016(-)	≤0.008/0.03(-)
Itraconazole	0.06/0.12(92.8)	0.06/0.12(94.3)	0.06/0.12(90.1)
Voriconazole	≤0.008/≤0.008(99.8)	≤0.008/≤0.008(100.0)	≤0.008/0.016(100.0)
Ravuconazole	≤0.008/≤0.008(99.8)	≤0.008/≤0.008(100.0)	≤0.008/≤0.008(100.0)
C. parapsilosis	(122)	(55)	(65)
Amphotericin B	1/1(97.5)	1/1(100.0)	1/1(96.9)
5-FC	0.12/0.5(100.0)	0.25/0.5(100.0)	0.12/0.25(100.0)
Fluconazole	1/2(97.5)	1/2(100.0)	1/8(100.0)
Ketoconazole	0.12/0.25(-)	0.12/0.25(-)	0.12/0.5(-)
Itraconazole	0.25/0.5(37.7)	0.25/0.5(30.9)	0.25/0.5(24.6)
Voriconazole	0.03/0.12(100.0)	0.03/0.06(100.0)	0.03/0.12(100.0)
Ravuconazole	0.03/0.12(100.0)	0.03/0.06(100.0)	0.03/0.12(100.0)
C. glabrata	(167)	(43)	(30)
Amphotericin B	1/2(86.8)	1/1(88.4)	1/2(73.3)
5-FC	0.06/0.12(100.0)	0.12/0.12(100.0)	0.12/0.12(100.0)
Fluconazole	8/64(52.1)	8/128(53.5)	8/64(50.0)
Ketoconazole	0.5/2(-)	0.5/2(-)	1/2(-)
Itraconazole	1/2(0.0)	1/2(0.0)	1/4(0.0)
Voriconazole	0.25/1(91.0)	0.25/2(88.4)	0.5/1(93.3)
Ravuconazole	0.25/1(92.8)	0.25/1(90.7)	0.5/2(86.7)
C. tropicalis	(59)	(34)	(59)
Amphotericin B	1/1(91.5)	1/1(97.1)	1/1(96.6)
5-FC	0.25/0.5(94.9)	0.25/>64(76.5)	0.25/0.5(93.2)
Fluconazole	1/2(96.6)	1/4(97.1)	1/2(100.0)
Ketoconazole	0.03/0.12(-)	0.03/0.12(-)	0.06/0.12(-)
Itraconazole	0.25/0.5(39.0)	0.25/0.5(29.4)	0.25/0.5(27.1)
Voriconazole	0.03/0.12(98.3)	0.06/0.25(100.0)	0.06/0.12(100.0)
Ravuconazole	0.03/0.12(98.3)	0.03/0.25(100.0)	0.03/0.12(100.0)
C. neoformans	(23)	(9)	(27)
Amphotericin B	1/1(-)	1/-(-)	1/1(-)
5-FC	8/16(-)	8/-(-)	8/16(-)
Fluconazole	16/32(-)	8/-(-)	16/32(-)
Ketoconazole	0.5/1(-)	0.5/-(-)	0.5/1(-)
Itraconazole	0.5/1(-)	0.5/-(-)	0.5/1(-)
Voriconazole	0.12/0.5(-)	0.12/-(-)	0.25/0.25(-)
Ravuconazole	0.12/0.5(-)	0.12/-(-)	0.12/0.25(-)

a. Breakpoint criteria have not been established by NCCLS; for comparative purposes, a susceptible breakpoint of ≤ 1 µg/ml was used (Diagn. Microbiol. Infect. Dis. 2004; 48:101).
b. 5-FC = 5-Fluorocytosine.

Table 3. Activities[a] of six antifungal agents to *Aspergillus fumigatus* and other *Aspergillus* spp. (SENTRY Program, 2003).

Species (no. tested)	MIC (µg/ml) 50/90%	MIC (µg/ml) Range	% at ≤ 1 µg/ml
A. fumigatus (52 strains)			
Amphotericin B	2/4	1-16	11.5
Fluconazole	256/>256	64->256	-
Ketoconazole	4/8	0.5-8	-
Itraconazole	1/2	0.25-2	84.6
Voriconazole	0.5/1	0.12-2	96.2
Ravuconazole	0.5/1	0.12-2	96.2
Other *Aspergillus* spp. (21 strains)			
Amphotericin B	2/4	1-4	9.5
Fluconazole	256/>256	64->256	-
Ketoconazole	2/8	0.5-8	-
Itraconazole	1/2	0.5-2	71.4
Voriconazole	0.5/2	0.06-4	81.0
Ravuconazole	1/2	0.25-4	76.2

a. Breakpoint criteria have not been established by NCCLS [2002]; for comparative purposes, the percent inhibited by ≤ 1 µg/ml was used (Antimicrob. Agents Chemother. 2002; 46:1032).

CONCLUSIONS

- The rank order of *Candida* spp. recovered during 2003 essentially remains unchanged from previous SENTRY Program monitoring periods (1997 - 1999).
- Ravuconazole and voriconazole displayed the greatest spectrum of activity and potency against *Candida* spp. and *Aspergillus* spp., regardless of geographic origin.
- These results are congruent with earlier reports, demonstrating that use of antifungals has had little appreciable impact on prevalence of resistance in the population of organisms surveyed.

REFERENCES

Diekema DJ, Messer SA, Hollis RJ, Jones RN, Pfaller MA. (2003). Activities of caspofungin, itraconazole, posaconazole, ravuconazole, voriconazole, and amphotericin B against 448 recent clinical isolates of filamentous fungi. *Journal of Clinical Microbiology* 41:3623-3626.

National Committee for Clinical Laboratory Standards. (2002). *Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-second edition. Approved document M27-A2*. Wayne, PA:NCCLS.

National Committee for Clinical Laboratory Standards. (2002). *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard. Approved document M38-A*. Wayne, PA:NCCLS.

Ostrosky-Zeichner L, Rex JH, Pappas PG, Hamill RJ, Larsen RA, Horowitz HW, Powderly WG, Hyslop N, Kauffman CA, Cleary J, Mangino JE, Lee J. (2003). Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. *Antimicrobial Agents and Chemotherapy* 47:3149-3154.

Pfaller MA, Diekema DJ, Jones RN, Sader HS, Fluit AC, Hollis RJ, Messer SA, The SENTRY Participants Group. (2001). International surveillance of bloodstream infections due to *Candida* species: Frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY Antimicrobial Surveillance Program. *Journal of Clinical Microbiology* 39:3254-3259.

Pfaller MA, Diekema DJ, Messer SA, Boyken L, Hollis RJ, Jones RN. (2004). In vitro susceptibilities of rare *Candida* bloodstream isolates to ravuconazole and three comparative antifungal agents. *Diagnostic Microbiology and Infectious Disease* 48:101-105.

Pfaller MA, Jones RN, Doern GV, Sader HS, Messer SA, Houston A, Coffman S, Hollis RJ, The SENTRY Participant Group. (2000). Bloodstream infections due to *Candida* species: SENTRY Antimicrobial Surveillance Program in North America and Latin America, 1997-1999. *Antimicrobial Agents and Chemotherapy* 44:747-751.

Pfaller MA, Messer SA, Hollis RJ, Jones RN, Diekema DJ. (2002). In vitro activities of ravuconazole and voriconazole compared with those of four approved systemic antifungal agents against 6,970 clinical isolates of *Candida* spp. *Antimicrobial Agents and Chemotherapy* 46:1723-1727.

Pfaller MA, Messer SA, Hollis RJ, Jones RN, Doern GV, Brandt ME, Hajjeh RA. (1998). In vitro susceptibilities of *Candida* bloodstream isolates to the new triazole antifungal agents BMS-207147, Sch 56592, and voriconazole. *Antimicrobial Agents and Chemotherapy* 42:3242-3244.

Pfaller MA, Messer SA, Hollis RJ, Jones RN, The SENTRY Participants Group. (2002). Antifungal activities of posaconazole, ravuconazole, and voriconazole compared to those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: Report from SENTRY Antimicrobial Surveillance Program, 2000. *Antimicrobial Agents and Chemotherapy* 46:1032-1037.