ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2007, p. 3014–3016 Vol. 51, No. 8
0066-4804/07/$08.00+0 doi:10.1128/AAC.00459-07

Cross-Resistance to Medical and Agricultural Azole Drugs in Yeasts from the Oropharynx of Human Immunodeficiency Virus Patients and from Environmental Bavarian Vine Grapes[▽]

Frank-Michael C. Müller,[1,2]* Andrea Staudigel,[2] Stefanie Salvenmoser,[1] Antje Tredup,[1] Rudolf Miltenberger,[3] and Josef V. Herrmann[3]

Pediatric Pulmonology and Infectious Diseases, Department of Pediatrics III, University Heidelberg, Heidelberg,[1] Department of Pediatrics, University Würzburg, Würzburg,[2] and Bavarian State Institute for Viticulture and Horticulture, Veitshöchheim,[3] Germany

Received 3 April 2007/Returned for modification 20 May 2007/Accepted 25 May 2007

Cross-resistance among *Candida albicans* isolates from the oropharynges of human immunodeficiency virus-infected patients ($n = 16$) and environmental yeast strains of various species ($n = 54$) to medical and agricultural azole drugs was observed. Precautions against the unnecessary widespread use of azoles in the environment and human medicine are strongly recommended to prevent patients from acquiring azole-resistant yeasts.

Fungi are important opportunistic pathogens in immunocompromised patients as well as in the environment (9). Azole drugs are in widespread use in agriculture and viticulture to control fungal growth on plants and fruits. Due to their good oral bioavailability, medical azoles are first-line antifungals for the treatment of human submucosal and invasive mycoses (3).

In this study, we have tested the in vitro activity of the medical azoles ketoconazole (KTC) and itraconazole (ITC) (Janssen, Beerse, Belgium) and fluconazole (FLC) and voriconazole (VRC) (Pfizer, Sandwich, United Kingdom) and the agricultural azoles fluquinconazole (FQZ) and penconazole (PCZ), tebuconazole (TCZ), and triadimenol (TDL) (Dr. Ehrenstorfer GmbH, Augsburg, Germany) against 16 clinical *Candida albicans* isolates, including 4 azole-susceptible and 12 azole-resistant strains, from the oropharynges of human immunodeficiency virus (HIV)-infected patients. Additionally, 54 environmental isolates were tested, including 14 *C. albicans* strains isolated from animals, 1 *Candida glabrata* strain from fodder beets, and 7 *Candida krusei* strains from conventionally grown grapes, draff, grass silage, silage, grist, and swill. Five *Candida lambica* strains from grapes, four from grapes treated with TDL and one from untreated grapes, were tested. One *Candida norvegensis* strain from TDL-treated grapes was tested, as was one *Candida rugosa* strain from feedingstuff. Nine *Candida stellata* strains from grapes, eight from grapes treated with TDL and one from untreated grapes used in wine growing, were tested. Three *Cryptococcus albidus* strains from untreated grapes, one *Pichia anomala* strain from conventionally grown grapes, one *Kloeckera apiculata* strain from TDL-treated grapes, and one *Rhodotorula* sp. strain from untreated grapes were tested. Nine *Saccharomyces cerevisiae* strains from grapes, seven from grapes treated with TDL and two from untreated grapes, and one *Saccharomycopsis guttulata* strain from total mixed feed for cattle were tested. *C. albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *Candida parapsilosis* ATCC 22013, and *Cryptococcus neoformans* ATCC 90112 served as controls.

The in vitro susceptibility testing for all azoles was performed in accordance with the guidelines in NCCLS document M27-A2, with slight modifications (7). The antifungals were diluted from 0.125 to 128 μg/ml for FLC; 0.03 to 64 μg/ml for KTC, ITC, and VRC; and 0.06 to 128 μg/ml for FQZ, PCZ, TCZ, and TDL. The drugs were diluted in high-resolution antifungal assay medium (Oxoid, Wesel, Germany). The MIC endpoints were determined spectrophotometrically at 540 nm with an MR 700 spectrophotometer (TECAN GmbH, Crailsheim, Germany). For the agricultural azoles, no breakpoints are defined; therefore, isolates for which MICs were >64 μg/ml were considered resistant. Cross-resistance in a single isolate was assumed when an elevated MIC of a given azole corresponded to an elevated MIC of another azole drug.

The *C. albicans* strains isolated from the oropharynges of HIV-infected patients before FLC exposure were susceptible to all azoles (Table 1). Strains isolated from HIV-infected patients already treated with FLC were resistant to all tested azoles. Almost all these isolates were cross-resistant to the agricultural azoles. All 12 isolates from FLC-treated HIV-infected patients were resistant to FLC, 2 were resistant to ITC, and 3 were resistant to VRC. MICs of TDL for five isolates and those of TCZ and FQZ for two isolates were increased relative to MICs for susceptible isolates.

Some environmental species are intrinsically resistant to FLC; however, 28 environmental strains were susceptible to all azoles. MICs of the agricultural azoles for 23 strains and MICs of the medical azoles for 19 strains were increased; furthermore, cross-resistance to medical and agricultural azoles in 16 isolates was observed.

For five *C. stellata* strains isolated from grapes from Bavar-

* Corresponding author. Mailing address: Pediatric Pulmonology and Infectious Diseases, Dept. of Pediatrics III, University Heidelberg, Im Neuenheimer Feld 153, D-69120 Heidelberg, Germany. Phone: 49-6221-56-8356. Fax: 49-6221-56-33853. E-mail: Frank-Michael_Mueller@med.uni-heidelberg.de.

▽ Published ahead of print on 4 June 2007.

ian vineyards that were treated with TDL, MICs of this drug were high. MICs of TDL for five *C. lambica* strains, isolated from grapes treated with TDL as well, were raised. These strains were cross-resistant to FLC (MIC, >64 μg/ml). The exposure to TDL most likely induced the azole resistance in these strains.

Thirteen of 14 *C. albicans* strains isolated from animals were susceptible to all azoles. MICs of agricultural azoles were higher than those of medical azoles. This finding was consistently observed for almost all tested isolates. Fewer isolates from the environment were resistant to medical azoles (15 strains out of 54) than were resistant to agricultural azoles (23 strains out of 54). MICs of TDL for almost all the 70 strains tested were elevated, and the MICs of this drug for half of the isolates exceeded 32 μg/ml.

Inherited and acquired azole resistance is relevant in human medicine and in agriculture. For the environmental yeast isolates from different sources investigated in this study, MICs of the agricultural azoles that are in widespread use to prevent fungal infections were often elevated. These isolates were often cross-resistant to the medical azoles presently in use. The converse was also true; FLC-resistant *C. albicans* isolates from the oropharynges of HIV-infected patients often demonstrated cross-resistance to the agricultural azoles.

All azoles, irrespective of their distinctive chemical structures and variable biological properties, interact with and inhibit the lanosterol 14-α-demethylase needed for transforming lanosterol into ergosterol in the cell membranes of the yeasts (4). In human medicine, azole derivatives other than those for plant protection are used. However, the agents have similar modes of action and can be affected by the same mechanisms of antifungal drug resistance. For example, azole resistance in *C. albicans* results from point mutations, the overexpression of the *ERG11* gene, and the overexpression of the efflux pumps encoded by *CDR1*, *CDR2*, and *MDR1* (12). FLC resistance in strains in HIV-infected patients was common in the 1980s and 1990s due to the use of FLC prophylaxis for the prevention of oropharyngeal candidiasis (5, 8, 11). Müller et al. reported cross-resistance to FLC, ITC, and VRC among oropharyngeal candidiasis isolates from HIV-infected patients (6). Goff et al. reported FLC resistance in yeast strains from HIV-negative patients (2), and Rowen et al. detected FLC resistance in isolates from preterm infants that were never exposed to azoles (10).

The use of large quantities of azole fungicides in plant protection is a potential risk factor for the development and/or transmission of resistance in human medicine. The induction of azole resistance in the yeast flora of humans by contact with residues through the food chain is possible and requires further attention (1).

The use of azoles in human medicine and in the environment requires restrictions to minimize the risk of acquired azole resistance in human yeast strains. Regular surveillance of cultures as well as in vitro susceptibility testing of human isolates is therefore strongly recommended.

This work was funded by the Universitätsbund Würzburg, Germany (Az. 01-31).

We are grateful to Monika Kruger, Leipzig, for providing the isolates from animals. Antifungal azole powders KTC and ITC were

TABLE 1. MICs of tested medical and agriculture azoles for yeast isolates from the oropharynges of HIV-infected patients and for environmental strains

Species (no. of isolates)	Source(s) or description of isolate(s)	Median (range) MIC_{50} [a] (μg/ml) of:							
		KTC	FLC	ITC	VRC	FLQ	PCZ	TCZ	TDL
C. albicans (4)	HIV-infected patients before FLC therapy	0.25 (0.25–0.5)	2.25 (0.5–8)	0.06 (0.03–0.06)	0.03 (0.03)	0.375 (0.25–64)	0.25 (0.25–8)	0.75 (0.5–8)	2 (2–8)
C. albicans (12)	HIV-infected patients during FLC therapy	2 (0.25–4)	128 (2–128)	0.75 (0.12–32)	1.5 (0.03–32)	64 (0.25–128)	12 (0.25–128)	24 (0.5–128)	64 (2–128)
C. albicans (14)	Animals	0.5 (0.25–4)	0.25 (0.12–256)	0.12 (0.06–0.5)	0.03 (0.03–32)	0.5 (0.12–32)	2 (1–64)	0.185 (0.06–64)	1.5 (1–32)
C. albicans (3)	Control strains	0.03 (0.03)	0.12 (0.06–0.5)	0.06 (0.06–0.12)	0.03 (0.03–0.06)	0.5 (0.25–1)	2 (1–2)	0.06 (0.06–64)	2 (0.5–2)
C. glabrata (1)	Feedingstuff	4 (4)	32 (32)	1 (1)	0.25 (0.25)	1 (1)	4 (4)	2 (2)	8 (8)
C. glabrata (1)	Control strain ATCC 90030	8 (8)	8 (8)	4 (4)	2 (2)	32 (32)	4 (4)	8 (8)	8 (8)
C. krusei (7)	Grapes and feedingstuff	8 (4–8)	64 (64)	1 (1)	1 (1–2)	128 (64–128)	128 (32–128)	64 (32–64)	64 (64–128)
C. krusei (1)	Control strain ATCC 6258	2 (2)	64 (64)	0.5 (0.5)	1 (1)	128 (128)	64 (64)	32 (32)	32 (32)
C. lambica (5)	Grapes	4 (2–4)	128 (64–256)	2 (2)	2 (1–8)	64 (64)	64 (64–128)	16 (8–16)	128 (128)
C. norvegensis (1)	Grapes	2 (2)	128 (128)	1 (1)	2 (2)	64 (64)	64 (64)	4 (4)	64 (64)
C. parapsilosis (1)	Control strain ATCC 22013	0.12 (0.12)	2 (2)	0.25 (0.25)	0.06 (0.06)	2 (2)	8 (8)	1 (1)	16 (16)
C. rugosa (1)	Feedingstuff	0.25 (0.25)	1 (1)	0.06 (0.06)	0.03 (0.03)	0.12 (0.12)	1 (1)	0.06 (0.06)	1 (1)
C. stellata (9)	Grapes	8 (2–8)	64 (32–64)	2 (2–4)	1 (1–2)	64 (32–64)	96 (64–128)	64 (16–64)	128 (128)
C. albidus (3)	Grapes	8 (8)	64 (32–256)	32 (2–32)	8 (2–16)	8 (8–128)	128 (128)	64 (8–128)	128 (128)
C. neoformans (1)	Control strain ATCC 90112	2 (2)	8 (8)	0.5 (0.5)	0.25 (0.25)	0.12 (0.12)	4 (4)	4 (4)	4 (4)
P. anomala (1)	Grapes	1 (1)	2 (2)	0.5 (0.5)	0.25 (0.25)	4 (4)	8 (8)	4 (4)	16 (16)
K. apiculata (1)	Grapes	0.25 (0.25)	0.5 (0.5)	0.25 (0.25)	0.03 (0.03)	0.12 (0.12)	0.5 (0.5)	0.06 (0.06)	0.25 (0.25)
Rhodotorula sp. (1)	Grapes	0.5 (0.5)	16 (16)	0.06 (0.06)	0.06 (0.06)	0.5 (0.5)	16 (16)	1 (1)	64 (64)
S. cerevisiae (9)	Grapes	2 (1–4)	8 (4–16)	2 (0.5–2)	0.5 (0.25–0.5)	0.25 (0.25–4)	2 (0.5–16)	1 (0.25–2)	8 (1–16)
S. guttulata (1)	Feedingstuff	2 (2)	1 (1)	0.5 (0.5)	0.12 (0.12)	64 (64)	8 (8)	4 (4)	16 (16)

[a] MIC_{50}, MIC at which 50% of the isolates tested are inhibited.

kindly provided by Janssen, Beerse, Belgium; FLC and VRC were provided by Pfizer, Sandwich, United Kingdom. PCZ, TCZ, and TDL were kindly provided by Dr. Ehrenstorfer GmbH (Augsburg, Germany). We thank Herbert Hof, Mannheim, Germany, Karl-Heinz Kuck, Leverkusen, Germany, and Gil Hollbrock, Heidelberg, Germany, for critical reading of the manuscript.

REFERENCES

1. **Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin.** 7 June 2001, posting date. Problematik der Entwicklung von Resistenzen humaner Mykosen gegenüber Azol-Antimykotika und eventueller Wechselwirkungen mit den als Fungizid eingesetzten Pflanzenschutzmitteln. Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Berlin, Germany. http://www.bfr.bund.de/cm/218/problematik_der_entwicklung_von_resistenzen_humaner_mykosen_gegenueber_azol_antimykotika.pdf/.
2. **Goff, D. A., S. L. Koletar, W. J. Buesching, J. Barnishan, and R. J. Fass.** 1995. Isolation of fluconazole-resistant Candida albicans from human immunodeficiency virus-negative patients never treated with azoles. Clin. Infect. Dis. **20:**77–83.
3. **Gupta, A. K., and E. Tomas.** 2003. New antifungal agents. Dermatol. Clin. **21:**565–576.
4. **Ji, H., W. Zhang, Y. Zhou, M. Zhang, J. Zhu, Y. Song, and J. Lu.** 2000. A three-dimensional model of lanosterol 14alpha-demethylase of Candida albicans and its interaction with azole antifungals. J. Med. Chem. **43:**2493–2505.
5. **Leen, C. L., E. M. Dunbar, M. E. Ellis, and B. K. Mandal.** 1990. Once-weekly fluconazole to prevent recurrence of oropharyngeal candidiasis in patients with AIDS and AIDS-related complex: a double-blind placebo-controlled study. J. Infect. **21:**55–60.
6. **Müller, F. M., M. Weig, J. Peter, and T. J. Walsh.** 2000. Azole cross-resistance to ketoconazole, fluconazole, itraconazole and voriconazole in clinical Candida albicans isolates from HIV-infected children with oropharyngeal candidosis. J. Antimicrob. Chemother. **46:**338–340.
7. **NCCLS.** 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts; M27-A2, vol. 17, no. 9. NCCLS, Wayne, PA.
8. **Ng, T. T., and D. W. Denning.** 1993. Fluconazole resistance in Candida in patients with AIDS: a therapeutic approach. J. Infect. **26:**117–125.
9. **Odds, F. C., N. A. Gow, and A. J. Brown.** 2001. Fungal virulence studies come of age. Genome Biol. **2:**REVIEWS1009.
10. **Rowen, J. L., J. M. Tate, N. Nordoff, L. Passarell, and M. R. McGinnis.** 1999. *Candida* isolates from neonates: frequency of misidentification and reduced fluconazole susceptibility. J. Clin. Microbiol. **37:**3735–3737.
11. **Ruhnke, M., A. Eigler, I. Tennagen, B. Geiseler, E. Engelmann, and M. Trautmann.** 1994. Emergence of fluconazole-resistant strains of *Candida albicans* in patients with recurrent oropharyngeal candidosis and human immunodeficiency virus infection. J. Clin. Microbiol. **32:**2092–2098.
12. **White, T. C., S. Holleman, F. Dy, L. F. Mirels, and D. A. Stevens.** 2002. Resistance mechanisms in clinical isolates of *Candida albicans*. Antimicrob. Agents Chemother. **46:**1704–1713.