

BACTERIOLOGICAL FINDINGS OF THE VULVAR SWAB SPECIMENS FROM GIRLS WITH VULVOVAGINITIS

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Summary. The available evidence suggests that a microbial ecosystem in prepubertal girls with clinical features of vulvovaginitis is complex regarding bacterial species and their mutual numerical ratio.

The aim of the study was to identify and compare a number of positive and negative bacteriological findings in girls with and without vulvovaginitis, in accordance with the criteria defined. Also, the study aims at analyzing the positive bacteriological findings regarding occurrence and frequency of some bacteria.

A microbiological study of the vulvar swab specimen in 160 pre-pubertal girls with vulvovaginitis and 30 asymptomatic girls, aged 2–12 years, was conducted in the period from January to September 2005 at the Department of Microbiology and Parasitology of the Public Health Institute in Nis. Sexually transmitted pathogens were excluded from the study.

In the target group, bacterial pathogens were isolated in vulvar swab specimens of 78 girls (48.75%). The most frequently isolated bacteria were fecal in origin: *Enterococcus faecalis* (17.50%), *Proteus mirabilis* (11.25%) and *Escherichia coli* (10.63%). Statistically significant differences between the target and control group regarding the number of positive findings ($\chi^2 = 10.57$, $p < 0.01$) and the number of isolates which were fecal in origin ($\chi^2 = 10.67$, $p < 0.01$) were high. *Staphylococcus aureus* was identified in 13 cases, *Streptococcus pyogenes* (group A), *Streptococcus agalactiae* (group B) and *Gardnerella vaginalis* were isolated in four patients, respectively and *Haemophilus sp.* only in one girl with vulvovaginitis.

In accordance with the obtained results, it can be concluded that vulvovaginitis in this age group is obviously not caused by specific pathogenic bacteria, and is usually associated with mixed growth of fecal organisms.

Key words: Vulvovaginitis, pre-pubertal girls, vaginal flora

Introduction

Vulvovaginitis is one of the most usual gynecological problems in pre-pubertal girls. The most common and troublesome symptoms of this disease are vaginal discharge, pruritus, irritation, erythema, and soreness (1,2,3). Vulvovaginitis encompasses a variety of disorders characterized by inflammation that may be secondary to multiple causes, including infection, allergy, and systemic disease. There is little literature regarding the vaginal micro-flora of pre-pubertal girls. Many microorganisms have been reported in several studies, but frequently the pediatrician does not know the pathogenic significance of an isolate reported in vaginal specimens of girls with vulvovaginitis. Therefore, it is difficult to determine whether isolated bacteria are part of the normal microflora or are the cause of the symptoms of vulvovaginitis.

The available evidence suggests that a microbial ecosystem in prepubertal girls with clinical features of vulvovaginitis is complex and variable regarding bacterial species and their mutual numerical ratio (4). The most prevalent aerobic organisms in this group of pa-

tients were commensal skin and rectal bacterial flora (e.g. *Staphylococcus epidermidis*, *Enterococcus* spp. and *Escherichia coli*), and *Lactobacillus* spp. The anaerobes such as *Peptococcus* spp, *Peptostreptococcus* spp, *Veillonella parvula*, *Eubacterium* spp, *Propionibacterium* spp, *Bacteroides* spp, and bacteria that present commensal oral flora as well as yeasts, such as *Candida albicans* were significantly more infrequent (2,5,6,7,8). Bacteria which are not sexually transmitted and are generally considered as pathogens include: *Streptococcus pyogenes* (group A), *Haemophilus influenzae*, *Staphylococcus aureus* and *Streptococcus pneumoniae* (9). *Candida* may be found in girls with predisposing factors, such as a recent administration of antibiotics, diabetes, or the wearing of diapers. Sexually transmitted bacteria species such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are important but rare causes of vulvovaginitis in prepubertal girls. A great number of studies have reported that a primary infective cause was also considered important in a symptomatic child, if a pure or predominant growth of a probable pathogen was isolated on the culture of the low vaginal swab (2, 10).

Aim

The aim of the study was to identify and compare a number of positive and negative bacteriological findings in girls with and without vulvovaginitis, in accordance with the criteria defined. In addition, the aim was to analyze positive bacteriological findings regarding the occurrence and frequency of some bacterial species.

Patients and Method

This microbiological study of the vulvar swab specimen in 160 prepubertal girls with vulvovaginitis and in 30 asymptomatic girls, aged 2-12 years, was conducted in the period from January to September 2005 in the Department of Microbiology and Parasitology of the Public Health Institute in Nis. The target group consisted of girls who complained of vaginal irritation or discharge and visited their general practitioners, pediatric outpatient clinics, or a pediatric gynecologist. The girls without symptoms of vulvovaginitis, with approval of their parents, were the control group. Two sterile cotton tip swabs from the vulva and vaginal introitus were taken: one swab was rubbed on a glass slide for Gram stain and was examined by microscopy for clue cells, blastoconidia, pseudohyphae, leucocytes, and bacteria according to the standard laboratory methods, while the other swab was immediately cultured onto a number of agar plates. Human blood, heated blood agar and Sabouroud's agar plates were incubated aerobically at 37°C for 48 hours and the isolates were identified using standard procedures. The patients were not routinely screened for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, or *Trichomonas vaginalis*. These sexually transmitted pathogens were sought only in cases of suspected sexual abuse, all of which were excluded from this study.

Positive bacteriological findings were based on the isolation of *Staphylococcus aureus*, *Streptococcus pyogenes* (group A), *Streptococcus agalactiae* (group B), *Enterococcus faecalis*, *Haemophilus* sp, *Candida* sp. and the members of the family *Enterobacteraceae* or *Gardnerella vaginalis* with pure or predominant growths and the presence of numerous polymorphonuclear leucocytes in the Gram stained smear. The bacteriological findings which showed mixed infection with two or more potentially pathogenic bacteria which appeared in a small number, with the presence of diphtheroids and coagulase negative staphylococci and the absence or a small number of polymorphonuclear leucocytes in vulvar smears were considered negative. The epidemiological data were collected and analyzed using standard protocols.

The statistically significant correlation between positive and negative bacteriological findings in the two groups was analyzed using Pearson's χ^2 test. The results are presented in tables and graphs.

Results

During the study period, in the target group of girls, bacterial pathogens were isolated in vulvar swab specimens of 78 girls, while the rest of 82 examined girls had negative bacteriological findings. A total of 48.75% cases were diagnosed as having predominantly infective contribution to genital inflammation. Among the total number of girls with vulvovaginitis, infection with a single bacterium was identified in 64 (40.00%) cases, while in 14 (8.75%) cases we found a mixed infection with two bacteria (Table 1). In control girls without symptoms, a positive bacteriological finding was found in only 5 (16.66%) examinees, each with a single bacterium. Therefore, it was determined that statistically significant differences in the number of positive findings between the target and control group ($\chi^2 = 10.57$, $p < 0.01$) were high.

Table 1. Number and percentage of bacteriologically examined vulvar swab specimens, negative and positive bacteriological findings, and number of girls with single and mixed culture bacterial isolates

Total number of bacteriologically examined vulvar swab specimens	160	(100%)
Total number of negative bacteriological findings	82	(51.25%)
Total number of positive bacteriological findings	78	(48.75%)
Number of girls with single bacterial isolates	64	(40.00%)
Number of girls with bacterial isolates in mixed culture	14	(8.75%)

The most frequently isolated bacteria were *Enterococcus faecalis* (17.50%), *Proteus mirabilis* (11.25%) and *Escherichia coli* (10.63%). These bacteria species are fecal in origin and appeared in 41.25% of all examinees. *Enterococcus faecalis* was isolated alone in 21 examinees, while in 7 cases it was associated with other bacteria species. *Proteus mirabilis* was found in 18 girls and 3 isolates appeared in the mixed culture. *Escherichia coli* was isolated alone in 8 girls, while in 9 girls it was mixed with other bacteria. *Proteus vulgaris*, *Klebsiella* sp., and *Morganella morganii* appeared all in a single girl and were associated with other bacteria. *Staphylococcus aureus* was identified in 13 patients, in mixed infections in 3 cases. *Streptococcus agalactiae* (group B) was isolated in four patients and *Haemophilus* sp. only in one girl with pure growths. *Streptococcus pyogenes* (group A) appeared as a single isolate or as mutually associated in two examinees, respectively. *Gardnerella vaginalis* was isolated with pure growth on the culture in 3 examinees and only in one case in co-infection with *Candida* sp. (Table 2 and Figure 1). However, in the control group, the isolated bacteria of fecal origin were found in only 10%. *Proteus mirabilis* was identified in two cases and *Enterococcus faecalis* was present in only one case. It was determined that statis-

tically significant differences in the number of isolates of fecal origin between the target and control group ($\chi^2 = 10.67$, $p < 0.01$) were high. In the control group, *Streptococcus agalactiae* (group B) and *Haemophilus* sp. were found in one girl, respectively.

Table 2. Survey of single bacterial isolates and bacterial isolates in mixed culture

Bacteria species	Total number of isolates	Number of single isolates	Number of isolates in mixed culture
<i>Enterococcus faecalis</i>	28	21	7
<i>Proteus mirabilis</i>	18	15	3
<i>Escherichia coli</i>	17	8	9
<i>Staphylococcus aureus</i>	13	10	3
<i>Streptococcus pyogenes</i> (group A)	4	2	2
<i>Streptococcus agalactiae</i> (group B)	4	4	0
<i>Gardnerella vaginalis</i>	4	3	1
<i>Proteus vulgaris</i>	1	0	1
<i>Klebsiella</i> sp.	1	0	1
<i>Morganella morganii</i>	1	0	1
<i>Haemophilus</i> sp.	1	1	0
Total number	92	64	28

Table 3. Survey of findings with and without polymorphonuclear leucocytes in Gram stained vulvar smears

	Positive bacteriological findings	Negative bacteriological findings	Total
Findings with polymorphonuclear leucocytes	78 (48.75%)	5 (3.125%)	83 (51.875%)
Findings without polymorphonuclear leucocytes	0	77 (48.125%)	77 (48.125%)
Total	78 (48.75%)	82 (51.25%)	160 (100.00%)

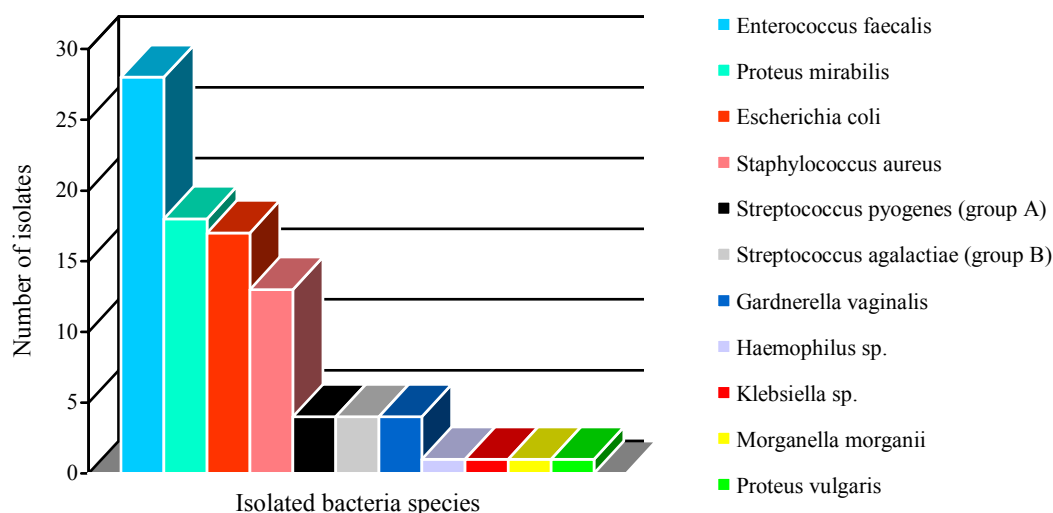


Fig. 1. Survey of bacteria isolated from vulvar swab specimens of premenarchal girls with vulvovaginitis

In the Gram stained smears of vulvar swabs we detected the presence of polymorphonuclear leucocytes in all examinees with a positive finding and in 5 examinees without isolates (Table 3). On the other hand, polymorphonuclear leucocytes were detected only in a small number in 4 control cases with a negative bacteriological finding. Thus, the finding of leucocytes in vulvar smears as an indicator of growth of bacterial pathogens had a high sensitivity and specificity.

Discussion

The premenarchal girls are anatomically, physiologically, and behaviorally at relative risk of vulvovaginitis. The major physiological factors in making vulvar and vaginal mucosa of pre-monarchal girls susceptible to infection include: a hypo-estrogenic hormonal milieu in a preadolescent girl, a thin mucosa, alkaline pH, lack of cornification, labial fat pads and pubic hair, small labia minora, and a close proximity of the rectum (10,11,12). Other non-physiological factors putting the girl at risk of vulvovaginitis are children's tendency to maintain poor local hygiene and explore their bodies, then spreading of respiratory bacteria from hand to perineum, local irritants, such as nylon underwear, and possible sexual abuse (13,14,15).

In the longitudinal survey by Jones *et al.* on consecutive premenarchal patients presenting with vulvovaginitis and/or vaginal discharge, it was concluded that non-specific vulvovaginitis with mixed fecal or skin bacterial flora is the most usual cause, because of a hypo-estrogenic hormonal milieu (8). Our study demonstrates that vulvovaginitis in prepubertal girls is frequently caused by infection. A total of 48.75% cases were diagnosed as having predominantly bacterial contribution to genital inflammation, while, in the control group of girls without symptoms, positive bacteriological finding was found in only 5 (16.66%) examinees, each with a single bacterium. It was determined that statistically significant differences in the number of

positive findings between target and control group were high ($\chi^2 = 10.57$, $p < 0.01$). Specifically, 41.25% of bacterial infections identified in the group of girls with vulvovaginitis were attributable to fecal organisms. The most frequently isolated bacteria in girls with vulvovaginitis were *Enterococcus faecalis* (17.50%), *Proteus mirabilis* (11.25%) and *Escherichia coli* (10.63%). On the other hand, in the control group, the isolated bacteria of fecal origin were found only in 10% ($\chi^2 = 10.67$, $p < 0.01$). Among the total number of girls with vulvovaginitis, infection with a single bacterium was identified in 64 (40.00%) cases, while in 14 (8.75%) cases we found a mixed infection with two bacteria.

Cuadros *et al.*, in a multicentre study, selected 74 girls aged 2 to 12 years with a clinical picture of vulvovaginitis and inflammatory cells on Gram stain, and isolated *Streptococcus pyogenes* and *Haemophilus* spp. in 47 and 12 cases, respectively. They concluded that pediatric inflammatory vulvovaginitis is mainly caused by pathogens of the upper respiratory tract and the most common risk factor for this infection is to have suffered an upper respiratory tract infection in the previous month (16). O'Brien *et al.* determined that common causes of vulvovaginitis in young girls include threadworms, *Streptococcus pyogenes* (group A) and *Haemophilus influenzae* (17). According to a great number of polymorphonuclear leucocytes in Gram stained smears of vulvar swab in pre-menarchal girls, Pena *et al.* (18) found a potentially pathogenic microorganism in 70 (28.7%) out of 262 patients. They isolated *Streptococcus pyogenes* (group A) in eight cases (3.0%) and *Candida albicans* in four cases (1.5%). Among uncertain etiologic agents, *Haemophilus influenzae* was the most frequently isolated (7.6%). In our study, *Staphylococcus aureus* appeared as a pathogen in 13 cases (8.13%), *Streptococcus pyogenes* (group A),

Streptococcus agalactiae (group B), and *Gardnerella vaginalis* were isolated in four patients (2.50%), respectively, and *Haemophilus* sp. only in one girl. Also, there were two isolates of *Candida* spp. In the control group, *Streptococcus agalactiae* (group B) and *Haemophilus* sp. were found in a single girl, respectively. In our study, we detected the presence of polymorph nuclear leucocytes in all examinees with a positive finding while, in the control group, polymorph nuclear leucocytes were found in a small number in only 4 girls with a negative bacteriological finding. Therefore, the finding of leucocytes in vulvar smears as an indicator of growth of bacterial pathogens showed a high sensitivity and specificity.

Diagnosing vaginal infection is confounded by the overlap between the normal flora and potential pathogens and, therefore, it is difficult to determine whether the bacteria isolated from patients' vaginal secretions are part of the normal microflora or the cause of symptoms of vulvovaginitis. In the study of Joishy *et al.*, a pure or a predominant growth of a probable pathogen in conjunction with symptoms of inflammation is considered diagnostic of a primary infective cause (19).

Conclusion

In accordance with the obtained results in this study and observations of research teams from other geographical area, it can be concluded that vulvovaginitis in this age group is obviously not caused by specific pathogenic bacteria. Childhood vulvovaginitis is not uncommon in general practice, and is usually associated with a mixed growth of fecal organisms. The current approach to the diagnosis of vulvovaginitis should be studied further.

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BAKTERIOLOŠKI NALAZ BRISA VULVE KOD DEVOJČICA SA VULVOVAGINITISOM

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Kratak sadržaj: *Mikrobni ekosistem vaginalne sluzokože kod devojčica sa kliničkim znacima vulvovaginitisa je kompleksan i varijabilan u pogledu bakterijskih vrsta i njihovog međusobnog brojčanog odnosa.*

Cilj studije je bio da se sagleda i uporedi broj pozitivnih i negativnih bakterioloških nalaza u grupi devojčica sa vulvovaginitisom i devojčica bez simptoma, rukovodeći se definisanim kriterijumima. Takođe, pozitivni bakteriološki nalazi su analizirani u pogledu pojave i učestalosti pojedinih bakterijskih vrsta.

Studija je obuhvatila bakteriološki nalaz 160 uzoraka brisa vulve devojčica sa vulvovaginitisom i 30 devojčica bez simptoma, starosne dobi od 2 do 12 godina, obrađenih na Sektoru za mikrobiologiju sa parazitologijom IZZZ u Nišu, u periodu od januara do septembra 2005. godine. Seksualno prenosivi mikroorganizmi su isključeni iz studije.

Pozitivan bakteriološki nalaz u ciljnoj grupi utvrđen je kod 78 devojčica (48,75%). Najčešće izolovane bakterije: E.faecalis (17,50%), P.mirabilis (11,25%) i E.coli (10,63%) su fekalnog porekla. Između ciljne i kontrolne grupe je utvrđena statistički značajna razlika u broju pozitivnih nalaza ($\chi^2 = 10,57$, $p < 0,01$) i broju izolata fekalnog porekla ($\chi^2 = 10,67$, $p < 0,01$). Staphylococcus aureus je identifikovan kod 13 devojčica, Streptococcus pyogenes (grupa A), Streptococcus agalactiae (grupa B) i Gardnerella vaginalis izolovani su po četiri puta, dok je Haemophilus sp. izolovan samo kod jedne devojčice sa vulvovaginitisom.

Na osnovu dobijenih rezultata, može se izvesti zaključak da vulvovaginitis u ovoj starosnoj grupi nije obavezno izazvan određenim patogenim bakterijama i da je obično povezan sa mešovitom crevnom florom.

Ključne reči: *Vulvovaginitis, prepubertetski period, vaginalna flora*