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Trends in Antifungal Susceptibility among *Candida* sp. Urinary Isolates from 1994 and 1998

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Antifungal susceptibilities were determined from 80 urinary isolates of *Candida* species collected in 1994 and 1998. Our findings demonstrate increasing geometric means of fluconazole MICs and fluconazole resistance in *Candida albicans* and *Candida tropicalis* (those for *Candida glabrata* were unchanged) within the 4-year span. Amphotericin B and voriconazole MICs remained constant.

Treatment of fungal infections has changed in the last decade due to the introduction of the newer triazole antifungal agents (1–3). With the ease of use of these agents, indiscriminate utilization has become common (1). While resistance to amphotericin B was rare, resistance to these drugs has been increasing in individuals receiving prolonged therapy (4, 6, 7). In this study, we compared antifungal susceptibilities in urinary tract isolates of common *Candida* species collected in 1994 and 1998 to monitor for changing trends in the general patient population.

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Forty urinary isolates of *Candida albicans*, *C. glabrata*, and *C. tropicalis* from 1994 (collected consecutively from inpatients for a possible study of colonization and stored at −70°C) and a comparable number from 1998 (collected from 15 January 1998 through 12 June 1998) were studied. Organisms were grown on inhibitory mold agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.). Species were identified by germ tube formation, CA50 test (Murex Diagnostics, Inc., Norcross, Ga.), and Yeast Biochemical Cards (bioMerieux Vitek, Inc., Hazelwood, Mo.). Control strains were obtained from the American Type Culture Collection (Rockville, Md.) and included *C. albicans* ATCC 24433 (MIC range, 0.25 to 1 μg/ml for amphotericin B and fluconazole) and *C. tropicalis* ATCC 750 (MIC ranges, 0.5 to 2.0 and 1.0 to 4.0 μg/ml for amphotericin B and fluconazole, respectively). All isolates were subcultured twice on Sabouraud dextrose agar prior to antifungal testing.

Fluconazole (Pfizer Inc., Groton, Conn.; compound UK-049,858) stock solution of 5,120 μg/ml in sterile water, amphotericin B (Bristol Myers-Squibb, Princeton, N.J.; type 1) stock solution of 1,600 μg/ml in dimethyl sulfoxide (DMSO; Sigma Chemical Co., St. Louis, Mo.), and voriconazole (Pfizer Inc.; compound UK-109,496) stock solution of 1,600 μg/ml in DMSO were prepared. Serial dilutions in RPMI 1640 medium (Sigma) resulting in test concentrations of 0.125 to 64 μg/ml for fluconazole and 0.03 to 16 μg/ml for amphotericin B and voriconazole were aliquoted into microtiter wells (100 μl each) and stored at −70°C until testing was done.

Susceptibility testing was performed by broth microdilution by utilizing the National Committee for Clinical Laboratory Standards M27-A method (8). A minimum of five colonies were suspended in 0.9% saline and adjusted to an 0.5 McFarland standard (corresponds to 1×10^6 to 5×10^6 CFU/ml) by using a Vitek colorimeter (bioMérieux, Vitek Inc.). This stock solution was diluted 1:50 in RPMI 1640 medium and then 1:20 to obtain a 2× test concentration. One hundred microliters of the 2× inoculum was pipetted to prepare antifungal dilutions in microwells to achieve a final concentration of 0.5×10^3 to 2.5×10^3 CFU/ml in a final test volume of 200 μl. Microwell plates were incubated at 35°C for 48 ± 2 h (mean ± standard deviation). The MIC was calculated by two independent observers as the lowest drug concentration with no growth for amphotericin B and an 80% reduction in growth for fluconazole and voriconazole (6, 9). All tests and controls were performed in duplicate. Final inoculum size was confirmed by subculture and colony count.

We studied 80 isolates of *Candida* species: *C. albicans* (n = 51), *C. tropicalis* (n = 11), and *C. glabrata* (n = 18). In 1994, 96% of *C. albicans* and all *C. tropicalis* isolates were susceptible to fluconazole. In 1998, fluconazole resistance was noted in 2 of 30 (6.7%) *C. albicans* isolates (MIC ≥ 64 μg/ml) and in 1 of 4 (25%) *C. tropicalis* isolates (MIC > 64 μg/ml); dose-dependent susceptibility (MIC = 16 to 32 μg/ml) was not observed. The MIC at which 50% of the isolates are inhibited (MIC_{50}) and geometric mean analysis for these two species increased two- to threefold during this time period (Table 1). Resistance and/or dose-dependent susceptibility was more prevalent among *C. glabrata* isolates, but the rate did not change.

Amphotericin B and voriconazole MICs remained constant. There was a significant correlation between fluconazole and voriconazole MICs (r^2 = 0.54; P = 0.01) but not with amphotericin B MICs.

The MICs of fluconazole and amphotericin B for all control strains were in the expected susceptibility range.

The epidemiology of *Candida* infections appears to be changing, with increasing prevalence of non-*C. albicans* species and the development of triazole resistance in ordinarily susceptible species (10, 11). The resistance has been reported most frequently for *C. albicans* oropharyngeal isolates from patients with advanced AIDS (11). Our findings show a trend towards increasing fluconazole MICs among *C. albicans* and *C. tropicalis* and a higher rate of fluconazole resistance over a 4-year period. In comparison, resistance among *C. glabrata* isolates was much more common but had already reached a steady state in 1994 and did not increase any further in 1998.

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TABLE 1. In vitro susceptibility of urinary *Candida* isolates to fluconazole, voriconazole, and amphotericin B in 1994 and 1998

Antifungal agent	Determination	*C. albicans*		*C. tropicalis*		*C. glabrata*	
		1994 (n = 21)	1998 (n = 30)	1994 (n = 7)	1998 (n = 4)	1994 (n = 12)	1998 (n = 6)
Fluconazole	MIC_{50} (range)[a]	0.25 (0.125–>64)	0.50 (0.25–>64)	1 (0.5–4)	2 (1–>64)	16 (4–>64)	16 (4–32)
	Geometric mean[b]	0.36	0.65	1.35	4.00	16.95	16.00
	% resistant[c]	4	6.7	0	25	66.7	66.7
Voriconazole	MIC_{50} (range)	0.03 (0.03–1)	0.03 (0.03–0.25)	0.06 (0.03–2)	0.06 (0.06–1)	0.5 (0.25–4)	0.5 (0.25–1)
	Geometric mean	0.05	0.04	0.13	0.15	0.6	0.56
Amphotericin B	MIC_{50} (range)	0.5 (0.5–1)	1 (0.5–1)	1 (1)	1 (1–2)	1 (1)	1 (1)
	Geometric mean	0.65	0.85	1.00	1.19	1.00	1.00

[a] MIC_{50}, range, and means are expressed in micrograms per milliliter.
[b] For calculation of the geometric mean, a MIC of >64 μg/ml was assumed to be 64 μg/ml.
[c] Percent resistant was defined as dose-dependent susceptibility (MIC of 16–32 μg/ml) or total resistance (MIC of ≥64 μg/ml).

These findings are not surprising because fluconazole resistance and/or dose-dependent susceptibility probably emerged in *C. glabrata* well before 1994 and remained relatively constant.

The MICs of voriconazole were low for all isolates tested. This finding most likely represents the extended potency of this newer antifungal agent as well as its limited use outside of clinical trials. However, although voriconazole remained highly effective against fluconazole-resistant strains, there was a significant correlation between voriconazole and fluconazole MICs. Whether this drug will remain effective against fluconazole-resistant strains after widespread use remains to be determined.

Amphotericin B MICs remained constant for all three species, and amphotericin resistance remained exceedingly rare. We did not observe any correlation between fluconazole and amphotericin B MICs.

The reasons for this trend in antifungal susceptibilities are unclear. The mechanisms of fluconazole resistance appear to be stepwise microevolutionary changes that occur during treatment (5). Whether our patients with higher MICs had received antifungal therapy in the past or these changes took place in response to selective pressure from widespread use of antifungal agents in the community is uncertain.

Although we studied only urine isolates, we suspect that similar trends may also be present in isolates from other sites. The clinical significance of these findings is unclear at this point since most of the MICs remain within the susceptibility range. Nevertheless, this trend is worrisome and requires close monitoring and better control of the use of antifungal agents.

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