MAJOR ARTICLE

Vaginitis Due to *Candida krusei:* Epidemiology, Clinical Aspects, and Therapy

Shivani Singh, Jack D. Sobel, Pallavi Bhargava, Dina Boikov, and Jose A. Vazquez
Division of Infectious Diseases, Wayne State University School of Medicine, Detroit, Michigan

Twelve women with vaginal *Candida krusei* infection were evaluated. In vitro antifungal susceptibility testing and molecular typing were performed. Patients infected with *C. krusei* frequently had refractory vulvovaginal signs and symptoms that were otherwise indistinguishable from vaginitis due to other yeasts. Patients were 32–63 years old and had previously received multiple courses of antimycotic agents, including fluconazole and miconazole. The most active azole in vitro was clotrimazole, with a 90% minimum inhibitory concentration of 0.25 μg/mL. Four of 6 patients treated with boric acid had clinical and mycological cure. Two dominant genotypes of *C. krusei* were identified via contour-clamped homogenous electrical field analysis. No major genotypic change was observed in successive isolates from the same patient in most cases, suggesting that these refractory cases were relapses. *C. krusei* is a rare but important cause of refractory vaginitis and is unique because of its intrinsic resistance to fluconazole.

Vulvovaginal candidiasis is one of the most common infections of the female genital tract. Most (80%–85%) cases are caused by *Candida albicans* [1–3], whereas the non-*albicans* species of *Candida* account for a mere 5%–20% of the cases. Most of these infections with non-*albicans* species of *Candida* are due to *Candida glabrata* (5%–10% of cases) or *Candida tropicalis* (<5% of cases) [3, 4]. *Candida krusei* is an unusual cause of fungal vaginitis. In fact, several investigators have questioned whether *C. krusei* is a true vaginal pathogen [5].

We present the results of an investigation of 12 patients with *C. krusei* vaginitis. Analysis included an in-depth chart review and in vitro antifungal susceptibility testing of 26 vaginal isolates of *C. krusei,* results of which were compared with the susceptibility test results for 8 nonvaginal *C. krusei* isolates. In addition, treatment outcome evaluation and molecular analysis were performed.

PATIENTS AND METHODS

Patients and yeast strains. The vaginitis clinic at Wayne State University (Detroit) was established in 1985 to evaluate women referred for chronic vaginitis symptoms. There are ~1200 patient visits per year, including an average of 200 new patients per year. Retrospective review of our records from 1987 through 2000 identified 12 women with *C. krusei* in their vaginal secretions. These 12 women had a total of 26 *C. krusei* isolates. Ten charts were available for review, and 9 patients had undergone complete follow-up.

Candida vaginitis was defined as the presence of vulvovaginal symptoms (discharge, pruritus, soreness, and burning) and signs (erythema, edema, and excoriation) of inflammation in patients for whom resolution of these symptoms and signs was accompanied by the achievement of cultures negative for *Candida* species following antimycotic therapy. In addition, other causes of vaginitis (i.e., bacterial vaginosis and trichomoniasis) were excluded.

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Informed consent was obtained from the patients, and guidelines for human experimentation of the US Department of Health and Human Services and those of the authors' institution were followed in the conduct of the clinical research.

Reprints or correspondence: Dr. Jose A. Vazquez, Div. of Infectious Diseases, Harper University Hospital, 3390 John R. Rd., 4 Brush Ctr., Detroit, MI 48201 (jvazquez@intmed.wayne.edu).

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For each patient, a detailed history was obtained, a bimanual examination with use of a speculum was performed, and swab samples from high in the vaginal tract were obtained for laboratory analysis. All patients received treatment, and long-term follow-up was done to evaluate response to therapy.

Laboratory procedures. Vaginal secretions were analyzed for pH, and amine testing and microscopy were performed with use of 10% KOH and normal saline. In addition, vaginal swabs were immediately inoculated on Sabouraud dextrose agar plates and incubated at 30.0°C for 48 h. Cultures positive for yeast were evaluated for germ-tube and chlamydospore formation. All yeast isolates were identified to the species level with the API 32C Yeast Identification Kit (bioMérieux). All fresh *Candida* isolates were stored in litmus milk at −70.0°C to await susceptibility testing and molecular strain delineation at a later date.

In vitro antifungal susceptibility analysis. Thirty-four *C. krusei* isolates, 26 vaginal and 8 nonvaginal, were evaluated by the broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS) M27-A standards [6]. Antifungal agents tested included clotrimazole, miconazole, fluconazole, voriconazole, itraconazole, caspofungin acetate, and amphotericin B (AmB). The MIC for AmB was defined as the lowest drug concentration that prevented any discernible growth. The MIC for other antifungals was defined as the lowest concentration that inhibited growth by 80% as detected visually; this result was confirmed by diluting 0.2 mL of drug-free control growth medium with 0.8 mL of medium to produce an 80% inhibition standard, in accordance with NCCLS guidelines.

Electrophoretic karyotyping. Strain delineation (i.e., genotyping) was performed via contour-clamped homogenous electrical field (CHEF) electrophoresis. Electrophoresis was performed on intact chromosomal DNA, prepared as described elsewhere [7]. Photographs of stained gels were examined visually. An isolate was considered to be a different strain if there was a difference in the position or number of $\geqslant$1 bands. A single-band difference was used to delineate genotypes, because a single-band difference between CHEF gel patterns for *Candida* isolates (unlike for prokaryotes) signifies a large and detectable size variation in chromosomal bands. All gels were analyzed in duplicate to assure reproducibility, as demonstrated elsewhere [7].

RESULTS

Chronic vulvovaginal symptoms were present for all the patients, although individual symptoms and signs were indistinguishable from vaginitis due to other yeasts. Patient ages ranged from 32 to 63 years, with a mean of 44 years. Seven of 10 patients were white. Four of 10 patients had undergone menopause. Nine of 10 patients reported recent exposure to antifungal drugs, including fluconazole for 6 patients. In specimens from 6 of 10 patients, only blastospores (budding yeast) were seen; in specimens from 3 patients, additional hyphal elements were observed.

One patient had a mixed culture with *C. krusei* and *Candida glabrata.* Three of 10 patients had different *Candida* species recovered sometime during follow-up. The other *Candida* species recovered during follow-up included *C. albicans, C. tropicalis, C. glabrata,* and *C. guilliermondii.*

In vitro susceptibility analysis. Clotrimazole was the most active azole, with an MIC_{50} of 0.125 μg/mL and an MIC_{90} of 0.25 μg/mL. The MIC_{90} of caspofungin, voriconazole, itraconazole, and AmB was 1.0 μg/mL. The MIC_{50} of fluconazole was 32 μg/mL, and the MIC_{90} was >64 μg/mL. The MIC_{50} of miconazole was 2.0 μg/mL, and the MIC_{90} was 4 μg/mL. There was no significant difference in the MIC_{50} values determined for the vaginal and nonvaginal isolates. Table 1 shows the comparison between the MIC_{90} of various antifungals for vaginal

Table 1. Comparison of in vitro susceptibilities of *Candida krusei* and *Candida albicans* vaginal isolates.

	C. albicans (n = 20)			*C. krusei* (n = 26)		
Antifungal agent	MIC range, μg/mL	MIC_{50}, μg/mL	MIC_{90}, μg/mL	MIC range, μg/mL	MIC_{50}, μg/mL	MIC_{90}, μg/mL
Clotrimazole	0.006–0.50	0.010	0.06	0.030–0.50	0.125	0.25
Caspofungin	0.050–1.00	0.250	0.50	0.060–2.00	0.500	1.00
Fluconazole	0.130–8.00	0.130	2.00	32 to >64	32	>64
Itraconazole	0.016–0.25	0.016	0.13	0.25–2.00	0.500	1.00
Voriconazole	0.006–0.13	0.030	0.03	0.25–1.00	0.250	1.00
Miconazole	0.010–0.13	0.013	0.03	1.00–4.00	2.000	4.00
Amphotericin B	0.030–0.50	0.120	0.25	0.25–1.00	1.000	1.00

NOTE. *C. albicans* isolates were recovered from women who experienced recurrent vaginal candidiasis [8].

Table 2. Antifungal therapy and outcome for 12 patients with *Candida krusei* vaginitis.

Patient	Outcome, by antifungal(s) administered							Treatment duration, weeks	Comments
	BA	CLZ	ITZ	KTZ	5-FC	AmB	Nystatin		
1	—	C	—	F	—	—	—	36	Infection cleared with 36 weeks of CLZ therapy
2	F	F/C	—	—	—	F	—	14	Infection failed to respond to 2 weeks of therapy with BA. Infection improved with 12 weeks of CLZ therapy but relapsed when treatment was terminated. Infection failed to respond to 2 weeks of therapy with AmB 3% cream. Cure was achieved with 14 weeks of CLZ.
4	C	—	—	—	—	—	—	4	Cure was achieved with 4 weeks of BA.
5	F/C	—	—	—	—	—	—	6	Infection failed to respond to 2 weeks of therapy with BA. Cure was achieved with 6 weeks of high-dose, twice-daily therapy with BA.
6	C	—	—	—	—	—	—	6	Cure was achieved with 6 weeks of therapy with BA. Recovered *C. tropicalis* after therapy.
8	F	F	F	—	—	F/C	—	4	Infection failed to respond to therapy with BA. Infection cleared with 6 weeks of therapy with CLZ but relapsed when treatment was terminated. Transient improvement with 12 weeks of therapy with AmB cream. Infection failed to respond to ITZ therapy.
9	—	—	F	C	—	—	—	1.4	Infection failed to respond to 2 weeks of therapy with ITZ. Cure was achieved with 10 days of KTZ therapy.
10	C	—	—	—	—	—	C	6	Cure was achieved with sequential treatment with BA for 2 weeks, then nystatin for 4 weeks. Coinfection with *Candida glabrata* not cleared.
12	—	—	—	—	C	—	C	4	*C. krusei* and *C. glabrata* recovered after FLZ therapy for *Candida guillermondii* vaginitis. Cure was achieved with combination therapy with FC for 2 weeks and nystatin for 4 weeks.

NOTE. Charts were not available for patients 3 and 11. Patient 7 was lost to follow-up. Dashes indicate that the drug was not given. 5-FC, flucytosine; AmB, amphotericin B; C, patient cured; CLZ, clotrimazole; F, infection failed to respond to therapy; ITZ, itraconazole; KTZ, ketoconazole.

isolates of *C. albicans* and *C. krusei*, which underscores that antifungals had higher MICs for *C. krusei*.

Treatment outcome. Table 2 shows treatment outcomes. In total, 7 courses of boric acid were provided to 6 patients [9]. Cure was achieved in 4 of these 6 patients. In addition, 4 prolonged daily courses of topically administered clotrimazole (duration of therapy, 6 weeks) were provided to 3 patients; cure was achieved for 2 of the patients. Three courses of topical 3% AmB were prescribed for 2 patients, but only 1 of the patients achieved a cure. Ketoconazole therapy had a successful result for 1 patient. Topically administered flucytosine, followed by maintenance therapy with topical nystatin, resulted in cure for 1 patient.

Overall, the duration of therapy for all the patients ranged from 10 days to 36 weeks (median, 6 weeks). The duration of follow-up for these patients ranged from 4.5 months to >6 years. For 1 patient, cultures remained persistently positive for *C. krusei* in spite of therapy with boric acid, AmB, clotrimazole, and itraconazole, although symptoms were suppressed.

Genotyping. All isolates revealed typical *C. krusei* genotypes [10], with 2 chromosomal bands at the lower molecular weight level, between 1.66 and 1.37 Mb, and 2–3 chromosomal bands at the higher molecular weights, between 2.7 and 3.13 Mb. Six different *C. krusei* genotypes were identified (table 3). The genotypes designated CK-1 and CK-2 were the most common strain types. Serial isolates of *C. krusei* from 8 patients were evaluated (table 4). For 5 of the 8 patients, isolates from serial samples revealed no change in genotype throughout follow-up. For 3 patients (patients 2, 9, and 11) a change in genotype identified in serial vaginal isolates. However, the differences in patterns were minor and were only a change in position of no more than 1 chromosomal band. Patient 2 demonstrated infection first with strain CK-2, then with strain CK-1, then again with genotype CK-2 (figure 1). Thus, the first and the last isolates had identical genotypes. For patient 9, isolates of 2 different genotypes, CK-2 and CK-5, were identified sequentially (figure 2). Patient 11 was also sequentially infected with 2 different genotypes of *C. krusei* (CK-2, then CK-1) (figure 2).

Table 3. Genotypic characteristics of *Candida krusei* vaginal isolates.

Genotype	No. of patients (n = 12	No. of isolates (n = 26)
CK-1	7	16
CK-2	3	5
CK-3	1	1
CK-4	1	1
CK-5	1	1
CK-6	1	2

Table 4. Genotypes and duration of follow-up for patients with *Candida krusei* vaginitis.

Patient	No. of isolates	Genotype(s) of isolates	Duration of follow-up
1	5	CK1, CK1, CK1, CK1, CK1	6 years, 5 months
2	3	CK2, CK1, CK2	7 months, 2 weeks
3	1	CK4	No chart
4	2	CK1, CK1	6 months, 1 week
5	3	CK1, CK1, CK1	3 months, 1 week
6	1	CK3	1 year, 8 months
7	1	CK1	No follow-up
8	2	CK1, CK1	1 year
9	2	CK2, CK5	1 year, 8 months
10	2	CK6, CK6	8 months
11	3	CK2, CK1, CK1	No chart
12	1	CK2	4 months, 2 weeks

DISCUSSION

C. krusei has, as our report illustrates, emerged as a true, albeit uncommon, cause of fungal vaginitis; the estimated annual incidence *C. krusei* vaginitis among all types of fungal vaginitis is ~1%. In spite of the acknowledged accrual bias, all the patients described in this report presented with chronic and treatment-resistant symptoms. In this series, *C. krusei* was predominately seen as a cause of vaginitis in comparatively older women; all of the women in this series were >30 years old (mean age, 44 years). We are unable to explain the epidemiologic trend toward infection in older women. Nevertheless, a possible pathophysiological explanation for the selection of *C. krusei* may be that these patients all experienced repeated episodes of vulvovaginal candidiasis and thus had been exposed to numerous courses of a wide array of antifungals, predominately azoles. As has been described for immunocompromised patients, it is possible that repeated exposure to antifungals, including topical agents, over a prolonged period of time may cause a shift in the vaginal mycoflora from the more drug-susceptible *C. albicans* to the less drug-susceptible *Candida* species, such as *C. krusei* [11, 12].

In addition, although it is a remote possibility and certainly not well evaluated, it may be possible that these women just happen to be colonized from the onset with a *C. krusei* isolate that they were exposed to in their environment. Because *C. krusei* is on occasion recovered from the environment and has well-known intrinsic fluconazole resistance, patients are now experiencing the consequences of being exposed to and infected with a highly resistant organism. We also evaluated the use of antifungals and/or fluconazole as a mechanism for selection of *C. krusei*. Unfortunately, almost all women with refractory yeast vaginitis referred to the vaginitis clinic had been previously exposed to at least 1 course of fluconazole and, very frequently, multiple courses of topical azoles.

In patients with chronic and recurrent fungal vaginitis, it should never be assumed that the yeast species responsible is invariably *C. albicans*. Signs and symptoms of vaginitis due to *C. krusei* appear to be indistinguishable from those for vaginitis due to other *Candida* species, an observation that emphasizes the need to obtain vaginal specimens for culture from all patients with refractory and recurrent disease. In addition, the presence on wet mount of blastospores only, as opposed to hyphae, should alert the practitioner to the increased likelihood of finding a non-*albicans* species of *Candida*. As predicted, the *C. krusei* isolates recovered from our patients were found to be highly resistant to fluconazole (MIC_{90}, >64 μg/mL). The isolates were also resistant to miconazole (MIC_{90}, 4 μg/mL), one of the most commonly used over-the-counter topical antifungal agents.

Vaginal *C. krusei* strains were most susceptible to clotrimazole on in vitro susceptibility testing. Although caspofungin is active against *C. krusei*, it is not available for oral or topical administration, nor has the US Food and Drug Administration (FDA) approved it for use in infections due to *Candida* species. Voriconazole and itraconazole also demonstrated favorable in vitro antifungal activity; however, they are not available as topical preparations, nor do they have FDA approval for systemic use in patients with vaginitis. AmB also showed in vitro activity against the *C. krusei* isolates. Fortunately, AmB may also be administered as a 3% topical preparation, although it also has not been approved by the FDA for this purpose.

Prolonged, not abbreviated, therapy with either topical boric

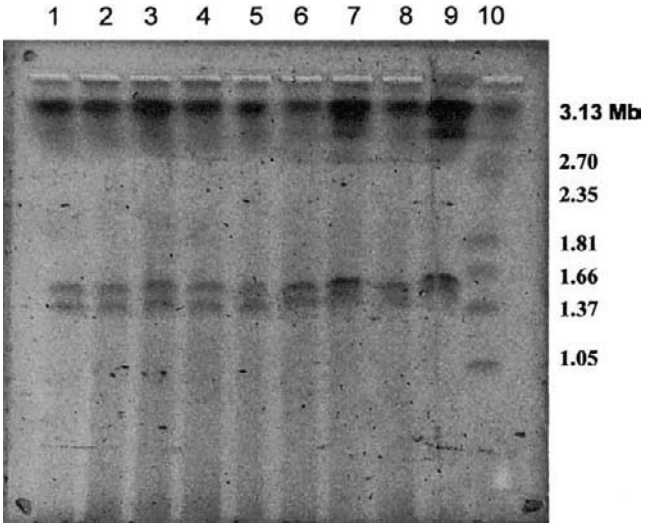

Figure 1. Electrophoretic karyotypes of serial isolates of *Candida krusei* recovered from patients 1, 2, and 3. *Lanes 1–5,* Patient 1; *lanes 6–8,* patient 2; *lane 9,* patient 3; *lane 10, Hansenula wingei* control.

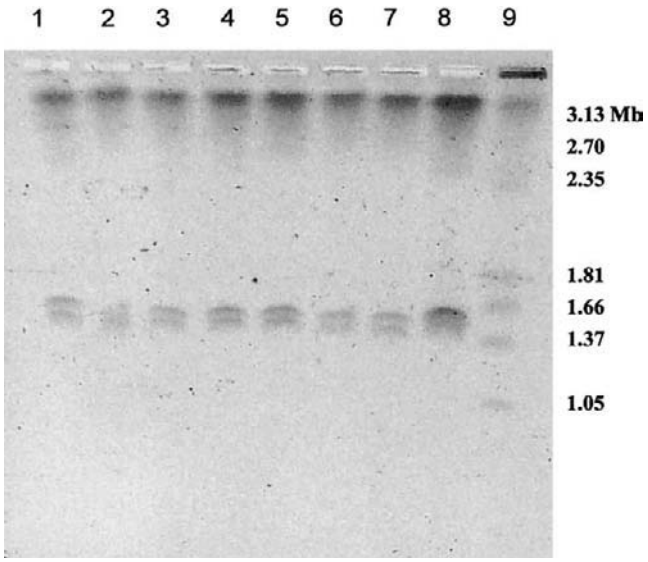

Figure 2. Electrophoretic karyotypes of serial isolates of *Candida krusei* recovered from patients 9, 10, 11, and 12. *Lanes 1–2,* Patient 9; *lanes 3–4,* patient 10; *lanes 5–7,* patient 11; *lane 8,* patient 12; *lane 9, Hansenula wingei* control.

acid or topical clotrimazole or oral therapy with either ketoconazole or itraconazole should be considered as first-line therapy for patients with *C. krusei* vaginitis. Therapy with all active antifungal agents should also be prolonged (duration, usually 2–6 weeks), regardless of the agent used. Unfortunately, these recommendations are based on a retrospective review of our clinical treatment experience; no other data are currently available in the literature.

The indolent nature of *C. krusei* vaginitis, along with the identical genotypes of consecutive isolates from the majority of patients, strongly suggest that recurrent infection is due to vaginal relapse, not reinfection. This pattern is similar to that seen in women who experience recurrent vaginitis due to *C. albicans,* as described elsewhere [8, 13]. However, how does one explain the consecutive appearance of a new nonidentical genotype that was observed in 3 of the 8 patients in our patient population? Given the rarity of *C. krusei* vaginitis or vaginal colonization, the possibility of being exposed to and infected with a new and different strain is unlikely.

One possible theory is that the same strain may be responsible for all relapses; however, small variations may occur in the original genotype, possibly as the resident strain undergoes evolutionary adaptive changes under antimycotic pressure. A second possibility is that $\geqslant 2$ strains of *C. krusei* may coexist in the vagina simultaneously, and, because of sampling error, 1 strain was recovered on the initial sample, and the second strain was recovered during serial sampling. Another possibility is that the initial infecting strain was replaced by an unrelated strain during a subsequent episode of vaginitis.

In conclusion, this is, to our knowledge, the first detailed description of a large series of patients with vaginitis caused by *C. krusei. C. krusei* should be considered as a cause of refractory vaginitis, especially in older white women who seek care for chronic vaginitis and whose infections respond poorly to conventional antimycotics. An early indication of infection with a non-*albicans* species of *Candida* may be found via routine microscopy, which will frequently reveal only blastospores on wet films. It is essential to identify the species of *Candida* in all patients with refractory infections, because the identification of *C. krusei* will influence the selection of antimycotic agents and the duration of therapy. Furthermore, as in most cases of recurrent vulvovaginal candidiasis, recurrence is likely due to vaginal relapses with the same genotype, rather than reinfection with a new strain.

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