Mixed vaginitis: clinical recommendations regarding presentation, diagnosis and treatment

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Abstract

Mixed vaginitis is caused by the simultaneous presence of at least two vaginal pathogens, contributing to an abnormal vaginal milieu and leading to vaginal symptoms and signs. However, associations between symptoms and microbes have not been clearly elucidated. Therefore, mixed vaginitis is an inflammatory condition that remains underrecognized. Mixed vaginitis generally involves the formation of mixed biofilms. The specific characteristics of mixed biofilms, especially their enhanced drug resistance and their ability to evade components of the host immune response, make them of high clinical importance. This review summarizes the relevant clinical data to improve clinical knowledge about mixed vaginitis.

Title Page

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Running Title:Mixed Vaginitis Clinical Recommendations

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between symptoms and microbes have not been clearly elucidated. Therefore, mixed vaginitis is an inflammatory condition that remains underrecognized. Mixed vaginitis generally involves the formation of mixed biofilms. The specific characteristics of mixed biofilms, especially their enhanced drug resistance and their ability to evade components of the host immune response, make them of high clinical importance. This review summarizes the relevant clinical data to improve clinical knowledge about mixed vaginitis.

Key words: Mixed Vaginitis, Candida, Bacterial vaginosis, Trichomoniasis, Aerobic vaginitis, Mixed biofilms

Introduction

Mixed vaginitis is caused by the simultaneous presence of at least two vaginal pathogens, contributing to an abnormal vaginal milieu and leading to the development of vaginal symptoms and signs(1). Nevertheless, simply identifying the presence of at least two vaginal pathogens in situ does not establish a cause–effect relationship with clinical symptoms and signs. For example, in patients with simple vaginitis, "vulvar pruritis" and "thick curdy discharge" are more likely to be reported by women with vulvovaginal candidiasis (VVC), while "thin white discharge" and "odor" are more commonly reported in women with bacterial vaginitis (BV)(2). Individual signs and symptoms have only limited value in the recognition of vaginitis. "Abnormal vaginal discharge," "dyspareunia," and "vaginal soreness" can occur with any kind of vaginitis. In addition, the presentation of mixed vaginitis can be atypical. Both pathogens require specific therapies for complete eradication(3). Therefore, in its simplest form, mixed vaginitis refers to the simultaneous presence of two or more potential pathogens in the lower genital tract, regardless of the clinical significance of the individual pathogens.

Today, approximately 20 lower genital tract-related infections have been recognized, such infections are caused by bacteria, fungi, protozoa, mycoplasma, and viruses(4). The majority of infections in the female reproductive tract (FRT) occur in the vagina and cervix. Numerous microorganisms are often linked to cervical infection, leading to cervicitis, including herpes simplex virus-2 (HSV-2), *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), Mycoplasma, and human papilloma virus (HPV)(5). The most common forms of vaginal infection include bacterial vaginosis (BV), trichomonas vaginalis (TV), vulvovaginal candidiasis (VVC), and aerobic vaginitis (AV). Mixed vaginitis in this review encompasses these 4 common types of vaginitis.

The signs and symptoms of mixed vaginitis are often atypical, diagnosis cannot always be established, treatment is complicated and the vaginal microbiota is more likely to be perturbed in contrast to single-type vaginitis. Moreover, mixed vaginitis can induce long-term symptoms with intermittent exacerbations, and recurrence after treatment is common, leading to repeat visits to physicians and higher healthcare costs. Therefore, the major goal of this review is to help improve clinicians' understanding of mixed vaginitis and discuss the therapeutic standard to reduce the disease burden and prevent associated complications.

Epidemiology

Although vaginitis is common, affecting millions of women every year, little information about the prevalence of mixed vaginitis is available. A literature review to assess the occurrence and frequency of mixed vaginitis revealed that most investigators reported coinfection rather than mixed vaginitis, and the proportion of mixed vaginitis infections ranged from 6.30% to 35.06%(6, 7). As noted above, one challenge is that studies have failed to correlate symptoms with microbe types. Therefore, most reports do not distinguish between mixed vaginitis and coinfection. The representative data are depicted in Table 1. The following factors are limitations that prevent the obtention of a clear picture of the actual prevalence of mixed vaginitis.

1. The types of vaginitis observed have not been concordant. Evaluations have traditionally focused on VVC, BV, and TV. Most studies have reported that VVC plus BV is the most prevalent form of vaginitis(2). Another condition, AV, was recently characterized by Donders in 2002(8). When AV is included, epidemiologic estimates shift considerably. Some studies indicated AV plus BV, VVC plus AV, and VVC plus BV were the most frequent coinfections(9). It is possible that some clinicians are unaware of AV, thus

sometimes misdiagnosing it as BV, affecting the epidemiological data.

2. There is great variability in the rates of infection in different populations. One study found a relatively low rate of mixed vaginitis (6.30%) in Brazil(6), while another found a higher rate (35.06%) in Shanghai(7). Research is required to demonstrate prevalence and outcomes in various populations, such as pregnant women, hypoestrogenic women, asymptomatic women, and so on.

3. The diagnostic criteria and tools to determine the prevalence of mixed vaginitis differ. The classical standards for vaginal infection diagnosis are physical examination, microscopy, and culture methods, which are usually performed in hospitals. Recent research has shown that some new molecular assays (Affirm VPIII, Aptima) for the diagnosis of mixed vaginitis have performed well, identifying proportions ranging from 9.26 to 27.23%(10, 11). In addition, the skill level of technicians is also an influencing factor(12).

4. The vaginal and cervical microbiome is an intricate ecosystem containing various normal and dysbiotic microbes in different ratios. At present, in the mixed vaginitis-related literature, only 4 common types of vaginitis are included. If one includes the cervical, but not strictly vaginal, pathogens such as HSV-2 virus, CT, NG, mycoplasma, and HPV may be included, and higher frequencies of mixed infections may be reported(13).

5. There is a lack of physician understanding and implementation of current guidelines(12). This is likely due, in part, to the fact that the majority of these infections are diagnosed empirically without objective data. Moreover, mixed vaginitis symptoms can be nonspecific and vary by patient. Empirical evidence in this population has likely led to many misdiagnoses.

Mechanism of mixed vaginitis

Polymicrobial infections generally involve the formation of mixed biofilms, dominated by bacteria and/or fungi, embedded in an extracellular matrix(14). It is even common to find mixed biofilms in the lower female reproductive tract in the clinical setting. The specific characteristics of mixed biofilms, especially their enhanced drug resistance and their ability to evade components of the host immune response, make them of high clinical importance. However, despite the importance of such mixed infections, mixed infection research, particularly research involving vastly diverse microorganism, is in its infancy(15). Bacteria and/or fungi can coexist within a host, and the nature of interspecies interactions can determine the fate of the microbial populations. They influence each other in diverse ways via synergistic or antagonistic interactions(16).

1. Medically antagonistic interactions between microorganism are common in the lower female reproductive tract. For example, studies on the vaginal microbiota have revealed that Lactobacillus species lower the local pH (by releasing lactic acid), which results in the inhibition of initial adherence of *Candida albicans* and *Gardnerella vaginalis* to the vaginal mucosal surface(17). Many environmental cues impact biofilm formation, such as hypoxia, elevated extracellular pH, body temperature, and elevated CO_2(18). A previous study reported that*Pseudomonas aeruginosa* killed *C. albicans* cells after attachment to *C. albicans* hyphae(19). The contemporaneous process may be dependent on the species present. Little is known about pathophysiological vaginal conditions during infection, but antagonistic interactions between probiotics and pathogens are more likely to occur than antagonistic interactions between pathogens.

2. Some synergistic relationships result in complex pathogenic processes, providing protection to one or both species in mixed-species biofilms. This occurs in the following ways: Cells of certain species can directly bind to cells of other species. For example, recent evidence has indicated that*Staphylococcus aureus* can "piggyback" on *C. albicans* hyphae to penetrate host cells, infiltrate deep tissues and participate in the pathogenic process of host cells(20). Similar synergies providing a protective microenvironment have also been observed; for example, the presence of a *C. albicans* biofilm enables the proliferation of anaerobic pathogens in an otherwise hostile, oxygen-rich environment. Moreover, the bacteria seem to induce the formation of these protective structures(21). A recent study has linked this protective interaction to enhanced drug resistance; when *C. albicans* and methicillin-resistant *Staphylococcus aureus* (MRSA) strains were grown together, the presence of *C. albicans* seemed to protect MRSA from eradication by vancomycin(22). Synergistic inter-

actions can also enhance virulence during infection(19). For example, higher host mortality was observed when *S. aureus* and *C. albicans* were introduced together at sublethal doses in a mouse peritonitis infection model than when either species was introduced alone(23). A limitation of these studies was that this interaction was evaluated not in the lower female reproductive tract. However, these observations illustrate the dynamic nature of polymicrobial infections in part. In other words, the contemporaneous process may be interdependent. The mechanisms behind these synergistic interactions have not been described.

3. We have focused on antagonistic versus synergistic interactions, but additional distinct interactions exist. Polymicrobial infections challenge the immune system in different ways compared with infection with a single organism. An host response to one pathogen may promote the proliferation of another pathogen. For example, coinfection with*Streptococcus agalactiae*significantly attenuated the hyphal development of *C. albicans* in vitro, but it may attenuate host vaginal mucosal TH17 immunity and contribute to mucosal colonization by *C. albicans* in vivo(24). A multicountry cross-sectional study reported that the factor independently associated with *S. agalactiae* was *C. albicans* presence(25). Similarly, another study suggested that *C. albicans* may suppress the local host immune response, allowing subclinical *P. aeruginosa* to proliferate, resulting in disease(26) (Figure 1). Thus, these interactions are highly complex, and the type of interaction that occurs often depends on a range of environmental, pathogenic and host factors. The mechanisms of mixed infections in vaginitis are unknown thus far, and further exploration is needed.

Clinical features

Mixed vaginitis has the following clinical features in contrast to simple vaginitis.

1. Regarding clinical features, mixed vaginitis is atypical. For example, some patients produce green-yellow, thin, purulent vaginal discharge; we found that this discharge was also observed in patients with single TV infection or those with AV mixed vaginitis. Clinical findings are thus unable to distinguish between single and mixed infections. In addition, mixed vaginitis can be characterized by single vaginitis, also can simultaneous presence of two or more potential vaginitis features(4, 9). Patients with AV plus BV reported a genital fish-like odor (indicator of BV) more frequently than those with single AV. Patients with AV plus VVC more often reported genital itching (indicator of VVC) than those with single AV(9). Symptoms varied among the patients with mixed vaginitis. The most frequently reported symptoms included a change in the characteristics of discharge (color, consistency, odor), genital itching, and burning pain.

2. Mixed vaginitis is hard to eradicate, and recurrence is frequent. For example, in a multicenter, prospective, open-label study, fenticonazole was evaluated in 101 women. On day 8, the eradication rate of mixed vaginitis (45%) was lower than that of single-pathogen infection (VVC 70%, TV 70%, BV 67%); 28 days later, the relapse rate was 23% in the mixed infection group and 0% in both the *C. albicans* and *T. vaginalis* groups(27). In addition, mixed infections lead to a high-dose therapeutic challenge. Liang Q reported that the complete eradication rate was significantly higher in the nifuratel-500 group than in the nifuratel-250 group among those with mixed vaginitis(28). A similar conclusion in an in vitro study showed that increasing fenticonazole concentrations can overcome potential interference between *C. albicans* and *S. aureus* or other bacterial species in mixed infections(29). This is likely due to the diverse behavior of the pathogenic vaginal flora that seems to affect the immune response of the host, making cure difficult.

Diagnosis of mixed vaginitis

A vaginitis diagnosis is made according to the presence of symptoms, clinical findings and microscopy examination (Gram-staining and wet-mount smears)(30). The key points in diagnosing mixed vaginitis are as follows(1):the simultaneous presence of at least two vaginal pathogens; an abnormal vaginal milieu due to the pathogens and, hence, symptoms and signs of vaginitis; and the requirement of specific therapies for both pathogens.

Since the diagnosis of mixed vaginitis is largely dependent on the diagnostic criteria for single vaginitis, the criteria to facilitate recognition of the coexistence of multiple pathogens are as follows.

TV: at least one of the following must be present: positivity on wet-mount smear, although the sensitivity

has been reported to be as low as 45–60%(31); positivity on culture, which has a higher sensitivity than microscopy but is not widely available in clinical settings; or positivity on a nucleic acid amplification test (NAAT), which has the highest sensitivity for the detection of TV in comparison to both microscopy and culture. The Guidelines Group recommends that the most effective tests to diagnose TV in women are NAATs(32). However, examination of wet-mount preparations is still commonly used in clinical practice.

VVC: at least one of the following must be present: the presence of yeast or pseudohyphae in vaginal discharge on wet-mount microscopy with either saline or 10–20% KOH solution (40–60% sensitivity); the presence of yeasts or pseudohyphae on gram staining (up to 65% sensitivity) of vaginal discharge; or positivity on culture, which is helpful in diagnosing recurrent or complicated vulvovaginal candidiasis because species other than *C. albicans* (e.g., *Candida glabrata* ,*Candida tropicalis*) may be present. Moreover, drug sensitivity testing should also be conducted. The Guidelines Group recommends that the current best test to diagnose Candida in women is microscopy(32) because positivity on microscopy indicates a large number of Candida, and hyphal formation is infrequently observed with only colonization.

BV: at least one of the following must be present: a Nugent score(33) >6; the Nugent score is considered the gold standard for studies and relies upon estimating the relative proportions of bacterial morphotypes on a Gram-stained vaginal smear to assign a score between 0 and 10. A score of <4 represents normal conditions, 4–6 represents intermediate infection, and >6 represents BV. The presence of three of four Amsel's criteria, including homogeneous, thin, white discharge that smoothly coats the vaginal wall; clue-cells on microscopic examination (prerequisite); pH of vaginal fluid >4.5; or vaginal discharge with a fishy odor before or after the addition of 10% KOH (whiff test). Amsel's criteria have a sensitivity of 60–72% for the diagnosis of BV compared to the Nugent score(32).

AV: The diagnosis of AV should be based on a combination of clinical features and microscopic findings(32). The clinical features are as follows: vulvar erythema; vulvar swelling; thinning of the vaginal mucosa; vaginal congestion; scattered bleeding points; and yellow-colored vaginal secretion, increased discharge or pruritus. The microscopic features are as follows: wet mount smears with a AV score [?]3(30). Accordingly, three main characteristics form the basis of an AV diagnosis: a variable amount of inflammation; thinning of the vaginal epithelium; and a disturbed bacterial community lacking the commonly observed high abundance of lactobacilli(34).

Amalgamative infection of the cervical and vagina should be recognized. Some cervical infections caused by pathogens, such as HSV-2, CT, NG, mycoplasma, and HPV(35), might occur concurrently with vaginitis, and symptoms of cervical cancer are generally obscured, increasing the complexity of diagnosis. Thus, coinfection with the pathogens mentioned above should be excluded in the diagnosis of mixed vaginitis.

Treatment

Compared to single vaginal infection, mixed vaginitis has atypical clinical manifestations, is hard to eradicate and often recurs. Therefore, mixed vaginitis poses a therapeutic challenge. Since the treatment of vaginitis is largely dependent on the pathogen, such infections may require treatment with multiple drugs. However, many countries have banned the availability of combination antimicrobial products for use in vaginitis, and little consideration has been given to the possibility or frequency of mixed vaginitis. Previously, a study confirmed that approximately 30% of women with vaginal symptoms failed to receive any kind of vaginitis diagnosis(36). With the development of laboratory-based diagnostics, including antigen detection, DNA probes, and PCR, recognition of the coexistence of multiple pathogens will increase. This phenomenon will increase the demand for polytherapy comprising multiple antimicrobials. Standard treatment for mixed vaginitis has not yet been established. The choice of multiple antimicrobials depends on the type of infection. Various guidelines for the treatment of different forms of mixed vaginitis state the following:(3, 32, 37-39): Mixed VVC infections (such as VVC plus BV; VVC plus TV; VVC plus AV) should be treated with topical or oral antifungal drugs along with treatment for other vaginitis. For example, oral or topical nitroimidazole is used for BV. Oral high-dose nitroimidazole is the first choice for TV treatment. Since treatment of AV with broad-spectrum antibiotics may increase the risk of recurrence and persistent infection in patients with

VVC, combined topical bactericide and antifungal drugs should be considered. Mixed AV infection (such as AV plus BV; AV plus TV) treatment is based on antibiotic targeting of aerobic pathogens associated with this condition. There are several regimens to treat AV plus BV, such as oral anti-aerobic drugs plus nitroimidazoles, oral anti-aerobic drugs plus topical nitroimidazole formulations and topical bactericides. For AV plus TV, oral anti-aerobic drugs plus nitroimidazoles are available. Mixed BV infections (such as BV plus TV) should be treated with oral nitroimidazole for BV plus TV; treatment should be provided as either two doses a day for 7 days or a single dose plus an intravaginal suppository. It should be noted that combination therapy is indicated for confirmed mixed vaginitis. In pathogen coinfection, although two pathogens may be identified, a potential pathogen may be present but may not be the cause of existing vaginal symptoms. One challenge is that individual signs and symptoms have shown only modest value in diagnosing mixed vaginitis. Therefore, how to identify at-risk subpopulations requires further consideration.

Although anti-infective treatments are available and are usually highly efficient in eradicating pathogenic microorganisms, the long-term efficiency is hampered by relapse(40). Mixed vaginitis usually has an intricate microecology. Therefore, in addition to the administration of antibiotics, the management of mixed vaginitis should target the recovery of the vaginal microecosystem and the immune system. Probiotics are recommended to maintain vaginal homeostasis and immune modulation(41). Combining lactobacilli probiotics with antibiotics may play an important role in strengthening the efficacy of the antibiotics and preventing the recurrence of mixed vaginitis. The main treatment objectives are the alleviation of symptoms, the elimination of pathogens, and eventually the recovery from disturbed to healthy lactobacilli-dominated vaginal flora.

In summary, mixed infections are largely ignored and poorly studied. Currently, mixed vaginitis has the characteristics of atypical signs and symptoms, a lack of conclusive diagnostic criteria, and little valid prevalence data. This review is of great significance for improving clinical awareness of mixed vaginitis, accurate diagnosis and appropriate treatment, and promoting recovery of the dynamic balance of the vaginal microecology to improve female reproductive health.

Disclosure of interests

The authors declare no conflict of interest.

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Table 1 Summary of representative data of mixed infections in the last 10 years

Year	Author	Area	Number	Rate of mixed infection	AV plus BV	VVC plus BV	TV plus BV	VVC plus AV	AV plus TV	VVC plus TV	Multiple infections	I
2020(11)	Schwebke JR	US	940	256 (27.23%)		147 (57.42%)	71 (27.73%)			15 (5.86%)	23 (15.65%)	4
2019(42)	Kamga YM	Cameroon	198	2(14.14%)		28(100.00%)	0(0.00%)					2
2019(43)	Sherrard J.	UK	186	15(8.07%)		14 (93.30%)				1 (6.70%)		1
2019(43)	Sherrard J.	UK	172	36 (20.93%)		36 (100.00%)						1
2019(44)	Khan Z	India	247	21 (8.50%)		21 (100.00%)	0 (0.00%)			0 (0.00%)		2

Year	Author	Area	Number	Rate of mixed infection	AV plus BV	VVC plus BV	TV plus BV	VVC plus AV	AV plus TV	VVC plus TV	Multiple infections	I
2019(45)	Abdul-Aziz M	Yemen	130	10 (7.69%)		9 (90.00%)				1 (10.00%)		2
2019(46)	Konadu DG	Ghana	332	74 (22.29%)		67(90.54%)	3(4.05%)			4 (5.41%)		2
2017(47)	Gaydos CA	America	1118	289 (25.85%)		195 (67.47%)	64 (22.15%)			7 (2.42%)	23 (7.96%)	4
2017(7)	Wand HX	Shanghai	4036	1415 (35.06%)		606 (42.83%)	471(33.27%)					1
2016(10)	Byun SW	Korea	108	10 (9.26%)								1
2016(48)	Wang ZL	Chongqing	830	184 (22.17%)	101 (54.90%)			48 (26.10%)	15 (8.20%)		20 (10.80%)	1
2013(49)	Jahic M	Sapna	96	30 (31.30%)	8 (26.70%)			13 (43.30%)	9 (30.00%)			0
2013(9)	Fan A	Tianjin	657	170 (25.88%)	31 (18.24%)	62 (36.47%)	18 (10.59%)	32(18.82%)	21(12.35%)	1 (0.58%)	5 (2.94%)	2
2012(50)	Bohbot JM	France	118	38 (32.20%)								0
2011(2)	Rivers CA	Birmingham	338	15 (4.44%)		15(100.00%)						2
2011(6)	Gondo F	Brazil	112	7 (6.30%)		7 (100.00%)						1

The "Number" in column 4 refers to the number of patients with vaginitis; The "rate of mixed infection" in column 5 refers to the ratio of mixed infections to total vaginitis; The rates in the following columns refer to the ratio of each item in mixed infection; The "Diagnostic criteria" in column 14 refers to RM and NAAT. RM: The reference methods for BV were Nugent's score and Amsel's criteria. The reference methods for VVC and TV were wet mount and culture. The reference method for AV was wet mount based on the criterion by Donders. NAAT: Nucleic acid amplification