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Trends in Species Distributions and Susceptibilities of Fungal Pathogens Recovered from European Medical Centers: A Report from the SENTRY Antimicrobial Surveillance Program (1999-2006)

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Objectives: To understand changes occurring with the prevalence of fungal pathogens in European patients and characterize key susceptibility (S) profiles of the predominant species utilizing results from the fungal surveillance component of the SENTRY Program (1999 to 2006). Development of standardized testing methodologies now allows the tracking of resistance (R) emergence to numerous old and new antifungal agents.

Methods: A total of 965 *Candida* spp. (CSP; 94.8%), 33 *Aspergillus* spp. (ASP; 3.2%), 8 *C. neoformans* (0.8%) and 12 other isolates (1.2%) from infected sterile-site sources (primarily bloodstream) were submitted from >20 European (EUR) medical centers to the central monitor (JMI Laboratories, IA) for identification and S testing. S for 5-fluorocytosine (FC), fluconazole (FLU), itraconazole (ITR) and voriconazole (VOR) were determined as part of the SENTRY Program for the intervals 1999-2001 and 2003 using NCCLS/CLSI reference methods and interpretive criteria (M38-A and M27-A2).

Results: Species rank order of CSP occurrence was: *C. albicans* (CA; 49.6%), *C. parapsilosis* (CP; 10.1%), *C. glabrata* (CG; 7.9%), *C. tropicalis* (CT; 6.9%), *C. krusei* (CK; 2.1%) and uncharacterized CSP (3.0%). Prevalence of CA declined slightly over the years 1999 to 2006 from 51 to 48%, whereas CP and CG both increased during the monitored interval (9.3 to 11.8% and 2.8 to 11.8%, respectively); CT and CK also increased slightly during the study (5.4 to 8.3% and 1.0 to 2.9%, respectively). Among ASP, *A. fumigatus* was predominant (63.6%). Comparison of MIC_{90} values for CA, CP and CT, respectively, demonstrated few differences between the intervals of 1999-2001 (13th ECCMID, abstract P713) and 2003 (44th ICAAC, abstract M1797) for FC (0.25-0.5, 0.12-0.5 and >64 mg/L), FLU (0.25-0.5, 1-2 and 2-4 mg/L), and ITR (0.06-0.12, 0.25-0.5 and 0.5 mg/L). Increases in MIC_{90} values (>= four-fold) of VOR were seen between these intervals with CP (0.015 to 0.06 mg/L), CG (0.25 to 2 mg/L) and CT (0.06 to 0.25 mg/L). CG also displayed the highest MICs against FLU (MIC_{90} values, 16-128 mg/L) and ITR (2 mg/L).

Conclusions: CSP accounted for 94.8% of fungal isolates recovered from patients in EUR medical centers from 1999-2006. While most were CA, increases were detected among other species, particularly CG, that displayed reduced S to azoles. The potential for emergence of species with R to recently marketed antifungal agents (echinocandins) warrants continued monitoring.

INTRODUCTION

Use of standardized antifungal testing methodologies such as those of the Clinical and Laboratory Standards Institute (CLSI) are allowing laboratorians to generate meaningful data to aid in patient care and to detect and track emergence of resistance to antifungal agents. These techniques also permit us to monitor the emergence of yeast and mould species with innate resistance profiles. The coupling of these technologies with international antimicrobial surveillance programs provides a critical resource for monitoring and assessing trends in pathogen prevalence, and in detecting changes in efficacy of marketed antifungal agents through the emergence of resistance strains or the unintended 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 European component of the SENTRY Antimicrobial Surveillance Program comparing temporal changes in species prevalence and the activity of currently marketed antifungal agents against older and more recent clinical isolates (1999-2001 and 2003). A total of 965 *Candida* spp. strains were tested by reference CLSI methods with susceptibilities to comparator agents interpreted by CLSI breakpoint criteria.

MATERIALS AND METHODS

Specimen Collection: A total of 965 *Candida* spp. (predominantly from blood stream infections), 33 *Aspergillus* spp. (ASP; respiratory tract infections), and 8 *C. neoformans* and 12 other isolates (any infected sterile body site source; all consecutive, non-duplicate) were submitted from ≥24 participating medical centers in Europe and forwarded to the central monitoring laboratory (JMI Laboratories, 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 *Candida* spp. strains were tested by the reference broth microdilution methods for yeasts as recommended by the CLSI M27-A2 and M27-S2 approved standards [2002 and 2005] using MOPS-buffered RPMI 1640 medium. Agents routinely tested included: 5-fluorocytosine, fluconazole, itraconazole, voriconazole and amphotericin-B. Interpretive criteria used for yeasts when testing fluconazole, itraconazole and flucytosine are those of CLSI [M27-S2]. A susceptible breakpoint of ≤1 mg/L was used for voriconazole as recently published. Quality control strains utilized included *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 [CLSI, 2005].

RESULTS

- The overall rank order of *Candida* spp. occurrence was: *C. albicans* (49.6%), *C. parapsilosis* (10.1%), *C. glabrata* (7.9%), *C. tropicalis* (6.9%), *C. krusei* (2.1%) and uncharacterized *Candida* spp. (3.0%; Table 1).
- Prevalence of *C. albicans* declined slightly over the years 1999 to 2006 from 51 to 48%, whereas *C. parapsilosis* and *C. glabrata* both increased during the monitored interval (9.3 to 11.8% and 2.8 to 11.8%, respectively); prevalence of *C. tropicalis* and *C. krusei* were variable during the study period (5.4 to 8.3% and 1.0 to 2.9%, respectively; Table 1).
- Among *Aspergillus* spp., *A. fumigatus* was the predominant species (63.6%).
- Comparison of MIC_{90} values for *C. albicans, C. glabrata* and *C. tropicalis*, respectively, demonstrated few differences between the intervals of 1999-2001 (13th ECCMID, abstract P713) and 2003 (44th ICAAC, abstract M1797) for 5-fluorocytosine (0.25-0.5, 0.12-0.5 and >64 mg/L), fluconazole (0.25-0.5, 1-2 and 2-4 mg/L), and itraconazole (0.06-0.12, 0.25-0.5 and 0.5 mg/L; Table 2).
- Increases in MIC_{90} values (≥ four-fold) of voriconazole were seen between these intervals with *C. parapsilosis* (0.015 to 0.06 mg/L), *C. glabrata* (0.25 to 2 mg/L) and *C. tropicalis* (0.06 to 0.25 mg/L; Table 2).
- Increases seen in prevalence of *C. glabrata* are most worrisome given the higher resistance rates seen with fluconazole, itraconazole and voriconazole (7.8, 73.3 and 4.4%, respectively); only 5-fluorocytosine provided complete (100.0% susceptible) coverage of this species (Table 3).
- Overall, voriconazole provided the broadest coverage against all tested *Candida* spp. (99.1% susceptible) and itraconazole the least (66.9%) at current CLSI breakpoints.

Table 1. Trends in prevalence of European *Candida* spp. among all recovered yeasts and moulds; SENTRY Antimicrobial Surveillance Program (1999 to 2006).

Organism (no. tested/% of total)	Testing Intervals; % of totals				
	1999-2000	2001-2002	2003-2004	2005-2006	All years
C. albicans (505/49.6)	51.4	50.5	49.0	48.0	49.6
C. parapsilosis (103/10.1)	9.3	9.2	10.1	11.8	10.1
C. glabrata (80/7.9)	2.8	5.4	9.8	11.8	7.9
C. tropicalis (70/6.9)	7.0	5.4	6.3	8.3	6.9
C. krusei (21/2.1)	1.4	2.2	2.9	1.0	2.1

Table 2. In vitro potency (MIC_{90} values) of antifungal agents tested against the leading Candida spp. for the intervals 1999-2001 and 2003 (SENTRY Program).

Organism (no. tested 1999-2001/2003)	MIC_{90} values (mg/L)			
	Fluconazole	5-fluorocytosine	Itraconazole	Voriconazole
C. albicans (299/175)	0.25/0.5	0.25-0.5/0.5	0.06-0.12/0.12	0.015/≤0.008
C. parapsilosis (25/55)	1-2/2	0.12-0.25/0.5	0.25-0.5/0.5	0.015/0.06
C. glabrata (70/43)	16-32/128	0.12/0.12	2/2	0.25-0.5/2
C. tropicalis (45/34)	2/4	128/>64	0.5/0.5	0.06/0.25

Table 3. In vitro susceptibilities of selected European *Candida* spp. isolates to five antifungal agents (SENTRY Program, 1999-2003).

Species (no. tested)	MIC (mg/L)		% by category[a]		
	50/90%	Range	Susceptible	Susceptible-dose dependent	Resistant
C. albicans (387)					
Amphotericin B	1/1	0.25-2	99.7[b]	-	-
5-FC[c]	0.12/0.5	≤0.03->64	98.2	(0.5)[d]	1.3
Fluconazole	0.25/0.5	≤0.12-64	99.0	0.7	0.3
Itraconazole	0.06/0.12	0.015-1	94.0	4.2	1.8
Voriconazole	≤0.008/≤0.015	≤0.008-0.5	100.0	0.0	0.0
C. parapsilosis (93)					
Amphotericin B	1/1	0.25-1	100.0[b]	-	-
5-FC	0.12/0.25	≤0.03-8	98.9	(1.1)	0.0
Fluconazole	1/2	0.25-16	98.9	1.1	0.0
Itraconazole	0.25/0.5	0.03-0.5	36.6	63.4	0.0
Voriconazole	0.03/0.06	≤0.008-0.5	100.0	0.0	0.0
C. glabrata (90)					
Amphotericin B	1/1	0.12-2	94.4[b]	-	-
5-FC	0.06/0.12	≤0.03-0.5	100.0	(0.0)	0.0
Fluconazole	8/32	0.25->128	60.0	32.2	7.8
Itraconazole	1/2	0.015->8	2.2	24.5	73.3
Voriconazole	0.25/1	≤0.008-8	93.3	2.3	4.4
C. tropicalis (64)					
Amphotericin B	1/1	0.25-2	98.4[b]	-	-
5-FC	0.25/>64	0.06->64	78.1	(1.6)	20.3
Fluconazole	1/4	0.12-32	95.3	4.7	0.0
Itraconazole	0.25/0.5	0.016-1	37.5	59.4	3.1
Voriconazole	0.06/0.25	≤0.008-0.5	100.0	0.0	0.0

a. Breakpoint criteria are those of CLSI M27-S2 [2006]; - = no breakpoints defined.
b. Breakpoint criteria have not been established by CLSI; for comparative purposes a susceptible breakpoint of ≤1 mg/L was used (Diagn. Microbiol. Infect. Dis. 2004; 48:101).
c. 5-FC = 5-Fluorocytosine.
d. Number in parenthesis = % intermediate [CLSI, 2006].

CONCLUSIONS

- *Candida* spp. accounted for 94.8% of fungal isolates recovered from patients in European medical centers from 1999-2006.
- While *C. albicans* was the most prevalent species, increases were detected during the monitored intervals among other species, particularly *C. glabrata*, that display reduced susceptibilities to azole agents.
- Among tested azole agents, voriconazole provided the broadest coverage against all tested *Candida* spp. (99.1% susceptible) and itraconazole the least (66.9%) at current CLSI breakpoints.
- The potential for emergence of species with resistance to recently marketed antifungal agents (e.g., echinocandins and azoles) warrants continued monitoring.

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