GYNECOLOGY

Recurrent vulvovaginal candidiasis

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Recurrent vulvovaginal candidiasis (RVVC) is a common cause of significant morbidity in women in all strata of society affecting millions of women worldwide. Previously, RVVC occurrence was limited by onset of menopause but the widespread use of hormone replacement therapy has extended the at-risk period. *Candida albicans* remains the dominant species responsible for RVVC, however optimal management of RVVC requires species determination and effective treatment measures are best if species-specific. Considerable progress has been made in understanding risk factors that determine susceptibility to RVVC, particularly genetic factors, as well as new insights into normal vaginal defense immune mechanisms and their aberrations in RVVC. While effective control of RVVC is achievable with the use of fluconazole maintenance suppressive therapy, cure of RVVC remains elusive especially in this era of fluconazole drug resistance. Vaccine development remains a critical challenge and need.

Key words: antifungals, antimycotics, *Candida* vaginitis, vaginal candidosis, vulvovaginal candidiasis

Originally termed "acute *Candida* vaginitis," vaginal infection by *Candida* species has transformed into the concept of vaginal candidiasis or candidosis in the United Kingdom, due to recognition of a wider spectrum of symptomatic and asymptomatic disease.[1] Subsequent recognition that the dominant site of inflammation and source of symptoms is the vulva led to the term "vulvovaginal candidiasis" (VVC). While most episodes of symptomatic disease appear as sporadic attacks of acute VVC, some women have more chronic or long-term daily manifestations and symptoms are infrequently diagnosed as mycotic in origin.[2] Yet another subgroup has emerged with recurrent episodes, being entirely asymptomatic between episodes (recurrent VVC [RVVC]). The entity of RVVC has been defined as at least 3 symptomatic episodes in the previous 12 months, although some investigators demand yet an additional episode, ie, 4 attacks.[3,4] This is an entirely arbitrary differentiation, not based on any data or study and likely women so identified by each definition are identical.

It has become impossible in any open, free society to perform epidemiologic studies and determine the frequency of women with RVVC.[4,5] This is, firstly, a consequence of the widespread availability of over-the-counter (OTC) antifungal agents. Procurement of the easily available, numerous highly effective topical antimycotic agents, although controversial, can be considered a boon to women's health. OTC availability clearly has given women access to rapid symptom relief, but is still not inexpensive and open access should have been accompanied by a diagnostic test that would have allowed a woman to correctly diagnose yeast as the cause of her symptoms. Regrettably self-diagnosis is unreliable, with significant overdiagnosis of VVC in the presence of extremely nonspecific and common symptoms. Numerous studies have shown the drawbacks of OTC availability of antifungal agents.[6] Fortunately, the excessive use and overuse of such topical agents has had infrequent adverse consequences, safety has been maintained, and drug resistance as a consequence of frequent use remains rare.

The inadequacies of self-diagnosis of VVC are compounded by practitioner overdiagnosis and underdiagnosis, with US standards comparable to those of syndromic methods in nonindustrialized countries. Current clinical approaches are too often based on empiricism and trial and error. These factors have contributed to poor data availability of the frequency of both acute VVC and RVVC.[5] Estimation of the prevalence of RVVC is similarly marred by physician diagnostic inaccuracies. A recent study using online computer technology reaffirmed a large earlier study reporting self-professed RVVC in 6-9% of women.[7,8]

Of note, in an expanding population of postmenopausal women isolated studies have supported the clinical impression that a growing number of older women remain at risk of VVC and RVVC as the result of hormone replacement therapy, especially vaginal topical use.[9] It is estimated that RVVC affects approximately 138 million women worldwide annually and 492 million over their lifetimes (David Denning, MD oral communication July 24, 2015). Unfortunately true population-based studies of RVVC are rare. Moreover, the natural history of RVVC over a lifetime has not been addressed. Foxman et al[8] obtained data on duration of symptoms linked with age in 247 women. Most women reported duration of RVVC to be 1-2 years although a substantial number had symptoms for 4 or 5 years and some very much longer, with risk and symptoms lasting decades.

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Given that *C albicans* remains responsible for >90% of episodes of

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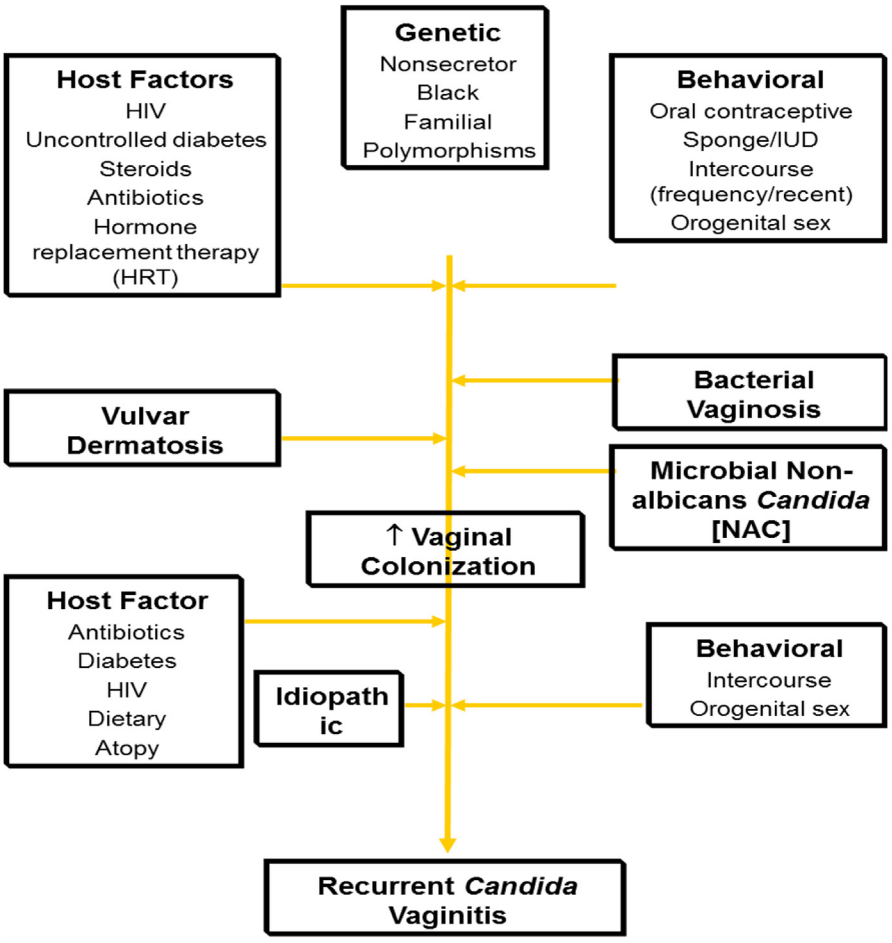
FIGURE
Pathogenesis of recurrent vulvovaginal candidiasis



IUD, intrauterine device; *RVVC*, recurrent vulvovaginal candidiasis.

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acute sporadic VVC in most studies worldwide, it is not surprising that *C albicans* similarly is responsible for the majority of infections in women with RVVC.[4,10-13] Approximately 85-95% of women with RVVC have azole-sensitive *C albicans* as the causative pathogen, implying that the host rather than the pathogen contributes dominantly to the pathophysiology of RVVC.[4,10] Of the non-*albicans Candida* species, *C glabrata* is the most frequently isolated species from the vagina in symptomatic and asymptomatic women.[4] Risk factors for *C glabrata* include type 2 diabetes, postmenopausal status, and older age.[4] The recent introduction of glycosuria-inducing agents to treat type 2 diabetics is also reported to increased prevalence VVC due to *C glabrata*.[14] In contrast to adults with invasive candidiasis and candidemia, azole exposure has not emerged as a risk factor for *C glabrata* in women with RVVC. It would appear that all non-*albicans Candida* species have substantially reduced capacity to express virulence characteristics and hence serve as vaginal pathogens. The implications are important in that the mere isolation of *C glabrata* and other non-*albicans* species in symptomatic women with vaginitis does not confirm causality. A cause-effect likelihood is far lower than that defined for *C albicans*, with likelihood of *C glabrata*, isolated from the vagina, being responsible for symptoms no higher that 20-30%.[15] Accordingly, in such women other causes for symptoms require active exclusion, before embarking on antifungal therapy, which is less likely to eradicate these low virulence, innocent bystanders also more resistant to antifungal agents.[16] Occasionally major geographic differences in *Candida* species distribution are reported and more reports usually indicate a higher frequency of *C glabrata* occurrence.[10]

Pathogenesis of RVVC

Candida blastospores (yeast) migrate from the lower gastrointestinal tract to the adjacent vestibule and vagina. This is a similar route taken by vaginal *Lactobacillus* species. Colonization of the vagina follows usually in low numbers after adherence of *Candida* to vaginal epithelial cells. Colonization resistance is poorly studied and understood but colonization is enhanced by an estrogen-influenced environment following menarche and declines in the postmenopause period. In healthy women not prone to RVVC, asymptomatic colonization may persist for months and years as yeasts live in symbiosis with vaginal microbiota. Acute symptomatic VVC follows a breakdown in this relationship and entails either a triggered overgrowth of *Candida* organisms or alteration in the host protective defense mechanisms, which act to maintain low numbers of yeast organisms and at the same time deliberately down-regulate the mucosal immune inflammatory response aimed at tolerating the presence of low numbers of yeast. An increased rate of vaginal colonization represents but 1 phase of susceptibility to RVVC and causes may be genetic, biologic, or behavioral as shown in the Figure. Description of *Candida* virulence factors is outside the scope of this review.

The innate immune system provides the first barrier against vulvovaginal *Candida* infections. Pattern recognition receptors on innate immune cells sense molecular moieties on the surface of yeast, and thereafter induce an intracellular signal within epithelial cells that stimulates production of effector molecules such as proinflammatory cytokines

or defensins. Toll-like receptors (TLRs) and C-type lectin receptors recognize numerous components of fungal cell walls. Single nucleotide polymorphisms in host genes are associated with an increased susceptibility to candidiasis. In recent years, it has become evident that interferon-gamma production, and interleukin (IL)-23-dependent, IL-17 and IL-22 signaling (IL-1/Th17 axis) are critical for protection against mucosal candidiasis in mice and possibly in human beings.[17-21]

Primary or idiopathic RVVC defines women in whom secondary precipitating events or triggering factors are not apparent and hence genetic factors are likely to play a dominant or exclusive mechanism. Nevertheless, even when acute VVC episodes are consistently linked to secondary identifiable triggering mechanisms or events it is likely that genetic susceptibility is still relevant in pathogenesis of RVVC attacks. A genetic basis for RVVC was first suggested by anecdotal reports based on family history occurrence and subsequently by blood group studies.[4] However more powerful were the descriptions of patients with chronic mucocutaneous candidiasis in which RVVC was a prominent clinical manifestation.[22-24] Several genes emerged in accompanying genetic studies including dectin, providing a likely explanation for RVVC. Unfortunately subsequent controlled studies in otherwise healthy women, without chronic mucocutaneous candidiasis, with idiopathic RVVC failed to confirm the role of these genes.[25] The only polymorphism emerging was a defect in epithelial cell membrane TLR2 due to substitution of amino acid proline by histidine.[25] Exactly how these genetic alterations predispose to RVVC is unclear. Hypotheses relate to *Candida* epithelial cell receptor function or density and vaginal mucosal immunological inflammatory response.[26,27] A common theory suggests that the normal regulated mucosal proinflammatory cytokine response, dependent on intracellular inflammasome regulation, is enhanced resulting in an exaggerated inflammatory reaction (hyperreactive) responsible for vulvovaginal symptoms.[28] Experimental animal studies strongly suggest that inflammasome and IL-17 activation are essential for discriminating between colonization and invasion with *C albicans* at the level of mucosa.[29] With each year, new genes emerge responsible for yet additional cytokines, more recently IL-22, that are critical in balancing resistance with tolerance to *Candida* and deficiencies thereof predisposing to RVVC.[20] The emergence of genetic information appears to have preceded understanding of normal vaginal defense mechanisms. Another polymorphism to emerge in women with RVVC relates to mannose—binding lectin (MBL), which binds to *Candida* organisms and activates complement enhancing opsonization and hence might reduce vaginal colonization.[30-35] Some investigators report reduced vaginal concentrations of MBL and impaired TLR receptor function in RVVC, both decreasing vaginal host defenses against *Candida* organisms. The role of MBL and TLR genetic polymorphisms remains controversial and has not been confirmed by all investigators.[36]

Clinical aspects of VVC

Cardinal symptoms include vulvovaginal pruritus, irritation, soreness, dyspareunia, and vaginal discharge. Clinical signs are best exemplified by vulva erythema, edema, excoriation, and fissure formation together with introital and vaginal erythema. A nonmalodorous clumpy white discharge is suggestive of VVC but is extremely nonspecific. Diagnosis should never be based on clinical findings alone given their lack of specificity.[37] RVVC, by virtue of the frequent, numerous acute recurrences or chronicity has a profound effect on the quality of life of affected women with additional systemic symptoms including depression and anxiety.[38,39]

Diagnosis of VVC

Vaginal pH is almost always normal in VVC and an elevated pH suggests an alternative diagnosis although mixed infections occasionally occur.[4] Traditional bedside laboratory tests, include saline and 10% potassium hydroxide microscopy, are still extremely useful providing rapid confirmation when positive, but these tests are now infrequently performed in an era where more expensive and complex tests have become a new but not necessarily superior diagnostic routine. Saline and KOH microscopy unfortunately have low sensitivity (40-70%) even in experienced hands. When clinical suspicion points to VVC in the presence of negative microscopy and normal vaginal pH, culture should be obtained. No new reliable, rapid, or immediately available tests to indicate the presence of yeast are available. DNA homology probes are useful, providing reasonably accurate results within several hours.[40,41] Polymerase chain reaction (PCR) methods to detect *Candida* genus presence as well as to determine specific species are now widely available.[42-44] This service is provided by commercial companies with reliable results available within several days. Whether or not PCR will replace the traditional gold standard viz culture methodology is unclear. PCR methods are undoubtedly more sensitive than culture but have not been shown to offer any advantage to the clinician in practice; moreover this technique is more expensive.[42]

Treatment of RVVC

Practitioners can offer reassurance and even assurances of high likelihood of symptomatic control but unfortunately not guarantee of cure (Table 1). As indicated in the pathophysiology section, susceptibility to recurrent symptomatic vaginitis likely has a genetic basis predisposing to enhanced vaginal colonization with an azole-susceptible strain of *C albicans*, and in the presence of secondary triggering mechanisms, the carrier state is transformed into a proinflammatory state by a host hyperactive local immune response. Currently, we have no mechanism or vehicle to control the host mucosal reaction other than by facilitating *Candida* antigen tolerance by keeping the vaginal fungal load at markedly reduced levels. The later status can almost always be achieved by long-term maintenance

TABLE 1
Treatment of recurrent vulvovaginal candidiasis

1. Regimens for *Candida albicans*, *C tropicalis*, *C parapsilosis*
   a. Oral
      1) Fluconazole induction 150 mg q 72 h for 3 doses
         Fluconazole maintenance regimen 150 mg q weekly for 6 mo
      2) Itraconazole induction 200 mg bid × 3 d
         Itraconazole maintenance regimen 100-200 mg/d for 6 mo
   b. Topical regimens:
      Induction
         Clotrimazole 1% vaginal cream for 7 nights
         Clotrimazole 2% vaginal cream for 3 nights
         Miconazole 2% vaginal cream for 7 nights
         Miconazole 4% vaginal cream for 3 nights
         Tioconazole 6.5% ointment for 1 night
         Terconazole 0.4% vaginal cream for 7 nights
         Terconazole 0.8% vaginal cream for 3 nights
         Terconazole 89 mg vaginal suppository for 3 nights
         Butoconazole 2% vaginal cream single dose
      Maintenance regimen
         Miconazole 1200 vaginal suppository once weekly for 6 mo
2. Regimens for *C glabrata*
   1) Boric acid vaginal suppository/capsule 600 mg daily for 14 d
      If necessary maintenance regimen (?)—no data to support.
   2) Nystatin—induction therapy 100,000-U suppository per vagina for 14 d
      Maintenance regimen in same dose should be considered
3. *C krusei*—any of the above regimens except fluconazole.
4. Azole-resistant *Candida* species (based on MIC studies)
   Boric acid (see above)
   Nystatin (see above)
   Amphotericin B vaginal cream/suppositories 5-10% nightly for 14 d
   Flucytosine cream 17% per vagina, nightly for 14 d
   Combination amphotericin B/flucytosine

bid, twice daily; *MIC*, minimal inhibitory concentration; *q*, every.

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suppressive regimens of antifungal drugs.[11-13,45-47]

Accordingly, once the diagnosis of RVVC is confirmed, accompanied by microbiologic confirmation of an azole-sensitive strain of *Candida*, but not *C glabrata* or *C krusei*, attempts should be made at identifying triggering or precipitating stimuli. Apart from antibiotics, other risk factors rarely emerge, but may include lowering intake of sugar or discontinuing oral contraceptives, although data to support these actions are anecdotal and proof of efficacy is minimal.[12,48-50] Routine screening for HIV and diabetes is not recommended unless other risk factors for these entities are present. Women whose recurrent episodes are precipitated exclusively by antibiotic administration can effectively prevent symptomatic recurrence using concurrent antifungal prophylaxis to extend through the antibiotic course. The most convenient regimen is oral fluconazole 150 mg with onset of antibiotic and then every 3-4 days to cover the duration at risk.

In the majority of women, no preventable triggering stimuli are forthcoming; ie, idiopathic RVVC and patients should receive an induction regimen of fluconazole, 150 mg every 72 hours for 3 doses, followed by once weekly fluconazole 150 mg for at least 6 months.[12,13] Several controlled studies have documented the therapeutic efficacy of this regimen: safety together with affordability, given generic availability.[11-13,51] During the period of maintenance treatment, breakthrough episodes of symptomatic vaginitis are rare (~5%) and vaginal cultures usually remain negative. Patients enjoy a prolonged period of normal life quality. On cessation of fluconazole, these same studies indicate that approximately 50% of women will develop a culture-positive episode of RVVC within 3-4 months, in the absence of a triggering event. The yeast so identified invariably remains fluconazole sensitive and is usually identical to the original strain isolated before suppressive therapy. In such women one can assume that the offending pathogen persisted vaginally in low numbers, hence the negative cultures when therapy is initially discontinued or that rapid reinfection from an extravaginal site occurred following fluconazole cessation. In any event, following vaginal recolonization the pathologic vaginal immune response was activated, having failed to moderate in the previous 6 months in spite of fluconazole therapy and a vaginal environment with no or minimal *Candida* presence. Explanations for both components are not yet evident. In the event of recurrence, the only available option is to recommend reinduction and suppressive maintenance with convenient weekly fluconazole for a further 6-12 months. Unfortunately, while experience indicates that successful future control is predictable while taking weekly fluconazole, cure rates after subsequent cessation of therapy are not available. Multiple grateful and content women taking long-term suppressive prophylaxis for several years are reported anecdotally. The long-term natural history of RVVC in women has not been reported; although menopause and accompanying estrogen deficiency likely predispose to reduced clinical expression of RVVC. On the other hand, approximately 40-50% of women remain in full clinical remission following the initial 6-month course of weekly fluconazole. The majority of these women remain culture negative indicating that the pathogenic original strain was eradicated and

TABLE 2
Future research areas in recurrent vulvovaginal candidiasis

- Vaginal and gastrointestinal microbiome of women with RVVC.
- Scientific studies on probiotics utilizing *Lactobacillus* species known to colonize vagina; persist and achieve functional goals.
- Additional genetic and other polymorphism studies identifying genetic factors responsible for susceptibility to RVVC and exploring how the changes influence infection susceptibility.
- Clarification of innate and adaptive vaginal mechanism responsible for host protection against *Candida.*
- Exploration of vaginal epithelial cell inflammasome to gain insights into the vaginal inflammatory response.
- Role of biofilm in pathogenesis of RVVC?
- Development of more potent fungicidal drugs, particularly agents with pharmacokinetic advantages including agents with prolonged half-life.
- Mechanisms of acquired azole-drug resistance in *C albicans.*
- Further progress in *Candida* vaccine development.

RVVC, recurrent vulvovaginal candidiasis.

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further recolonization failed to occur. Yet others become recolonized with a pathogenic strain and likely remain asymptomatic due to moderation of the hyperactive local immune putative defense mechanism, having achieved vaginal tolerance of the colonizing yeast strain.

This desirable reacquired physiologic state is the basis for recent attempts to achieve cure in women with RVVC by vaccine immunization.[52] Studies are currently underway to evaluate several vaccine antigen candidates.[50] A prophylactic vaccine directed at all at-risk women would need to induce a host immune response against fungal virulence components without altering the tolerance—inflammation balance of the vaginal environment. A more therapeutic vaccine would induce tolerance in women with RVVC. It should be emphasized that to date the most appropriate and effective antigen(s) to be incorporated in the experimental vaccines remains unknown mainly because of deficiencies in our knowledge of protective vaginal immune responses.

Non-*albicans Candida* species

The approach to RVVC in women with azole-resistant *Candida* species such as *C glabrata* and much less commonly *C krusei* or even *Saccharomyces* species does not require the aforementioned strategy, since recurrent diseases is not a function of genetic controlled immune hyperactivity but lack of susceptibility to azole drugs. There are no published data or long-term maintenance therapy for *C glabrata*. In past studies of RVVC, only women with *C albicans* were enrolled. The management of chronic and RVVC due to *C glabrata* remains complex and frustrating for patients and practitioners alike. Azole agents both oral and topical frequently fail.[4,15,53] Some success can be achieved with vaginal boric acid 600 mg daily for 7-14 days (60-70%). Failure to eradicate *C glabrata* in symptomatic women justifies additional efforts that include intravaginal nystatin, flucytosine, and amphotericin B and combinations thereof should be attempted.[54] There are however few published studies on the efficacy, safety, or even need for long-term maintenance regimen with boric acid or polyene agents.[55,56] Not infrequently chronic VVC and RVVC due to *C glabrata* occurs in women with uncontrolled type 2 diabetes and without control of hyperglycemia, eradication of *C glabrata* is unlikely. In all women with recurrent and chronic vaginal symptoms in whom non-*albicans Candida* species are isolated, it is important to establish their causality or role in causing symptoms because of the low virulence of these species.[16]

Fluconazole-resistant RVVC due to *C albicans*

In the last decade, isolated cases have been reported of women with RVVC who either fail to respond to induction therapy with fluconazole or who break through fluconazole maintenance suppressive therapy. After lack of compliance is excluded, fluconazole resistance should be considered.[57] Approximately 50 cases have been identified at the Wayne State University School of Medicine Vaginitis Clinic over the last 10 years, although widespread reports have not been forthcoming and there is no evidence of increased frequency of patients so diagnosed.[58] Epidemiologic studies reveal that almost all women diagnosed with fluconazole-resistant *C albicans* had experienced considerable previous exposure to fluconazole.[57] Confirmation of diagnosis requires laboratory in vitro susceptibility tests with readily available minimal inhibitory concentration test determination. Once confirmed, cross-resistance to other oral azoles should be determined since treatment alternatives to fluconazole are required. If ketoconazole and itraconazole are found to be active, ie, no cross-resistance, then either of these oral systemic agents can replace fluconazole for induction and maintenance therapy as described above, although comparative controlled studies are not available. Neither of these agents is as safe or as user-friendly.

In the event of azole cross-resistance, limited options exist for maintenance therapy. In this context induction and long-term therapy can be considered utilizing either vaginal nystatin or boric acid, with little published data of efficacy.[54,56]

Role of probiotics for RVVC

In layman literature, probiotic use to treat and prevent VVC is a long-standing tradition.[59,60] Scientific justification for

exogenous *Lactobacillus* replacement is based on the premise that *Lactobacillus* species exert a significant protective effect in vivo in reducing the virulence expression of *Candida* organisms colonizing the vagina as well as enhancing the vaginal epithelial cell immune defense mechanisms. There also exists the hypothesis that women with VVC and especially RVVC have reduced numbers of protective *Lactobacillus* species in situ, a premise entirely unproven in contrast to women with bacterial vaginosis.[61,62] Few good-quality studies have evaluated the microbiome of women with RVVC and data so derived show strikingly variable results.[61-63] Also supporting the benefit of *Lactobacillus* probiotic use is the indisputable observation that oral, systemic, or local vaginal antibacterial agents are a well-described trigger for the development of acute VVC.[4] Surprisingly, the mechanism whereby antibiotics induce acute VVC is unknown and the very same antimicrobials are not known to cause acute bacterial vaginosis where indeed protective *Lactobacillus* species are invariably absent. The paucity of scientific data and a sound rationale for the use of probiotics has not stopped the widespread use of probiotics. Regrettably good prospective randomized controlled studies have been extremely few in numbers, so proof of efficacy is sorely lacking.[64] This issue is further complicated by the poor quality and lack of standardization of probiotics available to women in the United States. Accordingly use of probiotics is not part of recommended regimens of experienced specialists. It is essential that as the quality of probiotics improves in the future, that well-designed studies be undertaken to resolve this issue.

While considerable progress in our understanding of RVVC is evident, enormous gaps in our knowledge continue. Current and future research challenges are suggested in Table 2. ■

REFERENCES

1. Sobel JD. Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. Am J Obstet Gynecol 1985;152:924.
2. Hong E, Dixit S, Fidel PL, Bradford J, Fischer G. Vulvovaginal candidiasis as a chronic disease: diagnostic criteria and definition. J Low Genit Tract Dis 2014;18:31-8.
3. Sobel JD, Faro S, Force RW, et al. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. Am J Obstet Gynecol 1998;178:203-11.
4. Sobel JD. Vulvovaginal candidosis. Lancet 2007;369:1961-71.
5. Rathod SD, Buffler PA. Highly-cited estimates of the cumulative incidence and recurrence of vulvovaginal candidiasis are inadequately documented. BMC Womens Health 2014;14:43-7.
6. Ferris DG, Nyirjesy P, Sobel JD, Soper D, Pavletic A, Litaker MS. Over-the-counter antifungal drug misuse associated with patient-diagnosed vulvovaginal candidiasis. Obstet Gynecol 2002;99:419-25.
7. Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JD. *Candida* vaginitis: self-reported incidence and associated costs. Sex Transm Dis 2000;27:230-5.
8. Foxman B, Muraglia R, Dietz JP, Sobel JD, Wagner J. Prevalence of recurrent vulvovaginal candidiasis In 5 European countries and the United States: results from an internet panel survey. J Low Genit Tract Dis 2013;17:340-5.
9. Fischer G, Bradford J. Vulvovaginal candidiasis in postmenopausal women: the role of hormone replacement therapy. J Low Genit Tract Dis 2011;15:263-7.
10. Ilkit M, Guzel AB. The epidemiology, pathogenesis, and diagnosis of vulvovaginal candidosis: a mycological perspective. Crit Rev Microbiol 2011;37:250-61.
11. Sobel JD. Recurrent vulvovaginal candidiasis: a prospective study of the efficacy of maintenance ketoconazole therapy. N Engl J Med 1986;315:1455-8.
12. Sobel JD, Wiesenfeld HC, Martens M, et al. Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. N Engl J Med 2004;351:876-83.
13. Donders G, Bellen G, Byttebier G, et al. Individualized decreasing-dose maintenance fluconazole regimen for recurrent vulvovaginal candidiasis (ReCiDiF trial). Am J Obstet Gynecol 2008;199:613-9.
14. Nyirjesy P, Zhao Y, Ways K, Usiskin K. Evaluation of vulvovaginal symptoms and *Candida* colonization in women with type 2 diabetes mellitus treated with canagliflozin, a sodium glucose co-transporter 2 inhibitor. Curr Med Res Opin 2012;28:1173-8.
15. Kennedy MA, Sobel JD. Vulvovaginal candidiasis caused by non-*albicans Candida* species: new insights. Curr Infect Dis Rep 2010;12:465-70.
16. Dennerstein G. Management of recurrent vaginitis. Drugs 1981;21:231-5.
17. Conti HR, Peterson AC, Brane L, et al. Oral-resident natural Th17 cells and $\gamma\delta$ T cells control opportunistic *Candida albicans* infections. J Exp Med 2014;211:2075-84.
18. Gladiator A, Wangler N, Trautwein-Weidner K, LeibundGut-Landmann S. Cutting edge: IL-17-secreting innate lymphoid cells are essential for host defense against fungal infection. J Immunol 2013;190:521-5.
19. Netea MG, Brown GD, Kullberg BJ, Gow NA. An integrated model of the recognition of *Candida albicans* by the innate immune system. Nat Rev Microbiol 2008;6:67-78.
20. De Luca A, Carvalho A, Cunha C, et al. IL-22 and IDO1 affect immunity and tolerance to murine and human vaginal candidiasis. PLoS Pathog 2013;9:e1003486.
21. Pietrella D, Rachini A, Pines M, et al. Th17 cells and IL-17 in protective immunity to vaginal candidiasis. PLoS One 2011;6:e22770.
22. Ferwerda B, Ferwerda G, Plantinga TS, et al. Human dectin-1 deficiency and mucocutaneous fungal infections. N Engl J Med 2009;361:1760-7.
23. van de Veerdonk FL, Plantinga TS, Hoischen A, et al. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. N Engl J Med 2011;365:54-61.
24. Usluogullari B, Gumus I, Gunduz E, et al. The role of human dectin-1 Y238X gene polymorphism in recurrent vulvovaginal candidiasis infections. Mol Biol Rep 2014;41:6763-8.
25. Rosentul DC, Delsing CE, Jaeger M, et al. Gene polymorphisms in pattern recognition receptors and susceptibility to idiopathic recurrent vulvovaginal candidiasis. Front Microbiol 2014;5:1-7.
26. Ben-Ali M, Corre B, Manry J, et al. Functional characterization of naturally occurring genetic variants in the human TLR1-2-6 gene family. Hum Mutat 2011;32:643-52.
27. Etokebe GE, Skjeldal F, Nilsen N, et al. Toll-like receptor 2 (P631H) mutant impairs membrane internalization and is a dominant negative allele. Scand J Immunol 2010;71:369-81.
28. Romani L. Immunity to fungal infections. Nat Rev Immunol 2011;11:275-88.
29. Gow NA, Netea MG, Munro CA, et al. Immune recognition of *Candida albicans* beta-glucan by dectin-1. J Infect Dis 2007;196:1565-71.
30. Babula O, Lazdane G, Kroica J, Ledger WJ, Witkin SS. Relation between recurrent vulvovaginal candidiasis, vaginal concentrations of mannose-binding lectin, and a mannose-binding lectin gene polymorphism in Latvian women. Clin Infect Dis 2003;37:733-7.
31. Wang M, Wang F, Yang J, et al. Mannan-binding lectin inhibits *Candida albicans*-induced cellular responses in PMA-activated THP-1 cells through Toll-like receptor 2 and Toll-like receptor 4. PLoS One 2013;8:e83517.
32. Babula O, Lazdāne G, Kroica J, Linhares IM, Ledger WJ, Witkin SS. Frequency of interleukin-4 (IL-4) -589 gene polymorphism and vaginal concentrations of IL-4, nitric oxide, and mannose-binding lectin in women with recurrent vulvovaginal candidiasis. Clin Infect Dis 2005;40:1258-62.
33. Liu F, Liao Q, Liu Z. Mannose-binding lectin and vulvovaginal candidiasis. Int J Gynaecol Obstet 2006;92:43-7.
34. Donders GG, Babula O, Bellen G, Linhares IM, Witkin SS. Mannose-binding lectin

gene polymorphism and resistance to therapy in women with recurrent vulvovaginal candidiasis. BJOG 2008;115:1225-32.
35. Wojtani MD, de Aguiar LM, Baracat EC, Linhares IM. Association between mannose-binding lectin and interleukin-1 receptor antagonist gene polymorphisms and recurrent vulvovaginal candidiasis. Arch Gynecol Obstet 2011;285:149-53.
36. Nedovic B, Posteraro B, Leoncini E, et al. Mannose-binding lectin codon 54 gene polymorphism and vulvovaginal candidiasis: a systematic review and meta-analysis. Biomed Res Int 2014;2014:738298.
37. Anderson MR, Klink K, Cohrssen A. Evaluation of vaginal complaints. JAMA 2004;291:1368-79.
38. Hurley R. Inveterate vaginal thrush. Practioners 1975;215:753.
39. Aballéa S, Guelfucci F, Wagner J, et al. Subjective health status and health-related quality of life among women with recurrent vulvovaginal candidosis (RVVC) in Europe and the USA. Health Qual Life Outcomes 2013;11:169-75.
40. Elie CM, Lott TJ, Reiss E, Morrison CJ. Rapid identification of *Candida* species with species-specific DNA probes. J Clin Microbiol 1998;36:3260-5.
41. Lowe NK, Neal JL, Ryan-Wenger NA. Accuracy of the clinical diagnosis of vaginitis compared with a DNA probe laboratory standard. Obstet Gynecol 2009;113:89-95.
42. Sobel JD, Akins RA. The role of PCR in the diagnosis of *Candida* vulvovaginitis—a new gold standard? Curr Infect Dis Rep 2015;17:488.
43. Cartwright CP, Lembke BD, Ramachandran K, et al. Comparison of nucleic acid amplification assays with BD affirm VPIII for diagnosis of vaginitis in symptomatic women. J Clin Microbiol 2013;51:3694-9.
44. Weissenbacher T, Witkin SS, Ledger WJ, et al. Relationship between clinical diagnosis of recurrent vulvovaginal candidiasis and detection of *Candida* species by culture and polymerase chain reaction. Arch Gynecol Obstet 2009;279:125-9.
45. Spinillo A, Colonna L, Piazzi G, Baltaro F, Monaco A, Ferrari A. Managing recurrent vulvovaginal candidiasis: intermittent prevention with itraconazole. J Reprod Med 1997;42:83-7.
46. Balsdon MJ, Tobin JM. Recurrent vaginal candidosis: prospective study of effectiveness of maintenance miconazole treatment. Genitourin Med 1988;64:124-7.
47. Roth AC, ilsom I, Forssman L, Wåhlén P. Intermittent prophylactic treatment of recurrent vaginal candidiasis by postmenstrual application of a 500 mg clotrimazole vaginal tablet. Genitourin Med 1990;66:357-60.
48. Donders GG, Mertens I, Bellen G, Pelckmans S. Self-elimination of risk factors for recurrent vaginal candidosis. Mycoses 2011;54:39-45.
49. Martin Lopez JE. Candidiasis (vulvovaginal). BMJ Clin Evid 2015.
50. Ray A, Ray S, George AT, Swaminathan N. Interventions for prevention and treatment of vulvovaginal candidiasis in women with HIV infection. Cochrane Database Syst Rev 2011;8:CD008739.
51. Rosa MI, Silva BR, Pires PS, et al. Weekly fluconazole therapy for recurrent vulvovaginal candidiasis: a systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol 2013;167:132-6.
52. Cassone A. Vulvovaginal *Candida albicans* infections: pathogenesis, immunity and vaccine prospects. BJOG 2015;122:785-94.
53. Davies S, Johnson E, White D. How to treat persistent vaginal yeast infection due to species other than *Candida albicans*. Sex Transm Infect 2013;89:165-6.
54. Fan S, Liu X, Wu C, Xu L, Li J. Vaginal nystatin versus oral fluconazole for the treatment for recurrent vulvovaginal candidiasis. Mycopathologia 2015;179:95-101.
55. Guaschino S, De Seta F, Sartore A, et al. Efficacy of maintenance therapy with topical boric acid in comparison with oral itraconazole in the treatment of recurrent vulvovaginal candidiasis. Am J Obstet Gynecol 2001;184:598-602.
56. Iavazzo C, Gkegkes ID, Zarkada IM, Falagas ME. Boric acid for recurrent vulvovaginal candidiasis: the clinical evidence. J Womens Health (Larchmt) 2011;20:1245-55.
57. Marchaim D, Lemanek L, Bheemreddy S, Kaye KS, Sobel JD. Fluconazole-resistant *Candida albicans* vulvovaginitis. Obstet Gynecol 2012;120:1407-14.
58. Kalkanci A, Güzel AB, Jabban II, Aydin M, Ilkit M, Kuştimur S. *Candida* vaginitis in nonpregnant patients: a study of antifungal susceptibility testing and virulence factors. J Obstet Gynaecol 2013;33:378-83.
59. Watson CJ, Pirotta M, Myers SP. Use of complementary and alternative medicine in recurrent vulvovaginal candidiasis—results of a practitioner survey. Complement Ther Med 2012;20:218-21.
60. Falagas ME, Betsi GI, Athanasiou S. Probiotics for prevention of recurrent vulvovaginal candidiasis: a review. J Antimicrob Chemother 2006;58:266-72.
61. Liu MB, Xu SR, He Y, et al. Diverse vaginal microbiomes in reproductive-age women with vulvovaginal candidiasis. PLoS One 2013;8:e79812.
62. Zhou X, Westman R, Hickey R, et al. Vaginal microbiota of women with frequent vulvovaginal candidiasis. Infect Immun 2009;77:4130-5.
63. Eloe-Fadrosh EA, Rasko DA. The human microbiome: from symbiosis to pathogenesis. Annu Rev Med 2013;64:145-63.
64. Pirotta M, Gunn J, Chondros P, Grover S, Hurley S, Garland S. The PAV trial: does *Lactobacillus* prevent post-antibiotic vulvovaginal candidiasis? Protocol of a randomized controlled trial [ISRCTN24141277]. BMC Fam Pract 2004;5:5.