

RESEARCH ARTICLE

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Analysing the Correlation between Vaginal pH Levels and the Prevalence of Specific Microbial Communities of Patients Attending a Tertiary Care Facility at Kamrup District of Assam

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Abstract

The vaginal microbiome plays a vital role in maintaining reproductive health. Vaginal pH serves as a simple yet reliable clinical marker reflecting microbial balance. A healthy vaginal environment is typically dominated by *Lactobacillus* species, which help maintain an acidic pH that inhibits colonization by pathogenic organisms. An increase in vaginal pH disrupts this balance and is associated with leukorrhea, infertility, preterm birth, and increased susceptibility to sexually transmitted infections (STIs). This study aimed to determine the association between vaginal pH and microbial community composition in women attending a tertiary healthcare center. A total of 50 vaginal swab samples were analysed using standard culture and biochemical methods, and vaginal pH was categorized as low (≤ 4.5), mid (4.6-5.5), or high (> 5.5). Elevated pH values were frequently associated with pathogenic organisms such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Gardnerella vaginalis*, whereas low pH corresponded to predominance of *Lactobacillus* species. *Candida albicans* was detected across a wide pH range. These findings demonstrate that vaginal pH is closely linked to microbial profiles, where high pH reflects dysbiosis and low pH indicates a healthy vaginal ecosystem. Routine monitoring of vaginal pH may therefore serve as a useful tool in guiding diagnostic and therapeutic decisions in gynecological practice.

Keywords: Vaginal pH, Microbiome, Microbial Communities, Vaginal Health, Bacteria

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INTRODUCTION

The vaginal microbiome is a highly dynamic ecosystem that plays a critical role in maintaining female reproductive health.^{1,2} It is primarily dominated by *Lactobacillus* species, which maintain a low vaginal pH through lactic acid production, creating an environment hostile to pathogenic microorganisms.^{3,4} Alterations in this balance, often characterized by an increase in vaginal pH, can disrupt microbial communities, predisposing women to bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and other reproductive tract infection.⁵⁻⁷

Recent studies suggest that dysbiosis of the vaginal microbiome is associated not only with infections but also with adverse reproductive outcomes such as infertility, spontaneous preterm birth, and increased susceptibility to sexually transmitted infections (STIs).⁸⁻¹² Even minor fluctuations in vaginal pH can trigger microbial shifts, serving as a predictive marker for BV or VVC.¹³⁻¹⁵ These findings highlight the importance of routine monitoring of vaginal pH as a low-cost, non-invasive clinical parameter.¹⁶ Globally, the prevalence of BV remains high, affecting up to 30% of reproductive-age women, with regional variations influenced by demographic, cultural, and geographic factors.^{11,17} In India, the burden of reproductive tract infections, particularly BV and VVC, is significant and remains underreported due to inadequate screening practices.¹⁸ Understanding the relationship between vaginal pH and microbial composition in symptomatic women is crucial for early detection and prevention of these conditions. The composition of the vaginal microbiome is shaped by host factors such as antibiotic exposure, hormonal changes, sexual activity, and immune responses.¹⁹⁻²¹ *Lactobacillus* species, especially *L. crispatus* and *L. jensenii*, are strongly associated with reproductive health as they produce lactic acid, bacteriocins, and hydrogen peroxide, which inhibit pathogen overgrowth.^{22,23} Pathogenic species like *Gardnerella vaginalis*, *Atopobium vaginae*, and enteric bacteria such as *E. coli* thrive in alkaline environments.^{1,6,24} These pathogens can form biofilms and persist even after treatment, leading to recurrent infections.^{7,8,12} There is growing literature supporting the role of pH-targeted therapies such as lactic acid gels

and probiotics in restoring vaginal microbiome balance.²⁵⁻²⁷ Clinical trials have demonstrated that lowering vaginal pH can suppress pathogenic growth and reduce recurrence rates of BV.^{27,28}

However, data from Northeast India, particularly Assam, are sparse, and regional studies are essential to understand population specific pattern.¹⁸ The present study aims to analyse the correlation between vaginal pH levels and microbial prevalence in symptomatic women attending a tertiary care hospital in Assam. This study addresses a significant gap in regional data and provides insights that could guide diagnostic and preventive strategies.

MATERIALS AND METHODS

This study was conducted after obtaining approval from the Institutional Ethics Committee of Gauhati Medical College & Hospital (Approval No: MC/190/2007/PT-II/MARCH.2023/9). All procedures involving human participants were performed in accordance with the ethical standards of the institutional research committee and relevant national guidelines. Written informed consent was obtained from all participants prior to sample collection. Participants were informed about the purpose of the study, procedures involved, potential risks and benefits, and their right to withdraw at any stage. Confidentiality and anonymity of patient data were strictly maintained throughout the study.

Data Source

Data were obtained from patients visiting the gynaecology and obstetrics department of Gauhati Medical College and Hospital containing laboratory and clinical records of female patients presenting with symptoms such as vaginal discharge, itching, and lower abdominal pain. Variables included presenting symptoms, pregnancy status, colour of the discharge, Microbial growth status, biochemical test results, colony characteristics, odour, measured vaginal pH, and identified microbial species along with antibiotic sensitivity test.

Sample inclusion criteria

Samples were included if they had complete data for vaginal pH and microbial

identification. Entries with missing or unclear microbial data or pH values were excluded. A total of 50 valid samples were analysed.

Microbial identification

Vaginal swab samples were collected aseptically from the posterior fornix and lateral vaginal walls using sterile cotton swabs. Each swab was immediately placed into sterile transport tubes and transported to the microbiology laboratory within two hours of collection. Vaginal pH was measured directly from vaginal secretions using sterile pH indicator strips (range 3.8-7.0), which were applied to the vaginal wall prior to swab collection.

For microbial isolation, swabs were inoculated onto MacConkey agar and nutrient agar for bacterial culture and Sabouraud dextrose agar for fungal culture. Bacterial cultures were incubated aerobically at 37 °C for 24-48 hrs, while fungal cultures were incubated at 28 °C for up to 72 hrs. Isolates were identified based on colony morphology, hemolytic pattern, pigmentation, odour, Gram staining, and standard biochemical tests including catalase, indole, citrate utilization, and glucose fermentation. Identification was performed according to Bergey's Manual of Systematic Bacteriology.

Vaginal pH categorization

Samples were categorized based on vaginal pH into three groups:

- **Low pH (≤ 4.5):** Typically, indicative of a healthy vaginal environment
- **Mid-range pH (4.6-5.5):** Transitional or early dysbiotic state
- **High pH (> 5.5):** Suggestive of dysbiosis and potential infection

Data analysis

Descriptive and inferential statistical methods were used to analyse the relationship between microbial communities and vaginal pH. Frequency distributions, cross-tabulations, and trend analyses were employed to interpret patterns. Pearson correlation coefficients and chi-square tests were used to evaluate the strength of association between pH levels and microbial prevalence.

RESULTS

Microbial distribution

The distribution of microorganisms isolated from the samples is summarized in Table 1. Both pathogenic and commensal organisms were detected. *Escherichia coli* was the most frequently isolated organism (24%), followed by *Klebsiella pneumoniae* (16%), *Lactobacillus* spp. (16%), *Candida albicans* (14%), *Staphylococcus aureus* (12%), *Gardnerella vaginalis* (10%), and *Streptococcus* spp. (8%). The detection of *Lactobacillus* spp. (Table 1) in 16% of samples indicates that only a minority of women retained a microbiome consistent with vaginal health, while the majority showed evidence of microbial imbalance or infection.

Vaginal pH patterns across microbial groups

A strong and consistent trend was observed between vaginal pH levels and microbial composition (Table 2). Samples dominated by *Lactobacillus* spp. exhibited a mean pH of 4.0 ± 0.1 , which falls well within the normal acidic range of a healthy vagina. None of these samples had pH values exceeding 4.5, confirming that *Lactobacillus* dominance is tightly linked with maintenance of vaginal acidity. In contrast, samples harbouring pathogenic organisms showed substantially elevated pH values. *Gardnerella vaginalis* showed the highest mean pH (7.0 ± 0.3), consistent with its established role in bacterial vaginosis and biofilm-associated alkalization of the vaginal environment.

Escherichia coli and *Klebsiella pneumoniae*, both enteric pathogens, were detected primarily in highly alkaline environments with mean pH values of 6.9 ± 0.3 and 6.8 ± 0.2 , respectively (Figure).

Staphylococcus aureus and *Streptococcus* spp. were also associated with elevated pH values (6.5 ± 0.2 and 6.3 ± 0.2 , respectively), indicating that these organisms preferentially colonize disrupted vaginal ecosystems.

Candida albicans, however, demonstrated a broader pH distribution. Although its mean pH was elevated (6.4 ± 0.2), it was also detected in some samples with relatively lower pH, suggesting that fungal overgrowth is influenced not only by

pH but also by host immunity, antibiotic use, and hormonal status. Overall, the data reveal a clear biological gradient:

As vaginal pH increases, protective *Lactobacillus* declines and pathogenic bacteria become dominant.

Relationship between pH elevation and dysbiosis

When samples were grouped according to pH category, nearly all specimens with pH >5.5 showed growth of potentially pathogenic bacteria or fungi, whereas samples with pH ≤4.5 were dominated exclusively by *Lactobacillus* spp. or showed no pathogenic growth.

This pattern demonstrates that vaginal alkalinization is strongly associated with dysbiosis, even though multiple organisms can coexist within this high-pH environment.

Statistical interpretation

A chi-square test comparing microbial presence with elevated vaginal pH (>4.5) yielded $\chi^2 = 0.000$, $P = 1.000$, indicating that when analysed as a binary variable, pH elevation alone did not significantly discriminate between individual microbial species. This likely reflects the overlapping ecological niches of pathogens in dysbiotic vaginal environments, where multiple organisms thrive simultaneously at high pH.

Pearson correlation analysis between mean vaginal pH and microbial groups revealed a moderate negative correlation ($r = -0.678$), suggesting a trend toward decreasing *Lactobacillus* dominance with increasing pH; however, this association did not reach statistical significance ($P = 0.094$), possibly due to the limited sample size.

DISCUSSION

The present study shows the importance of vaginal pH as a fundamental modulator of microbial community composition in symptomatic women. Boskey et al.¹ and Hillier et al.² showed that vaginal pH and hydrogen peroxide producing *Lactobacillus* are key determinants of microbial balance. Ravel et al.³ and Brotman⁴ confirmed that vaginal microbiota composition in reproductive-age women is largely shaped by *Lactobacillus* dominance, while Ma et al.⁵ emphasized that elevated pH disrupts this protective flora and

Table 1. Frequency of Isolated Microbial Species

Microbial Species	No. of Samples	% of Total Samples
<i>Escherichia coli</i>	12	24%
<i>Klebsiella pneumoniae</i>	8	16%
<i>Candida albicans</i>	7	14%
<i>Staphylococcus aureus</i>	6	12%
<i>Gardnerella vaginalis</i>	5	10%
<i>Streptococcus</i> spp.	4	8%
<i>Lactobacillus</i> (Normal Flora)	8	16%

Vaginal pH Range by Microbial Type

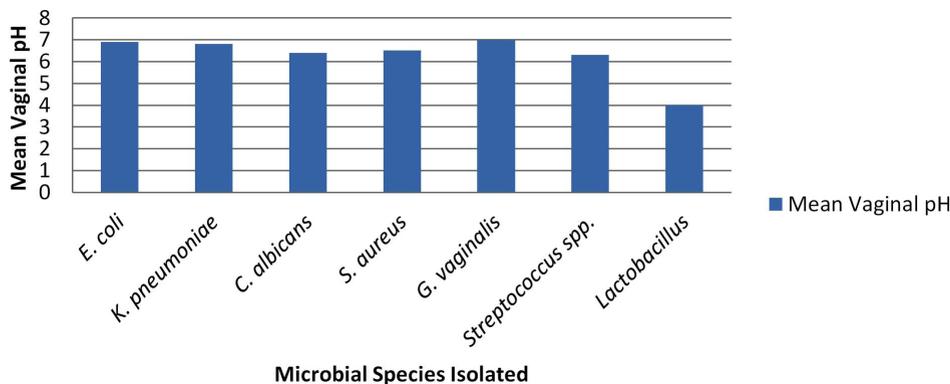


Figure. Vaginal pH Range by Microbial Type. Bar chart showing means pH values for each microbial group. Higher pH values are observed in samples with *E. coli*, *Klebsiella pneumoniae*, and *Gardnerella vaginalis*, whereas *Lactobacillus*-dominated samples show consistently lower pH

Table 2. Vaginal pH of the isolated Microbial Species

Microbial Species	Vaginal pH (Mean \pm Standard Deviation)	Above Normal Limit (>4.5)
<i>Escherichia coli</i>	6.9 \pm 0.3	Yes
<i>Klebsiella pneumoniae</i>	6.8 \pm 0.2	Yes
<i>Candida albicans</i>	6.4 \pm 0.2	Yes
<i>Staphylococcus aureus</i>	6.5 \pm 0.2	Yes
<i>Gardnerella vaginalis</i>	7.0 \pm 0.3	Yes
<i>Streptococcus</i> spp.	6.3 \pm 0.2	Yes
<i>Lactobacillus</i> (Normal Flora)	4.0 \pm 0.1	No

Mean vaginal pH associated with isolated microbial species

promotes dysbiosis. Verstraelen and Swidsinski⁶ described how biofilm formation contributes to bacterial vaginosis epidemiology and treatment challenges. Borges et al.⁷ further linked probiotics to restoration of vaginal health, and Machado and Cerca⁸ demonstrated that *Gardnerella vaginalis* biofilms persist in higher pH, explaining recurrent infections.

Mitra et al.⁹ associated vaginal microbial imbalance with HPV-related cervical disease. Tachedjian et al.¹⁰ highlighted evolutionary uniqueness of *Lactobacillus* dominance, while van de Wijgert and Jaspers¹¹ reviewed global health impacts of vaginal dysbiosis. Tachedjian et al.¹² then identified lactic acid as a key molecule in maintaining vaginal health. Ma et al.¹³ confirmed that acidic vaginal environments support protective microbial ecosystems in adolescents. Srinivasan and Fredricks¹⁴ provided an extensive overview of microbial dynamics, while Wilson et al.¹⁵ used computational models to show how small pH shifts can trigger transitions to BV or VVC.

Sobel¹⁶ originally described vulvovaginal candidiasis, noting its multifactorial nature. Amabebe and Anumb¹⁷ emphasized the physiological role of *Lactobacillus* in maintaining vaginal health, while Coudray and Madhivanan¹⁸ summarized bacterial vaginosis epidemiology in India. Muzny et al.¹⁹ updated the conceptual model of BV pathogenesis, Ceccarani et al.²⁰ linked microbiome and metabolome changes during genital infections, and Nunn et al.²¹ reviewed the impact of vaginal pH on microbial community structure.

Santiago et al.²² showed that *E. coli* adheres more readily to epithelial cells at high pH,

while France et al.²³ reviewed diversity in vaginal microbiota across demographics. Fettweis et al.²⁴ further connected dysbiosis and preterm birth risk. Souza et al.²⁵ presented compelling evidence that elevated pH and depletion of *Lactobacillus* spp. are linked with infertility and early pregnancy loss. Sousa et al.²⁶ demonstrated that lactic acid therapy can disrupt *Gardnerella* biofilms and restore balance. Armstrong-Buisseret et al.²⁷ compared lactic acid gel with metronidazole, showing similar long-term cure rates, while Tidbury et al.²⁸ confirmed better tolerability of lactic acid gels compared to antibiotics.

In summary, these studies collectively validate that vaginal pH acts as a central regulator of microbial community dynamics. Elevated pH favors pathogens like *E. coli*, *Klebsiella*, and *Gardnerella*, while low pH supports *Lactobacillus*-dominated health. In agreement with existing literature, our findings demonstrate vaginal pH as a non-invasive, cost-effective screening tool to guide diagnostics, monitor risk, and inform therapeutic strategies. Our findings support the use of vaginal pH as a non-invasive, cost-effective screening tool to guide diagnostics, monitor risk, and inform therapeutic strategies.

CONCLUSION

Vaginal pH is a vital, non-invasive marker of reproductive tract health. This study demonstrates a statistically significant correlation between pH values and microbial prevalence. Elevated pH values are associated with potentially pathogenic communities, while lower pH levels correspond to healthy flora. These insights are

valuable in clinical diagnostics and support the implementation of routine pH monitoring in gynecological assessments. Future studies should incorporate molecular diagnostics and longitudinal sampling to better understand the dynamics of microbiota shifts.

The study's limitations include the relatively small sample size and reliance on culture-based methods. Future research should incorporate metagenomic sequencing and longitudinal sampling to delineate microbial dynamics with precision.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

The study was approved by the Institutional Ethics Committee, Gauhati Medical College & Hospital (Approval No: MC/190/2007/PT-II/MARCH.2023/9).

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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