JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1999, p. 870–872 Vol. 37, No. 3
0095-1137/99/$04.00+0

In Vitro Susceptibilities of *Candida dubliniensis* Isolates Tested against the New Triazole and Echinocandin Antifungal Agents

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Received 15 October 1998/Returned for modification 24 November 1998/Accepted 12 December 1998

***Candida dubliniensis* is a newly recognized fungal pathogen causing mucosal disease in AIDS patients. Although preliminary studies indicate that most strains of *C. dubliniensis* are susceptible to established antifungal agents, fluconazole-resistant strains have been detected. Furthermore, fluconazole-resistant strains are easily derived in vitro, and these strains exhibit increased expression of multidrug resistance transporters, especially MDR1. Because of the potential for the development of resistant strains of *C. dubliniensis*, it is prudent to explore the in vitro activities of several of the newer triazole and echinocandin antifungals against isolates of *C. dubliniensis*. In this study we tested 71 isolates of *C. dubliniensis* against the triazoles BMS-207147, Sch 56592, and voriconazole and a representative of the echinocandin class of antifungal agents, MK-0991. We compared the activities of these agents with those of the established antifungal agents fluconazole, itraconazole, amphotericin B, and 5-fluorocytosine (5FC) by using National Committee for Clinical Laboratory Standards microdilution reference methods. Our findings indicate that the vast majority of clinical isolates of *C. dubliniensis* are highly susceptible to both new and established antifungal agents. Strains with decreased susceptibilities to fluconazole remained susceptible to the investigational agents as well as to amphotericin B and 5FC. The increased potencies of the new triazole and echinocandin antifungal agents may provide effective therapeutic options for the treatment of infections due to *C. dubliniensis*.**

Candida dubliniensis is a recently identified opportunistic yeast pathogen that is now recognized to be a minor constituent of normal human oral microbial flora. In previous studies *C. dubliniensis* was recovered from the oral cavities of 27% of human immunodeficiency virus (HIV)-infected individuals and 32% of AIDS patients with clinical symptoms of oral candidiasis (2, 6, 14–16). The role and incidence of *C. dubliniensis* in other infections has yet to be established. Despite this fact, there have been very few studies evaluating the in vitro susceptibilities of *C. dubliniensis* isolates to existing antifungal agents (15). Moran et al. (6) reported on the in vitro susceptibilities of 20 isolates of *C. dubliniensis* to fluconazole, itraconazole, ketoconazole, and amphotericin B. They found that the majority (80%) of *C. dubliniensis* isolates were susceptible to commonly used antifungal agents, including fluconazole. However, they did recover *C. dubliniensis* isolates with reduced susceptibilities to fluconazole (MIC range, 8.0 to 32 μg/ml) from AIDS patients with prior exposure to fluconazole. They also demonstrated that *C. dubliniensis*, unlike *Candida albicans*, was able to rapidly develop stable resistance to fluconazole following direct exposure to the drug in vitro (6). In these derivatives, fluconazole resistance was associated with increased expression of multidrug transporter genes, particularly *C. dubliniensis* MDR1 (7). These findings may have implications for antifungal drug treatment regimens and suggest that antifungal resistance may be a factor in the emergence of *C. dubliniensis* infection (15).

In the present study, we investigated the in vitro susceptibilities of 71 isolates of *C. dubliniensis* from 68 patients to several antifungal agents, including the triazoles BMS-207147, Sch 56592, and voriconazole and a representative of the echinocandin class of antifungal agents, MK-0991. We compared the in vitro activities of these new antifungal agents to those of established agents, including fluconazole, itraconazole, amphotericin B, and 5-fluorocytosine (5FC), by using National Committee for Clinical Laboratory Standards (NCCLS) reference broth microdilution methods (8). Our findings indicate that the vast majority of clinical isolates of *C. dubliniensis* are highly susceptible to both new and established antifungal agents. Isolates with decreased susceptibilities to fluconazole (MICs, 32 to 64 μg/ml) remained susceptible to the investigational agents as well as to amphotericin B and 5FC. The increased potencies of the new triazole and echinocandin antifungal agents may provide effective therapeutic options for the treatment of infections due to *C. dubliniensis*.

A total of 71 isolates (68 patients) of *C. dubliniensis* from the culture collection of the University of Dublin, Dublin, Ireland, were selected for testing. The isolates included the *C. dubliniensis* type strain, CD36 (CBS strain 7987); five previously described derivatives with decreased susceptibilities to fluconazole (MICs, ≥32 μg/ml) (6, 7), CD36-70, CD51-IIA, CD51-IIB, CD51-IIC, and CD57-B; and clinical isolates from a wide variety of geographic locations. The majority of the clinical isolates were oral isolates obtained from HIV-infected individuals or AIDS patients.

The isolates were identified as *C. dubliniensis* by their ability to produce abundant chlamydospores on rice agar-Tween medium (RAT medium; bioMérieux), by their inability to grow at 45°C (1), by indirect immunofluorescence with *C. dubliniensis* blastospore-specific polyvalent antiserum (12), by their characteristic DNA fingerprints following hybridization with the *C. albicans* mid-repeat sequence probes 27A and Ca3 (16),

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TABLE 1. In vitro susceptibilities of 71 *Candida dubliniensis* isolates tested against eight antifungal agents

Antifungal agent	MIC (μg/ml)			% S[a]
	Range	MIC_{50}	MIC_{90}	
Fluconazole	0.12–64	0.25	8.0	97
Itraconazole	0.015–0.5	0.06	0.25	100
BMS-207147	≤0.008–0.25	≤0.008	0.03	100
Sch 56592	0.015–0.12	0.03	0.06	100
Voriconazole	≤0.008–0.5	≤0.008	0.03	100
MK-0991	0.03–1.0	0.25	0.5	99
Amphotericin B	0.05–0.38	0.19	0.25	100
5FC	≤0.12	≤0.12	≤0.12	100

[a] % S, percentage of strains susceptible to the indicated antifungal agent at the following threshold concentration: fluconazole, <64 μg/ml; itraconazole, BMS-207147, Sch 56592, voriconazole, MK-0991, and amphotericin B, <1.0 μg/ml; or 5FC, <8.0 μg/ml.

and by hybridization with the *C. dubliniensis*-specific complex probe Cd25-1 (3).

Fluconazole (Pfizer Pharmaceuticals Group, New York, N.Y.), voriconazole (Pfizer), itraconazole (Janssen, Beerse, Belgium), BMS-207147 (Bristol-Myers Squibb, Wallingford, Conn.), Sch 56592 (Schering-Plough Research Institute, Kenilworth, N.J.), MK-0991 (Merck Research Laboratories, Rahway, N.J.), amphotericin B (Sigma Chemical Co., St. Louis, Mo.), and 5FC (Sigma) were all obtained as reagent grade powders from their respective manufacturers. Reference microdilution trays containing serial dilutions of the antifungal agents in RPMI 1640 medium (Sigma) were prepared in a single lot and were stored frozen at −70°C until used in the study.

Broth microdilution testing was performed according to NCCLS guidelines by the spectrophotometric method of inoculum preparation with an inoculum concentration of 0.5×10^3 to 2.5×10^3 cells per ml and RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma) (8). Yeast inocula (100 μl) were added to each well of microdilution trays containing 100 μl of antifungal drug solution (2× final concentration). Final concentrations of the antifungal agents were 0.008 to 8.0 μg/ml for voriconazole, BMS-207147, Sch 56592, itraconazole, and MK-0991 and 0.125 to 128 μg/ml for fluconazole and 5FC. Amphotericin B MICs were determined by using the Etest performed according to the instructions of the manufacturer (AB BIODISK, Solna, Sweden) (18). The microdilution trays and plates containing Etest strips were incubated in air at 35°C and were observed for the presence or absence of growth at 48 h. The MICs of 5FC, fluconazole, voriconazole, itraconazole, Sch 56592, and BMS-207147 were read as the lowest concentration at which a prominent decrease in turbidity (approximately 80% inhibition) relative to that of the growth control well was observed (8). The MICs of amphotericin B and MK-0991 were read as the lowest concentration at which complete growth inhibition was observed.

The interpretive criteria used for fluconazole (susceptible [S], <64 μg/ml), itraconazole (S, <1.0 μg/ml), and 5FC (S, <8.0 μg/ml) were those published by Rex et al. (13) and by the NCCLS (8). Interpretive criteria have not been defined for BMS-207147, voriconazole, Sch 56592, MK-0991, or amphotericin B. For purposes of comparison, the threshold concentration used for itraconazole (S, <1.0 μg/ml) was applied to these agents (Table 1).

Quality control (QC) was ensured by testing the NCCLS recommended QC strains (8) *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

In vitro susceptibility testing of the 71 isolates of *C. dubliniensis* revealed that 97% were susceptible to fluconazole, 100% were susceptible to itraconazole, and 100% were susceptible to 5FC at the recently published NCCLS MIC interpretive breakpoint concentrations (Table 1) (8, 13). The investigational agents were also highly active with ≥99% of isolates susceptible to BMS-207147, Sch 56592, voriconazole, and MK-0991 at a threshold concentration of <1.0 μg/ml. The MICs at which 90% of these isolates were inhibited (MIC_{90}) ranged from 0.03 μg/ml (BMS-207147 and voriconazole) to 0.5 μg/ml (MK-0991). Amphotericin B was also quite active, with 100% of the isolates being inhibited by a concentration of <1.0 μg/ml.

Five *C. dubliniensis* strains were noted to have decreased susceptibilities to fluconazole (MIC, ≥32 μg/ml) (Table 2). All five strains had previously been selected for resistance by subculturing on fluconazole-containing medium and had been shown to exhibit increased expression of multidrug resistance transporter proteins, especially MDR1, which mediate fluconazole resistance (6, 7). In Table 2, we compare the in vitro susceptibilities of these derivatives, plus the fluconazole-susceptible *C. dubliniensis* type strain, CD36, to itraconazole and the investigational triazole (BMS-207147, Sch 56592, and voriconazole) and echinocandin (MK-0991) antifungal agents.

As noted previously (6, 7), the MICs of itraconazole, as well as the newer triazole agents, remained quite low (≤1.0 μg/ml)

TABLE 2. Antifungal susceptibilities of the *Candida dubliniensis* type strain (CD36) and of strains with decreased susceptibilities to fluconazole tested against triazole and echinocandin antifungal agents

Isolate	Antifungal agent	MIC (μg/ml)
CD36	Fluconazole	0.25
	Itraconazole	0.06
	BMS-207147	0.008
	Sch 56592	0.03
	Voriconazole	0.03
	MK-0991	0.25
CD36-70	Fluconazole	64
	Itraconazole	0.12
	BMS-207147	0.06
	Sch 56592	0.06
	Voriconazole	0.5
	MK-0991	0.12
CD51-IIA	Fluconazole	32
	Itraconazole	0.06
	BMS-207147	0.03
	Sch 56592	0.06
	Voriconazole	0.25
	MK-0991	0.06
CD51-IIB	Fluconazole	32
	Itraconazole	0.12
	BMS-207147	0.06
	Sch 56592	0.12
	Voriconazole	0.5
	MK-0991	0.25
CD51-IIC	Fluconazole	64
	Itraconazole	0.25
	BMS-207147	0.06
	Sch 56592	0.12
	Voriconazole	0.5
	MK-0991	0.03
CD57-B	Fluconazole	32
	Itraconazole	0.5
	BMS-207147	0.25
	Sch 56592	0.25
	Voriconazole	0.25
	MK-0991	0.25

for all of the five strains (Table 2). Compared to that for the *C. dubliniensis* type strain, the MICs for these strains were elevated 2- to 32-fold for all of the triazoles tested, although in no instance was the increase in MIC considered sufficient to represent cross-resistance (7). Susceptibility to the MK-0991 echinocandin agent remained stable (MICs ranging from 0.03 to 0.25 μg/ml) regardless of the change in triazole susceptibility. Likewise, no cross-resistance to amphotericin B or 5FC was observed with these strains (data not shown).

In summary, we have confirmed and extended the observations of Moran et al. (6, 7) regarding the in vitro susceptibilities of *C. dubliniensis* isolates to the presently available antifungal agents. We have shown that *C. dubliniensis* isolates are generally quite susceptible to both established and investigational antifungal agents. The pattern of susceptibility to the newer triazole and echinocandin antifungal agents is similar to that observed for *C. albicans* (4, 5, 9–11). Importantly, all of the investigational triazoles and the echinocandin agent remained active against strains of *C. dubliniensis* with decreased susceptibilities to fluconazole. Given the demonstrated capacity of *C. dubliniensis* to become resistant to fluconazole by expression of multidrug resistance transporters (7), further clinical and in vitro studies of the antifungal susceptibility and resistance of this organism are warranted.

The excellent secretarial support of Kay Meyer is greatly appreciated.

This study was supported in part by Irish Health Research Board grant no. 41/96 and 05/97 (to D.C.C.), by Public Health Service grant DE10758 from the National Institutes of Health (to D.R.S.), and by grants from the Pfizer Pharmaceuticals Group and Bristol-Myers Squibb (to M.A.P.).

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