scientific reports

OPEN

Bacterial vaginosis, vaginal *Candida* colonization and antifungal susceptibility patterns in pregnant women of eastern Ethiopia: a prospective study

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Bacterial vaginosis (BV) and vaginal candidiasis are prominent causes of vaginal infections, leading to discomfort and negative pregnancy outcomes. Despite their significance, there is limited data on the prevalence, progression, and contributing factors of BV and vaginal *Candida* colonization among pregnant women in low-resource settings such as Ethiopia. Thus, the aim of this paper is to determine the prevalence and associated factors of BV and vaginal *Candida* colonization, and antifungal susceptibility patterns in Eastern Ethiopia. Exploratively, we assessed the relationship between these infections and adverse pregnancy outcomes among pregnant women. A total of 217 pregnant women, ranging from 12 to 22 weeks of gestation, were enrolled and followed until birth or pregnancy termination. Data on sociodemographic information, pregnancy history, and current conditions were gathered through interviews. Two vaginal swabs were collected for microbiological analysis, using Nugent scoring for BV, culture and Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry for *Candida* detection. Data were collected using Redcap and analyzed using STATA version 17. Multivariable logistic regression was employed to identify associated factors at a significance level of $p < 0.05$. This study indicated that 43% (95% confidence interval (CI): 36.6%, 50.2%) of the participants had BV or vaginal *Candida* colonization or both. The overall prevalence of BV and vaginal *Candida* colonization were 27.7% (95% CI: 21.8%, 34.1%) and 23.5% (95% CI: 18.4%, 29.6%), respectively. Factors associated with BV included antibiotic use (AOR = 9.47, 95% CI: 4.09–21.94) and vaginal douching (AOR = 6.93, 95% CI: 2.86–16.77). Similarly, antibiotic use (AOR = 4.18, 95% CI: 1.78–9.80) and vaginal douching (AOR = 5.48, 95% CI: 2.39–12.56) were significantly associated with *Candida* colonization. BV increased the likelihood of adverse birth outcomes by 1.89 times and preterm birth alone by 3.89 times. BV and vaginal *Candida* colonization are highly prevalent among pregnant women in Eastern Ethiopia and are associated with modifiable behavioral and clinical factors. The findings underscore the importance of improved understanding of vaginal microbiota dynamics during pregnancy and their potential links with adverse maternal and neonatal outcomes.

Keywords Bacterial vaginosis, Adverse pregnancy outcomes, Vaginal *Candida* colonization, Pregnant women, Ethiopia

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Vaginitis - inflammation or infection of the vagina - is one of the leading causes of vaginal discomfort and associated with different adverse pregnancy outcomes such as preterm birth and low birth weight, both major causes of neonatal mortality and morbidity[1]. The most common infectious causes of vaginitis are bacterial vaginosis (BV), vaginal candidiasis (VC), and trichomoniasis[2]. BV is a dysbiosis of the vaginal microbiota, marked by a decrease in the number or absence of lactobacilli. These metabolize glycogen to lactic acid thereby acidifying the vagina and inhibiting overgrowth by anaerobic bacteria. Key markers of BV include *Gardnerella*, *Prevotella*, and *Fannyhessea vaginae*[3,4].

Globally, approximately 50% of all vaginitis cases can be attributed to BV[5] whereas VC is the second most common cause, affecting approximately 20% of pregnant and 30% of non-pregnant women[6]. VC is primarily caused by *Candida albicans*[7], with *Nakaseomyces glabratus* (formerly *C. glabrata*), *C. tropicalis* and *Pichia kudriavzevii* (formerly *C. krusei)* being the most prevalent non-*albicans* species[8,9]. In addition to symptomatic VC, women may also harbor *Candida* species asymptomatically, referred to as vaginal *Candida* colonization (VCC). Increasing evidence suggests that VCC, like VC, can be associated with adverse pregnancy outcomes (APOs) such as preterm birth and low birth weight[10,11].

Globally, the prevalence of BV is high across different regions, ranging from 23% (Europe and Central Asia) to 29% (South Asia)[12]. The prevalence of BV among pregnant women in Africa varies by country, ranging from 20% to 29%[13]. Around 75% of all women experience at least one episode of VC during their childbearing years[14,15].

Several factors, such as multiple and/or new sexual partners, a history of sexually transmitted infections, vaginal douching, socioeconomic status, certain vaginal hygienic practices, and black ethnicity, and when in pregnancy have been identified as potential contributors to the development of BV[16]. Regarding VC, diabetes mellitus, HIV infection, a history of BV, sexual activity, contraceptive use, pregnancy, and the use of broad-spectrum antibiotics are known risk factors[17].

Despite their high prevalence, data on BV, VC, and especially VCC among pregnant women remain scarce in low-resource settings like Ethiopia. Furthermore, there is a lack of data on antifungal susceptibility patterns of *Candida* isolates from pregnant women in Ethiopia. This study was conducted to assess the prevalence, associated factors of BV and VCC, antifungal susceptibility patterns, and association between BV and/or VCC with adverse birth outcomes among pregnant women attending antenatal care in eastern Ethiopia.

Methods
Study settings
This study was conducted in selected health facilities within the Hararghe Health and Demographic Surveillance sites (HDSS) in Eastern Ethiopia. The HDSS consists of Kersa HDSS (rural and semi-urban), Haramaya HDSS (rural), and Harar HDSS (urban). The profile of the HDSS is described elsewhere[18,19]. In this study, we included pregnant women attending antenatal facilities in Kersa and Harar HDSS from December 2023 to November 2024.

Study design and population
The current study is part of the EthiOpian Pregnancy and Early Life Microbiome (EthiOMICS) study, a multicenter longitudinal cohort study of pregnant women enrolled from early pregnancy and followed until termination of pregnancy or delivery. The study protocol has been described previously[20]. In brief, healthy pregnant women attending health centers and hospitals within the Kersa and Harar HDSS for antenatal care were enrolled and seen at three visits. Current study only considers the baseline visit.

Women were included in case they were pregnant between 12 and 22 weeks of gestation as confirmed by obstetric ultrasound, residing within the HDSS catchment for at least 3 months, not planning to move out of the study area within the next two years, and had a singleton pregnancy. Women were excluded in case they used prolonged (more than 3 months) systemic antibiotics, antifungals, antivirals, or antiparasitic therapy, or had fetal anomalies. Details on sample size determination, sampling technique, and data collection techniques have been described in the EthiOMICS protocol[20].

Data and clinical sample collection procedures
Four trained midwives collected data on socio-demographic, reproductive health, vaginal hygiene and behavioral related factors of the women through a structured interview. No clinical investigation was done and data on symptoms such as vaginal discharge was collected through self-reporting by the participants. Upon completion

of the interviews, midwives collected two vaginal swabs from each participant. One was immediately placed in Amies transport media (Becton, Dickinson, USA) and transported to the medical microbiology laboratory of Haramaya University for microbiological analysis. The other swab was used for Gram staining followed by Nugent scoring to assess the presence of BV.

Birth outcomes

Birth outcomes were classified as normal or adverse. An adverse birth outcome was defined as any of the following seven birth outcomes: stillbirth, preterm birth, neonatal death, congenital anomaly, low birth weight, macrosomia, and small for gestational age[21]. Stillbirth was defined using a modified WHO criterion of fetal deaths occurring at ≥ 20 weeks' gestation[22]. Preterm birth referred to a live birth occurring before 37 completed weeks of gestation[23]. Neonatal death was defined as the death of a live-born infant within the first 28 days of life[24]. A congenital anomaly was described as a structural and/or functional abnormality/ies detected during pregnancy or of the newborn[25]. Low birth weight was defined as a birth weight of less than 2500 g (g)[26]. Macrosomia was defined as an infant weighing more than 4000 g at birth[27]. Small for gestational age indicated a birth weight below the 10th percentile for gestational age[28].

Sample processing and laboratory investigations

Bacterial vaginosis assessment

The smears from vaginal swabs were Gram-stained and examined under an oil immersion objective for assessment of the Nugent score[29]. Gram stain quality control was checked using control slides to assess reagent quality and staining efficacy. Gram positive (*Staphylococcus aureus* ATCC 25923) and Gram negative (*Escherichia coli* ATCC 25922) controls were used[30]. We used the following case definition: women with Nugent scores from 0 to 3 were classified as having a normal vaginal microbiota, scores of 4–6 as having an intermediate vaginal microbiota, and women with scores of 7–10 were classified as BV. The Nugent score was evaluated by two readers, with discrepancies resolved by a third.

Vaginal *Candida* carriage detection

Vaginal swabs were inoculated onto Sabouraud dextrose agar (SDA) (Becton Dickinson, New Jersey, USA) plates and incubated at 35–37 °C for 18–24 h. Then, a Gram stain smear was performed to check for the presence of yeast cells. *Candida* isolates were preliminarily identified by carbohydrate fermentation tests[31], and subculturing on Chromogenic Candida agar (TM Media, New Delhi, India). *Candida* isolates were identified based on colony color (*C. albicans*: light green, *N. glabratus*: cream to white, *P. kudriavzevii*: purple-pink, *C. tropicalis*: metallic blue). The final identification of all yeast isolates was performed by Matrix Associated Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) test at the Laboratory Bacteriology Research (LBR, Ghent University). All culture media were prepared following the manufacturer's instructions and stored at the refrigeration temperature (2–8 °C) after preparation. A case of VCC was defined as the isolation of any yeast species from a vaginal swab, irrespective of colony count or species.

Antifungal susceptibility testing

For yeast isolates, antifungal susceptibility testing was conducted at Haramaya University Medical Microbiology Laboratory using a modified disc diffusion method as described by the Clinical Laboratory Standard Institute (CLSI) guidelines[32]. A pure colony of an isolate was mixed with normal saline, adjusted to match the turbidity of a 0.5 McFarland standard, and a cotton swab moistened with the fungal suspension was streaked onto a Muller-Hinton agar plate (Becton, Dickinson, USA). Antifungal discs (amphotericin B (100 µg), clotrimazole (10 µg), fluconazole (25 µg), miconazole (10 µg), itraconazole (10 µg), and ketoconazole (10 µg)) (all Oxoid, UK) were placed on the agar plates using a dispenser and plates were incubated at 37 °C for 24 h. Finally, the resulting zones of inhibition were measured using a caliper and interpreted according to the CLSI guidelines (M44-A2)[33].

Data processing and analysis

The collected data was imported into STATA version 17 for cleaning and analysis. A descriptive statistical analysis was utilized to describe socio-demographics and associated factors, adverse pregnancy outcomes, the prevalence of BV and vaginal *Candida* colonization, the *Candida* species distribution and antifungal susceptibility patterns. Multivariable logistic regression models were built to identify factors associated with outcome variables (BV and vaginal *Candida* colonization). To do so, we first identified all (risk) factors with a p-value ≤ 0.25 in a bivariate analysis. Participants were categorized into four mutually exclusive groups (BV only, VCC only, BV + VCC, neither) based on the case definitions described above. These categories were used to report prevalence. For regression analyses, BV and VCC were modeled separately as binary outcomes; women with BV + VCC were therefore classified as positive for both BV and VCC. No regression analysis was performed for the BV + VCC category due to the low number of cases. Associated factors were subsequently modelled in a multivariable analysis. The variance inflation factor (VIF) was used to identify the degree of multicollinearity among the identified (risk) factors. The model goodness of fit was tested by the Hosmer-Lemeshow statistic. Variables with p-values < 0.05 in the multivariable models were considered statistically significant. As an exploratory analysis, bivariate associations between BV and vaginal *Candida* colonization (both separately and combined) with adverse birth outcomes were assessed, and adjusted odds ratios (AORs) were derived without additional covariates.

Declarations

Ethics approval and consent to participate

The study was performed per the World Medical Association Declaration of Helsinki and ethical approval has been granted by the Institutional Health Research Ethics Review Committee (IHRERC) of Haramaya University's College of Health and Medical Sciences (Ref. No. IHRERC/033/2022). All methods of the study were performed in accordance with relevant guidelines and regulations. The study's purpose, procedures, risks and benefits were explained to the head of each participating health institution and permission was granted. Informed written consent was obtained from all participants after providing a clear explanation of the study. Participants were informed of their right to decline or withdraw from the study at any time without any consequences to their care. Participants' confidentiality was ensured by omitting names and personal identifiers from all study documents and data entry. Women who were positive for BV and/or vaginal *Candida* were referred to attending healthcare professionals for appropriate treatment and follow up.

Results

Socio-demographic characteristics

The socio-demographic characteristics of the study population are summarized in Table 1. A total of 217 women with a mean age of 24.0 (± 4.9) years were included in the study. Most participants were urban residents (73.7%), married (97.7%), and had attended at least secondary school (60.4%). Nearly all pregnancies were planned (96.8%). At enrollment, 80.0% of women were attending their first antenatal care. The mean gestational age at enrolment was 18.25 (± SD = 3.35) weeks, ranging from 12 to 22 weeks.

Reproductive health, vaginal hygiene, and behavioral characteristics

The reproductive health, vaginal hygiene, and behavior characteristics of the study population are summarized in Table 2. Most respondents reported having only one sexual partner in current pregnancy (91.7%), and no history of stillbirth (93.1%). Vaginal douching, vaginal symptoms, and khat chewing were reported by 25.3%, 22.1%, and 34.1% of the respondents, respectively.

Prevalence of bacterial vaginosis and vaginal *Candida* colonization

Out of the 217 participants, 94 (43.3%) (95% CI: 36.6%, 50.2%) were found to have either BV or vaginal *Candida* colonization, or both, with 7.8% (17 out of 217) experiencing both conditions concurrently. According to Nugent scoring system, 60 (27.7%) (95% CI: 21.8%, 34.1%) of women had BV while 74 (34.1%) and 83 (38.3%) had an intermediate and normal vaginal microbiome, respectively. The overall prevalence of vaginal *Candida* colonization was 23.5% (95% CI: 18.4%, 29.6%).

Yeast detection and identification

Out of 217 samples inoculated on SDA medium, 51 (23.5%) yielded colonies that were confirmed as yeast cells upon further examination by microscopy after Gram staining. The presumptive (sugar fermentation assays and CHROMagar) and final (MALDI-TOF) identifications are shown in Fig. 1. The final MALDI-TOF identification revealed a total of 53 *Candida* isolates. Among these, *C. albicans* was the most prevalent yeast, accounting for 40 isolates (75.5%; 95% CI: 62.4, 85.1), followed by *P. kudriavzevii* with 6 isolates (11.3%; 95% CI: 5.3, 22.6), *N. glabratus* with 3 isolates (5.7%; 95% CI: 1.9, 15.4), and 2 isolates each (3.8%; 95% CI: 1.0, 12.8) of *Clavispora lusitaniae* and *Saccharomyces cerevisiae*.

Characteristics	Categories	Number	%
Age (in Years)	≤ 24	112	51.6
	25–35	97	44.7
	> 35	8	3.7
Residence	Urban	160	73.7
	Rural	57	26.3
Marital status	Married	212	97.7
	Single	1	0.5
	Divorced	4	1.8
Educational status	No formal education	81	37.3
	Primary school	5	2.3
	High school	116	53.5
	College and above	15	6.9
Pregnancy intent	Planned	210	96.8
	Unplanned	7	3.2
Number of antenatal visits at enrollment	1st	173	79.7
	2nd	41	18.9
	3rd	3	1.4

Table 1. Socio-demographic characteristics of the study population.

Characteristics	Categories	Number	(%)
Number of sexual partners*	1	199	91.7
	≥ 2	18	8.3
History of miscarriage/abortion	Yes	55	25.3
	No	162	74.7
History of stillbirth	Yes	15	6.9
	No	202	93.1
Vaginal symptoms (any)	Yes	48	22.1
	No	169	77.9
Vaginal discharge*	Yes	31	14.3
	No	186	85.7
Vaginal itching/pain*	Yes	19	8.8
	No	198	91.2
Vaginal burning*	Yes	9	4.2
	No	208	95.9
Lower abdominal pain*	Yes	17	7.8
	No	200	92.2
Genital ulcer*	Yes	4	1.8
	No	213	98.2
Vaginal douching*	Yes	55	25.3
	No	162	74.7
Take folate/folic acid supplements*	Yes	80	36.9
	No	137	63.1
Materials used for vaginal hygiene after sexual intercourse	Water	213	98.1
	Wipes	3	1.4
	Tissue	1	0.5
Chew khat*	Yes	74	34.1
	No	143	65.9
Gravida	1	129	59.4
	≥ 2	88	40.6

Table 2. Reproductive health, vaginal hygiene, and behavior of the study population. Legend. *During current pregnancy.

The sugar fermentation test correctly identified 79.2% of the isolates. All 33 *C. albicans* isolates were confirmed as such by Maldi-TOF. Out of 18 isolates that were presumptively identified as *N. glabratus/P. kudriavzevii*, only 9 were identified as such by Maldi-TOF (6 *P. kudriavzevii and 3 N. glabratus*). The remaining 9 were misidentified and included six *C. albicans*, one *C. lusitaniae* and two *S. cerevisiae* (Fig. 1, Panel A). All 33 *C. albicans* isolates identified by CHROMagar were correctly identified. In contrast, 2 out of 8 *P. kudriavzevii* isolates and 6 out of 9 *N. glabratus* isolates were wrongly identified by CHROMagar subculturing (Fig. 1, Panel B). In total, CHROMagar correctly identified 81.1% of isolates compared to MALDI-TOF.

Antifungal susceptibility patterns

Antifungal susceptibility testing was performed on 53 yeast isolates and is summarized in Fig. 2. Amphotericin B and fluconazole were generally effective against *C. albicans*, with susceptibility rates of 95.0% and 77.5%, respectively. *C. albicans*, however, showed high resistance rates to ketoconazole (80.0%) and miconazole (57.5%). Antifungal susceptibility testing for six *P. kudriavzevii* isolates showed high susceptibility to Amphotericin B (83.3%) and clotrimazole (66.7%). Resistance was notable for miconazole (50.0%) and ketoconazole (33.3%). All *P. kudriavzevii* isolates exhibited intermediate resistance to fluconazole (100%), and also itraconazole showed mostly intermediate resistance (83.3%). All *N. glabratus* isolates were resistant to miconazole and ketoconazole, 66.7% were resistant to itraconazole but all were susceptible to Amphotericin B. Additionally, all isolates of *S. cerevisiae and C. lusitaniae* were susceptible to the six antifungal they were tested against.

Birth outcomes

An overview of the birth outcomes is given in Table 3. Of the 217 women enrolled, birth outcomes were collected for 208 participants (95.9%). The majority of women gave birth to live neonates (94.7%), term babies (92.2%), and normal birth weight infants (76.6%). Eleven pregnancies (5.3%) resulted in abortion (1.9%), stillbirth (2.9%), or early neonatal death (0.5%).

Most of the births (92.4%) were through spontaneous vaginal delivery, and at health institutions (84.8%). The overall prevalence of adverse birth outcomes was 30.9 (63/204) (95% CI:24.6%, 37.7%); 20.6% of these (13/63) were preterm births.

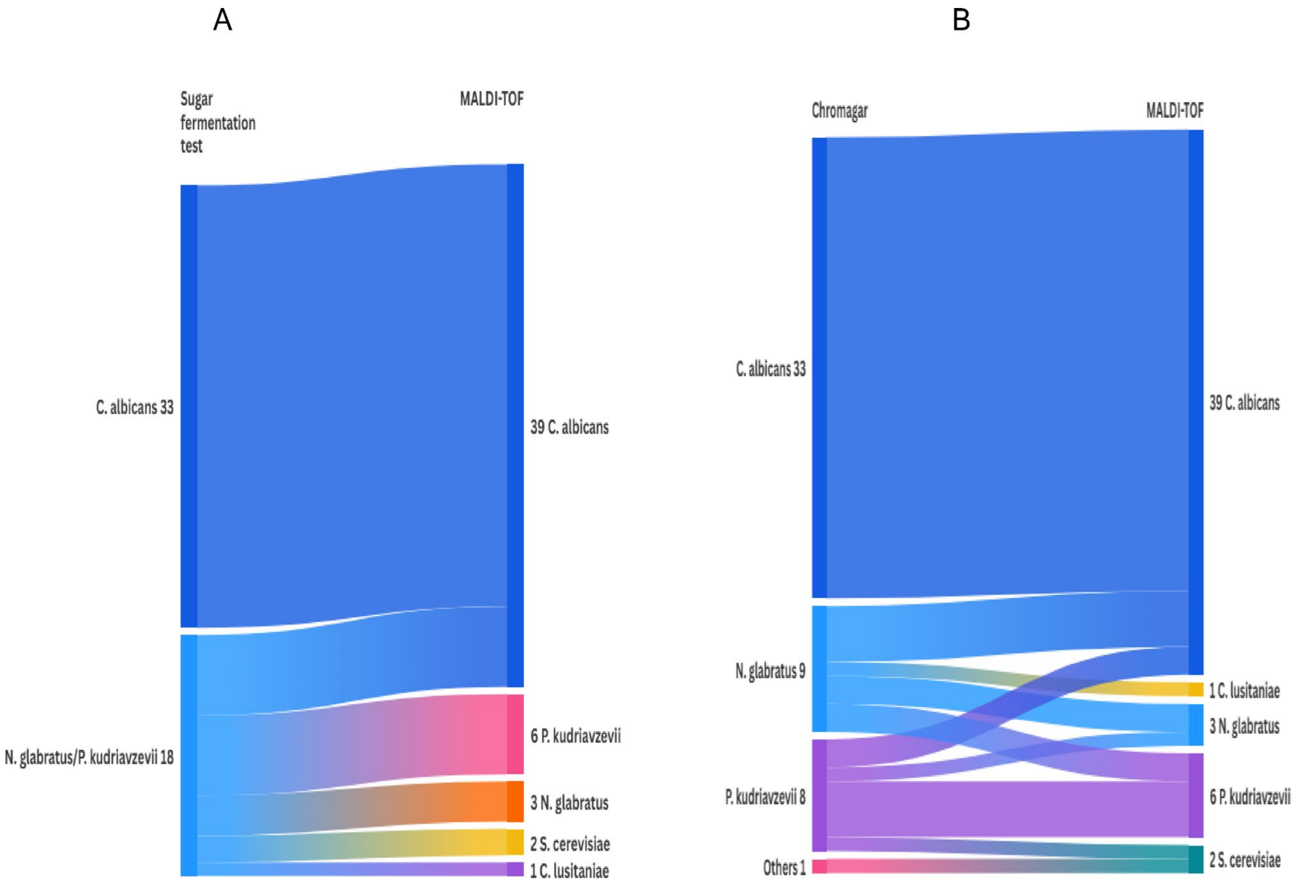

Fig. 1. Identification of yeast isolates by means of sugar fermentation test (**A**) or CHROMagar (**B**) compared to MALDI-TOF.

Associations of BV or vaginal *Candida* colonization with adverse birth outcomes

An overview of the association of BV and/or vaginal *Candida* colonization with adverse birth outcomes is given in Table 4. Stillbirth and early neonatal death; were not considered due to the low number of cases. Women positive for BV were 1.89 times (AOR = 1.89; 95% CI: 1.22, 4.97) higher odds for an adverse birth outcome and 3.89 times (AOR = 3.89; 95%CI: 1.24, 14.11) more likely to have a preterm birth compared to women without BV. However, BV was not statistically significant associated with low birth weight. Moreover, vaginal *Candida* colonization was not statistically associated with (any of the) adverse birth outcomes.

Factors associated with BV

The bivariate and multivariable analyses are shown in Table 5. In the bivariate analysis, residence, educational status, number of antenatal care visits, vaginal symptoms, history of miscarriage/abortion, vaginal douching, number of sex partners, and use of antibiotics were associated with BV at the $p < 0.25$ level. In the multivariable analysis, the use of antibiotics and vaginal douching were statistically significantly associated with BV ($p < 0.05$). Pregnant women who reported to vaginal douching were about seven (AOR = 6.93; 95% CI: 2.86, 16.77) times more likely to have BV compared to women who did not. Pregnant women who reported using antibiotics during the current pregnancy were almost ten (AOR = 9.47, 95% CI: 4.09, 21.94) times more likely to have BV compared to their counterparts.

Factors associated with vaginal *Candida* colonization

The bivariate and multivariable analyses are shown in Table 6. In the bivariate analysis, vaginal douching, chewing khat, vaginal symptoms, vaginal hygiene after each vaginal sexual intercourse, the use of antibiotics, a history of miscarriage/abortion, and gravidity were associated with vaginal *Candida* colonization at $p < 0.25$. In the multivariable analysis, only use of antibiotics and vaginal douching were statistically significantly with vaginal *Candida* colonization ($p < 0.05$). Pregnant women who reported to vaginal douching were about five (AOR = 5.48, 95% CI: 2.39, 12.56) times more likely to have vaginal *Candida* colonization as compared to women who did not. Moreover, pregnant women who reported using antibiotics during their current pregnancy were four (AOR = 4.18, 95% CI: 1.79, 9.80) times more likely to have vaginal *Candida* colonization as compared to their counterparts.

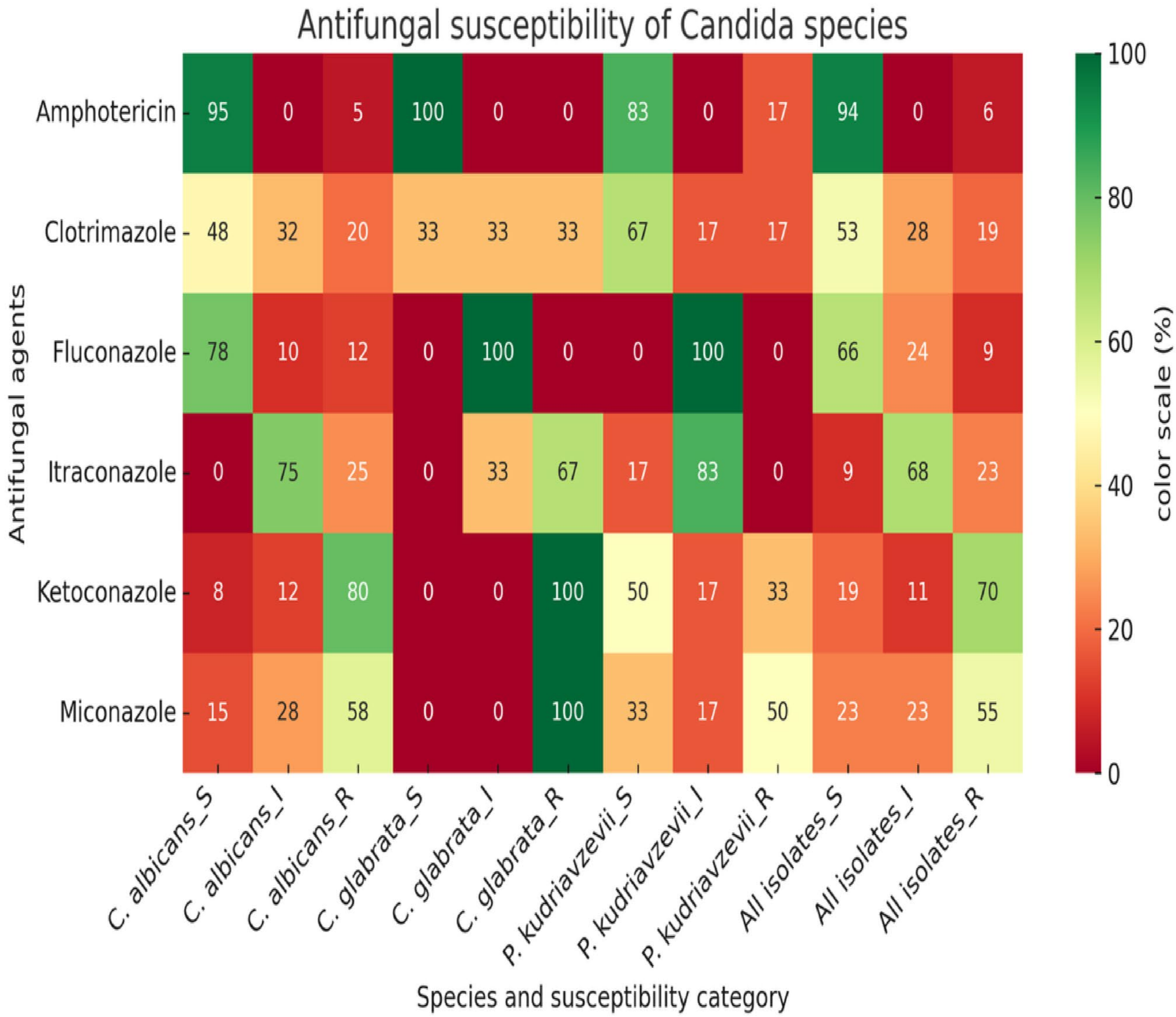

Fig. 2. Antifungal susceptibility patterns of *Candida* isolates. Legend: The colour coded graph shows patterns of susceptible (S), intermediate (I) and resistant (R) isolates, resistance pattern ranging from 0% (red) to 100% (green).

Discussion

This study aimed to assess the prevalence, and associated factors of BV and vaginal *Candida* colonization, antifungal susceptibility patterns, and association of BV and *Candida* colonization with adverse birth outcomes among pregnant women in eastern Ethiopia. Out of the 217 participants, 94 (43.3%) (95% CI: 36.6%, 50.2%) were found to have either BV or vaginal *Candida* colonization, or both, with 7.8% (17 out of 217) experiencing both conditions concurrently. The prevalence of BV and vaginal *Candida* colonization was 27.7% (95% CI: 21.81%, 34.11%) and 23.5% (95% CI: 18.35%, 29.57%), respectively. Major contributing factors for both BV and vaginal *Candida* colonization were antibiotic use and vaginal douching, and BV increased the likelihood of ABOs. *C. albicans* was the most prevalent yeast, accounting for 75.5% (95% CI: 61.7, 86.3) of isolates. Amphotericin B was the most effective antifungal agent against yeast isolates, with an overall sensitivity of 94.3% among isolates. Although 7.8% of women had both BV and VCC, regression analyses treated BV and VCC as separate binary outcomes, with co-infected women classified as positive for both; this group was not analyzed separately due to small numbers.

Prevalence of BV and vaginal Candida carriage

In this study, the prevalence of BV was 27.7% (95% CI: 21.81%, 34.11%). Our findings align with the prevalence of BV in sub-Saharan Africa reported by Park et al. (2024), which was 25.1% in pregnant women between 2000 and 2020[13]. Within the global context, our findings also align with the prevalence of BV among pregnant women: 33% in Latin America and the Caribbean, 26% in the Middle East and North Africa, and 29% in sub-Saharan Africa[12]. The BV prevalence in our study is also comparable to findings from other sub-Saharan Africa

Characteristics	Categories	Frequency	(%)
Birth outcome (208)	Live birth	197	94.7
	Stillbirth	6	2.9
	Abortion	4	1.9
	Neonatal death	1	0.5
Live birth based on gestational age (*n* = 197)	Term	182	92.2
	Preterm	13	6.6
Small for gestational age (*n* = 197)	Yes	0	0
	No	197	94.7
Birth weight (*n* = 197)	Normal	151	76.6
	Macrosomia	36	18.3
	Low birth weight	10	5.5
Total ABO#		63	30.8
Mode of birth (*n* = 197)	Vaginal	182	92.4
	Cesarean section	15	7.6
Place of delivery (*n* = 197)	Health center	48	24.4
	Health post	8	4.1
	Hospital	106	53.8
	Home	30	15.2
	Private health facility	5	2.5

Table 3. Birth outcomes among the pregnant women. Legend: # ABO; Adverse birth outcome.

Outcome	Exposure	% in outcome group	% in reference group	COR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
ABO	BV	39.3	60.7	1.98 (1.03–3.78)	**0.01**	**1.89 (1.22–4.97)**	**0.03**
ABO	VCC	30.4	69.6	1.54 (0.78–3.05)	0.21	1.51 (0.66–4.54)	0.34
PTB	BV	61.5	38.5	4.06 (1.27–12.98)	**0.04**	**3.89 (1.24–14.11)**	**0.02**
PTB	VCC	46.2	53.8	2.65 (0.85–8.29)	0.06	2.59 (0.72–9.54)	0.08
LBW	BV	40.0	60.0	1.56 (0.42–5.74)	0.501		
LBW	VCC	30.0	70.0	1.24 (0.31–4.99)	0.761		

Table 4. Associations of BV and/or vaginal *Candida* colonization with adverse birth outcomes. Legend. COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval; VCC, vaginal *Candida* colonization; PTB, preterm birth; LBW, low birth weight.

studies conducted in Zimbabwe (32.6%)[34] and Ghana (30.9%)[35]. However, it is lower than the prevalence reported in Sudan (49.8%)[36] and Ethiopia (48.6%)[37], but higher than that reported in other regions of Ethiopia, including Mekelle (20.1%)[4], Addis Ababa (19.4%)[38] and Harar City (21.4%)[39]. The variation in BV prevalence across studies can be attributed to several factors, including differences in diagnostic methods, study settings and population, and microbial ecology[40,41], socioeconomic status and healthcare access[42], as well as (sexual) behavioral and/or hygiene practices[43,44].

The prevalence of vaginal *Candida* colonization in our study was 23.5% (95% CI: 18.35%, 29.57%), which is comparable with findings from other Ethiopian regions, such as Debre Markos (25%)[45] and Bule Hora (26.8%)[46]. Moreover, our findings align with the regional prevalence of vaginal *Candid*a colonization in sub-Saharan Africa. In pregnant women, the prevalence was 29.2% between 1996 and 2020. The highest prevalence was observed in Eastern Africa (35%), followed by Western Africa (28%)[47]. The vaginal *Candida* colonization prevalence in our study was lower compared to other studies from Africa conducted in Lebanon (44.8%)[48], Nigeria (60.8%)[49], Kenya (90.38%)[50], and Saudi Arabia (70.2%)[51]. The variation in studies can be explained by several factors, including differences in the (gestational) age of the study population at inclusion[46,52], diagnostic methods[53], the use of antibiotics[54], environmental conditions[46], and healthcare and/or socioeconomic factors[55].

C. albicans was the predominant *Candida* species colonizing the vaginal Microbiome

In our study, *C. albicans* was the most prevalent yeast, accounting for 75.5% (95% CI: 61.7, 86.3) of isolates. Other species made up 24.5% of isolates (95% CI: 13.8, 38.3), with *P. kudriavzevii* (11.3%) and *N. glabratus* (5.7%) as the most common. *C. lusitaniae* and *S. cerevisiae* were also detected (3.8% each).

Our findings align closely with a recent systematic review and meta-analysis that pooled data from 17 studies across sub-Saharan Africa. The review reported that 71.7% of 2112 vaginal *Candida* isolates were *C. albicans*, with *N. glabratus* (40.9% of non- albicans), *C. tropicalis* (22.7%), and *P. kudriavzevii* (21.2%) being the leading non- albicans yeast[56]. Difference might be due to amongst others variations in diagnostic methods[53], as is also apparent from our findings comparing CHROMagar, sugar fermentation tests and MALDI-TOF MS

Characteristics		BV status		COR (95%CI)	p-value	AOR (95%CI)	p-value
		Positive N (%)	Negative N (%)				
Residence	Urban	50 (31.25)	110 (68.75)	1	0.150	1	0.460
	Rural	10 (17.54)	47 (82.45)	0.47 (0.22, 1.00)		1.87 (0.36, 9.81)	
Age (in years)	<=24	28 (25)	84 (75)	0.56 (0.13, 2.48)	0.435		
	25–34	29 (29.9)	68 (70.1)	0.71(0.16, 3.17)	0.373		
	>=35	3 (37.5)	5 (62.5)	1			
Educational status	No formal education	15 (18.5)	66(81.5)	0.26 (0.18, 0.83)	0.212	0.21 (0.62,1.67)	0.116
	Primary school	3 (60)	2 (40)	1.71 (0.22,13.41)	0.608	0.39 (0.03, 5.89)	0.495
	Secondary school	35 (30.2)	81(69.8)	0.49 (0.17, 1.47)	0.204	0.31 (0.06, 1.47)	0.139
	College and above	7(46.67)	8 (53.3)	1		1	
Number of ANC visit	1	51 (29.5)	122 (70.5)	1.63 (0.729, 6.04)	0.231	1.42 (0.68, 3.11)	0.376
	≥ 2	9 (20.5)	35 (79.5)	1		1	
Chewing khat*	No	33 (23.1)	110 (76.9)	1	0.381		
	Yes	27 (36.5)	47 (63.5)	1.91 (1.04, 3.53)			
Vaginal douching*	No	26 (16.1)	136 (83.9)	1	**0.001**	1	**0.001**
	Yes	34 (61.8)	21 (38.2)	8.47 (3.26, 16.23)		6.93 (2.86, 16.77)	
Vaginal burning*	No	52(25.5)	152(74.5)	1	0.401		
	Yes	8(61.5)	5(38.5)	4.68(1.90, 13.42)			
Lower abdominal pain*	No	50(25)	150(75)	1			
	Yes	10(58.8)	7(41.2)	4.29(1.55, 11.86)	0.305		
Genital ulcer*	No	57 (26.8)	156 (73.2)	1	0.371		
	Yes	3 (75)	1 (25)	8.21 (0.84, 80.55)			
Vaginal discharge*	No	50 (26.9)	136 (73.1)	1	0.536		
	Yes	10 (32.3)	21 (67.7)	1.29 (0.57, 2.94)			
Vaginal itching*	No	9 (47.4)	10 (52.6)	2.59 (0.99, 6.74)	0.404		
	Yes	51 (25.8)	147 (74.2)	1			
Vaginal symptoms #	No	51 (30.2)	118 (69.8)	1.87 (0.85, 4.15)	0.118	1.58 (0.96, 4.85)	0.119
	Yes	9 (18.8)	39 (81.2)	1		1	
History of miscarriage/abortion	No	20 (36.4)	35 (63.4)	1		1	
	Yes	40 (24.7)	122 (75.3)	1.74 (0.91, 3.36)	0.094	1.28 (0.89, 3.41)	0.681
History of antibiotics use##	No	16 (11.3)	125 (88.7)	1	**0.001**	1	**0.001**
	Yes	44 (57.9)	32 (42.1)	10.74 (5.38, 21.45)		9.47 (4.09, 21.94)	
Number of sex partners*	1	52 (26.1)	147 (73.9)	1	0.096	1	0.086
	≥ 2	8 (44.4)	10 (55.6)	2.26 (0.85, 6.04)		2.25 (0.81, 6.11)	
Gravida	1	40 (31)	89 (69)	1	0.482		
	≥ 2	20 (22.7)	68 (77.3)	0.65 (0.35, 1.22)			
Vaginal *Candida* colonization	Yes	17 (33.3)	34 (66.7)	1	0.299		
	No	43 (25.9)	123 (74.1)	1.43 (0.73, 2.82)			
Ever had sexual intercourse with another partner	Yes	8 (33.3)	16 (66.7)	1.36 (0.55, 3.36)	0.436		
	No	52 (26.9)	141 (73.1)	1			
Take folic acid supplements*	Yes	23 (28.8)	57 (71.2)	1	0.782		
	No	37 (27.0)	100 (73.0)	1.09 (0.82, 2.98)			

Table 5. Bivariate and multivariable analysis for factors associated with BV. Legend. *During current pregnancy; # Vaginal itching, genital ulcer, vaginal burning, and vaginal discharge; ##Systemic antibiotic use during current pregnancy for less than three months.

as identification methods. Our findings highlight that researchers should employ reliable laboratory diagnostic methods to detect non-albicans species.

Antifungal susceptibility patterns

According to the Ethiopian national guidelines for syndromic management of sexually transmitted infections (STIs), clotrimazole is recommended as the first-line treatment for vaginal discharge syndromes, with fluconazole listed as an alternative. In specific cases — particularly among women living with HIV — oral ketoconazole is recommended, based on the assumption that topical agents may be less effective in immunocompromised individuals[57].

Characteristics		Vaginal *Candida* colonization Positive N (%)	Negative N (%)	COR (95%CI)	P-value	AOR (95%CI)	P-value
Residence	Rural	9 (20.5)	35 (79.5)	0.80 (0.36, 1.80)	0.593		
	Urban	42 (24.3)	131(75.7)	1			
Educational status	No formal education	20 (24.7)	61(75.3)	2.13 (0.44, 10.30)	0.342		
	Primary school	1 (20)	4 (80)	1.63 (0.11,13.98)	0.711		
	Secondary school	28 (24.1)	88 (75.9)	2.07 (0.44, 5.47)	0.357		
	College and above	2 (13.3)	13 (86.7)	1			
Age of pregnant mother	<=24	29 (25.9)	83 (74.1)	1			
	25–34	21 (21.6)	76 (78.4)	0.79 (0.42, 1.50)	0.471		
	> 35	1 (12.5)	7 (87.5)	0.41 (0.05, 3.47)	0.412		
Number of ANC visit	1	39 (22.5)	134 (77.5)	1			
	≥ 2	12 (27.3)	32 (72.7)	1.29 (0.61, 2.74)	0.509		
Chewed khat*	No	30 (21)	113 (79)	1	0.224	1	0.394
	Yes	21 (28.4)	53 (71.6)	1.50 (0.78, 2.84)		0.70(0.30, 1.60)	
Vaginal douching	No	22(13.6)	140(86.4)	1	**0.001**	1	**0.001**
	Yes	29 (52.7)	26 (47.3)	7.10 (3.54, 14.21)		5.48 (2.39, 12.56)	
Vaginal itching*	No	43 (21.7)	155 (78.3)	1	0.512		
	Yes	8 (42.1)	11 (57.9)	2.62 (0.99, 6.92)			
Lower abdominal pain*	No	44 (22)	156 (78)	1	0.831		
	Yes	7 (41.2)	10 (58.8)	2.48 (0.89, 6.90)			
Vaginal discharge*	No	42 (22.6)	144 (77.4)	1	0.434		
	Yes	9 (29)	22(71)	1.40 (0.60, 3.28)			
Vaginal burning*	No	49 (23.6)	159 (76.4)	1.08 (0.22, 5.36)	0.411		
	Yes	2 (22.2)	7 (77.8)	1			
Genital ulcer*	No	50 23.5)	163 (76.5)	0.92 (0.09, 9.05)	0.326		
	Yes	1 (25.0)	3 (75.0)	1			
Vaginal symptoms#	No	43 (25.4)	126 (74.6)	1	0.206	1	0.208
	Yes	8 (16.7)	40 (83.3)	0.59 (0.25, 1.35)		0.47 (0.23, 1.63)	
Vaginal hygiene after sexual intercourse	No	21(15.1)	118 (84.9)	1	0.072	1	0.711
	Yes	30 (38.5)	48 (61.5)	3.51(1.83, 6.73)		0.84 (0.34, 2.08)	
Uses of antibiotics##	No	17 (12.1)	124 (87.9)	1	**0.001**	1	**0.001**
	Yes	34 (44.7)	42 (55.3)	5.90 (2.99, 11.64)		4.18 (1.79, 9.80)	
Ever had sexual intercourse with another partner	No	45 (23.3)	148(76.7)	1	0.852		
	Yes	6 (25)	18 (75)	1.09 (0.34, 2.44)			
History of miscarriage/abortion	No	39 (70.9)	16 (29.1)	1	0.112	1	0.132
	Yes	12 (7.4)	150 (92.6)	0.03 (0.01, 1.14)		0.02 (0.01, 1.01)	
Gravidity	1	21 (16.3)	108 (83.7)	1	0.112	1	0.202
	≥ 2	30 (34.1)	58 (65.9)	2.66 (1.39, 5.06)		2.64 (1.39, 5.09)	
Number of sexual partners*	1	45 (22.6)	154 (77.4)	1	0.304		
	≥ 2	6 (33.3)	12 (66.7)	1.71 (0.61, 4.82)			
BV	Yes	17 (28.3)	43 (71.7)	1	0.299		
	No	34 (21.7)	123 (78.3)	1.43 (0.73, 2.82)			
Take folic acid supplements*	Yes	23 (28.8)	57 (71.2)	1	0.165	1	0.385
	No	28 (20.4)	109 (79.6)	1.57 (0.83, 2.97)		1.52 (0.33, 1.53	

Table 6. Bivariate and multivariable analysis for factors associated with vaginal *Candida* colonization. Legend. *During current pregnancy; # Vaginal itching, genital ulcer, vaginal burning, and vaginal discharge; ##Systemic antibiotic use during current pregnancy for less than three months.

Our findings raise some concerns regarding the alignment between these national recommendations and our observed antifungal susceptibility patterns. Only 47.5% of the *C. albicans* isolates were susceptible to clotrimazole. This result is however higher than pooled estimates from a recent meta-analysis in Ethiopia (Melaku et al., 2025, unpublished), which reported resistance to clotrimazole to be 5% (95% CI: 1.0%, 9.0%) in vaginal *C. albicans* isolates from SSA.

Similarly, ketoconazole, recommended in HIV-associated candidiasis, demonstrated very poor performance in our study, with 80% resistance in *C. albicans* and 100% resistance in *N. glabratus*. These resistance levels are concerning, particularly in light of the Ethiopian guideline's exception for oral ketoconazole use in HIV-positive patients. The continued use of ketoconazole in such high-risk populations without local susceptibility data may lead to treatment failures and contributes to the broader problem of antifungal resistance.

Fluconazole, recommended as a second-line agent, showed 77.5% susceptibility in our *C. albicans* isolates. While this is relatively reassuring, it contrasts with meta-analytic estimates showing pooled resistance at 48% (95% CI: 22%, 73%) for *C. albicans*, and even lower for non-albicans species. These discrepancies may reflect geographic variability.

In contrast, amphotericin B was the most effective antifungal agent in our study, with 94.3% of isolates susceptible, including (nearly) all *C. albicans* and *N. glabratus* isolates, the resistance in *P. kudriavzevii* was 17%. This overall high level of susceptibility might be influenced, at least in part, by its limited use in routine syndromic care, where it is neither recommended nor widely available. However, its high efficacy also reflects its fungicidal mechanism of action (while azoles are fungistatic), which targets ergosterol directly, and the relatively low intrinsic resistance of *Candida* species to this compound[58]. Thus, while its preserved activity may be due to low selective pressure, drug-specific factors also play a significant role.

Our study also underscores the clinical implications of species distribution. *C. albicans* accounted for 75% of all isolates, followed by *P. kudriavzevii* (11.3%) and *N. glabratus* (5.7%). Both *P. kudriavzevii* and *N. glabratus* demonstrated high levels of resistance to commonly used azoles. Notably, all *N. glabratus* isolates were resistant to miconazole and ketoconazole, and two-thirds were resistant to itraconazole. *P. kudriavzevii* showed 50% resistance to miconazole and reduced susceptibility to several azoles. These findings are consistent with their well-documented intrinsic resistance profiles and highlight the risks of empiric azole therapy in areas with a significant burden of non-albicans species.

BV and vaginal *Candida* carriage were independently associated with vaginal douching and the use of antibiotics in pregnancy

Vaginal douching involves rinsing or washing out the vagina with a liquid solution, typically for hygienic or cultural reasons[59,60]. Women who reported vaginal douching – this was mostly done with plane water – were about sevenfold and sixfold more likely to have BV or carry *Candida* vaginally, respectively. Our findings align with meta-analysis done by Low et al.[61] and studies conducted in Tanzania[62] (for BV) and Cameroon[63] (for VCC). It is well-documented that vaginal douching disrupts the natural balance of the vaginal microbiota, increasing the risk for BV, VCC and preterm birth[59,64].

The use of antibiotics during their current pregnancy increased the odds of BV or VCC by nearly tenfold and fourfold, respectively. The association may be attributed to the impact of antibiotics on lactobacilli, as there is an inverse relationship between the presence of these protective lactobacilli and the development of vaginitis[65].

Raising awareness about the risks of vaginal douching and the misuse of antibiotics is critical for improving public health. Vaginal douching disrupts the natural vaginal microbiome, increasing the risk of BV and VCC, both of which can negatively impact birth outcomes. The inappropriate use of antibiotics contributes to antimicrobial resistance, complicating infection management and posing serious global health challenges.

BV was associated with adverse birth outcomes

In this study, the overall ABO prevalence was 30.9 (63/204) (95% CI:24.6%, 37.7%), which is in line with regional estimates reported in large-scale analyses of DHS data from sub-Saharan Africa (SSA). One review pooling data from 10 countries estimated an ABO prevalence of 29.7% (95% CI: 29.4%–30.0%)[66], while a more recent analysis from 2024 across 26 SSA countries reported a prevalence of 28.6% (95% CI: 28.4%–28.8%)[67]. Our finding also align with a study from Debre Berhan (Ethiopia) (28.3%) (Tadese et al., 2022), but is notably higher than those reported in Bale (Ethiopia) (21%)[68], Awi (Ethiopia) (19.4%)[69], and Hawassa (Ethiopia) (18.3%)[70]. The observed variation likely reflects differences in population characteristics, socio-economic conditions, healthcare access, and differences in the definition and classification of ABOs across studies.

In our study, women with BV had approximately twice the odds of any ABO and about four times the risk of preterm birth. This finding is supported by a previous meta-analysis, which reported that women with BV had more than twice higher odds of preterm birth[71]. Although our study only shows an association and not a causation, BV might be linked to preterm birth by ascending BV associated bacteria that lead tointra-amnionic infections and inflammation, increasing the risk of preterm labor and poor fetal growth[72]. However, recent evidence from an individual participant data meta-analysis suggests that antibiotic treatment of asymptomatic BV does not significantly reduce the risk of preterm delivery, highlighting the complexity of this association and the need for further mechanistic and interventional research[73].

To reduce the incidence of BV infection in pregnant women, various interventions could be considered, including raising awareness about the risks of BV, emphasizing the significance of early prenatal care, and preventing infections during pregnancy.

Strengths and limitations

This study contributes significantly to the understanding of BV and *Candida* colonization among pregnant women in Eastern Ethiopia, highlighting important contributing factors and guiding future research. The study was conducted within Health and Demographic Surveillance Sites, making it representative of similar settings. However, since recruitment happened in early pregnancy and at health facilities, women who were unaware of their pregnancy or had not sought antenatal care might have been missed. Additionally, the presence of vaginal discharge was based on self-report rather than clinical examination, which may have introduced reporting bias. Specific characteristics such as color, odor, and consistency were also not assessed. Moreover, the cross-sectional study design may limit its ability to determine causal relationships between variables. Finally, the small number of participants with both BV and VCC limited our ability to analyze this group separately in regression models.

Conclusions

In this prospective cohort study among pregnant women in Eastern Ethiopia, bacterial vaginosis and vaginal *Candida* colonization were found to be highly prevalent and frequently co-occurring, affecting over 40% of participants. Both conditions were strongly associated with modifiable behavioural factors, particularly vaginal douching and recent antibiotic use. C. *albicans* was the predominant species, but non-albicans yeasts—including intrinsically resistant species—accounted for one in four isolates, emphasizing the need for accurate speciation. Notably, in vitro antifungal resistance was high for several azoles commonly recommended in syndromic treatment guidelines, while amphotericin B retained broad activity. BV, but not *Candida* colonization, was independently associated with increased odds of adverse birth outcomes, including preterm birth.

These findings call for improved clinical awareness of local pathogen profiles, refinement of empirical treatment strategies, and deeper investigation into the mechanistic links between vaginal dysbiosis and pregnancy outcomes. A better understanding of the role of non-albicans species and emerging resistance patterns will be essential to guide diagnostics and antimicrobial stewardship in maternal health.

Data availability

The data sets generated during and/or analyzed during the current study are available in this manuscript.

Received: 6 June 2025; Accepted: 27 October 2025
Published online: 26 November 2025

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Acknowledgements
We would like to express our sincere gratitude to Haramaya University and Ghent University for funding and cofounding this study, respectively. We would like to thank Haramaya University Colleges of Health and Medical Sciences Medical Microbiology Laboratory, the Hararghe Health Research Laboratory, and Ghent University's Laboratory Bacteriology Research for providing the necessary laboratory facilities and technical support. Finally, we thank the study participants and all individuals who contributed in any way to the successful completion of this research.

Author contributions
The study concept and design were conceived by AKT, DAA, TW, AG, TAY, MD, FW, and NA. FW, ASN, TT, YMD, TG, KTR, AA, AAN, PC, FT, and TNA refined the study questionnaires, and study design, and supervised the data collection. The sample processing was done by FW, ASN, FT, BVE, LH and TT, and laboratory work was supervised by DAA and PC. Data analyses were conducted by FW and ASN with the help of statisticians. FW prepared the first draft of the manuscript with close support from PC and AKT. All authors critically revised and approved the final version of the manuscript.

Funding
This study was funded by Haramaya University (grant number: HUGG_2021_02_04_26). FW was partially funded by Ghent University (BOF grant number: 01W01223). The funding bodies had no role in the design of the study, data collection, data analysis, interpretation, or publication.

Declarations

Competing interests
The authors declare no competing interests.

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