FACULTY OF MEDICINE AND HEALTH SCIENCES

The role of *Gardnerella* and bacterial vaginosis in reproductive health of African women

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Preface

This Master's dissertation represents the finalization of my Master of Science in Biomedical Sciences. During these studies I have grown so much and have learned all the skills required for creating this dissertation. I hereby would like to acknowledge the people that made this possible. I would like to thank everybody that, throughout the past five years, has helped and inspired me to become the researcher and person that I am today.

Firstly, I would like to thank my promotor, Prof. Dr. Piet Cools, for the many hours he invested in training and supervising me during the past two busy years. I am grateful for the insightful feedback and the opportunities he offered me to investigate such an interesting subject in an international context. Prof. Cools was always available to answer my (many) questions and contaminated me with his enthusiasm for scientific research. Also, I would like to thank my co-promotor Dr. Souheila Abbeddou, for helping me with any questions I had and for including me in a productive study team. Additionally, I would like to thank Prof. Dr. Mario Vaneechoutte, who also gave me feedback when I came to a crossroads with this dissertation, but also for many other scientific outputs. He pushed me in my scientific writing and critical thinking skills. Furthermore, I send many thanks to Leen Van Simaey, who showed me around in the lab and taught me everything practical I needed to know to successfully conclude the experiments from this dissertation. Thank you for your patience and all the time you invested in explaining the workings of our laboratory. Without these people I would not have the results I am presenting in this dissertation. I also would like to thank all the other people of the LBR, for creating a welcoming environment at the lab, the chatty lunch breaks and the occasional spelling tips. I am glad to have been part of such a young team, where we supported each other and let each other ventilate about frustrations when experiments kept failing. I wish all fellow students a successful future and I hope we can stay in touch.

Apart from the support I received from fellow students at the laboratory I would also like to thank my friends from the Biomedical Sciences Hannah, Marian and Mareva, who followed the same major as me, for having my back the past two years. I knew that you were always available for whatever questions I had and will truly miss being in the same class together. Thank you for all the supportive advice and for being part of my biomedical experience. Furthermore, I would like to thank Jitske, Fauve, Sarah and Toon, also from the Biomedical Sciences. I truly appreciate your endless support and thank you for believing in what I was doing even when I was not confident about it. The laughs we shared together, in real life or through video-chat, pulled me through these odd academic years. Also to all of you I wish nothing but the best for the future and I am certain we will remain close.

Lastly, I would like to thank my mom and my grandparents. It is amazing how you put up with me when my stress levels went up. Thank you for your support during the past five years and for letting me chase my international dreams. You have always been there for me with comfort food, cuddles and inspirational peptalks.

Impact COVID-19

Initially, this Master's dissertation aimed to evaluate the effect of video-based health interventions on bacterial vaginosis and pregnancy outcomes in Dirashe district (Southern Ethiopia, Ethiopia). Unfortunately, the start-up of this study was delayed by six months due to the measures taken to prevent the further spread of SARS-CoV-2. Consequently, the focus of my dissertation switched to the AVEONS study and the initial project only makes up a small part of this dissertation. Results of the 'Arba Minch project' presented in this dissertation are only a fraction of what they were intended to be, since only one set of samples (acquired at the first visit of the pregnant women) could be analyzed. Data obtained from these samples could not yet be compared to samples from other timepoints, nor could they be brought in association with pregnancy outcomes, as the study participants had not given birth yet at the time my internship ended. Additionally, due to the strict traveling measures implemented by the government and Ghent University, my stay abroad in Arba Minch was cancelled.

Furthermore, due to COVID-19, all Biomedical students were not given the opportunity to present their poster at the Student Research Symposium. This was a missed opportunity for some feedback, for experiencing how research is presented at congresses and what questions are raised about your research and how to answer these questions. Last year, we also did not have the opportunity to defend our research protocol. This would have been a great moment to practice handling stress of public speaking and to receive some feedback to help us improve our presentation skills for the defense of the Master's dissertation this year.

Besides the deviations mentioned above, I personally did not experience any problems to finish this Master's dissertation and the internship connected to it. Prof. Cools provided many alternative opportunities for practical experiments in the lab, which made it possible for me to gain experience and to successfully complete this dissertation. However, I do believe that the COVID-19 measures taken during these past two academic years had a social impact on everyone, and this influenced productivity and motivation. With the support of the colleagues, friends and family around me I did the best I could to cope with the challenges presented to all of us.

Table of Contents

1. Summary

Bacterial vaginosis (BV) is the most common gynecological condition worldwide and is associated with multiple adverse outcomes, including preterm birth (PTB). It is a polymicrobial condition of which the pathogenesis is most importantly associated with vaginal colonization with *Gardnerella*. This Master's dissertation combines two studies concerning BV.

The first module of this Master's dissertation is part of a randomized clinical trial investigating the effect of video-based interventions on the health status and pregnancy outcomes of 688 pregnant Ethiopian women. One of these video-interventions is aimed at reducing vaginal practices that have been indicated as risk factors for BV. Vaginal smears taken at the first trimester of pregnancy were microscopically examined to determine the BV-status and vaginal *Candida* carriage. Within the analyzed subset of 151 samples the prevalence of BV and *Candida* carriage was 14.6% and 7.3%, respectively.

For the second module of this Master's dissertation, seven key species involved in BV were quantified in DNA-extracts of 331 pregnant Congolese women using species-specific qPCR assays. In this study population, *G. vaginalis* was the most prevalent *Gardnerella* species (33.8%). With univariate statistics, no significant association was found between the seven investigated species and the most important clinical signs of BV, and no evidence was found that one of the examined species could be used as an indicator of PTB. Based on hierarchical clustering, groups of women with a distinct pattern of species distribution were defined. These indicate that all *Gardnerella* species can be involved in (asymptomatic) BV.

2. Introduction

2.1 Importance and symptoms of bacterial vaginosis

Among women of reproductive age, bacterial vaginosis (BV) is the most common gynecological condition worldwide, with the prevalence in the general population ranging between 23 and 29 percent across regions[1]. This prominent condition is characterized by a disturbance in the vaginal microbial community (VMC), and can cause symptoms such as vaginal discharge, a vaginal malodor, itching, a burning sensation after sexual intercourse and pain[2]. However, about 50% of women with BV remains asymptomatic[3]. An important aspect of BV is that it is associated with an increased risk for the acquisition of sexually transmitted infections (STI) including HIV, and preterm birth (PTB)[4-6]. Worldwide, PTB affects nearly ten percent of all pregnancies and it is the number one cause of neonatal mortality[7,8]. Furthermore, PTB has a very high economic and social impact[9].

2.2 Risk factors for the acquisition of bacterial vaginosis

Multiple risk factors have been identified for BV, including sexual contact. Debate exists, however, about the sexually transmitted nature of BV[10]. BV is categorized as an STI by the Center for Disease Control and Prevention, while BV has also been reported in sexually non-experienced girls[11]. This contradicts that coital transmission is involved in disease acquisition. However, exposure to a new sexual partner is the most important risk factor for BV[12]. Therefore, it is bold to state that BV is an STI and it might be more accurate to instead categorize BV as a sexually enhanced disease[11]. Other risk factors include socio-economic status and etnicity[2], as, for example in North America, the prevalence of BV is higher in black and Hispanic women compared to other racial groups (such as whites or Asians)[1]. This gives rise to definitions of BV whereby an individual is more at risk for acquiring this condition based on ethnical and societal differences, since these are determining factors in VMC patterns. Recent antibiotic use is also indicated as a risk factor for BV, since this could affect the normal VMC. Furthermore, intravaginal hygienic practices are important risk factors for the acquisition of BV. Previous studies have indicated that the risk for acquiring BV is enhanced by intravaginal practices such as tampon use, vaginal douching, soaps and intravaginal use of herbs or household products[13]. Since these practices are common in Sub Saharan Africa[14], it is valuable to determine the effect of intervention studies that aim to reduce these vaginal practices.

2.3 Etiology and pathogenesis of bacterial vaginosis

The exact mechanisms involved in the onset of BV remain unclear, but it is hypothesized that multiple bacterial species play a role in causing BV[15]. Figure 1 shows a proposed model of the pathogenesis of BV based on what is known so far about this polymicrobial condition. The healthy human VMC of women of reproductive age is characterized by a predominance of bacteria of the genus *Lactobacillus*[16] (Figure 1a). This lactobacilli-dominated environment has a low pH due to production of lactic acid and hydrogen peroxide by lactobacilli, making the vagina a hostile environment for many pathogens. In women with BV, however, this lactobacilli-dominated VMC is replaced by a polymicrobial VMC. The bacterial species most strongly characterizing this VMC are anaerobic species such as *Gardnerella vaginalis*, *Atopobium vaginae* and *Prevotella bivia*[17]. *G. vaginalis* is considered one of the key pathogens in BV because it is most likely the first species to adhere to the vaginal epithelial cells[18].

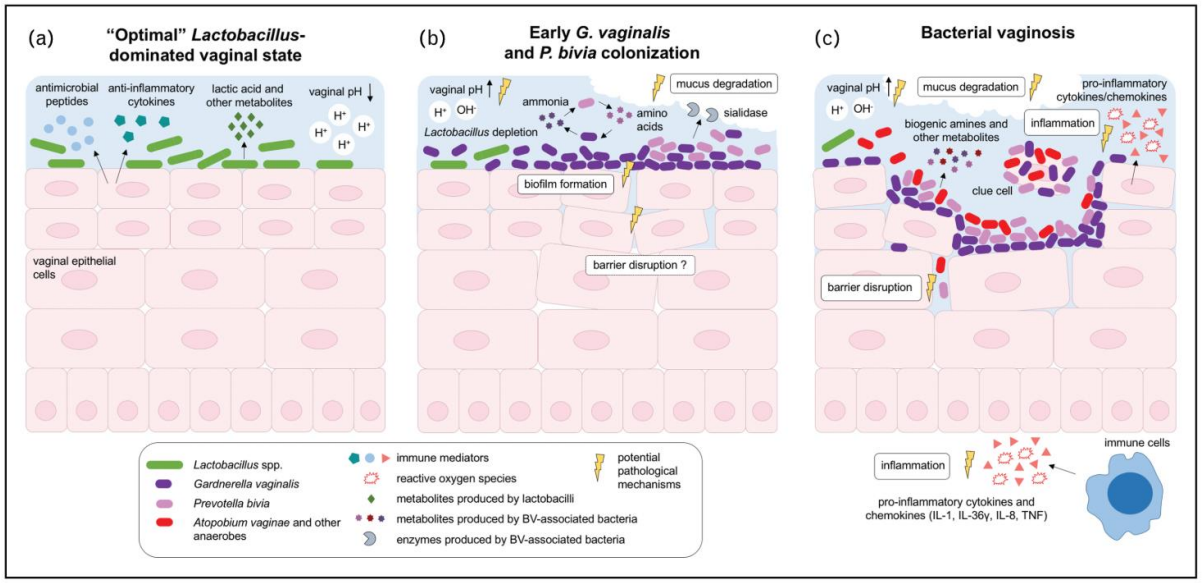

Figure 1. Depiction of a proposed model for bacterial vaginosis establishment and changes in the vaginal environment related to host-microbe interactions[15]. (A) *Healthy vaginal state, characterized by a predominance of bacteria of the genus Lactobacillus.* ***(B)*** *Initial colonization with Gardnerella vaginalis, leading to a deprivation of lactobacilli and enhanced opportunities for secondary colonizers, such as Prevotella bivia, to invade the vaginal niche.* ***(C)*** *Bacterial vaginosis, characterized by practically full replacement of lactobacilli by anaerobic secondary colonizers, such as Atopobium vaginae.*

Despite the indication that *G. vaginalis* is a primary colonizer in BV, its role in the pathogenesis of this condition remains dubious. On the one hand, it is virtually always present in women with BV[19], but it can also be found as a commensal in women with a healthy VMC or in women with asymptomatic BV[15,20,21]. This observation might be explained by the recent finding that *G. vaginalis* is not one single species[20]. Based on comparative genomics of 81 available genomes of *G. vaginalis*, it was shown that the genus *Gardnerella* actually contains at least 13 different species. Three of these species were published officially as *G. leopoldii*, *G. piotii* and *G. swidsinskii*, and *G. vaginalis* was reported as emended species. It can be hypothesized that women with a healthy VMC could be infected with a commensal *Gardnerella* species, while women with BV could be infected with a pathogenic *Gardnerella* species. Further research is required to investigate the specific role of the different *Gardnerella* species in reproductive health.

The recently proposed model of the pathogenesis of BV states that lactobacilli are suppressed upon infection with *Gardnerella* followed by further colonization with other pathogenic species[15] (Figure 1b). The deprivation of lactobacilli namely leads to a rise in vaginal pH, which enhances the colonization opportunities of other anaerobic species such as *P. bivia*. Together with *Gardnerella*, these species produce a number of enzymes that cause the degradation of the mucus, barrier disruption and biofilm-formation. For example, one of the enzymes produced by *Gardnerella*, as well as by *P. bivia*, is sialidase. It is hypothesized that variations in sialidase activity could explain the difference in virulence potential between the different *Gardnerella* species[22,23]. The sialidase enzyme breaks down sialic acid from the glycoproteins that make up the protective vaginal mucus layer and secretory immunoglobulins (IgA)[24,25]. The degradation of sialic acid by *Gardnerella* thus destroys the protective mucus layer and this could predispose the VMC toward dysbiosis by shifting the protective environment towards one that favors BV-associated organisms. The contribution of other virulence factors produced by *Gardnerella*, such as vaginolysin or phospholipase C, is also being investigated to determine which species are BV-related[26-28]. Another important aspect of *Gardnerella's* pathogenic potential is its ability to develop a vaginal biofilm[29], since this acts as a scaffold for other anaerobic species to adhere[18,26,30]. This biofilm forms an explanation for the nature of

clue cells, vaginal cells densely covered with bacteria, that are typically observed in BV[31]. Furthermore, the biofilm renders the involved bacteria more tolerable against high concentrations of hydrogen peroxide or lactic acid. *Gardnerella* thus plays a central role in BV due to its contribution to the diversity & survival of many other BV-associated bacterial species and its promotion of resistance to therapy, mainly because of biofilm formation.

When *Gardnerella* has caused a practically full replacement of the lactobacilli and a biofilm is established, secondary colonizers, such as *A. vaginae*, also invade the vaginal environment and play a role in the pathogenesis of BV (Figure 1c). *A. vaginae* is found in the vagina of almost all BV-positive women and is therefore considered a key species in the pathology of BV[32]. Several other bacterial species have also been identified as secondary colonizers in deep-sequencing studies, but their relevance in the pathogenesis of BV remains unclear[33]. Interestingly, also one unusual *Lactobacillus* species, i.e. *L. iners*, has been found to be predominant in BV[34]. Many bacterial species have thus been found in women with BV, but the exact role of each species in the pathogenesis of the condition remains to be elucidated.

2.4 Adverse sequelae associated with bacterial vaginosis

Globally, approximately ten percent of all neonates are born preterm (i.e. before 37 completed weeks of gestation) and PTB is the leading cause of neonatal mortality[8]. Additionally, PTB is associated with multiple (lifelong) adverse consequences and therefore also has a high morbidity burden[7]. These consequences include cerebral palsy, learning impairment and visual disorders. Hence, PTB causes a heavy load not only for thousands of families each year, but also for the global health system and the international economy. In 2005, the average cost per preterm infant was more than 50.000 dollars in the United States alone[35]. In most cases, PTB results from chorioamnionitis, although its etiology is multifactorial[36]. Chorioamnionitis, i.e. intra-uterine infection of the chorioamniotic membrane, can occur when vaginal organisms ascend up the genital tract[37]. In a healthy VMC, this is avoided thanks to the protective nature of the dominant lactobacilli. In BV, however, other anaerobic species colonize the vaginal niche and these bacteria have a higher chance of ascending the genital tract and so colonizing the placenta and giving rise to chorioamnionitis[38]. BV can thus be regarded as an important contributor to the global burden of PTB.

Next to PTB, BV is also associated with an increased risk for the acquisition of STIs, including HIV[6]. This could be explained by the increase of proinflammatory cytokines and chemokines associated with BV, which could enhance the risk for HIV-transmission. These proinflammatory molecules will establish themselves under the mucosa, where they could directly stimulate HIV-replication in latent viral reservoirs or could facilitate the trafficking and activation of CD4+ target cells. Normally, these CD4+ cells are not present in the cervicovaginal mucosa in large numbers, but they are recruited as a result of BV[39]. Additionally, BV could reduce the levels of antiviral factors and it has been shown that women with BV have lower innate anti-HIV activity of their cervicovaginal secretions[40]. Furthermore, BV has been associated with pelvic inflammatory disease and a wide array of other chronic health problems[2].

2.5 Progressing diagnostics of bacterial vaginosis

The current gold standard to diagnose BV in a research setting is the Nugent scoring system. This is a standardized evaluation method that was introduced in 1991 using a scoring system ranging from zero to ten[41]. This score is determined based on the microscopic examination of a Gram-stained vaginal smear. On this smear, the number of bacteria from three morphotypes is determined, i.e. *Lactobacillus* morphotype (Gram-positive rods) (Figure 2a), *Gardnerella* and

Bacteroides spp. morphotypes (Gram-negative to Gram-variable coccobacilli) (Figure 2b), and *Mobiluncus* morphotype (curved Gram-variable rods) (Figure 2c).

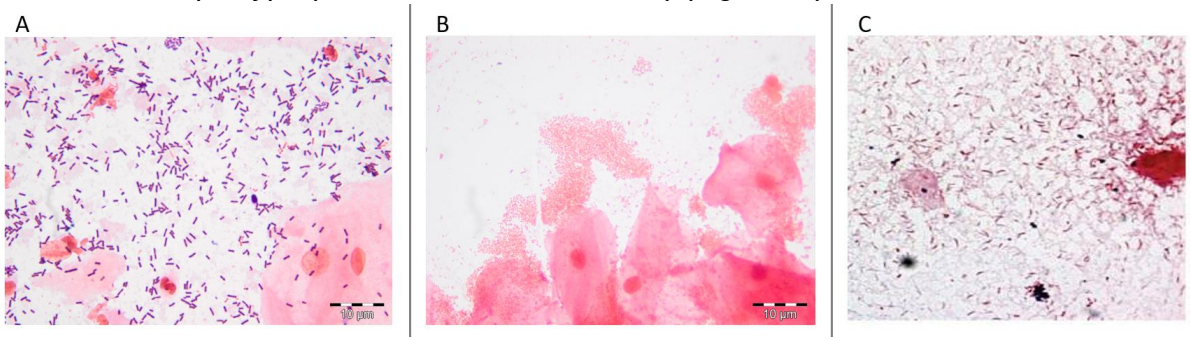

Figure 2. Examples of morphotypes observed on Gram-stained smears for Nugent scoring. (A) *Lactobacillus morphotype, Gram-positive rods (image obtained from Arba Minch study, described in this Master's dissertation).* ***(B)*** *Gardnerella morphotype, Gram-negative to Gram-variable coccobacilli (image obtained from Arba Minch study, described in this Master's dissertation).* ***(C)*** *Mobiluncus morphotype, curved Gram-variable rods (image obtained from Srinivasan et al. (2013)[42]).*

Table 1 shows the scoring table that is used for this method. The first column shows which score must be attributed to each of the morphotypes. In the following three columns the average number of bacteria of each of these morphotypes corresponding to this score is indicated. After all three morphotypes are evaluated and the corresponding score has been determined, these scores are added up to a total score. If this total score ranges between zero and three the sample is classified as BV-negative, a score between four and six is considered intermediate and if the score is above or equal to seven this is classified as BV-positive. However, this categorization into three rough BV-categories lacks resolution and does not provide specific information on the composition of the VMC.

Table 1. Nugent scoring system for Gram-stained vaginal smears.

Score	Number of bacteria of *Lactobacillus* morphotypes	Number of bacteria of *Gardnerella*/*Bacteroides* spp. morphotype	Number of bacteria of *Mobiluncus* morphotype
0	>30	0	0
1	5-30	<1	<1 or 1-4
2	1-4	1-4	5-30 or >30
3	<1	5-30	
4	0	>30	

In clinical practice, BV can be diagnosed based on the Amsel criteria[31]. Richard Amsel and colleagues put forward four characteristics linked to BV in 1983, and a woman showing minimum three out of these four characteristics is considered BV-positive. These criteria are (i) the presence of a thin, watery homogenous vaginal discharge, (ii) an elevated pH (> 4.5), (iii) a fishy odor of the discharge when treated with a 10% potassium-hydroxide solution (this procedure is called the Whiff test), and (iv) a rate of at least 20% clue cells. The advantage of these Amsel criteria is that these characteristics can easily be tested with standard methods of clinical microbiology. However, since up to 50% of BV-positive women do not experience symptoms, these criteria are not suitable to diagnose asymptomatic BV.

An alternative method of diagnosing BV that is being explored is based on molecular techniques. It was previously shown that the most important markers for BV are the presence of *G. vaginalis* and *A. vaginae* together with the absence of lactobacilli[19]. Detecting these

particular BV-associated organisms on cervical or vaginal samples with specific qPCR assays could provide better insights into a woman's BV-status.

2.6 Suboptimal treatment of (recurrent) bacterial vaginosis

Currently, the treatment of BV consists of broad-spectrum antibiotics[43]. In most cases, women are treated with metronidazole (a nucleic acid synthesis inhibitor) or clindamycin (a protein-synthesis inhibitor). Both antibiotics, however, only show a poor initial cure rate and have recurrence rates of up to 60%[43]. Moreover, these antibiotics are associated with several complications. Treatment with clindamycin can, for example, provoke pseudomembranous colitis, antibiotics-associated diarrhea and other gastrointestinal side effects[44], and the use of metronidazole is associated with nausea and vomiting[45]. The BV-associated biofilm plays an important role in treatment failure, since most bacteria are thought to be metabolically inactive in a biofilm[46,47]. Consequently, the bacteria do not respond to antibiotic products. Treatment failure could further be explained by the inability of the host to restore the lactobacilli-dominated VMC and by the intrinsic resistance of certain *Gardnerella* strains to antibiotic treatment. Namely, many *Gardnerella* strains now show resistance towards clindamycin and metronidazole[21,48], whereas some older studies show susceptibility of *Gardnerella* to these antibiotics. In a study from 1993 Kharsany *et al*., for example, showed that all 39 clinical isolated of *Gardnerella* were susceptible to clindamycin[49]. Antibiotic treatment is thus not ideal and novel alternative treatments are crucial to achieve better (long-term) cure rates.

To solve the issue of antibiotic resistance, many new concepts are being proposed. Potential new strategies include biofilm-disrupting agents, the use of antiseptics, disinfectants and vaginal acidification[18]. Additionally, the use of probiotics is being investigated and even vaginal microbiota transplants are being explored[46]. One promising alternative therapy in the field of bacterial infections is the use of bacteriophages, which are viruses that can kill bacteria in a species- or strain-specific way[50]. These bacteriophages produce peptidoglycan hydrolases (also called endolysins) towards the end of their lytic cycle to facilitate their release through cleavage of peptidoglycan in the bacterial cell wall[51]. The use of bacteriophage therapy could provide a highly specific and safe approach to control BV, with the elimination of specifically targeted bacteria. However, since no bacteriophages specifically targeting *Gardnerella* have been identified, the use of solely the endolysins is being explored. A genetically engineered endolysin was recently studied in samples from patients with BV and it was shown that *Gardnerella*-specific elimination from the biofilm is possible with the studied endolysin[47]. This novel endolysin strategy is thus a possible new treatment to combat (recurrent) BV.

2.7 Other infections influencing vaginal health

Besides BV, women can suffer many other vaginal infections, including female genital schistosomiasis, vulvovaginal candidiasis (VVC), or STIs such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, or *Trichomonas vaginalis*. VVC is the second most common gynecological condition affecting women of reproductive age[52]. Approximately 70-75% of women experience at least one episode of VVC during their lifetime, and it is estimated that 50% of these women will encounter recurrence[52,53]. Most VVC cases are caused by *Candida albicans*[54,55], and the remaining cases are mostly the consequence of infection with *C. glabrata*[52]. As part of the AVEONS study, our research group recently showed that, in a population of pregnant women in Bukavu (Democratic Republic of Congo (DRC)), VVC was significantly and independently associated with PTB[56]. There are thus many other vaginal infections potentially affecting reproductive health, and it would be of interest to investigate the interference of these conditions with BV.

3. Aims

The first goal of this dissertation is to report the prevalence of BV and vaginal *Candida* carriage among pregnant women in Dirashe district (Southern Ethiopia, Ethiopia). The first module of this Master's dissertation is part of a research study, the 'Arba Minch project', evaluating the effect of video-based education on the health status of pregnant mothers and their infants in this area. In this randomized controlled trial a group of pregnant women received educational videos about nutrition and hygiene, including one video specifically aimed at reducing vaginal practices that have been shown to be a risk factor for the acquisition of BV. It is hypothesized that there will be a positive effect of this innovative video-based health education on maternal knowledge and adherence to recommended maternal ante- and postnatal practices. Furthermore, it is hypothesized that informing pregnant women about good vaginal hygienic practices will result in a lower prevalence of BV in the last trimester of pregnancy and will have a positive effect on birth outcomes in the intervention group. This Master's dissertation plays a role in this project by performing quality control analysis and reporting the prevalence of BV in both the intervention group and the control group at baseline. Since VVC has also been indicated as a risk factor for adverse pregnancy outcomes, the baseline prevalence of vaginal *Candida* carriage was also determined.

The second goal of this dissertation is to examine the prevalence of *G. leopoldii*, *G. piotii*, *G. swidsinskii* and *G. vaginalis* among pregnant women in Bukavu (DRC), and to gain insights into the associations between these different *Gardnerella* species and clinical signs and symptoms. Furthermore we aimed to evaluate the associations between these different *Gardnerella* species and adverse pregnancy outcomes, and to suggest potential (molecular) markers for PTB. The second module of this dissertation, which is part of the 'AVEONS project', thus focuses on evaluating which specific bacterial species are present in the vaginal niche of women with and without BV to get new insights into the pathogenesis of this condition and to unravel the dubious role of *Gardnerella* in this pathogenesis.

4. Material and methods

4.1 Arba Minch Project (Dirashe district, Ethiopia)

4.1.1 Ethics

Ethical clearance was obtained for the study entitled 'Effects of video-based health education on health status of pregnant mothers and their infants (from 0 to 6 months) in Dirashe district (Southern Ethiopia, Ethiopia) - a cluster randomized controlled trial' from the Ethical Committee of the Faculty of Medicine and Health Sciences, University of Ghent (BC-06756), as well as from the Ethical Committee of Arba Minch University (reference number IRB/158/12). Subsequently, the study was registered at clinicaltrials.gov (reference number NCT04414527). All participating women signed an informed consent form.

4.1.2 Study design and population

This cross-sectional video-based intervention study, which is a collaboration project between Arba Minch University and Ghent University, was conducted in Dirashe district (Southern Ethiopia, Ethiopia). In this area, 688 pregnant women in their first trimester were recruited and will be followed during their last two trimesters of pregnancy and another six months postpartum. Neonates will also be followed during the first six months after birth. Women were recruited in March 2020 and from October 2020 to January 2021 by the local study team and data collectors who systematically conducted home screening visits in the study area. Newly pregnant women (gestational age < 16 weeks) living in rural kebeles (i.e. the smallest administrative units of Ethiopia) of Dirashe district were identified and invited to voluntarily

participate in the study. These women were enrolled in the study if i) they provided an informed consent form, ii) were at least 18 years old, iii) were permanently resident in the study area, iv) were available during the whole period of the study (twelve months), and v) accepted the intervention package (including home visits for data collection and morbidity follow up). Women with severe anemia (hemoglobin < 70 g/L), with undernutrition (body mass index < 18.5 kg/m^2), with a chronical illness such as tuberculosis or other chronic diseases, or with HIV were excluded from the study and were referred to a specialized treatment provided at the health center. In a cluster randomization, eight communities were assigned to the intervention cohort (receiving health-videos) and eight other communities were assigned to the control cohort (receiving standard counselling). Women received either treatment depending on their residency.

4.1.3 Intervention

The control cohort received antenatal care according to standard Ethiopian guidelines. This entailed a minimum of four antenatal care visits at the health centers and attending monthly forums (six in total) organized by the health extension workers for group counselling. In the intervention group, a total of nine educational videos about nutrition and health were additionally shown. One of these innovative videos focused on reducing possibly harmful vaginal hygienic practices, such as vaginal douching and the intravaginal use of soaps or herbs (Addendum 2), since these have been shown to be a risk factor for the acquisition of BV[13]. The video was produced locally and used a discussion format. The video was delivered to the women during home-based counselling visits at 16, 24, 32 and 36 weeks of pregnancy. This was performed by trained assistants, which were recruited from freshly graduated high school students to ensure a good quality of the behavior change component, but also through forum participation led by community nurses. It was displayed to the women using cordless projectors that could also be used in the absence of electricity.

Additionally, both groups received care according to national health care standards. This included iron-folic acid supplementation, treatment of any symptomatic health condition, and deworming in case of symptomatic complaints during their third trimester of pregnancy.

4.1.4 Sample collection

A baseline visit was performed at three months of pregnancy. Follow up visits were planned at six months and nine months of pregnancy. Post-partum, data will be collected from mother and child within two months after birth and at six months post-partum. Infant growth will be measured during monthly post-partum visits and for the monitoring of exclusive breast feeding a visit will be organized at three and five months post-partum. Data was collected offline and stored digitally on REDCap (https://redcap1.ugent.be/).

For this Master's dissertation, only vaginal smears from baseline collection were examined for the presence of BV and *Candida*. These vaginal smears were prepared immediately from vaginal swabs sampled from each woman, Gram-stained, locally evaluated by a laboratory technician and stored until shipment to the Laboratory for Bacteriology Research (LBR) (Ghent University, Ghent, Belgium) for quality control analysis, which is part of this Master's dissertation.

4.1.5 Laboratory techniques

4.1.5.1 Gram staining and Nugent scoring

Upon reception, vaginal smears were fixated by Ethiopian laboratory technicians in the parasitology laboratory at the College of Medicine and Health Sciences at Arba Minch University (Ethiopia) by briefly placing the smears through a flame. After this, the samples were

stained with a crystal violet dye, which causes all bacteria to take up the purple color. Next, iodine was added to the smears and they were decolorized with alcohol very briefly. Gram-positive bacteria have a thick cell wall and this short decolorizing step is not sufficient to abstract the purple dye from these bacterial cells. Lastly, the smears were colored again with safranin, causing the Gram-negative bacteria to take up this second dye. Gram-negative bacteria appear pink, while the Gram-positive bacteria remain purple. The remaining vaginal swabs were covered with ethanol and stored at room temperature until shipment to the LBR (Ghent University, Ghent, Belgium) for further analyses.

The BV-status of women was determined using the Nugent scoring system by evaluating five microscopic fields of each vaginal smear. In a first reading, all slides were scored locally by a trained team of laboratory technicians in the parasitology laboratory at the College of Medicine and Health Sciences at Arba Minch University (Ethiopia). A subset of the slides was scored at the LBR as part of this Master's dissertation in a second reading for quality control analysis. Additionally, the presence of *Candida* was examined on these Gram-stained vaginal smears. *Candida* infection can be recognized on Gram-stained vaginal smears by the presence of budding yeast cells and/or (pseudo)hyphae, which are long, tubular branching structures produced by *Candida* cells[57].

4.1.6 Data analysis

The BV-status of each analyzed sample was defined as a categorical variable, with three possible outcomes (i.e. positive, intermediate, negative). To calculate the prevalence and corresponding 95% confidence interval (CI) of each category in the analyzed subset of women, the online Epitools application (https://epitools.ausvet.com.au/ciproportion) was used with the Wilson confidence interval method. These analyses were repeated to determine the prevalence and corresponding 95% CIs of vaginal *Candida* carriage, which was defined as a binary variable (i.e. positive or negative).

4.2 AVEONS study (Bukavu, Democratic Republic of Congo)

4.2.1 Ethics

Ethical approval was acquired by the Internal Review Board of the Catholic University of Bukavu (reference number UCB/CIE/NC/016/2016), the Ministry of Public health (reference number 062/CD/DPS/SK/2017) and the Ethical Committee of Ghent University Hospital (reference number PA2014/003). An informed consent form was signed by all pregnant women participating in this study.

4.2.2 Study design and population

Samples used for this second part of this Master's dissertation were part of the AVEONS (Angamiza Vizuri (Swahili for 'stop') Early Onset Neonatal Sepsis) project, which is a research project investigating the causes of neonatal mortality in Bukavu (DRC). This AVEONS study was a longitudinal, prospective cohort study conducted in 2017 where pregnant women were seen between 16 and 20 weeks (visit 1 (V1)), between 36 and 38 weeks (V2) and at delivery. Newborns were followed from delivery until day seven of life. For this dissertation only samples from pregnant women at V1 and pregnancy outcomes were used.

Participants were recruited from January to October 2017. Pregnant women who came to the Provincial Referral Hospital of Bukavu (PRHB) for antenatal care were asked for their willingness to participate. Church announcements, radio advertisements, TV spots, posters and sessions at community leader's meetings were used to raise awareness of the existence of the AVEONS study. Interested eligible women were informed individually about the study

details. Pregnant women were considered eligible for inclusion into the study if (i) they were between 16 and 20 weeks pregnant, (ii) they accepted to be followed by a referral hospital team, (iii) were willing to deliver at PRHB, and (iv) agreed to be contacted by phone or other means. Women were excluded from the study when they planned to move out of Bukavu during their pregnancy, in case of twin pregnancies or a fetus with a visible malformation at ultrasound examination and if they had genital bleeding or used antibiotics during the 2 weeks before recruitment. An informed consent form was asked to be signed by all women accepting to participate and each participating woman was reimbursed for transport costs to and from the hospital.

4.2.3 Sample collection

At each prenatal visit (V1 and V2), the participants were questioned about sociodemographics, reproductive history, sexual behavior and vaginal symptoms. Next, a general physical examination was performed, including anthropometric measurements such as height and weight. Thereafter, a gynecological examination, including a speculum examination with a sterile non-moistened speculum, was performed. The vaginal mucosa and cervix were inspected for the presence of sores and tumors, and a diagnosis for vaginal infection was made according to the syndromic-based protocol issued by the Ministry of Public Health of DRC, which is based on WHO recommendations[58]. In case a pathological diagnosis was made based on this standard syndromic approach, treatment was prescribed. Empirical treatment consisted of a combination of clotrimazole (200 mg) and clindamycin (100 mg) (in one vaginal ovule) once a day for six days. When a woman was allergic to this treatment, metronidazole was given (in a vaginal ovule) once a day for six days. Also, during the gynecological examination, the vaginal pH was determined with indicator pH papers (Hilo indicator® pH paper). Additionally, an ultrasound examination was performed to determine the viability of the fetus and to measure cervical length. Furthermore, blood samples were collected in VacuTubes® red (without EDTA) and were used to screen for HIV and malaria, and to determine hemoglobulin concentrations. To diagnose urinary tract infections and bacteriuria, midstream urine was collected in a sterile container and tested with Multistix dispticks® to determine the presence of nitrite and white blood cells. Then, three vaginal swabs were taken from the midportion of the lateral vaginal wall. Lastly, cervicovaginal lavage (CVL) samples were acquired by rinsing the cervical mucosa with 5 mL of sterile physiologic water and collecting as much lavage as possible into a VacuTube®. These CVLs were then stored at -20 °C and later shipped with respect to the cold chain to the LBR. All women received routine antenatal care during their pregnancy. They were given a single dose of mebendazole (500 mg) against soil-transmitted helminths and a single dose of sulfadiazine-pyrimethamine (500 mg) against malaria. At delivery, the labor was monitored, and delivery features and pregnancy outcomes were collected by nurses and the senior assistant.

4.2.4 Laboratory techniques

4.2.4.1 Wet mount microscopy

In the local lab of the PRHB, a wet mount slide was prepared within 20 minutes after collection of a vaginal swab. The substances on the swab were mixed with 0.5 mL of saline and one droplet of this mixture was put on a glass slide and covered with a cover slip. The presence of *Trichomonas vaginalis*, *Candida*, white blood cells and clue cells was determined with microscopy.

4.2.4.2 Gram staining and Nugent scoring

At the PRHB, a vaginal swab was rolled on a glass slide and fixated under high temperatures by briefly holding the back of the slide into a flame. All fixated slides were then shipped to the

LBR and stained at the Department of Laboratory Medicine (Ghent University Hospital, Ghent, Belgium) with an automated Poly Stainer. These Gram-stained slides were used for the diagnosis of BV, using the Nugent scoring system as described previously.

4.2.4.3 DNA extraction and qPCR assays

DNA was extracted from the CVLs in the LBR using the RNAeasy PowerMicrobiome Kit (Qiagen) according to the manufacturer's instructions. These DNA extracts were stored at -20 °C until use.

As part of this Master's dissertation, the extracted DNA was used in qPCR assays to detect the presence of multiple bacterial species involved in the molecular diagnosis of BV, i.e. *G. leopoldii*, *G. piotii*, *G. swidsinskii*, *G. vaginalis*, *A. vaginae*, *L. crispatus* and *L. iners*. All primers used for these species-specific assays are listed in Table 2. The final qPCR mixtures contained these primers at a final concentration 0.7 µM (*A. vaginae*), 0.5 µM (*Gardnerella* species), 0.1 µM (*L. crispatus*) or 0.2 µM (*L. iners*), in 1X LightCycler 480 SYBR Green I master mix in HPLC water. A reaction volume of 10 µL was obtained by adding 2 µL of DNA extract of the CVLs, DNA from the type strain of the corresponding species (listed in Table 3) or HPLC water to 8 µL of the prepared qPCR mixture. All assays were carried out on a LightCycler 480 (Roche). Amplification of *A. vaginae* started with pre-incubation for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 20 s at 62 °C and 40 s at 72 °C. *G. leopoldii*, *G. piotii*, *G. swidsinskii* and *G. vaginalis* were amplified by pre-incubation for 5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 56 °C and 30 s at 72 °C. qPCR assays were performed for *L. crispatus* by pre-incubation for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, and for *L. iners* by pre-incubation for 10 min at 95 °C, followed by 40 cycles of 10 s at 95 °C, 20 s at 50 °C and 4 s at 72 °C. Of the amplified dsDNA, high resolution melting curves were generated by melting all dsDNA at 95 °C for 5 s, subsequently renaturating the DNA at 50 °C for 30 s (*A. vaginae*), at 55 °C for 1 min (*Gardnerella* species), or at 60 °C for 1 min (*L. crispatus* and *L. iners*), and finally increasing the temperature to 97 °C at a ramp rate of 0.02 °C per s.

Table 2. Primers used in this study.

Species	**Forward primer**	**Reverse primer**
Atopobium vaginae	CCCTATCCGCTCCTGATACC	CCAAATATCTGCGCATTTCA
Gardnerella leopoldii	GATACTGCACTGTATCGA	CAGTATCAATACCAGCC
Gardnerella piotii	AGCTGCTTACGATTATAGT	TTACTCATTCTAAGCTTAATAG
Gardnerella swidsinskii	ATTTAGTTAGATATTTGGCAA	TAGTCATATATTCCGCGC
Gardnerella vaginalis	TATTATAACTAAAGCTGCTG	CGCCACTATAGTCG
Lactobacillus crispatus	AGCGAGCGGAACTAACAGATTTAC	AGCTGATCATGCGATCTGCTT
Lactobacillus iners	GTCTGCCTTGAAGATCGG	ACAGTTGATAGGCATCATC

Table 3. Reference strains used in this study.
TSA, tryptic soy agar; CHOC, chocolate agar, NYC+HS, New York City agar + horse serum.

Species	Strain	Culture conditions
Atopobium vaginae	CCUG 38953^T	Anaerobe, 37 °C, TSA plates
Gardnerella leopoldii	UGent 09.48	Anaerobe, 37 °C, CHOC plates
Gardnerella piotii	UGent 18.01^T	Anaerobe, 37 °C, CHOC plates
Gardnerella swidsinskii	GS10234	Anaerobe, 37 °C, CHOC plates
Gardnerella vaginalis	GvB LMG7832^T	Anaerobe, 37 °C, CHOC plates
Lactobacillus crispatus	LMG 9479^T	Anaerobe, 37 °C, NYC+HS plates
Lactobacillus iners	FB123-CNA-4	Anaerobe, 37 °C, NYC+HS plates

Raw data were processed with the LightCycler 480 Software Version 1.5 (Roche). A specific cutoff Cq value, indicating the limit of detection, was determined for all species. For *A. vaginae*, *L. crispatus* and *L. iners* this value was set to 35, for *G. piotii* this was 34, for *G. vaginalis* this was 32, for *G. swidsinskii* this was 30, and for *G. leopoldii* this was 28. Samples with a Cq value equal to or higher than this specific cutoff value most likely represent cross contamination and were therefore not considered as positive samples. Additionally, cross contamination was evaluated based on melting temperatures.

4.2.5 Data analysis

For the analysis of prevalences and univariate statistics, the status of each bacterial species was defined as a binary variable (i.e. positive or negative) based on the results from the qPCR assays. The co-occurrence of species was investigated using a correlation matrix, which was created using the quantitative load of the bacterial species as continuous variables. These quantitative loads were also used as continuous variables for the creation of a clustered heatmap, which served for the investigation of the relationship between the different species as part of the VMC as a whole.

First, the prevalence of each of the bacterial species and the corresponding 95% CI were calculated with the online Epitools application (https://epitools.ausvet.com.au/ciproportion) using the Wilson confidence interval method. Second, a pairwise correlation matrix was generated based on the concentration of each of the bacterial species to analyze the co-occurrence of the different species. For this, the online tool available at heatmapper.ca (http://www.heatmapper.ca/pairwise/) was used. To determine the Pearson correlation coefficients (r) corresponding to this matrix, a Pearson correlation test was performed with the R programming language in Jupyter Notebook. Pearson correlation coefficients were considered negligible (r = 0.00 to r = 0.10), weak (r = 0.10 to r = 0.39), moderate (r = 0.40 to r = 0.69), strong (r = 0.70 to r = 0.89), or very strong (r = 0.90 to r = 1.00) according to Schober et al. (2018)[59]. Third, the associations between the bacterial species and clinical signs/symptoms, and PTB were determined by performing univariate analysis with the R programming language. For this, the status of each bacterial species was defined as an independent variable, while the clinical signs/symptoms of mother and neonate were defined as dependent variables. PTB was also defined as a dependent variable for these analyses. To determine which of the computed association were significant, logistic regression was performed for binary dependent variables, and linear regression was performed for categorical and continuous dependent variables. A p-value < 0.05 indicated significant outcome variables. Subsequently, the odds ratio was calculated for binary or categorical variable. The functions fisher.test() or odds.ratio() were used for this (in case of a binary or categorical variable, respectively) in the R programming language. Next, the function pheatmap() was used in the R programming language to create an annotated heatmap based on the hierarchical clustering of the women and the bacterial species. The log-transformed bacterial concentrations were used as input data for this heatmap. On it, four rough clusters of BV-positive women (as indicated by Nugent score) were visually defined and were recreated for detailed analyses

using the function cutree() in the R programming language. The dendrogram of the heatmap was split at a height (indicating dissimilarity) of 110 and the resulting clusters were used to assess associations with clinical signs and symptoms. Last, the dendrogram of the heatmap was split at a height of 125 to analyze less ramified clusters of women. For each cluster assay, regression analyses were repeated to calculate p-values for the computed associations and odds ratios were determined as described previously.

5. Results

5.1 Arba Minch project (Dirashe district, Ethiopia)

5.1.1 Description of the study population

A flowchart of the study, describing the number of participants included, is shown in Figure 3. A total of 851 women was screened for this study, of which 148 were screened in the first study phase (conducted in March 2020) and 703 in the second study phase (conducted from October 2020 to January 2021). During the first study phase, 98 of the 148 screened women (66.2%) were found eligible and were enrolled at three months pregnancy (visit 1). During the second study phase, 590 of 703 women (83.9%) were enrolled (visit 1). Of the total 688 vaginal smears available from the baseline visits, the first 151 smears (21.9%) were evaluated microscopically for this Master's dissertation. The other smears could not be examined due to time limitations. Of the 151 examined smears, 66 (43.7%) belonged to women in the control cohort, while the other 85 smears (56.3%) belonged to women from the intervention cohort. Due to time limitations, no sociodemographic data of the study population could be analyzed.

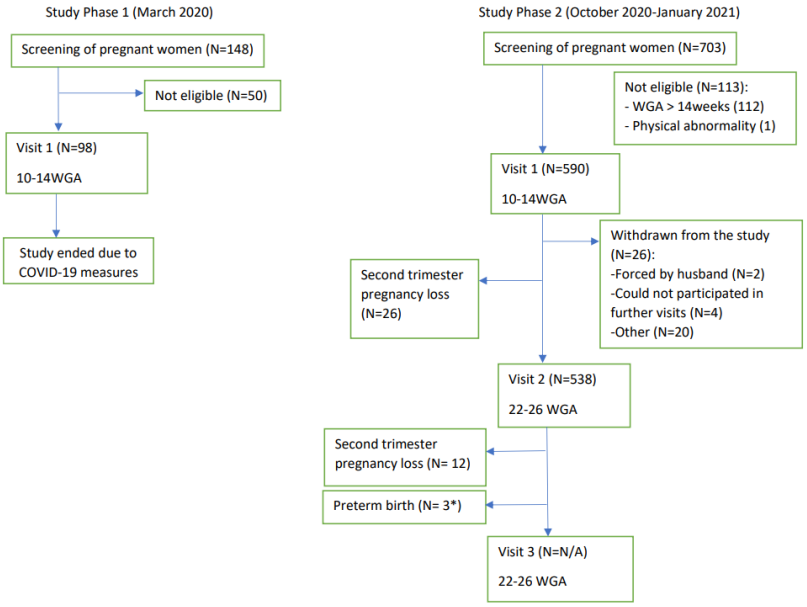

Figure 3. Flowchart of the Arba Minch study.
N, number of study participants; WGA, weeks of gestational age;
**, number on 14 May 2021 (study still ongoing); N/A, not available (study still ongoing).*

5.1.2 Prevalence of bacterial vaginosis and vaginal *Candida* carriage

Table 4 shows the total prevalence of each VMC-status and vaginal *Candida* carriage, as well as the distribution of these prevalences within the control and the intervention cohort. In the total subset of 151 smears, 14.6% was classified as BV-positive (Nugent score 7-10), 29.1% as intermediate (Nugent score 4-6) and 56.3% as BV-negative (Nugent score 0-3). The prevalence of BV within the control cohort was 19.7%, and 10.6% within the intervention cohort. Vaginal *Candida* carriage was observed in 7.3% of the 151 women, with a higher prevalence in the intervention cohort (8.2%) compared to the control cohort (6.1%).

Table 4. Pre** in the R programming language **valence of bacterial vaginosis and vaginal Candida carriage in pregnant women from Dirashe district (Southern Ethiopia, Ethiopia).
CI, confidence interval.

	Total prevalence (95% CI)	**Prevalence within control cohort (95%CI)**	**Prevalence within intervention cohort (95%CI)**
VMC-status			
BV	14.6 (9.8-21.1)	19.7 (11.9-30.8)	10.6 (5.7-18.9)
Intermediate	29.1 (22.5-36.8)	32.9 (23.9-43.5)	24.2 (15.5-35.8)
Healthy	56.3 (48.3-63.9)	56.5 (45.9-66.5)	56.1 (44.1-67.4)
Candida	7.3 (4.1-12.6)	6.1 (2.4-14.6)	8.2 (4.0-16.0)

5.2 AVEONS study (Bukavu, Democratic Republic of Congo)

5.2.1 Description of the study population

In Figure 4 a flowchart of the study is shown, describing the number of pregnant women and neonates withheld at each visit.

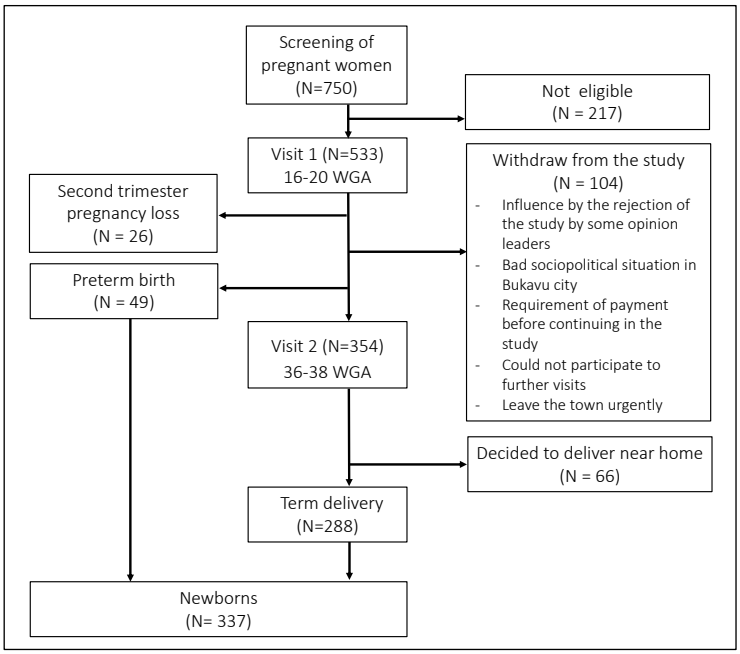

Figure 4. Flowchart of the AVEONS study[56].
N, number of study participants; WGA, weeks of gestational age.

Of the 750 women screened for participation in this study, 533 were found eligible and were enrolled as participants (at V1). Before V2, 104 (19.5%) of these women dropped out, mostly due to rejection of the study by some opinion leaders (who wrongly believed participants were given a substantial imbursement) and the socio-political conflicts in Bukavu during the study period. Of the 533 women included, a second trimester pregnancy loss was seen in 26 women (4.9%), and preterm birth occurred in 49 women (9.2%). In total, 354 women successfully completed V2, of which 66 (18.6%) dropped out of the study because they decided not to deliver at PRHB. The 288 other neonates (81.4%) were all born at term at PRHB.

Most women in this study population were unemployed (82.8%), lived below the threshold of poverty (72.5%), reached at least secondary school (88.0%), belonged to Christian religion (93.5%), and were from the Shi tribe (71.3%). Over half of the women were between 20 and 30 years of age (54.4%) and 73.8% of all participants had at least one previous baby. In total, 79.7% of the women experienced symptoms of BV, including homogenous watery vaginal discharge, vaginal itching, burning sensation after sexual intercourse and vaginal malodor. The median gestational age of this study population was 39 weeks (IQR = 38-40).

5.2.2 Prevalence of bacterial vaginosis

The prevalence of BV in this study population was previously assessed by means of the Nugent score, and is presented in Table 5[56]. A total of 417 vaginal smears were assigned a Nugent score, of which 54.6% was classified as BV-negative (Nugent score 0-3), 18.6% as having an intermediate VMC (Nugent score 4-6), and 26.8% as having BV (Nugent score 7-10).

Table 5. Prevalence of bacterial vaginosis in pregnant women from Bukavu (Democratic Republic of Congo).
CI, confidence interval.

VMC-status	Prevalence (95% CI)
BV	26.8 (23.2-30.8)
Intermediate	18.6 (15.5-22.2)
Healthy	54.6 (50.3-58.8)

5.2.3 Prevalence of the different species

As part of this Master's dissertation, species-specific qPCR assays were used to quantify seven bacterial species in the CVLs taken from 331 women. Table 6 shows the prevalence of each investigated species and the corresponding CIs. In this study population of 331 women, *G. vaginalis* was the most common *Gardnerella* species, with a prevalence of 33.8%. A total of 23.0% of women tested positive for *G. piotii,* 18.4% was positive for the vaginal presence of *G. swidsinskii*, and 14.5% was colonized with *G. leopoldii*. The most prevalent species overall was *L. iners* (75.8%). *L. crispatus* was only found in 41.1% of the women and *A. vaginae* showed a prevalence of 42.0% in this study population.

Table 6. Prevalence of the investigated bacterial species in pregnant women from Bukavu (Democratic Republic of Congo).
CI, confidence interval

Species	Prevalence (95% CI)
L. iners	75.8 (70.9-80.1)
A. vaginae	42.0 (46.8-47.4)
L. crispatus	41.1 (35.9-46.5)
G. vaginalis	33.8 (29.0-39.1)
G. piotii	23.0 (18.8-27.8)
G. swidsinskii	18.4 (14.6-23.0)
G. leopoldii	14.5 (11.1-18.7)

5.2.4 Correlations between the different species

A pairwise correlation matrix of the investigated bacterial species, with the corresponding Pearson correlation coefficients, is shown in Figure 5. In this study population, the strongest positive correlation was found between *G. swidsinskii* and *A. vaginae* (r = 0.403). Furthermore, a significant co-occurrence, although weak, was seen between *G. swidsinskii* and *G. vaginalis* (r = 0.155), *G. swidsinskii* and *G. piotii* (r = 0.234), *A. vaginae* and *G. leopoldii*, (r = 0.139) and *A. vaginae* and *G. piotii* (r = 0.126). A significant weak negative correlation was seen between *L. iners* and *L. crispatus* (r = -0.172), and a significant weak positive correlation was found between *L. iners* and *G. piotii* (r = 0.143).

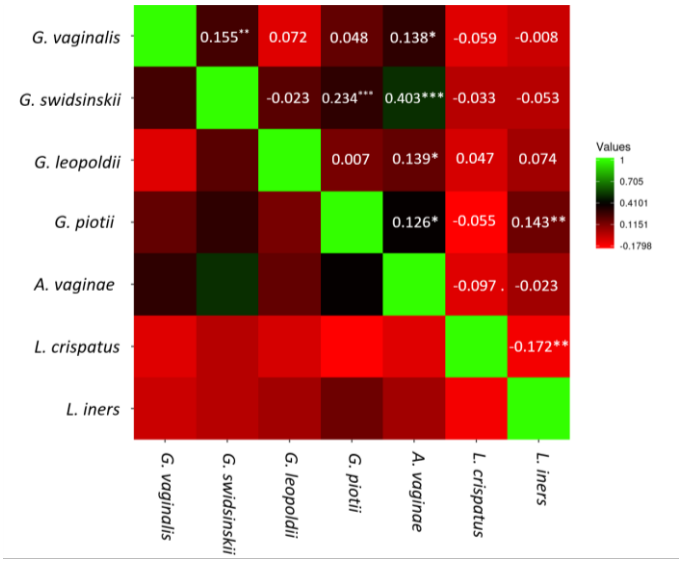

Figure 5. Pairwise correlation matrix with Pearson correlation coefficients. **** = p<0.001; ** = p<0.01; * = p<0.05; . = p<0.1.*

5.2.5 Clinical signs and symptoms

5.2.5.1 Univariate associations of the different species with clinical signs and symptoms

Associations between the presence of the investigated bacterial species and clinical signs and symptoms of BV were calculated with univariate statistics. Results of these statical analyses are shown in Addendum 3 to Addendum 9. Multiple clinical signs and symptoms were found to be significantly associated with the presence of a certain species at the $p < 0.05$ level.

Significant positive associations were found for *G. leopoldii* with Nugent score and with BV status (Addendum 3). For *G. piotii* a positive association was also found with vaginal pH and with dysuria (OR: 2.18; 95% CI: 1.20-3.92)) (Addendum 4). The strongest association was found for *G. piotii* with nitrite (determined with urine dipsticks), since women carrying vaginal *G. piotii* had a more than seven times higher odds for having a positive nitrite result compared to women without *G. piotii* (OR: 7.43, 95% CI: 1.92-34.77). Also, given that an odds ratio of 2.68 (95% CI: 1.01-6.74) was found for having hemoglobulin levels lower than 12.0 g/dL, women with *G. piotii* had a higher odds for anemia compared to women without *G. piotii*. Furthermore, the presence of *G. piotii* was associated with the last episode of vaginal malodor and with vulvar state. For *G. swidsinskii* a positive association was seen with Nugent score and with BV status, and an association was found between *G. swidsinskii* and speculum observations (Addendum 5). Nugent score and BV status were also positively associated with *G. vaginalis*, as was Whiff test, since women with *G. vaginalis* had an almost three times higher odds for a positive Whiff test compared to women without *G. vaginalis* (OR: 2.98; 95% CI: 1.32-6.91) (Addendum 6). Women with *A. vaginae* also had a higher odds for a positive Whiff test compared to women without *A. vaginae* (0R: 3.68; 95%CI: 1.61-8.85) (Addendum 7). Additionally, the presence of *A. vaginae* was significantly positively associated with Nugent score, BV status, and vaginal pH. For *L. crispatus* a significant negative association was found with Nugent score and with BV status, and a significant positive association was found with the observation of clue cells on wet mount (Addendum 8). A negative association with Nugent score was also found for *L. iners*, while a positive association was found between *L. iners* and the observation of *Candida* on wet mount and between *L. iners* and fever (OR: 3.71; 95% CI: 1.11-19.47) (Addendum 9). Additionally, a significant association was found between *L. iners* and the last episode of vaginal malodor.

5.2.5.2 Univariate associations of the investigated species with pregnancy outcomes.

Among the 331 women of whom a CVL sample was available, 172 gave birth at term and a total of 30 PTBs was observed. *L. iners*, the overall most common species in this study population, was found in 90.0% of women with PTB and 70.6% of women with term birth. The prevalence of the six other investigated species is not significantly different between both groups of women. Also, the four *Gardnerella* species were seen in no more than approximately one quarter of women, regardless of PTB. BV (Nugent score 7-10) was also equally prevalent in both groups of women (20.7% of women with PTB; 19.7% of women with term birth). The only significant association with PTB was found for *L. iners*, as women with *L. iners* had an almost four times higher odds of PTB compared to women without *L. iners* (OR: 3.73, 95% CI: 1.07-20.08).

5.2.5.3 Cluster analysis

In Figure 6 an annotated heatmap is shown with hierarchical clustering of women and of the investigated bacterial species, based on the log-transformed concentration of the bacterial species. Women graphically cluster together based on Nugent score and four clear clusters containing mainly BV-positive women can be defined: cluster 1 to cluster 4. These clusters have a distinct pattern of the distribution of *Gardnerella* species. Cutting the corresponding dendrogram of this heatmap at height 110 (Figure 7, left) resulted in six clusters in total: the four clusters that were visually defined as BV-positive based on the annotated Nugent score (cluster 1 to cluster 4) and two clusters that can be defined as BV-negative based on the annotated Nugent score (cluster 5 and cluster 6). When the dendrogram was split at a height of 125 (figure 7, right), two BV-positive clusters (cluster A and cluster B) and two BV-negative clusters (cluster C and cluster D) were found. Clusters C and D are identical to the BV-negative cluster 5 and cluster 6, respectively, that resulted from splitting the dendrogram at height 110.

The clusters that were visually indicated as BV-positive clusters based on Nugent score (i.e. cluster 1 to cluster 4), all contained *G. vaginalis* and *A. vaginae*, together with *G. swidsinskii* (cluster 1), *G. swidsinskii* and *G. piotii* (cluster 2), *G. piotii* (cluster 3) or *G. leopoldii* (cluster 4). In the clusters that mainly represent women without BV as visually defined by annotated Nugent score (i.e. cluster 5 and cluster 6), only two *Gardnerella* species were detected: *G. vaginalis* and *G. piotii*. In these clusters, mainly *L. iners* (cluster 5) or *L. iners* and *L. crispatus* (cluster 6) were observed.

Results from statistical analyses to determine associations between the different clusters and clinical signs and symptoms of BV and pregnancy outcomes are shown in Addendum 10 to Addendum 17. Women from cluster 1 showed a positive association with Nugent score and with BV status, as well as with vaginal discharge (OR: 2.44; 95% CI: 0.96-6.76) (Addendum 10). For cluster 2 a positive association was also found for Nugent score and for BV status, but also for *Candida*, for vaginal pH and for birthweight of the baby (Addendum 11). Nugent score and BV status were also significantly associated with cluster 3, and a positive association was also seen between cluster 3 and Whiff test (OR: 2.73; 95% CI 0.97-7.07) and between cluster 3 and *Trichomonas* (Addendum 12). Cluster 4 also showed a positive association with Nugent score and BV status (Addendum 13). Additionally, women from cluster 4 had a higher odds of a burning sensation after sexual intercourse compared to women not from cluster 4 (OR: 2.48; 95% CI: 1.08-5.78). For cluster 5 and for cluster 6 a negative association was found with Nugent score and BV status (Addendum 14 and Addendum 15). Furthermore, cluster 5

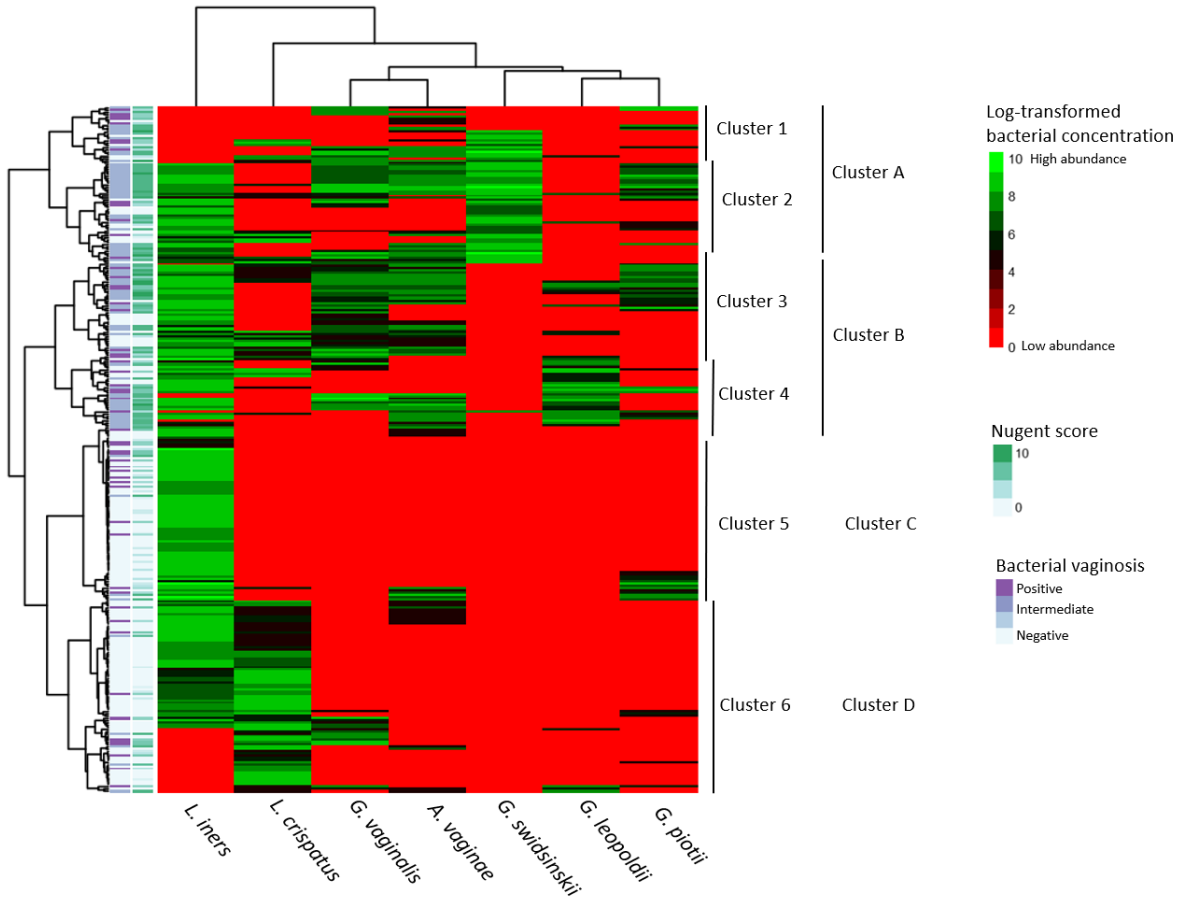

Figure 6. Annotated heatmap. *Heatmap with hierarchical clustering of women and bacterial species based on log-transformed concentration of bacterial species with annotation based on Nugent score and BV status. Each row depicts a woman's VMC profile and each column represents a bacterial species. The color scale ranges from red, indicating a low concentration of bacterial species, to green, indicating a high concentration of bacterial species.*

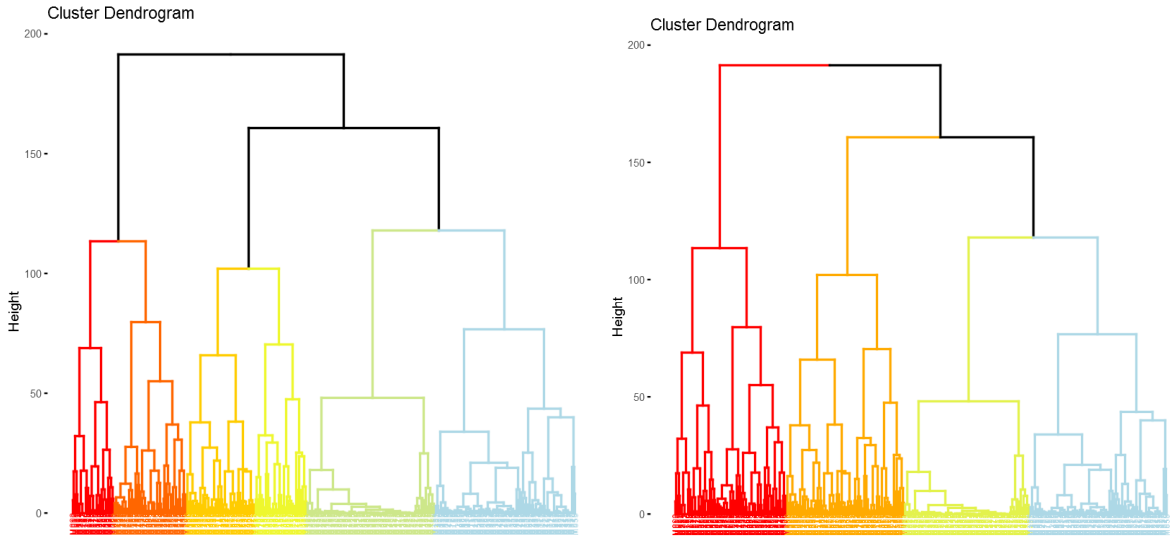

Figure 7. Dendrogram matching the annotated heatmap from Figure 6, split at height 110 (left) and 125 (right). (left) *Red, cluster 1; orange, cluster 2; light orange, cluster 3; yellow, cluster 4; green, cluster 5; blue, cluster 6, corresponding to the clusters 1 to 6 in Figure 6.* ***(right)*** *Red, cluster A; orange, cluster B; green, cluster C; blue, cluster D, corresponding to the clusters A to D in Figure 6.*

was negatively associated with Whiff test (OR: 0.17; 95% CI: 0.02-0.70) and positively associated with glycated keratin, with the state of vaginal secretions and with the presence of clue cells. For cluster 6, on the other hand, a negative association was observed with clue cells. Additionally, cluster 6 was significantly associated with speculum observations and the odds of dysuria were nearly two times higher for women from cluster 6 compared to women not from cluster 6 (OR: 1.74; 95% CI: 0.96-3.13). Also, for cluster A and for cluster B a positive association was seen with Nugent score and with BV status (Addendum 16 and Addendum 17). In addition, cluster A was positively associated with birthweight and cluster B was positively associated with Whiff test (OR: 3.39; 95% CI: 1.43-8.01) and with *Trichomonas*. Lastly, women from cluster B had a lower odds of having a baby with an infection during its first week of life compared to women not from cluster B (OR: 0.48; 95% CI: 0.22-0.99). Cluster C and cluster D are identical to cluster 5 and cluster 6, respectively. Hence, the results of analyses are identical to results described previously (Addendum 14 and Addendum 15).

6. Discussion

6.1 Arba Minch project (Dirashe district, Ethiopia)

The first aim of this dissertation was to report the prevalence of BV and vaginal *Candida* carriage among pregnant women in Dirashe district (Southern Ethiopia, Ethiopia) as part of the 'Arba Minch project'. Here, the BV-status of women in their first trimester of pregnancy was determined by means of the Nugent score[41].

We found an overall prevalence of BV of 14.6% (95% CI: 9.8-21.1%). This prevalence is lower than the prevalence found in the general population in Sub Saharan Africa of 25.0%[1]. So far, only few studies have determined the prevalence of BV in Ethiopia. In 2014, Mengistie et al. found a prevalence of BV (determined with Nugent score) of 19.4% among pregnant Ethiopian women, which is in line with results reported in this Master's dissertation[60]. In a hospital-based study, Mulu and colleagues reported an overall prevalence of BV of only 2.8%, with a higher prevalence among non-pregnant women (5.6%) compared to pregnant women (0.5%)[61]. However, the methodology of BV diagnosis was described rather vague and contradictory. In 2017, Bitew and coworkers documented a much higher BV prevalence (determined with Nugent score) of 48.6% in 210 women attending a hospital with vaginal complaints[62]. For vaginal *Candida* carriage a prevalence of 7.3% (95% CI: 4.1-12.6%) was found in this Master's dissertation, which is in line with the prevalence of 8.3% as reported by Mulu et al.[61]. However, Bitew and coworkers again reported a considerably higher prevalence of 41.4% among 210 participants[63]. Tsega and coworkers also found a higher prevalence of 25.0% among 384 pregnant women[64]. The mean prevalence of vaginal *Candida* carriage across previous studies among pregnant women in Sub Saharan Africa (Addendum 18) was 36.5%, which is also notably higher than the prevalence found in this Master's dissertation. However, since only a subset of the samples was analyzed due to time limitations, these results might not accurately represent the prevalence of BV and vaginal *Candida* carriage in this study population. Furthermore, the prevalence of vaginal *Candida* carriage was not a primary study outcome for this project and the study was therefore not designed accordingly for the reporting of this prevalence. Also, *Candida* was identified with microscopy for this Master's dissertation, whereas detection with qPCR has been proven to be be more sensitive.

6.2 AVEONS study (Bukavu, Democratic Republic of Congo)

The second aim of this Master's dissertation was to investigate the distribution of the different *Gardnerella* species and other relevant markers of BV, and bring this into relation with clinical signs and symptoms. Furthermore, we aimed to identify a possible (molecular) marker for PTB.

This part of this Master's dissertation was performed within the AVEONS project (Bukavu, DRC).

To our best knowledge, we report the prevalence of *G. leopoldii*, *G. piotii*, *G. swidsinskii* and *G. vaginalis* among pregnant women for the first time. In our study population of pregnant Congolese women, *G. vaginalis* was the most prevalent *Gardnerella* species (33.8%), followed by *G. piotii* (23.0%), *G. swidsinskii* (18.4%), and *G. leopoldii* (14.5%). In 2019, Hill and coworkers performed a deep-sequencing study, based on the cpn60 gene, investigating the newly described *Gardnerella* species in non-pregnant Canadian women[65]. They also reported *G. vaginalis* to be the most prevalent *Gardnerella* species (68.4%), but followed by *G. swidsinskii* (49.2%), *G. leopoldii* (26.2%) and *G. piotii* (25.2%). Interestingly, prevalences of the different *Gardnerella* species reported by Hill and coworkers are considerably higher than prevalences reported here. This observation could be explained by the fact that Hill and coworkers calculated these prevalences among only the samples that were *Gardnerella*-positive (301 samples), rather than among their total study population (413 samples). In their study, they also reported a co-occurrence between *G. swidsinskii* and *G. vaginalis*, and *G. piotii* and genomospecies 3, while *G. swidsinskii* and *G. leopoldii* did not occur together more often that what is estimated by chance. This is in line with findings reported here, since we also observed a positive correlation between *G. swidsinskii* and *G. vaginalis*, and not between *G. swidsinskii* and *G. leopoldii*. We did, however, also find a co-occurrence between *G. swidsinskii* and *G. piotii*. Additionally, we reported the prevalence of the different *Gardnerella* species among with PTB and women with term birth. Given that no significant difference was found between these two groups of women, not one of the four investigated *Gardnerella* species can serve as an indicator for PTB. However, literature states that BV is rather consistently associated with PTB[4], while in this study it was observed that BV is equally prevalent in women with and without PTB. This indicates our study population has a somewhat atypical distribution and this could possibly explain why no specific (molecular) marker for PTB could be identified in our study.

Here, we also report the prevalence of two lactobacilli, namely *L. crispatus*, a species associated with a healthy VMB, and *L. iners*, an atypical lactobacil which is often found in high numbers in women with vaginal dysbiosis[34]. Furthermore, we also quantified *A. vaginae* with a species-specific qPCR assay, since this is an important markers for a disturbed VMB[19]. In our study population of pregnant women from Bukavu, DRC, *L. iners* was the most prevalent species (75.8%), which is in line with results from numerous previous studies, as summarized by Vaneechoutte in 2017[16]. Remarkedly, a negative correlation was observed between *L. iners* and *L. crispatus* (r = -0.172), while a positive correlation was observed between *L. iners* and *G. piotii* (r = 0.143). This could suggest that *L. iners* is involved more in BV than in health, although the specific role of these species in the pathology of BV remains to be unraveled. For *A. vaginae* a co-occurrence was reported with *G. swidsinskii* (r = 0.403), with *G. leopoldii* (r = 0.139) and with *G. piotii* (r = 0.126), which is in line with the general knowledge that *A. vaginae* cannot survive without *Gardnerella*. Although the difference in prevalence of *L. iners* between women with (90.0%) and without PTB (70.6%) was significant (p = 0.036), the presence of *L. iners* cannot be considered indicative for PTB due to its high prevalence among women with term birth. These results are in line with results presented by Petricevic and colleagues, since they also report a significantly higher prevalence of *L. iners* among women with PTB compared to women with term birth[66]. In contrast, in the 2019 study performed by Fettweis and colleagues it is demonstrated that women who delivered at term showed significant decreases in *A.*

vaginae and *G. vaginalis*, while there was an increase of *L. iners* among these women[67]. Additionally, it was recently reported that the vaginal microbial oligotype *L. iners*/*Ralstonia solanacearum* is associated with a decrease in the risk of early spontaneous PTB[68]. These results therefore indicate that, in contrast to what we found, *L. iners* may be associated with a decreased risk of PTB.

Remarkedly, none of the seven investigated species showed a significant relationship with the most important clinical signs and symptoms of BV (including vaginal discharge, vaginal itching, burning sensation after sexual contact and vaginal malodor). We did, however, find an association between *G. piotii* and markers of urinary tract infection (dysuria and nitrite levels determined on urine dipstick). These observations, together with the fact that *G. piotii* is a sialidase-positive *Gardnerella* species, suggests that *G. piotii* might be more pathogenic. A significant association was also found between *G. piotii* and anemia (as determined by hemoglobin levels measured on Hemocue). This too is an interesting observation and it would be of meaning to investigate the relationship between these two factors in the future. Given that the AVEONS project was a cross-sectional study, no causal direction can be determined for this association, but it can be hypothesized that it is due to the uptake of iron by *G. piotii* that infected women develop anemia. On the other hand, it could also be so that women with anemia, having an impaired immune response, are more susceptible to infection with *G. piotii*. Furthermore, *G. piotii*, as well as *A. vaginae*, was positively associated with vaginal pH, which is in line with general expectations since it is known that the low protective vaginal pH is increased in BV. *A. vaginae* also showed a positive association with Whiff test, just like *G. vaginalis*, which is also in line with the fact that a positive Whiff test is something inherent of BV. It is also interesting to note that all four *Gardnerella* species and *A. vaginae* are positively associated with Nugent score and with BV-status, while both investigated lactobacilli are negatively associated with Nugent score. Additionally, *L. iners* was associated with *Candida*, which is interesting since VVC is also a prominent condition affecting reproductive health[56]. Furthermore, *L. iners* was positively associated with fever, which is of note since BV is not an inflammatory condition.

After hierarchical clustering, clear groups of women could be distinguished based on their distinct pattern of species distribution. Although no significant association was found between a certain *Gardnerella* species and the most important clinical signs and symptoms of BV, all *Gardnerella* species could be found among the clusters containing mainly BV-positive women (cluster 1 to cluster 4). This indicates that all *Gardnerella* species can be involved in (asymptomatic) BV and suggests that it is not a single *Gardnerella* species that causes BV, but it is likely that an interplay between the different species gives rise to (asymptomatic) BV. In clusters containing mainly women without vaginal dysbiosis (cluster 5 and cluster 6), *G. leopoldii* and *G. swidsinskii* were not observed. This might suggest these two species are more pathogenic than *G. piotii* and *G. vaginalis*, but further research is required to understand the exact role of all *Gardnerella* species in the pathogenesis of BV. When investigating the VMC as a whole with univariate analyses of the defined clusters, only few significant association were found with the most prominent clinical signs of BV. Cluster 1 and cluster 4, both defined as BV-positive clusters based on their annotated Nugent score, were positively associated with vaginal discharge and burning sensation after sexual intercourse, respectively. It is of note that *L. iners* was not observed in cluster 1, in contrast to the five other examined clusters, suggesting that *L. iners* might have a protective effect for vaginal discharge. For cluster 2, a significant positive association was found with vaginal pH, with *Candida* and with birthweight.

The latter is interesting given that cluster 2 is defined as a BV-positive cluster, making it seem as if women with BV deliver babies with a higher birthweight. Interestingly, the same observation was made for cluster A. Next, a positive association was found between cluster 3 and *Trichomonas*, another important pathogen affecting reproductive health. Moreover, a positive association was found between cluster 3 and Whiff test, while a negative association was found between cluster 5 and Whiff test. Given that cluster 3 is defined as a BV-positive cluster, while cluster 5 is a cluster containing mainly women without BV, these results are in line with our expectations. Furthermore, an association was found between clue cells and both cluster 5 and cluster 6. This would be interesting to further evaluate since cluster 5 and cluster 6 were both defined as BV-negative clusters, and yet cluster 5 shows a positive association with clue cells (a feature typically observed in BV), while none of the BV-positive clusters (cluster 1 to cluster 4) showed a significant association with clue cells. Cluster 6 was also significantly associated with dysuria, which is an interesting sign of urinary tract infection. In line with cluster 3, we observed a positive association between cluster B and Whiff test and between cluster B and *Trichomonas*, but also a negative association between cluster B and infection of the baby during its first week of life. This indicates that babies from women from cluster B are more protected against infections.

7. General conclusions

In this Master's dissertation, a prevalence of 14.6% was found for BV among pregnant Ethiopian women in their first trimester of pregnancy, and a prevalence of 7.3% was found for vaginal *Candida* carriage in this study population as part of the Arba Minch project. When evaluating BV in pregnant Congolese women in their first trimester of pregnancy as part of the AVEONS project, a prevalence of 26.8% was found. *G. vaginalis* was the most prevalent *Gardnerella* species in this study population, and *G. leopoldii* was the least prevalent species. Our results indicate that all *Gardnerella* species can be involved in BV, although only few significant associations were found between (groups of) the investigated bacterial species and the most important clinical signs and symptoms of BV. Also, not one of the seven examined species could be appointed as an adequate indicator for PTB.

Although BV is the most common gynecological condition in women of reproductive age, the exact mechanisms of its pathogenesis remain poorly understood. Overall, our findings are important to help clarify the role of *Gardnerella* in the pathogenesis of BV and further investigate the distribution of the different *Gardnerella* species to unravel the paradoxical occurrence of *Gardnerella* as both a commensal and a pathogen. Examining the presence of the different *Gardnerella* species among women and so identifying molecular markers of BV could also be of importance for the diagnosis of BV, since the current gold standard (Nugent score) is suboptimal.

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10. Poster

FACULTY OF MEDICINE AND HEALTH SCIENCES

GHENT UNIVERSITY

Distribution of *Gardnerella* species in pregnant women in Bukavu, DRC

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INTRODUCTION

Bacterial vaginosis (BV), a disturbance of the vaginal microbiome (VMB), is the most common vaginal condition worldwide and is associated with important adverse pregnancy outcomes[1]. The presence of *Gardnerella vaginalis* and *Atopobium vaginae*, and the absence of *Lactobacillus crispatus* are key markers of BV[2]. The role of *L. iners* remains enigmatic. Recently, we found that *G. vaginalis* comprises 13 different (genomo)species[3], but prevalences of these species are currently unknown. Here, we aimed to assess the prevalence of *G. vaginalis*, *G. swidsinskii*, *G. leopoldii*, *G. piotii*, *A. vaginae*, *L. crispatus* and *L. iners* in a population of pregnant women from Bukavu, Democratic Republic of the Congo (DRC).

METHODS

The BV status of 331 pregnant women was determined microscopically using the gold standard Nugent scoring (0-3, healthy VMB; 4-7, intermediate VMB; 8-10, BV). The vaginal pH was assessed using dipsticks. Clinical symptoms, including vaginal itching, dysuria, burning and vaginal smell, were assessed in a questionnaire. The presence of a biofilm was determined microscopically. DNA from cervicovaginal lavages was used to quantify the vaginal concentrations of *G. vaginalis*, *G. swidsinskii*, *G. leopoldii*, *G. piotii*, *A. vaginae*, *L. crispatus* and *L. iners* by means of qPCR.

Species	Prevalence (%)
L. iners	75.83
A. vaginae	41.99
L. crispatus	41.09
G. vaginalis	33.84
G. piotii	22.96
G. swidsinskii	18.43
G. leopoldii	14.50

Table 1: Prevalences of *Gardnerlla* species (*G. vaginalis*, *G. swidsinskii*, *G. leopoldii*, *G. piotii*), *Atopobium vaginae*, *Lactobacillus crispatus* and *L. iners*.

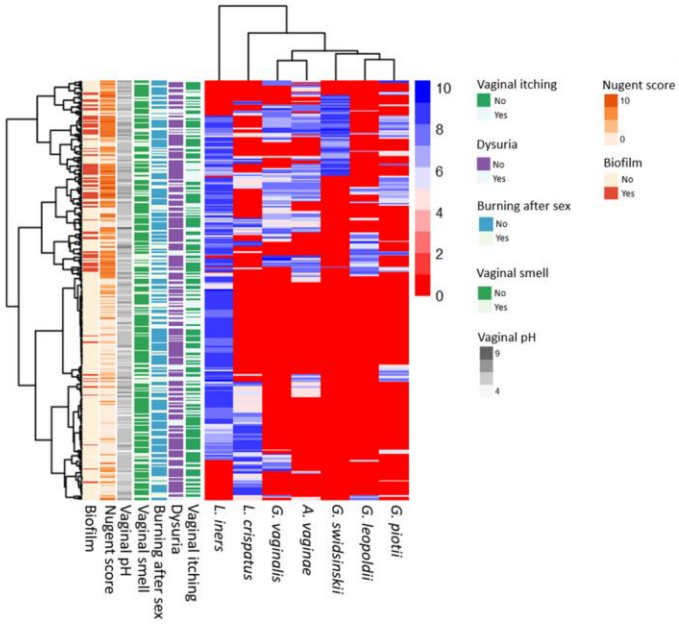

Figure 1: Heatmap with hierarchal clustering of women based on log-transformed concentration of bacterial species with annotation based on clinical symptoms, Nugent score and the presence of a biofilm.

RESULTS

The prevalence of the different bacterial species is shown in Table 1. *L. iners* was the most common colonizer, with a prevalence of 75.8%. Of the *Gardnerella* species *G. vaginalis* was most commonly found (33.8%) and *G. leopoldii* showed the lowest prevalence (14.5%).

Figure 1 shows a heatmap with the hierarchal clustering of women based on the log-transformed concentration of the bacterial species. Each row represents a woman's VMB profile and each column represents a species. We can distinguish clear clusters of women, where the *Gardnerella* species have a distinct distribution. Women also cluster together based on Nugent score, matching with clusters based on the presence of a biofilm.

CONCLUSION

This is the first study to examine the distribution of *Gardnerella* species amongst pregnant women. *L. iners* is the most prevalent species amongst pregnant women in Bukavu, DCR. *G. vaginalis* is the most common species of the genus *Gardnerella*. Clear groups of women are distinguishable with a unique pattern of species. These findings are important to further evaluate which specific species contribute to the pathogenesis of BV.

References:
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11. Addenda

11.1 Addendum 1: List of abbreviations

A. vaginae	*Atopobium vaginae*
AVEONS	Angamiza Vizuri Early Onset Neonatal Sepsis
BV	Bacterial vaginosis
C. glabrata	*Candida glabrata*
Cq	Quantification cycle
CI	Confidence interval
CVL	Cervicovaginal lavage
DRC	Democratic Republic of the Congo
dsDNA	double stranded DNA
EDTA	Ethylenediamine tetra-acetic acid
G. leopoldii	*Gardnerella leopoldii*
G. swidsinskii	*Gardnerella swidsinskii*
G. piotii	*Gardnerella piotii*
G. vaginalis	*Gardnerella vaginalis*
HIV	Human Immunodeficiency Virus
HPLC	High-performance liquid chromatography
IQR	Interquartile range
LBR	Laboratory for Bacteriology Research
L. crispatus	*Lactobacillus crispatus*
L. iners	*Lactobacillus iners*
OR	Odds ratio
P. bivia	*Prevotella bivia*
PTB	Preterm birth
PRHB	Provincial Referral Hospital of Bukavu, DRC
STI	Sexually transmitted infection
Tm	Melting temperature
qPCR	quantitative polymerase chain reaction
VMC	Vaginal microbial community
VVC	Vulvovaginal candidiasis
V1	Visit 1
V2	Visit 2
WHO	World Health Organization

11.2 Addendum 2: Script of intervention video on vaginal hygiene, Arba Minch project

W1, woman 1; W2, woman 2; HP, health practitioner

**W1 and W2 going to the Heath center for antenatal care*
HP: Do you experience any vaginal discomfort?
W1: I had itching on perineal area during my previous pregnancy, but not during this pregnancy
HP: What are the other problems you faced during your previous pregnancy?
W1: I also had some vaginal discharge and I got treatment in the clinic.

**HP asks W2 about any experience regarding vaginal discharge and her knowledge and practice of vaginal care*
W2: I did not have vaginal discharge during my previous pregnancy, and not during this pregnancy either. But, I had itching in between and when I was sick.
HP: How do you wash the perineal area and vagina in general during bathing/showering?
W1: I wash with water and soap. And when I have itching, I will wash it thoroughly when it is itching too much.
W2: When I wash it with cold water and/or swab with cloth it will go.

**W1 and W2 say that they wash inside the vagina using soap because they believe it should be cleaned there, and because they believe it will benefit their unborn baby*
HP: There are many reasons for vaginal discharge and itching. When the cause is infection, it might have an effect on your baby. First, whatever the cause may be, keep away from aggressive soap, detergents and any other form of washing intravaginally. It does not cure you and, very importantly, it will disturb the good bacteria that protect your vagina against infections. Also, you should know that our bodies have some bacteria that live in our intestines, on our skins and especially in mucosal areas such as the vagina, and keeping the good bacteria is important.
W1: What to do when we have discharge, can we treat it?
W2: I heard that small discharges are effects of the baby taking a shower in the womb, is this true?
HP: Naturally, there may be discharge during pregnancy. But, if the odor is changed and foul smelling and it is itching, its cause may be different types of infections. To prevent this, keep the perineal area clean but not with aggressive agents. You should keep the bacteria balanced:

1- Do not use aggressive agent such as soap intravaginally, lemon, detergents.
2- Use only water and natural soap on the perineal area
3- Take daily shower with warm water and soup. Moisture your body with ointments to prevent your skin from drying after bath. The water should not be hot or cold.
4- Put on clean underwear that should be cotton. Consulting the health workers will help. Also the use of substances such as cloth, herbs, should not be applied at all!
5- If it is an infection and it does not go away with all these hygienic practices, tell your doctor.

W1 and W2: Thank you for making it clear for us. We will practice it as per your advice.

11.3 Addendum 3: Univariate associations between *Gardnerella leopoldii* and clinical signs and symptoms

Addendum 3. Univariate associations between Gardnerella leopoldii and clinical signs and symptoms of mother and baby.
GL, Gardnerella leopoldii; n, number of study participants; OR, odds ratio; CI, confidence interval

	GL+ n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	21 (12.57%)	REF	0.333
Yes	26 (16.35%)	1.36 (0.70-2.67)	
Vaginal itching			
No	31 (16.15%)	REF	0.389
Yes	17 (12.50%)	0.74 (0.37-1.46)	
Dysuria			
No	33 (13.87%)	REF	0.586
Yes	14 (16.28%)	1.21 (0.56-2.48)	
Burning sensation after sex			
No	26 (12.44%)	REF	0.112
Yes	20 (19.23%)	1.67 (0.83-3.32)	
Last episode of burning			
1 day	1 (6.25%)	REF	0.640
2 days	4 (23.53%)	4.42 (0.37-241.79)	
2-7 days	6 (27.27%)	5.41 (0.55-275.22)	
7-14 days	1 (10.00%)	1.63 (0.02-139.25)	
>14 days	4 (19.05%)	3.42 (0.29-185.00)	
Sensation of vaginal smell			
No	30 (13.64%)	REF	0.221
Yes	15 (19.48%)	1.53 (0.72-3.16)	
Last episode of vaginal smell			
1 day	7 (28.00%)	REF	0.772
2 days	0 (0.00%)	0.00 (0.00-3.65)	
7-14 days	1 (8.33%)	0.24 (0.00-2.32)	
>14 days	0 (0.00%)	0.00 (0.00-2.90)	
Unknown	7 (24.14%)	0.82 (0.20-3.32)	
Whiff test			
Negative	40 (13.47%)	REF	0.070
Positive	8 (25.81%)	2.23 (0.80-5.61)	
State vaginal secretions			
Fine and homogenous	42 (14.14%)	REF	0.577
Thick	3 (18.74%)	1.40 (0.25-5.40)	
Thick and heterogenous	3 (17.65%)	1.30 (0.23-4.94)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	43 (14.10%)	REF	0.372
8,3-11,9 (anemia)	5 (20.83%)	1.60 (0.44-4.75)	

	GL+ n	OR (CI 0.95)	p-value
Fever			
No	39 (13.59%)	REF	0.089
Yes	9 (24.32%)	2.04 (0.79-4.87)	
Uterine contractions			
No	38 (15.14%)	REF	0.663
Yes	5 (12.50%)	0.80 (0.23-2.24)	
Antibiotics during 2w before visit			
No	41 (14.54%)	REF	0.904
Yes	7 (15.22%)	1.05 (0.37-2.61)	
Vulvar state			
Normal	47 (14.55%)	REF	0.325
Erythema	0 (0.00%)	0.00 (0.00-229.03)	
Postule	0 (0.00%)	0.00 (0.00-31.69)	
Leucorrhoea	0 (0.00%)	0.00 (0.00-14.52)	
Speculum			
Normal	42 (15.44%)	REF	0.222
Redness	4 (9.76%)	0.59 (0.15-1.78)	
Bleeding ex utero	1 (11.11%)	0.69 (0.02-5.34)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-213.79)	
Ulcerations	0 (0.00%)	0.00 (0.00-6.18)	
***Trichomonas* on wet mount**			
Negative	48 (14.77%)	REF	0.380
Positive	0 (0.00%)	0.00 (0.00-8.97)	
Unknown	0 (0.00%)	0.00 (0.00-225.08)	
***Candida* on wet mount**			
Negative	31 (13.03%)	REF	0.236
Positive	17 (18.68%)	1.53 (0.75-3.05)	
Unknown	0 (0.00%)	0.00 (0.00-260.62)	
Nitrite urine dipstick			
Negative	46 (14.47%)	REF	0.832
Positive	2 (16.67%)	1.18 (0.12-5.81)	
BV state visit 1			
Negative	11 (6.25%)	REF	**<0.001**
Intermediate	26 (28.57%)	5.95 (2.66-14.17)	
Positive	11 (18.64%)	3.42 (1.26-9.29)	
Nugent score visit 1			**<0.001**
Vaginal pH			0.088
White blood cells on wet mount			0.857
Clue cells on wet mount			0.633
Epithelial cells on wet mount			0.723
White blood cells urine dipstick			0.924
Glycated keratin			0.788
Length cervix			0.541

	GL+ n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	27 (14.36%)	REF	0.660
Yes	10 (12.35%)	0.84 (0.34-1.91)	
Evolution of the baby			
Well	9 (13.85%)	REF	0.303
Dead	1 (5.88%)	0.39 (0.01-3.23)	
Handicap	0 (0.00%)	0.00 (0.00-35.68)	
Preterm birth			
No	29 (16.86%)	REF	0.631
Yes	4 (13.33%)	0.76 (0.18-2.44)	
Birthweight			0.690

11.4 Addendum 4: Univariate associations between *Gardnerella piotii* and clinical signs and symptoms

Addendum 4. Univariate associations between Gardnerella piotii and clinical signs and symptoms of mother and baby.
GP, Gardnerella piotii; n, number of study participants; OR, odds ratio; CI, confidence interval

	GP+ n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	35 (20.96%)	REF	0.442
Yes	39 (24.53%)	1.22 (0.71-2.13)	
Vaginal itching			
No	43 (22.40%)	REF	0.810
Yes	32 (23.53%)	1.07 (0.61-1.85)	
Dysuria			
No	45 (18.91%)	REF	**0.006**
Yes	29 (33.72%)	2.18 (1.20-3.92)	
Burning sensation after sex			
No	46 (22.01%)	REF	0.554
Yes	26 (25.00%)	1.18 (0.65-2.11)	
Last episode of burning			
1 day	3 (18.75%)	REF	0.840
2 days	6 (35.29%)	2.30 (0.38-17.66)	
2-7 days	7 (31.82%)	1.99 (0.36-14.38)	
7-14 days	0 (0.00%)	0.00 (0.00-3.83)	
>14 days	7 (33.33%)	2.12 (0.38-15.46)	
Sensation of vaginal smell			
No	48 (21.82%)	REF	0.606
Yes	19 (24.68%)	1.17 (0.60-2.23)	
Last episode of vaginal smell			
1 day	11 (44.00%)	REF	**0.002**
2 days	2 (40.00%)	0.85 (0.06-8.88)	
7-14 days	2 (16.67%)	0.26 (0.02-1.64)	
>14 days	2 (33.33%)	0.65 (0.05-5.52)	
Unknown	2 (6.90%)	0.10 (0.01-0.54)	
Whiff test			
Negative	64 (21.55%)	REF	0.083
Positive	11 (35.48%)	2.00 (0.82-4.64)	
State vaginal secretions			
Fine and homogenous	66 (22.22%)	REF	0.322
Thick	3 (18.75%)	0.81 (0.14-3.06)	
Thick and heterogenous	6 (36.29%)	1.90 (0.56-5.87)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	64 (20.98%)	REF	**0.024**
8,3-11,9 (anemia)	10 (41.67%)	2.68 (1.01-6.84)	

	GP+ n	OR (CI 0.95)	p-value
Fever			
No	63 (21.95%)	REF	0.744
Yes	9 (24.32%)	1.14 (0.45-2.65)	
Uterine contractions			
No	60 (23.9%)	REF	0.217
Yes	6 (15.0%)	0.56 (0.18-1.45)	
Antibiotics during 2w before visit			
No	65 (23.05%)	REF	0.844
Yes	10 (21.74%)	0.93 (0.39-2.04)	
Vulvar state			
Normal	72 (22.29%)	REF	**0.032**
Erythema	0 (0.00%)	0.00 (0.00-136.21)	
Postule	0 (0.00%)	0.00 (0.00-18.78)	
Leucorrhoea	3 (100.00%)	inf (1.40-inf)	
Speculum			
Normal	62 (22.79%)	REF	0.839
Redness	10 (24.39%)	1.09 (0.45-2.45)	
Bleeding ex utero	2 (22.22%)	0.97 (0.10-5.26)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-132.45)	
Ulcerations	1 (20.00%)	0.85 (0.02-8.77)	
***Trichomonas* on wet mount**			
Negative	73 (22.46%)	REF	0.166
Positive	1 (25.00%)	1.15 (0.02-14.57)	
Unknown	1 (100.00%)	inf (0.09-inf)	
***Candida* on wet mount**			
Negative	53 (22.27%)	REF	0.593
Positive	21 (23.08%)	1.05 (0.56-1.92)	
Unknown	1 (100.00%)	inf (0.09-inf)	
Nitrite urine dipstick			
Negative	67 (21.07%)	REF	**0.001**
Positive	8 (66.67%)	7.43 (1.92-34.77)	
BV state visit 1			
Negative	18 (10.23%)	REF	**<0.001**
Intermediate	44 (48.35%)	8.14 (4.16)16.49)	
Positive	13 (22.03%)	2.47 (1.03-5.80)	
Nugent score visit 1			**<0.001**
Vaginal pH			**0.022**
White blood cells on wet mount			0.145
Clue cells on wet mount			0.307
Epithelial cells on wet mount			0.903
White blood cells urine dipstick			0.216
Glycated keratin			0.685
Length cervix			0.740

	GP+ n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	42 (22.34%)	REF	0.675
Yes	20 (24.69%)	1.14 (0.58-2.17)	
Evolution of the baby			
Well	14 (21.54%)	REF	0.296
Dead	5 (29.41%)	1.51 (0.36-5.65)	
Handicap	1 (50.00%)	3.55 (0.04-290.67)	
Preterm birth			
No	37 (21.51%)	REF	0.532
Yes	8 (26.67%)	1.32 (0.47-3.41)	
Birthweight			0.847

11.5 Addendum 5: Univariate associations between *Gardnerella swidsinskii* and clinical signs and symptoms

Addendum 5. Univariate associations between Gardnerella swidsinskii and clinical signs and symptoms of mother and baby.
GS, Gardnerella swidsinskii; n, number of study participants; OR, odds ratio; CI, confidence interval

	GS+ n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	29 (17.37%)	REF	0.620
Yes	31 (19.50%)	1.15 (0.63-2.10)	
Vaginal itching			
No	32 (16.67%)	REF	0.287
Yes	29 (21.32%)	1.35 (0.74-2.46)	
Dysuria			
No	45 (18.91%)	REF	0.589
Yes	14 (16.28%)	0.84 (0.40-1.67)	
Burning sensation after sex			
No	37 (17.70%)	REF	0.742
Yes	20 (19.23%)	1.11 (0.57-2.10)	
Last episode of burning			
1 day	5 (31.25%)	REF	0.501
2 days	2 (11.76%)	0.30 (0.02-2.29)	
2-7 days	5 (22.73%)	0.65 (0.12-3.58)	
7-14 days	1 (10.00%)	0.26 (0.00-2.94)	
>14 days	4 (19.05%)	0.53 (0.08-3.06)	
Sensation of vaginal smell			
No	39 (17.73%)	REF	0.402
Yes	17 (22.08%)	1.31 (0.65-2.59)	
Last episode of vaginal smell			
1 day	4 (16.00%)	REF	0.267
2 days	0 (0.00%)	0.00 (0.00-8.50)	
7-14 days	3 (25.00%)	1.72 (0.21-12.67)	
>14 days	3 (50.00%)	4.89 (0.48-52.75)	
Unknown	7 (24.14%)	1.65 (0.36-8.89)	
Whiff test			
Negative	52 (17.51%)	REF	0.259
Positive	8 (25.81%)	1.63 (0.60-4.06)	
State vaginal secretions			
Fine and homogenous	54 (18.18%)	REF	0.418
Thick	2 (12.50%)	0.64 (0.07-2.93)	
Thick and heterogenous	5 (29.41%)	1.87 (0.50-6.01)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	57 (18.69%)	REF	0.806
8,3-11,9 (anemia)	4 (16.67%)	0.87 (0.21-2.74)	

	GS+ n	OR (CI 0.95)	p-value
Fever			
No	52 (18.12%)	REF	0.366
Yes	9 (24.32%)	1.45 (0.57-3.40)	
Uterine contractions			
No	42 (16.73%)	REF	0.904
Yes	7 (17.50%)	1.06 (0.37-2.64)	
Antibiotics during 2w before visit			
No	55 (19.50%)	REF	0.300
Yes	6 (13.04%)	0.62 (0.20-1.57)	
Vulvar state			
Normal	59 (18.27%)	REF	0.183
Erythema	0 (0.00%)	0.00 (0.00-174.69)	
Postule	0 (0.00%)	0.00 (0.00-24.13)	
Leucorrhoea	2 (66.67%)	8.86 (0.45-527.96)	
Speculum			
Normal	54 (19.85%)	REF	**0.046**
Redness	6 (14.63%)	0.69 (0.23-1.78)	
Bleeding ex utero	0 (0.00%)	0.00 (0.00-2.12)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-157.78)	
Ulcerations	0 (0.00%)	0.00 (0.00-4.53)	
***Trichomonas* on wet mount**			
Negative	61 (18.77%)	REF	0.311
Positive	0 (0.00%)	0.00 (0.00-6.70)	
Unknown	0 (0.00%)	0.00 (0.00-168.98)	
***Candida* on wet mount**			
Negative	39 (16.39%)	REF	0.136
Positive	22 (24.18%)	1.62 (0.85-3.04)	
Unknown	0 (0.00%)	0.00 (0.00-199.38)	
Nitrite urine dipstick			
Negative	58 (18.24%)	REF	0.556
Positive	3 (25.00%)	1.49 (0.25-6.22)	
BV state visit 1			
Negative	17 (9.66%)	REF	**0.012**
Intermediate	34 (37.36%)	5.54 (2.77-11.45)	
Positive	9 (15.25%)	1.68 (0.62-4.28)	
Nugent score visit 1			**<0.001**
Vaginal pH			0.193
White blood cells on wet mount			0.632
Clue cells on wet mount			0.753
Epithelial cells on wet mount			0.195
White blood cells urine dipstick			0.365
Glycated keratin			0.819
Length cervix			0.893

	GS+ n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	34 (18.09%)	REF	0.365
Yes	11 (13.58%)	0.71 (0.31-1.54)	
Evolution of the baby			
Well	7 (10.77%)	REF	0.137
Dead	3 (17.65%)	1.76 (0.26-9.02)	
Handicap	1 (50.00%)	7.82 (0.09-656.94)	
Preterm birth			
No	41 (23.84%)	REF	0.210
Yes	4 (13.33%)	0.49 (0.12-1.54)	
Birthweight			**0.030**

11.6 Addendum 6: Univariate associations between *Gardnerella vaginalis* and clinical signs and symptoms

Addendum 6. Univariate associations between Gardnerella vaginalis and clinical signs and symptoms of mother and baby.
GV, Gardnerella vaginalis; n, number of study participants; OR, odds ratio; CI, confidence interval

	GV+ n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	58 (34.73%)	REF	0.884
Yes	54 (33.96%)	0.97 (0.60-1.57)	
Vaginal itching			
No	67 (34.90%)	REF	0.734
Yes	45 (33.09%)	0.92 (0.56-1.50)	
Dysuria			
No	85 (25.71%)	REF	0.190
Yes	24 (27.91%)	0.70 (0.39-1.23)	
Burning sensation after sex			
No	75 (35.89%)	REF	0.369
Yes	32 (30.77%)	0.79 (0.46-1.35)	
Last episode of burning			
1 day	7 (43.75%)	REF	0.202
2 days	8 (47.06%)	1.14 (0.24-5.56)	
2-7 days	4 (18.18%)	0.30 (0.05-1.53)	
7-14 days	3 (30.00%)	0.56 (0.07-3.75)	
>14 days	6 (28.57%)	0.52 (0.11-2.49)	
Sensation of vaginal smell			
No	72 (32.73%)	REF	0.163
Yes	32 (41.56%)	1.45 (0.82-2.57)	
Last episode of vaginal smell			
1 day	11 (44.00%)	REF	0.817
2 days	3 (60.00%)	1.87 (0.18-26.10)	
7-14 days	3 (25.00%)	0.43 (0.06-2.33)	
>14 days	3 (50.00%)	1.26 (0.14-11.40)	
Unknown	12 (41.38%)	0.90 (0.27-3.03)	
Whiff test			
Negative	94 (31.65%)	REF	**0.004**
Positive	18 (58.06%)	2.98 (1.32-6.91)	
State vaginal secretions			
Fine and homogenous	103 (34.68%)	REF	0.339
Thick	5 (31.25%)	0.86 (0.23-2.76)	
Thick and heterogenous	4 (23.53%)	0.58 (0.13-1.94)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	102 (33.44%)	REF	0.415
8,3-11,9 (anemia)	10 (41.67%)	1.42 (0.54-3.58)	

	GV+ n	OR (CI 0.95)	p-value
Fever			
No	100 (34.84%)	REF	0.538
Yes	11 (29.73%)	0.79 (0.34-1.74)	
Uterine contractions			
No	88 (35.06%)	REF	0.065
Yes	8 (20.00%)	0.46 (0.18-1.09)	
Antibiotics during 2w before visit			
No	98 (34.75%)	REF	0.390
Yes	13 (28.26%)	0.74 (0.34-1.53)	
Vulvar state			
Normal	108 (33.44%)	REF	0.180
Erythema	0 (0.00%)	0.00 (0.00-77.91)	
Postule	0 (0.00%)	0.00 (0.00-10.71)	
Leucorrhoea	3 (100.00%)	inf (0.81-inf)	
Speculum			
Normal	100 (36.76%)	REF	0.074
Redness	8 (19.51%)	0.42 (0.16-0.97)	
Bleeding ex utero	2 (22.22%)	0.49 (0.05-2.65)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-67.40)	
Ulcerations	1 (20.00%)	0.43 (0.01-4.44)	
***Trichomonas* on wet mount**			
Negative	109 (33.54%)	REF	0.141
Positive	2 (50.00%)	1.98 (0.14-27.62)	
Unknown	1 (100.00%)	inf (0.05-inf)	
***Candida* on wet mount**			
Negative	87 (36.55%)	REF	0.158
Positive	24 (26.37%)	0.62 (0.35-1.09)	
Unknown	1 (100.00%)	inf (0.04-inf)	
Nitrite urine dipstick			
Negative	107 (33.65%)	REF	0.566
Positive	5 (41.67%)	1.41 (0.34-5.29)	
BV state visit 1			
Negative	30 (17.05%)	REF	**<0.001**
Intermediate	57 (62.64%)	8.08 (4.40-15.17)	
Positive	22 (37.29%)	2.88 (1.41-5.85)	
Nugent score visit 1			**<0.001**
Vaginal pH			0.366
White blood cells on wet mount			0.835
Clue cells on wet mount			0.116
Epithelial cells on wet mount			0.657
White blood cells urine dipstick			0.292
Glycated keratin			0.546
Length cervix			0.132

	GV+ n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	66 (35.11%)	REF	0.095
Yes	20 (24.69%)	0.61 (0.31-1.12)	
Evolution of the baby			
Well	14 (21.54%)	REF	0.157
Dead	6 (35.29%)	1.97 (0.51-7.14)	
Handicap	1 (50.00%)	3.55 (0.04-290.67)	
Preterm birth			
No	65 (37.79%)	REF	0.245
Yes	8 (26.67%)	0.60 (0.21-1.50)	
Birthweight			0.394

11.7 Addendum 7: Univariate associations between *Atopobium vaginae* and clinical signs and symptoms

Addendum 7. Univariate associations between Atopobium vaginae and clinical signs and symptoms of mother and baby.
AV, Atopobium vaginae; n, number of study participants; OR, odds ratio; CI, confidence interval

	AV+ n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	58 (34.73%)	REF	0.573
Yes	60 (37.74%)	1.14 (0.71-1.84)	
Vaginal itching			
No	67 (34.90%)	REF	0.536
Yes	52 (38.24%)	1.15 (0.71-1.87)	
Dysuria			
No	85 (35.71%)	REF	0.805
Yes	32 (37.21%)	1.07 (0.62-1.83)	
Burning sensation after sex			
No	74 (35.41%)	REF	0.488
Yes	41 (39.42%)	1.19 (0.71-1.98)	
Last episode of burning			
1 day	8 (50.00%)	REF	0.860
2 days	9 (52.94%)	1.12 (0.23-5.42)	
2-7 days	3 (13.64%)	0.17 (0.02-0.92)	
7-14 days	1 (10.00%)	0.12 (0.00-1.24)	
>14 days	12 (57.14%)	1.32 (0.30-5.96)	
Sensation of vaginal smell			
No	75 (34.09%)	REF	0.241
Yes	32 (41.56%)	1.37 (0.78-2.42)	
Last episode of vaginal smell			
1 day	11 (44.00%)	REF	0.438
2 days	3 (60.00%)	1.87 (0.18-26.10)	
7-14 days	6 (50.00%)	1.26 (0.26-6.28)	
>14 days	3 (50.00%)	1.26 (0.14-11.4)	
Unknown	10 (34.48%)	0.67 (0.19-2.31)	
Whiff test			
Negative	98 (33.00%)	REF	**<0.001**
Positive	20 (64.52%)	3.68 (1.61-8.85)	
State vaginal secretions			
Fine and homogenous	105 (35.35%)	REF	0.238
Thick	5 (31.25%)	0.83 (0.22-2.68)	
Thick and heterogenous	9 (52.94%)	2.05 (0.68-6.31)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	107 (35.08%)	REF	0.294
8,3-11,9 (anemia)	11 (45.83%)	1.56 (0.61-3.93)	

	AV+ n	OR (CI 0.95)	p-value
Fever			
No	102 (35.54%)	REF	0.784
Yes	14 (37.84%)	1.10 (0.50-2.35)	
Uterine contractions			
No	96 (38.25%)	REF	0.058
Yes	9 (22.50%)	0.47 (0.19-1.07)	
Antibiotics during 2w before visit			
No	107 (37.94%)	REF	0.070
Yes	11 (23.91%)	0.51 (0.23-1.09)	
Vulvar state			
Normal	115 (35.60%)	REF	0.434
Erythema	1 (100.00%)	inf (0.05-inf)	
Postule	0 (0.00%)	0.00 (0.00-9.73)	
Leucorrhoea	2 (66.67%)	3.60 (0.19-214.13)	
Speculum			
Normal	103 (37.87%)	REF	0.419
Redness	11 (26.83%)	0.60 (0.26-1.30)	
Bleeding ex utero	1 (11.11%)	0.21 (0.00-1.57)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-64.31)	
Ulcerations	3 (60.00%)	2.45 (0.28-29.82)	
***Trichomonas* on wet mount**			
Negative	116 (35.69%)	REF	0.174
Positive	2 (50.00%)	1.80 (0.13-25.11)	
Unknown	1 (100.00%)	inf (0.05-inf)	
***Candida* on wet mount**			
Negative	84 (35.29%)	REF	0.538
Positive	34 (37.36%)	1.09 (0.64-1.85)	
Unknown	1 (100.00%)	inf (0.05-inf)	
Nitrite urine dipstick			
Negative	113 (35.53%)	REF	0.312
Positive	6 (50.00%)	1.81 (0.47-6.95)	
BV state visit 1			
Negative	31 (17.61%)	REF	**<0.001**
Intermediate	66 (72.53%)	12.19 (6.50-23.61)	
Positive	21 (35.59%)	2.57 (1.26-5.23)	
Nugent score visit 1			**<0.001**
Vaginal pH			**0.016**
White blood cells on wet mount			0.286
Clue cells on wet mount			0.352
Epithelial cells on wet mount			0.288
White blood cells urine dipstick			0.050
Glycated keratin			0.829
Length cervix			0.382

	AV+ n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	71 (37.77%)	REF	0.279
Yes	25 (30.86%)	0.74 (0.40-1.32)	
Evolution of the baby			
Well	20 (30.77%)	REF	0.553
Dead	6 (35.29%)	1.22 (0.32-4.25)	
Handicap	1 (50.00%)	2.22 (0.03-180.20)	
Preterm birth			
No	63 (36.63%)	REF	0.485
Yes	9 (30.00%)	0.74 (0.28-1.82)	
Birthweight			0.281

11.8 Addendum 8: Univariate associations between *Lactobacillus crispatus* and clinical signs and symptoms

Addendum 8. Univariate associations between Lactobacillus crispatus and clinical signs and symptoms of mother and baby.
LC, Lactobacillus crispatus; n, number of study participants; OR, odds ratio; CI, confidence interval

	LC+ n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	72 (43.11%)	REF	0.552
Yes	63 (39.62%)	0.87 (0.54-1.38)	
Vaginal itching			
No	86 (44.79%)	REF	0.113
Yes	49 (36.03%)	0.69 (0.43-1.12)	
Dysuria			
No	100 (42.02%)	REF	0.556
Yes	33 (38.37%)	0.86 (0.50-1.46)	
Burning sensation after sex			
No	92 (44.02%)	REF	0.207
Yes	38 (36.54%)	0.73 (0.44-1.22)	
Last episode of burning			
1 day	7 (43.74%)	REF	0.887
2 days	5 (29.41%)	0.55 (0.10-2.79)	
2-7 days	7 (31.82%)	0.61 (0.13-2.79)	
7-14 days	3 (30.00%)	0.56 (0.07-3.75)	
>14 days	9 (42.86%)	0.97 (0.22-4.37)	
Sensation of vaginal smell			
No	88 (40.00%)	REF	0.718
Yes	29 (37.66%)	0.91 (0.51-1.59)	
Last episode of vaginal smell			
1 day	7 (28.00%)	REF	0.612
2 days	3 (60.00%)	3.66 (0.34-52.89)	
7-14 days	5 (41.67%)	1.81 (0.33-9.61)	
>14 days	2 (33.33%)	1.27 (0.10-11.53)	
Unknown	11 (37.93%)	1.56 (0.43-5.92)	
Whiff test			
Negative	127 (42.76%)	REF	0.073
Positive	8 (25.81%)	0.47 (0.17-1.12)	
State vaginal secretions			
Fine and homogenous	128 (43.10%)	REF	0.078
Thick	3 (18.75%)	0.31 (0.05-1.14)	
Thick and heterogenous	5 (29.41%)	0.55 (0.15-1.73)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	123 (40.33%)	REF	0.190
8,3-11,9 (anemia)	13 (54.17%)	1.75 (0.70-4.46)	

	LC+ n	OR (CI 0.95)	p-value
Fever			
No	121 (42.16%)	REF	0.415
Yes	13 (35.14%)	0.74 (0.33-1.59)	
Uterine contractions			
No	104 (41.43%)	REF	0.671
Yes	18 (45.00%)	1.16 (0.55-2.39)	
Antibiotics during 2w before visit			
No	118 (41.84%)	REF	0.533
Yes	17 (36.96%)	0.82 (0.40-1.62)	
Vulvar state			
Normal	133 (41.18%)	REF	0.453
Erythema	1 (100.00%)	inf (0.04-inf)	
Postule	1 (50.00%)	1.43 (0.02-112.62)	
Leucorrhoea	0 (0.00%)	0.00 (0.00-3.51)	
Speculum			
Normal	117 (43.01%)	REF	0.064
Redness	15 (36.59%)	0.76 (0.36-1.58)	
Bleeding ex utero	2 (22.22%)	0.38 (0.04-2.04)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-51.96)	
Ulcerations	1 (20.00%)	0.33 (0.01-3.42)	
***Trichomonas* on wet mount**			
Negative	134 (41.23%)	REF	0.704
Positive	1 (25.00%)	0.48 (0.01-6.00)	
Unknown	1 (100.00%)	inf (0.04-inf)	
***Candida* on wet mount**			
Negative	102 (42.86%)	REF	0.417
Positive	33 (36.26%)	0.76 (0.44-1.28)	
Unknown	1 (100.00%)	inf (0.03-inf)	
Nitrite urine dipstick			
Negative	131 (41.19%)	REF	0.974
Positive	5 (41.67%)	1.01 (0.25-3.82)	
BV state visit 1			
Negative	90 (51.14%)	REF	**0.004**
Intermediate	23 (25.27%)	0.32 (0.18-0.58)	
Positive	22 (37.29%)	0.57 (0.29-1.08)	
Nugent score visit 1			**<0.001**
Vaginal pH			0.196
White blood cells on wet mount			0.256
Clue cells on wet mount			**0.009**
Epithelial cells on wet mount			0.844
White blood cells urine dipstick			0.367
Glycated keratin			0.122
Length cervix			0.304

	LC+ n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	75 (39.89%)	REF	0.378
Yes	37 (45.68%)	1.27 (0.72-2.21)	
Evolution of the baby			
Well	31 (47.69%)	REF	0.657
Dead	9 (52.94%)	1.23 (0.37-4.18)	
Handicap	0 (0.00%)	0.00 (0.00-6.17)	
Preterm birth			
No	72 (41.86%)	REF	0.594
Yes	11 (36.67%)	0.80 (0.32-1.91)	
Birthweight			0.117

11.9 Addendum 9: Univariate associations between *Lactobacillus iners* and clinical signs and symptoms

Addendum 9. Univariate associations between Lactobacillus iners and clinical signs and symptoms of mother and baby.
LI, Lactobacillus iners; n, number of study participants; OR, odds ratio; CI, confidence interval

	LI+ n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	132 (80.00%)	REF	0.193
Yes	116 (73.89%)	0.71 (0.40-1.23)	
Vaginal itching			
No	145 (76.72%)	REF	0.947
Yes	104 (77.04%)	1.02 (0.58-1.79)	
Dysuria			
No	180 (76.92%)	REF	0.853
Yes	67 (77.91%)	1.06 (0.57-2.03)	
Burning sensation after sex			
No	158 (76.70%)	REF	0.850
Yes	78 (75.73%)	0.95 (0.53-1.73)	
Last episode of burning			
1 day	11 (68.75%)	REF	0.383
2 days	11 (68.75%)	1.00 (0.17-5.78)	
2-7 days	18 (81.82%)	2.01 (0.35-12.52)	
7-14 days	9 (90.00%)	3.90 (0.34-214.32)	
>14 days	16 (76.19%)	1.44 (0.26-8.00)	
Sensation of vaginal smell			
No	168 (77.06%)	REF	0.323
Yes	55 (71.43%)	0.74 (0.40-1.41)	
Last episode of vaginal smell			
1 day	21 (84.00%)	REF	**0.026**
2 days	4 (80.00%)	0.77 (0.05-46.79)	
7-14 days	8 (66.67%)	0.39 (0.06-2.65)	
>14 days	5 (83.33%)	0.95 (0.07-56.15)	
Unknown	16 (55.17%)	0.24 (0.05-0.97)	
Whiff test			
Negative	223 (76.11%)	REF	0.334
Positive	26 (83.87%)	1.63 (0.59-5.64)	
State vaginal secretions			
Fine and homogenous	222 (75.77%)	REF	0.077
Thick	13 (81.25%)	1.38 (0.37-7.79)	
Thick and heterogenous	16 (94.12%)	5.10 (0.77-217.38)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	232 (77.08%)	REF	0.499
8,3-11,9 (anemia)	18 (75.00%)	0.89 (0.32-2.86)	

	LI+ n	OR (CI 0.95)	p-value
Fever			
No	213 (75.27%)	REF	**0.033**
Yes	34 (91.89%)	3.71 (1.11-19.47)	
Uterine contractions			
No	188 (76.11%)	REF	0.376
Yes	33 (82.50%)	1.48 (0.60-4.17)	
Antibiotics during 2w before visit			
No	218 (78.14%)	REF	0.175
Yes	31 (68.89%)	0.62 (0.30-1.35)	
Vulvar state			
Normal	244 (76.49%)	REF	0.186
Erythema	1 (100.00%)	inf (0.01-inf)	
Postule	2 (100.00%)	inf (0.06-inf)	
Leucorrhoea	3 (100.00%)	inf (0.12-inf)	
Speculum			
Normal	205 (76.49%)	REF	0.993
Redness	33 (80.49%)	1.27 (0.54-3.34)	
Bleeding ex utero	6 (66.67%)	0.62 (0.13-3.91)	
Yellowish plaques	1 (100.00%)	inf (0.01-inf)	
Ulcerations	4 (80.00%)	1.23 (0.12-61.47)	
***Trichomonas* on wet mount**			
Negative	246 (76.64%)	REF	0.244
Positive	4 (100.00%)	inf (0.02-inf)	
Unknown	1 (100.00%)	inf (0.01-inf)	
***Candida* on wet mount**			
Negative	173 (73.93%)	REF	**0.034**
Positive	77 (84.62%)	1.94 (1.00-3.98)	
Unknown	1 (100.00%)	inf (0.01-inf)	
Nitrite urine dipstick			
Negative	240 (76.43%)	REF	0.246
Positive	11 (91.67%)	3.38 (0.48-147.85)	
BV state visit 1			
Negative	141 (81.03%)	REF	**0.033**
Intermediate	67 (75.28%)	0.71 (0.37-1.39)	
Positive	40 (67.80%)	0.49 (0.24-1.02)	
Nugent score visit 1			0.209
Vaginal pH			0.120
White blood cells on wet mount			0.266
Clue cells on wet mount			0.662
Epithelial cells on wet mount			0.188
White blood cells urine dipstick			0.128
Glycated keratin			0.089
Length cervix			0.424

	LI+ n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	145 (77.96%)	REF	0.925
Yes	62 (78.48%)	1.03 (0.53-2.09)	
Evolution of the baby			
Well	48 (76.19%)	REF	0.386
Dead	14 (82.35%)	1.45 (0.34-8.93)	
Handicap	2 (100.00%)	inf (0.06-inf)	
Preterm birth			
No	120 (70.59%)	REF	**0.036**
Yes	27 (90.00%)	3.73 (1.07-20.08)	
Birthweight			0.233

11.10 Addendum 10: Cluster analysis of associations between the different bacterial species and clinical signs and symptoms – cluster 1

Addendum 10. Cluster analysis of associations between the different bacterial species and clinical signs and symptoms for cluster 1.
n, number of study participants; OR, odds ratio; CI, confidence interval

	Cluster 1 n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	8 (5.03%)	REF	**0.044**
Yes	17 (11.49%)	2.44 (0.96-6.76)	
Vaginal itching			
No	15 (8.29%)	REF	0.896
Yes	10 (7.87%)	0.95 (0.37-2.34)	
Dysuria			
No	16 (7.24%)	REF	0.491
Yes	8 (9.64%)	1.37 (0.48-3.55)	
Burning sensation after sex			
No	17 (8.72%)	REF	0.627
Yes	7 (7.07%)	0.80 (0.27-2.11)	
Last episode of burning			
1 day	2 (12.50%)	REF	0.225
2 days	2 (12.50%)	1.00 (0.06-15.62)	
2-7 days	1 (4.76%)	0.36 (0.01-7.55)	
7-14 days	0 (0.00%)	0.00 (0.00-8.57)	
>14 days	1 (5.00%)	0.38 (0.01-7.96)	
Sensation of vaginal smell			
No	19 (9.18%)	REF	0.581
Yes	5 (7.04%)	0.75 (0.21-2.19)	
Last episode of vaginal smell			
1 day	0 (0.00%)	REF	0.155
2 days	1 (20.00%)	inf (0.12-inf)	
7-14 days	1 (9.09%)	inf (0.06-inf)	
>14 days	1 (16.67%)	inf (0.10-inf)	
Unknown	3 (12.00%)	inf (0.41-inf)	
Whiff test			
Negative	23 (8.24%)	REF	0.801
Positive	2 (6.90%)	0.82 (0.09-3.66)	
State vaginal secretions			
Fine and homogenous	25 (8.90%)	REF	0.113
Thick	0 (0.00%)	0.00 (0.00-3.60)	
Thick and heterogenous	0 (0.00%)	0.00 (0.00-2.85)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	24 (8.42%)	REF	0.473
8,3-11,9 (anemia)	1 (4.17%)	0.47 (0.01-3.19)	

	Cluster 1 n	OR (CI 0.95)	p-value
Fever			
No	24 (8.92%)	REF	0.985
Yes	0 (0.00%)	0.00 (0.00-1.23)	
Uterine contractions			
No	20 (8.55%)	REF	0.986
Yes	0 (0.00%)	0.00 (0.00-1.20)	
Antibiotics during 2w before visit			
No	21 (7.95%)	REF	0.798
Yes	4 (9.09%)	1.16 (0.27-3.68)	
Vulvar state			
Normal	25 (8.25%)	REF	0.474
Erythema	0 (0.00%)	0.00 (0.00-432.31)	
Postule	0 (0.00%)	0.00 (0.00-60.52)	
Leucorrhoea	0 (0.00%)	0.00 (0.00-27.87)	
Speculum			
Normal	23 (8.91%)	REF	0.230
Redness	2 (5.13%)	0.55 (0.06-2.40)	
Bleeding ex utero	0 (0.00%)	0.00 (0.00-9.22)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-397.69)	
Ulcerations	0 (0.00%)	0.00 (0.00-16.25)	
***Trichomonas* on wet mount**			
Negative	25 (8.2%)	REF	0.528
Positive	0 (0.00%)	0.00 (0.00-17.73)	
Unknown	0 (0.00%)	0.00 (0.00-435.38)	
***Candida* on wet mount**			
Negative	21 (9.46%)	REF	0.150
Positive	4 (4.60%)	0.46 (0.11-1.43)	
Unknown	0 (0.00%)	0.00 (0.00-372.96)	
Nitrite urine dipstick			
Negative	25 (8.36%)	REF	0.991
Positive	0 (0.00%)	0.00 (0.00-4.67)	
BV state visit 1			
Negative	6 (3.61%)	REF	**0.003**
Intermediate	11 (12.79%)	3.89 (1.26-13.31)	
Positive	8 (14.55%)	4.50 (1.30-16.58)	
Nugent score visit 1			**0.004**
Vaginal pH			0.624
White blood cells on wet mount			0.410
Clue cells on wet mount			0.687
Epithelial cells on wet mount			0.754
White blood cells urine dipstick			0.685
Glycated keratin			0.933
Length cervix			0.360

	Cluster 1 n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	14 (8.05%)	REF	0.650
Yes	5 (6.41%)	0.78 (0.12-2.41)	
Evolution of the baby			
Well	4 (6.45%)	REF	0.284
Dead	0 (0.00%)	0.00 (0.00-5.64)	
Handicap	0 (0.00%)	0.00 (0.00-88.91)	
Preterm birth			
No	15 (9.49%)	REF	0.988
Yes	0 (0.00%)	0.00 (0.00-1.48)	
Birthweight			0.774

11.11 Addendum 11: Cluster analysis of associations between the different bacterial species and clinical signs and symptoms – cluster 2

Addendum 11. Cluster analysis of associations between the different bacterial species and clinical signs and symptoms for cluster 2.
n, number of study participants; OR, odds ratio; CI, confidence interval

	Cluster 2 n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	24 (15.09%)	REF	0.693
Yes	20 (13.51%)	0.88 (0.44-1.75)	
Vaginal itching			
No	23 (12.71%)	REF	0.261
Yes	22 (17.32%)	1.44 (0.72-2.85)	
Dysuria			
No	36 (16.29%)	REF	0.147
Yes	8 (9.64%)	0.55 (0.21-1.27)	
Burning sensation after sex			
No	29 (14.87%)	REF	0.687
Yes	13 (13.13%)	0.87 (0.39-1.82)	
Last episode of burning			
1 day	3 (18.75%)	REF	0.595
2 days	1 (6.25%)	0.30 (0.01-4.27)	
2-7 days	4 (19.05%)	1.02 (0.14-8.21)	
7-14 days	1 (10.00%)	0.49 (0.01-7.37)	
>14 days	2 (10.00%)	0.49 (0.04-4.94)	
Sensation of vaginal smell			
No	27 (13.04%)	REF	0.278
Yes	13 (18.31%)	1.49 (0.66-3.23)	
Last episode of vaginal smell			
1 day	4 (16.67%)	REF	0.542
2 days	0 (0.00%)	0.00 (0.00-8.12)	
7-14 days	2 (18.18%)	1.11 (0.09-9.51)	
>14 days	2 (33.33%)	2.41 (0.17-25.64)	
Unknown	5 (20.00%)	1.24 (0.23-7.25)	
Whiff test			
Negative	38 (13.62%)	REF	0.305
Positive	6 (20.69%)	1.65 (0.52-4.54)	
State vaginal secretions			
Fine and homogenous	38 (13.52%)	REF	0.065
Thick	2 (15.38%)	1.16 (0.12-5.64)	
Thick and heterogenous	5 (31.25%)	2.89 (0.75-9.66)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	41 (14.39%)	REF	0.761
8,3-11,9 (anemia)	4 (16.67%)	1.19 (0.28-3.81)	

	Cluster 2 n	OR (CI 0.95)	p-value
Fever			
No	37 (13.75%)	REF	0.159
Yes	8 (22.86%)	1.85 (0.68-4.61)	
Uterine contractions			
No	31 (13.25%)	REF	0.396
Yes	7 (18.42%)	1.48 (0.50-3.82)	
Antibiotics during 2w before visit			
No	40 (15.15%)	REF	0.512
Yes	5 (11.36%)	0.72 (0.21-1.98)	
Vulvar state			
Normal	43 (14.19%)	REF	0.081
Erythema	0 (0.00%)	0.00 (0.00-235.85)	
Postule	0 (0.00%)	0.00 (0.00-32.69)	
Leucorrhoea	2 (66.67%)	11.93 (0.61-712.41)	
Speculum			
Normal	39 (15.12%)	REF	0.163
Redness	5 (12.82%)	0.83 (0.24-2.31)	
Bleeding ex utero	0 (0.00%)	0.00 (0.00-4.96)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-219.25)	
Ulcerations	0 (0.00%)	0.00 (0.00-8.78)	
***Trichomonas* on wet mount**			
Negative	45 (14.75%)	REF	0.380
Positive	0 (0.00%)	0.00 (0.00-9.00)	
Unknown	0 (0.00%)	0.00 (0.00-225.41)	
***Candida* on wet mount**			
Negative	26 (11.71%)	REF	**0.033**
Positive	19 (21.84%)	2.10 (1.03-4.23)	
Unknown	0 (0.00%)	0.00 (0.00-294.15)	
Nitrite urine dipstick			
Negative	42 (14.05%)	REF	0.234
Positive	3 (27.27%)	2.29 (0.38-10.02)	
BV state visit 1			
Negative	12 (7.23%)	REF	**0.059**
Intermediate	27 (31.40%)	5.83 (2.65-13.50)	
Positive	5 (9.09%)	1.28 (0.34-4.15)	
Nugent score visit 1			**<0.001**
Vaginal pH			**0.019**
White blood cells on wet mount			0.166
Clue cells on wet mount			0.397
Epithelial cells on wet mount			0.404
White blood cells urine dipstick			0.483
Glycated keratin			0.838
Length cervix			0.663

	Cluster 2 n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	25 (14.37%)	REF	0.743
Yes	10 (12.82%)	0.88 (0.36-2.02)	
Evolution of the baby			
Well	6 (9.68%)	REF	0.100
Dead	3 (17.65%)	1.98 (0.29-10.75)	
Handicap	1 (50.00%)	8.72 (0.10-739.69)	
Preterm birth			
No	30 (18.99%)	REF	0.507
Yes	4 (13.79%)	0.68 (0.16-2.20)	
Birthweight			**0.016**

11.12 Addendum 12: Cluster analysis of associations between the different bacterial species and clinical signs and symptoms – cluster 3

Addendum 12. Cluster analysis of associations between the different bacterial species and clinical signs and symptoms for cluster 3.
n, number of study participants; OR, odds ratio; CI, confidence interval

	Cluster 3 n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	22 (13.84%)	REF	0.934
Yes	20 (13.51%)	0.97 (0.48-1.97)	
Vaginal itching			
No	25 (13.81%)	REF	0.915
Yes	17 (13.39%)	0.96 (0.46-1.96)	
Dysuria			
No	32 (14.48%)	REF	0.269
Yes	8 (9.64%)	0.63 (0.24-1.48)	
Burning sensation after sex			
No	29 (14.87%)	REF	0.376
Yes	11 (11.11%)	0.72 (0.31-1.56)	
Last episode of burning			
1 day	2 (12.50%)	REF	0.521
2 days	2 (12.50%)	1.00 (0.06-15.62)	
2-7 days	1 (4.76%)	0.36 (0.01-7.55)	
7-14 days	1 (10.00%)	0.79 (0.01-17.25)	
>14 days	4 (20.00%)	1.72 (0.21-21.81)	
Sensation of vaginal smell			
No	29 (14.01%)	REF	0.778
Yes	9 (12.68%)	0.89 (0.35-2.07)	
Last episode of vaginal smell			
1 day	4 (16.67%)	REF	0.127
2 days	2 (40.00%)	3.17 (0.20-39.21)	
7-14 days	1 (9.09%)	0.51 (0.01-6.11)	
>14 days	1 (16.67%)	1.00 (0.02-13.74)	
Unknown	1 (4.00%)	0.21 (0.00-2.40)	
Whiff test			
Negative	34 (12.19%)	REF	**0.026**
Positive	8 (27.59%)	2.73 (0.97-7.07)	
State vaginal secretions			
Fine and homogenous	37 (13.17%)	REF	0.754
Thick	3 (23.08%)	1.97 (0.33-8.15)	
Thick and heterogenous	2 (12.50%)	0.94 (0.10-4.37)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	37 (12.98%)	REF	0.287
8,3-11,9 (anemia)	5 (20.83%)	1.76 (0.48-5.27)	

	Cluster 3 n	OR (CI 0.95)	p-value
Fever			
No	34 (12.64%)	REF	0.235
Yes	7 (20.00%)	1.72 (0.59-4.46)	
Uterine contractions			
No	35 (14.96%)	REF	0.472
Yes	4 (10.53%)	0.67 (0.16-2.05)	
Antibiotics during 2w before visit			
No	36 (13.64%)	REF	0.682
Yes	5 (11.36%)	0.81 (0.23-2.26)	
Vulvar state			
Normal	40 (13.20%)	REF	0.582
Erythema	0 (0.00%)	0.00 (0.00-256.37)	
Postule	0 (0.00%)	0.00 (0.00-35.57)	
Leucorrhoea	1 (33.33%)	3.27 (0.05-64.21)	
Speculum			
Normal	34 (13.18%)	REF	0.520
Redness	4 (10.26%)	0.75 (0.18-2.31)	
Bleeding ex utero	2 (33.33%)	3.27 (0.29-23.85)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-257.06)	
Ulcerations	1 (25.00%)	2.19 (0.04-28.15)	
***Trichomonas* on wet mount**			
Negative	39 (12.79%)	REF	**<0.001**
Positive	2 (50.00%)	6.75 (0.48-95.66)	
Unknown	1 (100.00%)	inf (0.17-inf)	
***Candida* on wet mount**			
Negative	34 (15.32%)	REF	0.271
Positive	7 (8.05%)	0.48 (0.17-1.17)	
Unknown	1 (100.00%)	inf (0.14-inf)	
Nitrite urine dipstick			
Negative	39 (13.04%)	REF	0.190
Positive	3 (27.27%)	2.49 (0.41-10.95)	
BV state visit 1			
Negative	12 (7.23%)	REF	**0.020**
Intermediate	21 (24.42%)	4.12 (1.18-9.77)	
Positive	8 (14.55%)	2.18 (0.73-6.20)	
Nugent score visit 1			**<0.001**
Vaginal pH			0.457
White blood cells on wet mount			0.782
Clue cells on wet mount			0.463
Epithelial cells on wet mount			0.592
White blood cells urine dipstick			0.578
Glycated keratin			0.597
Length cervix			0.069

	Cluster 3 n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	29 (16.67%)	REF	0.063
Yes	6 (7.69%)	0.42 (0.14-1.09)	
Evolution of the baby			
Well	5 (8.06%)	REF	0.883
Dead	2 (11.76%)	1.51 (0.13-10.42)	
Handicap	0 (0.00%)	0.00 (0.00-68.21)	
Preterm birth			
No	20 (12.66%)	REF	0.728
Yes	3 (10.34%)	0.80 (0.14-2.99)	
Birthweight			0.070

11.13 Addendum 13: Cluster analysis of associations between the different bacterial species and clinical signs and symptoms – cluster 4

Addendum 13. Cluster analysis of associations between the different bacterial species and clinical signs and symptoms for cluster 4.
n, number of study participants; OR, odds ratio; CI, confidence interval

	Cluster 4 n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	13 (8.18%)	REF	0.250
Yes	18 (12.16%)	1.55 (0.69-3.59)	
Vaginal itching			
No	20 (11.05%)	REF	0.651
Yes	12 (9.45%)	0.84 (0.36-1.89)	
Dysuria			
No	24 (10.86%)	REF	0.758
Yes	8 (9.64%)	0.88 (0.33-2.13)	
Burning sensation after sex			
No	14 (7.18%)	REF	**0.019**
Yes	16 (16.16%)	2.48 (1.08-5.78)	
Last episode of burning			
1 day	1 (6.25%)	REF	0.785
2 days	4 (25.00%)	4.77 (0.40-261.89)	
2-7 days	5 (23.81%)	4.52 (0.43-235.89)	
7-14 days	1 (10.00%)	1.63 (0.02-139.25)	
>14 days	2 (10.00%)	1.64 (0.08-104.72)	
Sensation of vaginal smell			
No	19 (9.18%)	REF	0.143
Yes	11 (15.49%)	1.81 (0.73-4.27)	
Last episode of vaginal smell			
1 day	5 (20.83%)	REF	0.930
2 days	0 (0.00%)	0.00 (0.00-5.78)	
7-14 days	1 (9.09%)	0.39 (0.01-4.23)	
>14 days	0 (0.00%)	0.00 (0.00-4.61)	
Unknown	5 (20.00%)	0.95 (0.19-4.87)	
Whiff test			
Negative	26 (9.32%)	REF	0.064
Positive	6 (20.69%)	2.53 (0.77-7.18)	
State vaginal secretions			
Fine and homogenous	28 (9.96%)	REF	0.890
Thick	3 (23.08%)	2.70 (0.45-11.33)	
Thick and heterogenous	1 (6.25%)	0.60 (0.01-4.22)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	31 (10.88%)	REF	0.489
8,3-11,9 (anemia)	1 (4.17%)	0.36 (0.01-2.36)	

	Cluster 4 n	OR (CI 0.95)	p-value
Fever			
No	26 (9.67%)	REF	0.182
Yes	6 (17.14%)	1.93 (0.60-5.34)	
Uterine contractions			
No	24 (10.26%)	REF	0.959
Yes	4 (10.53%)	1.03 (0.24-3.27)	
Antibiotics during 2w before visit			
No	28 (10.61%)	REF	0.761
Yes	4 (9.09%)	0.84 (0.20-2.60)	
Vulvar state			
Normal	32 (10.56%)	REF	0.412
Erythema	0 (0.00%)	0.00 (0.00-329.80)	
Postule	0 (0.00%)	0.00 (0.00-45.92)	
Leucorrhoea	0 (0.00%)	0.00 (0.00-21.09)	
Speculum			
Normal	30 (11.63%)	REF	0.137
Redness	2 (5.13%)	0.41 (0.05-1.74)	
Bleeding ex utero	0 (0.00%)	0.00 (0.00-6.77)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-296.33)	
Ulcerations	0 (0.00%)	0.00 (0.00-11.97)	
***Trichomonas* on wet mount**			
Negative	32 (10.49%)	REF	0.470
Positive	0 (0.00%)	0.00 (0.00-13.39)	
Unknown	0 (0.00%)	0.00 (0.00-332.21)	
***Candida* on wet mount**			
Negative	20 (9.01%)	REF	0.255
Positive	12 (13.79%)	1.61 (0.68-3.66)	
Unknown	0 (0.00%)	0.00 (0.00-393.42)	
Nitrite urine dipstick			
Negative	32 (10.70%)	REF	0.612
Positive	0 (0.00%)	0.00 (0.00-3.51)	
BV state visit 1			
Negative	7 (4.22%)	REF	**0.002**
Intermediate	17 (19.77%)	5.55 (2.08-16.59)	
Positive	8 (14.55%)	3.84 (1.15-13.15)	
Nugent score visit 1			**<0.001**
Vaginal pH			0.666
White blood cells on wet mount			0.959
Clue cells on wet mount			0.837
Epithelial cells on wet mount			0.251
White blood cells urine dipstick			0.844
Glycated keratin			0.397
Length cervix			0.639

	Cluster 4 n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	19 (10.92%)	REF	0.431
Yes	6 (7.69%)	0.68 (0.21-1.87)	
Evolution of the baby			
Well	5 (8.06%)	REF	0.637
Dead	1 (5.88%)	0.72 (0.01-7.09)	
Handicap	0 (0.00%)	0.00 (0.00-68.21)	
Preterm birth			
No	20 (12.66%)	REF	0.728
Yes	3 (10.34%)	0.80 (0.14-2.99)	
Birthweight			0.507

11.14 Addendum 14: Cluster analysis of associations between the different bacterial species and clinical signs and symptoms – cluster 5/cluster C

Addendum 14. Cluster analysis of associations between the different bacterial species and clinical signs and symptoms for cluster 5/cluster C.
**, Cluster 5 and cluster C are identical; n, number of study participants; OR, odds ratio; CI, confidence interval*

	Cluster 5/C n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	47 (29.56%)	REF	0.532
Yes	39 (26.35%)	0.85 (0.50-1.49)	
Vaginal itching			
No	58 (32.04%)	REF	0.071
Yes	28 (22.05%)	0.60 (0.34-1.04)	
Dysuria			
No	63 (28.51%)	REF	1.000
Yes	23 (27.71%)	0.96 (0.52-1.74)	
Burning sensation after sex			
No	59 (30.26%)	REF	0.337
Yes	24 (24.24%)	0.74 (0.40-1.32)	
Last episode of burning			
1 day	4 (25.00%)	REF	0.685
2 days	3 (18.75%)	0.70 (0.08-5.12)	
2-7 days	5 (23.81%)	0.94 (0.16-5.83)	
7-14 days	2 (20.00%)	0.76 (0.06-6.89)	
>14 days	6 (30.00%)	1.28 (0.23-7.71)	
Sensation of vaginal smell			
No	56 (27.05%)	REF	0.877
Yes	18 (25.35%)	0.92 (0.46-1.75)	
Last episode of vaginal smell			
1 day	5 (20.83%)	REF	0.492
2 days	1 (20.00%)	0.95 (0.02-13.11)	
7-14 days	3 (27.27%)	1.41 (0.18-9.51)	
>14 days	0 (0.00%)	0.00 (0.00-4.61)	
Unknown	8 (32.00%)	1.77 (0.41-8.30)	
Whiff test			
Negative	85 (30.47%)	REF	**0.017**
Positive	2 (6.90%)	0.17 (0.02-0.70)	
State vaginal secretions			
Fine and homogenous	84 (29.89%)	REF	**0.043**
Thick	1 (7.69%)	0.20 (0.00-1.37)	
Thick and heterogenous	2 (12.50%)	0.34 (0.04-1.51)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	77 (27.02%)	REF	0.155
8,3-11,9 (anemia)	10 (41.67%)	1.93 (0.73-4.89)	

	Cluster 5/C n	OR (CI 0.95)	p-value
Fever			
No	81 (30.11%)	REF	0.070
Yes	5 (14.29%)	0.38 (0.11-1.06)	
Uterine contractions			
No	65 (27.78%)	REF	0.698
Yes	12 (31.58%)	1.20 (0.52-2.64)	
Antibiotics during 2w before visit			
No	75 (28.41%)	REF	1.000
Yes	12 (27.27%)	0.85 (0.42-2.01)	
Vulvar state			
Normal	85 (28.05%)	REF	0.896
Erythema	1 (100.00%)	inf (0.06-inf)	
Postule	1 (50.00%)	2.56 (0.03-201.90)	
Leucorrhoea	0 (0.00%)	0.00 (0.00-6.31)	
Speculum			
Normal	73 (28.29%)	REF	0.896
Redness	11 (28.21%)	1.00 (0.42-2.19)	
Bleeding ex utero	2 (33.33%)	1.27 (0.11-9.05)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-99.22)	
Ulcerations	1 (25.00%)	0.85 (0.02-10.72)	
***Trichomonas* on wet mount**			
Negative	87 (28.52%)	REF	0.183
Positive	0 (0.00%)	0.00 (0.00-3.87)	
Unknown	0 (0.00%)	0.00 (0.00-98.02)	
***Candida* on wet mount**			
Negative	67 (30.18%)	REF	0.172
Positive	20 (22.99%)	0.69 (0.37-1.26)	
Unknown	0 (0.00%)	0.00 (0.00-90.68)	
Nitrite urine dipstick			
Negative	86 (28.76%)	REF	0.302
Positive	1 (9.09%)	0.25 (0.01-1.79)	
BV state visit 1			
Negative	70 (42.17%)	REF	**<0.001**
Intermediate	5 (5.81%)	0.09 (0.03-0.22)	
Positive	11 (20.00%)	0.34 (0.15-0.74)	
Nugent score visit 1			**<0.001**
Vaginal pH			0.089
White blood cells on wet mount			0.129
Clue cells on wet mount			**0.002**
Epithelial cells on wet mount			0.241
White blood cells urine dipstick			0.112
Glycated keratin			**0.023**
Length cervix			0.471

	Cluster 5/C n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	47 (27.01%)	REF	0.180
Yes	28 (35.90%)	1.51 (0.82-2.77)	
Evolution of the baby			
Well	24 (38.71%)	REF	0.635
Dead	7 (41.18%)	1.11 (0.31-3.74)	
Handicap	0 (0.00%)	0.00 (0.00-8.90)	
Preterm birth			
No	42 (26.58%)	REF	0.621
Yes	9 (31.03%)	1.24 (0.46-3.13)	
Birthweight			0.167

11.15 Addendum 15: Cluster analysis of associations between the different bacterial species and clinical signs and symptoms – cluster 6/ cluster D

Addendum 15. Cluster analysis of associations between the different bacterial species and clinical signs and symptoms for cluster 6/cluster D.
**, Cluster 6 and cluster D are identical; n, number of study participants; OR, odds ratio; CI, confidence interval*

	Cluster 6/D n	**OR (CI 0.95)**	**p-value**
MOTHER			
Vaginal discharge			
No	45 (28.30%)	REF	0.299
Yes	34 (22.97%)	0.76 (0.44-1.31)	
Vaginal itching			
No	40 (22.10%)	REF	0.143
Yes	38 (29.92%)	1.50 (0.87-2.61)	
Dysuria			
No	50 (22.62%)	REF	**0.056**
Yes	28 (33.73%)	1.74 (0.96-3.13)	
Burning sensation after sex			
No	47 (24.10%)	REF	0.480
Yes	28 (28.28%)	1.24 (0.69-2.21)	
Last episode of burning			
1 day	4 (25.00%)	REF	0.680
2 days	4 (25.00%)	1.00 (0.15-6.77)	
2-7 days	5 (23.81%)	0.94 (0.16-5.83)	
7-14 days	5 (50.00%)	2.87 (0.42-22.01)	
>14 days	5 (25.00%)	1.00 (0.17-6.24)	
Sensation of vaginal smell			
No	57 (27.54%)	REF	0.347
Yes	15 (21.13%)	0.71 (0.34-1.39)	
Last episode of vaginal smell			
1 day	6 (25.00%)	REF	0.344
2 days	1 (20.00%)	0.76 (0.01-9.98)	
7-14 days	3 (27.27%)	1.12 (0.14-7.07)	
>14 days	2 (33.33%)	1.48 (0.11-13.84)	
Unknown	3 (12.00%)	0.42 (0.06-2.29)	
Whiff test			
Negative	73 (26.16%)	REF	0.373
Positive	5 (17.24%)	0.59 (0.17-1.65)	
State vaginal secretions			
Fine and homogenous	69 (24.56%)	REF	0.217
Thick	4 (30.77%)	1.36 (0.30-5.08)	
Thick and heterogenous	6 (37.50%)	1.84 (0.53-5.83)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	75 (26.32%)	REF	0.219
8,3-11,9 (anemia)	3 (12.50%)	0.40 (0.07-1.40)	

	Cluster 6/D n	OR (CI 0.95)	p-value
Fever			
No	67 (24.91%)	REF	1.000
Yes	9 (25.71%)	1.04 (0.41-2.44)	
Uterine contractions			
No	59 (25.21%)	REF	0.689
Yes	11 (28.95%)	1.21 (0.51-2.70)	
Antibiotics during 2w before visit			
No	64 (24.24%)	REF	0.349
Yes	14 (31.82%)	1.46 (0.67-3.04)	
Vulvar state			
Normal	78 (25.74%)	REF	0.533
Erythema	0 (0.00%)	0.00 (0.00-112.80)	
Postule	1 (50.00%)	2.87 (0.04-227.00)	
Leucorrhoea	0 (0.00%)	0.00 (0.00-7.10)	
Speculum			
Normal	59 (22.87%)	REF	**0.024**
Redness	15 (38.46%)	2.10 (0.96-4.49)	
Bleeding ex utero	2 (33.33%)	1.68 (0.15-12.08)	
Yellowish plaques	1 (100.00%)	inf (0.09-inf)	
Ulcerations	2 (50.00%)	3.35 (0.24-47.18)	
***Trichomonas* on wet mount**			
Negative	77 (25.25%)	REF	0.701
Positive	2 (50.00%)	2.95 (0.21-41.29)	
Unknown	0 (0.00%)	0.00 (0.00-115.78)	
***Candida* on wet mount**			
Negative	54 (24.32%)	REF	0.512
Positive	25 (28.74%)	1.25 (0.69-2.26)	
Unknown	0 (0.00%)	0.00 (0.00-121.83)	
Nitrite urine dipstick			
Negative	75 (25.08%)	REF	0.480
Positive	4 (36.36%)	1.70 (0.36-6.92)	
BV state visit 1			
Negative	59 (35.54%)	REF	**0.009**
Intermediate	5 (5.81%)	0.11 (0.03-0.30)	
Positive	15 (27.27%)	0.68 (0.32-1.39)	
Nugent score visit 1			**<0.001**
Vaginal pH			0.468
White blood cells on wet mount			0.257
Clue cells on wet mount			**0.019**
Epithelial cells on wet mount			0.956
White blood cells urine dipstick			0.605
Glycated keratin			0.151
Length cervix			0.105

	Cluster 6/D n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	40 (22.99%)	REF	0.275
Yes	23 (29.49%)	1.40 (0.73-2.65)	
Evolution of the baby			
Well	18 (29.03%)	REF	0.985
Dead	4 (23.53%)	0.75 (0.16-2.90)	
Handicap	1 (50.00%)	2.41 (0.03-195.83)	
Preterm birth			
No	31 (19.62%)	REF	0.080
Yes	10 (34.48%)	2.15 (0.81-5.45)	
Birthweight			0.051

11.16 Addendum 16: Cluster analysis of associations between the different bacterial species and clinical signs and symptoms – cluster A

Addendum 16. Cluster analysis of associations between the different bacterial species and clinical signs and symptoms for cluster A.
n, number of study participants; OR, odds ratio; CI, confidence interval

	Cluster A n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	32 (20.13%)	REF	0.339
Yes	37 (25.00%)	1.32 (0.75-2.35)	
Vaginal itching			
No	38 (20.99%)	REF	0.409
Yes	32 (25.20%)	1.27 (0.71-2.24)	
Dysuria			
No	52 (23.53%)	REF	0.537
Yes	16 (19.28%)	0.78 (0.39-1.50)	
Burning sensation after sex			
No	46 (23.59%)	REF	0.557
Yes	20 (20.20%)	0.82 (0.43-1.53)	
Last episode of burning			
1 day	5 (31.25%)	REF	0.228
2 days	3 (18.75%)	0.52 (0.07-3.39)	
2-7 days	5 (23.81%)	0.69 (0.12-3.83)	
7-14 days	1 (10.00%)	0.26 (0.00-2.94)	
>14 days	3 (15.00%)	0.40 (0.05-2.54)	
Sensation of vaginal smell			
No	46 (22.22%)	REF	0.625
Yes	18 (25.35%)	1.19 (0.59-2.30)	
Last episode of vaginal smell			
1 day	4 (16.67%)	REF	0.153
2 days	1 (20.00%)	1.24 (0.02-18.37)	
7-14 days	3 (27.27%)	1.84 (0.22-13.85)	
>14 days	3 (50.00%)	4.67 (0.46-50.30)	
Unknown	8 (32.00%)	2.31 (0.51-12.42)	
Whiff test			
Negative	61 (21.86%)	REF	0.486
Positive	8 (27.59%)	1.36 (0.50-3.93)	
State vaginal secretions			
Fine and homogenous	63 (22.42%)	REF	0.602
Thick	2 (15.38%)	0.63 (0.07-3.00)	
Thick and heterogenous	5 (31.25%)	1.57 (0.41-5.13)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	65 (22.81%)	REF	0.825
8,3-11,9 (anemia)	5 (20.83%)	0.89 (0.25-2.60)	

	Cluster A n	OR (CI 0.95)	p-value
Fever			
No	61 (22.68%)	REF	0.981
Yes	8 (22.86%)	1.01 (0.38-2.44)	
Uterine contractions			
No	51 (21.79%)	REF	0.831
Yes	7 (18.42%)	0.81 (0.28-2.02)	
Antibiotics during 2w before visit			
No	61 (23.11%)	REF	0.846
Yes	9 (20.45%)	0.86 (0.34-1.95)	
Vulvar state			
Normal	68 (22.44%)	REF	0.317
Erythema	0 (0.00%)	0.00 (0.00-135.07)	
Postule	0 (0.00%)	0.00 (0.00-18.63)	
Leucorrhoea	2 (66.67%)	6.85 (0.35-408.33)	
Speculum			
Normal	62 (24.03%)	REF	0.050
Redness	7 (17.95%)	0.69 (0.25-1.70)	
Bleeding ex utero	0 (0.00%)	0.00 (0.00-2.77)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-123.68)	
Ulcerations	0 (0.00%)	0.00 (0.00-4.91)	
***Trichomonas* on wet mount**			
Negative	70 (22.95%)	REF	0.250
Positive	0 (0.00%)	0.00 (0.00-5.19)	
Unknown	0 (0.00%)	0.00 (0.00-131.22)	
***Candida* on wet mount**			
Negative	47 (21.17%)	REF	0.392
Positive	23 (26.44%)	1.34 (0.71-2.46)	
Unknown	0 (0.00%)	0.00 (0.00-145.71)	
Nitrite urine dipstick			
Negative	67 (22.41%)	REF	0.716
Positive	3 (27.27%)	1.30 (0.22-5.60)	
BV state visit 1			
Negative	18 (10.84%)	REF	**<0.001**
Intermediate	38 (44.19%)	6.45 (3.25-13.21)	
Positive	13 (23.64%)	2.53 (1.05-5.99)	
Nugent score visit 1			**<0.001**
Vaginal pH			0.099
White blood cells on wet mount			0.521
Clue cells on wet mount			0.653
Epithelial cells on wet mount			0.364
White blood cells urine dipstick			0.392
Glycated keratin			0.906
Length cervix			0.819

	Cluster A n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	39 (22.41%)	REF	0.621
Yes	15 (19.23%)	0.82 (0.39-1.67)	
Evolution of the baby			
Well	10 (16.13%)	REF	0.419
Dead	3 (17.65%)	1.11 (0.17-5.17)	
Handicap	1 (50.00%)	5.01 (0.06-414.20)	0.320
Preterm birth			
No	45 (28.48%)	REF	0.108
Yes	4 (13.79%)	0.40 (0.10-1.26)	
Birthweight			**0.022**

11.17 Addendum 17: Cluster analysis of associations between the different bacterial species and clinical signs and symptoms – cluster B

Addendum 17. Cluster analysis of associations between the different bacterial species and clinical signs and symptoms for cluster B.
n, number of study participants; OR, odds ratio; CI, confidence interval

	Cluster B n	OR (CI 0.95)	p-value
MOTHER			
Vaginal discharge			
No	35 (22.01%)	REF	0.503
Yes	38 (25.68%)	1.22 (0.70-2.15)	
Vaginal itching			
No	45 (24.86%)	REF	0.787
Yes	29 (22.83%)	0.89 (0.50-1.57)	
Dysuria			
No	56 (25.34%)	REF	0.293
Yes	16 (19.28%)	0.70 (0.35-1.35)	
Burning sensation after sex			
No	43 (22.05%)	REF	0.385
Yes	27 (27.27%)	1.32 (0.73-2.39)	
Last episode of burning			
1 day	3 (18.75%)	REF	0.807
2 days	6 (37.50%)	2.52 (0.41-19.57)	
2-7 days	6 (28.57%)	1.17 (0.29-12.71)	
7-14 days	2 (20.00%)	1.08 (0.07-11.76)	
>14 days	6 (30.00%)	1.83 (0.31-13.67)	
Sensation of vaginal smell			
No	48 (23.19%)	REF	0.425
Yes	20 (28.17%)	1.30 (0.67-2.47)	
Last episode of vaginal smell			
1 day	9 (37.50%)	REF	0.231
2 days	2 (40.00%)	1.11 (0.08-11.75)	
7-14 days	2 (18.18%)	0.38 (0.03-2.49)	
>14 days	1 (16.67%)	0.34 (0.01-3.85)	
Unknown	6 (24.00%)	0.53 (0.13-2.12)	
Whiff test			
Negative	60 (21.51%)	REF	**0.002**
Positive	14 (48.28%)	3.39 (1.43-8.01)	
State vaginal secretions			
Fine and homogenous	65 (23.13%)	REF	0.726
Thick	6 (46.15%)	2.84 (0.76-10.25)	
Thick and heterogenous	3 (18.75%)	0.77 (0.14-2.91)	
Hemoglobin on Hemocue			
12,0-17,1 (no anemia)	68 (23.86%)	REF	0.900
8,3-11,9 (anemia)	6 (25.00%)	1.06 (0.33-2.94)	

	Cluster B n	OR (CI 0.95)	p-value
Fever			
No	60 (22.30%)	REF	0.060
Yes	13 (37.14%)	2.05 (0.89-4.56)	
Uterine contractions			
No	59 (25.21%)	REF	0.687
Yes	8 (21.05%)	0.79 (0.30-1.89)	
Antibiotics during 2w before visit			
No	64 (24.24%)	REF	0.703
Yes	9 (20.45%)	0.80 (0.32-1.83)	
Vulvar state			
Normal	72 (23.76%)	REF	0.837
Erythema	0 (0.00%)	0.00 (0.00-125.42)	
Postule	0 (0.00%)	0.00 (0.00-17.29)	
Leucorrhoea	1 (33.33%)	1.60 (0.03-31.18)	
Speculum			
Normal	64 (24.81%)	REF	0.799
Redness	6 (15.38%)	0.55 (0.18-1.42)	
Bleeding ex utero	2 (33.33%)	1.51 (0.13-10.84)	
Yellowish plaques	0 (0.00%)	0.00 (0.00-118.61)	
Ulcerations	1 (25.00%)	1.01 (0.02-12.84)	
***Trichomonas* on wet mount**			
Negative	71 (23.28%)	REF	**0.032**
Positive	2 (50.00%)	3.28 (0.23-46.00)	
Unknown	1 (100.00%)	inf (0.08-inf)	
***Candida* on wet mount**			
Negative	54 (24.32%)	REF	0.944
Positive	19 (21.84%)	0.87 (0.45-1.62)	
Unknown	1 (100.00%)	inf (0.08-inf)	
Nitrite urine dipstick			
Negative	71 (23.75%)	REF	0.728
Positive	3 (27.27%)	1.20 (0.20-5.19)	
BV state visit 1			
Negative	19 (11.45%)	REF	**<0.001**
Intermediate	38 (44.19%)	6.07 (3.09-12.29)	
Positive	16 (29.09%)	3.15 (1.38-7.17)	
Nugent score visit 1			**<0.001**
Vaginal pH			0.364
White blood cells on wet mount			0.853
Clue cells on wet mount			0.659
Epithelial cells on wet mount			0.688
White blood cells urine dipstick			0.760
Glycated keratin			0.302
Length cervix			0.263

	Cluster B n	OR (CI 0.95)	p-value
BABY			
Infection baby first week			
No	48 (27.59%)	REF	**0.038**
Yes	12 (15.38%)	0.48 (0.22-0.99)	
Evolution of the baby			
Well	10 (16.13%)	REF	0.822
Dead	3 (17.65%)	1.11 (0.17-5.17)	
Handicap	0 (0.00%)	0.00 (0.00-29.73)	1.000
Preterm birth			
No	40 (25.32%)	REF	0.596
Yes	6 (20.69%)	0.77 (0.24-2.13)	
Birthweight			0.059

11.18 Addendum 18: Prevalence of vaginal *Candida* carriage in pregnant women in Sub Saharan Africa

Addendum 18. Prevalence of vaginal Candida carriage in pregnant women in Sub Saharan Africa. *Adapted from Master's dissertation of De Keyser Karen (for references mentioned in this table please consult this work)69 . N, number of study participants. *, average is excluding % Candida found in this Master's dissertation.*

Country	Year	N	% *Candida*	Sample	Detection	Reference
Burkina Faso	1997	645	14.0	Vaginal	Microscopy	Meda et al. (1997)
Burkina Faso	2017	229	22.7	Vaginal	Culture	Sangaré et al. (2018)
Cameroon	2013	112	55.4	Vaginal	Culture	Toua et al. (2013)
Central African Republic	1999	481	46.6	Cervical	Microscopy	Blankhart et al. (1999)
DRC	2019	330	38.2	Vaginal	qPCR	Mulinganya et al. (2021)[56]
Ethiopia	2017	384	25.0	Vaginal	Culture	Tsega et al. (2019)[64]
Ethiopia	**2021**	**151**	**7.3**	**Vaginal**	**Microscopy**	**This Master's dissertation**
Gabon	1998	646	30.8	Vaginal	Microscopy	Bourgeois et al. (1998)
Ghana	2005	517	39.8	Vaginal	Microscopy + culture	Apea-Kubi et al. (2005)
Ghana	2019	589	36.5	Vaginal	Microscopy	Konadu et al. (2019)
Kenya	1996	291	26.2	Vaginal	Microscopy	Thomas et al. (1996)
Kenya	2000	289	42.0	Vaginal	Microscopy	Fonck et al. (2000)
Kenya	2000	334	55.0	Vaginal	Microscopy	Fonck et al. (2000)
Kenya	2013	104	42.7	Vaginal	Microscopy + culture	Nelson et al. (2013)
Kenya	2014	30	23.0	Vaginal	Microscopy	Jespers et al. (2014)
Mali	1999	549	39.0	Vaginal	Microscopy	Mulanga-Kabeya et al. (1999)
Mauritania	2018	200	26.0	Vaginal	Culture	Sy et al. (2018)
Nigeria	1981	187	20.9	Vaginal	Culture	Ekwempu et al. (1981)
Nigeria	2002	500	65.0	Vaginal	Culture	Akerele et al. (2002)
Nigeria	2003	230	37.8	Cervical + vaginal	Microscopy	Aboyeji et al. (2003)
Nigeria	2007	311	56.3	Vaginal	Microscopy + culture	Nwosu et al. (2007)
Nigeria	2010	100	26.0	Vaginal	Culture	Donbraye-Emmanuel et al. (2010)
Nigeria	2010	901	62.2	Vaginal	Culture	Akah et al. (2010)
Nigeria	2010	90	30.0	Vaginal	Microscopy	Okonkwo et al. (2011)
Nigeria	2014	100	36.0	Vaginal	Microscopy	Olowe et al. (2014)
Nigeria	2015	140	25.0	Vaginal	Culture	Nurat et al. (2015)
Nigeria	2017	288	60.8	Vaginal	Microscopy + culture	Nnadi et al. (2017)
Nigeria	2019	20	45.0	Vaginal	Microscopy	Mumuney et al. (2019)
Nigeria	2019	20	25.0	Vaginal	Microscopy	Mumuney et al. (2019)
Uganda	2015	456	45.4	Vaginal	Microscopy + culture	Mukasa et al. (2015)
South Africa	1989	193	38.3	Vaginal	Microscopy + culture	O'Farrell et al. (1989)
South Africa	2014	30	57.0	Vaginal	Microscopy	Jespers et al. (2014)
Sudan	2009	151	13.9	Vaginal	Microscopy	Ortashi et al. (2004)
Sudan	2014	200	16.6	Vaginal	Microscopy	Abdelaziz et al. (2014)
Tanzania	2009	2654	11.4	Vaginal	Microscopy	Msuya et al. (2009)
The Gambia	1984	100	35.0	Cervical	Culture	Mabey et al. (1987)
Togo	2018	126	48.0	Vaginal	Microscopy	Dakey et al. (2018)
Togo	2013	221	30.8	Vaginal	Microscopy + culture	Tchelougou et al. (2013)
Zimbabwe	2010	691	39.3	Vaginal	Microscopy	Kurewa et al. (2010)
Average*			**36.5**			