Microbial ecology of the lower genital tract in women with sexually transmitted diseases

George Creatsas and Efthimios Deligeoroglou

2nd Department of Obstetrics & Gynecology, University of Athens, Medical School, 'Arethaion' Hospital, 76 V. Sofias Ave, 11528 Athens, Greece

Sexually transmitted diseases (STDs) in women are of great concern to all health-care providers since many of them are preventable and/or treatable conditions which, if left untreated, could have serious sequelae such as pelvic inflammatory disease, infertility, cervical cancer, systemic disease, etc. They may also become a major public health problem when dealing with diseases such as hepatitis, etc., or in people with human immunodeficiency virus. We present here a comprehensive review of the common causes of STDs and their treatment.

Introduction

Sexually transmitted diseases (STDs) are infections acquired mainly by sexual contact. More than 448 million new cases of curable STDs [chlamydia, gonorrhoea, etc., excluding human immunodeficiency virus (HIV) infections] occur every year worldwide in people aged between 15 and 49 years. Most bacterial or parasitic infections can be easily treated, while viral infections may be treated but not completely eradicated. Early diagnosis of STDs may be difficult, since many do not have early symptoms and consequently therapy is delayed with adverse outcomes especially regarding future fertility. In the USA, almost half of the new cases each year are in the 15–24 year age group, even though this group comprises only 25% of the population engaged in promiscuous activity. Although a division between vulvovaginal and cervical infection is made, most of the pathogens infect both the vagina and cervix.

Vulvitis/vulvovaginitis

The most common gynaecological pathologies in neonates and children are vulvitis and vulvovaginitis, respectively (Koumantakis et al., 1997). Vulvitis is a local irritation of the external genitalia, mainly due to poor local hygiene. Mechanical or chemical irritation, trauma, etc., could also be the cause of the disease. Involvement of the vagina with inflammation of the vaginal mucosa, erythema and discharge is characterized as vaginitis (Farrington, 1997).

Potential risk factors for vulvitis and/or vulvovaginitis are (Syed & Braverman, 2004): (i) anatomical: the lack of labial fat pads and pubic hair, prepubertal hypo-oestrogenism resulting in thin, sensitive, vulvar skin and thin, atrophic, vaginal epithelium; (ii) the prepubertal hypo-oestrogenic vagina has a neutral pH and is an excellent environment for growth of bacteria; (iii) hygiene: poor hand washing, exposure to vulvar irritants (e.g. dirt, sand, soaps), inadequate cleansing of the vulva after voiding or defecation; (iv) foreign body in the vagina; (v) sexual abuse.

Patients usually present with vaginal discharge and/or erythema, which may be accompanied by pruritus. Other less common symptoms such as dysuria, pain, oedema, adhesion of the labia minora or vaginal spotting may also occur. Pathogens most commonly isolated from swab cultures obtained through the hymenal orifice include Candida species, group B haemolytic streptococci, Escherichia coli and enterococci (Deligeoroglou et al., 2004) (Table 1).

The management of the disease consists of giving instructions for correct and/or improvement of hygiene both locally and systematically (washing hands before and after touching the genitals) and administration of the specific antimicrobial/antifungal agent depending on the culture and antibiogram.

In the case of persistent non-specific vulvovaginitis, a 10–14 day course of oral antibiotics is indicated. Additionally, a 1 week use of topical antibacterial cream or a 1 month course of a nightly dose of amoxicillin, cephalosporin or trimethoprim/sulfamethoxazole may be suggested. A course of a small amount of nightly applied, oestrogen-containing cream (discontinued if signs of systemic absorption appear) may also prove beneficial (Blackwell et al., 1983).

Infections due to Neisseria gonorrhoeae, Chlamydia, human papillomavirus (HPV) and HIV and genital herpes in prepubertal girls should alert examining doctors to the possibility of sexual abuse (Hayman & Lanza, 1971).

Candidal vaginitis

Normally yeast species are part of the vaginal flora but infections may result from overgrowth, producing thick, white discharge; vaginal and sometimes vulvar pruritus are present with or without burning sensation and irritation. The pH is <4.5 (normal) while microscopic findings show budding yeast, pseudohyphae and/or mycelia. Candida species (albicans, torulopsis, glabrata, tropicalis) are usually...
increased coccobacilli, while the pH is.

microscopic findings are clue cells, decreased lactobacilli and
charge often accompanied by pruritus and irritation. The
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vaginal administration of metronidazole or clindamycin.

normal vaginal lactobacilli (Majeroni, 1998).
test'); (iv) vaginal pH greater than 4.5; and (v) absence of the
amine (fishy) odour when potassium hydroxide solution is
vary); (ii) presence of clue cells (greater than 20 %); (iii)

Gardnerella vaginalis
Pseudomonas species
Candida species
Trichomonas vaginalis
Enterobius vermicularis
Klebsiella species
Escherichia coli
Neisseria gonorrhoeae
Anaerobic bacteria
Prevotella melaninogenicum
Peptostreptococci
Chlamydia trachomatis
Mycoplasma hominis
Ureaplasma urealyticum

Aerobic bacteria
β-Haemolytic group A streptococci
β-Haemolytic group B streptococci
β-Haemolytic group C streptococci
β-Haemolytic group G streptococci
β-Haemolytic group F streptococci
Enterococci
Staphylococcus aureus
Haemophilus influenzae
Gardnerella vaginalis
Pseudomonas species
Candida species
Trichomonas vaginalis
Enterobius vermicularis
Klebsiella species
Escherichia coli
Neisseria gonorrhoeae

the cause of the disease. The recent use of antibiotics and
diabetes mellitus have been considered risk factors (Creatsas
Initial treatment consists of topical (in the form of cream
or vaginal suppositories) or oral antifungal agents, while
persistent infection warrants oral antifungals once a week
for 6 months (Mårdh et al., 2004).

Bacterial vaginosis

Overgrowth of the normally occurring anaerobic vaginal
organisms (Gardnerella vaginalis, Bacteroides species, Mobi-
luncus species) is the cause of bacterial vaginosis. The typical
findings and symptoms are grey, thin, fishy-smelling dis-
charge often accompanied by pruritus and irritation. The
microscopic findings are clue cells, decreased lactobacilli and
increased coccobacilli, while the pH is >4.5. Three of the
following criteria are needed to establish the diagnosis: (i)
homogeneous vaginal discharge (colour and amount may
vary); (ii) presence of clue cells (greater than 20 %); (iii)
amine (fishy) odour when potassium hydroxide solution is
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added to vaginal secretions (commonly called the ‘whiff test’); (iv) vaginal pH greater than 4.5; and (v) absence of the
normal vaginal lactobacilli (Majeroni, 1998).

Treatment may be oral with metronidazole twice a day for
7 days or clindamycin twice a day for 7 days, or topical with
vaginal administration of metronidazole or clindamycin.

Trichomonal vaginitis

Trichomonas vaginalis, a small, motile, flagellated, protozoan
parasite, is the cause of trichomonal vaginitis. Patients
commonly complain of discharge (frothy, malodorous,
greyish-yellow or yellow–green), itching, dysuria and post-
coital bleeding but sometimes may even be asymptomatic.
The micro-organism may be found on Papanicolaou smears
or wet prep. On speculum exam, the cervix is strawberry-like
with grossly visible punctate haemorrhages. Wet prep reveals
mobile flagellated organisms and an increased number of
white blood cells (Hassan & Creatsas, 2000).

Treatment with metronidazole orally in a single dose of 2 g
is usually sufficient. Sexual partner(s) must also be treated
with the same regimen at the same time. Patients should be
warned about possible side-effects of metronidazole, such as
nausea, vomiting and metallic aftertaste, and be instructed
to avoid alcohol for 24–48 h (Gallion et al., 2009). Screening
for other STDs (gonorrhoea, chlamydia) should also be

cervicitis

There is no consensus regarding the definition of cervicitis.
Most studies define cervicitis as the clinical presence of
mucopurulent discharge or friability at the endocervical os,
with previous studies focusing on vaginal microbial
ecology, while the cervical flora has not been sufficiently
investigated (Marrazzo & Martin, 2007). The correlation
between the microbial flora of the cervix, cervicitis and
other genital tract infections remains unexplored. Cervicitis
may be infectious or non-infectious. Infectious cervicitis is
usually endocervical and may be caused by N. gonorrhoeae,
Chlamydia trachomatis, herpes simplex virus (HSV), HPV
and Mycoplasma genitalium (Marrazzo & Martin, 2007).
Cervicitis is also associated with T. vaginalis, causing
erosion of the ectocervical epithelium, with the clinical
manifestation of the ‘strawberry cervix’ (Marrazzo &
Martin, 2007). Non-infectious cervicitis is found primarily
at the ectocervix and might be caused by local trauma,
radiation or malignancy.

The infectious aetiologies are significantly more common
than the non-infectious aetiologies (Lusk & Konecny, 2008).

HSV

HSV-1 mainly causes infections in the lips (herpes labialis),
mouth (gingivostomatitis), eye (conjunctivitis/keratitis),
face and central nervous system and is the cause of a small
proportion of neonatal and genital herpes infections, while
HSV-2 is the principal cause of neonatal and anogenital
infections. It is important to note that both types of HSV
can cause infection in all the above-mentioned body areas,
but each type has its own preferred site of latency after the
primary infection, i.e. the trigeminal ganglion for HSV-1
and the sacral ganglia for HSV-2. The virus may be silenced
by the defensive mechanism of the host in neuronal cells.

Table 1. Pathogens isolated from the cervical and vaginal area
in patients presenting with vulvovaginitis (adapted from
Deligeoroglou et al., 2004)

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>No. of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Haemolytic group A streptococci</td>
<td>160</td>
<td>9.0</td>
</tr>
<tr>
<td>β-Haemolytic group B streptococci</td>
<td>267</td>
<td>15.0</td>
</tr>
<tr>
<td>β-Haemolytic group C streptococci</td>
<td>36</td>
<td>2.0</td>
</tr>
<tr>
<td>β-Haemolytic group G streptococci</td>
<td>35</td>
<td>2.0</td>
</tr>
<tr>
<td>β-Haemolytic group F streptococci</td>
<td>36</td>
<td>2.0</td>
</tr>
<tr>
<td>Enterococci</td>
<td>178</td>
<td>10.0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>36</td>
<td>2.0</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>18</td>
<td>1.0</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>53</td>
<td>3.0</td>
</tr>
<tr>
<td>Pseudomonas species</td>
<td>17</td>
<td>1.0</td>
</tr>
<tr>
<td>Candida species</td>
<td>409</td>
<td>23.0</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>54</td>
<td>3.0</td>
</tr>
<tr>
<td>Enterobius vermicularis</td>
<td>17</td>
<td>1.0</td>
</tr>
<tr>
<td>Klebsiella species</td>
<td>53</td>
<td>3.0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>178</td>
<td>10.0</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Anaerobic bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevotella melaninogenicum</td>
<td>177</td>
<td>10.0</td>
</tr>
<tr>
<td>Peptostreptococci</td>
<td>53</td>
<td>3.0</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>14</td>
<td>0.8</td>
</tr>
<tr>
<td>Mycoplasma hominis</td>
<td>36</td>
<td>2.0</td>
</tr>
<tr>
<td>Ureaplasma urealyticum</td>
<td>67</td>
<td>3.7</td>
</tr>
</tbody>
</table>
There, it lies dormant and may be reactivated by hormonal changes or physical or emotional stress. When recurrences occur, the virus in a nerve cell (at their latency sites) becomes active and through the axon of the cell reaches the skin, where it infects cells locally entering a new replication cycle (Ryan & Ray, 2004).

Symptoms of HSV infection include watery blisters in the skin or mucous membranes (Sayed & Braverman, 2004). The virus is transmitted through contact with an infected area of the skin, while transmission during latency is possible although very rare. Symptoms from primary infection with HSV are usually more severe than those in recurrent disease because there are no antibodies against the virus. This first infection with oral herpes carries a low (about 1%) risk of aseptic meningitis developing.

Vertical transmission of the virus is possible, although the risk is low if the mother is asymptomatic during labour and delivery. In contrast, the risk is higher if the mother acquires the primary infection during pregnancy (Corey & Wald, 2009; Kimberlin, 2007).

Diagnosis is often clinical due to the characteristic vesicle formation. A viral culture and/or PCR, especially of the cerebrospinal fluid in cases of encephalopathy, provide a definitive solution to the differential diagnosis (Chayavichitsilp et al., 2009).

Primary infections of genital herpes can be treated with antiviral medications (acyclovir, 200 mg per os 5 times per day for 10 days; valaciclovir, 1 g per os b.i.d. for 10 days; famciclovir, 250 mg per os t.i.d. for 7–10 days) administered systemically and possible secondary bacterial infections with antibiotics. Recurrent infections normally resolve untreated but antiviral therapy shortens the duration and severity of symptoms. Regimens used are aciclovir per os 200 mg every 4 h for 5 days, valaciclovir per os 500 mg b.i.d. for 3 days, or famciclovir 1000 mg per os b.i.d. for 1 day (Hassan & Creatsas, 2000).

**C. trachomatis**

Chlamydiae are non-motile, obligately intracellular organisms. Three species may cause human disease: *C. trachomatis*, *Chlamydia pneumoniae* and *Chlamydia psittaci*. *C. trachomatis* may cause cervicitis, urethritis and pelvic inflammatory disease in women as well as proctitis and lymphogranuloma venereum. There is also a chance of vertical transmission causing conjunctivitis and pneumonia in the newborn. Diagnosis is usually delayed in women since the infection is asymptomatic in most cases. Routine screening of asymptomatic women at high risk for STDs (sexually active adolescents, history of STDs, etc.) is recommended because of the risk of pelvic inflammatory disease and its possibly serious sequelae (Marrazzo & Martin, 2007).

PCR is the standard test for diagnosis, as it is more sensitive than cell culture, ELISA or direct immunofluorescence.

Treatment for uncomplicated lower genital tract infection is with a single dose of 1 g per os of azithromycin or with a 7 day regimen of doxycycline (100 mg per os b.i.d.). When there is evidence of gonorrhea infection, treatment for presumed chlamydial infection is always indicated while pelvic inflammatory disease requires treatment for at least 2 weeks.

**N. gonorrhoeae**

*N. gonorrhoeae* is a Gram-negative coccobacillus that invades columnar and transitional epithelial cells, becoming intracellular. This is the reason why the vaginal epithelium is not involved. Infection with *N. gonorrhoeae* is frequently asymptomatic, while risk factors for gonococcal infection include: age under 25 years, past history of gonococcal infection, co-infection with other STDs, new or multiple sexual partners, lack of barrier protection and drug use (Marrazzo & Martin, 2007).

Symptoms of gonorrhoea may present as vaginitis or cervicitis. Cervicitis is usually in the form of profuse odourless, white-to-yellow vaginal discharge, with no signs of local irritation. The infection may involve the Bartholin and Skene glands, the urethra, and if ascends to the endometrial cavity and Fallopian tubes may cause pelvic inflammatory disease. Diagnostic tests include culture, nucleic acid hybridization tests and PCR. All patients found to be infected with *N. gonorrhoeae* should be tested for other sexually transmitted infections and their partners should be referred for evaluation and treatment.

Recommended treatment of uncomplicated gonococcal infection includes ceftriaxone 250 mg intramuscularly in a single dose, cefixime 400 mg orally in a single dose, single-dose injectible cefalosporin regimens, azithromycin 1 g orally in a single dose or doxycycline 100 mg orally twice a day for 7 days.

**HPV**

HPV is a DNA-containing virus and belongs to the papillomavirus family of viruses. There are over 200 genotypes of HPV and the virus is capable of infecting humans. More than 40 types of HPV are transmissible through sexual contact and may infect the anogenital region. Although some sexually transmitted HPV types may cause genital warts (HPV types 6 and 11 are responsible for about 90% of the cases), persistent infection with 'high-risk' HPV types (Table 2) may progress to precancerous lesions (cervix, vulva, anus) and invasive cancer. Transmission is typically through the skin or mucous membranes by contact with infected areas and involves penetrative vaginal or anal intercourse, but also oral, digital and non-penetrative genital contact.

The incubation period is 1–6 months and it is estimated that almost 70–75% of the population will contact some type(s) of the virus during their lifetime (Koutsky, 1997; Weaver, 2006).
HPV can infect and replicate in only the basal cells of stratified epithelium, which is accomplished through micro-abrasions or other epithelial trauma that exposes parts of the basement membrane (Schiller et al., 2010). Risk factors include sexually active women under 25 years of age (Table 3), a high number of sexual partners and coinfection with \textit{C. trachomatis} or HSV (Dunne et al., 2007). According to a study by Zheng et al. (2010), there is a significant association between infection with HPV and infection with \textit{C. trachomatis} or \textit{Ureaplasma urealyticum}. In the same study, no correlation was found between HPV infection and bacterial vaginosis, \textit{Streptococcus agalactiae}, \textit{Candida} or \textit{Trichomonas vaginalis}. According to a study by King et al. (2011), bacterial vaginosis is associated with increased odds for prevalent and incident HPV and with delayed clearance of the infection.

Infection from HPV is in most cases a self-limiting condition that subsides in almost 90% of infected females, roughly 2 years after the infection (about 70% within the first year) (Pagliusi & Aguado, 2004). In cases where the infection persists (about 5–10% of infected females) and if due to a high-risk virus type, there lies the risk of precancerous lesions of the cervix developing, which can progress to invasive cervical cancer (Parkin, 2006).

The introduction of the Papanicolaou procedure of cervical sampling and specimen examination, as a screening method for cervical cancer and precancerous conditions, has resulted in a dramatic fall in the incidence of invasive cervical cancer during the last 50–60 years (Sirovich & Welch, 2004; Koss, 1989).

Primary prevention of HPV infection is achieved through active immunization with the two vaccines available: (i) the quadrivalent vaccine (Gardasil) offering immunity to the two most common high-risk HPV types (16 and 18, responsible for about 70% of the cases of cervical cancer) and the two most common low-risk types which cause genital warts (6 and 11, responsible for almost 90% of genital warts) (Muñoz et al., 2004); and (ii) the bivalent vaccine (Cervarix) offering immunity to the same two most common high-risk HPV types (16 and 18) and possibly cross-protection against oncogenic non-vaccine HPV types, in particular HPV-45, which may be important in the prevention of cervical adenocarcinoma, which is currently not well served by screening (Szarewski, 2010).

In our Institution, the Division of Paediatric–Adolescent Gynecology and Reconstructive Surgery has joined the multicentric studies conducted during the development of both vaccines, and has recognized the safety and efficacy of both of them.

### Concluding remarks

Infection with STDs is a very sensitive matter, especially when it concerns adolescents and young adults. Most of these tend to neglect safe sex precautions and thus infection with STDs is common. Adolescents’ natural tendency for experimentation and having multiple sexual partners may lead to combined infections; for instance, an infection from \textit{C. trachomatis} and/or HSV may predispose to HPV coinfection through epithelial erosion that exposes part of the basement membrane. This type of combined STD infection may prove even harder to treat, partly due to the adolescents’ unwillingness to see a gynaecologist regularly and their tendency to seek medical help when the disease is symptomatic or has progressed.

The cost of medical care is also an issue for young people, and community/government facilities specifically aimed at them (with provision to be open in out of school hours) and with adequately educated personnel might be a solution to the problem. Since privacy is also a major concern for adolescents, appropriate measures should be taken to provide reassurance that medical confidentiality is kept in these facilities and that patients’ visits are programmed to avoid meeting other patients in the waiting room.

Further research is needed on the natural vaginal microflora of prepubertal and adolescent girls. HPV and HSV typing in children do not seem to be helpful, even in cases of investigating the possibility of sexual assault. Treatment indicated for adults should not be applied to very young girls unless clinical trials have already assessed its safety in this specific age group.

The possibility of sexual abuse must be investigated if a pathogen has been isolated, accompanied by a clinical examination by an expert in paediatric and adolescent gynaecology. Psychological consultation is recommended in certain cases.

As the majority of lower genital tract infections in prepubertal girls are non-specific, the clinical examination

### Table 2. HPV types and genital cancer (adapted from Kumar et al., 2007; Muñoz et al., 2006)

<table>
<thead>
<tr>
<th>HPV type</th>
<th>HPV type</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest risk</td>
<td>16, 18, 31, 45</td>
<td></td>
</tr>
<tr>
<td>Other high-risk</td>
<td>33, 35, 39, 51, 52, 56, 58, 59</td>
<td></td>
</tr>
<tr>
<td>Probably high-risk</td>
<td>26, 53, 66, 68, 73, 82</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. HPV prevalence by age in the USA, including 20 low-risk types and 23 high-risk types (adapted from Dunne et al., 2007)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14–19</td>
<td>24.5</td>
</tr>
<tr>
<td>20–24</td>
<td>44.8</td>
</tr>
<tr>
<td>25–29</td>
<td>27.4</td>
</tr>
<tr>
<td>30–39</td>
<td>27.5</td>
</tr>
<tr>
<td>40–49</td>
<td>25.2</td>
</tr>
<tr>
<td>50–59</td>
<td>19.6</td>
</tr>
<tr>
<td>14–59</td>
<td>26.8</td>
</tr>
</tbody>
</table>
should focus on the distinction between non-specific and infectious cases. Specific antimicrobial treatment is indicated in cases where a specific pathogen is isolated.

References


