

Candidiasis, Bacterial Vaginosis, Trichomoniasis and Other Vaginal Conditions Affecting the Vulva

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24.1 Introduction

The term “vulvovaginitis” encompasses a broad and heterogeneous number of conditions, which, contrarily to what the suffix “-itis” implies, are not necessarily infectious (e.g. cytolytic vaginosis [CyV]) or inflammatory (e.g. bacterial vaginosis [BV]).

The clinical or symptomatic approach to the management of vulvovaginitis is very limited; a significant number of women will not fit the “traditional” diagnosis of candidiasis, BV, or trichomoniasis. A proper approach, encompassing other neglected diagnoses, will reduce the number of women without a diagnosis (reported to be as high as 30%), and lead to more adequate treatments.

These entities, despite not life-threatening, can seriously contribute to impair the quality of life of affected women at all levels, and leave deep and long lasting psychosexual sequelae.

Candidiasis: Breaking the Myths

- A significant number of women will not fit the “traditional” diagnosis of candidiasis, BV, or trichomoniasis
- *Candida albicans* infection does not consist only of vaginal discharge. In fact, its mycelium can penetrate the vaginal mucosa, up to 10 layers of cells deep.
- Despite the long list of risk factors and associations with vulvovaginal candidiasis (VVC), most of the times a precipitating factor cannot be identified.
- Itch is the most notorious symptom of VVC—but most women with itching do not have a VVC.
- Identification of *Candida* in cultures, per se, cannot be assumed as a diagnosis of VVC, as it does not distinguish colonization from infection.

24.2 Vulvovaginal Candidiasis (or Candidosis)

It is debatable whether the symptomatic presence of fungi from the genera *Candida* in the vagina should be referred to as vulvovaginal “candidiasis” or “candidosis” (VVC), since inflammation is not always present, and “-osis” is the suffix usually used for fungal infections.

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24.2.1 Aetiology and Pathophysiology

Approximately 90% of the cases of VVC are caused by *C. albicans*. The next most common agent is *C. glabrata*; much less frequently (<1–2% of the total), *C. krusei*, *C. tropicalis*, *C. dubliniensis* and *C. parapsilosis*, among others, can also be involved [1]. The non-*albicans* *Candida* (NAC) species are more commonly encountered in cases of recurrent VVC (in up to 20%), and in diabetic or immunocompromised women.

Co-infection is uncommon (<10% of cases) and usually the involved species are *C. albicans* and *C. glabrata* [1].

Candida is commonly found in the form of blastospores in the mucous layer, as a commensal. Infection starts with adherence to vaginal epithelial cells, mediated by mannoproteins. For the species that form mycelium (*C. albicans*), it is a fundamental step in eliciting an immune response and in causing host cell damage. The mycelium, in a phenomenon mediated, among others, by candidalysin, penetrates the vaginal mucosa, up to 10 layers of cells deep [2].

The first line of defence, innate immunity, includes phagocytosis by neutrophils and macrophages, activation of the complement cascade, direct cellular killing, and promotion of opsonization via complement receptors. This process is mediated by the binding of mannose-binding lectin (MBL) to *Candida* and explains why women with mutations of the MBL gene, especially at the codon 54, are more prone to recurrent VVC [3].

Acquired immunity against *Candida* is mostly mediated by IgA secretion, as well as a Th2 type cytokine profile response. However, an impaired Th1 response has been associated with increased risk of recurrent VVC [4].

A normal lactobacillary flora is protective against VVC due to: (1) competition for nutrients, (2) competition for adhesion sites, (3) production of biosurfactants that decrease the adherence of *Candida*, (4) production of H₂O₂, (5) production of toxic peptides and fatty acids, (6) downregulation of genes responsible for the biofilm production, and (7) inhibition of filamentation [5]. Interestingly however, most

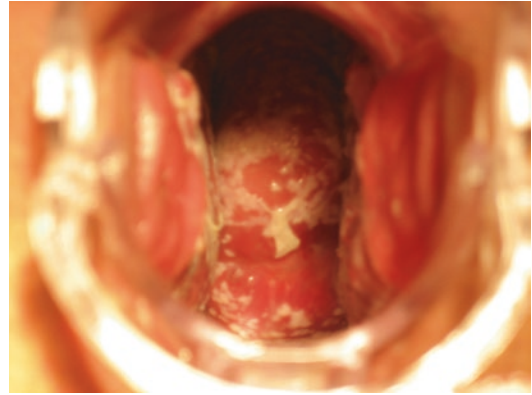


Fig. 24.1 Typical curdy white discharge of VVC (cultures were positive for *C. krusei*)

cases of VVC occur in women with normal flora [2] (Fig. 24.1).

24.2.2 Prevalence and Epidemiology

VVC is not a reportable disease, thus all prevalence data are based on studies performed in different settings, often not representative of the population, and looking for different outcomes [1].

It is considered the second most frequent cause of “vulvovaginitis” symptoms, after BV. *Candida* can be responsible for the symptoms of vulvovaginitis in up to 30% of cases [6]. However, the perception of its prevalence, from patients’ and health care providers’ point of view, is higher; most cases are treated with over-the-counter medication, without proper confirmation.

Vaginal *Candida* colonization is found in at least 10–20% of healthy, reproductive age, asymptomatic women [7]. It is higher in pregnant women (20–40%), especially in the third trimester, due to the high levels of estrogens, and in immunocompromised women [8]. The cumulative incidence of colonization over 1 year is as high as 70% [9].

Up to 75% of women will experience at least one episode during their life time [1]. Half of these will suffer at least one additional episode and 5–10% will have recurrent VVC (Table 24.1) [1, 6, 10].

There is no data suggesting an increase in the incidence of VVC over the years [2].

24.2.3 Classification

The majority of episodes of VVC (90%) are considered uncomplicated. When the episodes are recurrent, associated with severe symptoms, caused by NAC species, or occur in diabetic or immunocompromised patients, it is classified as complicated [10] (Table 24.1).

According to most societies' guidelines, recurrent VVC is defined as four or more symptomatic episodes in 1 year. Most do not refer the need for a laboratorial confirmation (wet mount, culture, Gram staining) of every episode. We support that confirmation is necessary, in order to exclude alternative diagnosis (cytolytic vaginosis, allergic vaginitis) or NAC species.

24.2.4 Risk Factors and Associations

Despite the long list of risk factors and associations with VVC (Table 24.2), most of the times a precipitating factor cannot be identified [11].

The presence of *Candida* is highly related to oestrogen levels, and, accordingly it is much

less common in prepubertal and postmenopausal women, and, contrarily, more common in pregnant women.

The role of oestrogens is complex: (1) promotes *Candida* proliferation and virulence, (2) induces immunological tolerance, and (3) promotes the production of glycogen by the vaginal epithelium cells. Progesterone, on one side, promotes *Candida* proliferation, but on the other has been shown also to inhibit its ability of forming biofilms and mycelium [12, 13].

The role of oral contraceptives in VVC is controversial. Modern low dose pills seem to pose a low risk, if any. Progestin-only pills and subdermal progestin implants seem to be associated with a lower risk of colonization, probably due to the relative state of hypoestrogenism they induce [14].

Copper intrauterine devices (IUDs) and, more recently, also levonorgestrel-releasing intrauterine systems (LVG-IUS) were associated with an increased risk of colonization by *Candida* [14, 15]. The formation of biofilms in the IUDs/LVG-IUS, to which *Candida* can adhere and act as a reservoir, is a possible explanation. However, this phenomenon does not seem to occur with the vaginal ring [16].

Glycogen, which is increased in cases of high levels of oestrogens, of steroids use, and in diabetic women, is the main growth substrate for *Candida* [12].

In women colonized with *Candida*, the use of antibiotics and the consequent destruction of the vaginal lactobacilli is a well-established risk factor [6] (Table 24.2).

Factors that can decrease the inflammatory response are associated with VVC. These can be genetic (MBL gene polymorphisms, diabetes) or acquired (HIV, drugs, low zinc levels).

Behavioural and environmental factors are the most complex ones to understand and with the most disparate results across studies. Higher temperatures and increased moisture, whether derived from the climate or local conditions (obesity, non-breathable clothes, etc.) seem to be related to increased rates of VVC. Some authors consider panty liners a risk factor for VVC, but systematic reviews do not support it [17].

Table 24.1 Classification of VVC

Uncomplicated	All 4 criteria must be met: <ul style="list-style-type: none"> • Sporadic or infrequent • Mild-to-moderate symptoms • Caused by <i>Candida albicans</i> • Non immunocompromised woman
Complicated	Any of these criteria present: <ul style="list-style-type: none"> • Recurrent VVC • Severe (extensive vulvar erythema, oedema, excoriations and fissures) • Non-albicans species involved • Women with diabetes, pregnancy, immunocompromising conditions (e.g. HIV infection), debilitation, or immunosuppressive therapy (e.g. corticosteroids)
Recurrent	<ul style="list-style-type: none"> • Four or more symptomatic episodes within 1 year • Each episode must have laboratorial confirmation (not recommended by all societies)

Table 24.2 Risk factors associated with VVC

Hormonal factors	Consequences/mechanisms of action
<ul style="list-style-type: none"> • Increased oestrogen levels (e.g. pregnancy) • Menopausal hormonal replacement therapy • Oral contraceptives (?) 	<ul style="list-style-type: none"> • ↑ Adherence of <i>Candida</i> to vaginal cells • ↑ Mycelium formation • ↓ Vaginal immunological response • ↑ Levels of vaginal glycogen • Direct stimulation of <i>Candida</i> proliferation
Metabolic factors	
<ul style="list-style-type: none"> • Obesity • Diabetes <i>mellitus</i> 	<ul style="list-style-type: none"> • ↑ Adherence of <i>Candida</i> do vaginal cells • ↑ Levels of vaginal glycogen • ↓ Vaginal immunological response
<ul style="list-style-type: none"> • Low zinc levels 	<ul style="list-style-type: none"> • ↓ Cellular immunity
Host factors	
<ul style="list-style-type: none"> • Immunosuppression (e.g. HIV) • Debilitating illnesses • Stress 	<ul style="list-style-type: none"> • ↓ Immunological defences
<ul style="list-style-type: none"> • Genetic polymorphisms (MBL gene, IL4 gene, NALP3, dectin-1 gene, CARD9, STAT1, STAT3) 	<ul style="list-style-type: none"> • ↓ Immunological defences
<ul style="list-style-type: none"> • ABO-Lewis nonsecretor blood type 	<ul style="list-style-type: none"> • ↓ Inhibition of adherence lectins • ↓ IgA levels • Carbohydrates metabolism abnormalities
<ul style="list-style-type: none"> • Atopy/allergy history 	<ul style="list-style-type: none"> • Excessive reaction to <i>Candida</i> antigens
<ul style="list-style-type: none"> • Black race 	<ul style="list-style-type: none"> • Genetic factors?
Drugs	
<ul style="list-style-type: none"> • Antibiotics (topical and systemic) (<i>mostly beta-lactamics, macrolides, and tetracyclines</i>) 	<ul style="list-style-type: none"> • Elimination of protective vaginal flora (lactobacilli)
<ul style="list-style-type: none"> • Steroids • Immunosuppressors • Chemotherapy 	<ul style="list-style-type: none"> • ↓ Immunological defences
Behavioural factors	
<ul style="list-style-type: none"> • Hot and humid climates • Tight and synthetic clothes • Patny-liners (?) • Obesity 	<ul style="list-style-type: none"> • ↑ Moisture and temperature
<ul style="list-style-type: none"> • Bad hygienic habits • Vaginal douching • Swimming pools rich in chloride 	<ul style="list-style-type: none"> • ↑ Spore load (from the intestinal reservoir) • ↑ Vaginal pH • Destruction of lactobacilli
<ul style="list-style-type: none"> • Dietary factors (rapid sugars) 	<ul style="list-style-type: none"> • ↑ Glycogen
<ul style="list-style-type: none"> • Sexual habits (frequent sexual intercourse, recipient oral sex) (<i>Controversial: early sexual debut, new or multiple partners, intercourse during menses</i>) 	<ul style="list-style-type: none"> • ↑ Spore load • Alteration of the flora by semen • Exposure to cytokines, antigens, prostaglandins, and antibodies from semen • Transmission from the oral mucosa (?)
<ul style="list-style-type: none"> • Copper IUDs/ LNG-IUS 	<ul style="list-style-type: none"> • Formation of biofilms, that increase adherence and act as reservoirs • Changes in the vaginal flora

VVC is not considered STI, but it is sexually related. Sexual intercourse and the deposition of semen in the vagina changes the pH, the interleukin and prostaglandin milieu, exposes the vagina to IgE and antibodies from the male partner, which can be responsible for the development of symptoms of VVC.

24.2.5 Signs and Symptoms

The reservoir of *Candida*, during an active episode of VVC is the vagina, which is devoid of itch receptors, thus the symptoms manifest only in the vulva. It is not known, if itch is caused by the infection itself or secondary to the contact with the

vaginal discharge. In severe cases, manifestations of VVC such as redness and oedema may extend up to the perianal area and the intercrural folds (Fig. 24.2). Satellite vulvar lesions can be present.

Itch is the most notorious symptom of VVC—but it must be kept in mind that most women with itching do not have a VVC [18]. Especially in cases of hypoestrogenism, in the absence of immunodepression, VVC is not likely to be the cause of itching.

Other symptoms can include entry dyspareunia, soreness, and irritation. The presence of NAC is usually asymptomatic; if not, it usually causes burning, rather than itching, and the symptoms tend to be milder.

Women can describe a more abundant, curdy, white and thick (cheese-like) or watery discharge, without an offensive smell (Fig. 24.1). It can also be absent, especially in chronic VVC.

Fissures and excoriations can cause vulvar pain and terminal or post-micturition dysuria—derived from the contact of urine with the lesions, rather than from urethral involvement. Oedema can be present, more exuberantly in the *labia minora*, and vulvar redness is seen in up to 40% of cases [2]. The vagina and cervix may be covered with a white, cheesy discharge (Fig. 24.2), but usually do not look inflamed and there is no cervicitis, but minor bleeding can be noticed if removal of the discharge plaque from the vaginal wall is attempted [2].



Fig. 24.2 Vulvovaginal candidiasis (VVC), with vulvar erythema in the interlabial sulci Courtesy of Professor Jacob Bornstein

The severity of symptoms can fluctuate along the menstrual cycle, worsening in the week prior to menses and improving during it. It can be explained by the vaginal epithelial cell proliferation and cytolysis, mediated by oestrogens and progesterone, respectively, which ultimately leads to increased levels of glycogen in the vagina. The raising of the pH mediated by the presence of blood and endometrial debris in the vagina leads to a decrease in the symptoms.

24.2.6 Complications

VVC, especially the complicated form, is a major cause of mental suffering, depression, low self-esteem, with impact on sexual and affective relations.

A large majority of newborns who are colonized with *C. albicans* during a vaginal delivery will develop oral candidiasis or napkin dermatitis during their first year of life. This can be diminished by treating colonized mothers during the 3rd trimester [2].

Petricovic et al. have found slight increases in preterm labour and risk of low birth weight rates, especially if colonization is present during the 2nd trimester of pregnancy [19].

Candida chorioamnionitis has been reported, but it is a very rare event and usually related to procedures such as in vitro fertilization, *cérclage*, or amniocentesis [20].

In one study, the presence of *Candida* was significantly associated with an increased risk of seroconversion in HIV negative women, with a seropositive male partner [21].

VVC has been associated with vulvodynia in several studies but most of the reported episodes are based on recall and not laboratory confirmed, thus making it unreliable. Nevertheless, long-term treatment with fluconazole is not an effective treatment of vulvodynia. *Candida* can, however, be the initial aggression to the vestibular mucosa and it has been shown that in vulvodynia patients the inflammatory response to it can be heightened [22].

24.2.7 Diagnosis

Clinical history and a vulvovaginal examination are fundamental parts of the diagnosis, but not sufficient. Self-diagnosis of VVC is wrong in up to 90% of cases and should not be encouraged [6, 23]. “Phone diagnosis” is also an unreliable approach.

The diagnosis is supported by wet mount microscopy (WMM), ideally using phase contrast microscopy [2] and, in some cases, by culture.

24.2.7.1 Microscopy

The most important tool in the diagnosis of VVC is microscopy, which should be performed systematically in women with suspected vulvovaginitis. Not only it allows the diagnosis of VVC, as it is the only way to identify the presence of mixed infections. WMM is cheaper than Gram staining and allows an immediate diagnosis.

There is evidence that collecting the sample from the anterior vaginal wall increases the likelihood of identifying *Candida* [24].

Candida in WMM can present as mycelium and/or blastospores. Mycelium includes both hyphae (filaments that compose the body of the fungus) and pseudohyphae (filaments composed of elongated budding cells that failed to detach) and their presence is highly suggestive of infection, rather than mere colonization (Fig. 24.3). However, some species, like *C. glabrata*, are unable to form mycelium. The blastospores of *C. glabrata* tend to cluster and to have the shape of a bowling pin or a “snowman” (Fig. 24.4). In the presence of clustering blastospores, in the absence of mycelium, NAC should be considered (Fig. 24.5) [4].

Care must be taken not to confuse sperm heads with blastospores. The former tend to be asymmetrical (larger where the tail was attached) and are bicolor (Fig. 24.6) [4].

Some authors recommend the addition of a drop of KOH to the slide, which dissolves the epithelial cells and leukocytes, but not the fungal structures, increasing the sensitivity of WMM to up to 85% [6, 24]. Other measures to try to improve the identification of these structures can include the staining with methylene blue [4].



Fig. 24.3 *C. albicans* (pseudohyphae) in wet mount (phase contrast 400×). The background flora is normal, despite some scarcity of lactobacilli. (phase contrast, 400×)

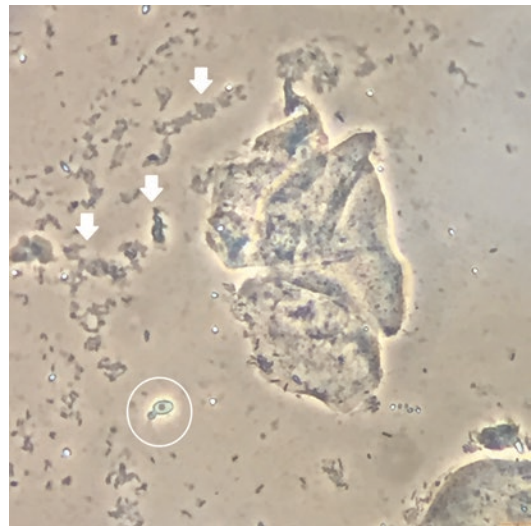


Fig. 24.4 “Snowman” typical of *C. glabrata* (inside the white circle). Anaerobic type background flora (white arrows). (phase contrast, 400×)

The Pap test is specific for the presence of *Candida*, but has a sensitivity of only 25%, making it useless as diagnostic tool. This comes as no surprise, since it is a cervical rather than a vaginal sample [6].

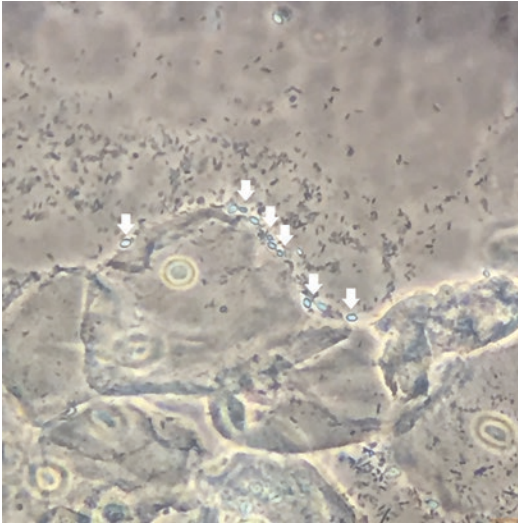


Fig. 24.5 Clustering of blastospores, typical of NAC (white arrows). (phase contrast, 400×)

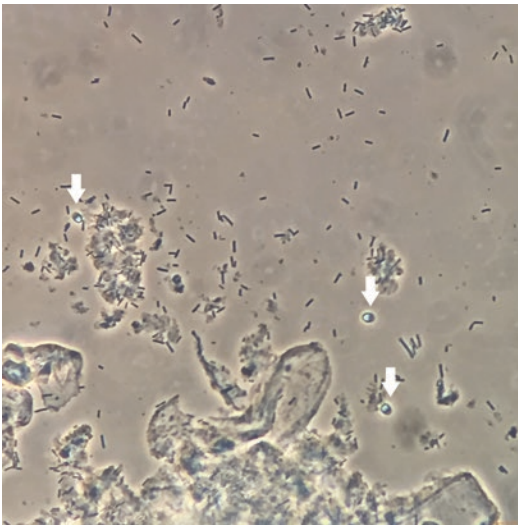


Fig. 24.6 Sperm heads, which can be mistaken by blastospores (white arrows). Notice that these are bicolor and asymmetrical. (phase contrast, 400×)

24.2.7.2 Cultures

Cultures are not needed routinely. Exceptions are recurrent episodes, microscopy negative or uncertain, or no response to treatment [2, 6]. Sensitivity tests are hardly ever needed, except for difficult NAC cases. *C. albicans* strains resistant to fluconazole are uncommon and very rarely pose a clinical problem.

Identification of *Candida* in cultures, per se, cannot be assumed as a diagnosis of VVC, as it does not distinguish colonization from infection.

The swabs can be transported in common transport means (Stuart or Amies)—or even without it, at room temperature, and should be cultivated within 6 h [4]. There seems to be no advantage between the available culture means (Sabouraud agar, Nickerson medium, or Microstix-Candida medium) for *Candida* [6].

24.2.7.3 pH

Candida is more often found with normal pH and is likely to be more symptomatic under those conditions. However, it may co-occur with BV or aerobic vaginitis (AV) (Fig. 24.4), which are typically associated with elevated pH. An elevated pH cannot rule out VVC, and at the same time, typical clinical presentation with a normal pH does not necessarily confirm the diagnosis [4].

However, the pH can guide the next steps in the management of the patient with symptoms suggestive of VVC: If microscopy is positive for *Candida* and the pH is increased, a mixed infection must be considered; if microscopy is negative and pH is normal, cultures are recommended [6].

24.2.7.4 Molecular Tests

The Affirm VPIII is a commercially available test, based on DNA probes, that detects *Candida*, *T. vaginalis*, and *G. vaginalis* in vaginal samples, returning the result in 45 min. The sensitivity and specificity for *Candida*, in comparison with the gold standard (culture) were 82.76% and 98.80%, respectively [25, 26].

The use of nucleic acid amplification tests (NAAT) techniques increases the detection rate of *Candida* in 12.5% [27] and can be an option in those cases of high grade of suspicion, in which wet mount and cultures are negative. In recurrent cases, when microscopy is positive but cultures are negative, molecular techniques lead to the diagnosis of three times more cases [28, 29].

More recently, the BD MAX system, a real time PCR test for the detection of the presence of DNA from lactobacilli, bacteria associated

with BV, *T. vaginalis*, and *Candida* (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*, *C. glabrata*, and *C. krusei*) was developed. Sensitivity for the different species of *Candida* ranged from 75–91% and specificity was higher than 90%. It showed to perform similarly in self-collected samples [30].

These tests allow for faster results, are less operator dependent, and can improve the diagnostic performance, but pose the risk of overdiagnosis (not distinguishing colonization from infection), not recognizing the full picture (mixed infections), and are still expensive.

24.2.8 Treatment

24.2.8.1 General Principles

Since the symptoms are mostly vulvar, women must be warned that topical medication must be applied inside the vagina, rather than (just) in the vulva. External, vulvar application can lead to transitory relief of symptoms, but not to cure.

Topical antifungals can be oil based and, consequently weaken condoms [10].

Oral azoles can interfere with oral anticoagulants (increasing its levels) and anticonvulsants, such as phenytoin.

There is no indication to treat sexual partners, unless they have symptoms themselves (balanitis); in the latter case, topical antifungals can be used. Redness, in some cases, may be associated with an allergic reaction to *Candida* antigens, rather than infection.

Persistence of symptoms can be the result of a contact dermatitis, resulting from the repeated and long lasting treatments, or from secondary lichen simplex *chronicus*. In some cases, allergy to propylene glycol, a common excipient in vaginal medications can develop.

24.2.8.2 Asymptomatic *Candida*

There is no reason to treat asymptomatic *Candida* in non-pregnant women, independently of being an incidental finding in a Pap test or other laboratorial exam, or being suspected during a clinical examination.

24.2.8.3 Uncomplicated VVC

These cases should be treated with short courses (1–7 days) of vaginal imidazoles (clotrimazole, miconazole, butoconazole, etc.) or single doses of oral triazoles (fluconazole or, alternatively, itraconazole), which have similar efficacy (>80%) and adverse effects profiles [10] (Table 24.3). The choice for oral or topical routes is according to patients' preferences. In some cases, women find a faster relief by using vaginal formulations, as it temporarily increases the pH.

There is no evidence that itraconazole is more effective than fluconazole [31, 32]. Topical polyenes, such as nystatin, have lower cure rates than topical imidazoles [33]. The limited data concerning the use of terbinafine, an allylamine antifungal suggests that it is efficacious if used topically, but not if orally [34, 35]. Data about ciclopirox olamine is also limited, but the few trials in which it was used showed it to be safe and effective.

24.2.8.4 Complicated VVC

Severe cases of VVC should be treated with more prolonged courses of topical antifungals or, if fluconazole is chosen, a second dose after 72 h is recommended (Table 24.3).

Topical, low potency corticosteroids (hydrocortisone) for a couple of days can be helpful in very symptomatic patients.

Cases of recurrent VVC are a truly clinical challenge. Reasonable attempts to control or correct risk factors should be tried: reducing sugar intake, stopping hormonal contraceptives or removing IUDs/LVG-IUSs, correcting excessive washing, stopping the use of tight or synthetic clothes, etc. Each measure should be sequentially tried and, if not efficacious, suspended—otherwise, these changes will just add suffering to someone who already has an impaired quality of life due to a chronic disease [4, 6].

In case of failure of these measures, self-treatment of the episodes or, preferably, suppressive treatment must be considered. The most commonly recommended treatment was proposed by Sobel [36] and consists of taking 150 mg of oral fluconazole weekly, for 6 months, after an induction in the first week (three times 150 mg in the first week). Donders proposed an alternative

Table 24.3 Treatment of VVC

Uncomplicated	Topical (vaginal)	<p>Clotrimazole 1% cream 5 g <i>id</i> for 7–14 days</p> <p>Clotrimazole 2% cream 5 g <i>id</i> for 3 days</p> <p>Clotrimazole 100 mg suppository, <i>id</i> for 7 days</p> <p>Clotrimazole 200 mg suppository, <i>id</i> for 3 days</p> <p>Clotrimazole 500 mg suppository, single application</p> <p>Miconazole 2% cream 5 g <i>id</i> for 7 days</p> <p>Miconazole 4% cream 5 g <i>id</i> for 3 days</p> <p>Miconazole 100 mg suppository, <i>id</i> for 7 days</p> <p>Miconazole 200 mg suppository, <i>id</i> for 3 days</p> <p>Miconazole 1200 mg suppository, single application</p> <p>Tioconazole 6.5% ointment 5 g single application</p> <p>Tioconazole 300 mg suppository, single application</p> <p>Butoconazole 2% cream (bioadhesive), 5 g in a single application</p> <p>Terconazole 0.4% cream 5 g <i>id</i> for 7 days</p> <p>Terconazole 0.8% cream 5 g <i>id</i> for 3 days</p> <p>Terconazole 80 mg suppository, <i>id</i> 3 days</p> <p>Econazole 150 mg tablets, <i>id</i> for 3 days</p> <p>Nystatin 100,000 units suppositories <i>id</i> for 14 days</p> <p>Ciclopirox olamine 1% cream 6 days</p>
	Oral	<p>Fluconazole 150 mg, single dose</p> <p>Itraconazole 200 mg <i>id</i>, 3 days</p> <p>Itraconazole 200 mg 2 <i>id</i>, one day</p> <p>Ketoconazole 200 mg 2 <i>id</i>, 5 days (hepatic toxicity)</p>
Pregnancy	Only topical azoles during 7 days	
Breastfeeding	Azoles excreted in milk; avoid during breastfeeding	
Severe VVC	Topical	Prolong treatment for 7–14 days
	Oral	Fluconazole 150 mg day 1 and day 4
Recurrent VVC	Fluconazole suppression for 12 months (ReCiDiF)	<p>Phase 1–200 mg <i>po</i> days 1, 4 and 7</p> <p>Phase 2–200 mg <i>po</i> once a week for 2 months</p> <p>Phase 3–200 mg <i>po</i> once every other week for 4 weeks</p> <p>Phase 4–200 mg <i>po</i> once a month for 6 months</p> <p>Transition from one phase to the other implies: Symptoms free, wet mount/culture negative</p>
	Fluconazole suppression for 6 months (Sobel)	150 mg <i>po</i> once a week (100, 150 or 200 mg on day 1, 4 and 7, prior to suppressive therapy)
	Depot medroxyprogesterone acetate	Intramuscular 150 mg every 12 weeks
Non-albicans VVC	Boric acid 600 mg vaginal capsules	<p>Vaginally <i>id</i> for 2 weeks</p> <p>Must be compounded by pharmacists</p> <p>Toxic if per mouth</p> <p>Maintenance regimen?</p>
	Gentian violet	<p>Useful in some cases</p> <p>Good antipruritic effect</p> <p>Messy</p>
	High dose fluconazole	800 mg <i>id</i> for 2–3 weeks
	Other options may include:	
<ul style="list-style-type: none"> • Flucytosine • Posaconazole • Voriconazole • Amphotericin B • Caspofungin • Micafungin 		

regimen, after the ReCiDiF trial, lasting 1 year and consisting of progressively increased intervals between fluconazole intake (see scheme in Table 24.3) [28]. In the later, while using the same total dosage of fluconazole and being comparable in terms of efficacy at 6 months, 77% of women were disease free at 12 months, comparing to only 42.9% in the Sobel's scheme [28, 36].

Episodes during suppressive therapy can be managed with topical antifungals; in occasional cases, antihistamines can be helpful.

Dennerstein reported good results in controlling the symptoms by using depo-medroxyprogesterone, probably by inducing a hypoestrogenic state [37]. In our hands, it has been an excellent option when other options fail.

It can be advisable that these patients have swabs with them, for self-collection and confirmation of the episodes.

In difficult cases, consider exclusion of HIV and diabetes.

In women with history of frequent or recurrent VVC after antibiotic intake, beta-lactamics, macrolides, and tetracyclines should be avoided. For urinary tract infections, alternative options include fosfomicine or norfloxacin. Additionally, prophylactic antifungals can be prescribed, to be taken at the beginning and halfway through the course of antibiotics [4].

The most common of the NACs, *C. glabrata*, is not sensible to the usual antifungals. One of the best alternatives is boric acid. Patients must be warned of its toxicity if ingested (gastrointestinal, mental disorders, seizures, anaemia, etc.).

C. krusei is intrinsically resistant to oral triazoles, however, it responds well to topical imidazoles, nystatin, boric acid, and ciclopirox olamine [2].

24.2.8.5 Candida in Pregnancy

Oral triazoles should not be used during pregnancy, as it has been related to spontaneous abortion and to a slight increase in the risk of Fallot's tetralogy.

As discussed previously, *Candida* can be associated with negative obstetrical outcomes and for that reason some authors recommend treatment of asymptomatic women. This recommendation, however, is not universal and more data is needed to support it.

24.2.9 Future Perspectives

Despite the role played by lactobacilli in the prevention of symptomatic VVC, its administration either as treatment or adjuvant of antifungal treatment, has not shown to be useful in acute or recurrent episodes [38]. This line of investigation, however, can prove to have some role in the future, especially if strains that naturally dominate the vagina of healthy women are used.

In the future, vaccines may be the answer to treat recurrent VVC.

24.2.10 *Saccharomyces cerevisiae*

The yeast *S. cerevisiae* can colonize the vagina of healthy women, and very rarely, become symptomatic. Its incidence, however, seems to be rising and, apparently it is not always associated with professional exposure (brewery or bakery) or with consumption of it as a food additive or probiotic [39, 40].

Symptoms are undistinguishable from those of VVC. It is, however, less susceptible to antifungals and is associated with high rates of recurrence. The diagnosis is confirmed by culture, but can be suspected when larger than usual blastospores are seen on WMM [4] (Fig. 24.7). Treatment options can include nystatin, amphotericin B, flucytosine, and boric acid.

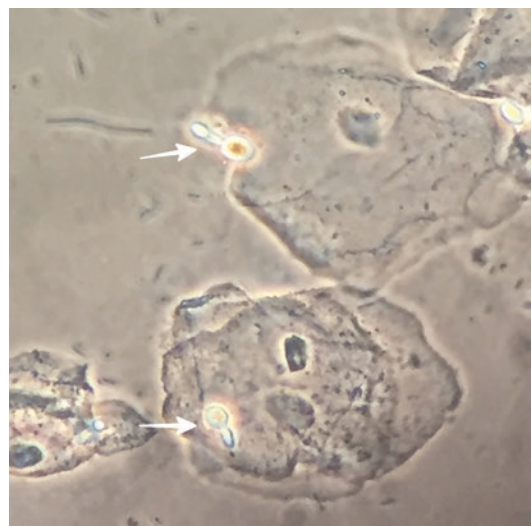


Fig. 24.7 Larger than usual budding blastospores (white arrows). Culture confirmed *S. cerevisiae*

24.3 Trichomoniasis

Trichomoniasis: Breaking the Myths

- Trichomoniasis is not rare—it is the most common non-viral STI and prevalence seems to be rising.
- The classical image of the “strawberry cervix” is seen in less than 5% of the cases with *T. vaginalis*
- Maternal *Trichomonas vaginalis* infection is not only a nuisance. It is a risk factor for low birth weight, premature rupture of membrane, preterm labour, and even intellectual disability. One study related *T. vaginalis* with neonatal death.

24.3.1 Aetiology and Pathophysiology

Trichomoniasis is caused by the extracellular protozoan *Trichomonas vaginalis*, which was first described in 1836, but forgotten in the medical literature for a century.

T. vaginalis is of piriform, round or amoeboid shape, with a distinctive elongated nucleus, four anterior flagella, and a large posterior axostyle to which an undulant membrane is attached. It is motile, with fast and erratic movements.

The amitochondriate and anaerobic parasite is capable of phagocytosing bacteria (including lactobacilli), epithelial cells, and erythrocytes (from where it gets iron and cholesterol), but can itself be phagocytosed by macrophages [41].

Its only natural hosts are humans, colonizing almost exclusively the genitourinary tract of both men and women. Infection sites in women include the vagina, endocervix, urethra, and para-urethral glands. Very rarely it can be found in the respiratory tract.

In adults, transmission is almost exclusively by sexual route; direct vaginal or urethral inoculation is needed. There are some reports of possible transmission by sharing the bath water, per fomites, or by genital manipulation by another

infected person. The parasite can survive outside of the human body for periods of around 3 h, if protected from desiccation [42].

T. vaginalis can be infected by double strand RNA viruses, which, as discussed ahead, can have severe implications in its virulence [43].

24.3.2 Prevalence and Epidemiology

Trichomoniasis is the most common non-viral STI and prevalence seems to be rising [44, 45]. Existing projections are likely to be an underestimation of the true prevalence and burden of the infection, as it is not a reportable disease. This leaves extensive gaps in the understanding of the dynamics of the infection and the true extent of its complications.

Different diagnostic techniques and baseline characteristics of the populations studied preclude comparisons between series. For instance, the very high prevalences found in STI clinics are unlikely to reflect the true picture for the general population [7].

According to World Health Organization, in 2008, in the age range 15–49 years, trichomoniasis led the list of curable STIs, with 276.4 million of new cases in that year: more than the sum of those of *Chlamydia trachomatis*, *Neisseria gonorrhoea* and syphilis. The estimated number of *T. vaginalis* infections at any moment was estimated at 187 million (Table 24.4).

The prevalence is much higher in females (5.6–22.0%) than in males (0.6–2.2%) (Table 24.4). Incidence, however, is similar in both sexes, ranging between 45.6 and 180.60/00 (promil) [45], meaning that the rate of transmis-

Table 24.4 Prevalence and incidence of *T. vaginalis* in 2008, according to the WHO. Adapted from Rowley et al. [45]

WHO region	Incidence (‰)		Prevalence (%)	
	Female	Male	Female	Male
African	146.0	164.8	20.2	2.0
Americas	177.7	180.6	22.0	2.2
South East-Asia	40.3	50.1	5.6	0.6
European	51.7	48.4	5.8	0.6
Eastern Mediterranean	64.0	66.1	8.0	0.8
Western Pacific	45.6	47.0	5.7	0.6

sion and acquisition is similar, but males spontaneously eliminate the infection much faster. It usually takes about 10–21 days in males, while in women it can persist for months or even years [46]. However, in men it too can become chronic or reinfection can occur. The zinc rich milieu of prostatic secretions can account for these differences; chronic infection in men is more likely in those with lower concentrations of zinc in the prostatic secretions [47].

Transmission is more effective from males to female than the other way round (*T. vaginalis* is positive in 66–100% of the female partners of infected men vs. 30–80% of the male partners of infected women [48]).

The incubation period is believed to range between 4 and 28 days [49].

Contrary to other STIs, the prevalence of *T. vaginalis*, at least in some settings, peaks in women after 40 years of age. Possible explanations include: (1) long-standing infection in reservoirs, such as the periurethral and subepithelial glands, (2) increasing resistance to metronidazole, (3) increased pH, due to the falling oestrogen levels, (4) sexual risk factors, and (5) screening artefact. The theory of sexual risk factors is supported by the fact that this peak was also described in men, and in women it only occurred in those who were also positive for high-risk human papillomavirus (HPV) [50].

24.3.3 Risk Factors and Associations

Despite geographical variations, racial, social, sexual, and behavioural factors for trichomoniasis have been identified, disentanglement of the various factors in order to distinguish true risk factors from mere associations is a nearly impossible task.

In the United States, the prevalence of trichomoniasis in women of black race was four times higher than in the general population and, more striking, ten times higher than white non-Hispanic women.

In the same study, positive correlations were found with lower educational level, poverty, greater number of sexual partners, increasing age, and vaginal douching [51]. Other reported

factors include early sexual debut, new or multiple sexual partners, having other STIs, incarceration, commercial sex, being an immigrant, intravenous drug use, cigarette smoking, and not using condoms [51, 52].

Sexual factors, per se, cannot account for the full explanation of the different prevalences found in different countries: for instance, European countries with rates of *Chlamydia* similar to those found in the United States have lower rates of TV [41].

One interesting association is that of *T. vaginalis* with BV, which is found in 60–80% of cases. The mechanism is not fully understood: on one side, *T. vaginalis* benefits from the increased pH and anaerobic environment associated with BV, and, on the other side, *T. vaginalis* may disturb the vaginal microbiome and shift it to the anaerobic side [7].

24.3.4 Signs and Symptoms

It is estimated that up to 80–85% of infected women and 77% of men are asymptomatic [41, 45, 48]. Many of the asymptomatic women eventually become symptomatic—sometimes in a very exuberant, highly symptomatic way. Given the long periods during which the infection can be asymptomatic, it is almost impossible to ascertain when infection was acquired.

When symptomatic, women can refer a yellow or green discharge, frequently with a foul smell. Urinary symptoms can be present (frequency, dysuria), as well as lower abdominal pain, dyspareunia, vulvar irritation, itching, and burning. Dysuria, unlikely in women with VVC is not only terminal or post-micturition, as there can be a real urethritis or cystitis (urethral involvement occurs in 90% of cases) [53]. Symptoms tend to worsen during menses, probably due to the increase in the number of parasites, associated with the phagocytosis of erythrocytes.

Some women may suffer from postcoital bleeding, as a consequence of the presence of erosions in the vaginal wall.

On physical examination, this discharge is frothy, more or less abundant and fluid (Fig. 24.8). Erosions, cervical friability, and vaginal enantema

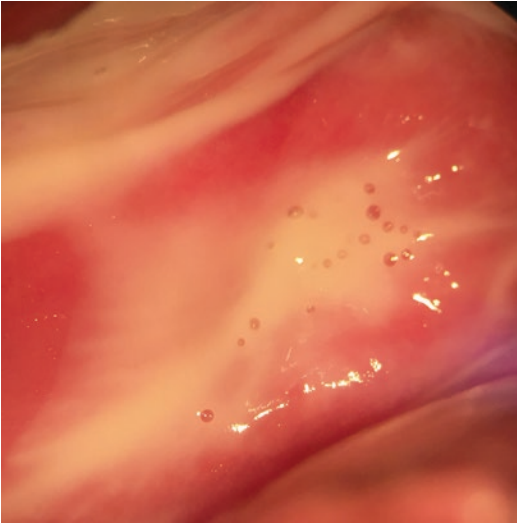


Fig. 24.8 Frothy vaginal discharge in a woman with *Trichomonas vaginalis* and bacterial vaginosis



Fig. 24.10 Strawberry cervix after the application of iodine (“leopard skin”)

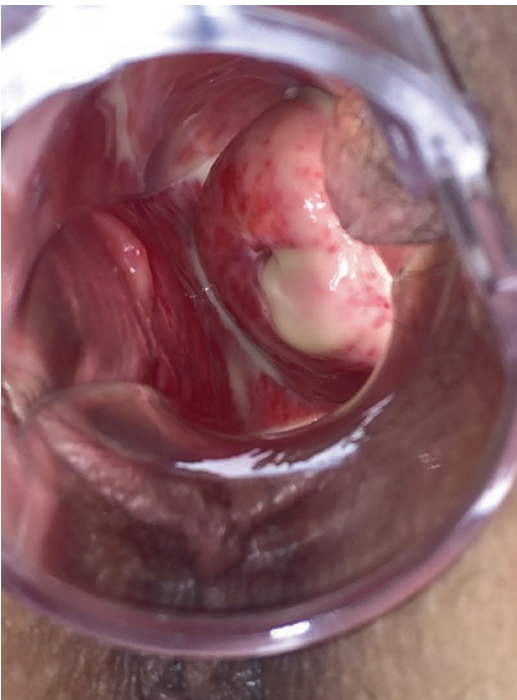


Fig. 24.9 Strawberry cervix, typical of a *Trichomonas* infection

can be noticed in the vaginal walls and the cervix. The classical image of the “strawberry cervix” is uncommon (<5%) (Fig. 24.9); after the application of Lugol’s solution it may gain the typical appearance of “Leopard skin” (Fig. 24.10).

Very rarely, *T. vaginalis* can be associated with the enigmatic condition of emphysematous vaginitis, in which air bubbles form in the vaginal walls, sometimes with crepitus during intercourse or speculum examination [54].

A normal gynaecological examination is not sufficient to exclude the presence of *T. vaginalis*.

24.3.5 Complications

For a long time, *T. vaginalis* was considered a nuisance STI. However, nowadays, it is acknowledged as being associated with severe complications, and considered a public health issue [41].

Maternal *T. vaginalis* infection is a risk factor for low birth weight (RR, 1.51; [95% confidence interval 1.32–1.73, $p < 0.001$]), premature rupture of membranes (RR, 1.41; [95% confidence interval, 1.10–1.82, $p = 0.007$]), preterm labour (RR 1.4 [95% confidence interval, 1.15–1.75; $p = 0.001$]) [55], and even intellectual disability [41]. One study related *T. vaginalis* with neonatal death (1.6% vs. 0.8%, $p = 0.005$) [56].

T. vaginalis infected with *M. hominis*, besides being more cytopathogenic, induce an increased production of IL1 β , IL6, IL8, and tumour necrosis factor (TNF) 1 α —a similar profile to that associated with preterm labour [57].

Newborns from infected mothers can have a self-limiting vaginitis or pneumonia, but sometimes treatment is required. The symptoms of vaginitis usually disappear as the levels of oestrogens wane.

T. vaginalis is a well-recognized risk factor for the transmission and acquisition of other STIs, namely HIV [58]. *T. vaginalis* increases the risk of acquisition and transmission by several ways: (1) by attracting inflammatory cells to the vagina, (2) by causing breaches in the integrity of the vagina and cervix (facilitating the access of HIV to the bloodstream), (3) by increasing IL-8, which increases HIV1 replication, and (4) by changing the vaginal flora towards BV [41].

Proper management of HIV patients should include regular screening of trichomoniasis (and BV), as it can be asymptomatic and still increase the HIV load in the vagina, as thus promote the transmis-

sion. Reducing *T. vaginalis* rates can be an effective, cost-effective measure to reduce HIV transmission, especially in populations such as in sub-Saharan Africa (Table 24.4), where it is believed to increase the risk of infection 2.1–2.8 times; this impact can be extensive to vertical transmission [59].

It has also been linked to increased risk of pelvic inflammatory disease, HSV2, persistence of HPV infection and cervical cancer, and false positive abnormal Pap tests [43, 44, 60]. Some authors claim it should be included in the carcinogenic agents list (increased risk of cervical and prostate cancer) [43].

T. vaginalis infections out of the genitourinary tract are very rare, but a few cases have been described, including a central nervous infection in a premature neonate, leading to death [61].

24.3.6 Diagnosis

Given the burden of complications associated with *T. vaginalis*, low threshold for testing and sensitive tests should be used (Table 24.5).

Table 24.5 Available tests for the diagnosis of *T. vaginalis*

Type of test		Sensitivity	Specificity	Notes
Wet mount microscopy		36–82%	100%	<ul style="list-style-type: none"> • Results in a few minutes • Easy and cheap • Low sensitivity • Exam must be performed immediately • Not useful in men
Pap test		56–76%%	>95%	<ul style="list-style-type: none"> • Not recommended for screening • Liquid based cytology performs better than classical cytology • Controversial if further testing is needed before treatment
Culture	Modified diamond medium InPouch (biomed diagnostics, White City, Oregon)	75–96%	>95%%	<ul style="list-style-type: none"> • Not easily available • Results in up to 7 days for diamond medium • Vaginal secretions preferred • In InPouch, most positive results occur within 3 days, but 17% only after that • InPouch has increased sensitivity over traditional culture mediums (?) • Useful if resistance tests are to be performed

Table 24.5 (continued)

Type of test		Sensitivity	Specificity	Notes
Immunochromatographic assays	OSOM (Sekisui, Framingham, MA)	82–95%	>95%	<ul style="list-style-type: none"> • Results in 10–15 min • Does not allow to test for other STIs • False positives possible • Self-testing possible (decreased sensitivity) [62]
DNA probes	Affirm VPIII (BD, Sparks, MD)	46.3–63%	>95%	<ul style="list-style-type: none"> • Results in <1 h • Concomitant test for <i>C. albicans</i>, <i>G. vaginalis</i> and <i>T. vaginalis</i> • Useful in settings where STIs are not prevalent • FDA approved
NAATs	AmpliVue (Quidel, San Diego, CA)	>90%	>95%	<ul style="list-style-type: none"> • Results in 45 min • Good agreement with PCR • False positives possible? FDA approved
	Solana (Quidel, San Diego, CA)	89.7%	>95%	<ul style="list-style-type: none"> • Results in >45 min • FDA approved
	Aptima <i>T. vaginalis</i> (Hologic, San Diego, CA)	>95%	>95%	<ul style="list-style-type: none"> • Results in <8 h • Possible cotest of CT/NG • FDA approved
	In-house real time PCR	76–98%	>95%	<ul style="list-style-type: none"> • Better performance if vaginal swabs are used
	XPert <i>T. vaginalis</i> (Cepheid, Sunnyvale, CA)	95% agreement with in-house PCR	100% agreement with in-house-PCR	<ul style="list-style-type: none"> • Results in 60 min • FDA approved
	BD ProbeTec Q ^x (BD, Sparks, Maryland)	>95%	>99%	<ul style="list-style-type: none"> • Results in <8 h • FDA approved
	BD MAX system (BD, Sparks, Maryland)	>90%	>99%	Results in 3 h

However, that is not the reality most of the times [63]. When comparing studies, it is important to keep in mind if the gold standard used was wet mount/cultures or the much more sensitive NAATs, as these can detect even non-viable protozoans and low loads of microorganisms.

Wet mount microscopy can allow the immediate diagnosis, besides being a cheap and easy technique. However, it relies on documenting motile protozoans (https://www.youtube.com/watch?v=pTL-_Q4S1Og). The observation should be performed immediately after collection of the sample; heating slightly the slide can increase the motility of the parasite. Immotile parasites are difficult to identify, as they have approximately the same size as a leucocyte.

Inflammation is not always present (Fig. 24.11), but when present it can be very exuberant. Wet mount microscopy has a rather low sensitivity (36–82%) [64–67], even if using phase contrast and with experienced examiner. The sensitivity may be even lower in asymptomatic women, thus it cannot be recommended as a screening test. However, it can be used in a “cascade” model for symptomatic women: If it is diagnostic for *T. vaginalis*, treatment can be provided immediately; if negative, further laboratory testing must be carried out. Nevertheless, it is an excellent tool to check for the commonly associated presence of BV (Fig. 24.12).

Determination of pH is not a diagnostic tool, but it can work as a triage test; in symptomatic



Fig. 24.11 *Trichomonas vaginalis* (inside white circle) (phase contrast, 400×)

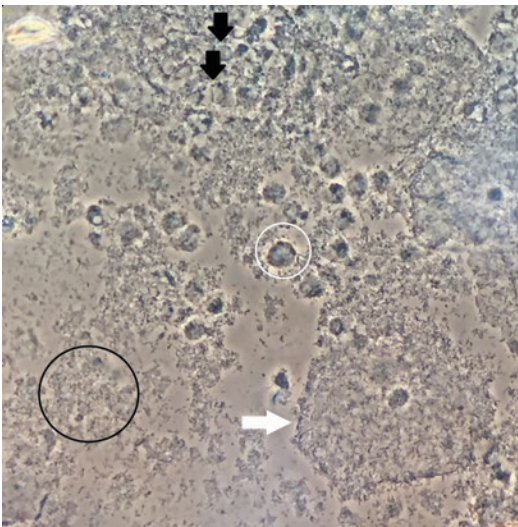


Fig. 24.12 *Trichomonas vaginalis* (white circle) and bacterial vaginosis (granular flora—black circle; clue cell—white arrow). Notice the presence of inflammation (black arrows), which is not a feature of BV (phase contrast, 400×)

trichomoniasis values are higher than 4.5 (frequently even higher than 5) [42]. The sensitivity of pH for the diagnosis of trichomoniasis is very high, but the predictive positive value is very low.

The Whiff test is frequently positive, as the coexistence *T. vaginalis* and BV is very high (60–80%). However, a negative test does not exclude the diagnosis.

The Pap test should not be performed with the objective of diagnosing trichomoniasis. It is controversial if the finding of *T. vaginalis* in a Pap test should be confirmed prior to treatment: Some defend that no further testing is needed if liquid based cytology was used (as specificity is high), while others only accept it in settings where the disease is very prevalent (>10%) [64], and others recommend systematic confirmation [10]. If the diagnosis was made in a conventional Pap test, confirmation should always be performed prior to treatment. In one study from 1972, comparing Pap test to cultures for the diagnosis of trichomoniasis, it was concluded that the former yielded high rates of false negative and false positive results, making it highly unreliable. It was assumed that the source of mistake was the rounded forms that the protozoan can assume, and that are difficult to distinguish from leukocytes [68].

Optimal tests for *T. vaginalis* in terms of sensitivity are NAATs, which can detect 3–5 times more infections than WMM [10]. These tests, however, are expensive and do not produce an immediate result—which is of utmost importance in STI clinics.

Vaginal and endocervical specimens (swabs or liquid based cytology mediums) are the best sampling options. With the use of urine samples, sensitivity drops slightly [66].

In the future, versatile platforms such as the XPert *T. vaginalis* (Cepheid, Sunnyvale, CA) [69] can gain terrain in this field: The results are rapid (60 min) and the cartridge system allows the performance of different analysis using the same platform.

Point of care tests such as OSOM (Sekisui, Framingham, MA) or Affirm VPIII (BD, Sparks, MD) may represent an intermediate option, with a good performance, but cheaper than NAAT tests and allowing faster results, without the need for complex laboratory facilities.

24.3.7 Treatment

Treatment must be considered in all cases, as even if asymptomatic; *T. vaginalis* must not be seen as a commensal. The objectives of treating

all cases are: the elimination of symptoms, stopping transmission, and reducing HIV risk.

As male partners of infected women have a high rate of infection [48], simultaneous treatment of those partners without need for confirmatory testing is mandatory.

The standard recommended treatment is a single dose of 2 g of metronidazole or tinidazole. Sexual abstinence for 1 week is recommended.

Patients must be advised to avoid alcohol consumption, as a disulfiram effect is possible for up to 24 h after taking metronidazole and 72 h after tinidazole; however, this risk may be overemphasized in the literature [70]. The most common secondary effects of nitroimidazoles are gastrointestinal symptoms (nausea, vomiting, metallic taste), headache, and dizziness). These are dose dependent and, for that reason, multi-dose schemes, are

better tolerated. On the other hand, the compliance is higher for single dose schemes [71].

The success rate of cure with oral metronidazole is high (>90%). Resistance, defined as a failure to eradicate the infection after two consecutive courses of treatment, is a raising problem, estimated to be of up to 5% [71]. For tinidazole it is lower (1%) [10]. Possible factors involved in resistance include: inactivation of metronidazole by vaginal bacteria, inadequate local concentration, and low zinc concentration [47]. It must be kept in mind, however, that many apparent failures of treatment are due to reinfection and non-compliance.

Zinc sulphate douches have been tried in small series, with good efficacy; however, it has been reported that it can cause cervical ulcerations [47] (Table 24.6).

Table 24.6 Treatment options for trichomoniasis

First choice	Metronidazole	<ul style="list-style-type: none"> • 2 g <i>per os</i>, single dose
	Tinidazole	<ul style="list-style-type: none"> • 2 g <i>per os</i>, single dose • Longer half-life • More expensive • Better intestinal tolerance
Alternative regimen	Metronidazole	<ul style="list-style-type: none"> • 500 mg 2 <i>id</i> for 7 days (similar cure rates to single dose schemes) • Better tolerance
	Metronidazole 750 mg/miconazole 200 mg	<ul style="list-style-type: none"> • Vaginal application 2 <i>id</i> for 7 days • Only if oral schemes not tolerated • Very limited data on efficacy (one study)
In case of allergy to nitroimidazoles	Desensitization	
Pregnancy	Metronidazole	<ul style="list-style-type: none"> • 2 g <i>per os</i>, single dose (avoid higher doses in pregnancy) • Consider multiple dose scheme if single dose not tolerated [71] • Treat all symptomatic cases • If asymptomatic, consider treatment only in the first trimester or after 37 weeks of gestation
	(Tinidazole)	<ul style="list-style-type: none"> • Avoid in the first trimester • Use in the second or third trimester only if no better options available
Breastfeeding women	Metronidazole	<ul style="list-style-type: none"> • 2 g <i>per os</i>, single dose (discard milk for 12–24 h) • 500 mg 2 <i>id per os</i> 7 days • 400 mg 3 <i>id per os</i> 7 days
	(Tinidazole)	<ul style="list-style-type: none"> • Avoid • 2 g <i>per os</i>, single dose (discard milk for 72 h)
HIV patients	Metronidazole	<ul style="list-style-type: none"> • 500 mg 2 <i>id</i> for 7 days

(continued)

Table 24.6 (continued)

Alternative treatments in resistant cases (always consider non-compliance or reinfection)	Metronidazole	<ul style="list-style-type: none"> • 800 mg 3 id for 5–7 days • 2 g id for 5–7 days ± intravaginal tinidazole
	Tinidazole	<ul style="list-style-type: none"> • 2 g id per os for 5–7 days • 2–3 g id per os for 14 days (same for partners)
	Options needing further studies: <ul style="list-style-type: none"> • Zinc sulphate 1% (± oral tinidazole) douches • Pessaries (paromomycin/furazolidone/6% non-oxynol-9) • Boric acid (vaginal) • Disulfiram • Nithiamide • Albendazole + nitroimidazole • Coenzyme B12 + nitroimidazole 	

The rate of clinical cure can be improved by using sensitivity tests and tailoring the treatment according to it (higher doses of metronidazole or changing to tinidazole).

Topical metronidazole is not recommended for treatment, as it is associated with very low rates of cure (<50%), probably due to the fact that only insufficient concentrations are achieved in the urethra and periurethral glands, which can act as reservoirs of the parasite. However, higher doses of vaginal metronidazole (750 mg), combined with miconazole can lead to cure rates comparable to oral metronidazole [72].

It is still uncertain which is the impact of the coexistence of BV and *T. vaginalis* on the results of treatment, despite that both situations usually respond to nitroimidazole therapy [7].

Treatment during pregnancy, in one study increased the risk of preterm delivery three times (RR 3.0; 95% confidence interval, 1.5–5.9) but this is still controversial and not confirmed in other studies [73, 74]. One of the theories sustains that the exposition of *T. vaginalis* infected with *M. hominis* to antibiotics leads to a massive release of the latter. These, once released, can infect the membranes and the amniotic fluid, thus initiating a preterm labour [73]. Treatment, however, reduces the risk of transmission to the newborn and decreases the risk of HIV infection. One reasonable option is treating all cases in the first trimester (to reduce HIV risk), the symptomatic cases at any time, and the asymptomatic ones after 37 weeks of gestational age (to reduce the risk of transmission, without increasing that of preterm labour) [53].

Women who test positive for *T. vaginalis* during pregnancy should be counselled to

consistently use condoms, besides the adequate treatment of all sexual partners.

A test of cure can be performed 2–3 weeks after the treatment; prior to that, NAATs can be positive due to the presence of non-viable organisms. In pregnant women, given that *T. vaginalis* is a risk factor for HIV, the CDC recommends retesting after 3 months. In HIV women, retesting is also recommended [10].

24.4 Dysbiosis

Vaginal Lactobacilli: Breaking the Myths

- Bacterial vaginosis is not the only cause for absent lactobacilli in non-hypoestrogenic women. Aerobic vaginitis is another cause, which is much less recognized
- Despite the important role of Lactobacilli in vaginal health, they are absent in nearly one third of asymptomatic women

Vaginal dysbiosis refers to the conditions in which there is a flora disturbance or imbalance, characterized by a severe depletion or even absence of lactobacilli [75]. Traditionally, it has been assumed that BV is “the” cause for absent lactobacilli in non-hypoestrogenic women. However, clinical practice, especially with the use of WMM, has shown that it is not necessarily true. An increased pH (a marker of lactobacilli depletion and, thus, dysbiosis) is found not only in BV, but also in con-

ditions in which aerobic, rather than anaerobic, bacteria predominate. Besides the overgrowth of aerobic bacteria, these women usually also have atrophy and inflammation (which are not part of BV) [76]. This entity was named aerobic vaginitis (AV) by Donders et al. in 2002 [77].

The non-recognition of AV may have led to serious drawbacks in clinical investigation, namely in the areas of preterm labour, sexually transmitted infections, and cervical dysplasia [78].

The concept of the essential role of the dominance of lactobacilli in the vaginal flora during reproductive age has been challenged, as it has been shown that it does not happen in nearly one third of asymptomatic women [79]. This is the rule in other species of mammals, in which lactobacilli represent only 1% of the vaginal microbiota, while one similar to that associated with BV dominates (*Gardnerella*, *Mobiluncus*, *Sneathia*, and *Prevotella*). Bacteriocins are not of exclusive production by lactobacilli; other bacteria such as *Streptococcus*, *Prevotella*, and *Corynebacterium* may fulfil that function [80].

24.4.1 Bacterial Vaginosis

Some authors have suggested that the condition should be named anaerobic vaginosis, rather than bacterial, as it overemphasizes a mere bacterial aetiology [81].

Bacterial Vaginosis (BV): Breaking the Myths

- BV may go unnoticed—it is asymptomatic in 50–85% of the cases
- BV is a sexually associated condition but not an STI, as no disease has been identified in the male partners, the condition cannot be attributed to a single microorganism, and treatment of the partner does not prevent recurrences in women.

24.4.1.1 Aetiology and Pathophysiology

BV is a polymicrobial syndrome, characterized by the absence of lactobacilli and overgrowth of a

variable mixture of predominantly anaerobic and facultative bacteria, which in low loads are part of the normal vaginal flora. These can include *G. vaginalis*, *Atopobium vaginalis*, *Mobiluncus*, *Prevotella*, *Megasphaera*, *Leptotrichia*, *Sneathia*, *Bifidobacterium*, *Dialister*, *Peptostreptococcus*, *Fusobacterium*, *Clostridium*, non-cultivable “BV-associated bacteria” (BVAB) 1–3, and *M. hominis*, among others, in loads 100–1000 times higher than the normal. Identifiable modifications in the microbiota precede the development of BV by weeks or months [82].

Given that these bacteria are sensitive to lactic acid and hydrogen peroxide, the initial step for the development of BV is likely to be the reduction or disappearance of lactobacilli. Several endogenous and exogenous factors, including phages and some *Mollicutes*, can be responsible for this [81, 83].

According to the model proposed by Nasioudis et al., it all starts with a transitory state of diminished lactobacillary dominance. During this stage, if the disturbing factor is enough to prevent the recovery of the lactobacilli or gives advantage to other bacteria in the competition for glycogen, a biofilm starts to be produced. The biofilm further deprives lactobacilli from having access to manganese, inhibiting their recovery, and allows the eviction of the already depressed innate immune system defences (low levels of H₂O₂ and lactic acid) [58, 84]. Additionally, the production of sialidase leads to the degradation of IgA, impairing the acquired immune system response. The immunological response is typically absent or scarce [58, 84].

24.4.1.2 Prevalence and Epidemiology

BV is the most common cause of symptomatic vulvovaginal discharge in childbearing age women, accounting for nearly half of the cases. It is rarely encountered in children and becomes less common during menopause. Prevalence is lower during pregnancy [85].

As BV is asymptomatic in 50–85% of the cases, when comparing epidemiological data, it must be checked whether it refers to microbiological or clinical (symptomatic) disease and how the diagnosis was established.

Global prevalence (symptomatic and asymptomatic) varies widely according to the population studied (12–55%), being consistently higher in black women (50–55%) [86, 87]. BV is much rarer in non-sexually experienced women, especially in those without any kind of sexual contact [88], but is very high (20–50%) in women with female partners [89].

24.4.1.3 Risk Factors and Associations

BV is a sexually associated condition (Table 24.7). It cannot be considered an STI, as no disease has been identified in the male partners, the condition cannot be attributed to a single microorganism, and treatment of the partner does not prevent recurrences in women. However, there is a growing evidence that BVAB can be exchanged during intercourse, and it has been isolated from the penis, seminal fluid, and even urine of male partners of women with BV. The bladder may act as a reservoir for recurrences [90]. Circumcision can decrease the load of BVAB and, consequently the risk of BV in partners [91].

Table 24.7 Risk factors and associations for BV

Demographic factors	<ul style="list-style-type: none"> • Lower educational level • Poverty • Black race • Childbearing age
Sexual factors	<ul style="list-style-type: none"> • New partner(s) • Multiple partner(s) • Higher number of lifetime sexual partners • Having female partner(s) • Receptive oral sex • Anal sex • Higher frequency of intercourse • Early sexual debut • Unprotected sex • Sharing sex toys
Behavioural factor	<ul style="list-style-type: none"> • Smoking • Vaginal douching • IUD • Not using oral contraceptives • Having an STI (HIV, HSV2, <i>C. trachomatis</i>, <i>M. genitalium</i>) • Uncircumcised partner • Antibiotic use • Stress • Dietary factors (increased fat, low calcium, folate, and vitamin A)

While women usually do not consider it an STI themselves, they can link sexual activity to the development or recurrence of symptoms [92].

Phages can be the “missing link” between BV and its association with sexual activity: hypothetically, a partner may transmit it, leading to the depletion of lactobacilli [81]. Interestingly, phages are promoted by smoking, which is also a risk factor for BV, by direct toxicity to lactobacilli, and by inducing hormonal changes [93].

While *L. crispatus* is associated with a lower risk for BV, *L. iners* can be identified during dysbiosis. It is uncertain whether it predisposes to dysbiosis or is part of the recovery process [82, 94]. Interestingly, female couples share lactobacilli strains [95].

Progesterone seems to play a protective role, as the rates of BV are lower and the cure rates higher during pregnancy, and less cases are detected during the luteal phase [96].

24.4.1.4 Signs and Symptoms

More than half of the women with BV type flora are asymptomatic. If not, symptoms tend to be mild, fluctuating and sometimes with a long-standing duration (weeks or months).

The most common symptom is an offensive fishy smell, which typically worsens with menses and sexual intercourse, as the release of volatile amines (putrescine and cadaverine) is increased in elevated pH. Sometimes women tend to wash more frequently or to use perfumed products, which paradoxically further worsen the smell.

The discharge associated with BV is yellow or grey, homogeneous, thin, and adherent to the vaginal walls (Fig. 24.13a). Mild burning or dysuria are sometimes described, but are not typical features of BV.

The vulvar and vaginal exam, apart from the presence of discharge, are usually unremarkable (Fig. 24.13b).

BV can be considered recurrent if a woman has 3 or more episodes during 1 year.

24.4.1.5 Complications

The risk of complications is independent of whether or not a woman is symptomatic.



Fig. 24.13 (a) Homogenous vaginal discharge in a woman with bacterial vaginosis. (b) At the vulva, the homogenous vaginal discharge can be easily detected in women with bacterial vaginosis. (c) Bacterial vagi-

nosis with all typical features: no lactobacilli, granular floor (white circle) and clue cells (white arrows) (phase contrast, 400 \times)

Most studies failed to distinguish BV from AV, as the diagnosis was frequently based on an increased pH and/or absence of lactobacilli. This can explain different results between studies and why, for instance, treatment failed to reduce the risk of preterm labour in women with the diagnosis of BV. The listed complications most likely represent those of abnormal vaginal flora or dysbiosis, rather than just of BV.

BV was first associated with adverse pregnancy outcomes in 1984, namely with preterm labour. Following that, other obstetrical and neonatal complications were added to the list: spontaneous abortion, low birth weight and increased neonatal morbidity [97].

Pelvic inflammatory disease, post-hysterectomy cuff infection and post C-section endometritis are also more common in women with BV. Interestingly, there are theories sustaining that one of the roles of vaginal lactobacilli may be the reduction of the infectious risks related to pregnancy and delivery [80].

The risk of acquiring an STI, including *C. trachomatis*, *N. gonorrhoea*, HSV2 and HIV infection, is double in women with BV [98]. *U. urealyticum*, *U. parvum* and *M. hominis*, but not *M. genitalium*, are frequently found associated with BV [99].

Additionally, it increases the risk of transmission of HIV to male partners [100]. Several

studies link BV to HPV persistence, cervical dysplasia and cancer.

More recently, there is increasing data relating BV with infertility and lower success rates of assisted reproduction techniques [101].

24.4.1.6 Diagnosis

BV should not be screened in asymptomatic women; it can be considered, but without enough evidence to recommend it, in pregnant women, prior to pelvic surgery or uterine cavity instrumentation, or in those with STIs, especially HIV.

Clinical Diagnosis

Clinical criteria allow the diagnosis of BV in settings of limited resources or expertise. To fulfil the Amsel criteria [102], 3 out of the following 4 must be fulfilled:

1. Homogeneous grey or white discharge coating the vaginal walls (Fig. 24.13a, b);
2. Vaginal pH increased (>4.5);
3. Fishy smell (before or after 10% KOH addition—Whiff test);
4. Clue cells on wet mount (cells with a heavy coating of bacteria, leading to blurring of the peripheral borders; must be present in >20% of cells) (Fig. 24.13c).

The sensitivity and specificity of the Amsel criteria is around 80 and 90%, respectively. The first three criteria are also frequently positive in women with trichomoniasis. The combination of only pH and Whiff test may perform better than the traditional criteria [103]. However, odour is too subjective to base a diagnosis on; in the future, it may be overcome by the use of “electronic noses” [104].

In our opinion, in settings where a microscope is available, it should be used for the proper diagnosis of BV by WMM, rather than just for the mere evaluation of the presence of clue cells.

pH

The pH based point of care tests available for self-testing have a sensitivity and specificity of 73% and 67%, respectively [105].

The VI-SENSE is a pH test, based on a strip placed in a panty liner; clinical trials showed good performance for BV or trichomoniasis, but not allowing distinction between both conditions [106].

Microscopy

In WMM, BV is characterized by absent lactobacilli, granular flora or abundant motile small, curved, rod shaped bacteria (*Mobiluncus*), absence of inflammation, and presence of clue cells (Fig. 24.13c).

WMM has several advantages relatively to Gram staining based Nugent score: (1) it is cheaper, (2) allows the diagnosis during the appointment, (3) requires less expertise, (4) allows the distinction of full-blown from partial BV (in which in the same slide areas of granular flora can coexist with areas with lactobacilli, and frequently clue cells represent <20% of the total number of epithelial cells) and the overcome of the problem of “intermediate scores” from Nugent score, and (5) the evaluation of the bacteria may be more accurate, since there is no fixation and washing of the slides.

The Nugent score, an improvement from the Spiegel score, was described in 1991 [107], and is considered the gold standard for the diagnosis of BV. It is based on the proportion of bacterial morphotypes, using Gram staining:

- Lactobacilli (large Gram-positive rods)—0 to 4 points (higher scores if less abundant);
- *G. vaginalis* and bacteroides (small Gram variable rods)—0 to 4 points (higher scores if more abundant);
- *Mobiluncus*-like morphotypes (curved Gram negative rods)—0 to 2 points (higher scores if more abundant);
- (Gram-positive cocci are not considered in this scoring system).

The diagnosis of BV is established if the final score is equal or higher than seven. If it is equal or lower than three, it is considered normal. A score between 4 and 6 is the troublesome “intermediate score”. It does not correspond to partial BV; part of the cases classified in this grey area,

which usually do not respond to metronidazole treatment, may represent AV. For this reason, Donders proposed that “undetermined flora” would be a more accurate description [108].

The Hay and Ison criteria [109], also based on Gram staining may allow a more complete and realistic evaluation of the vaginal flora:

- Grade 0—epithelial cells with no bacteria seen;
- Grade I—lactobacillus morphotypes only (normal flora);
- Grade II—reduced lactobacillus morphotypes with mixed bacterial morphotypes (intermediate flora);
- Grade III—mixed bacterial morphotypes with few or absent lactobacillus morphotypes (BV);
- Grade IV—epithelial cells covered with Gram-positive cocci only (AV type flora).

Cultures

G. vaginalis can be cultivated from the large majority of women with BV, but also from more than half of the women without it [110]. This translates into a high sensitivity, but a low positive predictive value and thus should not be used.

Pap Test

The Pap test is not intended for the diagnosis of vaginitis. The sensitivity for BV is low, and likely to be even lower when using liquid based cytology, due to centrifugation and filtering of the sample.

Enzymatic Tests

The OSOM BVBlue is a point of care test, based on the chromogenic determination of the presence of sialidase, which is produced by bacteria commonly involved in BV (*Gardnerella*, *Prevotella* and *Bacteroides*). The results are available within 10 min. The sensitivity of the test is variable (38–100%), while the specificity is systematically high (>90%) [111]. In AV, sialidase is also increased and it may explain part of the differences in sensitivity across studies.

Tests to evaluate the activity of proline aminopeptidase have also been designed, giving the results in 10 min. Sensitivity and specificity are higher than 90% [112]. It has also been evaluated in combination with pH and amines determination (FemExam).

Molecular Tests

The use of DNA tests in BV is very complex, as it is polymicrobial and most of the involved agents commonly colonize the vagina of healthy women in low loads. These techniques are usually highly sensitive, but with low predictive positive values. In the future, combinations of evaluation of the proportion of selected anaerobic bacteria and lactobacilli may increase the specificity of this methodology [113].

Nevertheless, the Affirm VPIII, which tests not only BV, but also *T. vaginalis* and *Candida*, has been shown to be a good alternative in settings where a microscope is not available [25, 26].

24.4.1.7 Treatment

BV can heal spontaneously [53]. Treatment aims at resolving the symptoms associated with BV; there is no indication to treat asymptomatic women. It can, however, be considered in high-risk populations for STIs, as it can reduce the risk of acquiring HIV, *T. vaginalis*, *C. trachomatis*, *N. gonorrhoea* and HSV2, and before pelvic surgery.

Therapeutic options for BV include oral and topical antibiotics (metronidazole, tinidazole and clindamycin), and antiseptics (dequalinium chloride) (Table 24.8). The rates of cure are high for all drugs and routes (around 80%), but relapses are common [10, 114].

The vaginal levels achieved with topical medication can be 30 times higher than those achieved with oral medication. This translates into cure rates equal or slightly higher than those achieved with the oral route, with the advantage of having less adverse effects. Combinations of oral and vaginal treatment can increase the cure rates [115].

Despite the perceived increased resistance to metronidazole and clindamycin, it does not seem yet to be a major clinical issue [85, 116]. Some *Gardnerella* strains have been shown to be intrinsically resistant to metronidazole [117]. Several of the bacteria involved in BV cannot be cultivated, thus making it impossible to test its susceptibility profiles.

The cure rates achieved with the antiseptic dequalinium chloride are non-inferior to those of clindamycin [116]. It has the advantages of being less toxic for lactobacilli, and not increasing the risk of VVC, contrarily to antibiotics [118].

Table 24.8 Treatment of BV

First line	Metronidazole tablets	500 mg <i>per os</i> 2 <i>id</i> 7 days
	Metronidazole gel 0.75%	5 g <i>id</i> intravaginally 5 days
	Clindamycin cream 2%	5 g <i>id</i> intravaginally 7 days
	Dequalinium chloride	10 mg tablets <i>id</i> intravaginally 6 days
Second line	Tinidazole	2 g <i>id</i> 2 days
	Tinidazole	1 g <i>id</i> 5 days
	Clindamycin	300 mg <i>per os</i> 2 <i>id</i> 7 days
	Clindamycin	100 mg vaginal ovules <i>id</i> for 3 days
Alternatives	Secnidazole	2 g <i>per os</i> , single dose
	Polyhexamethylene biguanide hydrochloride	1–2 <i>id</i> for 7 days
	Chlorhexidine	Vaginal douche once a day
	Rifaximin	25–100 mg <i>id</i> intravaginally
Recurrent BV	Metronidazole 0.75% gel	2 times/week for 4–6 months
	Triple phase regimen: Oral nitroimidazole, vaginal boric acid, and vaginal metronidazole	<ul style="list-style-type: none"> • Oral nitroimidazole for 7 days • Vaginal boric acid for 3 weeks • Vaginal metronidazole gel twice a week for 16 weeks
	Metronidazole 2 g + fluconazole 150 mg	Once a month
	Ascorbic acid	250 mg vaginal tablets <i>id</i> 6 days/month for 6 months

Additionally, it is not expected that bacteria develop resistance to it and can have some effect in other causes of “vaginitis” other than BV, thus making it at least partially useful for mixed infections, including AV, VVC and trichomoniasis [116]. However, long-term studies concerning recurrences are still lacking.

Women must be warned about the possible interaction between alcohol and nitroimidazoles. Clindamycin is associated with pseudomembranous colitis, and topical formulations may weaken latex condoms.

The use of H₂O₂ irrigations has resulted in contradictory results, but it seems to be less effective than metronidazole, and associated with a risk of development of severe lesions in the vaginal mucosa, due to its caustic effects [119].

The use of acidifying agents, though tempting, has not proven to be efficacious. Only the use of ascorbic acid has some clinical support [120].

There is no evidence that the use of probiotics in acute episodes, isolated or concomitantly with antibiotics improves the rate of success [121].

Pregnancy

Treatment of BV during pregnancy with metronidazole failed to decrease the risk of preterm labour and, in some studies, even lead to an

increase. On the contrary, in most series in which clindamycin was used showed a decrease in the rate of preterm labour [122–124]. Difference in terms of outcomes may be related to the antibiotics spectrum: clindamycin covers Gram-positive bacteria, frequently involved in AV. New studies with a more accurate classification of the vaginal flora may settle the question in the near future.

While treatment of symptomatic women during pregnancy is recommended, the available data does not allow the recommendation of screening and treating asymptomatic cases.

All first line schemes recommended for non-pregnant women can safely be used during all trimesters of pregnancy. There was some concern about the use of clindamycin during the second half of pregnancy, but it has been shown that there is no reason for that [10, 53, 125].

Recurrent BV

Antibiotic treatment is usually effective, but relapses are common, estimated at 50–70% at 6–12 months [126]. There is contradictory data associating *A. vaginae* and specific strains of *G. vaginalis* with failure of treatment and recurrences [85, 127, 128]. Bacterial strains that produce more sialidase have a higher potential of developing a biofilm and adhering to epithelial cells [129].

Higher loads of bacteria also seem to correlate with an increased risk of recurrence [130].

Recurrent episodes can be managed by using a different antibiotic/antiseptic from the previous episode, for a longer duration. The use of repeated options in the subsequent episodes is an acceptable approach [131].

Some patients will opt for a suppressive treatment. The use of metronidazole 0.75% gel twice a week for 4–6 months is one of the most commonly used regimens, with an efficacy of 70%. However, the rate of relapse severely increases once it is stopped and VVC is not uncommon during treatment (43.1%) [132]. A regimen in which 2 g of oral metronidazole and 150 mg of oral fluconazole monthly was used, in HIV1 women showed to be effective, and the risk of VVC was lower than in the placebo arm [133].

There is no advantage in the treatment of male partners, however this recommendation is not based on good quality studies [10, 134]. If the phages theory proves to be correct, the male treatment will not be effective indeed.

Using oral contraceptives continuously for 3 months is a strategy that can be tried; it may work both by reducing the levels of oestrogens and by eliminating the exposure to blood [85].

A triple sequential scheme, including oral metronidazole or tinidazole, vaginal boric acid, and vaginal metronidazole, has shown a cure rate of 65% at 28 weeks, but it has a failure rate of 50% at 36 weeks [135].

Recurrences/treatment failures seem to be associated with inability to eradicate the biofilm. Boric acid is one of the agents capable of destroying it; other options may include tobramycin, DNases, octenidine, and retrocyclin, among others [98].

The concept of using probiotics to restore the vaginal health is intuitive and appealing, however the studies have not supported its use. It does not seem to affect the cure rate, but may increase the time to recurrence in 50% [136, 137]. Failure to improve the vaginal flora with probiotics may be due to the use of inadequate species (the ones commercially available usually are not dominant ones in healthy women) and/or inability to colonize the vagina.

There is no data supporting the use of acidifiers of the vagina for the purpose of avoiding recurrences [138].

Future options may include oral or vaginal lactoferrin, which can act by immunomodulation, disruption of the cell membranes, and by binding to iron and thus making it unavailable for bacteria (without affecting lactobacilli, which rely on manganese for their metabolism) [126].

24.4.2 Aerobic Vaginitis, Desquamative Inflammatory Vaginitis, GBS Vaginitis

Aerobic Vaginitis: Breaking the Myth

- Group B streptococcus (GBS) infection and desquamative inflammatory vaginitis (DIV) are not separate entities. They are a part of aerobic vaginitis (AV).

In 2002, Donders et al. named a condition characterized by lack of lactobacilli, presence of aerobic (enteric) bacteria, and atrophy as aerobic vaginitis, thus stressing the main differences between this entity and its anaerobic, non-inflammatory, counterpart: BV [77]. Its most severe forms of presentation correspond to the desquamative inflammatory vaginitis (DIV), described by Gardner in 1968 [139] (probably the same as Scheffey's "exudative vaginitis" [140]). While DIV is an extreme form of the disease, AV shows the full spectrum of the disease [141].

Group B streptococcus (GBS) vaginitis is also included in the spectrum of AV. GBS is today considered one of the agents commonly found in women with AV.

Even though it is more and more recognized as a nosologic entity, unfortunately it still is not the rule. Failure to recognize it may be a missed opportunity to help women with otherwise "untreatable" or "unspecific" vaginitis, and to diminish the associated gynaecological and obstetrical risks associated with it.

As we consider these entities as subtypes of AV, AV will be used to discuss it as a whole.

24.4.2.1 Aetiology

The aetiology of AV remains unknown. Although the isolation of bacteria other than lactobacilli is one of the features of AV, it cannot be assumed straightforward that AV is an infection—its presence may be secondary to the abnormal local conditions. The most commonly isolated bacteria are *Streptococcus* spp., *S. aureus*, coagulase negative staphylococci, *E. coli* and *E. faecalis* [142].

The lack of lactobacilli, namely the H₂O₂ producing strains which are capable of reducing the levels of IL-1 β [143] can, partially, explain the inflammatory response found in AV.

Host immunity factors can also be involved, as not only the levels of IL-1 β are increased (higher than in women with BV), but also those of IL-6 and -8 (while in BV they are within normal range) [144, 145]. Sialidase, which leads to diminished local immunity by ultimately causing IgA proteolysis, is increased in both AV and BV [144].

The presence of intermediate or even parabasal cells suggests a role for hypoestrogenism. However, it must be a local phenomenon, given that it can be seen in women without further evidence of low oestrogens, and systemic treatment is ineffective.

Other theories include that AV/DIV might be localized forms of lichen planus, or that it is a consequence of low levels of vitamin D [142].

24.4.2.2 Prevalence and Epidemiology

Data concerning the epidemiology of AV is still scarce, however we are starting to see the broad picture. Comparing data on prevalence is not easy, as studies considered different grades of AV, some looked only into symptomatic women, and others considered only pregnant women.

In Europe, the prevalence rate of moderate or severe AV (msAV) seems to range between 7.4 and 12.0%, while it seems to be less common in South America (2.0–2.9%), and more common in Africa (11.0–25.8%) [142]. The prevalence is systematically lower in pregnant women (4.0–8.0%) [57], and in one study it has been shown to be very high in a cohort of 123 HIV positive women (26.8% with msAV and 20.4% with light forms) [146].

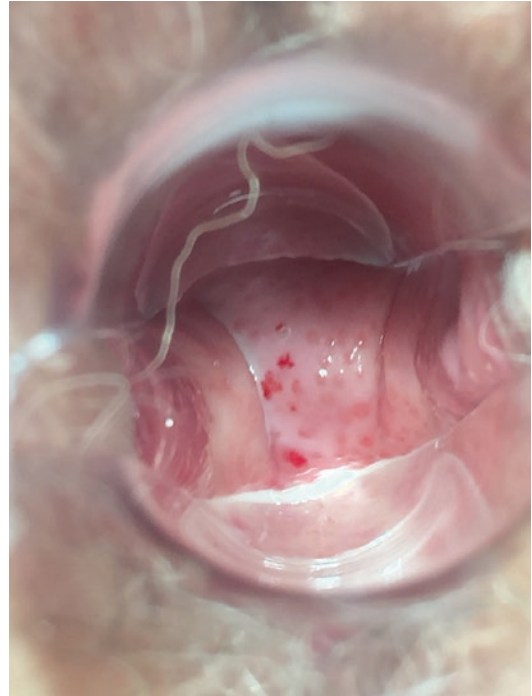


Fig. 24.14 Erosions of the vaginal walls in a patient with severe aerobic vaginosis

It can be found in women of all ages (not studied in children), but there seems to exist a peak in the peri-menopausal period.

24.4.2.3 Risk Factors and Associations

Up to the moment, the studies concerning risk factors and associations with AV are very limited. One Chinese study associated AV with being unmarried, using an IUD, long-term use of antibiotics, and vaginal douching; on the contrary, higher education and systematic use of condoms were protective [147]. The latter was not confirmed in a European study [78].

24.4.2.4 Signs and Symptoms

AV can range from asymptomatic to highly symptomatic. Symptoms can include yellowish or yellow-green vaginal purulent discharge (in 20% with a rotten smell—different from the fishy one typical of BV), dyspareunia, burning and stinging. The vagina and the vestibule can be reddish and with erosions (Fig. 24.14) [57, 77]. Symptoms can be present for months with variable intensity [142].

24.4.2.5 Complications

The number of studies linking AV with severe gynaecological and obstetrical complications has been growing the last years [142].

Among gynaecological complications, there are studies relating it to an increased risk of having an abnormal Pap test and of acquiring STIs. The presence of inflammation and erosions, the lack of lactobacilli, and the associated increased levels of sialidase can easily explain the increased risk for STIs; diagnosing and treating AV can be highly relevant in populations at elevated risk, namely in Africa [87, 118].

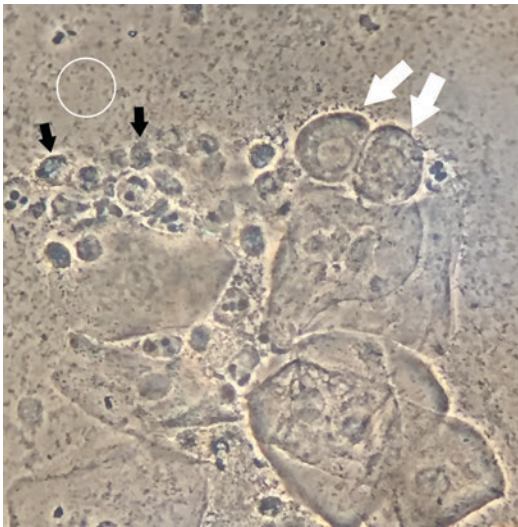


Fig. 24.15 Severe aerobic vaginosis/desquamative inflammatory vaginitis: absent lactobacilli, basal/parabasal cells (white arrows), cocci (white circle), and inflammation (black arrows) (phase contrast 400×)

In terms of obstetrics, it can be the explanation for the contradictory results found when relating flora changes to preterm labour. It has also been linked to premature rupture of membranes, chorioamnionitis and even neonatal sepsis.

The interleukin profile associated with these complications, as well as that associated with cervical intraepithelial neoplasia and cervical cancer are near perfect matches with that of AV [78, 142, 148].

24.4.2.6 Diagnosis

The diagnosis of AV is made with the aid of a microscope, ideally with phase contrast (Fig. 24.15). A composite score must be calculated, including: (1) relative quantity of lactobacilli; (2) presence of inflammation; (3) relative quantity of toxic leucocytes (active, swollen leucocytes); (4) presence of other bacteria and (5) proportion of parabasal cells (Table 24.9). WMM is a better tool than Gram staining, as the former allows a more precise evaluation of the lactobacillary grades.

Cultures are not useful, as it is not informative of the relative proportion of lactobacilli and other bacteria and colonization is common [113].

The pH is usually increased, in a more pronounced way than in BV [149].

Rumyantseva et al. have shown that molecular diagnosis, using real time PCR is feasible and, in the future, can be an option in settings where health care professionals are not trained to perform microscopy [113]. Enzymatic test has already been developed, but validation is still needed [150].

Table 24.9 AV score

Score	Lactobacillary grade	Inflammation (leucocytes)	Toxic leucocytes	Presence of other bacteria	Proportion of parabasal cells
0	I or IIa	≤10 per HPF	Absent	Unremarkable or cytolysis	<1%
1	IIb	>10 per HPF and ≤10 per cell	≤50%	Small coliform bacilli	1–10%
2	III	>10 per cell	>50%	Cocci or chains of cocci	>10%

Score <3—no AV; score 3 to 4—light AV; score 5 to 6—moderate AV; score >6—severe AV. Lactobacillary grades: I—only numerous pleomorphic lactobacilli; IIa—dominance of lactobacilli, but other bacteria present; IIb—dominance of other bacteria, but still some lactobacilli present; III—lactobacilli absent or severely depressed and dominance of other bacteria. Use 400× magnification. HPF high power field. Adapted from Donders et al. [145]

Table 24.10 Treatment of Aerobic Vaginosis. Donders et al. [142]

Antiseptics	Dequalinium chloride	<i>id</i> for 6 days
	Iodopovidone	(Transient effect, low impact on lactobacilli, washing effect?)
	Chlorhexidine	
Topical antibiotics	Clindamycin cream 2%	<i>id</i> for 2 weeks
	Rifaximin 25–100 mg	<i>id</i> for 6 days
Systemic antibiotics	Moxifloxacin 400 mg	<i>id</i> for 6 days (repeat if absence of microscopic cure)
Topical steroids	Hydrocortisone cream 10%	3–5 times/week (vaginal) advisable to concomitantly use antifungals?
Topical oestrogens	Estriol Estradiol	According to usual schemes
Probiotics	<i>L. acidophilus</i> + estriol 30 µg	<i>id</i> for 6–12 days
Other	Nifuratel 500 mg	<i>id</i> 10 days (topical or oral)

24.4.2.7 Treatment

Treatment of AV is not yet standardized and further studies are needed in this specific field. The treatment is guided by the microscopy findings: It must be attempted to correct the atrophy (topical oestrogens), the inflammation (topical steroids) and the presence of bacteria (antiseptics, topical and antibiotic antiseptics). In one study, probiotics have shown to increase the time to recurrence [136] (Table 24.10).

If antibiotics are chosen, preference should be given to topical formulations, to those active against Gram negative bacilli and Gram-positive cocci, and as innocuous as possible to lactobacilli. The use of the antiseptic dequalinium chloride was less associated with *Candida* at the end of the treatment, when compared to antibiotics [118].

24.5 Cytolytic Vaginosis, Lactobacillosis and “Leptothrix”

Cytolytic Vaginosis: Breaking the Myths

- Lactobacilli are not always protective—
Increase in lactobacilli may cause cytolytic vaginosis, a special form of sometimes symptomatic vaginal discharge
- Cytolytic vaginosis symptoms closely mimic those of vulvovaginal candidiasis

The dominance of the vaginal milieu by lactobacilli species and the consequent low pH is unique to the human species. Its main goal seems to be the protection against sexually transmitted infections (STIs) and, ultimately, the consequences of it in the offspring. In evolutionary terms, it may have happened as a consequence of the continuous ovarian cycle (and, consequently, an increased risk of exposure to STIs, when compared to other species) or due to the high consumption of starch, which occurred after the agricultural revolution [80, 151]. Other authors have highlighted the need for a protective environment during delivery, as due to the disproportion between the head and the pelvis, the likelihood of vaginal wall lacerations is higher than in other species. However, this unique situation can turn against the host [80].

Higher levels of oestrogens, as seen just before ovulation, are directly linked to the lactobacilli abundance, lower vaginal pH, thickening of the vaginal epithelium, and increased production of glycogen [80].

For still unknown reasons, sometimes the lactobacilli can be a cause of cytolysis (cytolytic vaginosis [CV]), can be increased in number (lactobacillosis), or longer than normal (previously denominated “leptothrix”). These are controversial entities, and it is still unknown whether or not they are part of the same spectrum. Some authors consider lactobacillosis and “leptothrix” as one single entity.

These are not infectious causes of “vaginitis”, but are important differential diagnosis of VVC,

which are commonly found in women with suspected “resistant” or recurrent candidiasis.

24.5.1 Cytolytic Vaginosis

24.5.1.1 Aetiology

It has not been fully understood what leads to the overgrowth of lactobacilli in some women or why does it lead to the destruction of the intermediate cells of the vaginal epithelium. It is highly likely that hormones play a role in the development of CV, as it is more common—or more symptomatic, during the luteal phase, pregnancy or perimenopause [152].

The cytolysis may be the consequence of the very low pH, due to the increased degradation of glycogen by the large number of lactobacilli.

Lactobacilli, except for *L. iners*, produce both L- and D-lactic acid (*L. iners* produces only the L-isomer); vaginal cells produce only the L-isomer. An imbalance in the local ratio between the two isomers, in favour of the L-isomer, can be involved in the development of VC, by altering the expression of the extracellular matrix metalloproteinase inducer [153].

Most women with symptomatic CV were previously treated with repeated courses of antifungals, which lead some authors to try to establish an association. However, this association is more likely to be due to the fact that they were previously misdiagnosed as *Candida* and thus treated as so.

24.5.1.2 Prevalence and Epidemiology

The prevalence of CV in asymptomatic women ranges between 1 and 7%, peaking during the reproductive years [152]. Despite the now acknowledged racial differences in terms of vaginal microbiome composition, it is not known if it has any impact in the prevalence of CV.

24.5.1.3 Risk Factors and Associations

One study suggested that less sexually active women were more likely to have CV, but it can be biased, as the comparison group was with sexual workers [154]. It has been suggested, also in

one isolated study, that women with CV are less likely to have an abnormal Pap test, but we could not confirm it [155].

24.5.1.4 Signs and Symptoms

VC mimics the symptoms of VVC; almost all patients were previously treated with antifungals, either for a suspected diagnosis performed by a physician or by self-diagnosis.

The clinical history most of the times is enough to raise a high level of suspicion for CV.

The most striking symptom is burning, rather than pruritus. The typical discharge is white, sometimes lumpy and abundant (Fig. 24.16); some women will describe that it smells like vinegar. Dysuria and dyspareunia may also be among the list of symptoms. Symptoms tend to be cyclic, aggravating in the luteal phase, peaking just before the menses. There is usually a great relief associated with menstruation or withdrawal bleeding. It is common that women report a transitory relief with the use of topical antifungals, but not oral formulations, probably due to a buffering effect.

The vulvar exam may be unremarkable, or some degree of erythema and/or oedema can be present. Fissures are an uncommon feature.

24.5.1.5 Complications

The very few studies about CV usually did not address the issue of complications. In one single study, we found that CV is much more prevalent



Fig. 24.16 Abundant, lumpy discharge in a woman with cytolytic vaginosis

in women with localized provoked vulvodynia, when compared to healthy controls (16.5% vs. 4.1%, $p = 0.000$) [156]. However, women with vulvodynia tend to have less frequent intercourse and, as referred before, CV has been found to be more common in less sexually active women [154]. On the other side, there is a biological plausibility that the chronic exposure of the vestibule to a very low pH, excessive amounts of H_2O_2 , or the association to a specific lactobacillus species can be involved in the development of some cases of vulvodynia. Ventolini et al. associated *L. gasseri* with vulvodynia, which can lead to the speculation that this can be a common link between the two entities [157].

24.5.1.6 Diagnosis

The diagnosis is made using the microscope (Table 24.12). It is characterized by the presence of an abundant number of lactobacilli, bare nuclei and cellular debris (in extreme cases, no intact cells are seen in the slide). Inflammation, clue cells, bacteria other than lactobacilli, and *T. vaginalis* are typically absent (Fig. 24.17). Although, per definition, the diagnosis implies the exclusion of the presence of *Candida*, given the typically low pH (<4.0–4.2) the coexistence of both is not rare.

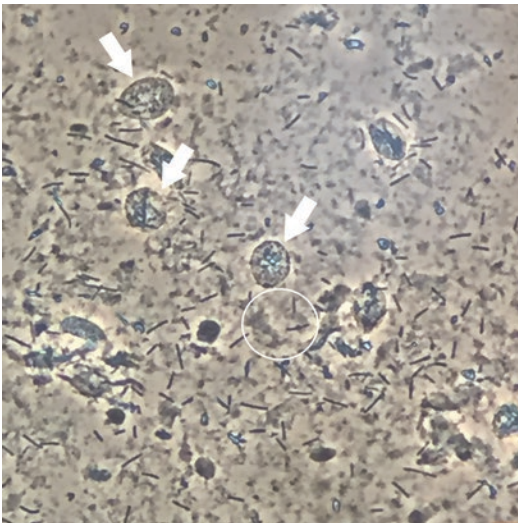


Fig. 24.17 Cytolytic vaginosis. Bare nuclei (white arrows), cellular debris (white arrows) and abundant lactobacilli

Cultures for fungi can be used to exclude the presence of *Candida*.

24.5.1.7 Treatment

The treatment consists of increasing the pH of the vagina (buffer effect), which will diminish the load of lactobacilli in the vagina and ameliorate the symptoms.

Vaginal irrigations with sodium bicarbonate are highly effective in controlling the symptoms. While some authors recommend sitz baths, in our experience irrigations are much more effective (Table 24.11). In recurrent cases, if a pattern is identified, treatment should be started 24–48 h prior to the anticipated moment of onset [159].

24.5.2 Lactobacillosis

This entity is characterized by the overgrowth of lactobacilli, without cellular lysis (Fig. 24.18). When symptomatic, it is very similar to CV. There is no epidemiological data in the literature concerning lactobacillosis.

The diagnosis is established by a normal or reduced pH, cultures negative for *Candida*, and, most important, by an increased number of lactobacilli in microscopy, without other types of

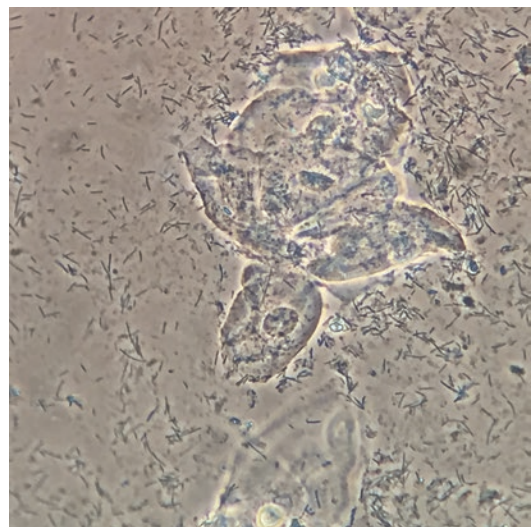


Fig. 24.18 Lactobacillosis (phase contrast 400×)

bacteria, cytolysis, inflammation, clue cells, or *T. vaginalis* (Table 24.12).

It is believed that it is self-limited, thus expectant management is an option. In cases in which treatment is needed, it is similar to that of CV (Table 24.11).

24.5.3 “Leptothrix”

Leptothrix is a controversial entity; even those who acknowledge its existence do not agree in how it should be designated (alternative names include “fusiform lactobacilli”) [160]. It is characterized

Table 24.11 Treatment of CV, lactobacillosis and leptothrix

Cytolytic vaginosis	Sodium bicarbonate	30–40 mg should be diluted in 1 L of warm water and vaginal irrigations performed daily for 2 weeks Maintenance, if needed, should be kept as the minimum required to control the symptoms
Lactobacillosis		
Leptothrix	Amoxicillin + clavulanate 500/125	First choice 3 <i>id</i> for 7 days
	Doxycycline 100 mg [158]	If allergic to penicillin 2 <i>id</i> for 10 days
	Nifuratel 200 mg	Alternative needing further tests 3 <i>id</i> for 7 days

Table 24.12 Wet mount characterization and differential diagnosis of vulvovaginitis

	Presence of parabasal/basal cells	Lactobacilli	Inflammation	Other	Notes
Candidiasis or candidosis	–	Normal	Can be present	Hyphae, pseudohyphae, spores	Inflammation, if present, usually without toxic leucocytes <i>C. glabrata</i> presents only as blastospores Presence of pseudohyphae is highly suggestive of infection rather than colonization Blastospores of <i>C. parapsilosis</i> and <i>S. cerevisiae</i> are larger than those of <i>C. glabrata</i>
Trichomoniasis	–/+	Frequently decreased	+ (frequently exuberant and with toxic leucocytes)	Motile protozoarians can be seen (10–20 by 2–14 μm)	Frequently associated with BV. If slide kept warm and microscopy performed immediately after collection, the likelihood of seeing motile TV is higher
Bacterial vaginosis	–	Decreased/absent	–	Clue cells	If inflammation is present, exclude other causes for it
Aerobic vaginitis	+	Decreased/absent	+	Toxic leucocytes can be present	–
Cytolytic vaginosis	–	Increased	–	Bare nuclei and cellular debris	–
Lactobacillosis	–	Increased and long	–	–	–
Leptothrix	–	Long	–	–	Lactobacilli longer than in lactobacillosis Can coexist with normal lactobacilli, BV, or even TV



Fig. 24.19 Leptothrix (phase contrast 400×)

by the presence of long, filamentous lactobacilli, sometimes with segmentation and/or sporulation (Fig. 24.19). Its size can be 8–15 times that of a normal lactobacillus [161]. It can totally dominate the flora or be associated with normal lactobacilli, lactobacillosis [158], or even BV type flora and *T. vaginalis*. In a Swedish study, its prevalence in a cohort of women without BV was 8%. These authors identified these bacteria as *L. gasseri* [160].

There is some speculation that these lactobacilli may be the consequence of the previous use of antibiotics or antifungals.

Horowitz et al. described women with cyclical symptoms of vulvar discomfort and vaginal discharge during the luteal phase, associated with the presence of long and serpiginous lactobacilli.

The diagnosis is easily made by microscopy, by the presence of the typical long bacteria, that should not be confused with *Candida* or *Actinomyces* (Table 24.12).

Treatment with oral antibiotics has proven to be highly effective, both in the control and symptoms and in the elimination of the bacteria [158]. The first choice is amoxicillin + clavulanate; in women allergic to penicillin, doxycycline is an alternative [158]. Nifuratel has also been tested, but with much higher rates of relapse [162] (Table 24.11).

In contrast to other authors' experience, we have seen recurrences, even after successful treatment.

24.6 Other Conditions to Consider in the Differential Diagnosis of Vaginal Discharge

Several other conditions can present in similar ways to vulvovaginitis and must be considered in the differential diagnosis.

Vaginal Atrophy: Breaking the Myth

“Vaginal atrophy” and “atrophic vaginitis” may be associated with vaginal discharge. These terms have recently been renamed: “genitourinary syndrome of menopause”. However, no minimum number of criteria was defined, the list of signs and symptoms is not specific, and some of the mainstays for its creation are highly debatable.

Postmenopausal vaginal atrophy can present in two ways: (1) without discharge (on microscopy there is paucity of epithelial cells and no inflammation) and (2) with purulent discharge (abundance of deep layer cells and inflammation—“atrophic vaginitis”). In 2014, the North American Menopause Society and the International Society for the Study of Women's Sexual Health proposed that “vaginal atrophy” and “atrophic vaginitis” should be replaced by “genitourinary syndrome of menopause”. However, we disagree with this concept, as it cannot be considered a syndrome (which, by definition, is a particular abnormality or condition), no minimum number of criteria was defined, the list of signs and symptoms is not specific, and some of the mainstays for its creation are highly debatable (like “atrophy” having a negative connotation or “vagina” not being a well accepted term) [163].

Vaginal involvement of erosive lichen planus can manifest itself also with by a purulent discharge, sometimes with more or less extensive formation of synechia.

Other causes to be considered include allergic and contact vaginitis, as well as cervical ectopy [53] (Table 24.13).

Table 24.13 Clinical characteristics and potential risks of different causes of vulvovaginitis

	pH	Whiff test	Variation of symptoms during ovarian cycle	Symptoms	Signs	Risks
Candidiasis	Normal (but can occur at any pH; most likely to be symptomatic at low pH)	-	Worsening during the luteal phase and improving with menses	Pruritus, terminal/post-micturition dysuria, dyspareunia. If burning is the main symptom consider <i>C. non-albicans</i> or alternative diagnosis	Erythema and enanema, fissures, oedema, white, curdy discharge, without foul smell	<ul style="list-style-type: none"> Limited data relating it to bad obstetrical outcomes Increased risk of HIV
Trichomoniasis	Increased (≥ 5)	+ (frequently)	Worsening of symptoms during menses	Can be asymptomatic Dysuria, burning, itching, irritation, discharge	Erythema, enanema, erosions, fissures, strawberry cervix, yellow or green purulent discharge (sometimes with bubbles), discharge can have a rotten smell	<ul style="list-style-type: none"> Adverse obstetrical outcomes STIs (HIV)
Bacterial vaginosis	Increased (>4.5)	+	Worsening of symptoms during menses	Fishy smell, mild burning	Grey or yellow, thin, adherent discharge	<ul style="list-style-type: none"> Adverse obstetrical and neonatal outcomes STIs Cervical dysplasia/cancer
Aerobic vaginitis	Increased (usually more than in BV: >5)	-	Worsening of symptoms during menses?	Rotten wood smell discharge; burning, stinging, dyspareunia	Yellow or green purulent discharge; erosions, enanema	<ul style="list-style-type: none"> Adverse obstetrical and neonatal outcomes STIs Abnormal pap tests
Cytolytic vaginosis	Lower than normal	-	Worsening during the luteal phase and marked improvement with menses	Burning, abundant discharge	White, curdy, abundant discharge	<ul style="list-style-type: none"> Vulvodynia (?)
Lactobacillosis	Lower than normal	-	Worsening during the luteal phase and marked improvement with menses	Burning	Normal/white discharge	<ul style="list-style-type: none"> Not reported
Leptothrix	Normal/low (?)	-	?	Asymptomatic most of time times Burning	Normal	<ul style="list-style-type: none"> Not reported

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