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A two year global evaluation of the susceptibility of *Candida species* to fluconazole by disk diffusion

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Abstract

The in-vitro activity of fluconazole against 46,831 yeast isolates collected over a two-year period from 57 laboratories in 33 countries worldwide was assessed using a disc diffusion method. *Candida albicans* was the organism isolated most frequently, accounting for 68.6% of the total number of isolates. *C. glabrata, C. tropicalis, C parapsilosis* and *C. krusei* and *Cryptococcus neoformans* represented 9.9, 4.7, 4.3, 1.9, and 1.4% of isolates respectively during the 2 year period and rates varied markedly between countries. In 1999 data blood isolates represented 4.9% of all isolates and intensive care unit isolates represented 9.9%. In both the 1998 and 1999 data, 99% of *C. albicans* were fully susceptible (S) to fluconazole, and 95.6% of all species of yeasts tested were S or susceptible-dose dependent (S-DD) to fluconazole. No emerging trends of resistance were noted with any of the *Candida* spp. tested as 96% of all isolates retained susceptibility (S or S-DD) to this agent.

1. Introduction

There has been a dramatic increase in the incidence of candidal infections over the past twenty years (Banerjee et al., 1991). A wide spectrum of mucosal and invasive diseases is caused by *Candida* species. Fungemia, which occurs most frequently in immunocompromised patients and patients receiving prolonged i.v. therapy or total parenteral nutrition, is the fourth most common nosocomial bloodstream infection in the United States, while oropharyngeal and esophageal candidiasis are common conditions in patients with AIDS (Jarvis & Martone, 1992). Unfortunately, there are only a limited number of drugs available for treating such infections. i.v. amphotericin B remains a suitable agent for invasive infections but there are problems with its administration and toxicity. The azoles have the advantages of oral administration and low toxicity. Ketoconazole was the first oral azole to be introduced into clinical medicine. However, it is not indicated for the treatment of invasive candidiasis in immunocompromised patients (Walsh & Pizzo, 1988) and although an effective agent for the treatment of mucocutaneous candidiasis, prolonged courses select for ketoconazole resistance (Horsburgh & Kirkpatrick, 1983). Fluconazole is available in both IV and oral forms with excellent bioavailability and is well tolerated. It has good activity against most yeasts with the exception of *C. krusei* which is intrinsically resistant and *C. glabrata* which is highly variable, and has been shown to be as efficacious as amphotericin B in the treatment of many candidal infections (Anaissie et al., 1996). There is concern that the use of this agent, particularly with repeated courses or long term prophylactic use, may select for infections caused by less susceptible strains of *C. albicans* and non-*albicans* spp. Treatment failures and isolation of *Candida* species with high MICs to fluconazole have been reported (Ng & Denning, 1993; Troillet et al., 1993; Rex et al., 1995, White & Goetz, 1994). This ongoing study was designed to monitor trends in isolation rates and fluconazole suscepti-

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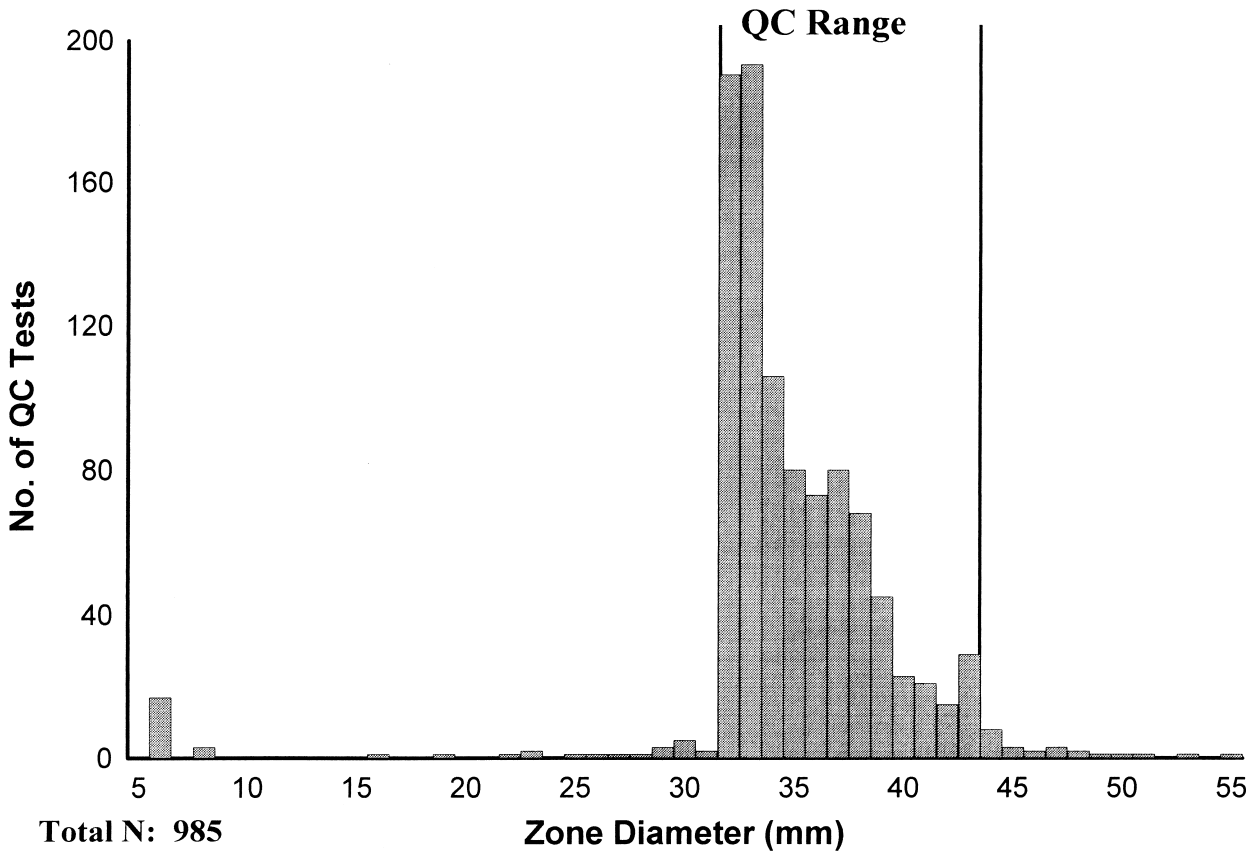

Fig. 1. Fluconazole QC zone diameter distribution with *C.albicans* ATCC 90028.

bility of significant clinical isolates of yeasts in 33 countries worldwide. Isolates from various body sites were consecutively isolated and tested, and results do not reflect any specific patient population or clinical indications.

For routine laboratories to generate susceptibility data, a simple and reliable testing method is needed. A disc diffusion test for fluconazole susceptibility of *Candida* species has been described and shown to provide accurate and reproducible results (Meis et al., 2000; Bille et al., 1997; Barry et al., 1996). This study, employing the disc diffusion method, is the largest ongoing global survey on isolation rates and fluconazole susceptibility to date. The 1999 data were collected from 48 institutions in 33 countries and compared to 1998 data which was reported previously (Meis et al., 2000).

2. Materials and methods

All investigators tested fresh clinically significant yeasts from all body sites and all in-hospital locations during the study period. Isolates were identified to genus/species by the institution's routine methodology.

The disc diffusion method has been described previously (Meis et al., 2000). Briefly, the test is based on the National Committee for Clinical Laboratory Standards (NCCLS) Document M2-A6 employing a 25 ug fluconazole disc (Becton Dickinson, Sparks, MD) and Mueller-Hinton agar supplemented with 2% glucose and 0.5 ug/mL methylene blue. Media was sourced locally at all sites. Inocula were adjusted to a 0.5 McFarland density standard. Plates were incubated aerobically at 35–37°C for 18–24 h and read by electronic image-analysis and interpreted and recorded with a BIOMIC Plate Reader System (Giles Scientific, 1999). Slow growing isolates, primarily *Cryptococcus*, were read after 48 h incubation.

Zone inhibition interpretive criteria for fluconazole disc testing (Meis et al., 2000; Bille, 1998) was based on zone diameters correlated with NCCLS recommended category breakpoints for the reference macrobroth dilution method (NCCLS, 1996). Fluconazole breakpoints were: susceptible (S) $\leq$ 8 μg/mL or $\geq$ 19 mm, susceptible-dose dependent (S-DD) = 16–32 μg/mL or 13–18 mm, and resistant (R) $\geq$ 64 μg/mL or $\leq$ 12 mm.

Quality control was performed once a week using *C. albicans* ATCC 90028 with a recommended acceptable performance range of 32–43 mm and an optional QC strain, *C. parapsilosis* ATCC 22019 with a range of 26–37 mm. Quality Control tests were required to be acceptable within 7 days of testing. Uncontrolled test results were automatically eliminated from analysis.

All test results were sent to Giles Scientific for analysis.

Table 1
Distribution of yeast isolates in 30 countries from Jan 1999–Feb 2000

Percent	C. albi	C. glab	C. trop	C. para	C. krus	Cr. neo	C. guil	Sacchar	C. lusi	C. kefy	Trichos	Other	Count
United Kingdom	75.6	9.1	3.1	4.0	1.8	0.1	0.6	1.1	0.5	0.3	0.1	3.7	6112
South Africa	64.8	9.8	3.2	3.3	2.3	5.0	0.5	0.2	0.4	0.3	0.4	9.8	5413
Netherlands	76.8	11.8	3.4	3.3	1.1	–	0.2	0.9	0.3	0.4	0.0	1.8	2330
Czech Republic	62.1	7.6	8.7	6.9	5.0	–	–	1.9	1.4	1.2	3.0	2.2	1751
Spain	68.9	13.0	2.8	5.2	2.0	–	0.3	0.2	0.3	0.1	0.1	7.1	1015
Hungary	60.4	10.6	8.5	4.4	5.3	–	0.5	–	0.9	1.2	–	8.2	962
Brazil	54.9	3.8	16.5	14.6	1.0	–	8.1	–	0.3	–	–	0.8	941
Belgium	66.4	11.0	2.9	2.5	3.9	–	0.7	–	–	0.7	–	11.9	909
Peru	76.5	10.4	–	–	1.8	–	–	–	–	–	–	11.3	719
Turkey	1.6	–	–	–	–	–	–	–	–	–	–	98.4	369
Greece	63.5	12.8	5.4	5.1	2.2	2.9	–	–	1.0	–	–	7.1	312
Slovakia	71.9	4.6	7.6	1.9	6.1	–	0.4	–	0.8	1.1	2.3	3.3	263
Australia	44.9	20.4	6.2	19.1	3.6	4.0	0.4	–	–	0.4	–	1.0	225
Colombia	34.4	3.3	9.3	3.8	–	38.8	2.7	0.5	0.5	1.1	0.5	5.1	183
Malaysia	72.7	5.5	5.5	12.6	1.1	–	–	–	–	–	–	2.6	183
Italy	80.8	8.7	5.8	2.9	0.6	–	–	0.6	–	–	0.6	–	172
Portugal	55.6	8.0	1.9	8.0	0.6	4.3	2.5	–	–	–	–	19.1	162
Germany	71.9	8.1	12.5	–	1.3	–	–	–	–	–	1.3	4.9	160
Argentina	53.2	0.6	17.3	2.6	1.9	0.6	–	–	–	–	–	23.8	156
South Korea	92.1	–	3.3	0.7	–	0.7	0.7	–	–	–	–	2.5	151
Thailand	61.5	7.7	13.8	3.8	3.1	–	0.8	–	–	–	–	9.3	130
Mexico	42.5	7.1	33.1	11.0	2.4	–	–	–	–	–	–	3.9	127
Taiwan	4.6	23.1	40.7	28.7	–	–	–	–	–	–	–	2.9	108
Ecuador	92.3	1.0	1.9	–	2.9	–	–	–	–	–	–	1.9	104
China	61.2	9.0	13.4	14.9	–	–	–	–	–	–	–	1.5	67
Sweden	76.8	19.6	–	1.8	1.8	–	–	–	–	–	–	–	56
Venezuela	49.1	1.8	25.5	3.6	–	–	–	–	–	–	–	20.0	55
Poland	98.0	–	–	–	–	–	–	–	–	–	–	2.0	51
Russia	56.8	–	–	–	–	–	–	–	–	–	–	43.2	37
Singapore	–	30.0	40.0	20.0	10.0	–	–	–	–	–	–	–	10
Percent	67.3	9.5	5.3	4.9	2.4	3.0	0.9	0.8	0.5	0.5	0.5	4.4	
Count	15630	2160	1158	1057	524	377	171	139	104	90	90	1733	23233

48 institutions

Zone diameter, susceptibility category (S, S-DD, R), MIC, and QC test results were recorded electronically. Patient and doctor names, duplicate test results (same patient-same species-same biotype results during any 7-day period) were automatically eliminated prior to analysis.

3. Results

The distribution of fluconazole zone diameters with quality control (QC) organism *C. albicans* ATCC 90028 is shown in Fig. 1. Ninety-three percent of all QC tests were within the tentative acceptable performance range for both *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019. QC test results were relatively consistent over time and results that were out-of-range tended to be just below the acceptable range for both quality control organisms. Specimens tested when QC tests performed out-of-control or when no quality control test was performed within 7 days were not included in this study.

A total of 23,233 yeast isolates were collected and tested in 1999 at the participating study sites in 30 countries. The distribution of yeasts species isolated by country is shown in Table 1. *C. albicans* was the species encountered most frequently, accounting for 67.3% of the isolates overall. *C. krusei* and *C. glabrata* accounted for 2.4% and 9.5% respectively and other yeast species represented approximately 20% of the isolates. Over the 2 year period 46,831 yeast were isolated; *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis* and *C. krusei* and *Cryptococcus neoformans* represented 9.9, 4.7, 4.3, 1.9, and 1.4% of isolates respectively, and these rates varied markedly between countries.

The susceptibility of individual species to fluconazole with 1998 and 1999 data are shown in Table 2. In both the 1998 and 1999 data, overall 98.6% of *C. albicans* were fully susceptible to fluconazole and 95.6% of all species of yeasts tested were S or S-DD to fluconazole. The countries isolating the highest percentage of fluconazole-resistant *C. albicans* isolates were China (9.8%), Russia (7.8%) and Ecuador (6.3%). *C. albicans* showed a 0.2% increase in susceptibility. *C. krusei*, *C. glabrata* and *C. parapsilosis* showed a decrease in susceptibility of 11.7, 3.4, and 1.5% respectively.

The distribution of fluconazole zone diameters for *C.*

Table 2
Comparison of 1998 and 1999 susceptibility information by organism

Organism	1998 Data			1999 Data			Change	
	Total N	% R	% S	Total N	% R	% S	% R	% S
Candida albicans	16,493	0.7	98.5	15,630	0.7	98.7	0.0	0.2
Candida glabrata	2,470	14.3	65.9	2,160	16.3	62.5	2.0	−3.4
Candida species	898	11.5	74.7	1,257	6.0	86.6	−5.5	11.9
Candida tropicalis	1,037	3.0	90.6	1,158	2.7	93.8	−0.3	3.2
Candida parapsilosis	952	1.5	94.7	1,057	1.8	93.2	0.3	−1.5
Candida krusei	370	37.8	23.2	524	58.4	11.5	20.6	−11.7
Other Yeast	722	13.0	77.7	386	13.2	71.8	0.2	−5.9
Cryptococcus neoformans	275	10.2	83.6	377	3.2	92.8	−7.0	9.2
Candida guilliermondii	111	4.5	82.9	171	4.7	82.5	0.2	−0.4
Saccharomyces sp.	36	0.0	80.6	139	3.6	88.5	3.6	7.9
Candida lusitaniae	115	3.5	91.3	104	3.8	94.2	0.3	2.9
Trichosporon sp.	67	3.0	95.5	180	2.8	88.3	−0.2	−7.2
Candida famata	19	31.6	36.8	55	3.6	74.5	−28.0	37.7
Rhodotorula sp.	33	90.9	6.1	35	85.7	5.7	−5.2	−0.4
Total	23,598	4.0	91.4	23,233	4.3	91.2	0.3	−0.2

1998 data: 6/98–12/98, 40 institutions.
1999 data: 1/99–2/00, 48 institutions.

albicans, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* showed a normal bell-shaped population distribution. The *C. albicans* isolates were tightly clustered well into the susceptible region. *C. krusei* showed a relatively tight cluster in the R/S-DD break-point region, but with a broad trailing into the susceptible region. Susceptible and S-DD isolates require clinical correlation studies to determine their "true" nature. *C. glabrata* showed a normal but broad distribution across all categories S/S-DD/R.

Fluconazole resistance for *C. albicans* and other species is shown by specimen type in Table 3, and for all specimen types by in-hospital location in Table 4. The only specimen types in which the non-albicans yeasts predominated were blood and cerebrospinal fluid (CSF). In 1999 data blood isolates represented 4.9% and intensive care unit isolates represented 9.9%. No major differences in resistance patterns were observed between in-hospital locations or specimen types (Tables 3 and 4). Yeasts were cultured most frequently from respiratory and urinary tract specimens. Lower respiratory tract accounted for 19%, urinary tract 11% and upper respiratory tract 10% of the isolates respectively. It is of interest that while *C. albicans* was the yeast most frequently isolated from patients with cutaneous and mucosal infections, the non-albicans species predominated in blood and CSF specimens. Of the blood isolates 38% were *C. albicans*, 18% *C. parapsilosis*, 17.5% *C. tropicalis*, and 10% *C. glabrata. C. krusei* accounted for only 1.7% of the blood isolates. Over 90% of the CSF isolates were cryptococci with most obtained from AIDS patients in South Africa.

The highest percentage of fluconazole-resistant *C. albicans* strains (1.65%) were isolated from upper respiratory

Table 3
Fluconazole resistance patterns by specimen type

Specimen Type	C. albicans		C. glabrata		C. tropicalis		C. krusei		Other	
	N	% R	N	% R	N	% R	N	% R	N	% R
Biliary Tract	25	0.00	4	0.00	3	0.00	1	100.00	2	0.00
Blood	430	1.16	113	14.16	199	7.54	19	36.84	371	2.70
CSF	13	0.00	3	0.00	0	0.00	1	100.00	268	0.37
Genital	1100	0.64	172	8.72	22	4.55	31	67.74	240	10.83
Lower G.I.	711	1.41	93	7.53	37	2.70	25	48.00	187	5.35
Lower Resp. Tract	3226	0.59	354	22.32	238	2.10	147	68.03	431	9.05
Misc./Other	6117	0.49	681	17.18	244	0.82	140	60.00	1428	5.88
Misc. Fluids	259	1.54	40	17.50	22	0.00	6	50.00	55	10.91
Skin/Soft Tissue	443	0.45	74	20.27	36	2.78	22	50.00	164	6.10
Upper Resp. Tract	1808	1.66	182	9.89	138	2.90	69	40.58	225	4.89
Urinary Tract	1498	0.13	444	17.34	219	0.91	63	60.32	390	3.59
Total	15630	0.70	2160	16.25	1158	2.68	524	58.40	3761	5.61

Unclassified specimen types are included in misc./other.
Jan 1999–Feb 2000, 48 institutions.

Table 4
Fluconazole resistance patterns by in-hospital location

Location	C. albicans		C. glabrata		C. tropicalis		C. krusei		Other	
	N	% R	N	% R	N	% R	N	% R	N	% R
Dermatology	149	1.34	8	0.00	16	6.25	3	100.00	145	3.45
Hematology/Oncology	1015	0.59	195	14.87	84	1.19	59	33.90	155	7.10
Medical	2246	0.22	354	17.80	162	3.09	107	72.90	561	3.03
Medical ICU	1365	0.51	222	19.82	138	5.07	48	43.75	307	4.23
Neo-Natal ICU	90	0.00	26	34.62	8	0.00	1	100.00	36	2.78
ObGyn	1043	0.77	189	7.41	19	0.00	27	62.96	103	2.91
Other	6150	1.17	802	16.21	545	2.39	192	57.29	1980	6.26
Outpatient	1467	0.48	159	20.13	40	2.50	33	57.58	217	14.29
Surgical	389	0.26	76	11.84	52	3.85	25	76.00	109	1.83
Surgical ICU	297	0.00	59	6.78	59	0.00	15	40.00	73	1.37
Urology	1419	0.07	70	24.29	35	2.86	14	85.71	75	4.00
Total	15630	0.70	2160	16.25	1158	2.68	524	58.40	3761	5.61

Unclassified results are included in other
Jan 1999–Feb 2000, 48 institutions.

tract specimens while the highest percentages of resistant candidal species (5–5.5%) were recovered from the lower respiratory tract. Interestingly, only 0.15% of *C. albicans* urinary isolates demonstrated resistance to fluconazole.

The medical and medical-ICU in-hospital locations provided the largest number of yeast isolates. Overall, the highest percentage of resistant yeasts (6.8%) was isolated from the neo-natal intensive care units. No resistant strains of *C. albicans* were encountered in either the neo-natal or surgical intensive care units.

4. Discussion

With the increasing incidence of yeast infections and emergence of resistant strains, it has become imperative for diagnostic laboratories not only to isolate and identify candidal species, but also to perform routine susceptibility testing. The NCCLS provides recommendations for broth reference MIC susceptibility testing of yeasts; however, that method is labor-intensive and difficult for most routine diagnostic laboratories. Correlation between the NCCLS standard reference method and the disk method was recently shown by Barry (Barry et al., 1996) and the disk method has the advantages of being easy to perform, accurate and low cost. The BIOMIC System (Giles Scientific, 1999) provided a cost effective means to electronically read plates and collect data. BIOMIC eliminated paper work, transcription errors, and provided improved intra and inter–laboratory reading consistency.

This is the largest global surveillance study of yeast isolation rates and fluconazole susceptibility reported to date. The 2-year study included 46,831 sequentially isolated fungal pathogens from the broadest geographic range of institutions (57 sites in 33 countries). In both the 1999 and 1998 data, *C. albicans* was the most commonly encountered species. Over the two year period there was a significant decrease (p-value is < 0.0001, difference of two binomial proportions) in the isolation rate of *C. albicans* from 69.9% in 1998 to 67.3% in 1999; a concomitant increase was seen in isolation frequency of the non-albicans species. This shift in species may be due in part to effective prevention of some *C. albicans* infections with prophylactic fluconazole therapy. Fortunately the isolation rates of the less susceptible *Candida* species remained relatively low with *C. glabrata* accounting for 9.6% and *C. krusei* 2.4%.

With the increasing use of fluconazole, particularly repeated courses or long-term prophylaxis with sub-therapeutic dosing, there is concern that fluconazole resistance may increase. Failure of fluconazole therapy in patients with oropharyngeal candidiasis caused by *C. albicans* with reduced susceptibility to fluconazole has been described (Boken et al., 1993; Sanguineti et al., 1993; White & Goetz, 1994). The fluconazole susceptibility results in this survey are similar to those reported from the analysis of our 1998 data by Meis et al., and those reported by Bille et al. using an earlier disc method in laboratories in 23 countries (Bille et al., 1997; Meis et al., 2000). In all three studies almost all *C. albicans* isolates (97.1–99.4%) were S or S-DD to fluconazole with resistance occurring predominantly in the non-*albicans Candida.* In our study, there was virtually no change in percentage of *C. albicans* strains found to be S or S-DD during 1998 (99.2% of 16,493) and 1999 (99.4% of 15,630) (Meis et al., 2000). In a study performed by Boschman et al. on significant clinical isolates and strains colonizing non-AIDS immunocompromised patients, only 88–90% of the *C. albicans* isolates tested were susceptible to fluconazole (Boschman et al., 1998). Interestingly, Boschman et al. noted a decrease in percentage of sensitive *C. albicans* isolates from sterile body sites from 100% during the period 1984 to 1992, to 72–90% over the period 1993 to 1997 (Boschman et al., 1998).

Higher resistance rates were observed, as expected, with a few non-*albicans Candida* species. Those exhibiting the

highest percentage of resistant strains were *C. krusei* and *C. glabrata.* Approximately 11.5% of the *C. krusei* strains appeared to be susceptible to fluconazole during the 1999 test period and 23% during 1998; these organisms are considered to be intrinsically resistant to fluconazole and we recommend that all isolates be reported as resistant until further clinical data can be obtained. *C. glabrata* and *C. krusei* accounted for only 9.6% and 2.4% of the total isolates respectively.

Non-*albicans Candida* isolated most frequently from blood were *C. parapsilosis* and *C. tropicalis* each representing 18%, and *C. glabrata* representing 10%. Other studies reported *C. tropicalis* as the most frequent non-*albicans Candida* species isolated from blood (Wey et al., 1988; Marina et al., 1991; Meunier-Carpentier et al., 1981) and often associated with total parenteral nutrition and catheter infections (Meunier-Carpentier et al., 1981). *C. krusei*, in line with other reports, accounted for only 1.7% of blood isolates (Meunier-Carpentier et al., 1981; Harvey et al., 1987). The recent SENTRY study of blood isolates in the Americas reported the second most common species was *C. glabrata* at 16%, with *C. parapsilosis* at 15% and *C. tropicalis* at 8% (Pfaller et al., 2000). The SENTRY study of blood isolates in Europe reported the second most common species was *C. parapsilosis* at 21% followed by *C. glabrata* at 12% and *C. tropicalis* at 6% (Pfaller et al., 1999). The recent NEMIS study reported *C. parapsilosis* at 29% was the second most common yeast species in the neonatal intensive care units; whereas *C. glabrata* and *C. tropicalis* were more common in surgical intensive care units at 24% and 19% respectively, with *C. parapsilosis* at 7% (Rangel-Frausto et al., 1999).

Results with *Cryptococcus neoformans* were included, although the disc method has not yet been validated for fluconazole susceptibility testing with a large number of these isolates. As with *Candida* species, factors other than an elevated MIC may be significant determinants of treatment failures (Witt, 1996.). There was a trend to increasing susceptibility of these organisms; while 84% were found to be susceptible in 1998, 92% of *Cryptococcus neoformans* were susceptible during 1999.

Fluconazole resistance overall remains very low with 95.6% of all yeast isolates being susceptible or S-DD. The fluconazole disc diffusion testing method was easy to perform and quality control performance was consistently acceptable. Only 7% of tests fell out of the acceptable performance range. The out-of-range values tended to be just below the acceptable range with a smaller cluster just above the acceptable range. It is suggested that the lower limit be expanded 1 mm to accommodate a more normal distribution. Disc diffusion provides a low cost, reproducible and accurate susceptibility test that can easily be used in routine hospital settings. After further validation, this method should help to guide clinical therapy, provide epidemiologic data, and reduce the number of misleading reports of drug resistance based on selective testing (Meis et al., 2000; Bille et al.,1997; Barry et al., 1996).

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Appendix

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