Ref: Ro Med J. 2021;68(Suppl6)
DOI: 10.37897/RMJ.2021.S6.15

# Modern investigation of cervico-vaginal infections (from microbiome disorders to pelvic inflammatory disease)

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## ABSTRACT

Vaginitis is the general term for disorders of the vagina caused by infection, inflammation, or changes in the normal vaginal flora, while cervicitis refers to inflammation of the uterine cervix. Symptoms of vaginitis include vaginal discharge, pruritus, odor, and/or discomfort. Women with cervicitis may have presenting symptoms such as purulent or mucopurulent (yellow) vaginal discharge, intermenstrual or postcoital bleeding, dysuria, vulvovaginal irritation and dyspareunia. The initial evaluation typically consists of a medical history, physical examination, microscopy examination of vaginal swab, and cervical tests for sexually transmitted infections. Evaluation of patient risk factors is important and should include a detailed discussion about the sexual history (number of partners, type of sexual activity, condom use, new sex partner, sex partner with a diagnosed sexually transmitted infection, sex partner with concurrent sex partners), vaginal hygiene (douching), and age (< 25). All women with symptoms suggestive of cervicitis or vaginitis undergo a physical examination that includes pelvic and vaginal evaluations. Vaginitis is often the result of the presence of infectious agents. The most common infections, such as bacterial vaginosis, Candida vulvovaginitis and trichomoniasis, account for over 90 percent of the vaginal infections. Cervicitis, typically acquired from sexually transmitted infections such as gonorrhea, chlamydia, and mycoplasma, can present with nonspecific vaginal symptoms.

**Keywords:** infection, vaginitis, cervicitis, pelvic inflammatory disease

## INTRODUCTION

Cervico-vaginal infections are one of the most common problems in clinical medicine. The diagnosis of vaginal discharge is based on a history, physical examination, and a few simple diagnostic tests. Some patients with cervicitis note a purulent vaginal discharge, deep dyspareunia, and spotting after intercourse, while others may be symptom-free. The most common infections are *Gardnerella vaginalis*, *Trichomona vaginalis*, *Chlamydia trachomatis*,

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*Article History:*
Received: 27 November 2021
Accepted: 22 December 2021

*Neisseria gonorrhoeae,* that accounts for aproximatively 90% of the cases of cervico-vaginitis with infectious origin (1).

## BACTERIAL VAGINOSIS

Bacterial vaginosis (BV) is the most common cause of vaginal discharge in women of childbearing age and is characterized by a shift in vaginal microbiota away from *Lactobacillus* species and towards diverse other bacterial species, including facultative anaerobe types. BV represents a complex change in the vaginal microbiota characterized by a reduction in concentration of the normally dominant hydrogen peroxide-producing lactobacilli and an increase in concentration of other organisms, especially anaerobic Gram-negative rods (2).

The major bacteria detected in women with BV are: *Gardnerella vaginalis, Prevotella species, Porphyromonas species, Bacteroides species, Peptostreptococcus species, Mycoplasma hominis, Ureaplasma urealyticum*, Mobiluncus, Megasphaera, Sneathia, and *Clostridiales species* (3).

In clinical practice, the diagnosis of BV in premenopausal women is usually based upon the presence of at least three of the Amsel criteria (4): characteristic vaginal discharge, elevated pH, clue cells, fishy odor.

Another option is the use of Nugent or Hay/Ison criteria to evaluate a Gram-stained smear of vaginal discharge as is the diagnostic standard used in research studies. If microscopy is not available, the diagnosis should be based upon findings on clinical examination. Amsel criteria for diagnosis of BV without available microscopy are: homogeneous, thin, grayish-white discharge that smoothly coats the vaginal walls, vaginal pH > 4.5, clue cells on saline wet mount, positive whiff-amine test, defined as the presence of a fishy odor when a drop of 10 percent potassium hydroxide (KOH) is added to a sample of vaginal discharge.

Gram stain of vaginal discharge is the gold standard for diagnosis of BV, but is mostly performed in research studies because it requires more time, resources, and expertise than Amsel criteria (2).

Commercial tests for diagnosis of BV are now increasingly used, given their availability increment. The Affirm VPIII test is an automated DNA probe assay for detecting G. vaginalis when present in high concentration. It takes less than one hour to perform it and is a fast option when findings from the physical examination suggest bacterial vaginosis but microscopy cannot be performed to look for clue cells (2).

The OSOM BVBlue system is a chromogenic diagnostic test based on the presence of elevated sialidase enzyme activity in vaginal fluid samples. This enzyme is produced by bacterial pathogens associated with BV including Gardnerella, Bacteroides, Prevotella, and Mobiluncus.

The BD Max system uses quantitative PCR measurement of BV-associated bacteria, scuh as *Gardnerella vaginalis, Atopobium vaginae, Megasphaera, L. crispatus, L. jensenii*, and a proprietary algorithm to provide a positive/negative assessment for the presence of BV (1).

The Aptima BV is a nucleic acid amplification test that measures the presence of Lactobacillus, *G. vaginalis* and *A. vaginae (1)*.

A urine test that uses fluorescence in situ hybridization to identify the BV biofilm on desquamated vaginal epithelial cells in urine sediment appears promising and is also under investigation (2).

## CHLAMYDIA TRACHOMATIS

*Chlamydia trachomatis* is the most common bacterial cause of sexually transmitted genital infections (5).

Although the majority of females with *Chlamydia trachomatis* infection are asymptomatic, the pathogen is an important cause for several common clinical syndromes: genitourinary tract infection (the cervix is the most commonly infected anatomic site and a proportion of females may also have infection of the urethra), cervicitis, dysuria-pyuria syndrome due to urethritis, pelvic inflammatory disease (PID), perihepatitis (Fitz-Hugh-Curtis syndrome), proctitis, conjunctivitis, pharyngitis, genital lymphogranuloma venereum (6).

Diagnosic techniques of *Chlamydia trachomatis* include nucleic acid amplification testing (NAAT), culture, antigen detection and genetic probes, while microscopy is not a useful diagnostic tool (5).

Nucleic acid amplification testing (NAAT) methodology consists of amplifying *Chlamydia trachomatis* DNA or RNA sequences using polymerase chain reaction (PCR), transcription-mediated amplification (TMA) or strand displacement amplification (SDA). These sensitive and specific tests have become the “gold standard” and are the preferred diagnostic method. For females who are undergoing speculum exam, NAAT can be performed on either endocervical or vaginal swabs. A first-catch urine specimen is also acceptable in females but might detect up to 10 percent fewer infections when compared with vaginal and endocervical swab samples. The XPert *Chlamydia trachomatis*/*Neisseria gonorrhoeae* (CT/NG) assay is an NAAT that can provide testing results for chlamydia (and gonorrhea) within 90 minutes. This test is US Food and Drug Administration (FDA) approved for use on endocervical or vaginal swabs and urine. Another CT/NG NAAT assay (binx io) uses electrochemical detection tech-

nology to provide results in approximately 30 minutes and is FDA approved for use on vaginal swabs, with sensitivity, specificity, positive predictive value, and negative predictive value for *Chlamydia trachomatis* of 96, 99, 91, and, respectively, 100 percent (5).

Genetic probe methods, *Chlamydia trachomatis* serology, antigen detection and culture aren't routinely recommended. Culture methods are now limited for research field, in reference laboratories, due to the expense and technical expertise required. *Chlamydia trachomatis* serology can support the positive diagnosis in clinical context, but is performed infrequently, not standardized and requires a high level of expertise for interpretation. Antigen detection requires invasive testing using a swab from the cervix or urethra (6).

## TRICHOMONAS VAGINALIS

Trichomoniasis is a genitourinary infection with the protozoan *Trichomonas vaginalis*. It is the most common non-viral sexually transmitted disease worldwide. *Trichomonas vaginalis* colonize the squamous epithelium in the urogenital tract: vagina, urethra, and paraurethral glands. Other less common sites include the cervix, bladder, Bartholin glands, and prostate (7).

Trichomoniasis ranges from an acute, severe inflammatory disease to an asymptomatic carrier state. Common signs and symptoms of acute infection include purulent, malodorous, thin discharge associated with burning, pruritus, dysuria, lower abdominal pain and dyspareunia.

In chronic infection, signs and symptoms are milder and may include pruritus and dyspareunia, with scanty vaginal secretion. Physical examination often reveals green-yellow, frothy, malodorous discharge and erythema of the vulva and vaginal mucosa. Punctate hemorrhages may be visible on the vagina and cervix (strawberry cervix) (8).

The diagnosis of *Trichomonas vaginalis* is based on laboratory testing (motile trichomonas on wet mount, positive culture, positive nucleic acid amplification test, or positive rapid antigen or nucleic acid probe test) that confirms the positive diagnostic (9).

Microscopy is often the first step in the diagnostic evaluation for trichomoniasis because microscopy is important in the evaluation of vaginal discharge. Microscopy is convenient and low cost, although less accurate than other tests. If the microscopic evaluation is positive for trichomonas, no further testing is indicated (8).

For women with nondiagnostic (or negative) wet-mount slides on microscopy, nucleic acid amplification tests (NAAT) are performed. If NAAT is not available, PID diagnostic kits, or culture are performed instead. The test choice is based on availability and cost. NAATs detect RNA by transcription-mediated amplification (polymerase chain reaction or reverse transcriptase), are highly sensitive and specific and have become the accepted gold standard for the diagnosis of *Trichomonas vaginalis (7).*

Rapid diagnostic kits can be useful in areas of high prevalence where microscopy or culture are not available. The Affirm VPIII Microbial Identification System test uses a DNA hybridization of the probe on a vaginal swab specimen, with results that are available in 45 minutes. The OSOM Trichomonas Rapid Test is a rapid antigen test that uses an immunochromatographic technology on a vaginal swab specimen and testing can be done at the point of care with results are available in 10 minutes (9).

Culture of vaginal secretions and cervical cytology have a lower grade of accuracy (7).

## NEISSERIA GONORRHOEAE

Gonorrhea, or infection with the gram-negative coccus *Neisseria gonorrhoeae*, is a major cause of morbidity among sexually-active individuals worldwide. Gonorrhea is a major cause of urethritis in men and cervicitis in women; the latter can result in PID, infertility, ectopic pregnancy, and chronic pelvic pain (10).

*Neisseria gonorrhoeae* can be identified using several diagnostic modalities. The sensitivity and specificity of these techniques vary widely. Overall, nucleic acid amplification testing (NAAT) is the most accurate and thus the preferred diagnostic test for both genital and extragenital infection. An additional advantage is that NAAT retains accuracy with the patient-collected specimens. Additional diagnostic tests include culture, microscopy, and other nucleic acid-based tests (11).

Use of nucleic acid amplification testing (NAAT) is recommended as the optimal method for the diagnosis of genital and extragenital infections caused by *N. gonorrhoeae* in women with and without symptoms. Compared with culture, commercially available NAAT offers rapid results (within hours) and enhanced sensitivity. NAAT methodology consists of amplifying *N. gonorrhoeae* DNA or RNA sequences using various techniques, such as polymerase chain reaction, transcription-mediated amplification or strand displacement amplification. NAAT can theoretically detect as little as one organism per sample, while the detection threshold of other methods is approximately 1000 organisms. Although the sensitivity of NAAT is generally better than culture, the sensitivity of the individual NAAT varies by NAAT and specimen type. NAAT can be

performed on a wide variety of urogenital samples including endocervical and vaginal samples, urine samples and urethral samples (from men only) (10).

Cultures for *N. gonorrhoeae* are processed on Thayer-Martin agar, which prevents the overgrowth of other endogenous flora. The main advantage of culture is to assess antibiotic susceptibilities, which is of particular importance when antibiotic resistant infection is suspected. Resistance of the gonococcus to several classes of antibiotics and the emergence of gonococcal resistance to the currently recommended classes of antibiotics (cephalosporins and macrolides) has highlighted the importance of maintaining culture capacity (11).

An enzyme immunoassay was developed to detect gonococcal antigens from cervical swab or urine specimens but is not widely used because its positive predictive value is only acceptable in populations with a high prevalence of infection (10).

## MYCOPLASMA GENITALIUM

*Mycoplasma genitalium* is a bacterium that is a common cause of cervicitis in females and also may be a cause of PID and is recognized as a sexually transmitted infection in females and an overall, cumulative evidence suggests its association with cervicitis. Cervicitis with *M. genitalium* frequently have no symptoms or are usually nonspecific, with the most commonly reported symptom being vaginal discharge or itching, dysuria, and pelvic discomfort; or, in case it ascends from the lower to upper genital tract after sexual transmission, it can cause signs or symptoms of PID, that include mild to severe pelvic pain, abdominal pain, abnormal vaginal discharge, and/or bleeding (12,13).

The diagnosis of *M. genitalium* infection can be made through detection of the organism using polymerase chain reaction or other, more recently developed nucleic acid amplification tests. The preferred specimens are a vaginal swab, endocervical swab, and urine sample (12).

Routine screening for *M. genitalium* in asymptomatic individuals is not recommended. Detection and treatment of infections due to *M. genitalium* in high-risk asymptomatic persons have not yet been demonstrated to prevent PID, infertility, adverse pregnancy outcomes (12).

Infection of the upper female genital tract leads to inflammatory damage, resulting in scarring, adhesions, and partial or total obstruction of the Fallopian tubes. This can result in loss of the ciliated epithelial cells along the fallopian tube lining, resulting in impaired ovum transport and increased risk for infertility and ectopic pregnancy. Additionally, adhesions can lead to chronic pelvic pain (15).

Ascending infection from the cervix causes PID. The main cause of PID is represented by sexually transmitted pathogens or bacterial vaginosis-associated pathogens that have colonized the lower genital tract and can ascend to the upper reproductive tract (uterus, fallopian tubes, and ovaries) (14,16).

PID is an acute and subclinical infection of the upper genital tract in women, involving any or all of the uterus, fallopian tubes, and ovaries; this is often accompanied by involvement of the neighboring pelvic organs. The main cause of PID is represented by sexually transmitted pathogens or bacterial vaginosis-associated pathogens (17).

Many cases of PID may cause no symptoms and only be suspected years later in the setting of tubal infertility, while the most common symptoms are abdominal and pelvic pain. The character of the pain is variable, and in some cases, may be quite subtle. The recent onset of pain that worsens during coitus or with jarring movement may be the only presenting symptom of PID. The onset of pain during or shortly after menses is particularly suggestive. On physical examination, most women with PID have abdominal tenderness on palpation, greater in the lower quadrants, which may or may not be symmetrical. Rebound tenderness, fever, and decreased bowel sounds are usually limited to women with more severe PID (14,15).

Acute cervical motion, uterine, and adnexal tenderness on bimanual pelvic examination are the defining characteristic of acute symptomatic PID. Purulent endocervical discharge and/or vaginal discharge is also common. However, significant lateralization of adnexal tenderness is uncommon in mild to moderate PID. PID is associated with higher rates of subsequent tubal infertility and ectopic pregnancy (14).

## CONCLUSIONS

PID is defined as an inflammation of the upper genital tract due to an infection in women. The disease affects the uterus, Fallopian tubes, and/or ovaries. It is typically an ascending infection, spreading from the lower genital tract. The majority of cases of PID are related to a sexually transmitted infection. The diagnosis of PID is primarily clinical and should be suspected in female patients with lower abdominal or pelvic pain and genital tract tenderness. During the patient's evaluation, other etiologies of pain, including ectopic pregnancy, should be considered in the differential diagnoses and should be ruled out. PID is treated with antibiotics to cover the primary pathogens, including *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Short-term complications include tubo-ovarian or pelvic abscess, while the long-term complications include ectopic pregnancy, infertility, and chronic pelvic pain. Early diagnosis and treatment can potentially prevent complications.

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*Conflict of interest:* none declared
*Financial support:* none declared