JOURNAL OF CLINICAL MICROBIOLOGY, July 1996, p. 1856–1858 Vol. 34, No. 7
0095-1137/96/$04.00+0

Strain Variation among and Antifungal Susceptibilities of Isolates of *Candida krusei*

Y. F. BERROUANE,[1]† R. J. HOLLIS,[2] AND M. A. PFALLER[2]*

Division of General Medicine, Clinical Epidemiology and Health Services Research, Department of Internal Medicine,[1] and Department of Pathology,[2] University of Iowa College of Medicine, Iowa City, Iowa

Received 18 December 1995/Returned for modification 12 March 1996/Accepted 19 April 1996

***Candida krusei* is an emerging pathogen that is well known for its propensity to develop resistance to fluconazole and other azoles. Despite the potential clinical significance of *C. krusei*, little is known of its epidemiology and genetic diversity as defined by the newer DNA-based typing methods. We investigated the genotypic diversity and antifungal susceptibility of 67 clinical isolates from 44 patients and 5 health care workers from six different medical centers. Strain delineation was performed by restriction endonuclease analysis of genomic DNA (REAG) with the restriction enzyme *Hin*fI followed by conventional electrophoresis. The susceptibility of the isolates to the antifungal agents amphotericin B, flucytosine, fluconazole, and itraconazole was determined by methods recommended by the National Committee for Clinical Laboratory Standards. The MICs at which 90% of the isolates were inhibited ranged from 1.0 μg/ml for itraconazole to 64 μg/ml for fluconazole. In general, isolates from a given patient, or epidemiologically related isolates from a single institution, were identical by molecular typing methods. Epidemiologically unrelated isolates were distinctly different by the REAG typing method employed. These data document the genetic diversity and antifungal susceptibility of clinical isolates of *C. krusei*.**

Candida krusei has been noted as an occasional pathogen among hospitalized patients, particularly those with hematologic malignancies (2–5, 8, 20, 21). Although recent reports have linked the apparent emergence of *C. krusei* to the widespread use of fluconazole (2, 21), it must be recognized that the pattern of colonization and infection due to this organism was apparent in certain medical centers well before the introduction of fluconazole (3, 5, 20).

Despite the potential clinical significance of *C. krusei*, little is known of its genetic diversity and molecular epidemiology as defined by the newer DNA-based typing methods (1, 6). The availability of these typing methods plus the development of standardized methods for antifungal susceptibility testing provides an opportunity to examine the genotypic diversity and antifungal susceptibility of clinical isolates of *C. krusei* from multiple anatomic sites and from several different medical centers.

The test organisms include 67 isolates of *C. krusei* isolated from 44 patients and 5 health care workers from six different medical centers located in Iowa, Oregon, Georgia, Texas, Virginia, and Brazil. The isolates were obtained from cultures of blood, urine, stool, peritoneal fluid, and sputum and from samples from the throat, tissue biopsy specimens, and hands. Nine patients accounted for 27 isolates (2 isolates from each of three patients, 3 isolates from each of four patients, 4 isolates from one patient, and 5 isolates from one patient), and the remaining 40 isolates were obtained from individual patients or health care workers. All isolates were identified as *C. krusei* by standard methods (19).

Molecular typing of all isolates was accomplished by restriction endonuclease analysis of genomic DNA (REAG) with the restriction enzyme *Hin*fI followed by conventional electrophoresis as described by Carlotti et al. with slight modifications (1). Briefly, cells were imbedded in agarose (SeaKem GTG agarose; FMC BioProducts, Rockland, Maine) and lysed by incubation with lyticase (L5263; Sigma, St. Louis, Mo.), Sarkosyl (1%; Sigma), and proteinase K (0.75 mg/ml; Sigma). For restriction endonuclease digestion, agarose inserts containing chromosome-sized DNA were incubated with *Hin*fI as recommended by the manufacturer (New England Biolabs, Beverly, Mass.). Conventional horizontal electrophoresis was performed in 1% agarose at 40 V over 26 h in Tris-borate-EDTA buffer (pH 8.5). After electrophoresis, the gels were stained with ethidium bromide, illuminated under UV light, and photographed.

Analysis of REAG profiles was performed by visual inspection of photographs of ethidium bromide-stained gels to detect similarities and differences. All bands had to match exactly in order for isolates to be classified as identical; any difference in a major or minor band was considered important. Banding patterns differing by one or two bands were termed similar and were grouped under the same DNA type (≥90% of bands shared by a pair of patterns). Isolates with ≥3 bands different (<90% of bands shared) were considered different DNA types (12, 17, 18).

Broth microdilution antifungal susceptibility testing was performed according to the standard guidelines proposed by the National Committee for Clinical Laboratory Standards, as described previously (7, 10, 11, 13, 16). Antifungal agents were obtained from their respective manufacturers and included amphotericin B, flucytosine, fluconazole, and itraconazole. Quality control was ensured by testing a strain of *Candida parapsilosis* (ATCC 22019) and a strain of *C. krusei* (ATCC 6258) recommended for this purpose (9).

Genetic discrimination among the 67 isolates of *C. krusei* was achieved by REAG analysis with *Hin*fI (Table 1 and Fig. 1). A total of 28 distinct REAG profiles (DNA types) were identified. Generally, each DNA type represented an individ-

* Corresponding author. Mailing address: Department of Pathology, 273 MRC, University of Iowa College of Medicine, Iowa City, IA 52242. Phone: (319) 335-8170. Fax: (319) 335-8348.

† Present address: Unite de Lutte contre Les Infections Nosocomiales, CHRU de Lille, Hopital A. Calmette, 59037 Lille Cedex, France.

TABLE 1. Distribution of DNA types and recovery sites of clinical isolates of *Candida krusei* from six different medical centers[a]

Medical Center	Patient no.	Source of isolate	Date of isolation[b]	DNA type
A	1	Stool	6-05-90	B
	2	Stool	6-06-90	B
		Stool	6-25-90	B
	3	Stool	11-12-90	B
	4	Stool	11-27-90	B
	5	Stool	12-31-90	B
		Urine	1-08-91	B
	6	Stool	2-15-94	B
		Stool	3-15-94	B
		Urine	4-12-94	B
	7	Stool	8-16-94	R
		Urine	9-26-94	R
		Urine	9-26-94	R
		Urine	9-26-94	R
		Urine	10-17-94	R
	8	Peritoneal fluid	7-29-94	X
	9	Stool	12-24-94	X
		Stool	12-28-94	X
	10	Blood	2-11-85	P
	11	Stool	8-15-88	W
	12	Blood	4-07-90	A
		Blood	4-17-90	A
		Blood	5-19-90	A
		Blood	5-25-90	A
	13	Stool	8-29-90	D
	14	Stool	11-25-91	X
	15	Throat	1-20-92	R
	16	Tissue	9-15-93	K
	17	Stool	8-17-94	Q
	18	Stool	12-05-94	S
		Urine	12-20-94	S
		Stool	12-20-94	T
	19	Stool	1-17-95	U
		Urine	1-24-95	V
		Stool	1-30-95	V
	20	Stool	12-19-90	B
	21	Blood	1-27-93	L
	22	Stool	7-30-89	R
	23	Throat	7-24-90	C
	24	Stool	10-16-90	E
	25	Stool	9-17-91	X
	26	Stool	3-10-92	J
	27	Sputum	11-25-85	Z
	28	Blood	4-03-89	Z
	29	NA	9-01-87	H
	30	Tissue	12-27-91	X
	31	Tissue	10-07-93	F
	32	Peritoneal fluid	11-08-91	L
B	33	Stool	9-00-92	N
	34	Blood	4-11-91	O
		Blood	4-16-91	O
		Blood	4-19-91	O
	35	NA	1-25-93	P
	36	NA	NA	P
C	37	Blood	8-00-91	CC
	38	Rectum	8-10-94	BB
	39	Rectum	10-31-94	R
	40	Rectum	12-05-94	R
	41 (HCW)	Hands	6-00-94	R
	42 (HCW)	Hands	7-00-94	R
	43 (HCW)	Hands	7-00-94	R
	44 (HCW)	Hands	4-00-94	AA
D	45	NA	NA	F
	46 (HCW)	Hands	NA	G
E	47	Stool	NA	Y
	48	Stool	NA	F
F	49	Vagina	NA	M

[a] Abbreviations: HCW, health care worker; NA, not available.
[b] Month-day-year. 00, day unknown.

ual patient and epidemiologically unrelated isolates of *C. krusei* had distinctly different REAG profiles (Table 1 and Fig. 1). Patterns of multiple isolates from a single patient were the same regardless of the anatomic site or date of the isolates (Table 1 and Fig. 1). Among the nine patients with two or more isolates, only patients 18 and 19 had more than one DNA type of *C. krusei* isolated from clinical specimens (Fig. 1).

In several instances the same DNA type of *C. krusei* was obtained from epidemiologically related patients. DNA type B included 11 isolates from seven patients hospitalized in the bone marrow transplant unit of medical center A. DNA types L and X included isolates from two and five patients, respectively, all hospitalized in the surgical service of medical center A. DNA type R included seven isolates from three patients hospitalized in the bone marrow transplant unit of medical center A and five isolates from two patients and three health care workers from an intensive care unit of medical center C. The clustering of isolates with the same DNA type within a given institution suggests possible nosocomial transmission in that institution.

The in vitro susceptibilities of the 67 *C. krusei* isolates to four antifungal agents are shown in Table 2. As expected, the fluconazole MICs were uniformly high for all isolates. Likewise, the decreased susceptibility (relative to those of other species of *Candida*) to both amphotericin B (MIC at which 90% of the isolates were inhibited [MIC_{90}] = 2.0 μg/ml) and flucytosine (MIC_{90} = 32 μg/ml) was consistent with previous values reported for this organism (10). Itraconazole was the most potent of the four antifungal agents tested; however, the MIC_{90}

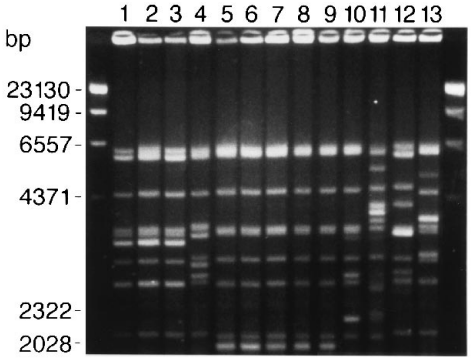

FIG. 1. Representative REAG profiles of *C. krusei* obtained by using *Hin*fI followed by conventional electrophoresis (in 1% agarose at 40 V for 26 h). Lanes: leftmost and rightmost, lambda phage DNA *Hin*dIII digest as molecular size standards; 1 to 3, type B from patient 6; 4, type Q from patient 17; 5 to 9, type R from patient 7; 10 and 11, types S and T, respectively, from patient 18; 12 and 13, types U and V, respectively, from patient 19.

TABLE 2. In vitro susceptibilities of 67 *Candida krusei* isolates to four antifungal agents as determined by broth microdilution testing

Antifungal agent	MIC (μg/ml)		
	Range	50%	90%
Amphotericin B	0.5–2.0	1.0	2.0
Flucytosine	4.0–32	16	32
Fluconazole	16–>128	32	64
Itraconazole	0.5–2.0	1.0	1.0

of 1.0 μg/ml is approximately fourfold higher than that observed for other species of *Candida* (14, 15).

The results of the present study confirm and extend the observations of previous investigators regarding the usefulness of DNA-based typing methods for strain delineation of *C. krusei* (1, 6). In agreement with Carlotti et al. (1), we found READ analysis with *Hin*fI to be an excellent means of delineating individual strains of *C. krusei*. With rare exceptions this typing method identified epidemiologically unrelated isolates as different yet was still able to identify multiple isolates from the same patient or epidemiologically related isolates from a nosocomial cluster as the same strain.

In general, each patient was infected or colonized with his or her own distinct DNA type. Multiple isolates from the same or different anatomic sites represented the same DNA type and support the suggestion by Carlotti et al. (1) of an endogenous origin for the infecting and colonizing *C. krusei* isolates. The inclusion of isolates from multiple institutions further emphasizes the tremendous strain diversity seen with *C. krusei.*

Despite the large number of strains identified, DNA typing was still able to identify clusters of epidemiologically related isolates with the same READ profile, suggesting possible nosocomial transmission. Although Carlotti et al. (1) did not find similar evidence of nosocomial transmission, the differences between the two studies may be due simply to the patient populations studied and the effectiveness of infection control practices at the different institutions.

Despite the well-known lack of susceptibility of *C. krusei* to fluconazole, only a limited amount of data regarding the in vitro susceptibility of *C. krusei* to antifungal agents, determined by the recently standardized testing methods (10, 13, 16), has been presented. The present study demonstrates a pattern of decreased susceptibility to amphotericin B and flucytosine as well as to the azole class of antifungal agents. Although a decreased level of susceptibility of *C. krusei* to amphotericin B is not widely appreciated, it is notable that the review of Goldman et al. (2) found that response rates of *C. krusei* infection were significantly better in patients who had received amphotericin B in doses of ≥1 mg/kg of body weight per day than in patients who had received lower doses. Furthermore, the in vitro studies of Merz et al. (5), Wingard et al. (21), and Pfaller and Barry (10) have all shown that isolates of *C. krusei* may be less susceptible to amphotericin B than isolates of *Candida albicans* from similar populations (2). Taken together, these data suggest that *C. krusei* may merit consideration as a multiply resistant nosocomial pathogen.

In summary, we have documented the genetic diversity and relatively decreased susceptibility of *C. krusei* to several antifungal agents. Although *C. krusei* may be considered an endogenous pathogen in most clinical settings, the potential for transmission of a strain from patient to patient in the hospital environment must be considered. The relative resistance of this organism to several antifungal agents, coupled with the potential for nosocomial transmission, suggests that *C. krusei* may well become a major nosocomial pathogen in certain hospital environments.

We thank Kay Meyer for secretarial assistance in the preparation of the manuscript.

This study was partially supported by a grant from Pfizer Pharmaceuticals—Roerig Division.

REFERENCES

1. **Carlotti, A., R. Grillot, A. Couble, and J. Villard.** 1994. Typing of *Candida krusei* clinical isolates by restriction endonuclease analysis and hybridization with CkF1,2 DNA probe. J. Clin. Microbiol. **32:**1691–1699.
2. **Goldman, M., J. C. Pottage, and D. C. Weaver.** 1993. *Candida krusei* fungemia: report of four cases and review of the literature. Medicine (Baltimore) **72:**143–150.
3. **Iwen, P. C., D. M. Kelly, E. C. Reed, and S. H. Hinrichs.** 1995. Invasive infection due to *Candida krusei* in immunocompromised patients not treated with fluconazole. Clin. Infect. Dis. **20:**342–347.
4. **McQuillen, D. P., B. S. Zingman, F. Meunier, and S. M. Levitz.** 1992. Invasive infections due to *Candida krusei*: report of ten cases of fungemia that include three cases of endophthalmitis. Clin. Infect. Dis. **14:**472–478.
5. **Merz, W. G., J. E. Karp, D. Schron, and R. Saral.** 1986. Increased incidence of fungemia caused by *Candida krusei*. J. Clin. Microbiol. **24:**581–584.
6. **Morace, G., M. Sanguinetti, L. Pagano, and B. Posteraro.** 1994. Epidemiological characterization of *Candida krusei* clinical isolates using molecular techniques. J. Mycol. Med. **4:**191–195.
7. **National Committee for Clinical Laboratory Standards.** 1992. Reference method for broth dilution antifungal susceptibility testing for yeasts. Proposed standard M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
8. **Pfaller, M. A.** 1996. Nosocomial candidiasis: emerging species, reservoirs, and modes of transmission. Clin. Infect. Dis. **22**(Suppl. 2)**:**589–594.
9. **Pfaller, M. A., M. Bale, B. Buschelman, M. Lancaster, A. Espinel-Ingroff, J. H. Rex, M. G. Rinaldi, C. R. Cooper, and M. R. McGinnis.** 1995. Quality control guidelines for National Committee for Clinical Laboratory Standards recommended broth macrodilution testing of amphotericin B, fluconazole, and flucytosine. J. Clin. Microbiol. **33:**1104–1107.
10. **Pfaller, M. A., and A. L. Barry.** 1995. In vitro susceptibilities of clinical yeast isolates to three antifungal agents determined by the microdilution method. Mycopathologia **130:**3–9.
11. **Pfaller, M. A., C. Grant, V. Morthland, and J. Rhine-Chalberg.** 1994. Comparative evaluation of alternative methods for broth dilution susceptibility testing of fluconazole against *Candida albicans*. J. Clin. Microbiol. **32:**506–509.
12. **Pfaller, M. A., R. J. Hollis, and H. S. Sader.** 1992. Chromosomal restriction fragment analysis by pulsed-field gel electrophoresis, p. 10.5.c.1-10.5.c.12. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
13. **Pfaller, M. A., S. A. Messer, and S. Coffmann.** 1995. Comparison of visual and spectrophotometric methods of MIC endpoint determinations by using broth microdilution methods to test five antifungal agents, including the new triazole D0870. J. Clin. Microbiol. **33:**1094–1097.
14. **Pfaller, M. A., S. A. Messer, and R. J. Hollis.** 1994. Strain delineation and antifungal susceptibilities of epidemiologically related and unrelated isolates of *Candida lusitaniae*. Diagn. Microbiol. Infect. Dis. **20:**127–133.
15. **Pfaller, M. A., S. A. Messer, and R. J. Hollis.** 1995. Variations in DNA subtype, antifungal susceptibility, and slime production among clinical isolates of *Candida parapsilosis*. Diagn. Microbiol. Infect. Dis. **21:**9–14.
16. **Rex, J. H., M. A. Pfaller, A. L. Barry, P. W. Nelson, and C. D. Webb for the NIAID Mycoses Study Group and the Candidemia Study Group.** 1995. Antifungal susceptibility testing of isolates from a randomized, multicenter trial of fluconazole versus amphotericin B as treatment of nonneutropenic patients with candidemia. Antimicrob. Agents Chemother. **39:**40–44.
17. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
18. **Voss, A., M. A. Pfaller, R. J. Hollis, J. Rhine-Chalberg, and B. N. Doebbeling.** 1995. Investigation of *Candida albicans* transmission in a surgical intensive care unit cluster by using genomic DNA typing methods. J. Clin. Microbiol. **33:**576–580.
19. **Warren, N. G., and K. C. Hazen.** 1995. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 723–737. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
20. **Wingard, J. R.** 1995. Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. Clin. Infect. Dis. **20:**115–125.
21. **Wingard, J. R., W. G. Merz, M. G. Rinaldi, T. R. Johnson, J. E. Karp, and R. Saral.** 1991. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N. Engl. J. Med. **325:**1274–1277.