

## Prepubertal Girls with Vulvovaginitis Clinical and Microbiological Results

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### Abstract

**Purpose:** To compare genital microbiological results between healthy controls and prepubertal girls who have vulvovaginitis.

**Method:** 50 prepubescent females with vulvovaginitis, ages 1 to 9, and 40 age-matched healthy controls. From November 2021 to May 2022, all study participants in Patna Medical College & Hospital had samples for microbiological culture taken from the introitus and the lower third of the vagina using sterile cotton swabs. Analysis of microbiological findings was done based on the type of bacteria and growth rate.

**Results:** The majority of vaginal microbiological swab results showed bacterial growth: 45 (90.3%) and 33 (80.8%) were comparable in the study and control groups, respectively (P=0.23). According to the results of the microbiological features in the case and control groups, respectively, 15 (30.7%) and 7 (21.4%) were thought to be probable causal factors (P=0.26). Streptococcus pyogenes was the most common pathogen in the study group (P=0.2); all other microorganisms discovered in the control group as either a pure or dominant growth were regarded as opportunistic.

**Conclusion:** Both healthy controls and prepubescent girls with vulvovaginitis had positive vaginal bacterial culture results. There was a higher prevalence of non-specific vulvovaginitis without a dominant/isolated pathogen than vulvovaginitis with a putative causal agent. Girls experienced higher clinical symptoms when the probable infectious agent was discovered.

**Keywords:** Prepuberty Girls, Clinical Symptom, Microbiological Characteristics, And Vulvovaginitis.

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### Introduction

In prepubertal females, vulvovaginitis is a common gynaecological issue that frequently creates anxiety in both the kid and her parents. Girls who are prepubescent are more likely to experience vulvovaginal

inflammation due to morphological, physiological, and behavioural reasons. [1,2].

Prepubescent girls with vulvovaginitis are often diagnosed based on their clinical

history and an examination of their external genitalia [1,2]. In typical clinical practise, further tools include the microscopic analysis and culture of vulvovaginal secretions [3,4]. Based on microbiological evidence, the majority of vulvovaginitis cases are thought to be nonspecific since mixed growth cultures are more frequently observed from vaginal swabs than an isolated pathogen [5,6].

Interestingly, it is challenging to evaluate whether an isolated species of bacterium can be the causative agent of vulvovaginal inflammation because there are few research on the normal vaginal microbiota in healthy prepubertal females [5]. The majority of opportunistic infections are thought to be a typical component of prepubescent females' vaginal microbiota. Consequently, while determining the source of inflammation and taking into consideration particular antibacterial therapy, vaginal bacterial cultures from patients with vulvovaginitis should be studied cautiously [3,4].

In this study, we sought to compare the microbiological results in prepubescent girls with vulvovaginitis to those in healthy controls.

### Method

From November 2021 to May 2022, Patna Medical College & Hospital conducted a prospective case-control study. The study was open to prepubescent girls with a probable case of vulvovaginitis. Every patient received a standard evaluation, which included taking their medical history, visually inspecting their vulvovaginal region, and taking bacterial samples as described below. The following symptoms and signs, including discomfort, itching, burning, soreness, dysuria, rash, white plaques, inflammation, and (or) discharge, were used to make the diagnosis of vulvovaginitis.

The study comprised patients who had vulvovaginitis and Tanner stage I pubertal development. The study excluded patients

who had a history of suspected sexual abuse or a suspected vaginal foreign body.

The consecutive age-matched Tanner stage I healthy girls who had routine screening at the Pediatric Outpatient unit during the study period were invited to take part as controls. For the control group, girls without vulvovaginitis symptoms or signs, other infectious diseases, antibiotic use during the previous six months, or immunosuppressive illnesses were chosen after receiving written informed consent from the patient and her parents/guardians.

The child's parents or guardians assisted in the "frog-leg" examination of the patients. Using sterile cotton swabs, samples were taken from the introitus and the lower part of the vagina for microbiological culture. The samples were then immediately put into Amies Transport Medium (Becton Dickinson), kept, and delivered to the microbiology lab within 20 hours. On 5% sheep blood agar, chocolate agar, and MacConkey agar plates, samples were inoculated directly. Sheep blood and chocolate agar plates were incubated at 35°C for 18–24 hours in an environment with 5% CO<sub>2</sub>, as were MacConkey agar plates. After an additional 24 hours of incubation, sheep blood (BBL) and chocolate agar (BBL) plates that yielded a negative culture result at the initial observation were reexamined.

Groupings based on the rate of bacterial growth. Negative specimens were those that showed no bacterial growth. When pure or mixed cultures were found, specimens were considered positive. Single isolated bacteria growing in culture were regarded as pure specimens, while several bacteria were regarded as mixed specimens. Specimens were categorised as follows based on the rate of growth in a Petri dish: Group II, high or very high growth; group I, sporadic or medium growth (one or two quadrants filled with developing bacterial culture) (3 or 4 quadrants filled with bacterial culture). Pure cultures or the predominate bacteria in a mixed culture

were thought to be possible inflammatory triggers, whilst other mixed culture results were thought to represent the normal vaginal microbiota. Pathogens, opportunistic pathogens, and nonpathogens were among the microorganisms identified.

The  $\chi^2$  test was used to process the statistical data and determine statistical significance, while Microsoft Excel 2010 and SPSS Statistics version 23 were used for statistical analysis. The statistical significance level ( $P < 0.04$ ) was determined to be the level of error set below 4%.

### Results

The study group included 50 prepubertal girls with ages ranging from 1 to 10 (mean,

4.4 $\pm$ 2.1 years), and the healthy control group included 40 girls with ages ranging from 3.8 $\pm$ 3.0 years on average. There was no statistically significant difference between the study's and the control groups' mean ages. The study participants ranged in age from 1 to 10 years, with a mean (SD) age of 4.1 ( $\pm$ 3.0) years. 50 girls in the study group had clinically confirmed vulvovaginitis; in 35 (72%) of these instances, the girls ranged in age from 2 to 5.

Vaginal discharge 33/50 (65.3%), redness 31/50 (61.4%), itching 21/50 (42.2%), and discomfort 17/50 (34.5%) were the most prevalent clinical signs of vulvovaginitis (Table 1).

**Table 1: The Relationship between the Clinical Signs of Vulvovaginitis and the Presence of Possible Infectious Agents in Vaginal Swabs**

Clinical Symptoms	Potential Causative Agents (n=15)		Microflora (n=35)		P Value	Total no of Patients (n=50)	
	n	%	n	%		n	%
Vaginal discharge	15	100%	17	50.1%	0.05	33	65.3%
Genital redness	8	56.1%	22	63.8%	0.18	31	61.4%
Itch	7	50.1%	11	33.2%	0.32	21	42.2%
Pain	7	50.1%	9	27.7%	0.30	17	34.5%
Burning	4	31.2%	5	16.6%	0.51	10	21.1%
White plaque	4	31.2%	3	11.0%	0.31	8	17.2%
Rash	3	25.1%	2	8.2%	0.47	6	13.4%
Soreness	3	25.1%	1	5.5%	0.21	5	11.4%
Dysuria	1	12.4%	3	11.0%	0.57	5	11.4%

All vaginal culture findings were positive for bacterial growth in the study and control groups, respectively, in 45 of 50 (90.3%) and 33/40 (80.8%) cases ( $P=0.23$ ; Table 2). With 15/50 (30.7%) instances in the study group and 8/40 (21.3%) cases in the control group, potential causal microorganisms

were equally distributed in both groups ( $P=0.26$ ). In both groups, there were more bacteria that were thought to be part of the vaginal microflora than there were probable disease-causing pathogens ( $P=0.03$ ; Table 2).

**Table 2: Results of the Vaginal Microbiological Swab (n=90)**

Variables	Study Cohort		Control Cohort		P-Value
	n	%	n	%	
Pure culture	9	19.1%	3	9.4%	0.14
Mixed culture					
1 From group II	5	11.4%	4	11.8%	0.61
More than 1 from group II	5	11.4%	8	21.3%	0.14
Only group I	24	48.0%	15	38.0%	0.46
Negative	4	9.5%	7	19.0%	0.15

<b>Potential Causative agent</b>	15	30.7%	8	21.3%	0.26
<b>Microflora</b>	15	69.1%	30	78.%	0.03

*Streptococcus pyogenes* was the most prevalent pathogen in the study group, appearing in 8/15 (56.5%;  $P=0.02$ ) samples with pure culture; in 4 cases, low-intensity growth in mixed culture was seen. The two most prevalent opportunistic dominating bacteria in the control group were *Enterococcus faecalis* and *Staphylococcus aureus* ( $P=0.8$ ). The enteric and skin bacteria, including *Escherichia coli*, *Enterococcus faecalis*, and *Enterococcus epidermidis* dominating, were identical in both groups' vaginal microbiota. No statistically significant difference was seen between the study's microorganisms and the control groups.

Except for the symptoms of vaginal redness and dysuria, which occurred equally frequently in both groups, clinical symptoms were more common among girls in the presence of a potentially infectious agent, however, this difference was not statistically significant (Table 1).

## Discussion

Pubertal status affects the vaginal microbiota. *Gardnerella Vaginalis*, *Diphtheroids*, and *Lactobacilli* are typically absent in prepubescent girls, allowing pathogenic and opportunistic bacteria to change the vaginal epithelium and result in vulvovaginitis [4–7]. Thus, it is clinically relevant if opportunistic microorganisms are present in vaginal swab culture during vulvovaginitis. Six symptomatic females in this investigation had opportunistic infections found in pure culture, while eight other girls had specific pathogens found. According to past findings, the most common complaints among vulvovaginitis-symptomatic girls were vaginal discharge, genital redness, itching, and discomfort [8–12].

In prepubertal girls, vulvovaginitis has been described as nonspecific in 25%–75% of instances [2,6,9,12], which was also true in the current investigation, where 59.5% of

symptomatic girls had the condition. Because the bacteria found may just be part of the normal vaginal microbiota, the vaginal swab culture results should be interpreted with utmost caution (Table 2). When an isolated, high-growing bacterium is found in pure culture, specific treatment for vulvovaginitis is suggested [1-3]. Reported that prevalent and fast-growing bacteria should be taken into account as potential inflammatory triggers and as such, warrant therapy [5]. Contrarily, our data indicate that the management strategy should be determined by the severity of the symptoms because isolated or dominating microorganisms are abundantly present in the control group as well. Only 19.0% of the healthy control subjects showed negative culture results (Table 2).

The most often discovered pathogen in females with vulvovaginitis [2,8,11,13] is *Streptococcus pyogenes*, which is occasionally observed concurrently with or following symptomatic pharyngitis. In prepubertal girls with vulvovaginitis, *Streptococcus pyogenes* was isolated from 8% to 47% of cases, which is consistent with our findings. It was isolated from 17.2% of cases in the study group but only once in the control group, so it is thought to be a part of the normal microflora and sporadic growth.

*Streptococcus pyogenes*-induced vulvovaginitis usually progresses rapidly and is characterised by seropurulent or blood-stained discharge, erythema, including in the perineal region, burning skin, and dysuria [3]. All study group females had abnormal purulent, thick discharge, and perineal erythema that were linked to a pure culture of *Streptococcus pyogenes*.

In prepubescent girls, *Haemophilus influenzae* has been identified as the second most often isolated pathogen responsible for vulvovaginitis [10]. The *Haemophilus*

influenza type b vaccination [14] and provides protection against respiratory tract infections as well as vulvovaginitis, may account for the decreased prevalence of *Haemophilus influenza* in our study.

*Candida albicans* was only found twice among control participants with modest growth intensity and only once in the research group, where it is a rare finding in the genitalia of prepubertal girls [2,5,12]. The excessive use of antifungal medications continues because many doctors continue to think that vulvovaginitis in prepubertal girls has a fungal origin, which raises the risk of resistance [6,15].

According to our findings and those of other studies, the cause-and-effect link between *Staphylococcus aureus* and vulvovaginitis is still up for debate [13]. Data from several trials showed that 2.4% to 6% of the patients were not positive for *Staphylococcus aureus* [14]. In our investigation, *Staphylococcus aureus* was found in 2 (3.1%) girls, however only 1 (1.05%) of them displayed vulvovaginitis symptoms. *Staphylococcus aureus* was frequently found in mixed cultures but was not regarded as the primary pathogen.

It is yet unknown what part faecal bacteria play in the aetiology of this disease. In girls with abnormal discharge, *Escherichia coli* and *Enterococcus fecalis* are typically the most prevalent opportunistic pathogens [16-18]. One-third of the sick females had bacteria of faecal origin, according to Randelovic et al. [2], and in nearly all of these instances, pure cultures were isolated. In contrast, we found faecal flora in 61.4% of the study group's female participants; dominance was only observed once. Yet, earlier findings are supported by the frequent occurrence of faecal flora in our cultures of healthy prepubertal females. [2,3,10,12] We think that inadequate hygiene, the vulva's anatomical proximity to the anus, and the propensity of faecal flora to survive in the vaginal environment

could all contribute to the frequent finding of faecal flora in vaginal cultures.

The capacity of faecal flora to endure in various pH conditions. In the cultures of healthy prepubertal females, Hammerschlag et al [19-21] found a significant incidence of diphtheroids, *Staphylococcus epidermidis*, and enteric bacteria. According to Jaquery et al. [22], healthy and vulvovaginitis-symptomatic girls both had the same vaginal microbiota, which is in line with our findings. In both the research and control groups, we discovered the same microbiota with a high incidence of skin and digestive bacteria. These bacteria are common because of their widespread distribution throughout the body and high vitality [23-25].

### Conclusion

Our data imply that both females with symptomatic vulvovaginitis and healthy control participants have positive vaginal bacterial culture results. In the majority of cases, mixed vaginal cultures without a dominant/isolated pathogen were discovered. The types of germs found in symptomatic and healthy girls were the same. *Streptococcus pyogenes* was the vulvovaginitis pathogen with the highest prevalence. Girls experienced more clinical symptoms when the possible infectious agent was found. In the majority of prepubertal girls, our study found that vulvovaginitis has an unspecific aetiology.

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