



## Article

# Bacteriological Profile and Risk Factors Among Patients with Recurrent Bacterial Vaginosis at Puskesmas Tanggul

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**Abstract:** Background: Bacterial vaginosis (BV) is the most common vaginal disease affecting approximately 30% of women aged between 15 and 44 years. Bacterial vaginosis (BV) can be caused by anaerobic bacteria, including *Gardnerella vaginalis*, *Prevotella* spp., *Bacteroides* spp., *Mobiluncus* spp. The current first-line BV therapy is oral metronidazole twice daily for 7 days, but the recurrent rate is more than 50% within 12 months after treatment. Risk factors for BV were associated with racial origin, smoking, level of education, vaginal douching, sexual behavior and hygiene behavior. Many microorganisms that cause BV, the high rate of recurrent BV on treatment with metronidazole and the many risk factors for BV were the reasons for this study. Objective: To determine the bacteriological profile and risk factors of recurrent bacterial vaginosis at the Tanggul health center. Method: This study used a descriptive study with etiological data taken from vaginal secretory specimen culture using Vitek 2 Compact, risk factor data taken from interviews and SRQ-20 questionnaire results. Result: A total of 5 specimens of vaginal secretions were identified as *E. coli* (83.3%) and 1 was identified as *K. pneumoniae* (16.6%). The description of the risk factors found were age over 46 years (66.7%), work as a housewife (66.7%) and as a farmer (33.3%), high school education level (66.7%), habit of changing underwear <3 times a day (100%), vaginal douching habits (50%), use of hormonal contraceptives (66.7%), frequency of sexual intercourse >2 times a week (33.3%) and high stress levels (66.7%). Conclusion: The etiological species of recurrent BV found were *E. coli* and *K. pneumoniae*. The risk factors found were age over 46 years, occupation, education level, habit of changing underpants <3x a day, habit of vaginal douching, use of contraception, frequency of intercourse and high stress level.

**Keywords:** Recurrent Bacterial Vaginosis; Bacteriological test; Risk Factor

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## 1. Introduction

Bacterial vaginosis (BV) is a vaginal disorder characterized by a high concentration of anaerobic bacteria in the vaginal flora, accompanied by a marked reduction in the concentration of *Lactobacillus* spp., increased vaginal discharge that is thin, grayish in color, and has a fishy odor. Bacterial vaginosis is the most common vaginal disorder, affecting approximately 30% of women aged 15–44 years in the United States. The prevalence of BV varies among populations worldwide, with Black women having a higher prevalence than White women [1]. Studies among Asian women, particularly in Nepal and Indonesia, have reported prevalence rates of 24.4% and 30.7%, respectively [2,3].

Anaerobic bacteria such as *Gardnerella vaginalis* (*G. vaginalis*), *Prevotella* spp., *Bacteroides* spp., and *Mobiluncus* spp. are associated with BV. A study by Selastri et al. in Makassar reported that 15.8% of patients with BV tested positive for *Mobiluncus*, while a study in Medan reported that 23.1% of patients were infected with *G. vaginalis* [3,4]. In addition to these pathogens, BV is influenced by several risk factors. Risk factors associated with BV include race, smoking, alcohol consumption, and vaginal douching. Bacterial vaginosis is more common among Black women, smokers, sexually active

women, and women who practice vaginal douching [3,5,6]. Educational level and hygiene practices have also been identified as risk factors for BV. Women with lower educational attainment and those who infrequently change their underwear have a higher risk of developing BV [6]. Sexual behaviors, such as having multiple sexual partners and having female sexual partners, have also been associated with an increased risk of BV [7].

The current first-line therapies for BV include oral metronidazole twice daily for 7 days, 2% intravaginal clindamycin cream once daily for 7 days, or intravaginal metronidazole gel once daily for 5 days (Workowski and Bolan, 2015). The cure rate following metronidazole therapy is estimated to be 70%–80%; however, the recurrence rate exceeds 50% within 12 months after treatment [8].

The high recurrence rate of BV has been attributed to several factors. First, the etiology of BV itself is not yet fully understood. Reinfection may also play a role in recurrent BV cases [7]. Resistance of *G. vaginalis* to metronidazole has also been associated with recurrent BV [9]. Another theory suggests that biofilm production by *G. vaginalis* and other bacteria may inhibit antibiotic penetration, thereby contributing to treatment failure and recurrence [10].

The high recurrence rate of BV following metronidazole treatment, the multiple risk factors associated with BV, and the absence of a single causative microorganism provide the rationale for this study. This study aimed to describe the bacterial profile and risk factors associated with recurrent BV among patients at Puskesmas Tanggul, Jember.

## 2. Materials and Methods

This study employed a descriptive observational design and was conducted from January to March 2020 at Tanggul Community Health Center. Of the 11 patients diagnosed with *bacterial vaginosis*, 6 continued to experience vaginal discharge and agreed to participate as study respondents. The respondents underwent speculum examination to diagnose recurrent *bacterial vaginosis* using the Amsel criteria, followed by culture of vaginal secretion specimens. The vaginal secretion specimens were placed in Amies transport medium and subsequently cultured for identification of the etiological species at Parahita Laboratory, Jember, using the VITEK 2 Compact system. Data on risk factors were obtained through direct interviews, and respondents completed the Self-Reporting Questionnaire-20 (SRQ-20) to assess stress levels.

## 3. Results and Discussion

### 3.1. Results

The diagnosis of *bacterial vaginosis* was established based on the Amsel criteria, with a diagnosis confirmed when at least 3 of the 4 criteria were positive. In this study, all six respondents met at least 3 of the 4 Amsel criteria and were therefore diagnosed with *bacterial vaginosis*. All respondents had a previous history of *bacterial vaginosis*, confirming that all six respondents had recurrent *bacterial vaginosis*.

The risk factor data of the respondents are presented in Table 1. One respondent (16.7%) was younger than 35 years, one respondent (16.7%) was aged 36–45 years, and four respondents (66.6%) were older than 45 years. Regarding occupation, four respondents (66.7%) were housewives and two (33.3%) were farmers. In terms of educational attainment, one respondent (16.7%) had not completed elementary school, one (16.7%) had completed elementary school, and four (66.6%) had completed senior high school.

None of the respondents reported a history of smoking (0%). All respondents (100%) reported changing their underwear fewer than three times per day. Three respondents (50%) reported practicing vaginal douching, while two respondents (33.3%) reported using feminine-area whitening soap.

Two respondents (33.3%) reported having sexual intercourse more than twice a week, three respondents (50%) reported having sexual intercourse once to twice a week, and one respondent (16.7%) reported no sexual intercourse. Regarding contraceptive use, four respondents (66.7%) used hormonal contraceptives, while two respondents (33.3%) did not use any contraceptive method. In terms of stress levels, four respondents (66.7%) had high stress levels, while two respondents (33.3%) had low stress levels.

**Table 1.** Risk Factor Data for Recurrent Bacterial Vaginosis Among Study Respondents

| Risk Factor                         | Category                           | Number (n) | Percentage |
|-------------------------------------|------------------------------------|------------|------------|
| Age                                 | ≤35                                | 1          | 16,7%      |
|                                     | 36-45                              | 1          | 16,7%      |
|                                     | >45                                | 4          | 66,7%      |
| Occupation                          | Farmer                             | 2          | 33,3%      |
|                                     | Housewife                          | 4          | 66,7%      |
| Educational level                   | Did not complete elementary school | 1          | 16,7%      |
|                                     | Elementary school                  | 1          | 16,7%      |
|                                     | Senior high school                 | 4          | 66,7%      |
| Smoking                             | Yes                                | 0          | 0%         |
|                                     | No                                 | 6          | 100%       |
| Frequency of changing underwear     | ≥3 times/day                       | 0          | 0%         |
|                                     | <3 times/day                       | 6          | 100%       |
| Vaginal douching                    | Yes                                | 3          | 50%        |
|                                     | No                                 | 3          | 50%        |
| Use of feminine-area whitening soap | Yes                                | 2          | 33,3%      |
|                                     | No                                 | 4          | 66,7%      |
| Frequency of sexual intercourse     | >2 times/week                      | 2          | 33,3%      |
|                                     | 1–2 times/week                     | 3          | 50%        |
|                                     | None                               | 1          | 16,7%      |
| Contraceptive method                | None                               | 2          | 33,3%      |
|                                     | Hormonal                           | 4          | 66,7%      |
|                                     | Intrauterine device (IUD)          | 0          | 0%         |
| Stress level                        | High                               | 4          | 66,7%      |
|                                     | Low                                | 2          | 33,3%      |

Culture results from the vaginal secretion specimens showed that five specimens (83.3%) were positive for *E. coli*, while one specimen (16.7%) was positive for *K. pneumoniae*. The data are presented in Table 2.

**Table 2.** Bacterial Species Identified in Recurrent Bacterial Vaginosis

| Bacterial Species    | Number (n) | Percentage (%) |
|----------------------|------------|----------------|
| <i>E. coli</i>       | 5          | 83,3%          |
| <i>K. pneumoniae</i> | 1          | 16,7%          |

### 3.2. Discussion

This study was conducted among patients diagnosed with bacterial vaginosis (BV) at Tanggul Community Health Center, Jember. Vaginal swabs were collected by a midwife at Tanggul Community Health Center, Jember. The specimens were then placed in Amies transport medium, stored in an ice box, and immediately transported to Parahita Laboratory, Jember. The transportation time to Parahita Laboratory was less than 3 hours. The entire process of bacterial culture and species identification was performed at Parahita Laboratory. Bacterial species identification was performed using the VITEK 2 Compact system. The bacterial species identified in the vaginal secretion specimens of the study respondents were *E. coli* (83.3%) and *K. pneumoniae* (16.7%). *E. coli* and *K. pneumoniae* are aerobic bacteria that, according to established theory, are not causative agents of BV.

The presence of aerobic bacteria such as *E. coli* and *K. pneumoniae* in patients with BV has also been reported in studies conducted by Ranjit et al. and Bitew et al. In the study by Ranjit et al., *E. coli* was found in 6.3% of respondents, while *K. pneumoniae* was found in 3.1% of respondents [3]. In the study by Bitew et al., *E. coli* was found in 28.5% of respondents and *K. pneumoniae* in 18.5% of respondents [3,6].

The diagnosis of BV in this study was established using the Amsel criteria. The Amsel criteria have been used to diagnose BV in primary healthcare facilities throughout Indonesia. This is based on the Regulation of the Minister of Health of the Republic of Indonesia No. 5 of 2014 concerning clinical practice guidelines for physicians in primary healthcare facilities. All six respondents in this study met at least 3 of the 4 Amsel criteria, confirming the diagnosis of BV.

Schwartz et al. identified limitations in diagnosing BV using the Amsel criteria. Their study found that more than 61% of BV diagnoses made by physicians were incorrect. In that study, 54 of the 80 BV diagnoses were based on the Amsel criteria. This may have been due to inadequate use of microscopy. Low-power microscopes and examinations without special staining leave considerable room for interpretation. Schwartz et al. stated that clinical diagnosis of vaginal infections based on history and physical examination, vaginal pH, wet-mount preparation, and the whiff test is insufficient and should be confirmed by microbiological testing [11,12].

The transport medium used in this study was Amies agar, and transportation to the laboratory was completed within less than 3 hours. According to a study by Van Horn et al., the use of Amies agar transport medium is appropriate for transporting vaginal swab specimens. Amies agar transport medium can maintain the viability of anaerobic bacteria for up to 24 hours at room temperature [13].

The instrument used for bacterial identification in this study was the VITEK 2 Compact system. According to Prihatini, the VITEK 2 Compact technology facilitates bacterial identification through a simple three-step examination process, providing identification and antibiotic susceptibility results that have been validated and interpreted according to the international standards of the Clinical and Laboratory Standards Institute (CLSI). The VITEK 2 system consists of two types of cards: ID cards for identification and AST cards for antibiotic susceptibility testing. Each card is equipped with a barcode to ensure maximum confidentiality. VITEK 2 cards contain 600 highly specific colorimetric substrates that enable differentiation between bacterial species, allowing approximately 98% of clinical isolates to be rapidly detected using this single system, including anaerobic bacteria. The reagent system is enclosed, thereby minimizing the possibility of contamination [14].

One possible mechanism explaining the presence of *E. coli* in this study is the ability of *E. coli* to associate with *G. vaginalis* biofilms. A recent study by Castro et al. reported that uropathogenic bacteria such as *E. coli* and *E. faecalis* can associate with *G. vaginalis* biofilms [15]. This in vitro study demonstrated that *E. coli* and *E. faecalis* were able to become incorporated into established *G. vaginalis* biofilms, with increasing cell concentrations. *E. coli* produces antigen 43, which supports its ability to adhere to other microorganisms and promote coaggregation [16].

The presence of *E. coli* in vaginal secretion specimens may also be associated with improper perineal hygiene practices. Cemek et al. reported that the presence of normal intestinal flora, including *E. coli*, in vaginal secretion specimens may result from fecal contamination. Such contamination can occur due to improper washing practices, particularly washing from back to front, which may facilitate the transfer of normal flora from the anus to the vagina [17].

*Klebsiella pneumoniae* is an opportunistic pathogenic bacterium that can cause vaginitis. In a study by Jebur and Hammoudi, *K. pneumoniae* was detected in 14.8% of pregnant women with vaginitis [18]. *Klebsiella pneumoniae* is normally found as part of the human flora in the respiratory tract and feces. The presence of *K. pneumoniae* in the vagina may therefore be associated with improper washing practices, resulting in the transfer of *K. pneumoniae* from feces to the vagina [19].

The proportion of respondents aged over 45 years (66.7%) was higher than that of respondents aged 45 years or younger (33.3%). This finding is consistent with studies conducted by Ocviyanti et al. and Bitew et al. Ocviyanti et al. reported that women over 40 years of age are at increased risk of BV, while Bitew et al. found that the highest proportion of BV occurred among women over 46 years of age [2,6]. Age over 40 years may represent a risk factor for BV due to the hypoestrogenic condition associated with this age group. Decreased estrogen levels lead to an increase in vaginal pH, which inhibits the growth of *Lactobacillus* spp. and creates favorable conditions for the growth of microorganisms associated with BV [20].

Regarding occupation, four respondents (66.6%) were housewives and two respondents (33.4%) were farmers. Housewives were also found to comprise 69.2% of respondents in the study by Ocviyanti et al.; however, no significant association was found between being a housewife and BV [2]. In contrast, Garba et al. reported that 52.6% of respondents were farmers and found a significant association between farming occupation and BV. The higher incidence of BV among farmers was associated with the use of traditional substances or medicines for vaginal care [21].

Four respondents (66.6%) had completed senior high school, compared with one respondent (16.7%) who had completed elementary school and one respondent (16.7%) who had not completed elementary school. This finding differs from the results of studies by Bahram et al. and Ibrahim et al., which reported that lower educational attainment was significantly associated with BV. This may be because women with higher levels of education have greater knowledge of medical treatments and are more likely to use them. Women with lower educational attainment are more likely to use traditional medicines or substances for vaginal care and may therefore be more susceptible to vaginal discharge [22,23]. However, other studies conducted by Bitew et al. and Li et al. found no significant difference in BV incidence according to educational level. In fact, Li et al. reported that 83% of women with BV were university students [6,24].

None of the respondents in this study reported smoking (0%). This finding is consistent with studies by Bradshaw et al. and Li et al., which reported no correlation between smoking and recurrent BV. In contrast, Smart et al. found that smoking significantly increased the incidence of BV. Chemicals contained in cigarette smoke, such as nicotine, cotinine, and benzo(a)pyrene diol epoxide (BPDE), may cause thinning of Langerhans cells in the cervical epithelium and suppress local immunity, thereby increasing the risk of BV [8,24,25].

All respondents in this study reported changing their underwear fewer than three times per day (100%). This finding is consistent with a study conducted by Ernawati, which reported that the frequency of changing underwear is a risk factor for BV. Women who did not change their underwear at least three times per day had an 8.3-fold higher likelihood of developing BV compared with women who routinely changed their underwear three times per day [26]. In contrast, Li et al. found that changing underwear every day was significantly associated with an increased incidence of BV. In addition to the frequency of changing underwear, other factors such as underwear cleanliness and

moisture may also influence the incidence of BV. Dirty and damp underwear may provide favorable conditions for the growth of microorganisms in the vaginal area [24]. Other factors, such as underwear material, have also been investigated. Klebanoff et al. reported no significant difference in BV incidence between women using cotton and nylon underwear [27].

Regarding vaginal douching practices, three respondents (50%) reported practicing vaginal douching. This finding is consistent with the study by Ranjit et al., which reported that women who practiced vaginal douching were significantly more likely to develop BV. Routine vaginal douching may disrupt the normal vaginal flora, particularly *Lactobacillus*, leading to an increase in vaginal pH and consequently contributing to the development of BV. Limited knowledge regarding appropriate vaginal hygiene and BV may be a major factor underlying the practice of vaginal douching. Women with limited knowledge may believe that vaginal douching improves vaginal cleanliness [3,28].

The use of feminine-area whitening soap was reported by two respondents (33.3%). No previous studies specifically investigating the use of feminine-area whitening soap were identified; however, studies examining the use of soap in the vagina have been conducted by Alcaide et al. and Ocviyanti et al. Ocviyanti et al. found that 32.6% of women who used soap in the vagina experienced BV, although the statistical analysis did not demonstrate a significant association. A significant association was reported by Alcaide et al., who found a correlation between daily use of soap in the vagina and BV [2,29].

In this study, two respondents (33.3%) reported having sexual intercourse more than twice per week, three respondents (50%) reported having sexual intercourse once to twice per week, and one respondent (16.7%) reported no sexual intercourse. A study by Anjani et al. showed that women who had sexual intercourse more than twice per week had a significantly increased prevalence of BV. One explanation for the increased prevalence of BV among sexually active women is the presence of semen in the vagina. Semen is alkaline and may increase vaginal pH. An increase in vaginal pH may disrupt the balance of normal vaginal flora. Another study by Ranjit et al. showed that unmarried women had a higher risk (100%) than married women (24.2%). This may be associated with tight clothing and inadequate attention to menstrual hygiene among unmarried women [3,30,31].

In this study, four respondents (66.7%) used hormonal contraceptives. This finding differs from the study by Indriana et al., which reported no association between BV and hormonal contraceptive use. In contrast, Bradshaw et al. reported that estrogen-containing contraception has a protective effect against BV. The use of estrogen-containing contraception was associated with a lower prevalence of BV compared with the use of other hormonal contraceptives and/or combined hormonal methods. One hypothesis explaining this association is that estrogen increases glycogen accumulation in vaginal epithelial cells, which serves as a substrate for *Lactobacillus* spp. to convert into lactic acid. Increased lactic acid production suppresses bacterial species associated with BV [8,32,33].

Another contraceptive method associated with BV risk is the intrauterine device (IUD). In this study, none of the respondents used an IUD (0%). This differs from the findings of Ernawati, who reported that IUD use was a risk factor for BV. Women using an IUD had a 60.9-fold greater risk of developing BV. This may be because an IUD is a foreign body within the uterus that can induce an inflammatory response and interfere with the physiology of the reproductive organs. In addition, IUD insertion is often preceded by vaginal disinfection, which may eliminate a substantial proportion of *Lactobacillus* spp. in the exposed area [26].

Stress levels in this study were assessed using the Self-Reporting Questionnaire-20 (SRQ-20). Four respondents (66.7%) had high stress levels. Similar findings were reported by Pratiwi et al., who used the SRQ-20 to assess stress levels and found that stress was significantly associated with BV. Stress resulting from life events may activate

neuroendocrine pathways, leading to impaired immune regulation and an increased risk of developing BV [34].

#### 4. Conclusions

The bacterial species identified in recurrent BV were *E. coli* and *K. pneumoniae*. The risk factors identified included age over 46 years, occupation, educational level, changing underwear fewer than three times per day, vaginal douching practices, use of hormonal contraceptives, sexual intercourse more than twice per week, and high stress levels.

Future researchers are recommended to increase the number of study respondents. A larger sample size would allow statistical analysis of the associated risk factors to be performed and would improve the validity and representativeness of the study.

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