

Vaginal microbiocenosis and cytology of prepubertal and adolescent girls: their role in health and disease

Lyubov A Matytsina, Donald E Greydanus, Yuriy A Gurkin

Plymouth, UK

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Background: Clinicians and investigators often do not appreciate the importance of vaginal microbiocenosis and vaginal cytology in the health of prepubertal and adolescent girls.

Data sources: Based on recent publications in human medicine and our own experience with vaginal cytology and microbiology in children and adolescent girls, we review the principles of vaginal microbiocenosis and cytology and their roles in disease prevention in prepubertal and adolescent girls.

Results: The main role of vaginal microbiocenosis and cytology in diagnosing as well as developing vulvovaginitis is demonstrated. Clinicians can identify states of vaginal health and disease by important well-known diagnostic tools, including vaginal cytology. *Lactobacilli* are infrequently observed in the prepubertal girls, but become more abundant in adolescent girls. Three basic types of vaginal smears are presented in addition to a classification of inflammatory urogenital diseases. Management of common types of vulvovaginitis is also considered.

Conclusions: This essential shift in vaginal biocenosis is important to prevent the growth of potentially pathogenic flora in the vagina. The detection of vaginal microbiocenosis problems and recognition of changing cytology in vaginal development can provide helpful clues to identifying and preventing vaginal diseases in this pediatric population.

Key words: cytology;
lactobacilli;
vaginal microbiocenosis;
vulvovaginitis

Introduction

Vaginal inflammatory and infectious diseases are the most common gynecological diseases found in prepubertal and adolescent girls. Microbiocenosis (biocenosis) refers to a group of interacting organisms that live in a specific habitat such as the vagina and form an ecological community. Cytological evaluation of vaginal cells is an important diagnostic method used in identifying vaginal disorders (Fig.).^[1-3] Such an analysis reveals vaginal epithelial status, presence or absence of inflammatory changes, and etiological factor(s) causing vaginal symptomatology.^[4-9] An examination of cervical cytology has proven useful in cervical cancer screening for adolescent and adult females.^[10,11]

This article focuses on vaginal microbiocenosis and cytology. The vaginal microbiological picture can be divided into two groups: 1) normal or non-pathogenic agents (normal flora or microbiocenosis),^[7] and 2) potentially dangerous or conditionally pathogenic microorganisms. A decrease of immunological health of an individual can lead to some organisms becoming pathogenic.^[1,12-15]

Role of *lactobacilli*

Vaginal microorganisms are divided into aerobes, facultative anaerobes and obligate (strict) anaerobes. The vaginal *lactobacilli* flora consists of 11 strains of *lactobacilli* (gram-positive bacilli); the dominant strain in quantity (42.9%) is *Döderlein's bacillus* (*Lactobacillus acidophilus*). The role of this *lactobacilli* flora is to prevent the presence of potentially pathogenic flora by producing an acidic environment that has a pernicious effect on these other flora.

Author Affiliations: Obstetrics, Gynecology, Perinatology and Pediatric & Adolescent Gynecology, Donetsk Medical University, Ukraine, Peninsula Medical School, Plymouth, UK (Matytsina LA); Pediatrics & Human Development, Michigan State University College of Human Medicine, Michigan State University/Kalamazoo Center for Medical Studies, 1000 Oakland Drive, Kalamazoo, Michigan, USA (Greydanus DE); Pediatric Gynecology, St-Peterburgh Pediatric Academy, St-Peterburgh, Russia (Gurkin YA)

Corresponding Author: Lyubov A Matytsina, Obstetrics, Gynecology, Perinatology and Pediatric & Adolescent Gynaecology, Donetsk Medical University, Ukraine, Peninsula Medical School, Plymouth, UK (Tel: 44 1872 274501; Fax: 44 1872 274501; Email: lubore@mail.ru; lyubov.matytsina@yahoo.com)

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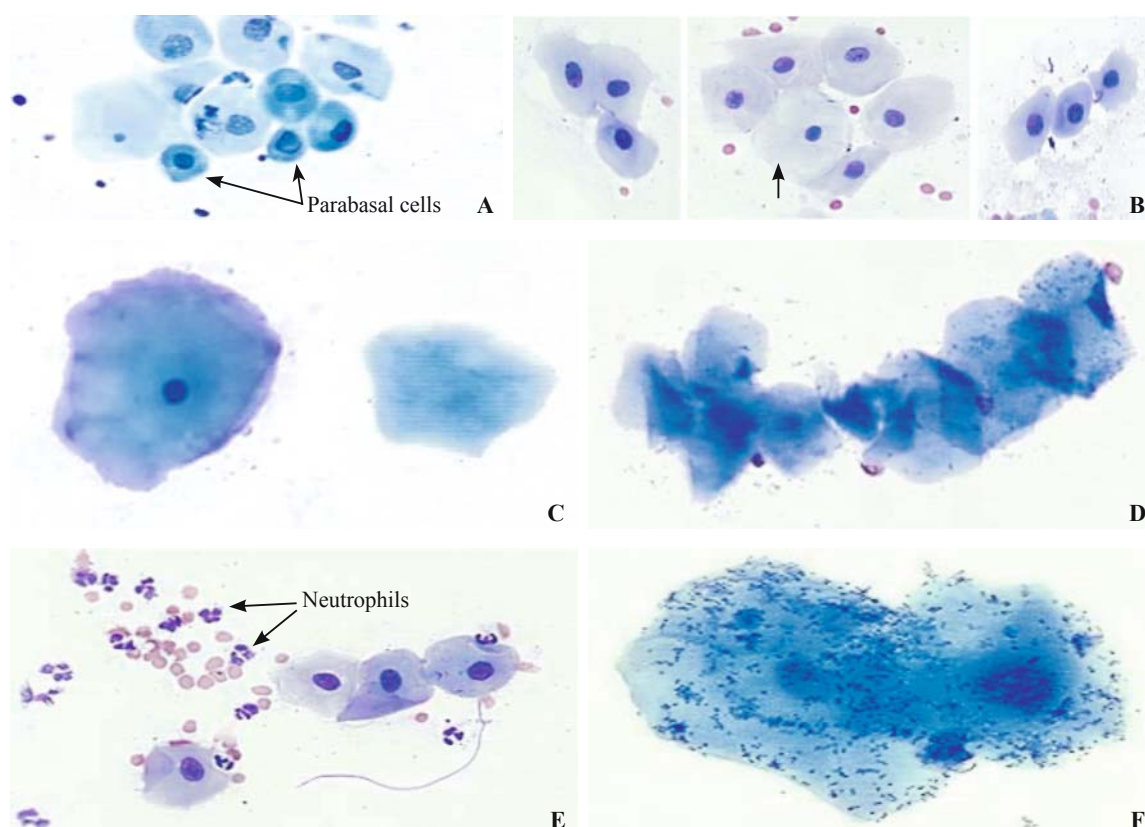


Fig. Classification of vaginal epithelial cells. **A:** parabasal cells: the smallest epithelial cells seen on a typical vaginal smear. They are round or nearly round and have a high nuclear to cytoplasmic ratio. **B:** Intermediate cells vary in size and shape, but typically have a diameter 2 to 3 times that of parabasal cells. All of the cells are typical intermediates except for the one cell in the middle panel (arrow), which might be classified as a superficial cell (small, dark nucleus). Note that the intermediate cells vary in size and shape: round outlines (small intermediates), a polygonal shape (large intermediates). **C:** Superficial cells are the largest cells seen on a vaginal smear. They are polygonal in shape and distinctly flat, sometimes having the appearance of being rolled up. Their nuclei are either absent or pyknotic (very small and dark). Superficial cells are often seen in large sheets or strings, as seen below with fully cornified cells. **D:** Aside from the epithelial cells described above, a number of other cells are seen on vaginal smears, for example: leukocytes/neutrophils, erythrocytes. **E:** a group of intermediate cells associated with neutrophils and red blood cells (erythrocytes). Bacteria are often seen on vaginal smears in huge numbers, covering cells and spilling onto the background. The minute dark specks covering the superficial cells in the image below are bacteria (**F**).^[3]

In the prepubertal girls, the vaginal environment is alkaline or neutral in which the vaginal vestibule and mucosa are pink, tender, and devoid of overt vaginal secretion or fluid. Vaginal cytology reveals few epithelial cells and leukocytes; cytology or culture reveals cocci along with nitrobacteria and corynebacteria. *Lactobacilli* are absent or in low numbers due to low ovary endocrine function. As sex hormone secretion begins in the peripubertal period and increases as puberty progresses, the vagina becomes inhabited by increasing amounts of *lactobacilli*. The vaginal milieu becomes acidic and physiologic leukorrhea occurs.^[1,4]

Other vaginal flora

A variety of flora can be found in the vagina at

different ages and in different stages of health, growth, or disease.^[7,15,16] An important group is the cocci bacteria. The biological feature of cocci lies in the fact that young cocci are intensively colored by Gram staining, and when the cocci become older they turn to be gram-negative. Some of the cocci cultures belong to normal microbiological growth in skin, and mucus membranes of the respiratory tract, the gastrointestinal tract, and the genitals. However, some of the cocci flora in the vagina can become pathogenic flora. The *staphylococci* in the vagina include *Staphylococci aureus*, *epidermidis*, and *micrococcus* (saprophytic). The *streptococci* include *Hemolytic* (green) *streptococci*, *Enterococci* (*Fecal streptococci*), and *Streptococcus pneumoniae*. Environmental niches of *Peptostreptococci* (anaerobic cocci) are the oral cavity, intestine, and genitals.

Bacillus flora is also conditionally pathogenic and can grow in the vagina. *Diphtheroids* are gram-positive bacilli that belong to the *Corynebacteria* group. Some *Diphtheroids* represent normal flora in the respiratory tract and mucus membranes of the skin and genitals. *Enterobacteria* are gram-positive bacilli that include *Intestinal bacilli*, *Proteus*, *Enterobacteria* (blue pus bacilli), and *Klebsiella*. They make up a considerable part of normal flora of the intestine and take part in providing its normal gastrointestinal functioning; however, they can become pathogenic flora when found in the vagina.

Gardnerella vaginalis and other *Gardnerella* species are small gram-positive bacilli (sometimes found in the form of *coccobacilli*) that are facultative anaerobes.^[7,15,17] *Gardnerellas* are seen in healthy women (their frequency fluctuates from 47% to 68%) and girls aged from 2 months to 15 years without any clinical manifestations. Thus, *Gardnerella vaginalis* are considered as co-inhabitants of the vagina, though some experts feel that *Gardnerella* species can be pathogenic in some cases.^[7,15-17]

Leptothrix are gram-variable bacilli that can have the form of coccobacilli; they are facultative anaerobes growing in the vagina and do not grow on ordinary culture media. These bacteria are found in nature in streams of slowing running water polluted by various industries such as paper or milk factories. *Acinetobacter* are aerobic, thick, short, gram-positive bacilli that have the form of diplococci; in gram stains, they appear similar to *gonococcal* and *meningococcal* bacteria. *Acinetobacter* can be seen in the skin, mucus membranes, respiratory tract, and the urogenital tract in humans. Patients with immunodeficiency can develop sepsis in association with other microbes.^[1]

Vaginal cytology and microbiology

There are three basic vaginal smear types in prepubertal and adolescent girls: first type (normal state of vaginal biocoenosis), second type (reflection of non-specific vulvovaginitis), and third type (reflection of specific vulvovaginitis) (Table 1).^[18] This classification is recommended for clinicians caring for prepubertal and adolescent girls, providing a helpful picture of the clinical-laboratory status of the vagina and its disorders in this pediatric population.

First type

In the first type classification of the vaginal smear (normocoenosis), there is a normal state of vaginal health. The quantity of leukocytes is rather negligible,

and presented mainly by granulocytes; phagocytosis is not marked. Also, the flora is lean, containing mainly of a small quantity of gram-positive cocci (diplococci).

Second type

The smear called the second type represents an intermediate picture in which there is a transition state from normocoenosis to a non-specific vaginitis picture. There is a low content of leukocytes (mainly granulocytes) and epithelial cells, and *Döderlein's bacillus* occur with girls of adolescent age. This type has considerable quantity of pathogenic and potentially pathogenic microbiologic flora with absence or minimal clinical characteristics of vaginal genital inflammation. The quantity of degeneratively changed cells is small.

Third type

The third type of vaginal smear reflects marked, specific vulvovaginitis. The quantity of leukocytes is more than 15, mainly neutrophils and macrophages; phagocytosis and mucous reaction are marked. The quantity of degenerative and reactively changed cells is considerable. The quantity of shed epithelium up to age of 9 years is often 9-12, and 10 to 20 for older adolescents. *Döderlein's bacillus* as a rule are absent. The flora is mixed and represented in considerable quantity. Further evaluation reveals a specific etiology such as *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Candida albicans*, anaerobic bacteria reflective of bacterial vaginosis, or others (Table 1).^[5,19-22]

Vaginal inflammation

Diagnostic methods used to identify vaginal pathology in prepubertal and adolescent girls are clinical evaluation and specific laboratory tests, including saline as well as potassium hydroxide preparations and microbiology; a major tool in the diagnosis is vaginal cytology (Fig.).^[6,7] Vaginal inflammation is noted by exudative, degenerative, reparative, and defensive changes of cell composition (Table 2).

A classification of inflammatory urogenital disorders noted in prepubertal and adolescent girls is shown in Table 3. The specific diagnosis is based on a detailed history, genital examination, recto-abdominal examination, vaginoscopy, and laboratory evaluation of vaginal contents, including microscopic and microbiological testing looking for bacterial, viral, parasitic, and helminthic causes. In the medical history, the clinicians should pay attention to the beginning of the disorder, its course, and any possible connection to other disorders the patient may also

Table 1. Vaginal biocoenosis in prepubertal and adolescent girls (Matytsina LA, Kuznetsova YI, 2004)^[18]

Smear type	Readings	Age					Nosology
		1-5 y	6-10 y	11-15 y	16-17 y	Adolescent girls having sexual life	
First type (normocoenosis)	Leukocytes	0-2	3-5	5-7	to 10	to 10	Normal state of vaginal biocoenosis
	Mucus	1-2	1-2	1-2	1-2	1-2	
	Epithelium*	1-3	4-6	5-6	to 10	to 10	
	Flora	cocci (+)	cocci (+)	mixed	cocci (+)	mixed	
Second type	Leukocytes	7-10	10-15	10-15	to 15	to 15	State of non-specific vulvovaginitis
	Mucus	3	3	3	3-4	3-4	
	Epithelium*	3-4	4-5	5-6	To 10	to 10	
	Flora	cocci, mixed	cocci, mixed	cocci, mixed	cocci, mixed	cocci, mixed	
Third type	Leukocytes	>15	>15	>15	>20	>20	State of gonorrhoea, trichomoniasis, chlamydia, bacterial vaginosis, candidiasis vulvovaginitis, others
	Mucus	3	3	3	3-5	3-5	
	Epithelium*	4-8	8-10	10-20	10-20	>20	
	Flora	cocci, mixed	cocci, mixed	cocci, mixed	cocci, mixed	cocci, mixed	

*: number of epithelial cells found in the vaginal smear.

Table 2. Exudative changes of cells

Types of inflammatory reactions	Typical characteristics of cell composition
Acute inflammation	Destroyed, "naked" leukocyte nuclei are prevalent; leukocytes are mainly neutrophils
Semi-acute and chronic inflammation	Eosinophils and neutrophils noted
Virus, Chlamydia infections, other infections	Leukocytes and macrophages prevail

Table 3. Classification of inflammatory urogenital diseases with girls

Inflammatory
Nonspecific
Nonspecific bacterial vaginosis
Specific
Gonorrhoea
Trichomoniasis
Chlamydia infection
Bacterial vaginosis
Mycoplasmiasis
Diphtheritic vulvovaginitis
Genital tuberculosis*
Candidal vulvovaginitis
Viral vulvovaginitis (cytomegalovirus, herpes simplex virus, others)
Non-virus
Vaginal foreign body
Enterobius vermicularis
Masturbation
Others (i.e., impaired metabolism, allergy)

*: It is rarely seen in developed countries, for example, in the United States.

have.^[12] The most common disorder in children is non-specific vulvovaginitis. The bacteria that can lead to vulvovaginitis include *Staphylococci*, *Streptococci*, *Enterococci*, *Proteus*, *Klebsiella*, and others.^[5,7,12]

Table 4. Vulvovaginitis treatment^[8,9,12-14,22-48]

Non-specific vulvovaginitis
Improve genital hygiene
White cotton underpants and loose skirts
Avoid nylon tights and close fitting blue jeans
Good hand washing
Sits baths
A and D ointment
Seek consultation for severe and/or chronic cases
Foreign body vaginitis
Removal of the foreign body
Irrigation of the vagina with warm water* and/or miscellaneous antiseptic solutions (furacilinum [Activetex] solution 1:5000, ethacridin lactate [Rivanolum] 1:5000, or 5% streptocide emulsion); can also use lidocaine jelly 2%
Masturbation
Education and/or therapy to stop the masturbation
Enterobius vermicularis (pinworm)
Oral antihelminth therapy† (as mebendazole)
Irrigation of the vagina with herbal tinctures (camomile, salvia, sage, calendula); sits baths in warm water or Burow's solution (aluminum acetate)
Specific vulvovaginitis
Specific antibiotics for bacterial infections (i.e., <i>Neisseria gonorrhoeae</i> , <i>Chlamydia trachomatis</i> , <i>Streptococcus pyogenes</i> , <i>Staphylococcus aureus</i> , <i>Shigella</i> , <i>Streptococcus pneumoniae</i> , <i>Hemophilus influenzae</i> , etc)
Antifungal agents for <i>Candida albicans</i> (i.e., clotrimazole, miconazole, others)
Antiparasitic agent (nitroimidazole) for <i>Trichomonas vaginalis</i> (i.e., metronidazole, tinidazole, or ornidazole)
Antiviral agents for viral infections (i.e., oral acyclovir, famciclovir or valacyclovir for herpes simplex)
1% lindane shampoo or 1% permethrin creme for <i>Pediculosis pubis</i> (due to <i>Phthirus pubis</i> or crab lice)
Ensure healthy diet
A and D ointment

*: foreign body should be removed by irrigation with water; †: should be given to everyone in the household at the same time.

Management of vulvovaginitis

Management of vulvovaginitis is dependent on the specific etiology and underlying factors.^[8,9,12,23-29] Not all infections are caused by only one microbe (despite Koch's postulate: "one microbe—one disease"), and there can be some blurring of clinical features from one cause to another. Foreign bodies as a cause of vulvovaginitis are usually discovered because of foul smell and/or dark bloody discharge from the vagina. Some foreign bodies such as wads of toilet tissues are quite difficult to discover because they cannot be detected by rectal examination or ultrasound.

Methods of transmission of specific urogenital infections do change as puberty occurs. In the sexually active adolescent girl, sexually transmitted infections may occur.^[6,13] This is not the case in a prepubertal child, unless sexual abuse has occurred. Improper hygiene becomes more of a causative factor in children. Table 4 outlines treatment of vulvovaginitis of various causes.^[8,9,13,14,30-48]

Conclusion

This article reviews the principles of vaginal microbiocoenosis in prepubertal and adolescent girls. Clinicians can identify states of vaginal health and disease by using important diagnostic tools including vaginal cytology. *Lactobacilli* are infrequent in the prepubertal girls, but become more abundant in the adolescent girls. This important shift in vaginal biocoenosis is important to prevent the growth of potentially pathogenic flora. Three basic types of vaginal smears are presented in addition to a classification of inflammatory urogenital diseases. Management of common types of vulvovaginitis is also considered.

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