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The prevalence and risk factors of vaginal Candida species and group B Streptococcus colonization in pregnant women attending antenatal care at Hawassa university comprehensive specialized hospital in Hawassa City, Southern Ethiopia

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Abstract

Background The global prevalence of vaginal candidiasis and group B streptococcus (GBS) colonization among pregnant women is significant and these pathogens are associated with adverse maternal and neonatal outcomes, including preterm birth, stillbirth, and neonatal infections.

Objective This study aimed to determine the magnitude and risk factors for vaginal *Candida* and GBS in pregnant women who were attending antenatal care at Hawassa University Compressive Specialized Hospital from July October 2021.

Method A Hospital-based, cross-sectional study was conducted using microscopy, culture, germ tube, and biochemical tests on vaginal swab samples from 110 volunteer pregnant women. A structured questionnaire was used to collect data on perceived risk factors. Data was analyzed using SPSS version 22, and an odds ratio at a 95% confidence interval with p < 0.05 was used to interpret the risk factors.

Results Candida species was identified in 33 (30%) pregnant women, whereas, GBS colonization was not detected in any of them. Of the vaginal Candida species, 17 (51.52%) were Candida albicans and 16 (48.48%) were non-albicans Candida. Symptomatic vaginal candidiasis was diagnosed in only four women. The most important predictors of vaginal Candida colonization were parity of two and underwear replacement once a day.

Conclusion Based on these findings, screening for vaginal candidiasis and prophylactic treatment should be considered for young, multiparous, pregnant women in their third trimester, if supported clinically.

Keywords Candida albicans, Colonization, GBS, Hawassa, Pregnant women



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Introduction

Reduction of maternal and child mortality is a key component of the global sustainable development goals [1]. Despite efforts, this issue persists, with thousands of women dying yearly from pregnancy, childbirth, and postpartum complications. In 2020, a total of 287,000 women worldwide died due to maternal causes, which translates to an average of 223 maternal deaths for every live birth [2]. Developing countries bear the brunt of these figures, with Sub-Saharan Africa and southern Asia accounting for 80% of five million under-5 deaths in 2020, despite only making up 53% of global live births [3]. Ethiopia is one of six countries responsible for over half of maternal deaths globally [4, 5]. Vulvovaginal candidiasis (VVC) and group B streptococcal (GBS) diseases, are among the major causes of maternal and child morbidity and mortality.

Candida species are part of the human microflora that inhabit mucosal surfaces such as the gastrointestinal and urogenital tracts [6]. They usually live on the skin and mucosal surfaces of the body, including the mouth, throat, gut, and vaginal mucosa, without causing harm [7]. However, some Candida species can cause opportunistic infections when the host immunity is weakened. Candidiasis can range from superficial skin disorders to life-threatening conditions [8, 9]. Candida albicans is the most common cause of vaginitis and vulvovaginal infection [10]; however, 75% of infected women are asymptomatic [11]. Virulence factors of Candida species and host physiology/immunity determine infection severity [12, 13].

The key virulence factors of Candida include adhesins, calcineurin, and extracellular enzymes [7, 10, 14]. Adhesins help Candida recognize and attach to host cells, facilitating colonization and biofilm formation [15]. Calcineurin plays a role in the fungus's response to stress, enhancing its ability to survive in the host. Extracellular (secreted) enzymes, such as lipases and phospholipases, damage host cell membranes and evade immune responses [16]. Candida also adapts to its environment during infection, with morphological changes that allow it to thrive in different tissue niches. Morphological changes in C. albicans help it adapt to different biological niches due to environmental shifts [17]. Each form of Candida exhibits varying levels of virulence, which is important for understanding its pathogenic potential and addressing public health concerns related to infections [18].

Genetics, pregnancy, diabetes, antibiotics, immunosuppressant, oral contraceptives, vaginal estrogen, hygiene, sexual behavior, humid weather, and feminine hygiene products can increase symptomatic infections [10]. Pregnancy is a well-known risk factor for vulvovaginal candidiasis, with 10–15% of pregnant women

experiencing colonization, and this figure rising to 30% in the third trimester [19]. Progesterone and estrogen levels increase during the third trimester of pregnancy. Progesterone reduces neutrophil anti-Candida activity, whereas estrogen weakens the ability of vaginal epithelial cells to inhibit *Candida albicans* growth [20]. Symptoms of vulvovaginal candidiasis (VVC) include itching, whitish discharge, odor, edema, pain, and vulvar redness. Untreated VVC increases the risk of abortion, premature delivery, and bloodstream infections in preterm infants [21–23].

In addition to Candida species, another significant threat to newborns is Streptococcus agalactiae, commonly known as Group B Streptococcus (GBS) [24]. It is an encapsulated gram-positive bacterium that inhabits the lower gastrointestinal and urogenital tracts in 20-30% of healthy adult women [25, 26]. During pregnancy, approximately 10-30% of women experience vaginal colonization with GBS, and 60% of their infants acquire this organism through the birth canal [27]. Infection by invasive strains can cause maternal, fetal, and early onset neonatal diseases (days 0-6), leading to maternal death, stillbirth, and/or neonatal death. Genital colonization is a major risk factor for early neonatal sepsis. GBS vaginal colonization varies between 12 and 27% worldwide; however, the prevalence varies from place to place [26].

There are limited published data on the prevalence of both VVC and GBS colonization rates in pregnant women in Ethiopia, particularly in Hawassa. In one study conducted in Debre Markos Hospital, Amhara region of Ethiopia, 96 of the 384 (25%) pregnant women investigated were positive for VVC [28]. *Candida albicans* (56.25%) was the predominant cause, and contraceptives and prolonged antibiotic use were associated risk factors. Similarly, 24 of 126 (19%) pregnant women were positive for GBS vaginal colonization in Jimma, Ethiopia [29]. In another study conducted in Addis Ababa, 41 of 281 (14.6%) pregnant women were positive for GBS vaginal colonization [30].

The paucity of data on the role of these pathogens in maternal and child morbidity and mortality in the southern region of Ethiopia, particularly in Hawassa, prompted this work. This study aimed to answer the following research question: What is the prevalence of vaginal *Candida* species and GBS colonization among pregnant women attending antenatal care at HUCSH? What are the key risk factors associated with vaginal *Candida* species and GBS colonization in this population? How do the prevalence rates of vaginal *Candida* species and GBS colonization compare between different age groups and socioeconomic statuses among pregnant women? The findings in this work can be used to identify and prioritize high-risk populations for targeted screening and treatment as well as serve as baseline data for further more

comprehensive studies. Moreover, it can also inform local health policies and guidelines for informed-planning of educational campaign during antenatal care to raise awareness and improve preventive measures for better maternal and neonatal health in the community.

Materials and methods

Description of the study area

This study was conducted at the Hawassa University Compressive Specialized Hospital (HUCSH), Hawassa, among randomly selected pregnant women attending the antenatal care clinic during July 2021 and October 2021. Hawassa City, the administrative center of the Sidama Regional State of Ethiopia, 275 km south of Addis Ababa, and is located within the geographic 7^O3'43.38" N latitude and 38^O28'34.86" E longitude, and an altitude of 1,656-2,137 m above sea level. The annual average minimum and maximum temperatures are 13.0 °C and 29.2 °C, respectively, with a mean rainfall of 953.5 mm [31]. HUCSH, a tertiary hospital, and serves approximately 12 million people in the region.

Study design

A hospital-based cross-sectional study was conducted by microbiological laboratory culture of vaginal swab specimens with the aim of determining the prevalence of VVC and GBS colonization among pregnant volunteer women attending the antenatal care clinic at HUCSH. Moreover, a questionnaire-based survey of the sociodemographic, economic, and medical backgrounds of pregnant women was performed to assess the association with perceived risk factors.

Sample size

A total of 110 volunteer pregnant women were enrolled based on a non-probabilistic, convenient sampling approach. This method allowed for efficient recruitment of pregnant women attending antenatal care at the hospital, ensuring accessibility to the target population. Given the limited resources and time for the study, the rapid data collection facilitated by this method was essential in addressing pressing public health concerns related to maternal and neonatal health. While the findings may not be generalizable to the broader population, they provided valuable context-specific insights relevant to the local community.

Questionnaire survey

The study subjects were contacted during a visit to the antenatal care clinic by the attending nurse along with the principal investigator. The objectives of the study were explained and formal written consent was obtained from the volunteers. After this, the prepared questionnaire was administered through face-to-face interviews

to collect sociodemographic backgrounds and other relevant medical histories, including a history of recent use of contraceptives.

Inclusion criteria

Pregnant women aged 18–50 years old, who met the following criteria were included in the study. They were not critically ill, had not recently taken antibacterial or antifungal drugs recently, were HIV negative, and voluntarily consented to participate in the study after being informed the study objectives. These women were attending antenatal care clinic at HUCSH during the study period.

Exclusion criteria

Pregnant women younger than 18 years (likely to have complications) and those beyond 37 weeks of gestation, those who were HIV positive, critically ill patients, and those who were not willing to participate in the study were excluded. The descriptions "not being critically ill" refers to patients who were stable and did not require intensive medical intervention, while complications is defined in terms of specific medical conditions that could affect the study outcomes, such as severe preeclampsia or active infections requiring hospitalization.

Specimen collection

Vaginal swabs were collected according to the standard operating procedures (SOP) of the HUCSH laboratory. Briefly, specimens were collected by the attending midwifery nurse using sterile swabs following aseptic procedures [32]. Two vaginal specimens were collected (one for fungal culture and the other for GBS culture) in separate sterile test tubes containing Amie's transport medium with charcoal, and immediately transported to the microbiology laboratory for immediate processing [33]. During vaginal swab collection, several precautionary aseptic measures were exercised to ensure accurate results and minimize contamination. First and foremost, the trained midwifery nurse, adhered to strict aseptic techniques, beginning with thorough hand washing with soap and water and use of alcohol-based hand sanitizer. Sterile swabs and collection tubes with transport medium were used, all checked for sterility prior to use. Before collection, the pregnant women were informed about the procedure, ensuring they understand the importance of the sample and are comfortable. They were asked if they had menstruation, sexual intercourse in the past 24–48 h at the time of the sample collection to further reduce the risk of contamination. During the collection, the swab was gently inserted into the vaginal canal, rotating it lightly against the vaginal walls to collect an adequate sample without causing discomfort to the patient.

Laboratory culture media and reagents

All microbiological culture media and reagents used in this study were prepared following the instructions of the manufacturer (Merck, Germany).

Candida species isolation and putative identification

The vaginal specimens were inoculated by streaking with a wire loop on Sabouraud's dextrose agar (SDA) medium supplemented with chloramphenicol (0.05 g/ml) and cyclohexamide (0.5 g/l) and incubated at 37 °C for 24–48 h [34]. At the end of the incubation period, typical creamy, white colonies were picked separately for purification by repeated subculturing. The purified isolates were stored in an SDA slant at refrigerator temperature (4 °C) until further confirmation by microscopic examination and germ tube test. SDA is effective for the growth of various *Candida* species, making it a standard method for detection.

The germ tube test [35] was performed to differentiate *C. albicans* from non-albicans *Candida* (NAC) by taking a well-isolated colony and transferring it into a sterile test tube containing 0.5 ml human serum and incubating it at 37 °C for 3 h. After this, a loopfull of the suspension was placed on a clean sterile microscopic slide and covered with cover glass for microscopic examination under a 40X objective, and the presence or absence of a germ tube was noted. The germ tube test is primarily used to differentiate *Candida albicans* from other *Candida* species. Isolates with a germ tube were putatively identified as *C. albicans*, and those without a germ tube were categorized as NAC [36].

Isolation and identification of group B Streptococcus (GBS)

This was performed according to the method described before [37]. The vaginal specimen was inoculated in Todd-Hewitt broth and aerobically incubated at 37 °C overnight. After 18–24 h, a loop full of broth was streaked onto 5% sheep blood agar plates and incubated for 24 h. The plates were then checked for growth and hemolysis. Negative culture plates were incubated for a further 18–24 h and re-examined. GBS colonies are gray and mucoid, with a small zone of beta-hemolysis. Colony morphology, Gram staining, and biochemical tests (catalase, Christie, Atkins, Munch-Petersen, and bacitracin) were performed to confirm and identify GBS. GBS is CAMP factor-positive and bacitracin-resistant [37].

Data analysis and presentation

Data were entered into Microsoft Excel 2007 and exported to SPSS Version-26 for analysis. Descriptive statistics, frequencies, and percentages were used to summarize data in tables. The $\rm X^2$ test was used to compare the proportion of the study subjects who were positive for the target pathogens among the different

sociodemographic groups. The observed differences in the proportion of positives for the target pathogens among the sociodemographic groups were considered statistically significant at a p-value < 0.05. The crude odds ratio and 95% confidence interval were determined, and for those that showed a statistically significant difference, a further adjusted odds ratio was considered to determine statistical significance.

Ethical considerations

Ethical clearance and approval were obtained from the institutional review board (IRB) of Hawassa University, College of Medicine and Health Sciences, after the submission of the proposal through an official letter of collaboration from the Department of Biology. Verbal and written informed consent was obtained from the study participant's pregnant women before data collection. Additionally, the confidentiality of information was assured throughout the study. Participants who tested positive for the pathogens received follow-up care, which included counseling and information on treatment options.

Results and discussions

Sociodemographic characteristics of the study participants

A total of 110 volunteer, pregnant women were included in the study. The sociodemographic backgrounds of the study participants are presented in bar charts bellow (Fig. 1). The majority (95/110 or 86.36%) of them were in the age group of 18–33 years old, urban dwellers (71/110 or 64.5%), secondary and above educational level (64/110 or 58.18%), house wives (49/110 or 44.45%) and a monthly income of 2501–5000 ETB (Ethiopian currency, Birr) earners.

The sample size in this study was smaller than the 384 pregnant women included in a similar study conducted in the Amhara region of Ethiopia [28]. Another study conducted in Tigray, northern Ethiopia, also involved group B Streptococcus vaginal colonization among 150 pregnant women, a sample size larger than that of the present study [38]. Similarly, a study on the prevalence of vaginal and anorectal colonization among 126 pregnant women was reported in the Jimma, Oromiya region of Ethiopia [29]. We acknowledge the limitations using a small sample size and non-probabilistic convenience sampling to assess the prevalence of vaginal Candida and Group B Streptococcus (GBS) colonization in pregnant women. These methods may reduce statistical power, increasing the risk of overlooking true effects and resulting in limited generalizability, as the findings might not accurately represent the broader population [39]. Nonetheless, the sociodemographic composition of the study participants with regard to age structure, marital status, occupation,

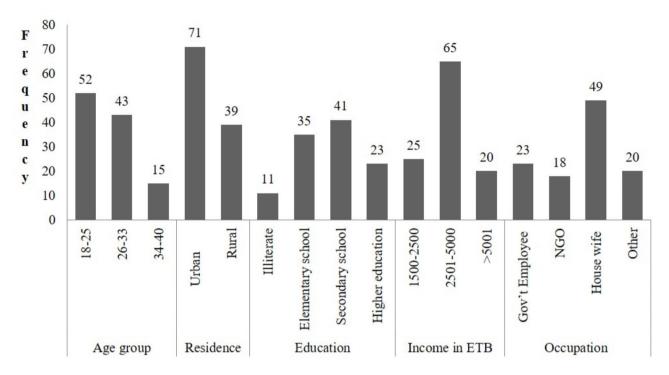


Fig. 1 Sociodemographic backgrounds of the study participants in the survey of prevalence and risk factors of vaginal candidiasis and group B streptococcus colonization in pregnant women attending antenatal care clinic in Hawassa, Ethiopia, 2021

and monthly income status is more or less similar to previous studies in Ethiopia.

Clinical and behavioral characteristics of the pregnant women

The majority of study participants were parous, consisting of 37(33.6%) with one child, 25 (22.7%) with two children, eight (7.3%) with three children, and four (3.6%) with four or more children (Table 1). On the other hand, 36(32.7%) were nulliparous. In terms of the trimester of gestation, most (58.2%) were in their third trimester and 32(29.1%) were in their second trimester. Concerning contraceptive use, 76(69.1%) participants said yes, of which 27 (35.5%) used injections, 24 (31.6%) used Norplant, and 15 (19.7%) used pills. The majority (80%) had no history of chronic diseases, fever (97.3%), flank pain (99.1%), burning sensation during urination (96.4%), obesity (94.5%), or antibiotic use (83.6%). Different criteria have been proposed for the diagnosis of vaginal candidiasis [40-42]. The most common consensus is that signs and symptoms should be substantiated by evidence of a positive vaginal swab culture. Based on this, only four women (3.4%) had symptomatic candidiasis with discomfort and pain on urination and a positive vaginal swab culture.

Considering behavioral features, most participants said they had the habit of consuming sweet foods (54%), beverages (50%), and milk (50%), but not alcohol (98.2%). Regarding hygiene, 69(62.7%) participants practiced

forward wiping after toilet use, and 76(69.1%) replaced their underwear once a day (Table 1). Clinical and sociodemographic variables were included in the present study as perceived risk factors, based on previous reports [28, 43, 44].

Magnitude of vaginal candidiasis and group B Streptococcus colonization

Of the 110 pregnant women who participated in the study, 33 (30%) were positive for vulvovaginal *Candida* colonization based on culture on Sabouraud's dextrose agar (SDA). Among these, 17 (51.52%) were *Candida* albicans and 16 (48.48%) non-albicans *Candida* species. Group B streptococcus was not detected in the specimens from any of the study participants (Fig. 2).

The overall vaginal *Candida* species colonization rate among pregnant women in the present study (30%) was higher than the 25% reported in a similar study conducted in Debre Markos, Ethiopia [28]. This is also higher than the 22.7% vaginal *Candida* colonization rate reported by Burkina Faso [45]. However, it is similar to the 30.6% colonization rate in pregnant women in Ho City, Ghana [23]. In contrast, it is lower than the 36.5% vaginal colonization rate reported in Ghana [46] and 90.38% reported in a study of 104 pregnant women in Beirut, Lebanon [47]. Likewise, vaginal *Candida* colonization in pregnant women, higher than that in the present study, was also reported elsewhere in Aftrica, including 43.8% in Libiya [48] and 55.4% in Cameroon

Table 1 Clinical and other behavioral characteristics of pregnant women who participated in the study of vaginal candidiasis and group B streptococcus carrier state in Hawassa, 2021

Variables	Category	Frequency	%
Parity	Zero	36	33
	One	37	34
	Two	25	23
	Three	8	7.3
	Four or more	4	3.6
Gestational period	1st Trimester	14	13
	2nd Trimester	32	29
	3rd Trimester	64	58
Contraceptive use	Yes	76	69
	No	34	31
Type of contraceptive	Norplant	24	31.6
used	Pills	15	19.7
	Loop	10	13.2
	Injection	27	35.5
History of antibiotics	Yes	18	16
usage	No	92	84
Chronic diseases	Presence	17	16
	Absence	93	85
Flank pain	Yes	1	0.9
	No	109	99
Fever	Yes	3	2.7
	No	107	97
Burning during urination	Yes	4	3.6
	No	106	96
Presence of obesity	Yes	6	5.5
	No	104	95
Eating sweet food	Daily	23	21
	Weekly	60	55
	Not at all	27	25
Drinking sweet food	Daily	21	19
	Weekly	55	50
	Not at all	34	31
Drinking milk	Daily	38	35
	Weekly	65	59
	Not at all	7	6.4
Alcohol consumption	Yes	2	1.8
	No	108	98
Wiping direction after	Forward	69	63
toilet	Backward	41	37
Frequency of underwear	> Once a day	34	31
replacement	Once a day	76	69

[49]. The variation in colonization rates among the studies could be due to several factors, including differences in population, geographic locations, culture, and other sociodemographic factors [26].

In agreement with the present study (51.2%), several studies have reported the predominance of *Candida albicans* in vaginal swab samples. *C. albicans* accounted for 54 (56.25%) of the 96 *Candida* species isolates in a similar

study in Debre Markos [28], 58% of 139 isolates in Turkey [50], 64.04% of 85 isolates in Nepal [51], 80.7% of 52 isolates in Argentina [52], and 92.3% of 90 *Candida* species isolates in Brazil [53]. In contrast, a small number of studies have reported the predominance of non-albicans *Candida* species in vaginal colonization among symptomatic and asymptomatic pregnant women [23, 45, 47].

Regarding GBS, none of the samples yielded positive isolates in the present study. Approximately 10-35% of pregnant women are naturally colonized with GBS in the vagina and/or rectum [54]. However, colonization rates vary by region of the world, study population, specimen collection, and culturing techniques [26]. Few published studies have investigated the incidence of vaginal GBS colonization in pregnant women in Ethiopia. The reported colonization rates ranged from 7.3% [55] to 14.6% [30] in Addis Ababa, 17.4% in Gondor [56], and 19% in Jimma, southwestern Ethiopia [29]. The absence of GBS in the present study may be due to the smaller sample size and differences in methods. The work was conducted in a laboratory with limited facility such as incubators with unreliable CO2 enrichment supply. Therefore, insufficient CO₂ during incubation, could have led to suboptimal growth of GBS, potentially resulting in false-negative results. CO₂ influences the pH levels of the culture environment, which can affect the viability and recovery of GBS [39].

The distribution of vaginal *Candida* species colonization with sociodemographic category

Of the women with vaginal *Candida* colonization, the majority (17 of 33 or 51.5%) were within the age group of 18–25 years old, were urban dwellers (51.5%), housewives (54.5%), elementary (33.3%), secondary (33.3%), and earners of 2501–5000 Ethiopian Birr (66.7%) monthly income (Table 2). In terms of parity, vaginal *Candida* was isolated predominantly from multiparous women, with two (36.4%), one (21.2%), and three (15.2%) children. Likewise, most of the pregnant women with vaginal *Candida* species colonization were in the third (63.6%) and second (27.3%) trimesters, and 63.6% had a history of contraceptive use (Table 2).

In agreement with the present study [28], reported a higher vaginal *Candida* species colonization in younger age group (18–33 years old) than in older age groups. This might be attributed to the increased secretion of reproductive hormones, which are known to enhance vaginal yeast colonization [19]. This may also be because younger age groups are sexually active and more likely to use contraceptives that enhance vaginal *Candida* colonization [57]. Similar findings have been reported in other countries [23, 51, 58]. In contrast to the present study, higher vaginal Candida species colonization in rural than in urban dwellers has been reported [59, 60].

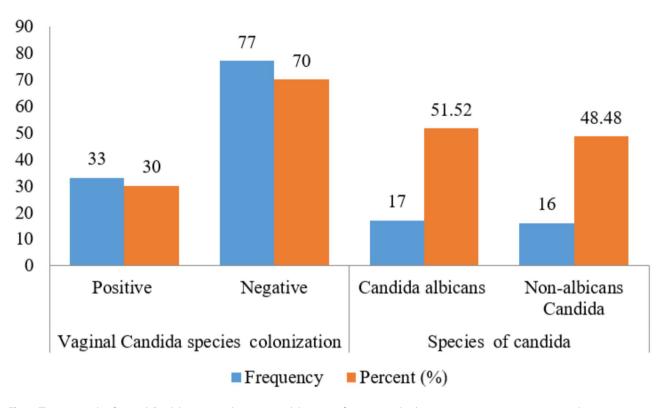


Fig. 2 The magnitude of vaginal Candida species colonization and the types of species involved in pregnant women visiting antenatal care at a tertiary Hospital in Hawassa, Ethiopia

This finding in the present case may be explained by the fact that urban dwellers are more likely to use contraceptives than rural dwellers, since birth control services are more accessible in urban than rural locations. In the present study, the majority of pregnant women with *Candida* species colonization were unemployed housewives (54.5%) with low incomes (66.7%), which is in agreement with a previous report [49]. Unemployed individuals and low-income earners may be unable to afford the basic amenities required to maintain personal hygiene to prevent candidiasis.

The relationship between vaginal Candida species colonization and educational level may also be explained by the same reasoning. Education may increase awareness regarding adherence to personal hygiene, which reduces vaginal Candida species colonization. However, it will also likely enhance the use of contraceptives. Thus, there is a trade-off between personal hygiene and the use of contraceptives in relation to educational and awareness levels. In this study, Candida species colonization was equally higher in women with primary and secondary education levels than in illiterate women. The majority were contraceptive users (63.6%). In terms of parity, multiparous women with two children (36.4%) showed the highest Candida species colonization. This finding is in agreement with previous reports from Ethiopia [28] and India [61]. In contrast, a study in Nigeria reported a decline in vaginal colonization with an increase in parity [21].

Hormonal changes during pregnancy and childbirth, particularly fluctuations in estrogen and progesterone, can alter the vaginal microbiome, creating an environment conducive to Candida overgrowth. Additionally, the immune system adapts during pregnancy, which may affect its ability to maintain a balanced vaginal flora [62]. Shifts in vaginal pH towards a more alkaline state can further promote Candida growth, as the yeast thrives in less acidic conditions [63]. Multiparous women may also experience multiple changes in their vaginal microbiota due to previous pregnancies, leading to a less stable environment [64]. Additionally, behavioral factors such as changes in personal hygiene practices or stress levels related to parenting may increase susceptibility to infections. Finally, physical changes from childbirth, including alterations in the vaginal epithelium and microtraumas, can increase susceptibility to infections like candidiasis. Together, these factors explain the increased colonization of Candida in this population.

An increase in vaginal colonization by *Candida* species was observed during the gestational period in this study, which is consistent with previous reports [65, 66]. This is explained by the increase in reproductive hormones that enhance vaginal *Candida* colonization with advancing gestation period [67].

Table 2 Frequency distribution of vaginal *Candida* species colonization among pregnant women by their sociodemographic groups in Hawassa, Southern Ethiopia, 2021

Sociodemographic	Category	Frequency	%
Variables		of Candida colonization	
Ago group (voars)	18–25	17	51.5
Age group (years)		17	
	26–33 34–40	3	39.4 9.1
Tatal	34-40		
Total		33	100
Residence	Urban	17	51.52
	Rural	16	48.48
Total		33	100
Educational Status	Illiterate	4	12.1
	Elementary	11	33.3
	Secondary	11	33.3
	Higher education	7	21.2
Total		33	100
Occupation	Gov't Employee	5	15.2
	Private	1	3
	NGO	5	15.2
	Housewife	18	54.5
	Daily labour	3	9.1
	Student	1	3
Total		33	100
Monthly income in Ethiopian	1500-2500	8	24.2
Birr (ETB)	2501-5000	22	66.7
	> 5001	3	9.1
Total		33	100
Parity	Zero	9	27.3
,	One	7	21.2
	Two	12	36.4
	Three	5	15.2
Total		33	100
Gestational period	1st trimester	3	9.1
	2nd trimester	9	27.3
	3rd trimester	21	63.6
Total		33	100
Contraceptive use	Yes	21	63.6
	No	12	36.4
Total		33	100

Factors associated with vaginal *Candida* species colonization in pregnant women

In bivariate analysis, age group 31–35 years (COR: 2.67, 95% CI, 0.53–13.42, p = 0.23), urban residence (COR: 2.66, 95% CI, 1.14–6.17, p = 0.023), parity of two (COR = 0.31, 95% CI, 0.10–0.91, p = 0.03), three (COR = 0.33, 95% CI, 0.07–1.62, p = 0.17)], absence of chronic disease (COR = 0.27, 95% CI, 0.06–1.24, p = 0.09) and replacement of underwear once a day (COR, 0.22, 95% CI, 0.07–0.68, p value 0.01) were associated candidate variables with vaginal *Candida* species at p value < 0.25. However, in multivariate analysis, only a parity of two (AOR, 0.24

95% CI 0.07–0.88, p value = 0.03) and replacement of underwear once a day (AOR, 0.18, 95% CI, 0.05–0.72, p value = 0.02) showed a statistically significant association with a higher likelihood of vaginal *Candida* species colonization (Table 3).

As discussed in the previous section, vaginal Candida colonization is higher in multiparous (multigravida) than nulliparous (primigravida) pregnant women. However, variations have been reported among multiparous women [19]. The results of the present study are consistent with these findings. In a study conducted in Nigeria, a decline in vaginal Candida species colonization was reported with an increase in gravidity [21]. The assumption is that experience will encourage pregnant women to take medical and other measures, such as strict adherence to personal hygiene, to minimize vaginal colonization with subsequent pregnancies. However, it is not clear why Candida colonization is higher in pregnant women with two children than in those with a higher number of children or those with one child. Underwear replacement once a day is more likely to be associated with vaginal Candida colonization than underwear replacement more than once a day, considering the hot humid weather of the study location. Wearing tight underwear in hot and moist climates has previously been reported as a likely risk [68].

Limitations of the study

We acknowledge that this study has important limitations. Firstly, the small sample size and the use of a non-probabilistic convenience sampling method may limit the generalizability of our findings. We recognize that convenience sampling may introduce biases that could affect our results. These constraints were primarily due to shortage of time and a lack of funding. Additionally, our laboratory facilities were not equipped with advanced technologies, and the incubators provided unreliable CO_2 enrichment. These factors may have contributed to an underrepresentation of the true prevalence of GBS in the population studied.

Conclusion

The overall incidence of vaginal *Candida* colonization in the pregnant women was 30% (33/110). The majority of the women with vaginal *Candida* (29/33 or 87.9%) were asymptomatic. Group B streptococcus was not detected in any of the samples. The most common cause of vaginal candidiasis among the study subjects was Candida albicans (17/33, 51.52%), while non-albicans Candida (NAC) species accounted for 16 (48.48%) of the cases. 'Parity of two' and 'underwear replacement once a day' were the only two factors that showed a statistically significant association with a higher incidence of vaginal candidiasis in pregnant women. The findings of a 30%

Table 3 Additional file 1. Tabulated results of statistical analysis of factors associated with vaginal Candida spp. and GBS colonization. Association of variables with vaginal candidiasis in preparat women attending antenatal care clinic in Hawassa. Southern Ethiopia

Virgin V	Variables	VC+ve VC-ve Total COR/95%	P-value	AOR (95% CI)	-d
10,002 20,0007 12,002 20,0007 12,002 20,0007 12,002 20,0007 12,0007 20,0007 12,0007 20,0007 12,0007 20,00		N (%) N (%)			value
11 11 11 11 11 11 11 1	Age Group	35 (67.2) 52 (47.3)		1.12(0.38-	0.84
1,000, 150	18–25	30 (69.77) 43 (39.1)		3.29)	0.29
174256 174526 1	26-30	11 (84.62) 13 (11.8)		2.83(0.41-	0.20
1,000,000,000,000,000,000,000,000,000,0	36-40	(0.05)		0.09(0.002-	
1,14,1259 2,1544 3,195,10 4,104 3,104 4,104 3,104 4,104 3,104 4,104 3,104 4,10		0.49 (0.03-8.25	œ	3.54)	
	Residence	22 (56.41) 39 (35.5)	1	1.91(0.70–5.24) 0.21	0.21
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Table 3 (continued)

prevalence of vaginal Candida, coupled with the absence of GBS colonization, suggest several important implications for clinical practice and public health. Routine screening for Candida should be integrated into antenatal care, particularly for women with a parity of two, who are at higher risk. Targeted education on hygiene practices, such as daily underwear changes, can help reduce moisture retention and lower the likelihood of candidiasis. Furthermore, these findings can inform public health policies by promoting guidelines that include Candida screening and hygiene education to enhance maternal and neonatal health outcomes. Additionally, further research is warranted to investigate the factors contributing to the absence of GBS colonization using recommended standard methods, which may provide insights into microbial interactions during pregnancy. We recommend that future research on the prevalence of vaginal Candida and GBS colonization focus on larger, more diverse populations to enhance generalizability. Specifically, studies should explore the relationships between risk factors, such as parity and hygiene practices, and candidiasis incidence. To improve upon the limitations of our current study, employing probabilistic sampling methods and adopting a longitudinal design would allow for a more representative sample and the assessment of causal relationships over time. These enhancements could provide invaluable insights that inform clinical practice and public health strategies aimed at improving maternal health outcomes.

List of acronyms and abbreviations

GBS Group B Streptococcus

HUCSH Hawassa University Comprehensive Specialized Hospital

NAC Non-albicans Candida VC Vaginal Candidiasis VVC Vulvoyaginal candidiasis

Acknowledgements

N = 1 and participants, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women negative for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis, NC + v = p pregnant women positive for vaginal candidiasis.

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Author contributions

AM conceived the idea and supervised the work and participated in the data analysis and writing up of the manuscript, AE developed the proposal and executed the laboratory work; TA supervised the laboratory work and participated in the analysis of the data and write-up of the manuscript. All authors have read and approved the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding and main author upon reasonable request.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participation

Ethical clearance and approval were obtained from the institutional review board (IRB) of Hawassa University, College of Medicine and Health Sciences. Permission was requested and obtained from Hawassa University Comprehensive Specialized Hospital. Verbal and written informed consent was obtained from the study participant's pregnant women before data collection. Additionally, the confidentiality of information was assured throughout the study.

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