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Susceptibilities of *Candida* Species Isolated from the Lower Gastrointestinal Tracts of High-Risk Patients to the New Semisynthetic Echinocandin LY303366 and Other Antifungal Agents

GEORGE G. ZHANEL,[1,2,3,4]* JAMES A. KARLOWSKY,[1,2,3] SHERYL A. ZELENITSKY,[2] MICHAEL A. TURIK,[5] AND DARYL J. HOBAN[1,3]

Department of Medical Microbiology[1] and Faculty of Pharmacy,[2] University of Manitoba, and Departments of Clinical Microbiology[3] and Medicine,[4] Health Sciences Centre, Winnipeg, Manitoba, Canada, and Lilly Research Laboratories, Indianapolis, Indiana[5]

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Fifty-two percent of stool specimens collected from 1,200 high-risk patients were colonized with yeasts, primarily *Candida albicans* (53.6%) and *Candida glabrata* (35.7%). Susceptibilities to all antifungal agents tested, including LY303366, were similar to those reported previously for *Candida* species isolated from blood.

Over the past 20 years, the incidence of *Candida* species causing systemic infection in compromised hosts has been steadily increasing (3). It has been suggested that the source of, or reservoir for, these infections may be the lower gastrointestinal tract in patients receiving antibiotics or antifungal agents and/or patients who are at high risk of infection (e.g., oncology, transplantation, dialysis, burn, and intensive care unit patients) (1, 4). The scientific literature contains a wealth of susceptibility data describing fungemic *Candida* species isolates from patients with a variety of medical and surgical conditions. There are, however, a lack of data describing the antifungal susceptibilities of *Candida* species colonizing the lower gastrointestinal tract of high-risk patients. We addressed the paucity of data in this area first by determining the frequency with which high-risk patients in Canadian hospitals carry yeast species in their gastrointestinal tract and then by performing antifungal susceptibility testing with clinically available antifungal agents as well as the new investigational echinocandin, LY303366, to determine if differences exist between the susceptibilities of isolates collected from the gastrointestinal tract and those previously reported for blood isolates.

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Between October 1995 and November 1996, 12 laboratories across Canada (Moncton Hospital, Moncton, New Brunswick; Victoria General Hospital, Halifax, Nova Scotia; Hôpital Universitaire de Sherbrook, Sherbrook, Quebec; Hôpital Maisonneuve-Rosemont, Montreal, Quebec; Hospital St. Luc, Montreal, Quebec; Mount Sinai Hospital, Toronto, Ontario; Toronto General Hospital, Toronto, Ontario; Sunnybrook Health Sciences Centre, North York, Ontario; Health Sciences Centre, Winnipeg, Manitoba; Regina General Hospital, Regina, Saskatchewan; University of Alberta Hospital, Edmonton, Alberta; Foothills Hospital, Calgary, Alberta) collected 100 consecutive stool specimens each, 1 stool specimen per patient, from hospitalized high-risk patients whose stools had been submitted to the respective hospital laboratories for *Clostridium difficile* toxin and/or culture testing. High-risk patients included those in oncology, transplantation, dialysis, burn, and intensive care units. Stool specimens were frozen following collection and shipped to a central reference laboratory (Health Sciences Centre), where they were thawed and plated on Inhibitory Mold Agar. Inhibitory Mold Agar plates were incubated at 35°C for 24 to 48 h. Up to five yeast morphotypes were selected from each plate, identified to the species level by using colony morphology, germ tube formation, and API 20C AUX (bioMerieux, Hazelwood, Mo.) (3), and then stocked for subsequent antifungal susceptibility testing.

All yeast species were subcultured twice onto Sabouraud agar prior to susceptibility testing. Antifungal MICs were determined by using the standard M27-T microbroth dilution format of the National Committee for Clinical Laboratory Standards (6). Stock solutions of LY303366 (Eli Lilly, Indianapolis, Ind.), amphotericin B (Bristol-Myers Squibb, St. Laurent, Quebec, Canada), ketoconazole (Janssen/Ortho, North York, Ontario, Canada), and itraconazole (Janssen/Ortho) were prepared in dimethyl sulfoxide. 5-Fluorocytosine (5FC; Hoffman-La Roche, Mississauga, Ontario, Canada) and fluconazole (Pfizer Canada, Kirkland, Quebec, Canada) stock solutions were prepared in water. The MIC doubling dilution ranges tested were 0.0313 to 64 μg/ml for LY303366, 0.0313 to 64 μg/ml for fluconazole, 0.0313 to 16 μg/ml for ketoconazole, 0.0313 to 64 μg/ml for itraconazole, 0.0313 to 64 μg/ml for 5FC, and 0.0313 to 16 μg/ml for amphotericin B. MIC end points for fluconazole, ketoconazole, itraconazole, 5FC, and amphotericin B were determined as described by the National Committee for Clinical Laboratory Standards (6). LY303366 MICs were defined as the lowest concentrations of LY303366 that inhibited 100% of visible growth (7). MICs were determined after 48 h of incubation at 35°C.

Of the 1,200 stool specimens cultured from high-risk patients, 624 (52%) grew yeast species. Seven hundred seventy morphologically distinct yeasts were identified from the 624 pos-

* Corresponding author. Mailing address: Health Sciences Centre, MS673-820 Sherbrook St., Winnipeg, Manitoba R3A 1R9, Canada. Phone: (204) 787-4902. Fax: (204) 787-4699. E-mail: ggzhanel@pcs.mb.ca.

itive stool cultures. Seven-hundred forty-two of the 770 (96%) isolates were *Candida* species (Table 1). The 770 isolates consisted of 53.6% *Candida albicans*, 35.7% *Candida glabrata*, 3.1% *Saccharomyces cerevisiae*, 2.3% *Candida parapsilosis*, 2.0% *Candida tropicalis*, 1.3% *Candida lusitaniae*, 1.0% *Candida krusei*, 0.5% *Rhodotorula rubra*, and 0.4% *Candida kefyr* (Table 1). The percent isolation ranges for *C. albicans* and *C. glabrata* were 35 to 63% and 28 to 44%, respectively. The mean number ± standard deviation of morphologically distinct yeasts isolated per hospital site was 64 ± 17 with a range of 47 to 81.

The results of antifungal susceptibility testing with LY303366, fluconazole, ketoconazole, itraconazole, 5FC, and amphotericin B are presented in Table 1 as MICs at which 50% and 90% of the isolates were inhibited (MIC_{50}s and MIC_{90}s, respectively) as well as MIC ranges. The MIC_{90}s of LY303366 were ≤0.25 μg/ml for *C. albicans* and 0.5 μg/ml for *C. glabrata*, which accounted for almost 90% of yeast isolates. LY303366 was also active against *C. tropicalis*, *C. krusei*, and *C. kefyr* but less active against *C. parapsilosis* and *R. rubra* (Table 1). Fluconazole MICs of ≥64 μg/ml were detected for 12 (4%) of the 275 *C. glabrata* isolates, 1 (7%) of the 15 *C. tropicalis* isolates, and 2 (25%) of the 8 *C. krusei* isolates tested. 5FC MICs of ≥32 μg/ml were detected for 6 (1.5%) of the 413 isolates of *C. albicans*, 1 (6.7%) of the 15 *C. tropicalis* isolates, and 1 (12.5%) of the 8 *C. krusei* isolates tested. Amphotericin B MICs were ≤1 μg/ml for all 770 yeast isolates tested.

Previous work has shown that up to 40% of healthy individuals and up to 75% of immunocompromised patients are colonized with yeasts (5). Our study demonstrated that 52% of high-risk patients tested were colonized with yeasts. Surprisingly, 35% of *Candida* species isolated in the present study were *C. glabrata*. A previous study of fungemic isolates at our hospital demonstrated that *C. glabrata* accounted for between 0 and 11% of *Candida* species isolates from 1976 to 1996 (3). The reason(s) for the disparity in *C. glabrata* isolation between these two sites is unclear.

LY303366 demonstrated activity against *C. albicans* (MIC_{90}, 0.25 μg/ml) and *C. glabrata* (MIC_{90}, 0.5 μg/ml) but was less active against *C. parapsilosis*. Yeast susceptibilities to all antifungal agents tested, including LY303366, were similar to those reported previously for yeasts isolated from blood (7, 8). As an example, our previous study describing the antifungal susceptibilities of *Candida* species bloodstream isolates at a Canadian tertiary care hospital demonstrated LY303366 MICs of ≤0.32 μg/ml for all *C. albicans* (99 isolates), *C. glabrata* (18 isolates), and *C. tropicalis* (10 isolates) isolates tested and less activity against *C. parapsilosis* (MIC_{90}, 5.12 μg/ml) (8). The same study also identified a fluconazole MIC of ≥64 μg/ml for only a single strain (1 of 10 strains of *C. tropicalis*) of *Candida* species, 5FC MICs of ≥32 μg/ml for no strains, and amphotericin B MICs of ≤1 μg/ml for all strains (8).

LY303366 demonstrated activity against many of the *Candida* species examined, including strains for which the fluconazole MICs were ≥64 μg/ml. Antifungal agents, such as LY303366, with activity against fluconazole-resistant isolates, in the absence of significant toxicity, need to become clinically available. In addition, the recent suggestion that the currently used LY303366 MIC end point (total inhibition of visible growth) in RPMI 1640 underestimates the activity of LY303366 may suggest even greater promise for this agent than was originally thought (2, 7). In conclusion, antifungal susceptibilities of *Candida* species colonizing the lower gastrointestinal tract are similar to those of blood isolates. In addition, LY303366 demonstrated excellent antifungal activity against lower gastroin testinal tract-colonizing isolates of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. kefyr*.

TABLE 1. Activities of LY303366, fluconazole, ketoconazole, itraconazole, 5FC, and amphotericin B against yeast species isolated from the lower gastrointestinal tract of seriously ill patients

Yeast (no. of isolates)	Antifungal agent	MIC (μg/ml)		
		50%	90%	Range
C. albicans (413)	LY303366	0.125	0.25	≤0.0313–0.25
	Fluconazole	0.125	0.25	≤0.0313–8
	Ketoconazole	≤0.0313	0.125	≤0.0313–1
	Itraconazole	0.125	0.25	≤0.0313–1
	5FC	0.125	0.5	≤0.0313–>64
	Amphotericin B	0.25	0.5	0.0625–0.5
C. glabrata (275)	LY303366	0.25	0.5	≤0.0313–1
	Fluconazole	8	32	0.5–64
	Ketoconazole	0.25	0.5	≤0.0313–1
	Itraconazole	0.25	1	≤0.0313–2
	5FC	0.0625	0.0625	≤0.0313–2
	Amphotericin B	1	1	0.0625–1
S. cerevisiae (24)	LY303366	1	2	0.25–2
	Fluconazole	0.5	2	≤0.0313–4
	Ketoconazole	0.25	1	≤0.0313–2
	Itraconazole	0.25	2	≤0.0313–4
	5FC	0.0625	0.25	≤0.0313–0.5
	Amphotericin B	1	1	0.125–1
C. parapsilosis (18)	LY303366	4	4	2–8
	Fluconazole	0.25	0.5	0.0625–1
	Ketoconazole	0.0625	0.25	≤0.0313–0.25
	Itraconazole	0.125	0.25	≤0.0313–0.5
	5FC	0.0625	0.125	≤0.0313–0.5
	Amphotericin B	1	1	0.25–1
C. tropicalis (15)	LY303366	0.5	1	0.125–1
	Fluconazole	0.5	4	0.0625–>64
	Ketoconazole	0.0625	0.125	≤0.0313–2
	Itraconazole	0.25	0.5	0.0625–8
	5FC	0.125	0.25	0.0625–64
	Amphotericin B	1	1	0.5–1
C. lusitaniae (10)	LY303366	0.5	2	0.125–2
	Fluconazole	0.25	0.5	0.125–1
	Ketoconazole	0.0625	0.25	≤0.0313–0.25
	Itraconazole	0.25	0.25	0.0625–0.25
	5FC	≤0.0313	0.0625	≤0.0313–0.125
	Amphotericin B	1	1	0.5–1
C. krusei (8)	LY303366			0.5–1
	Fluconazole			4–32
	Ketoconazole			0.125–2
	Itraconazole			0.5–4
	5FC			0.5–32
	Amphotericin B			0.5–1
R. rubra (4)	LY303366			32
	Fluconazole			0.125–>64
	Ketoconazole			0.125–0.5
	Itraconazole			0.25–0.5
	5FC			≤0.0313–0.25
	Amphotericin B			0.5–1
C. kefyr (3)	LY303366			0.25–0.5
	Fluconazole			0.125–0.25
	Ketoconazole			≤0.0313–0.125
	Itraconazole			0.25–0.5
	5FC			0.0625
	Amphotericin B			1

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