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Prevalence, Associated Factors, and Antifungigram of Vulvovaginal Candidiasis among Pregnant Women Receiving Antenatal Care At Lira Regional Referral Hospital

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Abstract

Background: Vulvovaginal candidiasis during pregnancy is a worldwide problem because of its association with premature labor, preterm membrane rupture, spontaneous abortion, early delivery, newborn infection, postnatal morbidity, and maternal mortality. This study aimed to determine the prevalence and associated factors and establish an antifungigram of vulvovaginal candidiasis among pregnant women at Lira Regional Referral Hospital.

Methods: A hospital-based cross-sectional study was conducted on 138 pregnant women at Lira Regional Referral Hospital, recruited using systematic random sampling techniques. Microscopy and SDA supplemented with 2% chloramphenicol were used to diagnose vulvovaginal candidiasis due to growth and detection of different *Candida species*. Sugar assimilation test and Germ tube test was used to differentiate between C. *albicans* and non-*albicans species*. Antifungigram was performed using the disk diffusion method to evaluate the current resistance pattern. Associated factors were collected using a questionnaire. Data were analyzed using SPSS version 25.0. Logistic regression analysis was used, where a p-value <0.05 was considered significant.

Results: The prevalence of VVC was 40.6%, with epidemiological shift towards non-*albicans species* dominating the infection profile at 65.07% compared to *C. albicans* (34.93%). Participants' average age was 25.54 years, SD of ± 4.692, and the most significant factors were parity (AOR, 4.33; $p < 0.001$), prolonged antibiotic use (AOR, 4.88; $p < 0.001$), gestational age (AOR, 0.12; $p = 0.003$), previous VVC (AOR, 3.22; $p = 0.043$), and history of miscarriage (AOR, 7.42; $p = 0.002$). However, those who were over 25 years old, and had a history of UTIs were significantly more likely to develop VVC at bivariate level. No statistically significant differences were observed between educational level and occupation. Clotrimazole exhibited 90%–98% sensitivity across both *C. albicans* and non-*albicans species (NACs).* However, nystatin resistance was alarming at 80% among non-*albicans candida species* and 70.6% in *C. albicans*.

Conclusion: The prevalence of vulvovaginal candidiasis among pregnant women at Lira regional referral hospital remains significantly higher than global averages, driven by high parity, long-term antibiotic use, and a prior history of VVC. A profound, non-traditional shift was established toward NACs. Clotrimazole is a highly effective therapeutic option, showing a high sensitivity across both *C. albicans* and non-*albicans species.* Nystatin showed a high resistance to NACs and *C. albicans,* presenting a serious obstacle to normal treatment.

Keywords: Prevalence, associated factors, antifungigram, vulvovaginal candidiasis, pregnant women.

Introduction

The World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) define vulvovaginal candidiasis (VVC) as an opportunistic, yeast-like fungal infection of the female lower genital [13, 75]. VVC remains the most prevalent fungal disease worldwide, accounting for 90% cases of all infectious vaginitis [54, 73].

Vulvovaginal candidiasis is caused by *Candida species* mainly *Candida albicans,* which accounts for about 85-95% of VVC infections, and non-*albican candida* (NAC) species, which account for 10-20%. However, current studies indicate a profound epidemiological shift characterized by the displacement of *C. albicans* by NAC species. This shift is driven by widespread, unregulated use of over-the-counter antifungal agents, which has eliminated sensitive *C. albicans* species, creating an ecological niche for less sensitive NAC species. NAC species possess specialized cell-surface adhesins that form a multi-layered extracellular matrix biofilms which shield underlying fungal cells from both host immune cells and topically applied antifungals [28, 34, 80, 4, 42].

Globally, approximately 30-50% of pregnant women experience VVC annually (WHO; 2021, [68]. In India, VVC cases was reported at 45.18% and 44.8% VVC cases were reported among pregnant Lebanese women [27, 71]. In Africa, the burden of VVC is particularly high with the estimated total prevalence at 29.2% where the highest regional burden was reported in Eastern Africa at 35% [56]. In Kenya, the prevalence of VVC was 42.7% slightly lower than the prevalence from two difference studies in Tanzania at 42.9% and 65.6% respectively [35, 48]. In Uganda, the prevalence of VVC among pregnant women has remained consistently high, reported at 45.4% in 2015 and 42.0% in 2023 [46, 62].

The risk of developing VVC is higher in pregnant females ranging from 30% to 50% compared to roughly 20% in non-pregnant women [20, 40, 42]. These discrepancies are due to heightened progesterone which reduces the inflammatory activity by causing a shift in the activity of Th1 (pro-inflammatory) cells to Th2 (anti-inflammatory) by decreasing the production of protective cytokines, such as IL-2 and interferon gamma (INFγ). Concurrently, a hyperestrogenic environments stimulates glycogen accumulation in the vaginal epithelium and increases vaginal wall receptors. This provides an abundant carbon source and adherence sites that accelerate fungal colonization and biofilm development [80, 21, 12].

Clinically, VVC is characterized by a non-specific symptoms such as odorless curdy white discharge, intense pruritus, vaginal discharge, dyspareunia, itching, inflammation (redness), and dysuria [51, 69]. In pregnancy, persistent and inadequately treated VVC causes chronic vaginal inflammation which weakens gestational tissues, predisposing patients to premature rupture of membranes (PROM), chorioamnionitis, spontaneous abortion, preterm birth, and invasive neonatal candidiasis [40, 51, 62]. Consequently, laboratory confirmation by culture distinguishing active disease ($\geq 10^5$ CFU/ml) from benign colonization is essential to halt this cycle [3, 49].

In addition, management is increasingly complicated by antifungal resistant due to the availability of over-the-counter antifungals with the resistance to polyene arising primarily through mutations in ergosterol biosynthesis genes causing *Candida* species to substitute ergosterol with alternative sterol precursors, effectively preventing nystatin binding as well as resistance to Azole which is mediated by point mutations within *ERG11* that reduce drug binding affinity, and major facilitator superfamily efflux pumps which actively eject the drug from the fungal cell [65, 69]. In Uganda, these resistance profiles present a serious public health challenge with the current national guidelines from the Ministry of Health rely heavily on empirical, low-cost topical imidazoles (clotrimazole and miconazole) and polyenes (nystatin) for pregnant women, as systemic azoles are contraindicated due to teratogenic risks [43, 12].

Current guidelines rely heavily on studies of non-pregnant populations, leaving pregnant women a uniquely vulnerable group at risk of mismanagement. This study addressed these urgent gaps by determining the VVC prevalence, associated factors, and emerging resistance patterns. Hence, context-specific antenatal care protocols and antifungal stewardship policies will ultimately reduce the burden of VVC-related complications for mothers and neonates.

Study purpose

To determine the prevalence, associated factors and establish the antifungigram of vulvovaginal candidiasis among pregnant women receiving antenatal care at Lira Regional Referral Hospital.

Specific objectives of the study

1. To determine the prevalence of vulvovaginal candidiasis among pregnant women receiving antenatal care at Lira Regional Referral Hospital.
2. To identify the factors associated with vulvovaginal candidiasis among pregnant women receiving antenatal care at Lira Regional Referral Hospital.

3. To isolate and identify *Candida albicans* and *non-albican candida* species causing vulvovaginal candidiasis in pregnant women receiving antenatal care at Lira Regional Referral Hospital.
4. To establish the antifungigram of *candida albicans* and *non-albican candida* species causing vulvovaginal candidiasis among pregnant women receiving antenatal care at Lira Regional Referral Hospital.

Justification of the study

Vulvovaginal candidiasis remains a profound public health challenge among pregnant women due to its direct association with adverse maternal and neonatal outcomes [11, 61]. While [62] found that 42.0% of pregnant women in Uganda suffer from VVC, this masks significant regional disparities driven by distinct socioeconomic and clinical environments. Currently, a critical epidemiological data gap exists regarding the Lango sub-region of Northern Uganda, where localized data on VVC prevalence, associated factors, and institutional antifungal susceptibility profiles remain a dearth. Lira Regional Referral Hospital (LRRH) serves as a strategic and vital site for investigating this phenomenon since it manages a uniquely dense, low-resource, and highly diverse antenatal care population drawn from multiple surrounding districts. This cohort faces unique regional challenges, including limited healthcare access, high reliance on empirical syndromic management, and widespread, unregulated over-the-counter access to self-medicated antifungals hence consequently accelerating the selection pressure for resistant non-*albicans Candida* (NAC) species within this community. This study directly addresses these urgent gaps by determining the current VVC prevalence, identifying associated factors, and establishing an up-to-date antifungigram for both *Candida albicans* and NAC species isolated from patients at LRRH. These findings will help to design specific antenatal protocols, curb local drug resistance, and improve maternal-fetal outcomes in Northern Uganda.

Research Methodology

Research design and setting

A facility-based, cross-sectional research design using quantitative approaches of data collection was employed to evaluate the prevalence, associated factors, and establishing the antifungigram of *Candida* species causing vulvovaginal candidiasis among pregnant women seeking antenatal care at Lira Regional Referral Hospital. This approach is highly effective for determining the VVC prevalence and identifying the associated factors within a specific point in time [67].

Target population

All pregnant women receiving antenatal care from the antenatal clinic of the Lira Regional Referral Hospital at the time of data collection.

Sample size determination

We applied the Kish-Leslie formula for determining the single population proportion [36].

$$n_0 = \frac{Z^2 pq}{e^2}$$

Where;

Z is the standard normal deviation corresponding to 95% confidence interval set at 1.96,

n_o = Minimum required sample size of pregnant women getting antenatal care,

e = Acceptable margin of error, set at 5% (0.05).

p = the estimated baseline prevalence of VVC in pregnant women. The rate of reported VVC-related symptoms among pregnant women at Lira Regional Referral Hospital remains was 10.0% (0.1) [39]. P =10.0% (0.1).

1-p = the probability of pregnant women not having VVC; hence, 1-p = 90.0% (0.9).

The calculated sample size $n = \frac{1.96^2 \times 0.10 \times 0.90}{(0.05)^2}$

The researcher recruited a sample size of 138 participants

Sampling technique

Pregnant women who received antenatal care at the time of data collection were carefully selected using a systematic random sampling technique. This sampling technique ensured that every participant had an equal opportunity for selection, thereby reducing selection bias [66]. We determined the interval (k) by dividing the estimated monthly population of pregnant women attending the ANC clinic (N = 500), according to facility ANC registers. Five hundred was divided by the allocated sample size of the study, that is, $k = \frac{N}{no}$, where *k* is the sampling interval, *N* is the total number of ANC attendants, and *no* is the calculated sample size, $k = \frac{500}{138}$, $3.64 \approx 4$. A sampling interval of four (k = 4) was established. The lottery method was used on each clinic day to select

the initial participant [18]. Four identical pieces of paper, numbered sequentially from 1 to 4, were placed in an opaque container and thoroughly mixed. The researcher blindly selected one piece of paper at the start of the clinic session. Number two (2) was drawn, the second (2^{nd}) pregnant woman who registered at the clinic and met the inclusion criteria served as the initial participant. Thereafter, every fourth (4^{th}) consecutive pregnant woman who checked into the clinic was systematically recruited until the target sample size of 138 participants was achieved.

Participant's selection criteria (inclusion criteria & exclusion criteria)

This study included all pregnant women aged 15–49 years receiving antenatal care from the antenatal clinic of the LRRH. Those aged < 18 years were classified as emancipated minors. This study excluded pregnant women who refused to provide consent, unwell pregnant women, those who were mentally incompetent, those who took antifungal agents within 2 weeks prior to the interview, and those with a history of vaginal bleeding.

Data collection tools

An interviewer-administered, self-designed, and semi-structured questionnaire was used to gather the primary data from the participants. The questionnaire comprised Section A: socio-demographic data, which was filled out by the participants; Sections B and C: identification and prevalence of *Candida species* causing vulvovaginal candidiasis in pregnant women using different culture media; and Section D: Candida species susceptibility to widely used antifungals. Sections B, C, and D were laboratory based and filled out by the researcher and laboratory technician. Section E: Maternal-associated factors, was filled out by participants. The socio-demographic, and maternal associated factors were adapted from validated reproductive tract infection survey instruments developed by the World Health Organization [76] and previously evaluated regional vulvovaginal candidiasis (VVC) questionnaires [46, 71]. The questionnaire was originally drafted in English and subsequently translated into *Lango* by a native-speaking health linguist. To verify conceptual equivalence and preserve the original medical meaning of each item, an independent medical translator back-translated the *Lango* version into English. Discrepancies in phrasing or interpretation were resolved through a consensus committee before finalizing the tool.

Quality control

Validity

The self-designed data collection tool adapted from the World Health Organization [76, 46, 71] was submitted to three research experts (1 senior medical microbiologists, 1 clinical obstetrician, and 1 public health researcher). The reviewers graded each item in Sections A (Socio-demographics) and E (Maternal associated factors) on a 4-point Likert scale measuring relevance, clarity, and simplicity. A formal Content Validity Index (CVI) was computed by calculating the proportion of items given a rating of 3 or 4. Only items achieving an individual CVI score of ≥ 0.80 were retained, establishing robust content validity [78].

Reliability

The data collection tool was pre-tested on the 5% of the calculated sample size (n = 7) participants at Kampala International University-Teaching Hospital. Pilot data from the socio-demographic factors, clinical factors and obstetrical factors were analyzed using Statistical Package for Social Sciences to compute Cronbach's alpha coefficients. The overall scale achieved an alpha value of alpha = 0.78. Because these coefficients exceeded the standard academic threshold of (0.70), the tool was verified as highly reliable and internally uniform [72].

Adjustments made on the data collection tool

The literal *Lango* translation for curdy white vaginal discharge initially lacked clarity for some participants. Following recommendations from the linguistic consensus committee, the phrasing was adjusted to include culturally specific descriptors that accurately describe an odorless, thick, milk-clotted discharge, ensuring high response accuracy.

During the pre-test, asking patients if they had a history of antibiotic use proved too broad and led to recall errors. The question was adjusted to a specific, closed-ended format with verification prompts: *"Have you taken any systemic or topical antibiotic medications within the past 14 days?*

Sample collection and processing

A total of 138 samples of high vaginal swabs were collected from eligible pregnant women aged 15 to 49 years attending the antenatal clinic at Lira Regional Referral Hospital. For each participant, two separate high vaginal swabs (HVS) specimens were obtained by a trained midwife by gently rotating a sterile cotton wool swab (Aptima® Multitest swab specimen collection kit) against the vaginal fornix posteriorly. After collection, the swabs the swabs were immediately inserted into their original transport tubes containing 2 mL of Amies transport medium to preserve

sample viability and prevent drying. Each tube was labeled with the participant's unique study identification number, initials, and the collection date and later transported in a cooler box which was maintained between 2°C and 8°C to the microbiology laboratory at Kampala International University Western Campus for diagnostic processing [46, 10, and 7]. One HVS was used for microscopic examination by gram staining, whereas the second HVS was cultured in Sabourad's Dextrose Agar (SDA) containing 2% chloramphenicol and incubated for 48 hours at 37^0C.

Identification of *Candida species* causing vulvovaginal candidiasis

Candida albicans and *non-albican candida* species were identified based on the cultural characteristics of Sabouraud dextrose agar, microscopic characteristics (Gram staining and germ tube), and sugar assimilation tests.

Direct Gram staining

A few drop of sterile normal saline was added to the first HVS in the tube and shaken. A small amount was removed using a sterile wire loop and placed on a clean glass slide to make a smear. The smear was then allowed to air dry and then fixed by passing it over the flame. This was then flooded with crystal violet for one minute followed by Lugol's iodine for one minute and 95% ethanol for 10 seconds for decolorization. The decolorized smear was then flooded with safranin for 30 seconds and then washed with tap water. This was then examined under the high-power objective lens with immersion oil. Gram positive budding yeast cells or pseudohyphae were considered positive for gram staining as per [33, 41].

Standard control smears were prepared using a reference bacterial and fungal strains from [41] where they mixed a known Gram-positive organism (*Stap. aureus* ATCC 25923 mixed with *Candida albicans* ATCC 90028) and a known Gram-negative organism (*E. coli* ATCC 25922) were stained concurrently on a single control slide with each testing cycle. The staining run was considered valid only if the reference organisms exhibited their expected staining characteristics under the microscope.

Cultivation on Sabouraud dextrose agar supplemented with 2% chloramphenicol

A second high vaginal swab was streaked onto Sabouraud's Dextrose Agar (SDA) containing 2% chloramphenicol. The inoculated plates were incubated at 37 ºC for 48 hours and were checked for the presence of creamed-colored pastry colonies, which were indicative of *Candida species*. Mixed growth was observed and distinct colonies were subcultured again on SDA to obtain pure

colonies after 24 hours of further incubation at 37 ºC. For quality control, we sub-cultured a mixed growth, using a fresh sterile loop for every single streak to ensure true isolation and the resulting pure plate were re-checked for uniform colony morphology. We also incubated an uninoculated SDA plate from each batch to confirm for any growth and we used the one which showed no growth. Negative Control was done where we inoculated a plate containing 2% chloramphenicol with E. *coli*, and we used the plate which showed no growth.

Sugar Assimilation Test

Yeast Nitrogen basal media was prepared according to manufacturer's instructions (HiMedia Laboratories Pvt. Ltd) i.e. 13.5 grams of dehydrated media was suspended in 200 ml of distilled water and mixed well. This was followed by supplementing the basal media with 5 grams of the respective carbohydrates solution via a 0.22µm syringe filter, and 2% Agar to enable solidification. The mixture was then boiled to ensure complete dissolution, and later sterilized by autoclaving at 115°C for 10 minutes to eliminate contaminants. For quality control, sugar-free control was used where one plate was created with only Yeast Nitrogen Base and Agar without sugar and inoculated with yeast and were incubated at 30°C for 48 hours in order to check for growth, the used plates remained clear. After sterilization, the media was aseptically transferred into 90 mm Petri plates at a thickness of 4 mm and allowed to solidify. The yeast cells from purified SDA plates were washed in sterile saline to prevent false positives and were further inoculated on the sterile solid media containing each sugar and incubated at 30 °C for 48 hours and observed for growth to determine their assimilation patterns. Incubated plates were checked daily and the maximum period for interpretation of results was six days. Glucose was used as a positive control since all the *Candida species* assimilated this carbohydrate. Assimilation was interpreted positively (+) if there was growth in the plate containing the carbohydrate and negative (-) if there was no growth.

To ensure quality control, a sugar-free control plate consisting solely of YNB and 2% agar without any carbohydrate supplement was prepared. This plate was inoculated with the target yeast suspension and incubated at 30°C for 48 hours. The test run was considered valid only if this control plate remained completely clear of any growth, confirming that yeast's growth was driven exclusively by the added sugar. A parallel glucose-supplemented YNB plate was inoculated alongside each clinical isolate. The assay was considered invalid if the glucose control failed to yield microbial growth [14, 60, 77].

Germ Tube Test

Sterile cryovial fluid (125 mL) was mixed with freshly prepared human serum (0.5 mL). A yeast colony from the purified SDA plate colonies were added to the serum via a sterile wire loop, and the cover was loosely placed to provide an aerobic environment. For 2 hours, the cryovial was kept in an incubator at 37°C. A drop of the serum yeast culture was placed on a glass slide using a Pasteur pipette, covered with a coverslip, and examined microscopically under ×40 objective lens with low light to increase contrast. Isolates demonstrating short, lateral, filamentous extensions (germ tubes) protruding from the mother yeast cell without any constriction or narrowing at their point of origin were interpreted as positive for *C. albicans* or *C. dubliniensis*. Cells lacking these parallel-walled protrusions or displaying distinct basal constrictions were classified as negative and recorded as NAC species. All germ tube-positive isolates were subcultured onto fresh SDA plates and incubated at an elevated temperature of 45°C for 24 hours. Isolates demonstrating growth at this high temperature were definitively confirmed as *Candida albicans*, whereas isolates showing total growth inhibition (no growth) were classified as *Candida dubliniensis.* Quality control was ensured*,* a parallel test tube containing the same serum batch was inoculated with a known reference strain, *C. albicans* ATCC 90028. The analytical run was validated only if this control tube demonstrated abundant germ tube formation at 2 hours and colony propagation during the subsequent 45°C thermal challenge. The analytical run was validated only if this control tube demonstrated abundant germ tube formation at 2 hours and colony propagation during the subsequent 45°C thermal challenge. Digital thermometers, both the 37°C and 45°C incubators were audited twice daily to verify strict temperature maintenance within ±0.5°C. [58, 59, 53].

Factors associated with vulvovaginal candidiasis among pregnant women

Participants' socio-demographic factors included age, education level, marital status, occupation, and income level. Associated factors: Clinical factors: HIV status, hypertensive status, previous VVC episodes, and UTI status. Obstetric factors such as gestational age, parity, previous cesarean section, current pregnancy complications, prolonged antibiotic use and history of VVC were captured using the interviewer-administered questionnaire. Data on HIV status was confirmed from the ANC cards, gestational age was calculated using the Last Normal Menstrual Period or Ultrasound data from the hospital records, and hypertensive status were checked from the ANC

cards rather than relying solely on self-reporting and VVC previous episode was based on previous clinical diagnosis.

Antifungal susceptibility test for the isolated *Candida species*

In this method, a yeast colony with a 24-hour incubation culture was picked from the purified SDA plate using a sterile inoculating wire loop. It was emulsified in 2 mL of 0.85% sterile sodium chloride and mixed on the vortex mixer to create a homogeneous suspension. The turbidity of the suspension was assessed using a spectrometer set at 625nm and corrected to a 0.5 McFarland standard number using sterile saline. Any extra moisture was wiped away by rolling the sterile cotton swab within the tube above the fluid level. To completely cover the Mueller Hinton-glucose agar plate, streaks were applied in four separate directions (at 90-degree angles) until the entire surface of the plate was covered. The plates were allowed to dry at 35°C until the agar surface was completely free of moisture droplets to avoid antifungal agent's diffusion.

Discs containing the antifungal agents (Nystatin (100 U), Clotrimazole (50 µg), and Miconazole (50 µg)) (Bio-Rad Laboratories Pvt Ltd., France) were placed on the surface of inoculated Muller-Hinton Agar supplemented with 2% glucose and gently pushed to achieve full contact with agar using a pair of flame-sterilized forceps as documented by the CLSI document M44-A2 [17]. Until adequate growth occurred, the plates were incubated at 35°C for 24 hours. After a 24-hour incubation period, plates were read as soon as feasible, and the findings were noted. Using a ruler, the diameters of the zones of inhibition for antifungal discs were measured in millimeters. The 15-15-15 Minute Rule was used where, the time between preparing the suspension and inoculating the plates was limited to 15 minutes, antifungal discs were applied within 15 minutes of plate drying, and plates were transferred to the incubator within 15 minutes of disc placement [57].

Data collection procedures

The ANC of LRRH was the study base for these operations. A trained research assistant (registered midwife) working at ANC recruited study participants who met the eligibility requirements for the research and obtained comprehensive details on the study design and objectives. Before giving consent and completing a self-administered questionnaire, a research assistant read a consent statement in the language they understood and offered them the opportunity to ask questions. They were given the opportunity to complete the questionnaire after providing consent, and they then received advice regarding specimen collection. To collect specimens, participants were placed in

a private room to obtain samples. Demographic data and factors associated with the participants were entered into the questionnaire.

Data processing and analysis plan

The researcher cross-checked the raw data to identify inconsistencies and discrepancies. The data obtained from each questionnaire were directly imported into the Statistical Package for the Social Sciences (SPSS) version 25.0, where any missing information found during entry was verified and fixed by referring back to the questionnaire using its unique number. Before conducting the data analysis, the researcher performed daily data cleaning to check for incompleteness, inconsistency, and spelling to minimize entry mistakes.

Data analysis

The collected data were analyzed using SPSS Version 25.0. The analyzed data are presented as frequencies and percentages. They are presented in the form of frequency tables, bar graphs, histograms, and pie charts. Means and standard deviations were used to summarize continuous data values. Odds ratios (OR) were used to describe findings. Bivariate and multivariate logistic regression analyses were used to assess the association between the participants' demographic characteristics, associated factors, and VVC prevalence. A p-value less than 0.25 at 95% CI during the bivariate analysis was further calculated using multivariate logistic regression to avoid the effect of confounding. Finally, a p-value < 0.05, at 95% CI, was considered statistically significant. The sensitivity of *Candida* species to antifungal medications was determined using ANOVA.

Ethical consideration

Ethical approval was obtained from the Kampala International University, Western Campus Research and Ethics Committee, which granted formal approval under approval number KIU-2024-396. Administrative clearance to conduct the study and access patient records was obtained from the Office of the Hospital Director and the Head of the Obstetrics and Gynecology Department at Lira Regional Referral Hospital.

The trained research assistants provided a detailed, verbal explanation of the study's purpose, scope, and procedures to the participants. All eligible pregnant women aged 18 years and older provided the written, signed, or thumbprint informed consent. Informed assent was obtained directly from participants aged below 18 years. Participants were assured that their involvement

in this research was completely voluntary. They were informed of their absolute right to withdraw from the study at any stage of the interview or laboratory collection without providing a reason. Furthermore, participants were free to decline to answer any specific questions within the questionnaire and this resulted in no penalties, loss of benefits, or compromise to the routine antenatal care.

No direct identifiers, such as names, telephone numbers, or hospital registration numbers, were recorded on the questionnaires or laboratory logbooks. Instead, each participant was assigned a unique, sequential alphanumeric study identification code. Filled questionnaires, laboratory data logs, and signed consent forms, were stored in a secure filing cabinet accessible exclusively to the principal investigator. Electronic data spreadsheets were protected on a password-protected computer.

The collection of HVS using unlubricated speculums may have caused brief physical discomfort or mild anxiety hence, the examinations were conducted in a highly private clinic room, and the midwife maintained continuous communication with the patient during the procedure. Participants received direct diagnostic benefits through free laboratory testing for VVC. Participants with positive findings were directed to the attending hospital clinicians for targeted, safe, and cost-effective antifungal therapies. Each participant was given 2,000 UGX token as an appreciation for their time and participation in this study.

Results

Prevalence of vulvovaginal candidiasis among symptomatic pregnant women

According to the data from (figure 1), we found that of the 138 samples examined, 56 tested positive for Candida, as demonstrated by the microbial growth on Sabouraud dextrose agar supplemented with 2% chloramphenicol. Therefore, the prevalence of vulvovaginal candidiasis among pregnant women receiving antenatal care at Lira Regional Referral Hospital was 40.6%.

Factors associated with vulvovaginal candidiasis in symptomatic pregnant women

Social demographic characteristics of study participants.

The study included 138 participants. With an average age of 25.54 years and a standard deviation of ± 4.692 years, the ages varied from 18 to 45 years. Most participants (46.4%) were aged 25–34 years. Nearly three-quarters (72.4%) of the participants had only primary education, while only 4.4% had both tertiary and non-formal education. In terms of marital status, only a small

percentage of participants (2.9%) were single, whereas the majority (71.7%) were married. Peasant farming accounted for 67.4% of the participants' economic activities, whereas only 0.7% reported having a formal job. These data support the occupational findings, which showed that only 16% of participants made more than 100,000 UGX per month, while more than half (53.6%) made less than 50,000 UGX. Data from (Table 1).

Bivariate and multivariate analysis of the socio-demographic factors associated with Vulvovaginal candidiasis among study participants.

From table 2, there was no factors found to be statistically significantly associated with VVC at multivariate level., However, pregnant women who were aged between 25-34 were 10.9 times more likely to have VVC (COR=10.9, p=0.004) as well as those aged 35 years and above were 9.8 times more likely to experience VVC (COR=9.80, p=0.021) as compare to pregnant women who were under 20 years of age. However, it remained insignificance when adjusted ($p > 0.05$) suggesting that age was a confounding factors. Participants who were single were significantly less likely to have VVC compared to those who were married (COR 0.22, p=0.002). However, it didn't show any significance when adjusted (p=0.187), this mean that high risk of VVC among married participants could be due to other factors. Compare to participants earning more than > 100,000 UGX monthly, participants who earned ≤ 100,000 UGX were 3.13 times more likely to have VVC (COR= 3.133, p=0.037). Our study found no statistically significant difference in VVC prevalence based on education level and the participants' occupation (all p-values > 0.05).

Maternal clinical factors of VVC among study participants.

Of the participants, 47.1% reported using antibiotics for more than 2 weeks, indicating a high proportion of chronic antibiotic use. The majority of participants (47.8%) had suffered from urinary tract infections (UTIs), which was the most commonly reported medical illness among participants, while the least common illnesses reported were HIV/AIDS (1.4%) and hypertension (5.1%) (Table 3).

Bivariate and Multivariate analysis of the maternal clinical factors associated with Vulvovaginal candidiasis among study participants.

From table 4, Pregnant women who reported having UTIs were 4.67 times more likely to have VVC than those without (COR=4.67, p<0.001). However, it remains statistically non-significance

when adjusted (AOR=2.15, p=0.124). This suggests that having a UTI is not an independent risk factor for VVC in this specific study population. There was no statistical significance between HIV status, and hypertension, and VVC experience among study participants.

Maternal obstetrical factors of VVC among study participants.

The majority of participants (90.6%) had never had a cesarean section, indicating that they had not undergone any significant obstetric interventions. Even though the fewest participants (16.7%) were in their third trimester, the majority (65.2%) were in their first trimester, 31.2% were pregnant for the first time, the majority (42.0%) had one to three children in the past, and 26.8% had four or more. Approximately 18.8% of the participants reported having health problems related to their current pregnancy, compared to 81.9% who reported no complications at all. Of the participants, 47.1% reported using antibiotics for more than 2 weeks, indicating a high proportion of chronic antibiotic use. (Table 5).

Bivariate and Multivariate analysis of maternal obstetrical factors associated with Vulvovaginal candidiasis among study participants

According to table 6, our study found that participants who were in their second trimester (13-28 weeks) were significantly less likely to have VVC compared to those in the first trimester (AOR 0.03, p<0.001). Pregnant women with 4 or more children were 4.33 times more likely to have VVC (AOR 4.33, p<0.001) as compare to participants who had 1 to 2 deliveries were 88% less likely to have VVC than those who had never delivered (AOR 0.12, p=0.003). This could possibly be due to cumulative physiological or hormonal changes. Participants who were on antibiotic therapy for more than 2 weeks within one month prior to this study have nearly 5 times higher odds of developing VVC (AOR 4.88, p<0.001). Pregnant women who reported having a history of VVC were 3.22 times more likely to have a current infection (AOR 3.22, p=0.043) as compare to their counterpart. Although significant in the bivariate analysis (COR 3.31, p=0.004), participant with current pregnancy complication were likely to have VVC though are not an independent cause since it remains insignificance when adjusted (AOR 1.95, p=0.215).

Isolates of *Candida species* associated with vulvovaginal candidiasis in pregnant women receiving antenatal care at Lira Regional Referral Hospital

Cultural features of the isolated *Candida species* on Sabouraud Dextrose Agar

Macroscopic features on SDA found the presence of various distinct colonies of different species of *Candida*, i.e., the white, round, raised colonies with a smooth texture were recorded (n=53, 34.93%), cream, irregular raised rough texture colonies (n=37, 25.34%), cream, round with wrinkled edges, raised smooth colonies (n=19, 13.01%), white, small and concentric raised smooth and shiny colonies (n=29, 19.86%), and cream, round raised smooth colonies (n=8, 6.85%) (Table 7).

Sugar assimilation characteristics of the isolated *Candida species*

Candida species with white, round, raised (convex), and smooth colonies were able to assimilate glucose, maltose, sucrose, and xylose but did not assimilate lactose and cellobiose. This implicate a highly characteristic of *Candida albicans* (34.9%). While candida species with cream-colored, irregular, raised, and rough colonies and those white, small and concentric, raised, smooth, and shiny colonies were able to assimilate only glucose but didn't assimilate the other sugars (25.3% & 19.9%). We also found that candida species with cream-colored, round with wrinkled edges, raised, and smooth were able to assimilate glucose, maltose, sucrose, xylose, and cellobiose but didn't assimilate lactose (13.0%). Candida species with cream-colored, round, raised, and smooth colonies were able to assimilate Glucose, Maltose, Sucrose, and Xylose but didn't assimilate lactose and cellobiose (Table 8).

Gram staining technique

Majority 65.07% (90/138) of the isolates purple budding yeast oval cells (Gram-positive), while 34.93% (48/138) were showed pink non-budding yeast cells **(**Figure 2)

Germ Tube Test

The difference distinct colonies from sub-cultured SDA were tested for Germ tube to isolate *candida albicans* from *non-albicans candida* species (NAC). The plate containing the white, round, raised colonies with a smooth texture (n=51, 34.93%) were positive for germ tube while other colonies didn't show positive for Germ tube indicating *non-albicans species* (n=95, 65.07%). Therefore, the frequency of *albicans species* was 51/146 (34.93%) compared to *non-albicans*

species 95/146 (65.07%). (Figure 3). The positive germ tube were further incubated at 45^0C for 24 hours and all the incubated plates had growth indicating *candida albicans.*

Antifungigram of *candida albican* and *non-albican candida* species causing VVC

The antifungal susceptibility pattern of all *Candida* isolates was similar, with the majority of species-specific isolates being susceptible to clotrimazole, with susceptibility rates ranging from 90% to 98%, and miconazole showing moderate efficacy, ranging from 36.8% to 90%. In contrast, nystatin showed very high levels of resistance, ranging from 58.6% to 80.0% across all tested species. Therefore, it is most resistant to antifungal agents. *Candida albicans* exhibited a very high resistance rate to nystatin (70.6%), yet a high susceptibility rate to clotrimazole (98%). *Non-albicans candida* species showed strong susceptibility to clotrimazole ranging from (90.0-96.6%) and miconazole ranging from (64.9-90.0%). However, nystatin resistance among *NAC*s was ranged 58.6% to 80.0%. Although only 15.8% NACs were resistant to miconazole (Table 9).

Table 1: Social demographic characteristic of the study participants

Variables	Category	Frequency (N)	Percentage (%)
Participant's age group in years	Less than 20	16	11.6
	20–24	46	33.3
	25–34	64	46.4
	35 and above	12	8.7
Education status	Non-formal	6	4.4
	Primary level	100	72.4
	Secondary level	26	18.8
	Tertiary level	6	4.4
Marital status	Single	4	2.9
	Cohabiting	32	23.2
	Married	99	71.7
	Separated/Divorce	3	2.2
Occupation	Employed	1	0.7
	Business	29	21.0
	Peasant farmer	93	67.4
	Unemployed	15	10.9
Average monthly income	Less than 50,000 UGX	74	53.6
	50,000 to 100,000 UGX	42	30.4
	Above 100,000 UGX	22	16.0

Table 2: Bivariate and multivariate analysis of the socio-demographic factors associated with Vulvovaginal candidiasis among study participants

Variables	Categories	Proportion of VVC		COR(95% CI	P-value	AOR (95% CI)	P-value
		Growth N(%)	No growth N(%)				
Age group in years	Less than 20 (Ref)	2(3.6)	14(17.1)	1.00	--	1.00	--
	20-24	8(14.3)	38(46.3)	1.47(0.27-8.06)	0.654	1.12(0.18-6.91)	0.902
	25-34	39(69.6)	25(30.5)	10.9(2.19-54.4)	**0.004***	5.21(0.84-32.1)	0.076
	35 and above	7(12.5)	5(6.1)	9.80(1.41-68.1)	**0.021***	4.15(0.50-34.3)	0.187
Education status	Primary level (Ref)	38(67.9)	62(75.6)	1.00	--	1.00	--
	Non-formal	2(3.6)	4(4.9)	0.82(0.14–4.65)	0.816	0.51 (0.07–3.52)	0.495
	Secondary level	13(23.2)	13(15.9)	1.63(0.69–3.84)	0.264	1.25 (0.41–3.78)	0.692
	Tertiary level	3(5.4)	3(3.7)	1.63(0.31–8.51)	0.562	0.94 (0.12–7.21)	0.954
Marital status	Married (Ref)	47(83.9)	52(63.4)	1.00	--	1.00	--
	Single	6(10.7)	30(36.6)	0.22(0.08–0.58)	**0.002***	0.44 (0.13–1.48)	0.187
	Separated/Divorce	3(5.4)	0(0.0)	9.64 (0.48–190)	0.138	6.12 (0.21–178)	0.301
Occupation	Peasant farmer (Ref)	36(64.3)	57(69.5)	1.00	--	1.00	--
	Employed	1(1.8)	0(0.0)	4.81 (0.19–121)	0.341	2.15 (0.04–112)	0.680
	Business	15(26.8)	14(17.1)	1.70(0.74–3.89)	0.211	1.32 (0.48–3.62)	0.591
	Unemployed	4(7.1)	11(13.4)	0.58(0.17–1.93)	0.372	0.74 (0.18–3.12)	0.684
Average monthly income	>100,000 ugx (Ref)	6(10.7)	16(19.5)	--	1.00	--	1.00
	≤ 100,000 ugx	50(89.3)	66(80.0)	3.13(1.07-9.20)	**0.037***	2.04(0.61-6.80)	0.248

Statistically significant at p < 0.05, Reference = 1. COR is crude odds ratio and AOR is adjusted odds ratio.

Table 3: Maternal clinical factors of VVC among study participants

Variables	Category	Frequency (N)	Percentage (%)
Antibiotic used for over 12 months	Yes	65	47.1
	No	73	52.9
Medical history of the participants			

Urinary Tract Infection	Yes	66	47.8
	No	72	52.2
HIV positive	Yes	2	1.4
	No	136	98.6
Hypertension	Yes	7	5.1
	No	131	94.9

Table 4: Bivariate and Multivariate analysis of the maternal clinical factors associated with Vulvovaginal candidiasis among study participants.

Variables	**Categories**	**Proportion of VVC**		**COR(95% CI**	**P-value**	**AOR (95% CI)**	**P-value**
		Growth N(%)	**No growth N(%)**				
Urinary Tract Infections	No (Ref)	17(30.4)	55(67.1)	1.00	--	1.00	--
	Yes	39(69.6)	27(32.9)	4.67(2.25-9.69)	**<0.001***	2.15(0.81-5.72)	0.124
HIV positive	No (Ref)	54(96.4)	82(100.0)	1.00	--	1.00	--
	Yes	2(3.6)	0(0.0)	7.69 (0.36–163)	0.191	3.12 (0.06–165)	0.582
Hypertension	No (Ref)	52(92.9)	79(96.3)	1.00	--	1.00	--
	Yes	4(7.1)	3(3.7)	2.03(0.44–9.37)	0.366	1.45 (0.28–7.52)	0.658

Statistically significant at p < 0.05, Reference = 1. COR is crude odds ratio and AOR is adjusted odds ratio.

Table 5: Maternal obstetrical factors of VVC among study participants

Variables	**Category**	**Frequency (N)**	**Percentage (%)**
Gestational age	< 13 weeks	90	65.2
	13-28 weeks	25	18.1
	28 weeks and above	23	16.7
Parity	First pregnancy	43	31.2
	1-3	58	42.0
	4 and above	37	26.8
Previous C-section	Yes	13	9.4

	No	125	90.6
Current pregnancy complications	Yes	26	18.8
	No	112	81.9
Previous VVC	Yes	31	22.5
	No	107	77.5
Antibiotic used for over 12 months	Yes	65	47.1
	No	73	52.9

Table 6: Bivariate and Multivariate analysis of maternal obstetrical factors associated with Vulvovaginal candidiasis among study participants

Variables	**Categories**	**Proportion of VVC**		**COR(95% CI**	**P-value**	**AOR (95% CI)**	**P-value**
		Growth N(%)	**No growth N(%)**				
Gestational age	< 13 weeks (Ref)	41(73.2)	49(59.7)	--	1.00	--	1.00
	13-28 weeks	1(1.8)	24(29.3)	0.05(0.01–0.38)	**0.004***	0.03 (0.00–0.26)	**<0.001***
	28 weeks and above	14(25.0)	9(11.0)	1.86(0.73–4.74)	0.195	1.41 (0.44–4.49)	0.563
Parity	1st pregnancy (Ref)	17(30.3)	41(50.0)	--	1.00	--	1.00
	1-3	3(5.4)	40(48.8)	0.18(0.05-0.66)	**0.01***	0.12(0.03-0.48)	**0.003***
	4 and above	36(64.3)	1(1.2)	4.68(11.3-66.5)	**<0.001***	4.33(5.14-34.5)	**<0.001***
Previous C-section	No (Ref)	48(85.7)	77(93.9)	1.00	--	1.00	--
	Yes	8(14.3)	5(6.1)	2.57(0.79–8.30)	0.116	1.84 (0.42–8.06)	0.418
Current pregnancy complications	No (Ref)	32(57.1)	80(81.7)	1.00	--	1.00	--
	Yes	24(42.9)	2(18.3)	3.31(1.47–7.46)	**0.004***	1.95 (0.68–5.58)	0.215

Antibiotic used for > 2 weeks	No (Ref)	15(26.8)	58(70.3)	1.00	--	1.00	--
	Yes	41(73.2)	24(29.3)	6.61(3.11-14.1)	**<0.001***	4.88(1.92-12.4)	**<0.001***
Previous VVC	No (Ref)	33(58.9)	74(90.2)	1.00	--	1.00	--
	Yes	23(14.3)	8(2.4)	6.45(2.65–15.7)	**<0.001***	3.22 (1.04–10.0)	**0.043***

Statistically significant at p < 0.05, Reference = 1. COR is crude odds ratio and AOR is adjusted odds ratio.

Table 7: Colony characteristics of the isolates on SDA from various samples

Color	Shape	Elevation	Texture	Total (N)	Percent (%)
White	Round	Raised (convex)	Smooth	53	34.93
Cream	Irregular	Raised	Rough	37	25.34
Cream	Round with wrinkled edges	Raised	Smooth	19	13.01
White	Small & concentric	Raised	Smooth and shiny	29	19.86
Cream	round	Raised	Smooth	8	6.85

Table 8: Utilization of sugars by various isolates of *Candida species*

Isolates (*Candida species*)	Glu	Mal	Lac	Suc	Xyl	Cel	Proportion
White, Round, Raised (convex), Smooth	+	+	-	+	+	-	51(34.9%)
Cream, Irregular, Raised, Rough	+	-	-	-	-	-	37(25.3%)
Cream, Round with wrinkled edges, Raised, Smooth	+	+	-	+	+	+	19(13.0%)
White, Small & concentric, Raised, Smooth & shiny	+	-	-	-	-	-	29(19.9%)
Cream, round, Raised, Smooth	+	+	-	+	+	-	10(6.9%)

Legend: Glu: Glucose; Mal: Maltose; Lac: Lactose; Suc: Sucrose, Xyl: Xylose, Cel: *Cellobiose*

Table 9: Showing Antifungal profile of candida species performed by Disc diffusion method

Candida spp		**Nystatin**			**Clotrimazole**			**Miconazole**		
(n = 146)		**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
C. albicans (n = 51)	**N (%)**	11(21.6)	4(7.8)	36(70.6)	50(98.0)	1(2.0)	-	30(58.8)	19(37.3)	2(3.9)
***Non-albican candida spp* (n=95)**										
Cream, irregular, raised, rough (n = 37)	**N (%)**	12(32.4)	2(5.4)	23(62.2)	35(94.6)	2(5.4)	-	24(64.9)	13(25.1)	-
Cream, round wrinkled edge, smooth (n = 19)	**N (%)**	5(26.3)	1(5.3)	13(68.4)	18(94.7)	-	1(5.3)	7(36.8)	9(47.4)	3(15.8)
White, small & concentric, raised, smooth & shiny (n = 29)	**N (%)**	9(31.0)	3(10.4)	17(58.6)	28(96.6)	1(3.4)	-	20(70.0)	8(27.6)	1(2.4)
Cream, round, raised, smooth (n = 10)	**N (%)**	2(20.0)	-	8(80.0)	9(90.0)	1(10.0)	-	9(90.0)	1(10.0)	-

Legend: S- Susceptible, **I**- Intermediate, **R**- Resistant

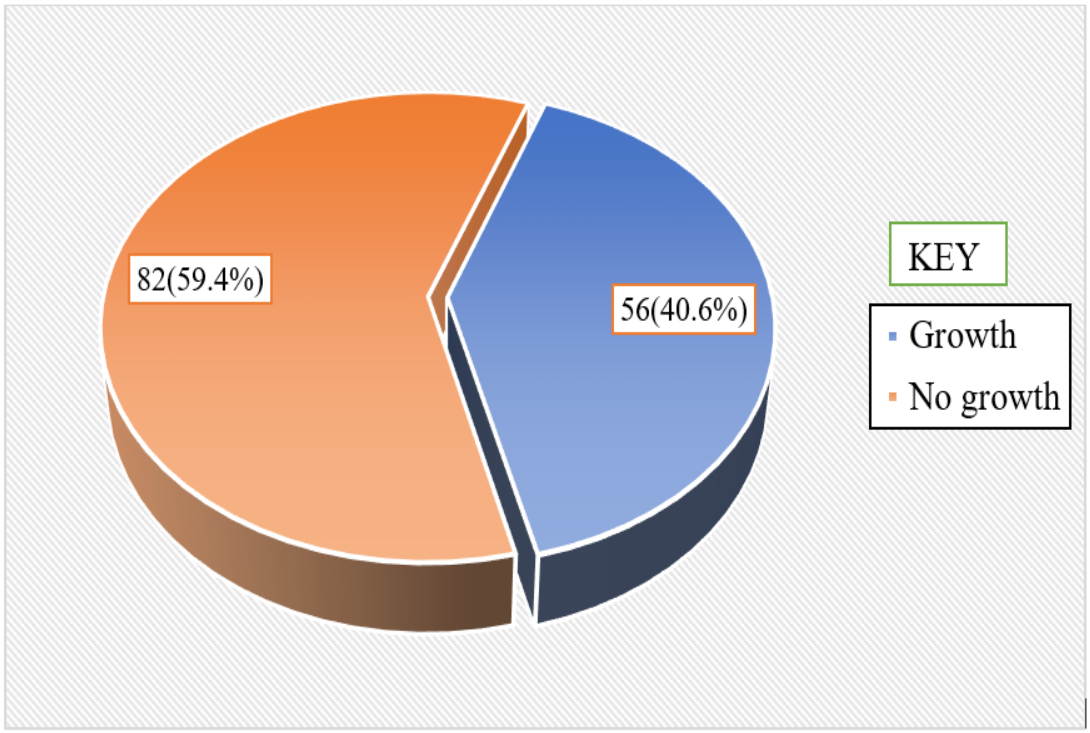


Figure 1: Prevalence of vulvovaginal candidiasis among pregnant women at LRRH

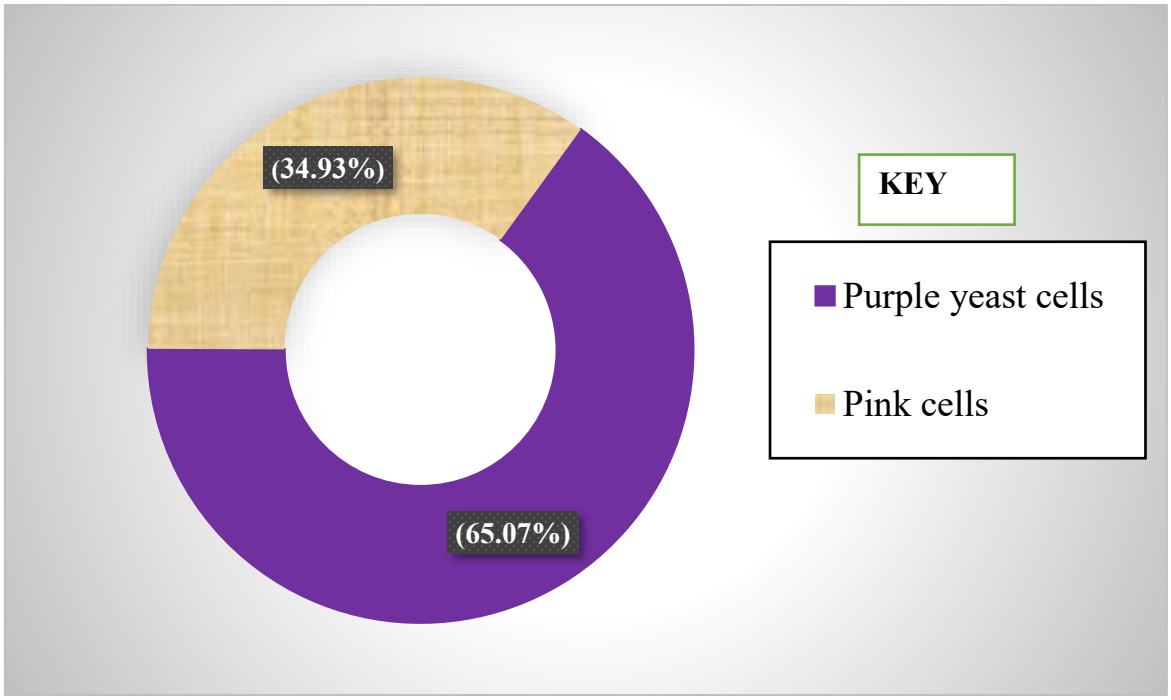


Figure 2: Gram stain technique for candida species

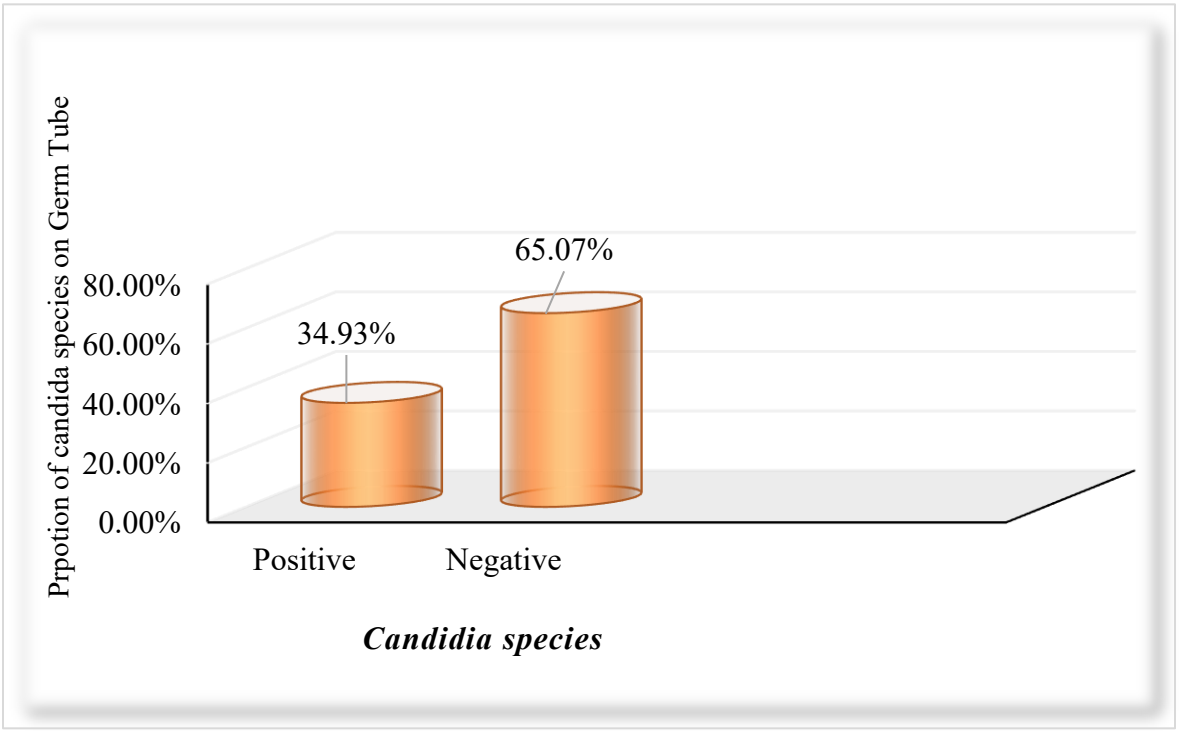


Figure 3: Showing Germ Tube test

Discussion

Prevalence of Vulvovaginal candidiasis among symptomatic pregnant women receiving antenatal care

According to our findings, the prevalence of vulvovaginal candidiasis among pregnant women receiving antenatal care at Lira Regional Referral Hospital was 40.6%, indicating that nearly four out of every ten pregnant women were affected by *candida* species. This high burden agrees with the report from Kamuli General Hospital in Eastern Uganda by [62] where they reported prevalence rate of VVC at 40.2%. Within the East African region, our findings are highly consistent with the 42.7% reported in Kenya and 42.9% in Tanzania [35]. These consistent regional figures reflect shared socioeconomic, and clinical risk profiles unique to East African antenatal women.

Concurrently, our result exceeded the broader continental averages. A meta-analysis carried out in Africa pooled the overall VVC prevalence among pregnant women at 29.2%, though it highlighted a pronounced sub-regional peak in Eastern Africa at 35% [44]. Consequently, the reported VVC prevalence rate in Lira Regional Referral Hospital surpassed the prevalence rate reported across Western Africa at 28%, and Northern Africa at 5% [44] including the reported prevalence from the middle belt of Ghana at 36.5% [38], Cameroon at 28.2% as reported by [25], Bukavu, Democratic Republic of the Congo at 38.2% [47], Burkina Faso at 22.71% [64]. Furthermore, our results significantly exceeded finding Ethiopia, where rates was 26.8% in Southern Ethiopia [30] and 30% in Hawassa [24] as well as the findings of [10] at a Medical Analysis Laboratory in Franceville, Gabon where the prevalence of VVC was low at 28.52%.

Conversely, substantially higher VVC prevalence rates among pregnant women have been reported globally. For instance, prevalence rates was up to 50.4% in Egypt [23], 51.6% in Yemen [6], and over 60% in Nigeria [53, 19]. Globally, our findings were higher than those in Papua New Guinea [38] and asymptomatic cohorts in India which reported 35% prevalence rate [63], but lower than the 67.8% prevalence recorded among symptomatic pregnant women in Brazil [58].

The wide variations in VVC prevalence across these studies can be largely attributed to differences in diagnostic methodologies. Methodologically, our study utilized culture on Sabouraud Dextrose Agar supplemented with chloramphenicol, which is highly sensitive for candida species isolation. In contrast, studies relying strictly on wet mount microscopy or Gram staining frequently reports

lower prevalence due to lower diagnostic sensitivity. Conversely, studies utilizing molecular methods yield much higher prevalence rates as was explained by [63].

Factors associated with vulvovaginal candidiasis in pregnant women receiving antenatal care

Socio-demographic factors associated with vulvovaginal candidiasis among pregnant women.

Our study found that the prevalence of vulvovaginal candidiasis among pregnant women was not independently associated with any socio-demographic factors at the multivariate level. This lack of independent association mirrors the findings of [8] and [74], who also reported no significant multivariate association between socio-demographic factors and VVC prevalence. However, these results contrast the findings from other studies where they found that age, marital status, income level and education as the main VVC associated factors. The lack of independent significance in our multivariate level suggest that clinical, or obstetrical factors may exert a more profound influence than socio-demographic variables alone.

At bivariate level, unadjusted crude analysis showed that maternal age was strongly associated with VVC; pregnant women aged 25-34 years and those aged 35 years and older were 10.9 and 9.8 times more likely to contract VVC, respectively, compared to those under 20 years. This high unadjusted prevalence among older participants mirrors findings by [34, 55] in Nigeria, [25] in Cameroon, and [53] in Northwest Nigeria. Biologically, older pregnant women often have higher number of pregnancies, resulting in prolonged exposure to estrogen and progesterone shifts that promote candida species colonization. Conversely, this pattern contradicts findings from Yemen [6], and Far North Region of Cameroon [50], where the younger pregnant women (<25 years) were the most vulnerable, likely due to immature vaginal defense mechanisms. The loss of statistical significance for age in our adjusted model confirms it as a confounding factor.

Marital status and income level similarly displayed significant associations only at the bivariate level. Bivariate analysis found that single participants were significantly less likely to have VVC than married women. This agrees with [35], who found high prevalence among cohabiting women, but opposes [32], who noted a higher prevalence of vulvovaginal candidiasis among single and widowed participants. This increased risk among married participants may be tied on the regular frequency sexual intercourse which serves as a predisposing factor during pregnancy. Furthermore, participants who earned ≤ 100,000 UGX were 3.13 times more likely to have VVC as compare to participants earning more than > 100,000 UGX monthly. This matches the observation from [6, 19]

which linked low socioeconomic status to VVC. Lower income limit pregnant women from managing their vaginal health effectively, leading to *candida* species overgrowth.

In contrast, maternal education level and occupation showed no statistical significant difference in VVC prevalence at all analysis level. This creates a distinct disparity with findings from [5, 6], where illiterate participants faced the highest risk of experiencing VVC. It simultaneously contradicts findings from [27, 70, 19], where they both found the highest VVC incidence among women with tertiary education. This imply that VVC affects pregnant women regardless of their employment status and educational attainment but rather due to other factors.

Maternal clinical related factors associated with vulvovaginal candidiasis among pregnant women.

At bivariate level, pregnant women with a reported history of urinary tract infections (UTIs) were 4.67 times more likely to have VVC than those without UTIs, however it lost its significance when adjusted to multivariate level. This crude association aligns with [27], who observed that participants suffering from UTIs often reports VVC symptoms. Empirical antibiotics treatment of UTIs during pregnancy eradicates protective vaginal microbiota triggering dysbiosis and the subsequent overgrowth of opportunistic *Candida* species. This was supported by [30] indicating that UTIs itself is not an associating VVC factors.

Surprisingly, our study found no statistically significant association between VVC prevalence and maternal comorbidities, specifically HIV status and gestational hypertension. These strongly contradicts many studies, including studies from [16, 31], which identify HIV-induced immunosuppression as the most associating factors for *candida* species overgrowth. Conversely, the studies from [26, 30] have consistently demonstrated that HIV is an independent associated factor for *Candida* species colonization disagreeing with our study. This can be attributed to Uganda's highly successful Prevention of Mother-to-Child Transmission protocols. Universal antenatal screening and robust adherence to Antiretroviral Therapy among HIV-positive pregnant women likely suppressed viral loads and maintained high CD4+ T-lymphocyte counts, thereby preserving mucosal immunity and preventing opportunistic *Candida* proliferation.

Maternal obstetrical factors associated with vulvovaginal candidiasis among pregnant women.

Regarding gestational age, our study found that participants in their second trimester (13-28 weeks) were significantly less likely to contract VVC than those in the first trimester (< 13 weeks).

This aligns with [41], who reported a first trimester VVC peak of 53.57%. This early trimester burden of VVC is driven by initial high peak level of maternal estrogen which elevate vaginal glycogen content and create an optimal carbon source for candida growth. Conversely, our findings contrasts with findings from North-west Nigeria [53] and Ghana [38], where VVC prevalence rose continuously into the third trimester. Our results also differ from [55], who observed a second-trimester peak (56.7%), and [8], who reported no statistically significant variations across trimesters.

Maternal parity also emerged as a powerful factors associated with VVC. Grand multiparous women (≥ 4 children) faced 4.33 times higher odds of developing VVC compared to primigravidae women, low-parity participants (1-3 children) were 88% less likely to be infected than primigravidae women. This finding is strongly supported by studies in India [71], Nigeria [53, 52], and Ethiopia [24], which uniformly identify multiparity as a significant predisposing factor for VVC. This suggest that multiple pregnancies cause long-term physiological changes to the vaginal environment making it easier for *Candida* species to colonize permanently. In contrast, [38] in Ghana reported that women with more than four deliveries had a lower risk of VVC, directly contradicting the positive association observed in our study.

Participants who were on antibiotic therapy for more than 2 weeks within one month have nearly five-fold higher odds of developing VVC. The findings aligns findings from Bbule Hhora University Teaching Hospital, Ethiopia [30], Cameroon [25], and other global studies [27, 45, 8, 5]. Prolonged antibiotic exposure disrupts the delicate vaginal ecosystem by eradicating protective, hydrogen peroxide-producing *Lactobacillus* species, thereby eliminating competitive inhibition and allowing yeast to flourish. However, this finding contradicts [38], who observed no association between antibiotic use and VVC prevalence.

Finally, a history of prior VVC infections significantly elevated current gestational risk, with previous sufferers being 3.22 times more likely to present with an active infection. This strongly concurs with findings by [79, 22, 5, and 30]. This high rate of recurrence is tied to the capacity of *Candida* species to form biofilms protecting fungal cells from antifungal agents, allowing latent yeast reservoirs to persist and reactivate under the hormonal influences of pregnancy. Participant with current pregnancy complication were likely to have VVC though are not an independent cause since it remains insignificance when adjusted. This nuances the claim by [2] that VVC is associated with complications.

Isolation and Identification of *Candida* species causing vulvovaginal candidiasis in pregnant women

The variations in *Candida* species distribution between our study and other studies are highly attributable to the specific diagnostic methodologies employed. In this study, phenotypic differentiation of *C. albicans* from NAC species relied on a robust combination of macroscopic colony characteristics on Sabouraud Dextrose Agar supplemented with chloramphenicol, carbohydrate assimilation tests, and the germ tube test (GTT). On SDA, *Candida* isolates displayed characteristic white-to-cream, round, smooth, and raised colonies. Among these, 34.93% were definitively confirmed as *C. albicans* based on a positive germ tube test and specific sugar assimilation profiles. This multi-phenotypic approaches aligns with the classic diagnostic methods by [77, 37, and 29].

The inclusion of the germ tube test provided an estimated specificity of 99% for differentiating *C. albicans* from NAC species, mitigating the diagnostic limitations of older studies that relied solely on basic microscopy or carbohydrate assimilation assay. The high *C. albicans* rate (84%) reported by [41] was derived using rapid carbohydrate assays without germ tube confirmation. Conversely, when the germ tube test was utilized by [58], high *C. albicans* rates (80.9%) are still observed, validating that true regional variances exist alongside methodological ones.

The used of germ tube test in our study as a rapid, accessible, and reliable diagnostic tool for *C. albicans* isolation matches the methods utilized by [77, 14]. This protocol is further supported by [9] and Zida et al. (2017). Notably, our positive germ tube isolation rate (34.93%) was lower than that of Zida et al. (2017), who documented a 59.36% rate, highlighting differences in the baseline distribution of the target species within our respective study populations.

Conversely, the microscopic examination via Gram staining revealed that 65.07% of the total isolates presented as classic Gram-positive (purple), oval, budding yeast cells, confirming their status as *Candida* species. The remaining 34.93% of samples exhibited pink, non-budding cellular morphologies. In general, *Candida* cells consistently retain crystal violet to appear Gram-positive, the appearance of pink, non-budding structures typically points to non-candidal elements, bacterial cells rather than viable yeast. This overall microscopic prevalence rate of true budding yeasts contrasts studies reporting high *Candida* species, [25] at 71.79%, [58] at 80.9%, and [47], who reported a 91.0% prevalence using direct microscopic techniques in the Democratic Republic of Congo.

However, our study was not able to perform definitive species-level speciation among the 65.07% NAC isolates. This contrasts with more epidemiological studies, such as the findings by [10] in Franceville, Gabon, which successfully speciated 11 distinct *Candida* species, [10] found a higher representation of *C. albicans* (82.73%) over NAC (17.27%), they were able to map the exact distribution of NAC species: *C. famata* (4.02%), *Candida spp.* (3.61%), *C. rugosa* (3.21%), *C. lipolytica* (1.61%), *C. parapsilosis* (1.61%), *C. glabrata* (1.21%), *C. tropicalis* (0.80%), *C. krusei* (0.40%), *C. dubliniensis* (0.40%), and *C. sphaerica* (0.40%).

Furthermore, detailed speciation in studies by [41, 70, and 61] consistently identified *Candida glabrata* as the second most prevalent isolated species following *C. albicans*. Because our phenotypic protocol grouped these organisms collectively as NAC, future studies in this region should incorporate molecular techniques to identify the specific non-*albicans* subspecies driving this high 65.07% prevalence in Lira Regional Referral Hospital.

Antifungal susceptibility of *Candida* species Isolated from pregnant women with Vulvovaginal candidiasis.

In this study, clotrimazole emerged as the most effective antifungal agent, demonstrating a robust susceptibility rates of 90% among *non-albicans candida* species and 98% for *candida albicans* species. This high sensitivity matches findings by [1], who reported a sensitivity rate of 87.2% in *candida albicans.* It also mirrors findings from [37], where nearly all *non-albicans candida* species isolates were sensitive to clotrimazole as well as observations by [80] in Chinese pregnant women.

Conversely, our findings strongly contradict a study finding by Zida et al. (2017) in Burkina Faso and [34] in India, who reported a much lower susceptibility rates of 33.5% and 70.96% respectively alongside a high resistance rates of 66.5% and 29%. Furthermore, [29], reported a 20% resistant rate which opposes our negligible resistance profile. The high effectiveness of Clotrimazole suggests that most candida species have not yet developed significant resistance, making it the ideal first-line treatment in Lira regional referral hospital.

In contrast, miconazole exhibited a wider variation in susceptibility, ranging from 36.8% to 90% across all tested isolates, implying that nearly up to 10% of these isolates have already acquired resistance. This susceptibility profiles is consistent with [41] which reported 93.2% sensitivity and matches the low 7.5% resistance rate recorded by [29]. However, our findings deviated from Zida et al. (2017), where a high miconazole resistance rate of 52.3% were reported. The clear disparities

in miconazole sensitivity across various studies are likely driven by the specific proportional distribution of emerging NAC species versus *C. albicans* within each distinct study population.

Alarmingly, nystatin demonstrated an extensive therapeutic failure in our study, with a high resistance ranging from 58.6% to 80.0% across all *candida* species, where *C. albicans* specifically showed 70.6% resistance rate. This profound resistance is heavily supported by [25], who recorded 100% nystatin resistance in Cameroon, and [74], who found that less than 4% of their isolates were susceptible to nystatin. Conversely, our findings opposes the high sensitivity rate documented by [1] at 87.2% and [33] at 94%, as well as finding from [15], who reported 100% sensitivity rate across NAC species. Nystatin is widely available, affordable, and frequently used without a prescription, this self-medication drive the emergence of resistance strains of candida species, supporting the severe nystatin resistance observed in this study which points toward the emergence of heavily resistant *Candida* strains within LRRH [22].

Recommendations

- Nystatin as a first-line treatment for VVC in pregnancy especially in Northern Uganda should be re-evaluated, hence clinical guidelines should prioritize Clotrimazole due to its superior 98% efficacy rate and ensuring its availability in the health facility within northern Uganda.
- Laboratory capacity should be strengthened at regional hospitals by providing resources for biochemical testing or molecular identification to address the increasing emergence of NAC.
- We also recommend that there should be routine culture and susceptibility testing be incorporated in ANC
- We recommend that clinicians should exercise caution when prescribing antibiotic therapy to pregnant women especially if the treatment duration will be for 2 weeks.
- We further recommend that pregnant women with high parity (4+ children), in their first trimester, previous VVC, with a history of UTIs or are undergoing antibiotic treatment should be closely monitored and encouraged to seek early treatment.
- We recommend future researcher to utilize a longitudinal design to track how *Candida* colonization changes throughout the entire course of pregnancy.
- Need for multicenter studies to confirm findings integrating antifungal stewardship into maternal health program.

- Further studies should also employ Polymerase Chain Reaction and sequencing to precisely identify the specific species within the NAC group, as different NAC species have varying levels of drug resistance.

Conclusion

The prevalence of vulvovaginal candidiasis among pregnant women at Lira regional referral hospital remains significantly higher than global and Africa pooled averages, driven by a clinical associated factors such as first-trimester status, high parity (≥4 children), prolonged antibiotic use, previous VVC history, and concurrent UTIs, conversely, being a first-time mother or in the second trimester provides a protective effect. Driven by a profound, non-traditional shift toward non-*albicans candida* species (NACs). Regarding therapeutics outcome, Clotrimazole remains a highly effective therapeutic option to both *C. albicans* and non-*albicans candida* species making it a primary local treatment for VVC in pregnant populations, the discovery of massive nystatin resistance particularly among the dominant non-*albicans candida* species presents a severe obstacle to standard empirical treatment protocols. This underscores an urgent need to update local guidelines away from nystatin and toward species-specific management.

Study Limitations

- The incubation times of *Candida* species are affected by frequent power fluctuations. This was resolved by working throughout the day when the generator was running.
- The study was conducted at Lira Regional Referral Hospital indicating that most vulnerable pregnant women who could not afford or access hospital care during the study period were excluded, potentially skewing the estimated community prevalence.
- There was a possibility of a potential recall bias regarding monthly income, previous history of VVC, and prior antibiotic use, where participants might inaccurately report sensitive information or forget past clinical events, potentially affecting the accuracy of the identified associated factors.
- Since the identification of *Candida* species relied on phenotypic methods, such as SDA, sugar assimilation test and Germ Tube tests which seem standard in regional settings, they are less sensitive than molecular techniques.

- This study design was purely cross-sectional in nature, this study design captures data at a single point in time, which precludes the ability to account for the recurrent nature of vaginal candidiasis throughout the entire course of pregnancy.

List of abbreviations

ANC: Antenatal Clinic

ANOVA: Analysis of Variance

CFU: Colony Forming Unit

CLSI: Clinical and Laboratory Standards Institute

HVS: High Vaginal Swab

IBM: International Business Machine

KIU-WC: Kampala International University Western Campus

LRRH: Lira Regional Referral Hospital

MIC: Minimum Inhibitory Concentration

MoH: Ministry of Health

SOP: Standard Operating Procedure

SPSS: Statistical Package for Social Sciences

UCG: Uganda Clinical Guideline

VVC: Vulvovaginal Candidiasis

WHO: World Health Organization

Declaration

Ethical approval and consent to participate

Ethical approval was obtained from the Kampala International University, Western Campus Research and Ethics Committee, which granted formal approval under approval number KIU-2024-396. The study was conducted in strict accordance with the ethical principles of the Declaration of Helsinki. Administrative clearance to conduct the study and access patient records

was obtained from the Office of the Hospital Director and the Head of the Obstetrics and Gynecology Department at Lira Regional Referral Hospital. The trained research assistants provided a detailed, verbal explanation of the study's purpose, scope, and procedures to the participants. All eligible pregnant women aged 18 years and older provided written, signed, or thumbprint informed consent. For participants aged below 18 years, written informed consent was obtained from their parents or legal guardians, alongside direct informed assent from the minors.

Participants were assured that their involvement in this research was completely voluntary. They were informed of their absolute right to withdraw from the study at any stage of the interview or laboratory collection without providing a reason. Furthermore, participants were free to decline to answer any specific questions within the questionnaire, and this resulted in no penalties, loss of benefits, or compromise to the routine antenatal care. No direct identifiers, such as names, telephone numbers, or hospital registration numbers, were recorded on the questionnaires or laboratory logbooks. Instead, each participant was assigned a unique, sequential alphanumeric study identification code. Filled questionnaires, laboratory data logs, and signed consent forms were stored in a secure filing cabinet accessible exclusively to the principal investigator. Electronic data spreadsheets were protected on a password-protected computer.

The collection of HVS using unlubricated speculums may have caused brief physical discomfort or mild anxiety; hence, the examinations were conducted in a highly private clinic room, and the midwife maintained continuous communication with the patient during the procedure. The packaging, handling, and transportation of the human samples were done in strict compliance with standard biosafety and national laboratory transport regulations to maintain sample integrity and safety. Participants received direct diagnostic benefits through free laboratory testing for VVC. Participants with positive findings were directed to the attending hospital clinicians for targeted, safe, and cost-effective antifungal therapies. Each participant was given a 2,000 UGX token as an appreciation for their time and participation in this study.

Consent for publication

Not applicable

Competing interest

No, we declare that the authors have no competing interests or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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Availability of data and materials

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Author contributions

AB: Study design and initial manuscript draft preparation; VS: Conceptualizing, Data collection and manuscript writing support; NR: Editing the manuscript and data collection and data interpretation; CA: Data curation and initial draft of the manuscript to the final form. NSW: Edited the final copy of the Manuscript. All authors reviewed the manuscript and approved the final version of the manuscript.

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References

1. Aboagye, G., Waikhom, S., Asiamah, E. A., Tettey, C. O., Mbroh, H., Smith, C., Osei, G. Y., Asafo Adjei, K., & Asmah, R. H. (2025). Antifungal susceptibility profiles of Candida and non-albicans species isolated from pregnant women: Implications for emerging antimicrobial resistance in maternal health. *Microbiology Spectrum*, *13*(7), e00787-25. https://doi.org/10.1128/spectrum.00787-25
2. Aguin, T. J., & Sobel, J. D. (2015). Vulvovaginal Candidiasis in Pregnancy. *Current Infectious Disease Reports*, *17*(6), 30. https://doi.org/10.1007/s11908-015-0462-0
3. Ajani, T. A., Elikwu, C. J., Tuta, K. E., Shonekan, O. A., Okangba, C. C., Anaedobe, C. G., Ebeigbe, E., Osinowo, A. O., Makanjuola, O. B., Ogunbiyi, A. I., Sadare, O. A., Oluwasola, T.

O., & Nwadike, V. U. (2024). Candida species causing vulvovaginitis among patients in Babcock University Teaching Hospital, Ilishan-Remo, Ogun State: A retrospective cross-sectional study. *Journal of Clinical Sciences*, *21*(1), 32. https://doi.org/10.4103/jcls.jcls_38_23

4. Al-Hatami, S. M. M., Al-Moyed, K. A. A., Al-Shamahy, H. A., Al-Haddad, A. M., & Al-Ankoshy, A. A. M. (2021). Vulvovaginal Candidiasis: Prevalence, Species Distribution And Risk Factors Among Non-Pregnant Women, In Sana'a, Yemen. *Universal Journal of Pharmaceutical Research*. https://doi.org/10.22270/ujpr.v6i3.613
5. Ali, M., Edrees, W. H., Al-Shehari, W. A., Xue, G., Al-Hammadi, S., Qasem, E. A., Chaulagain, R. P., & Lal, N. (2024). Antifungal susceptibility pattern of Candida species isolated from pregnant women. *Frontiers in Cellular and Infection Microbiology*, *14*, 1434677. https://doi.org/10.3389/fcimb.2024.1434677
6. Al-Rukeimi, A. A., Al-Hatami, S. M. M., AL-Danany, D. A., Al-Shamahy, H. A., & Rukeimi, R. A. A. A. (2020). Prevalence And Risk Factors Associated With Vulvovaginal Candidiasis During Pregnancy In Sana'a, Yemen. *Universal Journal of Pharmaceutical Research*. https://doi.org/10.22270/ujpr.v5i3.407
7. Bashir, G., Altaf, I., Khurshid, R., Ahmed, T., Ali, A., & Zaffar, S. (2023). Identification and pattern of antifungal susceptibility of Candida species isolated from cases of vaginitis in a tertiary care hospital in India. *Iranian Journal of Microbiology*, *15*(2), 318–324. https://doi.org/10.18502/ijm.v15i2.12484
8. Bender, R. A., Çalışkan, Ş., Önal, B., Aslancan, R., & Çalışkan, E. (2021). Treatment methods for vulvovaginal candidiasis in pregnancy. *Journal of Medical Mycology*, *31*(3), 101138. https://doi.org/10.1016/j.mycmed.2021.101138
9. Bhumbla, U., & Gupta, A. (2021). A comparative study for production of germ tube in Candida albicans of various pulmonary samples, by different methods in a tertiary care hospital of south west Rajasthan. *International Journal Of Community Medicine And Public Health*, *8*(12), 5968. https://doi.org/10.18203/2394-6040.ijcmph20214598
10. Bignoumba, M., Onanga, R., Bivigou Mboumba, B., Gafou, A., Mouanga Ndzime, Y., Lendamba, R. W., Mbombe Moghoa, K., & Kassa Kassa, R. F. (2019). Vulvovaginal candidiasis among symptomatic women of childbearing age attended at a Medical Analysis Laboratory in Franceville, Gabon. *Journal de Mycologie Médicale*, *29*(4), 317–319. https://doi.org/10.1016/j.mycmed.2019.100895

11. CDC. (2024, May 16). *Preventing Candidiasis*. Candidiasis. https://www.cdc.gov/candidiasis/prevention/index.html
12. CDC. (2025, September 12). *Treatment of Candidiasis*. Candidiasis. https://www.cdc.gov/candidiasis/treatment/index.html
13. Centers for Disease Control and Prevention. (2021, July 22). Vulvovaginal Candidiasis - STI Treatment Guidelines.
14. Chiang, S. J. F., Chien, M.-K., Tsai, C.-Y., Hsiao, J.-C., Koo, F.-H., Yen, Y.-F., Chou, Y.-C., & Cheng, C.-C. (2024). A Simple, Fast, and Reliable Method for the Identification of Candida albicans. *Environmental Health Insights*, *18*, 11786302241272398. https://doi.org/10.1177/11786302241272398
15. Chokoeva, A., Kouzmanov, A., Ivanova, Z., Zisova, L., Amalie, G., Petleshkova, P., Miteva-Katrandzhieva, T., Krasteva, M., & Uchikova, E. (2016). Investigation On Antifungal Susceptibility Of Candida Yeasts In Pregnant Patients With Confirmed Vulvovaginal Candidiasis And Their Newborns.. *Akusherstvo I Ginekologiia*, *55*(4), 20–29.
16. Chongtham, U., Athokpam, D. C., & Singh, R. M. (2022). Isolation, Identification and Antifungal Susceptibility Testing of Candida Species: A Cross-sectional Study from Manipur, India. *JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH*. https://doi.org/10.7860/JCDR/2022/55695.16248
17. Clinical and Laboratory Standards Institute (CLSI). (2024). *Quality control for commercially prepared microbiological culture media and staining reagents; Approved standard* (CLSI document M22-A4). Clinical and Laboratory Standards Institute.
18. Cochran, W. G. (1977). *Sampling techniques* (3rd ed.). John Wiley & Sons.
19. Disha, T., & Haque, F. (2022). Prevalence and Risk Factors of Vulvovaginal Candidosis during Pregnancy: A Review. *Infectious Diseases in Obstetrics and Gynecology*, *2022*, 6195712. https://doi.org/10.1155/2022/6195712
20. Dong, Z., Fan, C., Hou, W., Rui, C., Wang, X., Fan, Y., Zhao, L., Wang, Q., Wang, Z., Zeng, X., Feng, S., & Li, P. (2022). Vaginal Exposure to Candida albicans During Early Gestation Results in Adverse Pregnancy Outcomes via Inhibiting Placental Development. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.816161
21. Duarte, G., Linhares, I. M., Kreitchmann, R., Tristão, A. D. R., Traina, E., Canti, I., Takimura, M., & Andrade, J. Q. (2024). Vulvovaginitis in pregnant women. *Revista Brasileira de*

Ginecologia e Obstetrícia / RBGO Gynecology and Obstetrics, *46*, e-FPS03. https://doi.org/10.61622/rbgo/2024FPS03

22. Edrees, W. H., Al-Asbahi, A. A., Al-Shehari, W. A., & Qasem, E. A. (2020). Vulvovaginal Candidiasis Prevalence Among Pregnant Women In Different Hospitals In Ibb, Yemen. *Universal Journal of Pharmaceutical Research*. https://doi.org/10.22270/ujpr.v5i4.431
23. ElFeky, D. S., Gohar ,Noha Mahmoud, El-Seidi ,Eman Ahmad, Ezzat ,Mona Mahmoud, & and AboElew, S. H. (2016). Species identification and antifungal susceptibility pattern of Candida isolates in cases of vulvovaginal candidiasis. *Alexandria Journal of Medicine*, *52*(3), Article 3. https://doi.org/10.1016/j.ajme.2015.10.001
24. Eskezia, A., Teklemichael, A. M., & Alemayehu, T. (2025). 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. *BMC Pregnancy and Childbirth*, *25*(1), 299. https://doi.org/10.1186/s12884-025-07402-9
25. Eyong, I. M., Iwewe, Y. S., Yangsi, T. T., Abas, A., Katoua, F. N., & Njamen, T. N. (2023). Vulvovaginal Candidiasis in Pregnant Women Attending the Garoua Regional Hospital (Cameroon) and Antifungals Susceptibility Profile of Isolates. *Asian Research Journal of Gynaecology and Obstetrics*, 151–159.
26. Gedefie, A., Shimeles, G., Motbainor, H., Kassanew, B., & Genet, C. (2025). Vaginal colonization and vertical transmission of Candida species: Prevalence and associated factors among pregnant women and their neonates at public health facilities of Northeast Ethiopia. *BMC Pregnancy and Childbirth*, *25*(1), 22. https://doi.org/10.1186/s12884-024-07103-9
27. Ghaddar, N., El Roz, A., Ghssein, G., & Ibrahim, J.-N. (2019). Emergence of Vulvovaginal Candidiasis among Lebanese Pregnant Women: Prevalence, Risk Factors, and Species Distribution. *Infectious Diseases in Obstetrics and Gynecology*, *2019*, e5016810. https://doi.org/10.1155/2019/5016810
28. Gharaghani, M., Ahmadi, B., Sisakht, M. T., Ilami, O., Aramesh, S., Mouhamadi, F., Barati, Z., Roozmeh, S., Mohammadi, H., & Nouripour-Sisakht, S. (2017). *Identification of Candida Species Isolated from Vulvovaginal Candidiasis Patients by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) in Yasuj Southwestern Iran*

(Research Article No. 11; Issue 8, p. e65359). Jundishapur Journal of Microbiology. https://doi.org/10.5812/jjm.65359

29. Gubran, A. N. M., Al-Baghdadi, M. A. A., & Al-Haidary, N. M. (2026). Vulvovaginal candidiasis and antifungal susceptibility pattern of isolated Candida spp. Among women in Aden Governorate, Yemen. *BMC Infectious Diseases*, *26*(1), 232. https://doi.org/10.1186/s12879-025-12471-4
30. Hussen, I., Aliyo, A., Abbai, M. K., & Dedecha, W. (2024). Vaginal candidiasis prevalence, associated factors, and antifungal susceptibility patterns among pregnant women attending antenatal care at bule hora university teaching hospital, Southern Ethiopia. *BMC Pregnancy and Childbirth*, *24*(1), 619. https://doi.org/10.1186/s12884-024-06844-x
31. Jafarzadeh, L., Ranjbar, M., Nazari, T., Naeimi Eshkaleti, M., Aghaei Gharehbolagh, S., Sobel, J. D., & Mahmoudi, S. (2022). Vulvovaginal candidiasis: An overview of mycological, clinical, and immunological aspects. *Journal of Obstetrics and Gynaecology Research*, *48*(7), 1546–1560. https://doi.org/10.1111/jog.15267
32. Jombo, G. T. A., Akpera, M. T., Hemba, S. M., & Eyong, K. I. (2011). Symptomatic vulvovaginal candidiasis: Knowledge, perceptions and treatment modalities among pregnant women of an urban settlement in West Africa. *African Journal of Clinical and Experimental Microbiology*, *12*(1). https://doi.org/10.4314/ajcem.v12i1.61045
33. Kandeel, A., & Elmitwalli, A. (2017). Prevalence, Risk Factors and Antifungal Susceptibility of Vulvovaginal Candadiasis among Saudi Females. *The Egyptian Journal of Medical Microbiology*, *26*(2), 47–52. https://doi.org/10.12816/0046228
34. Kavitha.Y, D., S, D. M., V, D. A., & Babu, D. H. (2018). A study on Vulvovaginal candidiasis among non-pregnant women. *Tropical Journal of Pathology and Microbiology*, *4*(5), Article 5. https://doi.org/10.17511/jopm.2018.i05.05
35. Kawenyera, L. (2023). Factors Associated With Vaginal Candidiasis Among Pregnant Women At Kawaala Health Centre Iv, Kampala District. A Cross-Sectional Descriptive Study. *Student's Journal of Health Research Africa*, *4*(9), Article 9. https://doi.org/10.51168/sjhrafrica.v4i9.408
36. Kish, L. (1965). *Survey sampling*. John Wiley & Sons.
37. Kollam, & S, D. Thanooja. (2021). Prevalence of Candida albicans /Non albicans species in clinically suspected cases of vulvo vaginitis in antenatal women and their susceptibility to

commonly used antifungals. *Journal of Medical Science And Clinical Research*, *09*(02). https://doi.org/10.18535/jmscr/v9i2.45

38. Konadu, D. G., Owusu-Ofori, A., Yidana, Z., Boadu, F., Iddrisu, L. F., Adu-Gyasi, D., Dosoo, D., Awuley, R. L., Owusu-Agyei, S., & 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), 341. https://doi.org/10.1186/s12884-019-2488-z
39. Lira Regional Referral Hospital. (2024). *Health Management Information System (HMIS) annual antenatal clinic department report* (Report No. LRRH-HMIS-2024-V2). Ministry of Health, Republic of Uganda.
40. Levina, J., Ocviyanti, D., & Adawiyah, R. (2024a). Management of Vulvovaginal Candidiasis in Pregnancy. *Indonesian Journal of Obstetrics and Gynecology*, 115–121. https://doi.org/10.32771/inajog.v12i2.1990
41. Maftei, N.-M., Arbune, M., Georgescu, C. V., Elisei, A. M., Iancu, A. V., & Tatu, A. L. (2023b). Vulvovaginal Candidiasis in Pregnancy—Between Sensitivity and Resistance to Antimycotics. *Journal of Xenobiotics*, *13*(3), Article 3. https://doi.org/10.3390/jox13030023
42. Messina, A., Mariani, A., Brandolisio, R., Tavella, E., Germano, C., Lipari, G., Leo, L., Masturzo, B., & Manzoni, P. (2024). Candidiasis in Pregnancy: Relevant Aspects of the Pathology for the Mother and the Fetus and Therapeutic Strategies. *Tropical Medicine and Infectious Disease*, *9*(5), Article 5. https://doi.org/10.3390/tropicalmed9050114
43. Ministry of Health. (2023). Uganda Clinical Guidelines 2023: National guidelines for management of common conditions. Republic of Uganda. health.go.ug
44. Mohamed, A. O., Mohamed, M. S., Mallhi, T. H., Hussain, M. A., Jalloh, M. A., Omar, K. A., Alhaj, M. O., & Ali, A. A. M. M. (2022). Prevalence of vulvovaginal candidiasis among pregnant women in Africa: A systematic review and meta-analysis. *The Journal of Infection in Developing Countries*, *16*(08), 1243–1251. https://doi.org/10.3855/jidc.15536
45. Mohammadi, S., Keshavarzi, F., Manouchehri, B., Lahoorpour, F., & Javaheri, M. R. (2020). Etiologic factors of Candida vulvovaginitis in pregnant women. *Journal of Gorgan University of Medical Sciences*, *22*(2), 104–109.
46. Mukasa, K. J., Herbert, I., Daniel, A., Sserunkuma, K. L., Joel, B., & Frederick, B. (2015). Antifungal Susceptibility Patterns of Vulvovaginal Candida species among Women Attending

Antenatal Clinic at Mbarara Regional Referral Hospital, South Western Uganda. *British Microbiology Research Journal*, *5*(4), 322–331. https://doi.org/10.9734/BMRJ/2015/13804

47. Mulinganya, M. G., De Keyser, K., Mongane, I. J., Kampara, M. F., De Vulder, A., Boelens, J., Duyvejonck, H., Hendwa, E., Kujirakwinja, B. Y., Bisimwa, B. G., Rodriguez, A., Vaneechoutte, M., Callens, S., & Cools, P. (2024). Second trimester vaginal Candida colonization among pregnant women attending antenatal care in Bukavu, Democratic Republic of the Congo: Prevalence, clinical correlates, risk factors and pregnancy outcomes. *Frontiers in Global Women's Health*, *5*. https://doi.org/10.3389/fgwh.2024.1339821
48. Mushi, M. F., Mmole, A., & Mshana, S. E. (2019). Candida vaginitis among symptomatic pregnant women attending antenatal clinics in Mwanza, Tanzania. *BMC Research Notes*, *12*(1), 775. https://doi.org/10.1186/s13104-019-4793-z
49. Musinguzi, B., J. Sande, O., Mboowa, G., Baguma, A., Itabangi, H., & Achan, B. (2023). Laboratory Diagnosis of Candidiasis. In T. Askun (Ed.), *Candida and Candidiasis*. IntechOpen. https://doi.org/10.5772/intechopen.106359
50. Nadia, N. A. C. (2023). Prevalence and Antifungal Resistance Profile of Vulvovaginal Candidiasis Infections in Pregnant Women in Maroua, Far North Region of Cameroon. *International Archives of Medical Microbiology*, *5*(1), 018. https://doi.org/10.23937/2643-4008/1710018
51. Nalumbejja, L. H., & Wolinga, S. (2024). Prevalence And Associated Factors Of Vaginal Candidiasis Among Pregnant Women Attending Antenatal Clinic. A Cross-Sectional Study At Jinja Regional Referral Hospital, Jinja City. *SJ Gynecology and Obstetrics Africa*, *1*(5), Article 5. https://doi.org/10.51168/b4184j61
52. Nelson, M., Wanjiru, W., & Margaret, M. (2013). Identification and Susceptibility Profile of Vaginal Candida Species to Antifungal Agents among Pregnant Women Attending the Antenatal Clinic of Thika District Hospital, Kenya. *Open Journal of Medical Microbiology*, *3*(4), Article 4. https://doi.org/10.4236/ojmm.2013.34036
53. Nnadi, D. C., & Singh, S. (2017). The prevalence of genital Candida species among pregnant women attending antenatal clinic in a tertiary health center in North-west Nigeria. *Sahel Medical Journal*, *20*(1), 33. https://doi.org/10.4103/1118-8561.204333
54. Nurat, A. A., Babalola, G. O., Shittu, M. O., Tijani, M. A., & Adekola, S. A. (2015). Detection and Epidemiology of Vulvovaginal Candidiasis among Asymptomatic Pregnant Women

Attending a Tertiary Hospital in Ogbomoso, Nigeria. *International Journal of Biomedical Research*, *6*(7), 518. https://doi.org/10.7439/ijbr.v6i7.2242

55. Nyoyoko, V., Christopher, M., Antia, U., & Eyo, I. (2022). Prevalence of vulvovaginal candidiasis in pregnant women. *Microbes and Infectious Diseases*. https://doi.org/10.21608/mid.2021.87167.1175
56. Osman Mohamed, A., Suliman Mohamed, M., Hussain Mallhi, T., Abdelrahman Hussain, M., Ali Jalloh, M., Ali Omar, K., Omar Alhaj, M., & Makki Mohamed Ali, A. A. (2022). Prevalence of vulvovaginal candidiasis among pregnant women in Africa: A systematic review and meta-analysis. *Journal of Infection in Developing Countries*, *16*(8), 1243–1251. https://doi.org/10.3855/jidc.15536
57. Paladine, H. L., & Desai, U. A. (2018). Vaginitis: Diagnosis and Treatment. *American Family Physician*, *97*(5), 321–329.
58. Pereira, L. C., Correia, A. F., da Silva, Z. D. L., de Resende, C. N., Brandão, F., Almeida, R. M., & de Medeiros Nóbrega, Y. K. (2021). Vulvovaginal candidiasis and current perspectives: New risk factors and laboratory diagnosis by using MALDI TOF for identifying species in primary infection and recurrence. *European Journal of Clinical Microbiology & Infectious Diseases*, *40*(8), 1681–1693. https://doi.org/10.1007/s10096-021-04199-1
59. Pratyusha Yakasiri, K., & Siddabathuni, S. (2020). Performance analytics of the rapid germ tube test in speciation matrices of vaginal yeast isolates. *International Journal of Research in Medical Sciences*, 8(9), 3241-3246. doi.org
60. Purnamasari, I., Ervianti, E., Damayanti, D., Prasetyo, B., Astari, L., Endraswari, P., Listiawan, M., & Prakoeswa, C. (2022). Pattern of Candida Species Isolated from Patient with Vulvovaginal Candidiasis in Pregnancy. *Berkala Ilmu Kesehatan Kulit Dan Kelamin*, *34*, 178–183. https://doi.org/10.20473/bikk.V34.3.2022.178-183
61. Ramírez-Lozada, T., Espinosa-Hernández, V. M., Frías-De-León, M. G., & Martínez-Herrera, E. (2019). Update of Vulvovaginal Candidiasis in Pregnant and Non-pregnant Patients. *Current Fungal Infection Reports*, *13*(4), 181–190. https://doi.org/10.1007/s12281-019-00357-3
62. Richard, M., & Extension, K. P. (2023). *Prevalence and Factors Associated with Vulvovaginal Candidiasis among Pregnant Women Who Attended Antenatal Clinic at Kamuli General Hospital*. *9*, 12–26.

63. Roy, M., Majumdar, T., & Ray, J. (2024). Vulvovaginal candidiasis in pregnant women attending a tertiary care centre in North-Eastern India. *Indian Journal of Medical Microbiology*, *52*, 100738. https://doi.org/10.1016/j.ijmmb.2024.100738
64. Sangaré, I., Sirima, C., Bamba, S., Zida, A., Cissé, M., Bazié, W. W., Sanou, S., Dao, B., Menan, H., & Guiguemdé, R. T. (2018). Prevalence of vulvovaginal candidiasis in pregnancy at three health centers in Burkina Faso. *Journal de Mycologie Médicale*, *28*(1), 186–192. https://doi.org/10.1016/j.mycmed.2017.08.006
65. Satora, M., Grunwald, A., Zaremba, B., Frankowska, K., Żak, K., Tarkowski, R., & Kułak, K. (2023). Treatment of Vulvovaginal Candidiasis—An Overview of Guidelines and the Latest Treatment Methods. *Journal of Clinical Medicine*, *12*(16), 5376. https://doi.org/10.3390/jcm12165376
66. Saunders, M., Lewis, P., & Thornhill, A. (2019). *Research methods for business students* (8th ed.). Pearson Education
67. Setia, M. S. (2016). Methodology series module 3: Cross-sectional studies. *Indian Journal of Dermatology*, 61(3), 261-264.
68. Shekhany, K. A. M. (2021). Isolation and genotyping of Candida albicans involved in vaginal candidiasis among pregnant women in Sulaymaniyah and Erbil cities. *Zanco Journal of Medical Sciences (Zanco J Med Sci)*, *25*(1), 493–502. https://doi.org/10.15218/zjms.2021.012
69. Siddiqui, R. (2021). *Comparison of Species Distribution, Antifungal Susceptibility and Virulence Factors in Pregnant and Non-pregnant Women with Vulvo-vaginal Candidiasis*. *10*(4).
70. Sule-Odu, A. O., Akadri, A. A., Oluwole, A. A., Osinupebi, O. A., Andu, B. A., Akiseku, A. K., & Lawal, A. I. (2020). Vaginal Candida infection in pregnancy and its implications for fetal well-being. *African Journal of Reproductive Health*, *24*(3). https://www.ajrh.info/index.php/ajrh/article/view/2388
71. Sutaria, P., Cholera, M., & Donga, S. B. (2019). A prevalence study of vaginal candidiasis among pregnant women. *International Journal of Advances in Medicine*, *6*(3), 922–926. https://doi.org/10.18203/2349-3933.ijam20192265
72. Taber, K. S. (2018). The use of Cronbach's alpha when developing and reporting research instruments in science education. *Research in Science Education*, 48(6), 1273-1296.

73. Tsega, A., & Mekonnen, F. (2019). Prevalence, risk factors and antifungal susceptibility pattern of Candida species among pregnant women at Debre Markos Referral Hospital, Northwest Ethiopia. *BMC Pregnancy and Childbirth*, *19*. https://doi.org/10.1186/s12884-019-2494-1
74. Waikhom, S. D., Afeke, I., Kwawu, G. S., Mbroh, H. K., Osei, G. Y., Louis, B., Deku, J. G., Kasu, E. S., Mensah, P., Agede, C. Y., Dodoo, C., Asiamah, E. A., Tampuori, J., Korbuvi, J., & Opintan, J. A. (2020). Prevalence of vulvovaginal candidiasis among pregnant women in the Ho municipality, Ghana: Species identification and antifungal susceptibility of Candida isolates. *BMC Pregnancy and Childbirth*, *20*(1), Article 1. https://doi.org/10.1186/s12884-020-02963-3
75. World Health Organisation. (2021). Guidelines for the management of symptomatic sexually transmitted infections.
76. World Health Organization (WHO). (2015). *Laboratory diagnosis of sexually transmitted infections, including reproductive tract infections*. Geneva: World Health Organization.
77. Yilmaz, K., DemiRay, T., Köroğlu, M., HatiPoğlu, H., Kiliç, Ü., Karabay, O., & AltindiŞ, M. (2020). Rapid Differentiation of Candida albicans from Non-albicans Species by Germ Tube Test Directly From Blood Culture Bottles. *Mediterranean Journal of Infection Microbes and Antimicrobials*. https://doi.org/10.4274/mjima.galenos.2019.2019.37
78. Zamanzadeh, V., et al. (2015). Design and position of content validity indexing for questionnaire scales in health sciences. *Journal of Caring Sciences*, 4(2), 165-171.
79. Zeng, X., Zhang, Y., Zhang, T., Xue, Y., Xu, H., & An, R. (2018). Risk Factors of Vulvovaginal Candidiasis among Women of Reproductive Age in Xi'an: A Cross-Sectional Study. *BioMed Research International*, *2018*(1), 9703754. https://doi.org/10.1155/2018/9703754
80. Zhai, Y., Liu, J., Zhou, L., Ji, T., Meng, L., Gao, Y., Liu, R., Wang, X., Li, L., Lu, B., & Cao, Z. (2018). Detection of Candida species in pregnant Chinese women with a molecular beacon method. *Journal of Medical Microbiology*, *67*(6), 783–789. https://doi.org/10.1099/jmm.0.000740