



PREVALENCE OF BACTERIAL VAGINOSIS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN AMONG PREGNANT WOMEN AT TERTIARY CARE HOSPITAL

Microbiology

Grace Lalruatfeli	Junior Resident, Department of Microbiology, Government Medical College, Amritsar, Punjab, India
Shailpreet Kaur Sidhu*	Associate Professor, Department of Microbiology, Government Medical College, Amritsar, Punjab, India *Corresponding Author
Suparna Grover	Professor, Department of Obstetrics & Gynaecology, Government Medical College, Amritsar, Punjab, India
Loveena Oberoi	Professor, Department of Microbiology, Government Medical College, Amritsar, Punjab, India
Kanwardeep Singh	Professor, Department of Virology, Government Medical College, Amritsar, Punjab, India
Maninder Kaur	Research Scientist, Department of Virology, Government Medical College, Amritsar, Punjab, India
Maasha	Senior Resident, Department of Microbiology, Government Medical College, Amritsar, Punjab, India

ABSTRACT

Background: Vaginal infections in pregnant women are health problems that lead to serious medical complications and consequences. Infectious vaginitis includes bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and Trichomoniasis are common disorders in women. This study aimed to investigate and determine antimicrobial susceptibilities of the causative agents of vaginal infections in pregnant women. **Aim:** To isolate and identify the aerobic bacterial pathogens associated with vaginitis among pregnant women and to determine the antimicrobial susceptibility pattern. **Material and Methods:** A prospective study of 80 pregnant women was done over a period of 6 months in a tertiary care hospital to study microbiological profile of vaginitis. Duplicate high vaginal swabs were collected from each patient. BV was diagnosed by either Amsel's criteria or Nugent grading system and cultured on blood agar, MacConkey and chocolate agar for aerobic bacterial culture. Antibiotic Susceptibility of the isolated bacteria was performed by using Kirby-Bauer method. Fungal culture was performed on Sabouraud's Dextrose Agar media to detect Vulvovaginal candidiasis. **Results:** Out of 80 patients, 43(53.75%) women were positive for aerobic vaginitis, out of which BV and VVC were found to be 26(60.47%) and 13(30.23%) while mixed infections was found in 4(9.3%) of the infected patients. The highest rate of infections was reported in 3rd Trimester (53.85%) and most common symptoms were discharge, lower abdominal pain and vulval itching. **Conclusion:** Hence, BV was the most common vaginitis among pregnant women. Adequate investigation and prompt treatment will reduce the morbidity and harmful effects of these prevalent infections to both mother and fetus.

KEYWORDS

Vaginitis, Bacterial vaginosis, Vulvovaginal candidiasis, Antimicrobial susceptibility

INTRODUCTION

Vaginal infections are an important women's health problem associated with negative impacts on sexual and family lives and has a tendency of increasing prevalence worldwide [1]. Vaginitis refers to a non-specific inflammation of the vagina characterized by watery discharge with burning and itching of the vulva. The three common types of vaginitis includes Bacterial vaginosis (BV), Vulvovaginal candidiasis (VVC) and Trichomoniasis. Bacterial vaginosis is the most common vaginal dysbiosis that affects women of childbearing age and comprises around 50% of all cases of vaginitis worldwide. It has recently emerged as a global health issue especially in pregnant women because of its adverse outcomes and documented reported prevalence of 6%-32% from different regions of the world [2]. Various studies have shown the impact of BV on mother and baby as well as overall reproductive health of the women [3].

In healthy women, the vaginal environment is a balanced ecosystem characterized by presence of various species of lactobacilli. These bacteria inhibit the growth of other microorganisms through various mechanisms including production of organic acids, hydrogen peroxide, antimicrobial substances, competition for mannose and glycoprotein receptors and adhesion to the epithelium [2]. Bacterial vaginosis is a condition characterized by raised vaginal pH and milky discharge in which normal vaginal flora (Lactobacillus) is replaced by pathogenic mixed flora of aerobic, anaerobic and microaerophilic species. Bacterial vaginosis can be diagnosed clinically using Amsel's criteria [4] or through the laboratory using the Nugent scoring system [5]. A combination of both Amsel's clinical criteria and Nugent grade scoring system of Gram stain is better for an accurate diagnosis of BV [6]. There is enough evidence through various studies that bacterial vaginosis in pregnancy is associated with increased risk of severe complications and adverse pregnancy outcomes including premature

rupture of membranes (PROM), chorioamnionitis, preterm delivery, spontaneous abortion and low birth weight [3]. A meta-analysis by Leitch et al showed that BV significantly increases chances of preterm delivery in both asymptomatic women and those with preterm labour symptoms [7].

Aerobic vaginitis (AV), to emphasize its difference from BV, is inflammation of the vaginal epithelium and characterized by abnormal vaginal microflora containing aerobic and enteric bacteria [8]. In case of aerobic vaginitis facultative intestinal pathogens like *Escherichia coli*, *Klebsiella* spp., *Acinetobacter* spp., group B *Streptococci*, *Staphylococcus aureus* and *Enterococcus* spp. replace lactobacilli in vagina [9]. The global problem of AV is strongly linked to the emergence of antimicrobial resistance. Women with AV can develop an adverse outcome following antibiotic treatment. Aerobic vaginitis is often misdiagnosed and is not easy to treat due to the developing spread of multidrug-resistant bacterial strains [10]. These infections if not treated or ignored could debilitate the patient and could become a source of infection for the neonates. Hence the present study is designed to isolate and identify the aerobic bacterial pathogens associated with vaginitis among pregnant women and to determine the antimicrobial susceptibility pattern.

MATERIAL AND METHODS

A prospective study was conducted in the Department of Microbiology, Government Medical College, Amritsar over a period of six months i.e September 2023 - February 2024. A total of 80 high vaginal swabs were collected from pregnant women visiting Obstetrics and Gynecology Department of Guru Nanak Dev Hospital, Amritsar by following inclusion criteria. Antenatal women of second trimester onwards presenting with one or more of the following symptoms: Abnormal vaginal discharge, lower abdominal pain,

Itching or irritation in vagina and dyspareunia were included in the study. Antenatal women who are <18yrs of age, 1st Trimester and with any known obstetric complications (PROM, antepartum hemorrhage, Intrauterine death, severe anemia, cervical cerclage and genital malignancy) were excluded for this study.

Sample Collection, Handling and Transportation

Two vaginal swabs were collected aseptically from posterior vaginal fornix/lateral wall of the vagina using sterile rayon tipped applicator stick and dipped into a sterile tube containing sterile physiological saline and taken to the laboratory within 30 minutes of collection for laboratory processing. One swab was used for analysis of Amsel's criteria and the second swab for Gram staining and cultured on MacConkey Agar, Blood Agar and chocolate agar to isolate aerobic bacteria.

RESULTS

Among a total of 80 pregnant women included in the study, 43 (53.75%) women were positive for infectious vaginitis, out of which 26 (60.47%) was bacterial vaginosis (diagnosed by Amsel's and Nugent's criteria). VVC and mixed infections were found to be 13 (30.23%) and 4 (9.3%) respectively.

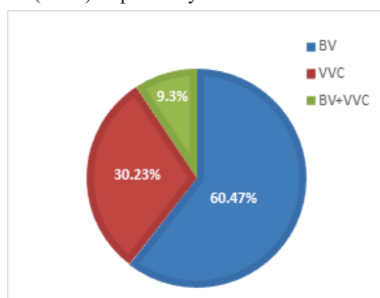


Figure 1: Prevalence Of Aerobic Vaginitis

Infection rate was highest among the age group 30-39yrs (61.54%) followed by 20-29yrs (26.92%). The higher rate of infection was found in 3rd Trimester 14(53.85%) of pregnancy and 12(46.15%) was found in 2nd Trimester (Table1).

Table 1: Age And Trimester Wise Distribution Of Bacterial Vaginosis

Characteristics	Infection		
	BV (n=26)	VVC (n=13)	BV+VVC (n=4)
Age of Women (years)			
≤ 19	2 (7.69%)	1 (7.69%)	1 (25%)
20-29	7(26.92%)	4 (30.77%)	1 (25%)
30-39	16 (61.54%)	7 (53.85%)	1 (25%)
>40	1 (3.85%)	1 (7.69%)	1 (25%)
Trimester distribution			
2nd Trimester	12 (46.15%)	5 (38.46%)	2 (50%)
3rd Trimester	14 (53.85%)	8 (61.54%)	2 (50%)

Regarding symptoms, all the BV positive women had complaint of vaginal discharge out of which 13 (50%) had malodour, 25 (96.15%) had abdominal pain, 18 (69.23%) had vulval itching and 6 (23.08%) had dyspareunia (Table 2).

Table 2: Clinical Manifestation Of Bacterial Vaginosis & Vulvovaginal Candidiasis In Pregnant Women

Presenting complaints	BV (n=26)	VVC (n=13)	BV+VVC (n=4)
Vulval itching	18 (69.23%)	10 (76.92%)	2 (50%)
Dyspareunia	6 (23.08%)	2 (15.38%)	1 (25%)
Lower abdominal Pain	25 (96.15%)	11 (84.62%)	3 (75%)
Discharge	26 (100%)	13 (100%)	4 (100%)
Colour			
White	12 (46.15%)	8 (61.54%)	1 (25%)
Yellow	12 (46.15%)	5 (38.46%)	3 (75%)
Consistency			
Watery	8 (30.77%)	3 (23.08%)	2 (50%)
Thick	17 (65.38%)	10 (76.92%)	2 (50%)
Odour			
Normal	11 (42.31%)	10 (76.92%)	3 (75%)
Malodorous	13 (50%)	3 (23.08%)	1 (25%)

Among 20 Bacterial isolates, K.pneumoniae (45%) and E.coli (40%) were the commonest organisms seen. Antimicrobial susceptibility Testing were carried out for all bacterial isolates (Figure - 2). The most prevalent organism of the study K.pneumoniae reported maximum sensitivity to Piperacillin/Tazobactam (80%), Carbapenems (70%) followed by Aminoglycosides (40%). The second most prevalent organism E.coli showed maximum sensitivity to Carbapenems (87.5%) and Aminoglycosides(62.5%). Strains of S.aureus showed high susceptibility to Linezolid (100%) and Vancomycin (100%) (Table 3).

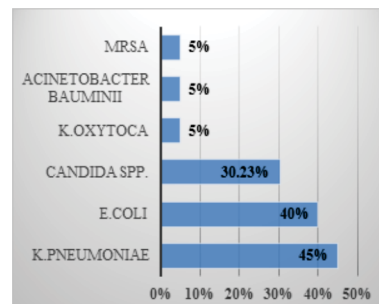


Figure 2: Distribution Of Isolates From Aerobic Vaginitis

Table 3: Antibiotic Sensitivity Pattern Of Bacterial Isolates By Kirby-bauer Method

Antibiotic	Gram Negative Isolates (19)			Gram Positive Isolates (1)	
	Klebsiella spp. (10)	E. coli (8)	Acinetobacter spp. (1)	Antibiotic	S. aureus (1)
Amp (%)	-	0 (0.0)	0 (0.0)	Amp (%)	0 (0.0)
Cip (%)	4 (40)	3 (37.5)	0 (0.0)	Cip (%)	0 (0.0)
Genta (%)	4 (40)	5 (62.5)	0 (0.0)	Genta (%)	1 (100)
Ak (%)	4 (40)	5 (62.5)	0 (0.0)	Ak (%)	1 (100)
Cz (%)	2 (20)	2 (25)	0 (0.0)	Cl (%)	1 (100)
Ctn (%)	1 (10)	3 (37.5)	0 (0.0)	E (%)	1 (100)
A-clav (%)	0 (0.0)	0 (0.0)	0 (0.0)	A-clav (%)	0 (0.0)
Pctz (%)	8 (80)	5 (62.5)	0 (0.0)	Lz (%)	1 (100)
Mero (%)	7 (70)	7 (87.5)	0 (0.0)	Cx (%)	0 (0.0)
Poly-B (%)	10 (100)	8 (100)	1 (100)	Vanco (%)	1 (100)

* Amp = Ampicillin, Cip = Ciprofloxacin, Genta = Gentamycin, Ak = Amikacin, Cl = Clindamycin, Cz = Ceftazidime, E = Erythromycin, Ctn = Ceftriazone, Lz = Linezolid, A-Clav = Amoxycillin & Potassium Clavulanate, Pctz = Piperacillin-Tazobactam, Mero = Meropenem, Van = Vancomycin, Poly-B = Polymyxin-B, Cx = Cefoxitin

DISCUSSION

In our study, the overall prevalence of infectious vaginitis was found to be 43(53.75%). Bacterial vaginosis 26(60.47%) was found to be most prevalent among study patients followed by VVC and mixed infection which were found to be 13(30.23%) and 4(9.3%) respectively. Similarly, Shrestha et al. [11] concluded that BV was more common (53.6%) and Afrakhteh [12] also showed 62.7% of bacterial vaginitis in a similar study. However, the rate of BV in this report is much higher than a study by Lamichhane et al which showed 40% cases of vaginitis in which bacterial vaginosis was 39.13% followed by VVC (30.43%) and mixed infection (30.43%)[8]. Higher prevalence rates of Bacterial vaginosis were also reported from other Sub-Saharan countries such as Kenya, Botswana and Zimbabwe [13].

According to the present study BV was found to be more prevalent in the age group 30-39yrs (61.54%), followed by 20-29yrs (26.92%), similarly reported in a study by Aseigbu et al [2]. This study revealed that the highest prevalence of BV occurred in women of reproductive age. These women being the most sexually active age group the highest rate of pregnancies and sexually transmitted diseases also being shown

by many studies [8]. Whereas a study by Zahrah Adnan et al shows 40-45yrs female has the highest percentage of Bacterial vaginosis which may be related due to low activity of hormones such as estrogen hormone and low secretion of glycogen, elevated pH to alkaline when advanced age which encourage pathogens [14]. More than half of women with BV were from urban areas 20 (76.92%) and majority were housewives. These findings are in agreement with a study done in India by (Prosper et al, 2012) stated that BV during pregnancy are more common among housewives and women living in urban area [15].

The highest frequency of vulvovaginal candidiasis in our study was observed in the age group of 30-39yrs (53.85%) followed by the age group of 20-29yrs (30.77%). The mean age of the study participants was 31 years, which correlates with the mean age of 31.5 years reported by Sasikala et al. [16] and Rati et al [17]. Reason for the high incidence in this age group includes low levels of protective cervical antibodies, increased sexual activity and new influence of reproductive hormones that may lead to increased susceptibility to reproductive tract infections. On the contrary, a higher mean age of 37.3 years was reported by Amar et al [18].

Our study observed a slight increase of BV in the gestational age of 3rd Trimester (53.85%) which is conflicting with the findings of Awoniyi et al. that prevalence decreased with advancement of pregnancy [3]. It has been suggested that prevalence rate of different trimesters could be probably be related to variation in individual responses from effect of endogenous sex hormones [19].

In this study, the symptoms of discharge with yellow (46.15%), thick (65.38%) and malodorous (50%), lower abdominal pain (96.15%) and vulval itching (69.23%) were found to be associated with the presence of BV. Similarly, a study performed in Nigeria by (Ibrahim et al, 2018) [20] showed yellowish discharge (80%), watery (75%) and malodorous (75%), lower abdominal pain (67.5%) and vulval itching (17%) were associated with BV, another study done by Dennis, 2015 [21] also reported a significant association between BV and vaginal symptoms (discharge, vulval itching and lower abdominal pain).

Another finding of current study was the determination of bacterial isolates of aerobic vaginitis and their antibiotic resistance. A total of 20 bacterial isolates were recovered of which 45% and 40% were *Klebsiella pneumoniae* and *E.coli* respectively. These findings are similar to a study conducted in India in which a greater predominance of Enterobacteriaceae isolated were reported whereas in contrast to study done by Yalew et al where Staphylococci were the highest prevalent bacteria in aerobic vaginitis women [22,5]. In accordance with our study, Serrettiello et al also reported that overall sensitivity rates of Enterobacteriaceae to Carbapenems and Piperacillin/tazobactam and moderately sensitive to Aminoglycosides [23]. The resistance to Cephalosporins, ciprofloxacin, penicillin and amoxicillin/clavulanic acid was high for both *E. coli* and *K. pneumoniae*. The higher rate of multi-drug resistant isolates may be due to unprudent use of antibiotics which is in particular true for developing countries where antibiotics are prescribed irrationally to the patients [24].

CONCLUSION

Pregnant women are at a risk of vaginal infections especially in the second and third trimesters. It is recommended that antenatal health care facilities should incorporate screening of vaginitis among pregnant women to prevent the complications of pregnancy. Proper use of antibiotics should be encouraged and abuse of antibiotics should be in check.

REFERENCES:

- Workowski, K. A., & Berman, S. M. (2010). Sexually transmitted diseases treatment guidelines, 2010.
- Aduloju, O. P., Akintayo, A. A., & Aduloju, T. (2019). Prevalence of bacterial vaginosis in pregnancy in a tertiary health institution, south western Nigeria. *Pan African Medical Journal*, 33(1).
- Bhakta, V., Aslam, S., & Aljaghawi, A. (2021). Bacterial vaginosis in pregnancy: prevalence and outcomes in a tertiary care hospital. *African journal of reproductive health*, 25(1), 49-55.
- Amsel, R., Totten, P. A., Spiegel, C. A., Chen, K. C., Eschenbach, D., & Holmes, K. K. (1983). Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *The American journal of medicine*, 74(1), 14-22.
- Nugent, R. P., Krohn, M. A., & Hillier, S. L. (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *Journal of clinical microbiology*, 29(2), 297-301.
- Ahmed, M., Admassu Ayana, D., & Abate, D. (2022). Bacterial Vaginosis and Associated Factors Among Pregnant Women Attending Antenatal Care in Harar City, Eastern Ethiopia. *Infection and Drug Resistance*, 3077-3086.
- Leitch, H., Bodner-Adler, B., Brunbauer, M., Kaider, A., Egarter, C., & Husslein, P.

- (2003). Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis. *American journal of obstetrics and gynecology*, 189(1), 139-147.
- Yalew, G. T., Muthupandian, S., Hagos, K., Negash, L., Venkatraman, G., Hagos, Y. M., ... & Saki, M. (2022). Prevalence of bacterial vaginosis and aerobic vaginitis and their associated risk factors among pregnant women from northern Ethiopia: A cross-sectional study. *PLoS one*, 17(2), e0262692.
- Lamichhane, P., Joshi, D. R., Subedi, Y. P., Thapa, R., Acharya, G. P., Lamsal, A., & Upadhyaya, S. (2014). Study on types of vaginitis and association between bacterial vaginosis and urinary tract infection in pregnant women. *IJBAR*, 5(06), 305-7.
- Kareem Raheem, Z., & Abdulhamid Said, L. (2023). Antibiotic susceptibility profile of bacteria causing aerobic vaginitis in women in Iraq. *Archives of Razi Institute*, 78(1), 31-43.
- Shrestha, S., Tuladhar, N. R., Basnyat, S., Acharya, G. P., Shrestha, P., & Kumar, P. (2011). Prevalence of vaginitis among pregnant women attending Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu, Nepal. *Nepal Med Coll J*, 13(4), 293-6.
- Sharami, S. H., Afrakhteh, M., & Shakiba, M. (2007). Urinary tract infections in pregnant women with bacterial vaginosis. *Journal of Obstetrics and Gynaecology*, 27(3), 252-254.
- Bitew, A., Abebaw, Y., Bekele, D., & Mihret, A. (2017). Prevalence of bacterial vaginosis and associated risk factors among women complaining of genital tract infection. *International Journal of Microbiology*, 2017.
- Adnan, Z., Dhaher, H., & Marjani, M. F. (2020). Prevalence of Aerobic Bacterial Vaginosis and Trichomonas Vaginalis Associated with Socioeconomic Factors among Women in Misan Governorate. *Indian Journal of Forensic Medicine & Toxicology*, 14(1), 745-752.
- Faris, G., & Nasir, S. (2018). Prevalence, risk factors and clinical presentations of bacterial vaginosis among group of pregnant women in labor ward at maternity teaching hospital in Sulaimani city. *Journal of Sulaimani Medical College*, 8(2), 109-117.
- Sasikala, G., Agatha, D., Janagond, A. B., & Thenmozhi Valli, P. R. (2013). Characterization of Candida and its antifungal susceptibility pattern from patients with vaginal candidiasis in a tertiary care hospital in South India.
- Rati, R., Patel, J., & Rishi, S. (2015). Vulvovaginal candidiasis and its antifungal susceptibility pattern: single center experience. *International Journal of Medical Research and Review*, 3(1), 72-78.
- Amar, C. S., Ashish, J., Vinay Hajare, V. H., Sreekantha, S., Yogesh, B., & Vinodchandran, V. (2013). Study of prevalence and antifungal susceptibility of candida.
- Morison, L., Ekpo, G., West, B., Demba, E., Mayaud, P., Coleman, R., ... & Walraven, G. (2005). Bacterial vaginosis in relation to menstrual cycle, menstrual protection method, and sexual intercourse in rural Gambian women. *Sexually transmitted infections*, 81(3), 242-247.
- Ibrahim, S. M., Bukar, M., Galadima, G. B., Audu, B. M., & Ibrahim, H. A. (2014). Prevalence of bacterial vaginosis in pregnant women in Maiduguri, North-Eastern Nigeria. *Nigerian journal of clinical practice*, 17(2), 154-158.
- Konadu, D. G., Owusu-Ofori, A., Yidana, Z., Boadu, F., Iddrisu, L. F., Adu-Gyasi, D., ... & Asante, K. P. (2019). Prevalence of vulvovaginal candidiasis, bacterial vaginosis and trichomoniasis in pregnant women attending antenatal clinic in the middle belt of Ghana. *BMC pregnancy and childbirth*, 19, 1-10.
- Sidhu S.K, Pal K, Devi P, Malhotra S, Malhotra A, Soneja S (2017). Etiology of vaginal infections and antimicrobial resistance pattern of aerobic bacterial isolates in women of reproductive age group attending a tertiary care hospital. *Asian Pacific Journal of Health Sciences*, 4(4), 15–18. <https://doi.org/10.21276/apjhs.2017.4.4.5>
- Serrettiello, E., Santella, B., Folliero, V., Iervolino, D., Santoro, E., Manente, R., ... & Boccia, G. (2021). Prevalence and antibiotic resistance profile of bacterial pathogens in aerobic vaginitis: a retrospective study in Italy. *Antibiotics*, 10(9), 1133.
- Bhavana, A. M., Kumari, P. H. P., Mohan, N., Chandrasekhar, V., Vijayalakshmi, P., & Manasa, R. V. (2019). Bacterial vaginosis and antibacterial susceptibility pattern of asymptomatic urinary tract infection in pregnant women at a tertiary care hospital, Visakhapatnam, India. *Iranian Journal of Microbiology*, 11(6), 488.