FACULTY OF MEDICINE AND HEALTH SCIENCES

Vaginal carriage of *Candida* sp., *Enterobacter cloacae* complex and *Klebsiella pneumoniae* in pregnant women in Bukavu, Democratic Republic of the Congo: prevalence, risk factors, symptoms and adverse pregnancy outcomes.

De Keyser Karen
Student number: 01504513

Supervisor(s): Prof. dr. Piet Cools, Prof. dr. Steven Callens, Prof. dr. Mario Vaneechoutte

A dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Medicine in Medicine

Academic year: 2019 – 2020

GHENT UNIVERSITY

Acknowledgement

For me, writing this master thesis was much more than an obligatory ‘task’ to fulfill the requirements of my Master degree. I became passionate about science in all its aspects and especially in the wonderful world of global maternal-neonatal health and vaginal infections. At the beginning, I was scared as infectiology and microbiology was not my cup of tea. However, I am not one to back down from a challenge and wanted to have a solid grasp of the subject. Luckily, I had the good fortune of meeting many wonderful people who motivated and supported me to fulfill this goal. Soon, the LBR lab was my second home and each day I became more excited about all the different vaginal micro-organisms and how they influence the women’s reproductive health. After gathering all data at the lab, I was thrilled as all the pieces of the puzzle slowly begun to fit together in the data-analysis. So now, a lot of vaginal samples, qPCRs and excel files later, I am proud to present this master thesis, which was not possible without the support of many, lovely, people.

First of all, I want to express my gratitude to Prof. dr. Piet Cools. He guided me each step of the way with his contagious enthusiasm. Whatever problem I was facing, I could always count on his support and empathy. Furthermore, I want to thank him for the insightful comments and perspectives that he always provided during our discussions. It was an honor.

Furthermore, I want to thank my other supervisors Prof. dr. Mario Vaneechoutte and Prof. dr. Steven Callens for sharing their passion and knowledge about the vaginal microflora and maternal-neonatal infections. I also want to express my gratitude to Dr. Guy Mulinganya for letting me participate in the AVEONS project, his support along the way and all the data I was able to use thanks to his excellent work in Bukavu. Moreover, writing this master thesis was not possible without Maud Claeys and Annelies De Vulder, who already did a lot of sublime research about this topic. Apart from their excellent scientific work, they provided me with a lot of tips and tricks and were always willing to listen, whenever needed.

Research is a team sport. At the beginning of this master thesis, I could not even handle a pipette, never heard of a ‘vortex’ and I certainly did not know how to carry out a qPCR successfully. Luckily, I was surrounded with lovely and sympathetic people at the LBR. I really want to thank Antonio Rodriguez Sanchez, Hans Duyvejonck, Leen Van Simaey and Tessa Gryp for their patience and guidance at the lab. Carrying out all the different laboratory tests was not possible without your help.

Last but not least, I want to express my gratitude to my lovely parents, brothers and friends. The words ‘Kan die da?’ will never sound the same at home. Thanks for the unconditional support, for cheering me up when another (and another) lab test failed and for the patience and empathy when I could not be there when you needed me. You know who you are.

How something you feared, can become something wonderful…

List of abbreviations

AE	Alkaline extraction
ALB	Alkaline lysis buffer
AldA	Aldehyde dehydrogenase
AOR	Adjusted odds ratio
APO	Adverse pregnancy outcome
AVEONS	Angamiza Vizuri Early Onset Neonatal Sepsis
BLAST	Basic Local Alignment Search Tool
BMI	Body Mass Index
Bp	Base pair
BV	Bacterial vaginosis
C	Endocervical swab
Candida	*Candida* species
C. albicans	*Candida albicans*
C. dubliensis	*Candida dubliensis*
C. famata	*Candida famata*
C. glabrata	*Candida glabrata*
C. inconspicua	*Candida inconspicua*
C. kefyr	*Candida kefyr*
C. krusei	*Candida krusei*
C. tropicalis	*Candida tropicalis*
CDC	Center for Disease and Control
CI	Confidence interval
COR	Crude odds ratio
Cq	Quantification cycle
CRP	C-reactive protein
Cu	Culture
CVL	Cervicovaginal lavage
D	Delivery
DRC	Democratic Republic of the Congo
dsDNA	Double strand DNA
E. aerogenes	*Enterobacter aerogenes*
E. cloacae	*Enterobacter cloacae* complex
E. coli	*Escherichia coli*
E. sakazakii	*Enterobacter sakazakii*
EONS	Early-onset neonatal sepsis
EtBr	Ethidium Bromide
GBS	Group B *Streptococcus*
Hb	Hemoglobin
HIC	High-income country
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
HPV	Human papillomavirus
HRM	High resolution melting
Hsp60	Heat shock protein 60
IRS	Inhibitor removal substance
ITS	Internal transcribed spacer
KOH	Potassium hydroxide
K. pneumoniae	*Klebsiella pneumoniae*
K. variicola	*Klebsiella variicola*
LBR	Laboratory of Bacteriology Research
LBW	Low birth weight
LIC	Low-income country
LONS	Late-onset neonatal sepsis
LOQ	Limit of quantification
M	Microscopy
Maldi-TOF	Matrix Assisted Laser Desorption/ Ionization Time-of-flight Analyzer
NaOH	Sodium hydroxide
NMR	Neonatal mortality rate
N	Number of samples
NTC	Negative template control
OR	Odds ratio
p	Percentile
PBS	Phosphate buffered saline
PD	Post-delivery
PhoE	Outer membrane phosphate porin
PRBH	Provincial Referral Hospital of Bukavu
PTB	Preterm birth
qPCR	Quantitative polymerase chain reaction
R	Rectal swab
RCT	Randomized controlled trial
RE	Roche DNA extraction
Ref.	Reference
ROM	Rupture of membranes
RT-PCR	Real-time polymerase chain reaction
S. cerevisiae	*Saccharomyces cerevisiae*
SA	South-Asia
SDG	Sustainable Development Goal
SDS	Sodium dodecyl sulfate
SES	Socio-economic status
SSA	Sub-Saharan Africa
T	Type strain
TAE	Tris acetate EDTA
Tm	Melting temperature
U5MR	Under five mortality rate

V	High vaginal swab
V1	Visit 1
V2	Visit 2
VLIR	Vlaamse Interuniversitaire Raad
UTI	Urinary tract infection
VVC	Vulvovaginal candidiasis
WBC	White blood cell
WGA	Weeks of gestational age
WHO	World Health Organisation

Table of contents

Abstract

Background

In sub-Saharan Africa, 3% of the neonates die within the first month of their life. Preterm birth (PTB) and early-onset neonatal sepsis (EONS) are the two leading causes of neonatal mortality. The vaginal microflora plays a substantial role in both adverse pregnancy outcomes (APO). *Candida* sp. (*Candida*) has been associated with an increased risk of PTB. Therefore, our aim was to investigate the prevalence of vaginal *Candida* carriage, as well as associated risk factors, symptoms and APOs in pregnant women in Bukavu (Democratic Republic of the Congo (DRC)). Furthermore, in Bukavu (DRC), *Enterobacter cloacae* complex (*E. cloacae*) and *Klebsiella pneumoniae* (*K. pneumoniae*) were suggested as the two principle pathogens causing EONS. EONS is typically thought to be caused by vertically transmitted vaginal pathogens during delivery. In order to elucidate the pathogenesis of EONS in Bukavu (DRC), the prevalence of vaginal *E. cloacae* and *K. pneumoniae* carriage in pregnant women, as well as associated risk factors, symptoms and APOs were investigated.

Methodology

In a prospective hospital-based study in Bukavu (DRC), a total of 533 women were followed during pregnancy and delivery. A complete clinical examination of these women and their neonates was performed. Furthermore, a gynaecological examination was carried out and questionnaires were used to obtain information about the sociodemographic situation, reproductive health history, sexual behavior, vaginal hygiene practices and vaginal symptoms of the pregnant women. Vaginal *Candida*, *E. cloacae* and *K. pneumoniae* carriage rates were assessed on a subset of 330 pregnant women, based on the availability of a cervicovaginal lavage (CVL). Vaginal *Candida* carriage was determined by means of microscopic examination of wet mount and Gram stained vaginal smears, as well as by qPCR (CVL). Vaginal *E. cloacae* and *K. pneumoniae* carriage rates were quantified on CVLs by means of newly developed and validated in-house qPCR assays. Different multivariate logistic regression models were built for vaginal *Candida*, *E. cloacae* and *K. pneumoniae* carriage, to identify risk factors, symptoms and adverse pregnancy outcomes.

Results

The vaginal *Candida*, *E. cloacae* and *K. pneumoniae* carriage rates were 38.18% (95% CI: 33.10 - 43.53%), 42.42% (95% CI: 37.21 - 47.81%) and 12.12% (95% CI: 9.03 – 16.09%), respectively. The concentration of *E. cloacae* was low for all *E. cloacae* positive women (< 2.5log3 *E. cloacae*/ml CVL), while *Candida* and *K. pneumoniae* concentrations ranged respectively from 3.95log2 to

5.25log6 *Candida*/ml CVL and from 3.63log2 to 6.25log6 *K. pneumoniae*/ml CVL. Among *Candida* colonized women, *Candida albicans* (91%) was by far the most prevalent. Vaginal *Candida* carriage was significantly and independently associated with risk factors, in particular the use of pit toilets (AOR: 2.39) and intermediate vaginal microflora (AOR: 3.54), and with adverse pregnancy outcomes, namely meconium-stained amniotic fluid (AOR: 2.76) and PTB (AOR: 7.21). After stratifying women according to the concentration of vaginal *Candida*, only high *Candida* concentrations were significantly associated with PTB (AOR: 3.60). Clinically, vaginal *Candida* carriage was independently associated with vaginal discharge (AOR: 2.30), vaginal itching (AOR: 2.70) and burning sensation after sexual intercourse (AOR: 3.06). The microscopic detection rate of *Candida* on wet mount and Gram stained vaginal smear was a function of the *Candida* concentration, as defined by qPCR. Vaginal *E. cloacae* carriage was significantly and independently associated with the following risk factors: previous dysuria (AOR: 6.26), previous premature delivery (AOR: 13.43) and borderline significantly with anal sexual intercourse (AOR: 6.85, p=0.054). Vaginal *K. pneumoniae* carriage was significantly and independently associated with risk factors, particularly intermediate vaginal microflora (AOR: 3.01) and previous abortion (AOR: 2.04), and with neonates showing signs of EONS (APO) (AOR: 11.76).

Conclusions

The prevalence of vaginal carriage of both *Candida* and *E. cloacae* was high, but *E. cloacae* concentrations were consistently very low. The prevalence of vaginal *K. pneumoniae* carriage was rather low, with varying concentrations. *Candida*, *E. cloacae* and *K. pneumoniae* were each associated with (a history) of APOs. Clinical and microscopy-based screening and treatment of pregnant women with vaginal *Candida* carriage should be explored as a mean to diminish PTB in Bukavu (DRC).

Samenvatting

Achtergrond

In sub-Saharisch Afrika sterft 3% van de neonaten tijdens de eerste maand van hun leven. Vroeggeboorte (PTB) en neonatale sepsis (EONS) zijn de twee belangrijkste oorzaken van dergelijke neonatale sterfte. De vaginale microflora speelt een belangrijke rol in beide negatieve zwangerschapsuitkomsten (APO). *Candida* sp. (*Candida*) is geassocieerd met een verhoogd risico op vroeggeboorte. Hierbij was ons doel om de prevalentie van vaginaal *Candida* dragerschap, alsook geassocieerde risicofactoren, symptomen en negatieve zwangerschapsuitkomsten te onderzoeken bij zwangere vrouwen in Bukavu (Democratische Republiek Congo (DRC)). Verder werden *Enterobacter cloacae* complex (*E. cloacae*) en *Klebsiella pneumoniae* (*K. pneumoniae*) in voorgaand onderzoek gesuggereerd als de twee voornaamste verwekkers van EONS. Er wordt algemeen aangenomen dat EONS wordt veroorzaakt door opstijgende vaginale bacteriën tijdens de bevalling. Met het oog op het ontrafelen van de pathogenese van EONS in Bukavu (DRC), werden de prevalentie van vaginaal *E. cloacae* en *K. pneumoniae* dragerschap in zwangere vrouwen, alsook geassocieerde risico factoren en negatieve zwangerschapsuitkomsten onderzocht.

Methode

In een prospectieve studie in het plaatselijk ziekenhuis te Bukavu (DRC) werden 533 vrouwen gevolgd gedurende hun zwangerschap en bevalling. De vrouwen en hun neonaten ondergingen allen een volledig klinisch onderzoek. Voorts werd een gynaecologisch onderzoek uitgevoerd bij de zwangere vrouwen en werd aan de hand van vragenlijsten informatie bekomen over hun socio-demografische situatie, reproductieve voorgeschiedenis, seksueel gedrag, vaginale hygiëne en vaginale klachten. De prevalentie van vaginaal *Candida*, *E. cloacae* en *K. pneumoniae* dragerschap werd bepaald in een subset van 330 zwangere vrouwen, gebaseerd op de beschikbaarheid van een cervicovaginale lavage (CVL). Vaginaal *Candida* dragerschap werd nagegaan aan de hand van microscopisch onderzoek op wet mount en Gram gekleurde vaginale uitstrijkjes, alsook door middel van qPCR (CVL). *E. cloacae* en *K. pneumoniae* werden gekwantificeerd op CVLs met behulp van nieuw ontwikkelde en gevalideerde qPCR testen. Verschillende multivariabele regressie modellen werden opgesteld voor vaginaal *Candida*, *E. cloacae* en *K. pneumoniae* dragerschap, met het oog op identificatie van geassocieerde risicofactoren, symptomen en negatieve zwangerschapsuitkomsten.

Resultaten

De prevalentie van vaginaal dragerschap van *Candida*, *E. cloacae* en *K. pneumoniae* was respectievelijk 38.18% (95% BI: 33.10 - 43.53%), 42.42% (95% BI: 37.21 - 47.81%) en 12.12% (95% BI: 9.03 – 16.09%). De concentratie van *E. cloacae* was eerder laag bij alle *E. cloacae* positieve vrouwen (< 2.5log3 *E. cloacae*/ml CVL), terwijl voor *Candida* en *K. pneumoniae* concentraties werden gevonden in een range van 3.95log2 tot 5.25log6 *Candida*/ml CVL en van 3.63log2 tot 6.25log6 *K. pneumoniae*/ml CVL. Bij *Candida* positieve vrouwen was *Candida albicans* (91%) het meest voorkomende species. Vaginaal *Candida* dragerschap was significant en onafhankelijk geassocieerd met enkele risicofactoren, meer bepaald het gebruik van latrines (AOR: 2.39) en intermediaire vaginale microflora (AOR: 3.54), alsook met negatieve zwangerschapsuitkomsten, namelijk meconium houdend amnionvocht (AOR: 2.76) en vroeggeboorte (AOR: 7.21). Na een stratificatie volgens *Candida* concentratie, waren louter hoge *Candida* concentraties significant geassocieerd met vroeggeboorte (AOR: 3.60). Klinisch gezien was vaginaal *Candida* dragerschap onafhankelijk en significant gerelateerd met vaginale afscheiding (AOR: 2.30), vaginale jeuk (AOR: 2.70) en een branderig gevoel na seksueel contact (AOR: 3.06). Het aantal microscopisch gedetecteerde gevallen van *Candida* op Gram gekleurde uitstrijkjes steeg naargelang de *Candida* concentratie, bepaald via qPCR, toenam. Vaginaal *E. cloacae* dragerschap was significant en onafhankelijk geassocieerd met volgende risicofactoren: vroeggeboorte in de reproductieve voorgeschiedenis (AOR: 13.43), eerder ervaren dysurie (AOR: 6.26), en borderline-significant met anaal seksueel contact (AOR: 6.85, p=0.054). *K. pneumoniae* dragerschap was significant en onafhankelijk gerelateerd aan risicofactoren als intermediaire vaginale microflora (AOR: 3.01) en abortus in de voorgeschiedenis (AOR: 2.04), alsook met neonaten met klachten passend bij EONS (AOR: 11.76).

Conclusie

De prevalentie van vaginaal *Candida* en *E. cloacae* dragerschap was hoog, echter, *E. cloacae* concentraties waren consistent laag. De prevalentie van *K. pneumoniae* daarentegen was eerder laag, met variërende concentraties. *Candida*, *E. cloacae* en *K. pneumoniae* waren allen geassocieerd met een (voorgeschiedenis) van negatieve zwangerschapsuitkomsten. Een klinische en microscopie-gebaseerde benadering en behandeling van zwangere vrouwen met vaginaal *Candida* dragerschap dient verder onderzocht te worden om het aantal vroeggeboortes in Bukavu (DRC) te verminderen.

1. Introduction

1.1 Neonatal mortality, an important global health issue

Between 1990 and 2015, a remarkable progress in the improvement of child survival has been made. However, the decline in neonatal mortality rate (NMR), defined as the number of neonatal deaths within 28 days of birth per thousand liveborn babies, has lagged remarkably compared to post-neonatal mortality rates **(Figure 1)** (1-8). In 2018, 2.50 million children died in their first month of life (equivalent to 7000 neonates per day), representing 45% of total deaths among children under five years (7). Three quarters of these neonatal deaths happened in the first week of life, with the highest risk of death in the first day of life (2, 3, 6, 7, 9-11).

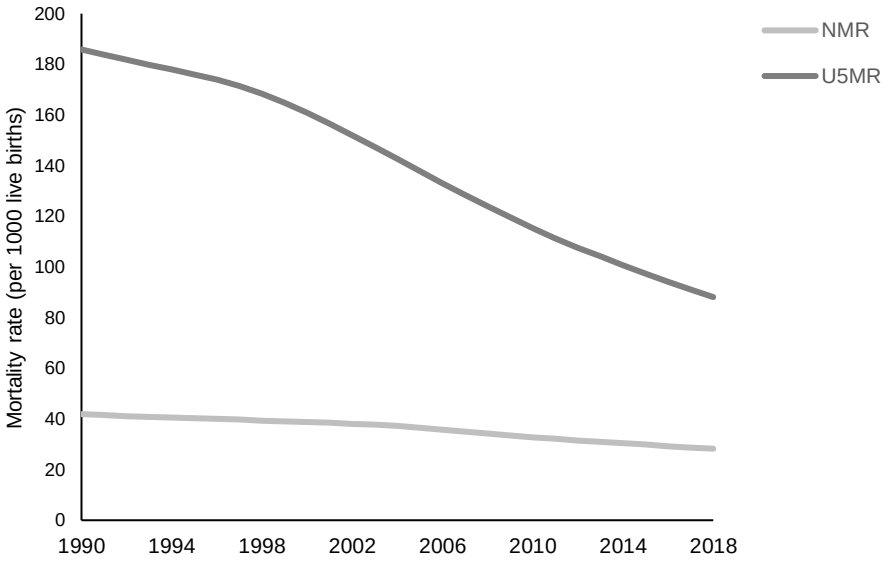


Figure 1. Comparison between global neonatal mortality rate and the global under five years mortality rate in the period 1990-2018. Between 1990 and 2018 the U5MR declined remarkably, but the NMR stagnated. NMR: neonatal mortality rate. U5MR: Under five mortality rate. Figure based on data from Unicef 2018 (7) .

Furthermore, NMRs vary dramatically between regions and countries **(Figure 2)** (2). More than two thirds of all neonatal deaths worldwide occur in low-income countries (LIC) in sub-Saharan Africa (SSA) and South-Asia (SA) (1, 2, 5, 9, 11, 12). The neonatal deaths in SSA account for almost half of the world's child deaths (12). In the Democratic Republic of the Congo (DRC), an NMR of 28.30 has recently been estimated by Unicef (7). This equity gap between low- and high-income countries continues to increase, partially because research mainly focuses on the 1% deaths in high-income countries (HIC). Closing this gap could prevent approximately 0.75 million deaths (11, 13).

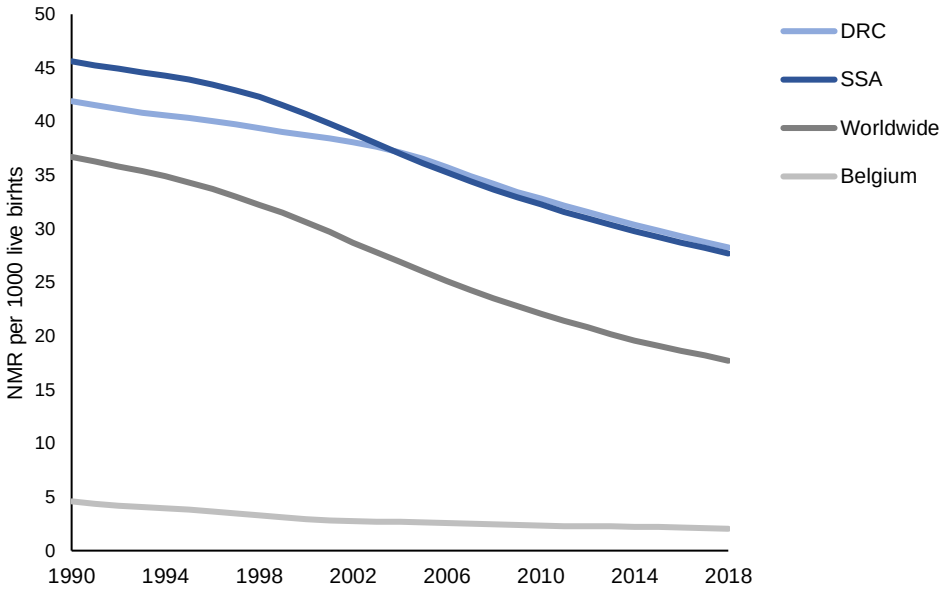


Figure 2. Evolution of the neonatal mortality rate in Belgium, the Democratic Republic of the Congo, sub-Saharan Africa and worldwide from 1990 to 2018. A notable equity gap in NMRs between low-income countries (DRC) and high-income countries (Belgium) is noted. DRC: Democratic Republic of the Congo. NMR: Neonatal mortality rate. SSA: sub-Saharan Africa. Figure composed based on data from Unicef 2018 (7).

The sustainable development goals (SDG) are a blueprint set up by the United Nations General Assembly to achieve a better and more sustainable future for everyone. The objective of one of the seventeen goals is to ensure good health and well-being for every person on earth (SDG 3). Within this sustainable development goal, one specific target on neonatal mortality aims to reduce neonatal mortality to at least as low as twelve neonatal deaths per thousand livebirths for each country (1, 4, 5, 7, 14, 15). In contrast to the previously formulated Millennium Development Goals - aiming to reduce under five mortality rates (U5MR) by two thirds - country specific absolute goals were imposed by the SDG (6). Currently, approximately sixty countries, mainly LICs in SSA, are of track in achieving this target (16) and must substantially accelerate reductions in NMRs in order to achieve this target (14).

1.2 Causes of neonatal mortality in the Democratic Republic of the Congo

The main causes of neonatal mortality in DRC are shown in **Figure 3** (7). The large majority of neonatal deaths (more than 80%) are due to complications related to preterm birth (PTB), intrapartum events such as birth asphyxia, or infections such as sepsis or pneumonia (1, 2, 9-12, 17).

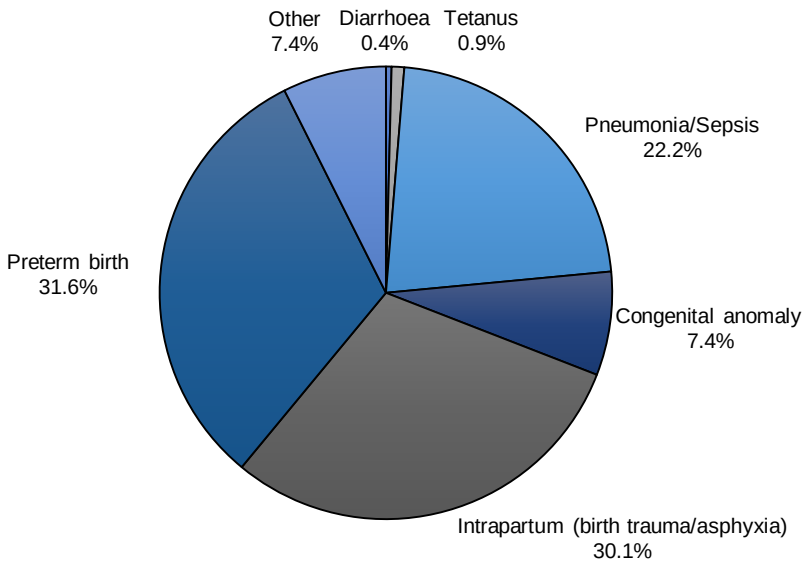


Figure 3. The main causes of neonatal mortality in the Democratic Republic of the Congo. Preterm birth, intrapartum events (birth trauma and asphyxia) and neonatal infections (sepsis and pneumonia) are the most common causes of neonatal mortality in the Democratic Republic of the Congo. Figure composed based on data from Unicef 2018 (7).

Preterm birth

PTB is defined by WHO (World Health Organisation) as birth before 37 weeks of gestational age (WGA) or fewer than 259 days from the first date of a women's last menstrual period (18). The global prevalence of PTB in 2010 was estimated to be 11.10%, ranging from 5% in northern Europe to 18% in SSA (19-21). DRC belongs to the top ten of countries with the highest prevalence of PTB, i.e. 14.90% (20).

The proportion of neonatal deaths due to prematurity is inversely correlated with neonatal mortality rates. HIC tend to have proportionally more PTBs, but survival rates among preterm neonates are much higher in these countries. Particularly, in HIC, half of the number of babies born at 24 WGA will survive, compared to LIC, where this 50% survival rate is barely reached at 32 WGA. The major survival gap between HIC and LIC is probably the result of a lack of appropriate neonatal care (13, 22).

PTB has a multifactorial etiology. Vaginal ascending infections account in up to 40% of all cases of PTB (19-26). It has become increasingly clear that vaginal carriage of *Candida* spp. (*Candida* – see below, section 3.3) may play a more important role than previously thought (27-33). Evidence about the association between bacterial vaginosis (BV) and PTB suggests that microorganisms appear to ascend from the female vaginal tract to the choriodecidual space before the expanding membranes seal the endometrial cavity near mid-pregnancy (19, 24-27, 34-37). When the intrauterine organisms are not cleared within four to eight weeks after colonization, a

subclinical, chronic infection of the myometrium, fetal membranes and amniotic fluid can occur (24-26, 34, 36). In the course of pregnancy, a host defense develops against the intrauterine microorganisms and results in chorioamnionitis. A pro-inflammatory environment with cytokines and inflammatory cells develops and induces labor (19, 20, 23-26, 34, 36-38). Although *Candida* itself is seldom identified as a cause of chorioamnionitis, it is assumed as a disrupter of the normal vaginal microflora, favoring the carriage of ascending, pathogenic bacteria (39-41).

Early-onset neonatal sepsis

Neonatal sepsis is a clinical syndrome caused by bacteria accessing the blood circulation, leading to unspecific systemic clinical manifestations, including temperature instability (hyperthermia, hypothermia), neurologic impairments (lethargy, seizures, irritability), feeding intolerance, respiratory distress (apnea, tachypnea), cardiac failure (bradycardia, hypotension) and a deviant skin (purpura, petechiae, pallor, cyanosis) (42-45). According to the golden standard, diagnosis is based on established symptoms and an associated positive blood culture (42-48).

Neonatal sepsis is categoriszed as either early-onset neonatal sepsis (EONS) or late-onset neonatal sepsis (LONS), based on the timing of the infection. EONS is defined by the onset of signs and symptoms within the first week after birth. Among very-low birth weight infants (<1500 g), the cut-off for EONS is restricted to infection occurring in the first 72 hours of life. The term LONS compromises the other cases of neonatal sepsis that occurred after the previously explained time points, until the age of one month (42-45, 47).

A high prevalence of EONS is determined in LIC, which stands in sharp contrast to that in HIC. Every year, an estimated 6.90 million neonates require treatment for possible serious bacterial infections in SA an SSA (49). In 2017, the mortality rate due EONS was 2.50 per 1000 live births worldwide. This rate was 4.20 deaths per 1000 live births in SSA (DRC: 4.40 deaths per 1000 live births), but decreased to 0.50 deaths per 1000 live births in Europe (Belgium: 0.10 deaths per 1000 live births) (50). Apart from this mortality rate, EONS is an important cause of several long-term morbidities, e.g. neurodevelopmental impairments (cerebral palsy), endocarditis with valve damage and thrombosis (42-45, 48).

EONS is typically caused by bacterial pathogens transmitted vertically from mother to infant before or during childbirth, after rupture of the membranes. In HIC, Group B *Streptococcus* (GBS or *Streptococcus agalactiae*) and *Escherichia coli* (*E. coli*) are the leading causes of EONS (51). Despite major advances in the clinical approach towards EONS, including the administration of intrapartum prophylaxis for women carrying GBS between 35-37 WGA, EONS has still a non-negligible poor outcome (42-44, 46, 48, 52-56).

In SSA, the etiology of EONS is largely unknown. In Bukavu (DRC), a previous study in the AVEONS project determined that *Enterobacter cloacae* complex (*E. cloacae*[1]) and *Klebsiella pneumoniae* (*K. pneumoniae*), two Gram-negative gastro-intestinal commensals, are the principal microorganisms causing EONS. This inconsistency in causative pathogens may be problematic as administered empirical antibiotics in DRC are based on the resistance patterns of GBS and *E.* coli, as recommended in the WHO guidelines (58).

1.3 Aims of this master thesis

AVEONS is a research project investigating the causes of neonatal mortality in Bukavu (DRC). DRC is one of the poorest countries in the world, despite the wealth in natural resources and potential for economic development (7). In order to diminish the NMR significantly, it is critical to create and implement interventions based on an estimation of the local causes of death (2).

In order to reduce the NMR in DRC, more information about the causes of neonatal mortality need to be gathered. As *Candida* carriage is thought to contribute to PTB (27-33), prevalence rates of vaginal *Candida* carriage were determined by molecular techniques and associations with adverse pregnancy outcomes (APO) as PTB and low-birth weight (LBW) were determined. In order to get more insights in the dynamics of vaginal *Candida* carriage, associated risk factors and symptoms were investigated. Secondary, the clinical utility of Gram stain and wet mount microscopy was examined.

In order to design and implement prevention strategies for EONS in DRC, the pathogenesis of *E. cloacae* and *K. pneumoniae* EONS has to be better understood. As mother-to-neonate transmission of vaginal pathogens is a prerequisite in the pathogenesis of GBS EONS (51), vaginal *E. cloacae* and *K. pneumoniae* carriage rates were examined to verify if similar mechanisms could be applied for *E. cloacae* and *K. pneumoniae* EONS. Furthermore, associated risk factors, symptoms and APOs such as EONS, were assessed.

[1] Several closely-related species (*Enterobacter cloacae*, *Enterobacter asburiae*, *Enterobacter hormaechei*, *Enterobacter kobei*, *Enterobacter ludwigii* and *Enterobacter nimipressuralis*) are addressed as the '*Enterobacter cloacae* complex', because no routinely applicable identification methods are available to distinguish them (57) . For brevity and readability, we will address the species of this complex as '*E. cloacae*' for the remainder of this master thesis.

2. Material and methods

This master thesis is part of an overarching project, called AVEONS, an acronym for Angamiza Vizuri (Swahili for 'Stop') Early Onset Neonatal Sepsis, which is a PhD study conducted in Bukavu (Democratic Republic of the Congo (DRC)) and funded by the VLIR (Vlaamse Interuniversitaire Raad). My contribution to this research project consists of extended Nugent scoring of Gram stained vaginal smears (59), the DNA extraction of cervicovaginal lavages (CVL), the development and validation of new in-house qPCR assays for *E. cloacae* an *K. pneumoniae*, performing qPCR assays of *Candida*, *E. cloacae* an *K. pneumoniae* on CVL DNA extractions and carrying out all statistical analyses described in the current master thesis.

2.1 Ethics

Ethical approval for this research was granted by the Internal Review Board of The Catholic University of Bukavu (reference number UCB/CIE/NC/016/2016), by the Ministry of Public Health of the Democratic Republic of the Congo (reference number 062/CD/DPS/SK/2017) and by the Ethical Committee of Ghent University Hospital (reference number PA2014/003), prior to patient recruitment.

Written informed consent was obtained from each pregnant woman after being informed about the study details and research aims. The main investigator was responsible for translating the template forms into local languages and verifying the accuracy of the translation by performing an independent back translation. The participants received a copy of the informed consent to keep at home.

To maintain participant confidentiality, a coded number was used for all specimens, laboratory forms and in all data analysis. All study-related information was stored securely at the study sites.

2.2 Study design

The primary objective of the AVEONS project is to determine the pathogens causing EONS, as well as the risk factors and antimicrobial drug sensitivity of these pathogens, in pregnant women in Bukavu (DRC). Furthermore, one of the secondary objectives was to investigate the role of vaginal microflora in pregnant women regarding adverse pregnancy outcomes (APO).

AVEONS was a longitudinal, prospective cohort study, conducted at the department of obstetrics and gynaecology in the Provincial Referral Hospital of Bukavu (PRHB). Bukavu is the capital city of the province South Kivu in DRC and is divided into 3 urban areas: Kadutu, Ibanda and Bagira.

The pregnant women included in the AVEONS study were seen at three moments: between 16 and 20 weeks of gestational age (WGA) (Visit 1: V1), between 35 and 37 WGA (Visit 2: V2) and during delivery. After delivery, their neonates were observed for one week. Both prenatal visits, as well as delivery and neonatal follow-up, were carried out at PRHB. Individual transportation costs were covered by the study project.

Between the two main prenatal visits, pregnant women could come to PRHB for care in case of any adverse health outcomes. Furthermore, pregnant women were called three times between the two prenatal visits to ensure that no adverse health outcomes were arisen. Apart from this AVEONS study, the pregnant women continued to follow antenatal care like usual. A small card with all appointments was given to each woman and a reminder phone call and SMS was sent to the patient or someone close in her neighbourhood.

2.3 Study population

Between January and October 2017, pregnant women seeking antenatal care at PRHB were asked to participate in the AVEONS project. In order to create awareness, church announcements, posters, radio/television spots and community leaders' speeches were harnessed to attempt participants.

Pregnant women who were interested in contributing to this study, were informed about the research project in detail and were asked to sign an informed consent. After consenting, women were screened for eligibility using the inclusion and exclusion criteria, listed in **Table 1**. When the pregnant woman met the inclusion criteria, she was enrolled in the study cohort.

Table 1. Inclusion and exclusion criteria used in the AVEONS research project.

Inclusion criteria	Exclusion criteria
Being able to consent and accept to participate in the study (parental approval if under 18 years)	Being unable to adhere to study procedures
Pregnancy between 16-20 weeks of gestational age with alive fetus	Planning to move out of Bukavu city during pregnancy
Accepting to be followed during pregnancy at PRHB and willing to deliver at PRHB	Any condition that prevent the women from appropriate follow up
Accepting to be contacted by phone or other means as reminder	Twin pregnancy
	Genital bleeding during this pregnancy
	Antibiotics administration two weeks before recruitment

PRHB: Provincial Referral Hospital of Bukavu

2.4 Clinical and sampling procedures

Clinical procedures and questionnaires

At each visit a fixed pattern of procedures was carried out. Firstly, the participants were asked to complete questionnaires (in local language) in order to gather data about the sociodemographic situation, reproductive history, sexual behavior, vaginal hygiene practices and vaginal signs and symptoms.

Next, a general clinical examination was performed, followed by a gynaecological examination with a sterile, non-moistened speculum. Hereby, the vaginal and cervical mucosa was carefully inspected and the vaginal pH was determined by means of indicator pH papers (Hilindicator® pH paper).

Furthermore, the potential clinical diagnosis of bacterial vaginosis (BV) was assessed by the African clinician using the Amsel criteria. Briefly, BV was diagnosed if three of the following four criteria were present: (i) a rate of at least 20% clue cells (vaginal epithelial cells covered with bacteria) observed on wet mount microscopy (see local laboratory procedures), (ii) a 'fishy' odor elicited after mixing vaginal secretions with 10% KOH (potassium hydroxide), (iii) vaginal secretions with an elevated pH of > 4.5 and (iv) a thin, white, skim-milk-like homogeneous vaginal discharge (60).

After each visit, treatments were prescribed if pathology was identified. The syndromic approach is the standard of care in Bukavu (DRC) to manage a vaginal infection (guidelines issued by the Ministry of Public Health of DRC) (61). This means that women experiencing abnormal vaginal discharge, vaginal malodor, itching and/or a burning sensation after sexual intercourse, were considered as symptomatic for BV or vaginal infections with *Candida* or *Trichomonas*, and treated empirically with a combination of 200 mg clotrimazole and 100 mg clindamycin, in one vaginal ovule (brand name Femaclin®), for six days. In case of allergy against clotrimazole and/or clindamycin, a metronidazole ovule was given once a day for six days (61). This syndromic approach is commonly applied in developing countries (62).

Furthermore, urinary tract infections (UTI) and other pathologies were approached according to local protocols. UTIs were treated with antibiotics, more specifically with 2 x 500/125 mg amoxicillin and clavulanic acid for 3-7 days. In case of malaria, a combination of 20 mg arthemeter and 120 mg lumefantrine was given in a course of 24 tablets. In case of amoebiasis and/or giardiasis, treatment consisted of 2 g tinidazole in one single dose.

Finally, chemoprophylaxis was offered to all pregnant women at 24 WGA as part of the usual antenatal care. They received a single dose of 500 mg mebendazole (brand name Vermox®), a

single dose of 500 mg sulfadoxine-pyrimethamine (brand name Fansidar®) and folic acid, respectively against soil-transmitted helminths, malaria and anemia.

Sampling procedures as part of the routine prenatal care

As part of the routine prenatal care, blood samples were collected in VacuTubes® red (without EDTA). The blood samples were used to screen for HIV and malaria (V1) and to determine the hemoglobin (Hb) concentration (V1 and V2). Serum was taken from the blood sample and tested with rapid tests, namely Alere Determine™ HIV-1/2® (Abbott), Malaria AG P.f/pan® (Bioline) and Hemocue® Hb201+ (Hemocue AB), for the detection of respectively HIV, malaria and anemia. Next, midstream urine was collected in a sterile container and tested for the presence of white blood cells and nitrite with Multistix® dipsticks (Siemens), indicating UTI and bacteriuria. Furthermore, a dry vaginal swab (Copan) was taken by gently rolling the top of the swab against the lateral vaginal wall and dipping it in the posterior fornix, in order to obtain a well moistened swab. Afterwards, the swab was gently rolled on a glass slide for wet mount microscopy (see laboratory procedures).

At the end of each visit, an obstetrical ultrasound examination was performed to confirm pregnancy and viability, as well as to assess the cervical length and gestational age.

Study specific sampling procedures

At each prenatal visit, three vaginal swabs (one dry swab for Gram stain microscopy and two Amies swabs with transport medium for culturing and molecular analysis) were taken by gently rolling the top of the swab against the midportion of the lateral vaginal wall and dipping it in the posterior fornix, in order to obtain a well moistened swab. Subsequently, a rectovaginal swab was collected by sampling consecutively the vaginal wall and rectal mucosa (1.5-2 cm beyond the anal sphincter). Moreover, a cervicovaginal lavage (CVL) was carried out by rinsing the cervical and vaginal mucosa with 5 ml of physiologic water and collecting the lavage into VacuTube® red. In this way, the water rinsed the cervicovaginal mucosa and contained elements of the superficial layer of the vaginal mucosa. Finally, a stool sample was taken for parasitological examination.

At delivery, the labor was followed carefully, and clinical parameters of the neonates were collected by nurses and the senior assistant. After childbirth, nose, ear and umbilical cord of each neonate were sampled using an Amies swab for microbial culturing. A blood sample (collected in a VacuTube® purple, with EDTA) for microbial culturing was taken from the neonates showing signs of sepsis in the first three days after birth (EONS).

2.5 Laboratory procedures

Wet mount microscopy

Within a maximum of twenty minutes after collecting the dry vaginal swab, a wet mount slide was prepared by mixing the substances on the swab with 0.50 ml of saline. One droplet of this suspension was gently rolled on a glass slide and covered with a cover slip. Microscopy under 10x/40x magnification was performed in order to detect motile *Trichomonas vaginalis*, *Candida* (budding cells and/or hyphae (long, tubular branching structures produced by *Candida*)), white blood cells and clue cells (epithelial cells covered by bacteria - one of the four Amsel criteria (60), used for the clinical diagnosis of BV). Wet mount microscopy was performed at the local lab in PRHB (Bukavu, DRC).

Gram stain microscopy

Apart from wet mount microscopy, Gram stain microscopy was performed on one of the vaginal dry swabs taken from each woman. At PRHB (Bukavu, DRC), each vaginal swab was gently rolled on a glass slide and fixated by holding the back of the slides (i.e. the side not containing the cells) briefly into a flame. These fixated slides were stored in boxes until shipment to the Laboratory for Bacteriology Research (LBR) (Ghent University, Ghent, Belgium) for further examination.

As part of this master thesis, heat fixated slides were Gram stained at the Department of Laboratory Medicine (Ghent University Hospital, Ghent, Belgium) by an automated Poly Stainer. Briefly, slides were immersed in a crystal violet dye for 1 minute, in an iodine solution for 1 minute, in alcohol for 20 seconds and finally in safranin for 1 minute. Slides were rinsed with water for two seconds after each immersion step.

These Gram stained vaginal smears were subsequently used for the laboratory diagnosis of BV, using the Nugent score (59). A total of five fields per Gram stained slide were scored on microscopy, using a 40x/100x magnification, according to the Nugent scoring system for laboratory-based diagnosis of BV (59). Furthermore, these Gram stained smears were also evaluated for the presence of *Candida* cells and hyphae, Gram-positive/negative cocci and clue cells. For quality control, all slides were scored single-blinded by two individuals. In case of discrepancy in categorization (i.e. normal vaginal microflora, intermediate microflora and BV according to Nugent scoring (59)), slides were reassessed by the two reviewers and discussed. **Figure 4** shows a microscopic image with 100x magnification of Gram stained *Candida* with and without hyphae.

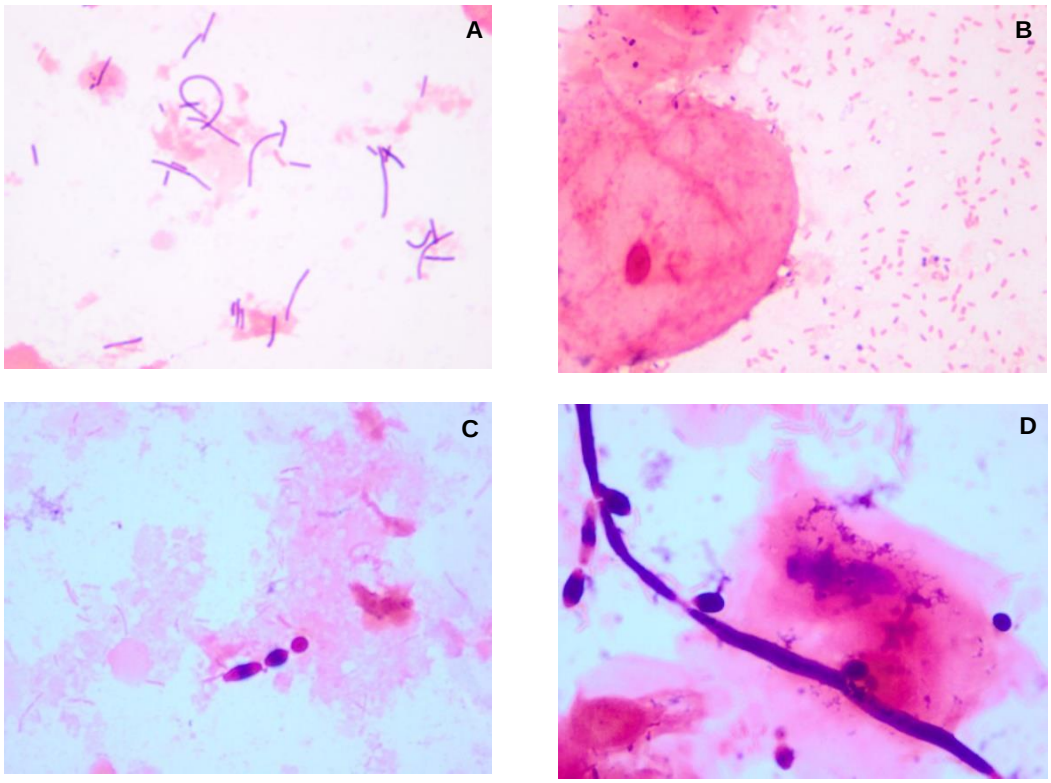


Figure 4. Microscopic images (100x magnification) of Gram stained vaginal smears. A: Healthy, normal vaginal microflora. B: Bacterial vaginosis. C: *Candida* cells (blastopores). D: *Candida* cells with hyphae (long, tubular branching structures produced by *Candida*). Adapted from [De Vulder, *et al*. 2019] (63).

Molecular quantification of *Candida*, *Enterobacter cloacae* and *Klebsiella pneumoniae* in cervicovaginal lavages

Quantitative Polymerase Chain Reaction (qPCR), also known as Real-Time polymerase chain reaction (RT PCR), is the golden standard to quantify bacterial and yeast species in clinical samples. Hence, this technique was used in this master thesis and executed at LBR (Ghent University, Ghent, Belgium).

DNA extraction of CVL

CVLs were chosen over vaginal swabs for DNA extraction and subsequent species quantification, as these DNA extracts will be used in the future to determine the carriage of the human papilloma virus (HPV, the causative agent of cervical cancer). The RNeasy PowerMicrobiome Kit® (Qiagen) was used to extract DNA according to the manufacturer's instructions. Briefly, the CVLs were first thawed at room temperature. After vortexing, 250 µl of CVL was gently pipetted into a beating tube. Subsequently, a strong lysis buffer, consisting of 650 µl guanidine thiocyanate and 6.50 µl β-mercaptoethanol (Fluka Chemie GmbH), was added. Afterwards, in order to increase bacterial and yeast cell lysis, the suspension was subjected to bead beating during 10 minutes by horizontally securing the beating tube to a vortex adapter. To further increase DNA yield from

bacterial and yeast cells, the suspension was incubated at 95 °C for 10 minutes (64). After incubation and centrifugation (1 min at 13 000 x g), the supernatant was gently removed and pipetted into a new collecting tube. A total of 150 µl inhibitor removal substance (IRS) was added to the supernatant to neutralize PCR inhibitors, followed by an incubation of 5 min at 4 °C. Next, 650 µl guanidine hydrochloride, a binding salt for nucleic acids, and 650 µl of 100% ethanol, were used to purify the DNA from the lysate. After vortexing, the supernatant was loaded to an MB RNA Spin Column. By means of centrifugation (1 min at 13 000 x g), the DNA was bound to the Spin Filter membrane. Subsequently, 650 µl isopropanol and 650 µl ethanol-containing wash buffer, were added to desalt the column before the elution step. After centrifugation (1 min at 13 000 x g), 100 µl RNase-Free Water was inserted in the Spin Column and DNA was solubilized from the Spin Filter membrane into the RNase-Free Water by centrifuging (1 min at 13 000 x g). This DNA extract was stored at -20 °C, until use in the different qPCR assays.

Preparation of standard dilution series

In order to prepare a qPCR standard dilution series for the generation of standard curves (to absolutely quantify the microorganisms), *C. albicans* (ATCC 90028^T), *E. cloacae* (LMG 02783^T) and *K. pneumoniae* (ATCC 13883^T) were first cultured aerobically overnight on blood agar plates (Biomerieux) at 37 °C. One colony of each plate was identified with Maldi-TOF (Matrix Assisted Laser Desorption/Ionization Time-of-flight Analyzer) for quality control purposes. Afterwards, DNA was extracted with the High Pure PCR Template Preparation Kit® (Roche). Briefly, from each plate, all colonies were harvested and suspended in 200 µl phosphate buffered saline (PBS). After adding 200 µl Tissue Lysis Buffer, containing chaotropic salts that weaken hydrophobic interactions and increase membrane permeability, and 40 µl proteinase K, an enzyme that lyses the cell wall, the suspensions were incubated for 1 h at 55 °C. Afterwards 2 µl mutanolysin (25 U/µl) was added and the whole sample was incubated for 15 min at 37 °C in order to stimulate cleavage of the peptidoglycan layer of the bacteria. Furthermore, 200 µl of Binding Buffer was added to purify the DNA from the lysate, before incubating the suspension for 10 min at 70 °C. After incubation, 100 µl of isopropanol was added to remove the remaining salt residue. Next, the whole sample was brought into a filter tube and centrifuged for 1 min at 8000 x g. Subsequently, 500 µl Inhibitor Removal Buffer and 500 µl Wash Buffer were added to neutralize possible inhibitors and to wash the filter. Finally, DNA was eluted by adding 200 µl Elution Buffer.

The DNA concentration of the Roche extracts was determined by spectrophotometric analysis (Nanodrop, Thermo Scientific) and converted from ‘ng DNA per ml DNA extract’ to ‘number of bacterial or yeast chromosomes per ml DNA extract’ based on the GC% content, genome size of

the type strain and the molecular weight of A, T, G and C. The DNA concentration of the Roche extracts was 20.50 ng/µl (260/280 = 1.16, 260/320 = 0.37), 315.25 ng/ml (260/280 = 1.83, 260/320 = 2.04) and 41.50 ng/ml (260/280 = 1.88, 260/320 = 2.5), resulting in a total of respectively 7.25log8 *C. albicans* cells/ml, 6.19log10 *E. cloacae* cells/ml and 7.16log9 *K. pneumoniae* cells/ml.

Tenfold dilution standard series of *C. albicans* (ATCC 90028^T), *E. cloacae* (LMG 02783^T) and *K. pneumoniae* (ATCC 13883^T) were prepared by diluting the DNA extracts with a 25% Calf Thymus DNA suspension (Sigma-Aldrich). The tenfold dilution standard series of *Candida dubliniensis, Candida famata, Candida glabrata, Candida guilliermondi, Candida inconspicua, Candida kefyr, Candida krusei, Candida lipolytica, Candida lusitania, Candida metapsilosis, Candida nivariensis, Candida norvegensis, Candida orthopsilosis, Candida parapsilosis, Candida tropicalis*, and *Saccharomyces cerevisiae* were already available at LBR (Ghent University, Ghent, Belgium).

Candida qPCR

To detect *C. albicans* in the CVL DNA extracts, an established *Candida* specific qPCR assay, available at LBR (Ghent University, Ghent, Belgium), was carried out (65). The ITS4 (TCC TCC GCT TAT TGA TAT GC) and ITS86 (GTG AAT CAT CGA ATC TTT GAA C) primers, designed by White et al. (1990) (66) and targeting the ITS2 (Internal Transcribed Spacer) region in the genome of *Candida,* were used (65). As these primers amplify the ITS2 region in all *Candida* species, melting curve analysis was necessary to make a distinction between the different *Candida* species (67).

The qPCR reactions were performed in a final volume of 10 µl, containing 5 µl Roche LC480 high resolution melting (HRM) mix® (Roche), 0.50 µM of both forward primer (ITS4) and reverse primer (ITS86), 3 µM of $MgCl_2$ (Roche), 1.30 µl PCR-grade H_2O (Roche) and 2 µl template DNA or 2 µl PCR-grade H_2O as negative control.

Thermal cycling was carried out on a LightCycler® 480 (Roche). The cycling conditions were as follows: pre-incubation at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 20 s; primer annealing at 55 °C for 30 s and primer extension at 72 °C for 30 s. Subsequently, a melting curve was generated with the following protocol: 5 s at 95 °C, 1 min at 60 °C, followed by a gradual increase in temperature from 60 °C to 97 °C, using a ramp rate of 0.02 °C per s. Finally, a cooling step of 30 s at 40 °C was carried out.

The standard dilution series of *Candida albicans* (ATCC 90028^T) was implemented in order to generate a standard curve for extrapolation of the concentration. Furthermore, to distinguish the different *Candida* spp., a log2 dilution of *Candida dubliniensis, Candida famata, Candida glabrata,*

Candida guilliermondi, Candida inconspicua, Candida kefyr, Candida krusei, Candida lipolytica, Candida lusitania, Candida metapsilosis, Candida nivariensis, Candida norvegensis, Candida orthopsilosis, Candida parapsilosis, Candida tropicalis and *Saccharomyces cerevisiae* was added. Finally, 10 negative template controls (NTCs) were applied in each qPCR run. The standard dilution series and all samples were run in duplicate.

Enterobacter cloacae and Klebsiella pneumoniae qPCR

As part of this master thesis, we developed new in-house qPCR assays for the quantification of *E. cloacae* and *K. pneumoniae.*

Primer selection

First, a literature study was performed to identify previously described *E. cloacae* and *K. pneumoniae* qPCR assays in order to identify already validated primers. Published primers were first critically assessed for specificity using the nucleotide Basic Local Alignment Search Tool (BLAST) (68). The specificity of *E. cloacae* and *K. pneumoniae* primers was determined by considering the amount of *E. cloacae* or *K. pneumoniae* matches per hundred hits, their query cover and the accordance for both the forward and reverse primer. The amplicon length was also determined.

Subsequently, primers that seemed specific, were further analyzed for the presence of secondary structures using mFOLD, an online tool that predicts secondary structures of oligonucleotides and the stability of these structures under a given temperature, Mg^{2+} and Na^{+} concentration (69). The formation of hairpins (especially at the 3' end) was considered as interfering with the primer function, hence, these primers were excluded for further wet-lab testing. Finally, the two best scoring primer pairs for both *E. cloacae* and *K. pneumoniae* resulting from this *in silico* analysis were purchased for further testing and validation.

Reference DNA used for validation

Apart from the *E. cloacae* and *K. pneumoniae* type strains, six *E. cloacae* and five *K. pneumoniae* strains, isolated from neonates with EONS, belonging to the same population and admitted to the same hospital (PRBH, Bukavu, DRC) as the AVEONS study, were cultured for validation of the qPCR assay. Moreover, other species related to *E. cloacae* and *K*. *pneumoniae* were grown to test the specificity of the primers. All the cultured strains are listed in **Table 2**.

Table 2. List of all the cultured strains used to validate the *Enterobacter cloacae* and *Klebsiella pneumoniae* assay.

Genus	Species	Strain	DNA extraction	Reference
Enterobacter sp.	*E. cloacae*	LMG 02783^T	RE	
	E. cloacae	ATCC23355	AE	
	E. cloacae	CIP 103441	AE	
	E. cloacae	CRP12BA1SC	AE	Claeys et al. (2019) (58)
	E. cloacae	CRP14MC1RSC	AE	Claeys et al. (2019) (58)
	E. cloacae	CRP5MC1RSC	AE	Claeys et al. (2019) (58)
	E. cloacae	CRP30BA1SC	AE	Claeys et al. (2019) (58)
	E. cloacae	CRP17MC1SC	AE	Claeys et al. (2019) (58)
	E. cloacae	CRP42BA1SC	AE	Claeys et al. (2019) (58)
	E. intermedia	CIP105566^T	AE	
	E. sakazakii	LM W212	AE	Kim et al. (2007) (70)
	E. aerogenes	ULB 6101-02 BE1	AE	
	E. cowanii	CIP 107300^T	AE	
	E. gergoviae	LMG 05739^T	AE	
	E. amnigena	LMG 02784^T	AE	
Klebsiella sp.	*K. pneumoniae*	ATCC 13883^T	RE	
	K. variicola	CCUG 47534^T	AE	
	K. oxytoca	CCUG 29683A	AE	
	K. pneumoniae	CRP48BA1SC	AE	Claeys et al. (2019) (58)
	K. pneumoniae	CRP75BA1SC	AE	Claeys et al. (2019) (58)
	K. pneumoniae	CRP80MC1SC	AE	Claeys et al. (2019) (58)
	K. pneumoniae	CRP81BA1RSC	AE	Claeys et al. (2019) (58)
	K. pneumoniae	CRP119MC1SC	AE	Claeys et al. (2019) (58)

All cultured strains were re-identified using MALDI-TOF MS. Roche DNA extraction was used for the *E. cloacae* and *K. pneumoniae* type strain. All the other strains were extracted by means of alkaline lysis. AE: alkaline extraction. Maldi-TOF: Matrix Assisted Laser Desorption/Ionization Time-of-flight Analyzer. RE: Roche DNA extraction. T: Type strain.

All strains were cultured aerobically overnight on blood agar plates (Biomerieux) at 37 °C. As part of quality control, they were re-identified at LBR (Ghent University, Ghent, Belgium) using Maldi-TOF according to the manufacturer's protocol (direct spot method). Except for the *E. cloacae* and *K. pneumoniae* type strains (Roche extraction, see standard dilution series), the bacterial DNA of the cultured *Enterobacter* sp. and *Klebsiella* sp. strains was extracted using alkaline lysis. Briefly, a single colony was picked up from the blood agar plate and dissolved in 20 µl alkaline lysis buffer (ALB) (0.25% SDS (Sodium Dodecyl Sulfate) and 0.05 N NaOH (Sodium Hydroxide)). The cell membrane was disrupted by SDS, by denaturizing the protein components and solubilizing the phospholipid component, leading to lysis and release of the cell contents. The cell wall itself was

broken down by NaOH. After suspending the colony in ALB, the mixture was heated for 15 min at 95 °C to release the chromosomal DNA from the bacteria. Subsequently, the tubes were briefly centrifuged and 180 µl sterile HPLC (High Performance Liquid Chromatography) water was added to neutralize the pH. Finally, the tubes were centrifuged during 5 min at 13 000 x g to spin down the bacterial cell debris. The supernatant was used as DNA extract.

Testing of primers and optimal annealing temperature

To wet-lab test the two best-scoring primers from the *in silico* analysis and simultaneously determine the optimal annealing temperature, a gradient PCR with DNA from the cultured *E. cloacae* (LMG 02783^T) and *K. pneumoniae* (ATCC 13883^T) type strains was carried out. The PCR reactions were performed in a final volume of 10 µl, containing 5 µl FASTSTART mastermix® (Roche), 0.50 µM of both forward and reverse primers (ENBCLO_F_1/ENBCLO_R_1, ENBCLO_F_2/ENBCLO_R_2, KLEPNE_F_1/KLEPNE_R_1 and KLEPNE_F_2/KLEPNE_R_2, for respectively the *Enterobacter cloacae* PCRs and *Klebsiella pneumoniae* PCRs), 2.50 µl PCR-grade H_2O (Roche) and 2 µl template DNA or 2 µl PCR-grade H_2O as negative control.

Thermal cycling was carried out on an Applied Biosystems® Verti® 96-well thermal cycler (Thermo Fisher). The cycling conditions were as follows: pre-incubation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s; primer annealing at 50-52-54-56-58-60 °C and 48-51-54-57-60-63 °C for 40 s, for respectively *Klebsiella pneumoniae* PCR and *Enterobacter cloacae* PCR, and primer extension at 72 °C for 30 s. A final extension step for 5 min at 72 °C was added.

The amplified DNA was evaluated by agarose gel electrophoresis. A 1% agarose gel (200 ml) was prepared by solubilizing 2 g of agarose in 200 ml TAE 1x buffer (Tris Acetate-EDTA) by means of boiling. When the solution was cooled down until 55 °C, 4 µl of 10 mg/ml EtBr (Ethidium Bromide) was added. After rotating the beaker to mix EtBr, the gel was gently poured out in the holder with well combs. Finally, the gel was placed during 15 min at room temperature and 15 min at 4 °C to stiffen.

Before loading the PCR products in the slots, 1.50 µl of sample buffer (5xSB) was added to 6 µl of sample. A total of 5 µl of this diluted sample was loaded in the corresponding slots. As a reference, 4 µl of a 100 base pairs (bp) ladder (GeneRuler, Thermofisher) was pipetted into a slot. The agarose gel was run for 25 min at 160 mV on a electrophoresis system (Biorad). Afterwards, a photograph of the gel was made under UV light using the Gel Doc XR$^+$ system® (Biorad).

To profoundly determine the specificity of the finally selected primers, a PCR with DNA from several *Enterobacter* and *Klebsiella* species was carried out (**Table 3**). As a control, the *E. cloacae*

(LMG 02783^T) and *K. pneumoniae* (ATCC 13883^T) type strains were also included. The PCR reactions were performed in a final volume of 10 µl, containing 5 µl FASTSTART mastermix® (Roche), 0.50 µM of both forward and reverse primers (ENBCLO_F_1/ ENBCLO_R_1 and KLEPNE_F_2/ KLEPNE_R_2 for respectively *E. cloacae* PCR and *K. pneumoniae* PCR), 2 µl PCR-grade H_2O (Roche) and 2 µl template DNA or 2 µl PCR-grade H_2O as negative control.

Thermal cycling was carried out on an Applied Biosystems® Verti® 96-well thermal cycler (Thermo Fisher). The cycling conditions were as follows: pre-incubation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s; primer annealing at 60 °C and 63 °C for 40 s, for respectively *K. pneumoniae* PCR and *E. cloacae* PCR, and primer extension at 72 °C for 30 s. Finally, a final extension step for 5 min at 72 °C was added. The amplified DNA was evaluated by agarose gel electrophoresis, according to the manufacturer's protocol (see above).

Enterobacter cloacae and *Klebsiella pneumoniae* qPCR assays

Finally, the appropriate PCR protocol was translated into a ResoLight-based qPCR assay. The qPCR reactions were performed in a final volume of 10 µl, containing 5 µl Roche LC480 high resolution melting (HRM) mix®, 0.50 µM of both forward primer (ENBCLO_F_1/KLEPNE_F_2) and reverse primer (ENBCLO_R_1/KLEPNE_R_2), 3 µM of $MgCl_2$ (Roche), 1.30 µl PCR-grade H_2O (Roche) and 2 µl template DNA or 2 µl PCR-grade H_2O as negative control. The HRM mix contains ResoLight as a dsDNA (double strand DNA) saturating dye.

Thermal cycling was carried out on a LightCycler® 480 (Roche). The cycling conditions for *E. cloacae* were as follows: pre-incubation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s; primer annealing at 63 °C for 30 s and primer extension at 72 °C for 10 s. For *K. pneumoniae*, following cycling conditions were used: pre-incubation at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 15 s; primer annealing at 60 °C for 40 s and primer extension at 72 °C for 30 s. Subsequently, a melting curve was generated with the following protocol: 5 s at 95 °C, 1 min at 55 °C, followed by a gradual increase in temperature from 60 °C to 97 °C, using a ramp rate of 0.02 °C per s. Finally, a cooling step of 30 s at 40 °C was carried out.

The standard dilution series of *E. cloacae* (LMG 02783^T) or *K. pneumoniae* (ATCC 13883^T), as well as DNA from three *E. cloacae* or *K. pneumoniae* strains, isolated from neonates showing signs of EONS in Bukavu (DRC), were added in each qPCR run. Furthermore, 10 NTC were used in every qPCR run. The standard dilution series and samples were all run in duplicate.

Results were analyzed with the standard LightCycler® 480 Software, version 1.5 (Roche). For each sample, a melting curve was generated and concordance with the melting curves of the standard samples or isolated strains from Bukavu (DRC) was considered. If a specific melting curve was established, quantitation cycles (Cq) values were used to quantify *E. cloacae* or *K. pneumoniae* concentration. A mean Cq value was calculated if the difference between the Cq values of the duplicates was less than 0.50. If not, the lowest Cq value was considered for further analysis. Afterwards, an assumption of the concentration was calculated by extrapolation. A limit of quantification (LOQ) was estimated for the three qPCR assays based on the average between the Cq value of the last reliable amplification of the diluted standard series and the Cq value of the first unreliable amplification.

2.6 Data management and analysis

All raw data were entered twice into CSPro 7.1. by two independent data entry clerks. Both data entries were then compared for inconsistencies and a final database was exported to SPSS. IBM SPSS Statistics software version 25.0 (IBM Corp.) was used for the statistical analysis of the data.

All analyses were carried out on the subset of 330 pregnant women, according to the availability of a CVL. The prevalence of vaginal carriage of *Candida*, *E. cloacae* and *K. pneumoniae* was reported as percentages with a 95% confidence interval (CI). Furthermore, for each of the three investigated species in this current master thesis, several multivariate models were built in order to identify risk factors, signs and symptoms and adverse pregnancy outcomes.

The presence of *Candida*, *E. cloacae* and *K. pneumoniae* in the vaginal microflora, determined by means of qPCR, were used as dependent variables. The independent variables were divided into three different groups: risk factors, signs and symptoms and APOs. For clarity, all the risk factors considered in univariate analysis were divided into categories, more particularly sociodemographic characteristics, living circumstances, medical history, usus, reproductive health, prevention in current pregnancy, sexual behavior, toilet hygiene and vaginal hygienic practices. Signs and symptoms were defined as general and vaginal signs and symptoms, indicated by the pregnant woman and obtained by clinical examination. The various mentioned vaginal symptoms were the presence of abnormal discharge, itching, burning sensation after sexual intercourse and vaginal malodor. APOs were characterized as events around delivery and neonatal outcomes.

The mean outcome measures in this master thesis were preterm birth (PTB), low birth weight (LBW) and signs of early-onset neonatal sepsis (EONS) (abnormal general state, respiratory

insufficiency and aberrant temperature). According to the World Health Organization (WHO), PTB was defined as birth before 37 WGA. Neonates born before 32 WGA were considered as very preterm and those between 32 and 36 WGA as moderate preterm (18). LBW was defined as a birth weight less than 2500 g (18). The diagnosis of EONS was suspected when neonates showed signs of generalized sepsis like abnormal general state, respiratory distress and fever, hence, a blood culture was taken.

In order to build the multivariate models, firstly, an univariate logistic regression for all independent variables was carried out. For each variable, the crude odds ratio (COR) with the 95% CI, and a p-value were calculated. BV was considered as an important confounder for the analysis of vaginal *Candida* carriage, so univariate logistic regressions, stratified for BV, were performed.

Afterwards, three multivariate models were created for *Candida*, *E. cloacae* and *K. pneumoniae*, according to the different groups of independent variables. In order to not overfit our multivariate models, variables were restricted in order to the number of cases positive for respectively *Candida*, *E. cloacae* and *K. pneumoniae,* i.e. maximum one degree of freedom per 10 cases (71). Variables included in the multivariable models were selected as follows: Firstly, independent variables found to be significantly associated ($p \leq 0,05$) with *Candida*, *E. cloacae* or *K. pneumoniae* carriage were considered to be included in the respective multivariable models. Subsequently, only one of two variables that were thought to be possibly related (e.g. white blood cells on Gram stain and on urine dipstick), was kept, to avoid collinearity. Furthermore, variables were excluded from the multivariate models after establishing an obvious, explanatory link between the independent variable and the carriage of *Candida*, *E. cloacae* and/or *K. pneumoniae* (e.g. *Candida* on Gram stain microscopy and *Candida* on qPCR). Consequently, antibiotic administration in the past two weeks and BV were included in each multivariate model, as they are generally considered as important confounding factors regarding the vaginal microflora. Parity was added to the multivariate model aiming to establish an association between APOs and the carriage of *Candida*, as parity has been shown to be an important confounder for PTB (72). The final selection was based on clinical relevance/expertise. The multivariate models were obtained and presented with the adjusted OR (AOR) with the 95% CI and p-value.

As the syndromic approach is the standard of care in Bukavu (DRC), additional univariate logistic regressions were performed to assess the typical symptoms for BV and *Candida* carriage. A regression between *Candida* on qPCR and BV on Gram stain microscopy, stratified for respectively BV on Gram stain microscopy and *Candida* on qPCR, and their statistic significant

symptoms, as established in previous univariate analyses, was performed. For each variable, the COR with the 95% CI and a p-value were obtained.

Furthermore, the link between the concentration of *Candida* on qPCR and the presence of yeast cells/hyphae on microscopy was examined. A log transformation of the *Candida* concentrations was performed in order to obtain a normal distribution, after which three less or more equal concentration groups were created: low – moderate – high concentration. The same protocol was carried out for wet mount microscopy. Finally, two histograms were plotted to determine the relationship between *Candida* concentration, established on qPCR, and the presence of *Candida* on Gram stain/wet mount microscopy. Afterwards, univariate logistic regressions were carried out to investigate the association between the different concentration groups of *Candida* and significant APOs, as established in previous univariate analyses. For each variable, the COR with the 95% CI and a p-value were obtained.

Subsequently, the impact of Femaclin® (200 mg clotrimazole and 100 mg clindamycin) administration was examined by comparing the presence of yeast cells/hyphae on microscopy at V1 and V2. Moreover, the impact of Femaclin® administration on APOs was investigated by performing univariate logistic regressions between the presence of *Candida* at V1, stratified for Femaclin® administration at V1, and significant APOs, as established in previous univariate analyses.

Finally, the association between hyphae on Gram stain microscopy at V1 and APOs was examined by performing univariate logistic regressions.

For all analyses, the significance level was set at 0.05.

3. Results

3.1 Study participants

Flowchart of the study participants (AVEONS)

At baseline, 750 pregnant women were screened (**Figure 5**). After screening for eligibility, using the in/exclusion criteria, 533 pregnant women were enrolled in the study protocol (V1: Visit 1). Furthermore, from the 533 women examined at V1, 104 women (19.51%) withdrew from the cohort. The main reason for this drop-out was probably the worsening sociopolitical situation in the city of Bukavu (Democratic Republic of the Congo (DRC)) during the study period (around election time), leading to increased insecurity and difficulty to travel safely to and from the hospital. Another 26 women dropped out of the study, because of pregnancy loss (unknown cause) in the second trimester. Together, only 354 pregnant women were seen again at visit 2 (V2). Finally, 66 women decided to deliver at home, because of the tense atmosphere in the city of Bukavu (DRC), resulting in 288 term births at Provincial Referral Hospital of Bukavu (PRHB). Together with the 49 preterm neonates, a total of 337 neonates were followed during the first week of their life. A total drop-out of 196 pregnant women was observed in the AVEONS project.

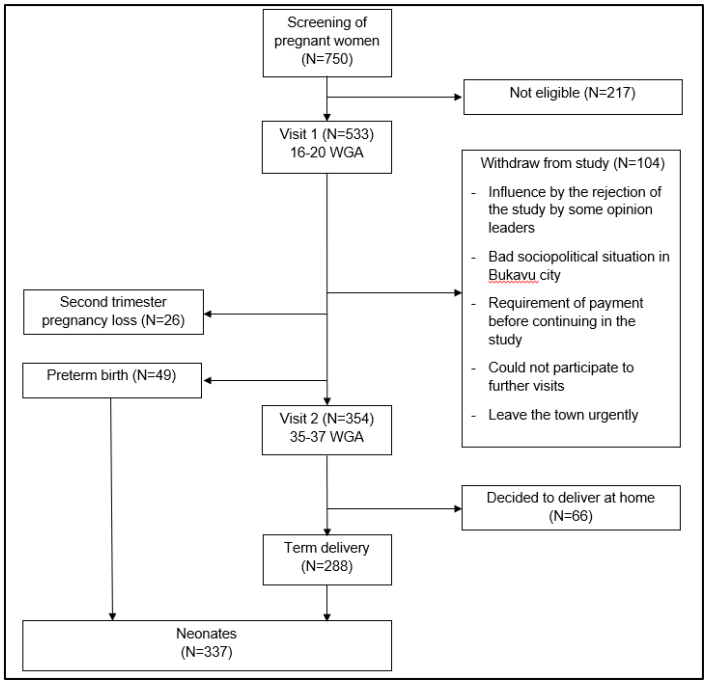


Figure 5. Flowchart of the AVEONS study. N: number of people. WGA: weeks of gestational age.

Characteristics of the study population

In this master thesis, a subset of 330 pregnant women was created, based on the availability of a cervicovaginal (CVL) sample. The mean age of these included pregnant women was 28.40 (95% CI: 27.77-29.03) years old. The pregnant women lived in three districts of Bukavu: Kadatu (34.67%), Ibanda (44.59%) and Bagira (20.74%). The majority of the participants were married

(94.86%), lived below the threshold of poverty (72.50%), belonged to Christian religion (61.70%), were from the Shi tribe (67.10%), had at least one baby (76.97%) and reached at least secondary school (89.66%).

The mean gestational age at V1 was 19.70 (95% CI: 19.4-20.1) weeks. Vaginal symptoms were frequently reported at this time point: 48.80% of the pregnant women experienced vaginal discharge, 41.50% reported vaginal itching, 26.50% mentioned dysuria, 33.20% experienced a burning sensation after sexual intercourse and 25.90% reported a vaginal malodor. Bacterial vaginosis (BV), determined by means of microscopy of a Gram stained vaginal smear at V1, occurred in 27.90% of the pregnant women.

Half of the neonates (50.50 %) were female. Considering the AVEONS research project (N=533), preterm birth (PTB) occurred in 49 (9.19%) cases, while EONS was suspected in 10 neonates (2.97%). In this master thesis, containing a subset of 330 pregnant women, 204 neonates were observed during the first week of their life. A total of 30 (14.85%) of these neonates were born preterm, more precisely, 2 (0.99%) between 28 and 32 weeks of gestational age (WGA) and 28 (13.86%) between 32 and 36 WGA. Low birth weight (LBW) was observed in 3.43% (N=7) of the neonates. A total of 2.94% (N=6) of the neonates showed signs of generalized sepsis in the first week of life, hence, blood cultures were taken.

3.2 *Enterobacter cloacae* and *Klebsiella pneumoniae* qPCR assay validation

The *in silico* analysis of already published *Enterobacter cloacae* (*E. cloacae)* and *Klebsiella pneumoniae* (*K. pneumoniae*) primer pairs is shown in **Addendum 1 and 2**, respectively. The two best scoring primer pairs for both *E. cloacae* and *K. pneumoniae*, resulting from this *in silico* analysis, are shown in **Table 3** and were purchased for further testing and validation.

Table 3. **Primers specific for *Enterobacter cloacae* and *Klebsiella pneumoniae* withheld after *in silico* analysis for further wet lab testing.**

Species	Primer name	Primer sequence (5'-3')	Targeted gene	Amplicon size (bp)	Reference
Enterobacter cloacae	ENBCLO_F_1	CTG CGT CAG ATC GTG TCC AA	Hsp60	44	Ohad et al. (2014) (73)
	ENBCLO_R_1	CGT TGT AAC CGT AGT TAC CTT CAC C			
	ENBCLO_F_2	AAA TCC CTT TGC TGT GCC CTG	AmpC	657	Hoffmann et al. (2012) (74)
	ENBCLO_R_2	CCA GGC GTA ATG CGC CTC TTC			
Klebsiella pneumoniae	KLEPNE_F_1	CCG CGG ACT ATC TCG ACT ATA T	aldA	192	Trung et al. (2018) (75)
	KLEPNE_R_1	CGA TGG CAT TAT TGG GCG TAA ATT			
	KLEPNE_F_2	GTG CGA TGC GGT CTT TG	phoE	398	Kaushik et al. (2012) (76)
	KLEPNE_R_2	GGG CGA ACT GAA CTG ATG			

After *in silico* analysis, the two best scoring primer pairs were purchased for further wet-lab testing. aldA: gene for aldehyde dehydrogenase. AmpC: chromosomal gene of the Bush type 1 cephalosporinase of *E. cloacae*. Bp: base pairs. Hsp60: heat shock protein 60. phoE: Outer membrane phosphate porin.

Results from the gradient PCRs showed that ENBCLO_F_1/ENCLO_R_1 was the most efficient primer pair for *E. cloacae* amplification, as the brightest bands (indicating most amplification) were observed with this primer pair **(Addendum 3.1)** (73). Additionally, the most efficient annealing temperature for this primer pair was 63 °C **(Addendum 3.1)**. Concerning the *K. pneumoniae* primers pairs, KLEPNE_F_2/KLEPNE_R_2 showed slightly brighter bands. The most efficient annealing temperature was found to be 60 °C **(Addendum 3.3)** (76).

Subsequently, primer pairs ENBCLO_F_1/ENCLO_R_1 and KLEPNE_F_2/KLEPNE_R_2 were further tested for specificity. The PCR, using ENBCLO_F_1/ENCLO_R_1 as primer pair, yielded some amplification for *Enterobacter sakazakii* (*E. sakazakii*) and *Enterobacter aerogenes* (*E. aerogenes*) **(Addendum 3.2)**. The KLEPNE_F_2/KLEPNE_R_2 primer pair also amplified DNA of *Klebsiella variicola* (*K. variicola*) **(Addendum 3.3)**. Other tested *Enterobacter* species (i.e., *Enterobacter intermedia*, *Enterobacter cowanii*, *Enterobacter gergoviae* and *Enterobacter amnigena*) and *Klebsiella* species (i.e., *Klebsiella oxytoca* and *Klebsiella aerogenes*) showed no amplification on agarose gel **(Addendum 3.2 and 3.3, respectively)**.

Afterwards, PCR protocols were translated into qPCR assays. Using melting curve analysis, the *E. cloacae* qPCR assay was able to differentiate between *E. cloacae* (melting temperature (Tm): 83.96 °C), *E. sakazakii* (Tm: 84.15 °C) and *E. aerogenes* (Tm: 83.02 °C). Melting temperatures of *E. cloacae* and *E. sakazakii* were closely related, but amplification of *E. sakazakii* was rather weak. The melting temperature of *K. pneumoniae* (Tm: 92.11 °C) allowed to discriminate with amplification of *K. variicola* (Tm: 90.50 °C).

The limit of quantification (LOQ) was estimated for both the *E. cloacae* and *K. pneumoniae* qPCR assay. This LOQ was pragmatically determined based on the average between the Cq value of the last reliable amplification of the diluted standard series and the Cq value of the first unreliable amplification. Particularly, the LOQ was 32.56 and 37.11, for respectively *E. cloacae* and *K. pneumoniae*. Moreover, all negative template controls (NTC) gave either no amplification or nonspecific amplification (primer dimer) for both the *E. cloacae* and *K. pneumoniae* qPCR assay.

3.3 Vaginal carriage rates of *Candida*, *Enterobacter cloacae* and *Klebsiella pneumoniae*

A total of 330 CVLs were collected from the 533 pregnant women. The vaginal carriage rates of *Candida, E. cloacae* and *K. pneumoniae* were determined with qPCR on DNA extracted from these 330 CVLs. **Table 4** represents the vaginal carriage rates of *Candida, E. cloacae* and *K. pneumoniae* with the 95% confidence interval. A total of 38.18% of the pregnant women were colonized with *Candida*. Melting curve analysis was used to differentiate between the multiple *Candida* spp. and *Saccharomyces cerevisiae* (*S. cerevisiae)*, which was also detected by the

Candida qPCR (67). The vast majority of the pregnant women were colonized with *Candida* spp. (96.80%), while *S. cerevisiae* was found in the other 3.20% of the cases. Among the *Candida* spp., *Candida albicans* was identified as the most prevalent one (91.00%). The remaining non-albicans *Candida* spp. (9.00%) were *Candida famata* (*C. famata*), *Candida glabrata* (*C. glabrata*), *Candida dubliensis* (*C. dubliensis*), *Candida inconspicua* (*C. inconspicua*), *Candida kefyr* (*C. kefyr*), *Candida krusei* (*C. krusei*) and *Candida tropicalis* (*C. tropicalis*). Vaginal *E. cloacae* and *K. pneumoniae* carriage were observed in, respectively, 42.24% and 12.12% of the pregnant women.

In the context of linearity, the term '*Candida*', used in this master thesis, covers the detected *Candida* spp. as well as the four cases of *S. cerevisiae*.

Table 4. Vaginal carriage rates of *Candida*, *Enterobacter cloacae* and *Klebsiella pneumoniae*.

Species	n / 330	Prevalence (% and (95% CI))
Yeast	126	38.18 (33.10-43.53)
C. albicans	111	33.64 (28.75-38.90)
C. famata	2	0.61 (0.17-2.18)
C. glabrata	1	0.30 (0.05-1.70)
C. dubliensis	3	0.91 (0.31-2.64)
C. inconspicua	1	0.30 (0.05-1.70)
C. kefyr	1	0.30 (0.05-1.70)
C. krusei	2	0.61 (0.17-2.18)
C. tropicalis	1	0.30 (0.05-1.70)
S. cerevisiae	4	1.21 (0.47-3.07)
E. cloacae	140	42.42 (37.21-47.81)
K. pneumoniae	40	12.12 (9.03-16.09)

This table shows the amount of cases, the prevalence and corresponding 95% CI for each species. *C. albicans*: *Candida albicans*. *C. famata*: *Candida famata*. *C. glabrata*: *Candida glabrata*. *C. dubliensis*: Candida dubliensis. *C. inconspicua*: *Candida inconspicua*. *C. kefyr*: *Candida kefyr*. *C. krusei*: *Candida krusei*. *C. tropicalis*: *Candida tropicalis*. CI: Confidence interval. *E. cloacae*: *Enterobacter cloacae*. *K. pneumoniae*: *Klebsiella pneumoniae*. N: number of positive cases. *S. cerevisiae*: *Saccharomyces cerevisiae*.

A distinction between quantifiable and non-quantifiable samples was made based on the LOQ of the qPCR assays (35.12, 32.56 and 37.11 for, respectively, the *Candida*, *E. cloacae* and *K. pneumoniae* qPCR assay). **Table 5** shows the quantitative aspects of vaginal carriage of *Candida, E. cloacae* and *K. pneumoniae.* The concentration of *Candida* in the CVLs was sufficiently high to quantify, whereas the concentration of *E. cloacae* in the samples appeared to be too low to quantify. *K. pneumoniae* was quantifiable in approximately two thirds of the samples.

Table 5. Quantitative aspects of vaginal *Candida, Enterobacter cloacae* and *Klebsiella pneumoniae* carriage.

Species	Average concentration (cells/ml)	Quantifiable (n/N)
Candida	3.95log2 - 5.25log6	126/126
C. albicans		111/111
C. famata		2/2
C. glabrata		1/1
C. dubliensis		3/3
C. inconspicua		1/1
C. kefyr		1/1
C. krusei		2/2
C. tropicalis		1/1
S. cerevisiae		4/4
E. cloacae	< 2.5log3	0/140
K. pneumoniae	3.63log2 - 6.25log6	26/40

A distinction between quantifiable and non-quantifiable samples was made based on the LOQ of the qPCR assays (35.12 Cq, 32.56 Cq and 37.11 Cq for, respectively, the *Candida*, *E. cloacae* and *K. pneumoniae* qPCR assay). *C. albicans*: *Candida albicans*. *Candida famata*. *C. glabrata*: *Candida glabrata*. *C. dubliensis*: Candida dubliensis. *C. inconspicua*: *Candida inconspicua*. *C. kefyr*: *Candida kefyr*. *C. krusei*: *Candida krusei*. *C. tropicalis*: *Candida tropicalis*. Cq: quantification cycles. *E. cloacae*: *Enterobacter cloacae*. *K. pneumoniae*: *Klebsiella pneumoniae*. LOQ: limit of quantification. n: number of quantifiable cases. N: number of all positive cases. qPCR: quantitative polymerase chain reaction. *S. cerevisiae*: *Saccharomyces cerevisiae*.

3.4 *Candida*: risk factors, signs and symptoms and adverse pregnancy outcomes

Addendum 4 represents the univariate associations between vaginal *Candida* carriage (determined by means of qPCR) and risk factors, signs and symptoms and adverse pregnancy outcomes (APO).

3.4.1 Independent risk factors associated with vaginal *Candida* carriage

Our multivariate model on risk factors for *Candida* carriage, adjusted for antibiotic use, is documented in **Table 6**. A positive association was observed with women who lived less than five years with their husband (Adjusted odds ratio (AOR): 1.92; 95% CI: 1.13-3.24; p=0.015), the use of pit toilets (compared to a flush toilet) (AOR: 2.39; 95% CI: 1.26-4.54; p=0.008) and an intermediate vaginal microflora (determined by Nugent scoring (59), compared to a healthy vaginal microflora) (AOR: 3.54; 95% CI: 1.82 - 6.91; p<0.001).

Table 6. Multivariate regression model showing the association between vaginal *Candida* carriage (qPCR) and risk factors.

	n	Number of *Candida* positive women (%)	Crude OR (95% CI)	p-value	Adjusted OR (95%CI)	p-value
Duration of life with husband	**314**	**119 (37.9)**				
≤5 years	166	73 (44.0)	1.74 (1.10-2.77)	**0.02**	1.92 (1.13-3.24)	**0.015**
>5 years	148	46 (31.1)	Ref.	-	Ref.	-
Known serologic HIV state of pregnant woman	**315**	**122 (38.7)**				
Yes	212	72 (34.0)	Ref.	-	Ref.	-
No	103	50 (48.5)	1.83 (1.14-2.96)	**0.01**	1.63 (0.94-2.81)	0.083
Type of toilet of pregnant woman	**330**	**126 (38.2)**				
Toilet with bowl and flush	81	20 (24.7)	Ref.	-	Ref.	-
Other types[1]	249	106 (42.6)	2.26 (1.29-3.97)	**0.01**	2.39 (1.26-4.54)	**0.008**
BV on Gram stain at V1[2]	**326**	**125 (38.4)**				
No BV	176	50 (28.5)	Ref.	-	Ref.	-
Intermediate	59	35 (59.4)	3.68 (1.99-6.79)	**<0.001**	3.54 (1.82-6.91)	**<0.001**
BV	91	40 (44.0)	1.98 (1.17-3.35)	**0.011**	1.76 (0.95-3.25)	0.070
Gram + cocci on Gram stain at V1	**326**	**125 (38.4)**				
Yes	31	17 (54.9)	2.10 (1.00-4.43)	**0.05**	1.64 (0.70-3.82)	0.260
No	295	108 (36.7)	Ref.	-	Ref.	-
Hemoglobin on Hemocue® at V1[3]	**328**	**125 (38.2)**				
Anemia (<11 Hb)	12	8 (66.7)	3.40 (1.00-11.54)	**0.05**	3.70 (0.97-14.08)	0.055
Normal (≥11 Hb)	316	117 (37.1)	Ref.	-	Ref.	-
Antibiotic administration two weeks before V1	**328**	**124 (37.8)**				
Yes	46	19 (41.3)	1.19 (0.63-2.24)	0.60	1.27 (0.61-2.66)	0.520
No	282	105 (37.2)	Ref.	-	Ref.	-

Vaginal *Candida* carriage was significantly positively associated with women who lived less than five years with their husband, the use of pit toilets and an intermediate vaginal microflora. Bold numbers indicate a p-value ≤ 0.05. [1]Squat latrine and pit latrine. [2]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV) (59). [3]Device that measures the hemoglobin concentration by means of spectrophotometry. BV: bacterial vaginosis. CI: confidence interval. Hb: hemoglobin. N: number of samples. OR: odds ratio. Ref.: reference. V1: visit 1 (16-20 weeks of gestational age).

Stratification for bacterial vaginosis

To investigate whether BV was a confounding factor regarding the associations between vaginal *Candida* carriage and the different risk factors, data were stratified for the presence of BV, before carrying out univariate logistic regressions for risk factors. **Addendum 7** represents univariate associations between vaginal *Candida* carriage, stratified for BV, and risk factors.

Living together with the husband for less than five years (Crude odds ratio (COR): 1.98; 95% CI: 1.13-13.02; p=0.017), knowledge of the HIV state of the pregnant women (COR: 1.93; 95% CI: 1.09-3.41; p=0.024) and non-employment (COR: 1.88; 95% CI: 1.10-3.23; p=0.022) were significantly associated with vaginal *Candida* carriage without the presence of BV. Concomitant

BV and vaginal *Candida* carriage were significantly associated with the use of pit toilets (compared to a flush toilet) (COR: 3.09; 95% CI: 1.09-8.75; p=0.034).

3.4.2 Symptoms associated with vaginal *Candida* carriage

Our multivariate model on *Candida* signs and symptoms, adjusted for BV and antibiotic administration, is shown in **Table 7**. A positive association was observed between vaginal *Candida* carriage and experienced vaginal discharge (AOR: 2.30; 95% CI: 1.25-4.23; p=0.008), vaginal itching (AOR: 2.70; 95% CI: 1.57-4.97; p=0.001) and a burning sensation after sexual intercourse (AOR: 3.06; 95% CI: 1.69-5.54; p<0.001). Furthermore, positive associations were found with weight loss during pregnancy (AOR: 3.24; 95% CI: 1.21-8.65; p=0.019), women with an arm circumference between 22.5-27 cm (compared to arm circumference >27 cm) (AOR: 2,34; 95% CI: 1,21-4,53; p=0.012), the presence of 5-30 white blood cells (WBC) per field on wet mount microscopy (compared to 1-4 WBC per field) (AOR: 2.12; 95% CI: 1.17-3.86; p=0.014) and thick and heterogenous vaginal secretions established during speculum examination at V1 (AOR: 4.62; 95% CI: 1.39-15.30; p=0.012).

Table 7. Multivariate regression model showing the association between vaginal *Candida* carriage (qPCR) and signs and symptoms.

	n	Number of *Candida* positive women (%)	Crude OR (95% CI)	p-value	Adjusted OR (95%CI)	p-value
Cough at V1	**327**	**125 (38.2)**				
Yes	72	20 (27.8)	Ref.	-	Ref.	-
No	255	105 (41.2)	1.82 (1.03-3.23)	**0.04**	1.75 (0.85-3.58)	0.129
Vaginal discharge at V1	**326**	**124 (38.0)**				
Yes	159	83 (52.2)	3.36 (2.10-5.37)	**<0.001**	2.30 (1.25-4.23)	**0.008**
No	167	41 (24.6)	Ref.	-	Ref.	-
Vaginal itching at V1	**328**	**125 (38.1)**				
Yes	136	79 (58.1)	4.40 (2.74-7.08)	**<0.001**	2.70 (1.57-4.97)	**0.001**
No	192	46 (24.0)	Ref.	-	Ref.	
Burning after sexual contact at V1	**313**	**118 (37.7)**				
Yes	104	64 (61.5)	4.59 (2.78-7.59)	**<0.001**	3.06 (1.69-5.54)	**<0.001**
No	209	54 (25.8)	Ref.	-	Ref.	-
Weight evolution during pregnancy[1]	**330**	**126 (38.2)**				
Weight loss	87	38 (43.7)	2.212(1.06-4.65)	**0.04**	3.24 (1.21-8.65)	**0.019**
Stable weight or ≤5 kg weight gain	189	74 (39.2)	1.84 (0.94-3.61)	0.08	2.14 (0.89-5.19)	0.091
> 5kg weight gain	54	14 (26.0)	Ref.	-	Ref.	-
Arm circumference (cm) at V1	**328**	**124 (37.9)**				
<22	25	9 (36.0)	1.47 (0.58-3.70)	0.42	0.95 (0.26-3.50)	0.944
22-27.5	202	87 (43.1)	1.97 (1.18-3.31)	**0.01**	2.34 (1.21-4.53)	**0.012**
>27.5	101	28 (27.8)	Ref.	-	Ref.	-

White blood cells per field on wet mount microscopy at V1	**330**	**126 (38.2)**				
0	0	0 (0.0)	-	-		
1-4	178	51 (28.7)	Ref.	-	Ref.	-
5-30	132	63 (47.8)	2.274 (1.419-3.643)	**0.00**	2.12 (1.17-3.86)	**0.014**
30+	20	12 (60.0)	3.735 (1.442-9.676)	**0.01**	1.62 (0.41-6.41)	0.495
State of vaginal secretions at V1	**330**	**126 (38.2)**				
Normal[2]	297	99 (33.4)	Ref.	-	Ref.	-
Abnormal[3]	33	27 (81.9)	9.00 (3.60-22.51)	**<0.001**	4.62 (1.39-15.30)	**0.012**
BV on Gram stain[4] at V1	**326**	**125 (38.4)**				
No BV	176	50 (28.5)	Ref.	-	Ref.	-
Intermediate	59	35 (59.4)	3.68 (1.99-6.79)	**<0.001**	2.46 (1.12-5.43)	**0.025**
BV	91	40 (44.0)	1.98 (1.17-3.35)	**0.011**	1.44 (0.74-2.81)	0.28
Antibiotic administration two weeks before V1	**328**	**124 (37.8)**				
Yes	46	19 (41.3)	1.19 (0.63-2.24)	0.60	1.12 (0.60-2.09)	0.732
No	282	105 (37.2)	Ref.	-	Ref.	-

Vaginal *Candida* carriage was significantly positively associated with vaginal discharge, vaginal itching, a burning sensation after sexual intercourse, weight loss during pregnancy, women with an arm circumference between 22.5-27 cm, the presence of 5-30 white blood cells per field on wet mount microscopy and abnormal vaginal secretions during speculum examination at V1. Bold numbers indicate a p-value ≤ 0.05. [1] Weight evolution determined based on the difference between the last known weight before conception and weight measured in the study at V1. [2] Fine and homogeneous secretions. [3] Thick and heterogeneous secretions. [4]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV) (59). BV: bacterial vaginosis. CI: confidence interval. N: number of samples. OR: Odds ratio. Ref.: reference. V1: visit 1 (16-20 weeks of gestational age).

Stratification for bacterial vaginosis

To investigate whether BV was a confounding factor regarding the associations between vaginal *Candida* carriage and complaints, data were stratified for the presence of BV, before carrying out univariate logistic regressions for signs and symptoms. **Addendum 7** represents univariate associations between vaginal *Candida* carriage, stratified for BV, and signs and symptoms.

Vaginal discharge (COR: 3.97; 95% CI: 2.25-7.03; p<0.001), vaginal itching (COR: 3.78; 95% CI: 2.15-6.65; p<0.001), burning sensation after sexual intercourse (COR: 5.41; 95% CI: 2.93-9.97; p<0.001), weight loss during pregnancy (COR: 3.22; 95% CI: 1.23-8.43; p=0.017), presence of 30+ WBC per field on wet mount microscopy (compared to 1-4 WBC per field) (COR: 3.80; 95% CI: 1.71-12.33; p=0.026) and thick and heterogenous vaginal secretions established during speculum examination (COR: 9.06; 95% CI: 2.94-27.96; p<0.001) were significantly associated with vaginal *Candida* carriage without presence of BV. Concomitant BV and vaginal *Candida* carriage were significantly associated with vaginal itching (COR: 5.27; 95% CI: 2.13-13.05; p<0.001), vaginal burning (COR: 2.71; 95% CI: 1.11-6.63; p=0.028), thick and heterogenous vaginal secretions established during speculum examination (COR: 7.11; 95% CI: 1.44-35.12; p=0.016) and the presence of Gram-positive cocci (COR: 3.21; 95% CI: 1.08-9.54; p=0.035).

Comparison between bacterial vaginosis and Candida, based on symptoms

Vaginal malodor is a typical symptom of bacterial vaginosis

Table 8 documents univariate associations between BV (defined by Nugent scoring (59)) and symptoms, stratified by the presence of *Candida* (qPCR). In the *Candida*-negative stratum, BV was only significantly associated with malodor (COR: 2.28; 95% CI: 1.09-4.77; p=0.028). Other symptoms as vaginal discharge (COR: 1.67; 95% CI: 0.88-3.19; p=0.119), vaginal itching (COR: 1.35; 95% CI: 0.68-2.68; p=0.391) and thick and heterogenous vaginal secretions established during speculum examination (COR: 1.48; 95% CI: 0.26-8.33; p=0.119) were not significantly associated with BV. In the *Candida*-positive stratum, no significant symptoms were associated with BV.

Table 8. Univariate associations between vaginal symptoms and bacterial vaginosis (defined by Nugent scoring (59)), stratified for the presence of *Candida* (qPCR).

	No *Candida*				*Candida*			
	n	**BV + women[1] (%)**	**Crude OR (95% CI)**	**p-value**	**n**	**BV + women[1] (%)**	**Crude OR (95% CI)**	**p-value**
Vaginal discharge at V1	**198**	**51 (25.8)**			**123**	**39 (31.8)**		
Yes	75	24 (32.0)	1.67 (0.88-3.19)	0.119	82	25 (30.5)	Ref.	-
No	123	27 (22.0)	Ref.	-	41	14 (34.2)	1.18 (0.53-2.63)	0.681
Vaginal itching at V1	**199**	**51 (25.7)**			**124**	**40 (32.3)**		
Yes	57	17 (29.9)	1.35 (0.68-2.68)	0.391	78	29 (37.2)	1.88 (0.83-4.27)	0.130
No	142	34 (24.0)	Ref.	-	46	11 (24.0)	Ref.	-
Vaginal malodor at V1	**176**	**49 (27.9)**			**116**	**35 (30.2)**		
Yes	41	17 (41.5)	2.28 (1.09-4.77)	**0.028**	33	12 (36.4)	1.49 (0.63-3.51)	0.361
No	135	32 (23.8)	Ref.	-	83	23 (27.8)	Ref.	-
State of vaginal secretions at V1	**200**	**51 (25.5)**			**125**	**40 (32.0)**		
Normal[2]	194	49 (25.3)	Ref.	-	99	31 (31.4)	Ref.	-
Abnormal[3]	6	2 (33.4)	1.48 (0.26-8.33)	0.119	26	9 (34.7)	1.16 (0.47-2.89)	0.748

Vaginal malodor seemed to be a typical symptom of bacterial vaginosis, as only this symptom is significantly positively associated with bacterial vaginosis. Bold marks reflect a p-value ≤0.05. [1]Nugent score of 7-10 (59). [2]Fine and homogeneous secretions, [3]Thick and heterogeneous secretions. CI: confidence interval. N: number of samples. OR: odds ratio. Ref.: reference. V1: visit 1 (16-20 weeks of gestational age).

Vaginal discharge, itching and a burning sensation are typical symptoms of vaginal *Candida* carriage

Table 9 documents univariate associations between vaginal *Candida* carriage (qPCR), stratified by the presence of BV (defined by Nugent scoring (59)), and symptoms. In the BV-negative stratum, vaginal *Candida* carriage was significantly associated with vaginal discharge (COR: 3.97; 95% CI: 2.25-7.03; p<0.001), vaginal itching (COR: 3.78; 95% CI: 2.15-6.65; p<0.001), a burning sensation after sexual intercourse (COR: 5.41; 95% CI: 2.93-9.97; p<0.001) and thick and heterogenous vaginal secretions established during speculum examination (COR: 9.06; 95% CI:

2.94-27.96; p<0.001). In the BV-positive stratum, vaginal itching (COR: 5.27; 95% CI: 2.13-13.05; p<0.001), burning after sexual intercourse (COR: 2.71; 95% CI: 1.11-6.63; p=0.028) and thick and heterogenous vaginal secretions established during speculum examination (COR: 7.11; 95% CI: 1.44-35.12; p<0.001) were associated with vaginal *Candida* carriage.

Table 9. Univariate associations between vaginal symptoms and *Candida* (qPCR), stratified for the presence of bacterial vaginosis (defined by Nugent scoring (59)).

	No BV[1]				BV[1]			
	n	***Candida* + women (%)**	**Crude OR (95% CI)**	**p-value**	**n**	***Candida* + women (%)**	**Crude OR (95% CI)**	**p-value**
Vaginal discharge at V1	**231**	**84 (36.4)**			**90**	**39 (43.4)**		
Yes	108	57 (52.8)	3.97 (2.25-7.03)	**<0.001**	49	25 (51.1)	2.01 (0.86-4.72)	0.11
No	123	27 (22.0)	Ref.		41	14 (34.2)	Ref.	-
Vaginal itching at V1	**232**	**84 (36.3)**			**91**	**40 (44.0)**		
Yes	89	49 (55.1)	3.78 (2.15-6.65)	**<0.001**	46	29 (63.1)	5.27 (2.13-13.05)	**<0.001**
No	143	35 (24.5)	Ref.	-	45	11 (24.5)	Ref.	-
Burning after sexual contact at V1	**221**	**80 (36.2)**			**87**	**37 (42.6)**		
Yes	70	44 (62.9)	5.41(2.93-9.97)	**<0.001**	33	19 (57.6)	2.71 (1.11-6.63)	**0.028**
No	151	36 (23.9)	Ref.	0	54	18 (33.4)	Ref.	-
State of vaginal secretions at V1	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Normal[2]	213	68 (32.0)	Ref.	-	80	31 (38.8)	Ref.	-
Abnormal[3]	21	17 (81.0)	9.06 (2.94-27.96)	**<0.001**	11	9 (81.9)	7.11 (1.44-35.12)	**0.016**

Vaginal discharge, itching and a burning sensation were typical symptoms of *Candida* carriage, as a significant positive association between these symptoms and vaginal *Candida* carriage was established. Bold marks reflect a p-value ≤0.05. [1]Nugent score of 7-10 (59). [2]Fine and homogeneous secretions, [3] Thick and heterogeneous secretions. CI: confidence interval. N: number of samples. OR: odds ratio. Ref.: reference. V1: visit 1 (16-20 weeks of gestational age).

3.4.3 Adverse pregnancy outcomes associated with vaginal *Candida* carriage

The multivariate model built to investigate the association between vaginal *Candida* carriage, adjusted for parity, BV and antibiotic use, and APOs is shown in **Table 10**. Women with vaginal *Candida* carriage at V1 had an 18-fold higher chance of giving birth to a neonate with normal birthweight (compared to a neonate with low birthweight) (AOR: 18.31; 95% CI: 1.56-214.87; p=0.021). Furthermore, vaginal *Candida* carriage was associated with the presence of meconium-stained amniotic fluid (AOR: 2.76; 95% CI: 1.22-6.25; p=0.015) and PTB (AOR: 7.21; 95% CI: 2.18-23.81; p=0.001).

Table 10. Multivariate regression model showing the association between vaginal *Candida* carriage (qPCR) and adverse pregnancy outcomes.

	n	Number of *Candida* positive women (%)	Crude OR (95% CI)	p-value	Adjusted OR (95%CI)	p-value
Low birthweight	**203**	**77 (38,0)**				
Yes (<2500 g)	7	1 (14.3)	Ref.	-	Ref.	-
No (≥2500g)	196	76 (38.8)	3.80 (0.45-32.18)	0.221	18.31 (1.56-214.87)	**0.021**
Amniotic fluid type at delivery	**204**	**78 (38.3)**				
Clear	162	54 (33.4)	Ref.	-	Ref.	-
Meconium[1] (fresh or old)	42	24 (57.2)	2.67 (1.33-5.33)	**0.006**	2.76 (1.22-6.25)	**0.015**
Duration of rupture of membranes	**199**	**78 (39.2)**				
≤6 hours	192	72 (37.5)	Ref.	-	Ref.	-
>6 hours	7	6 (85.8)	10.00 (1.18-84.75)	**0.035**	2.19 (0.21-22.62)	0.51
PTB	**202**	**77 (38.2)**				
Yes (<37w)	30	16 (53.4)	2.08 (0.95-4.55)	0.067	7.21 (2.18-23.81)	**0.001**
No (≥37w)	172	61 (35.5)	Ref.	-	Ref.	-
Parity	**330**	**126 (38.2)**				
0 children	76	27 (35.5)	1.02 (0.57-1.84)	0.94	Ref.	-
1-2 children	114	50 (43.9)	1.45 (0.87-2.41)	0.15	1.68 (0.67-4.22)	0.27
>3 children	140	49 (35.0)	Ref.	-	2.99 (1.24-7.21)	**0.015**
BV on Gram stain at V1[2]	**326**	**125 (38.4)**				
No BV	176	50 (28.5)	Ref.	-	Ref.	-
Intermediate	59	35 (59.4)	3.68 (1.99-6.79)	**<0.001**	3.18 (1.35-7.51)	**0.008**
BV	91	40 (44.0)	1.98 (1.17-3.35)	**0.011**	2.57 (1.22-5.45)	**0.014**
Antibiotic administration 2 weeks before V1	**328**	**124 (37.8)**				
Yes	46	19 (41.3)	1.19 (0.63-2.24)	0.60	1.14 (0.47-2.75)	0.780
No	282	105 (37.2)	Ref.	-	Ref.	-

Candida carriage was significantly positively associated with the presence of meconium-stained amniotic fluid and preterm birth. Bold marks reflect a p-value ≤0.05. [1]A dark greenish mass that accumulates in the bowel during fetal life and is discharged shortly after birth. [2]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV) (59). CI: confidence interval. N: number of samples. OR: odds ratio. PTB: preterm birth. Ref.: reference. V1: visit 1 (16-20 weeks of gestational age). W: weeks.

Stratification for bacterial vaginosis

To investigate whether BV was a confounding factor regarding the associations between vaginal *Candida* carriage and APOs, data were stratified for the presence of BV, before carrying out univariate logistic regressions for APOs. **Addendum 7** represents univariate associations between vaginal *Candida* carriage, stratified for BV, and APOs.

PTB (COR: 2.93; 95% CI: 1.09-7.88; p=0.033), meconium-stained amniotic fluid (COR: 4.22; 95% CI: 1.79-9.95; p=0.001) and prolonged ruptured membranes (>6 h) (COR: 9.66; 95% CI: 1.09-85.25; p=0.041) were significantly associated with vaginal *Candida* carriage, in absence of BV. Concomitant BV and vaginal *Candida* carriage were not significantly associated with APOs.

Associations between Candida concentration and adverse pregnancy outcomes

Pregnant women with established vaginal *Candida* carriage (as assessed by means of qPCR), were stratified into three less or more equal groups, according to the *Candida* concentration, i.e. low (3.95log2 *Candida* cells/ml - 8.68log3 *Candida* cells/ml), moderate (9.48log3 *Candida* cells/ml – 1.85log5 *Candida* cells/ml) and high concentration (2.24log5 *Candida* cells/ml – 5.25log6 *Candida* cells/ml) **(Addendum 8)**. The prevalence of PTB, meconium-stained amniotic fluid and prolonged ruptured of the membranes in each of these *Candida* concentration categories, as well as the corresponding univariate logistic regression analyses are shown in **Table 11**.

An increasing prevalence of PTB was observed in function of the *Candida* concentration, i.e. 11.20% (no *Candida*), 16.00% (low concentration), 19.40% (moderate concentration) and 31.30% (high concentration). Also, regarding prolonged ruptured membranes, the prevalence was a function of the *Candida* concentration, i.e. 0.80% (no Candida), 4.00% (low concentration), 5.60% (moderate concentration) and 17.60% (high concentration). The univariate analyses showed that only high concentrations of *Candida* were significantly associated with PTB (COR: 3.60; 95% CI: 1.09-11.90; p=0.035) and prolonged ruptured membranes (>6 h) (COR: 25.71; 95% CI: 2.50-264.26; p=0.006). Inversely, meconium-stained amniotic fluid was significantly associated with pregnant women carrying low (COR: 2.82; 95% CI: 1.06-7.50; p=0.037) to moderate (COR: 3.00; 95% CI: 1.28-7.05; p=0.012) concentrations of *Candida*.

Table 11. Univariate associations between adverse pregnancy outcomes and vaginal *Candida* carriage, divided according to concentration (qPCR).

	n	PTB n (%)	Crude OR (95% CI)	p-value	n	Meconium[1] AF n (%)	Crude OR (95% CI)	p-value	n	≥6h ROM n (%)	Crude OR (95% CI)	p-value
Candida	**202**	**30**			**204**	**42**			**199**	**7**		
Negative	125	14 (11.2)	Ref.	-	126	18 (14.3)	Ref.	-	121	1 (0.8)	Ref.	-
Low concentration[2]	25	4 (16.0)	1.51 (0.45-5.04)	0.503	25	8 (32.0)	2.82 (1.06-7.50)	**0.037**	25	1 (4.0)	5.00 (0.30-82.74)	0.261
Moderate concentration[3]	36	7 (19.4)	1.91 (0.71-5.18)	0.201	36	12 (30.0)	3.00 (1.28-7.05)	**0.012**	36	2 (5.6)	7.06 (0.62-80.22)	0.115
High concentration[4]	16	5 (31.3)	3.60 (1.09-11.90)	**0.035**	17	4 (23.5)	1.85 (0.54-6.30)	0.327	17	3 (17.6)	25.71 (2.50-264.26)	**0.006**

Only high concentrations of *Candida* were found to be significantly associated with PTB and prolonged rupture of membranes (≥6 hours). Inversely, meconium-stained amniotic fluid was associated with pregnant women carrying low to moderate concentrations of *Candida*. Bold marks reflect a p-value ≤0.05. [1]A dark greenish mass that accumulates in the bowel during fetal life and is discharged shortly after birth. [2]Low concentration: 3.95log2 *Candida* cells/ml - 8.68log3 *Candida* cells/ml. [3]Moderate concentration: 9.48log3 *Candida* cells/ml – 1.85log5 *Candida* cells/ml. [4]High concentration: 2.24log5 *Candida* cells/ml – 5.25log6 *Candida* cells/ml. AF: amniotic fluid. CI: confidence interval. N: number of samples. OR: odds ratio. PTB: preterm birth. Ref.: reference. ROM: rupture of membranes.

Microscopy as a tool to detect vaginal Candida carriage

The relationship between vaginal *Candida* carriage rates, as assessed by means of microscopy on wet mount and Gram stained vaginal smears, and *Candida* concentration, as assessed with qPCR on CVLs, is shown in **Figure 6**. *Candida* positivity on Gram stain microscopy was established as a function of the *Candida* concentration. A total of 48.58% (17/35), 75.39% (49/65) and 80.00% (20/25) of the vaginal smears were positive on Gram stain microscopy in the low, moderate and high concentration group, respectively.

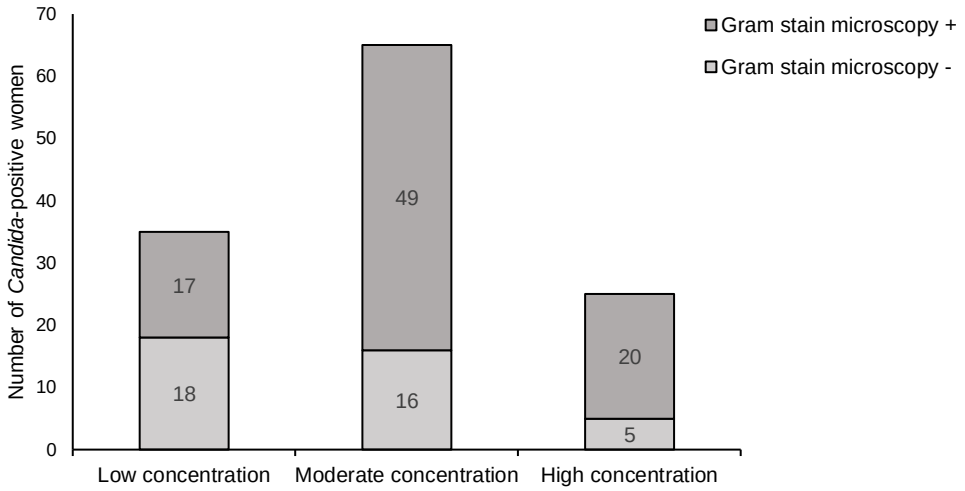


Figure 6. Histogram showing the relationship between concentration of *Candida* (established by qPCR) and the presence on Gram stain microscopy. *Candida* positivity on Gram stain microscopy was found to be a function the *Candida* concentration: the higher the *Candida* concentration, the more *Candida* cells were detected on Gram stain microscopy. A total of 48.58% (17/35), 75.39% (49/65) and 80.00% (20/25) of the vaginal smears were positive on Gram stain microscopy in the low, moderate and high concentration group, respectively. Low concentration: 3,95log2 *Candida* cells/ml - 8.68log3 *Candida* cells/ml. Medium concentration: 9.48log3 *Candida* cells/ml – 1.85log5 *Candida* cells/ml. High concentration: 2.24log5 *Candida* cells/ml – 5.25log6 *Candida* cells/ml.

The relationship between *Candida* concentration and the presence of *Candida* cells and/or hyphae on wet mount microscopy is shown in **Figure 7**. Compared to the category of low *Candida* concentration, with wet mount positivity established in only 42.11% (16/38) of the cases, the positivity was markedly higher in the moderate and high concentration categories (73.44% (47/64) and 66.67% (16/24), respectively).

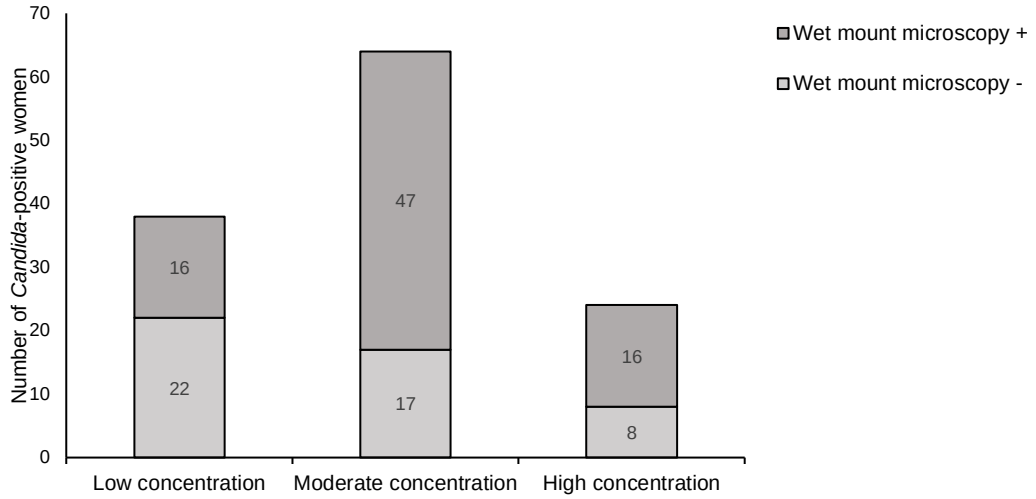


Figure 7. Histogram showing the relationship between concentration of *Candida* (assessed by means of qPCR) and the presence on wet mount microscopy. The *Candida* positivity on wet mount microscopy was found to be a function the *Candida* concentration: the higher the *Candida* concentration, the more *Candida* cells were detected on wet mount microscopy. A total of 42.11% (16/38), 73.44% (47/64) and 66.67% (16/24) of the vaginal smears were positive on wet mount microscopy in the low, moderate and high concentration group, respectively. Low concentration: 3,95log2 *Candida* cells/ml - 8.68log3 *Candida* cells/ml. Medium concentration: 9.48log3 *Candida* cells/ml – 1.85log5 *Candida* cells/ml. High concentration: 2.24log5 *Candida* cells/ml – 5.25log6 *Candida* cells/ml.

Effect hyphae on adverse pregnancy outcomes

It is suggested that hyphae may increase APOs, compared to blastopores. To investigate this assumption, univariate associations between hyphae and APOs were compared to univariate associations between general *Candida* carriage and APOs **(Table 12)**. No substantial effect of hyphae on APOs was documented.

Table 12. Comparison of univariate associations between general *Candida* presence (qPCR) and adverse pregnancy outcomes, and univariate associations between hyphae[1] (Gram stain microscopy) and adverse pregnancy outcomes.

	n	*Candida* + women (%)	Crude OR (95% CI)	p-value	n	Hyphae[1] + women (%)	Crude OR (95% CI)	p-value
PTB	**202**	**77 (38.2)**			**202**	**28 (13.9)**		
Yes	30	16 (53.4)	2.08 (0.95-4.55)	0.067	30	7 (23.4)	2.19 (0.84-5.72)	0.11
No	172	61 (35.5)	Ref.	-	172	21 (12.3)	Ref.	-
Meconium[1] amniotic fluid	**204**	**78 (38.3)**			**204**	**29 (14.3)**		
Yes	42	24 (57.2)	2.67 (1.33-5.33)	**0.006**	42	9 (21.5)	1.94 (0.81-4.64)	0.138
No	162	54 (33.4)	Ref.	-	162	20 (12.4)	Ref.	-
Duration of rupture of membranes	**199**	**78 (39.2)**			**199**	**29 (14.6)**		
≥6 hours	7	6 (85.8)	10.00 (1.18-84.75)	**0.035**	7	4 (57.2)	8.91 (1.89-42.17)	**0.006**
<6 hours	192	72 (37.5)	Ref.	-	192	25 (13.1)	Ref.	-

No substantial effect of hyphae on adverse pregnancy outcomes was documented. Bold marks reflect a p-value ≤0.05. [1]Long, tubular branching structures produced by *Candida*. [2]A dark greenish mass that

accumulates in the bowel during fetal life and is discharged shortly after birth. CI: confidence interval. N: number of samples. PTB: preterm birth. OR: odds ratio. Ref.: reference.

Effect of treatment with Femaclin®

Femaclin® has an impact on vaginal *Candida* carriage rates

Vaginal *Candida* carriage was determined at V1 and V2 by means of Gram stain microscopy. Femaclin® (200 mg clotrimazole and 100 mg clindamycin) was offered to the majority (88.60%) of *Candida* positive women at V1. **Figure 8** shows the evolution over time of positive *Candida* samples. The amount of *Candida* carriage diminished after administration of Femaclin® at V1. Inversely, vaginal carriage of *Candida* increased, when no medication was offered to the pregnant women.

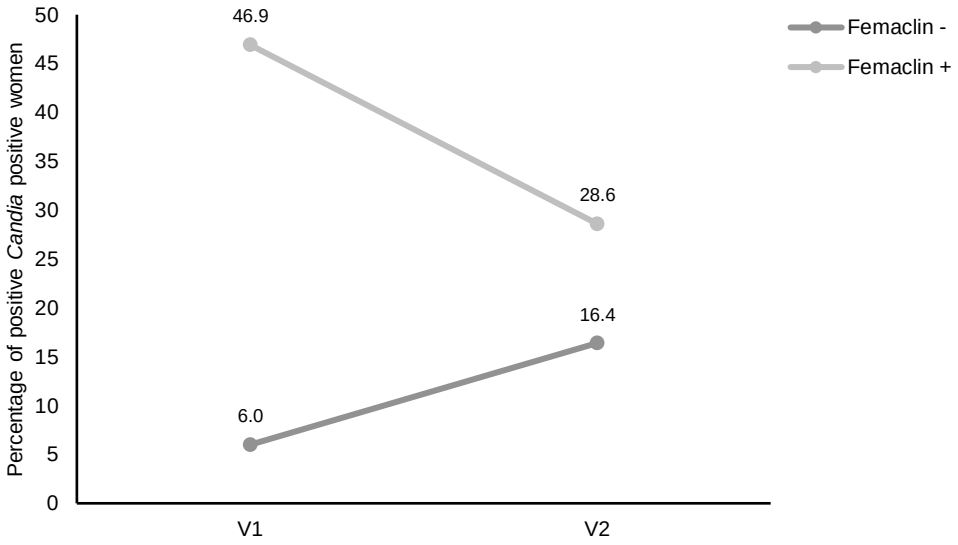


Figure 8. Comparison between vaginal *Candida* carriage at visit 1 and visit 2 in women treated and not treated with Femaclin®[1]. The amount of *Candida* carriage diminished when Femaclin® was administered at V1 (from 46.9% to 28.6%). Inversely, vaginal carriage of yeast increased when no medication was offered to the pregnant women (from 6.0% to 16.4%). Vaginal *Candida* carriage was assessed by means of microscopy of Gram stained vaginal smears. [1]200 mg clotrimazole and 100 mg clindamycin. V1: visit 1 (16-20 weeks of gestational age). V2: visit 2 (35-37 weeks of gestational age).

Femaclin® administration has no impact on adverse pregnancy outcomes

To verify the impact of Femaclin® on APOs, univariate logistic regressions were carried out between vaginal *Candida* carriage (qPCR), stratified for the administration of Femaclin®, and APOs, as shown in **Table 13**. APOs were proportionally more frequently present after Femaclin® administration, but no significant increase in APOs was established.

Table 13. Univariate associations between adverse pregnancy outcomes and *Candida* (qPCR), stratified for the administration of Femaclin®[1].

	No Femaclin®[1]				Femaclin®[1]			
	n	*Candida* + cases (%)	Crude OR (95% CI)	p-value	n	*Candida* + cases (%)	Crude OR (95% CI)	p-value
PTB	**105**	**18 (17.1)**			**96**	**59 (61.5)**		
Yes	10	2 (20.0)	1.23 (0.24-6.36)	0.801	20	14 (70.0)	1.61 (0.56-4.64)	0.38
No	95	16 (16.8)	Ref.		76	45 (59.2)	Ref.	-
Meconium[2] amniotic fluid	**106**	**18 (17.0)**			**97**	**60 (61.9)**		
Yes	19	8 (42.2)	5.60 (1.82-17.23)	**0.003**	23	16 (69.6)	1.56 (0.57-4.26)	0.386
No	87	10 (11.5)	Ref.	-	74	44 (59.5)	Ref.	
Duration of rupture of membranes	**102**	**18 (17.7)**			**96**	**60 (62.5)**		
≥6 hours	1	1 (100.0)	Ref.	-	6	5 (83.4)	3.18 (0.36-28.39)	0.3
<6 hours	101	17 (16.9)	-	-	90	55 (61.2)	Ref.	-

Adverse pregnancy outcomes (APO) were proportionally more frequently present when Femaclin® was administered, but no significant increase in APOs was established. Bold marks reflect a p-value ≤0.05. [1]200 mg clotrimazole and 100 mg clindamycin. [2]A dark greenish mass that accumulates in the bowel during fetal life and is discharged shortly after birth. CI: confidence interval. N: number of samples. OR: odds ratio. PTB: preterm birth. Ref.: reference.

3.5 *Enterobacter cloacae*: risk factors, signs and symptoms, adverse pregnancy outcomes

Addendum 5 represents the univariate associations between vaginal *E. cloacae* carriage and risk factors, signs and symptoms and adverse pregnancy outcomes.

3.5.1 Independent risk factors associated with vaginal *Enterobacter cloacae* carriage

Our multivariate model on risk factors for *E. cloacae* carriage, adjusted for BV and antibiotic administration, is documented in **Table 14**. A positive association was observed with women with a previously preterm neonate (AOR: 13.43; 95% CI: 1.11-162.76; p=0.041) and women who received a treatment for dysuria before V1 (AOR: 6.26; 95% CI: 1.53-25.59; p=0.011).

Table 14. Multivariate regression model showing the association between vaginal *Enterobacter cloacae* carriage and risk factors.

	n	Number of *E. cloacae* positive women n (%)	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Extension of the labia in the past[1]	**318**	**136 (42,8)**				
Yes	40	23 (57,5)	1,98 (1,10-3,87)	**0,047**	1,44 (0,17-11,86)	0,735
No	278	113 (40,7)	Ref.	-	Ref.	-
Antibiotic administration two weeks before V1	**328**	**139 (42.4)**				
Yes	46	17 (37.0)	Ref.	-	Ref.	-
No	282	122 (43.3)	1.30 (0.68-2.48)	0.423	3.46 (0.43-27.50)	0.24
Previous premature delivery	**330**	**140 (42.5)**				
Yes	20	13 (65.0)	2.68 (1.04-6.94)	**0.041**	13.43 (1.11-162.76)	**0.041**
No	310	127 (41.0)	Ref.	-	Ref.	-

Utilization of mosquito net during pregnancy	**324**	**138 (42.6)**				
Yes	287	128 (44.6)	2.17 (1.02-4.66)	0.046	4.45 (0.45-4.25)	0.203
No	37	10 (27.1)	Ref.	-	Ref.	-
Age of first sexual contact	**268**	**113 (42.2)**				
≤18 years	126	44 (35.0)	Ref.	-	Ref.	-
>18 years	142	69 (48.6)	1.76 (1.08-2.88)	0.024	1.85 (0.50-6.88)	0.356
Anal sexual intercourse[2]	**329**	**140 (42.6)**				
Yes	32	22 (68.8)	3.337 (1.526-7.301)	**0.003**	6.85 (0.97-48.52)	0.054
No	297	118 (39.8)	Ref.	-	Ref.	-
Previous treatment for dysuria[3]	**89**	**41 (46.1)**				
Yes	34	21 (61.8)	2.83 (1.17-6.84)	**0.021**	6.26 (1.53-25.59)	**0.011**
No	55	20 (36.4)	Ref.	-	Ref.	-
BV on Gram stain at V1[4]	**326**	**139 (42.6)**				
No BV	176	78 (44.3)	Ref.	-	Ref.	-
Intermediate	59	28 (47.5)	1.14 (0.63-2.05)	0.680	0.35 (0.6-1.94)	0.229
BV	91	33 (36.3)	0.72 (0.43-1.20)	0.210	1.82 (0.46-7.27)	0.397

Vaginal *E. cloacae* carriage was significantly positively associated with a previous premature delivery and a previos treatment for dysuria. Bold marks reflect a p-value ≤0.05. [1]A cultural tradition. [2]Information about frequency and timing was not known. [3]Painful urination. [4]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV) (59). BV: bacterial vaginosis. CI: confidence interval. *E. cloacae*: *Enterobacter cloacae*. N: number of samples. OR: odds ratio. Ref: reference. V1: visit 1 (16-20 weeks of gestational age).

3.5.2 Symptoms associated with vaginal *Enterobacter cloacae* carriage

The multivariate *E. cloacae* model aiming to identify the signs and symptoms independently associated with vaginal *E. cloacae* carriage, adjusted for BV and antibiotic administration, is shown in **Table 15**. Cough (AOR: 1.86; 95% CI: 1.03-3.36; p=0.04) and the presence of 5-30 WBC per field on wet mount at V1 (compared to 1-4 WBC) (AOR: 0.50; 95% CI: 0.30-0.83; p=0.008) were found to be significantly and negatively associated with vaginal *E. cloacae* carriage. A positive association was observed with estimated fetal weight percentiles (p) between p10 and p90 at V1 (compared to >p90) (AOR: 2.17; 95%CI, 1.05-4.48; p=0.04).

Table 15. Multivariate regression model showing the association between vaginal *Enterobacter cloacae* carriage and experienced symptoms.

	n	Number of *E. cloacae* positive women (%)	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Antibiotic administration 2 weeks before V1	**328**	**139 (42.4)**				
Yes	46	17 (37.0)	Ref.	-	1.01 (0.49-2.09)	0.97
No	282	122 (43.3)	1.30 (0.68-2.48)	0.423	Ref.	-
BV on Gram stain[1]	**326**	**139 (42.6)**				
No BV	176	78 (44.3)	Ref.	-	Ref.	-
Intermediate	59	28 (47.5)	1.14 (0.63-2.05)	0.680	1.15 (0.60-2.23)	0.67
BV	91	33 (36.3)	0.72 (0.43-1.20)	0.210	0.71 (0.40-1.24)	0.229

Cough	**327**	**138 (42.3)**				
Yes	72	23 (32.0)	Ref.	-	Ref.	-
No	255	115 (45.1)	1.75 (1.01-3.04)	**0.047**	1.86 (1.03-3.36)	**0.040**
White blood cells per field on wet mount	**330**	**140 (42.5)**				
0	0	0 (0.0)	-	-	-	-
1 -4	178	84 (47.2)	Ref.	-	Ref.	-
5-30	132	45 (34.1)	0.58 (0.36-0.92)	**0.021**	0.50 (0.30-0.83)	**0.008**
30+	20	11 (55.0)	1.39 (0.54-3.46)	0.509	1.07 (0.40-2.83)	0.897
Estimation of fetal weight centiles[2]	**315**	**132 (42.0)**				
<p10	44	16 (36.4)	1.14 (0.56-2.35)	0.716	Ref.	-
P10_p90	148	75 (50.7)	2.06 (1.25-3.37)	**0.004**	2.17 (1.05-4.48)	**0.04**
>p90	123	41 (33.4)	Ref.	-	0.96 (0.46-2.01)	0.91

Vaginal carriage of *E. cloacae* was significantly negatively associated with cough and the presence of 5_30 white blood cells per field on wet mount microscopy. Bold marks reflect a p-value ≤0.05. [1]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV) (59). [2]Determined based on ultrasound examination. BV: bacterial vaginosis. CI: confidence interval. *E. cloacae*: *Enterobacter cloacae*. N: number of samples. OR: odds ratio. P: percentile. Ref: reference. V1: visit 1 (16-20 weeks of gestational age)

3.5.3 Adverse pregnancy outcomes associated with vaginal *Enterobacter cloacae* carriage

No significant associations between carriage of *E. cloacae* and APOs were found, hence, no multivariate model was set up.

3.6 *Klebsiella pneumoniae*: risk factors, signs and symptoms, adverse pregnancy outcomes

Addendum 6 represents the univariate associations between vaginal *K. pneumonia* carriage and risk factors, signs and symptoms and adverse pregnancy outcomes.

3.6.1 Independent risk factors associated with vaginal *Klebsiella pneumoniae* carriage

Table 16 contains our multivariate model on risk factors for *K. pneumoniae* carriage. A positive association was observed with intermediate vaginal microflora carriage (determined by Nugent scoring, compared to a healthy vaginal microflora (59)) (AOR: 3.01; 95% CI:1.29-7.00; p = 0.011) and women with a previous naturally aborted pregnancy (AOR: 2.04; 95% CI: 1.00-4.14; p=0.05).

Table 16. Multivariate regression model showing the association between vaginal *Klebsiella pneumoniae* carriage and risk factors.

	n	Number of *K. pneumoniae* positive women (%)	Crude OR (95% CI)	p-value	Adjusted OR (95%CI)	p-value
Hemoglobin on Hemocue®[1]	**328**	**40 (12.2)**				
Anemia (<11 Hb)	12	4 (33.4)	3.89 (1.12-13.57)	**0.03**	3.24 (0.81-12.85)	0.095
Normal (≥11 Hb)	316	36 (11.4)	Ref.	-	Ref.	-
BV on Gram stain at V1[2]	**326**	**39 (12.0)**				
No BV	176	14 (8.0)	Ref.	-	Ref.	-
Intermediate	59	13 (22.1)	3.27 (1.44-7.45)	**0.005**	3.01 (1.29-7.00)	**0.011**
BV	91	12 (13.2)	1.76 (0.78-3.98)	0.18	1.46 (0.62-3.45)	0.388

Previous abortion[3]	**330**	**40 (12.2)**				
Yes	108	19 (17.6)	2.58 (1.32-5.04)	**0.01**	2.04 (1.00-4.14)	**0.050**
No	222	21 (9.5)	Ref.	-	Ref.	-
Previous fetal death in utero[4]	**329**	**40 (12.2)**				
Yes	21	6 (28.6)	3.22 (1.17-8.87)	**0.02**	2.42 (0.82-7.12)	0.108
No	308	34 (11.1)	Ref.	-	Ref.	-

Vaginal carriage of *K. pneumoniae* was significantly positively associated with an intermediate vaginal microflora and a previous naturally aborted pregnancy. [1]Device that measures the hemoglobin concentration by means of spectrophotometry. [2]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV) (59). [3]Miscarriage, the natural death of the embryo or fetus in the first trimester. [4]Intrauterine fetal demise is the natural death of the fetus after 20w of gestation. BV: bacterial vaginosis. CI: confidence interval. *K. pneumoniae*: *Klebsiella pneumoniae*. N: number of samples. OR: odds ratio. Ref.: reference. V1: visit 1 (16-20 weeks of gestational age).

3.6.2 Symptoms associated with vaginal *Klebsiella pneumoniae* carriage

Our multivariate model on *K. pneumoniae* signs and symptoms, adjusted for BV and antibiotic administration, is shown in **Table 17**. A positive association was observed between vaginal *K. pneumoniae* carriage and an increased heart frequency (≥110 bpm).

Table 17. Multivariate regression model showing the association between vaginal *Klebsiella pneumoniae* carriage and experienced symptoms.

	n	Number of *K. pneumoniae* positive women (%)	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Cardiac frequency at V1	**329**	**40 (12.2)**				
<110 bpm	318	36 (11.4)	Ref.	-	Ref.	-
≥110 bpm	11	4 (36.4)	4.48 (1.25-16.04)	**0.02**	4.14 (1.08-15.95)	**0.040**
BV on Gram stain at V1[1]	**326**	**39 (12.0)**				
No BV	176	14 (8.0)	Ref.	-	Ref.	-
Intermediate	59	13 (22.1)	3.27 (1.44-7.45)	**0.005**	2.75 (1.17-6.50)	**0.021**
BV	91	12 (13.2)	1.76 (0.78-3.98)	0.18	1.85 (0.81-4.22)	0.142
Antibiotic administration 2 weeks before V1	**328**	**39 (11.9)**				
Yes	46	6 (13.1)	1.13 (0.45-2.87)	0.79	Ref.	-
No	282	33 (11.8)	Ref.	-	1.07 (0.40-2.88)	0.900

Vaginal *K. pneumoniae* carriage was significantly positively associated with an increased heart frequency (≥110 bpm). Bold marks reflect a p-value ≤0.05. [1]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV) (59). Bpm: beats per minute. BV: bacterial vaginosis. CI: confidence interval. *K. pneumoniae*: *Klebsiella pneumoniae*. N: number of samples. OR: odds ratio. Ref.: reference. V1: visit 1 (16-20 weeks of gestational age).

3.6.3 Adverse pregnancy outcomes associated with vaginal *Klebsiella pneumoniae* carriage

The multivariate *K. pneumoniae* model built to investigate the association between *K. pneumoniae* carriage and APOs, adjusted for BV, is shown in **Table 18**. A positive association was observed with a performed blood culture during the first week of neonatal life (AOR: 11.76; 95% CI: 1.89-73.30; p=0.008) and caesarean section (AOR: 4.08; 95% CI: 1.59-10.48; p=0.003).

Table 18. Multivariate regression model showing the association between vaginal *Klebsiella pneumoniae* carriage and adverse pregnancy outcomes.

	n	Number of *K. pneumoniae* positive women (%)	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Blood culture during first week of neonatal life	**203**	**28 (13.8)**				
Not done	197	24 (12.2)	Ref.	-	Ref.	-
Done[1]	6	4 (66.7)	14.42 (2.51-82.98)	**0.003**	11.76 (1.89-73.30)	**0.008**
BV on Gram stain at V1[2]	**326**	**39 (12.0)**				
No BV	176	14 (8.0)	Ref.	-	Ref.	-
Intermediate	59	13 (22.1)	3.27 (1.44-7.45)	**0.005**	3.87 (1.25-12.00)	**0.019**
BV	91	12 (13.2)	1.76 (0.78-3.98)	0.18	3.47 (1.25-9.59)	**0.017**
Type of labor	**204**	**18 (8.9)**				
Eutocic[3] (with episiotomy)	167	18 (10.8)	Ref.	-	Ref.	-
Dystocic[4]	1	0 (0.0)	-	-	-	-
Caesarean section	36	10 (27.8)	3.18 (1.32-7.67)	**0.01**	4.08 (1.59-10.48)	**0.003**

Vaginal carriage of *K. pneumoniae* was significantly positively associated with abnormal lung auscultation and caesarean section. Bold marks reflect a p-value ≤0.05. [1]Blood culture taken, as neonate is suspected for EONS. [2]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV) (59). [3]Delivery without medical intervention. Episiotomy: an incision through the area between the vagina and the anus to make the vaginal opening larger for childbirth. [4]Difficult delivery. BV: bacterial vaginosis. CI: confidence interval. *K. pneumoniae*: *Klebsiella pneumoniae*. N: number of samples. OR: odds ratio. Ref.: reference. V1: visit 1 (16-20 weeks of gestational age).

4. Discussion

In 2018, approximately 2.50 million children died in the first month of their life, mostly from preventable causes (7, 11). The two leading causes were preterm birth (PTB) and neonatal sepsis (EONS), each accounting for roughly one third of the cases (7, 11). A total of 79% of the global neonatal deaths occur in sub-Saharan Africa (SSA) and South-Asia (10, 17). In SSA, the highest neonatal mortality rates (NMR) are reported in West and Central Africa, averaging 30.20 deaths per 1000 live births. The annual NMR in this region is more than nine times higher compared to high-income countries with 3.00 deaths per 1000 livebirths (17). Despite the substantial gap in NMR between high- and low-income countries, research mainly focuses on the neonatal deaths in high-income settings (11, 13). This master thesis aimed to contribute to bridge this gap and focused on revealing risk factors and pathophysiologic mechanisms of PTB and EONS, the two main causes of neonatal death, in Bukavu (Democratic Republic of the Congo (DRC)).

PTB is considered as a multi-factorial event. Particularly, vaginal ascending infections remain the major associated factor in up to 40% of all cases of PTB (27, 29, 77). It has become increasingly clear that vaginal carriage of *Candida* may play a more important role than previously thought (27-33). *Candida* itself is seldom identified as a cause of chorioamnionitis, but is assumed to disrupt the normal vaginal microbiome, favoring the carriage of pathogenic bacteria (39-41). Therefore, our first aim in this master thesis was to clarify the role of *Candida* on adverse pregnancy outcomes (APO) in 330 pregnant women in Bukavu (DRC).

Previous research has suggested that *Enterobacter cloacae* (*E. cloacae*) and *Klebsiella pneumoniae* (*K. pneumoniae*) are the principal micro-organisms causing EONS in Bukavu (DRC) (58). Therefore, our second aim was to get insights into the pathogenesis of EONS in Bukavu (DRC), by investigating the prevalence of vaginal *E. cloacae* and *K. pneumoniae* carriage in 330 pregnant women by molecular techniques.

4.1 *Candida*: prevalence, risk factors, symptoms and adverse pregnancy outcomes

4.1.1 Vaginal carriage of *Candida*

Candida may live in the vagina as a commensal, in symbiosis with lactobacilli (78). Asymptomatic *Candida* carriage can continue for several months or even last for multiple years (79). This vaginal *Candida* carriage is found in approximately 20% of the non-pregnant women. It rises up to 30-40% in pregnancy, especially in the second and third trimester. Physiologic changes, linked to pregnancy, enhance virulence factors and may explain the increased amount of *Candida* carriage. Particularly, increased concentrations of reproductive hormones, like estrogen, will increase the amount of glycogen and provide a carbon source for *Candida* organisms. Furthermore, estrogen

facilitates the adherence of yeasts to the vaginal wall and enhances mycelial transformation (39, 40, 78-80). Finally, decreased cell-mediated immunity can increase vaginal *Candida* carriage (39).

Based on our findings, the prevalence of vaginal *Candida* carriage in pregnant women in Bukavu (DRC) was 38.18% (95% CI: 33.10-43.53). To our best knowledge, no previous study investigated vaginal *Candida* carriage rates in pregnancy in DRC. An overview of the prevalence of vaginal *Candida* carriage in pregnant women, living in SSA, is listed in **Table 19** (81-114). According to the overview in **Table 19**, the mean vaginal *Candida* carriage prevalence in SSA is 36.40%, similar to our findings (81-114).

However, divergent prevalence rates were established in the several studies in SSA. Differences in prevalence rates in comparison to our findings could be explained by several reasons. First, in this master thesis, vaginal *Candida* carriage rates were assessed by means of microscopic examination of wet mount and Gram stained vaginal smears, as well as through quantitative polymerase chain reaction (qPCR) of cervicovaginal lavages (CVL). In contrast, all other studies listed in **Table 19** used microscopy and/or culture. It is known that qPCR is more sensitive compared to culturing techniques (88, 90, 108-110) or microscopy (81, 93, 96, 98, 101, 102, 107, 113).

Second, differences in prevalence rates may be explained by the different inclusion and exclusion criteria of the studies. For example, Akah et al. (2010) and Fonck et al. (2000) only included symptomatic pregnant women to determine the vaginal *Candida* carriage rate (83, 92). Furthermore, the weeks of gestational age (WGA) were frequently not mentioned, so it could be assumed that enrolment could have happened at every moment in pregnancy (93, 103, 112, 114). As prevalence rates of *Candida* carriage tend to increase when pregnancy advances (39), higher prevalence rates may be expected in studies assessing *Candida* in the second or third trimester, compared to our study, conducted early in pregnancy (16-20 WGA).

Table 19. Prevalence rates of vaginal *Candida* carriage in pregnant women in sub-Saharan Africa.

Country	Year	n	% *Candida*	Sample	Detection	Reference
Burkina Faso	1997	645	14,0	V	M	Meda et al. (96)
Burkina Faso	2017	229	22,7	V	Cu	Sangaré et al. (109)
Cameroon	2013	112	55,4	V	Cu	Toua et al. (112)
Central African Republic	1999	481	46,6	C	M	Blankhart et al. (87)
DRC	**2019**	**330**	**38,2**	**V**	**P**	**This master thesis**
Ethiopia	2015	214	9,3	V	M	Mulu et al. (101)
Gabon	1998	646	30,8	V	M	Bourgeois et al. (86)
Ghana	2005	517	39,8	V	Cu + M	Apea-Kubi et al. (85)

Ghana	2019	589	36,5	V	M	Konadu et al. (91)
Kenya	1996	291	26,2	V	M	Thomas et al. (113)
Kenya	2000	289	42,0	V	M	Fonck et al. (92)
Kenya	2000	334	55,0	V	M	Fonck et al. (92)
Kenya	2013	104	42,7	V	Cu + M	Nelson et al. (95)
Kenya	2014	30	23,0	V	M	Jespers et al. (93)
Mali	1999	549	39,0	V	M	Mulanga-Kabeya et al. (100)
Mauritania	2018	200	26,0	V	C	Sy et al. (110)
Nigeria	1981	187	20,9	C	C	Ekwempu et al. (90)
Nigeria	2002	500	65,0	V	C	Akerele et al. (84)
Nigera	2003	230	37,8	C + V	M	Aboyeji et al. (82)
Nigeria	2007	311	56,3	V	Cu + M	Nwosu et al. (103)
Nigeria	2010	100	26,0	V	C	Donbraye-Emmanuel et al. (88)
Nigeria	2010	901	62,2	V	C	Akah et al. (83)
Nigeria	2010	90	30,0	V	M	Okonkwo et al. (104)
Nigeria	2014	100	36,0	V	M	Olowe et al. (105)
Nigeria	2015	140	25,0	V	Cu	Nurat et al. (108)
Nigeria	2017	288	60,8	V	Cu + M	Nnadi et al. (114)
Nigeria	2019	20	45,0	V	M	Mumuney et al. (102)
Nigeria	2019	20	25,0	V	M	Mumuney et al. (102)
Uganda	2015	456	45,4	V	Cu + M	Mukasa et al. (99)
South Africa	1989	193	38,3	V	Cu + M	O'Farrell et al. (106)
South Africa	2014	30	57,0	V	M	Jespers et al. (93)
Sudan	2009	151	13,9	V	M	Orthashi et al. (107)
Sudan	2014	200	16,6	V	M	Abdelaziz et al. (81)
Tanzania	2009	2654	11,4	V	M	Msuya et al. (98)
The Gambia	1984	100	35,0	C	Cu	Mabey et al. (97)
Togo	2018	126	48,0	V	M	Dakey et al. (89)
Togo	2013	221	30,8	V	Cu + M	Tchelougou et al. (111)
Zimbabwe	2010	691	39,3	V	M	Kurewa et al. (94)
Average prevalence			**36.4**			

Based on our findings, the prevalence of vaginal *Candida* carriage of pregnant women in Bukavu (Democratic Republic of the Congo) is 38.18%. The mean prevalence of vaginal *Candida* carriage in sub-Saharan Africa is 36.40%, similar to our findings. This master thesis was excluded to measure the average prevalence, as qPCR is considered as more sensitive compared to culturing techniques (115). A correction for HIV was conducted. C: cervical sample. Cu: culture. M: microscopy. N: study participants. V: high vaginal swab. Year: year of publication of the study.

In our study, *Candida albicans* (*C. albicans*) was found to be the predominant species, occurring in 91% of the *Candida* positive women. The remaining non-albicans species were *Candida dubliensis, Candida famata, Candida glabrata, Candida inconspicua, Candida kefyr, Candida krusei* and *Candida tropicalis,* all detected in low prevalence. *Saccharomyces cerevisiae* (*S. cerevisiae*) was also present in 1.21% of the samples. These findings are in agreement with previous research, showing that 85–95% of the yeast strains, isolated from the vagina, belong to

the *C. albicans* species (39, 78, 80). In less than 10% of cases, non-albicans *Candida* species, especially *Candida glabrata, Candida krusei*, *Candida parapsilosis, Candida tropicalis* and, in rare cases, *S. cerevisiae*, cause vulvovaginal candidiasis (symptomatic vaginal *Candida* carriage, VVC) (39, 78, 80). In particular, Mukasa and coworkers found that 45.50% of the 456 examined pregnant women in Uganda carried *Candida* vaginally, of which 78.95% belonged to the species *C. albicans* (116). *Candida* colonization of the vagina requires adherence to the vaginal epithelial cells. *C. albicans* is able to adhere more efficiently compared to non-albicans species (78, 79), which may explain the higher prevalence of this species.

4.1.2 Risk factors associated with vaginal *Candida* carriage

In this master thesis, the use of pit toilets (compared to flush toilets) (AOR: 2.39), living together with the husband for less than 5 years (compared to longer than 5 years) (AOR: 1.92) and an intermediate vaginal microflora (compared to a healthy vaginal microflora) (AOR: 3.54) were all established as independent risk factors for vaginal *Candida* carriage.

Women living less than 5 years with their husband were almost two times more likely to carry *Candida* vaginally. It may be possible that these women were sexually more active. This could explain the higher rates of *Candida* carriage as the incidence of vaginal *Candida* carriage dramatically increases in the second decade of life, corresponding with the onset of sexual activity. From the age of forty, the incidence of vaginal *Candida* carriage declines gradually. During sexual intercourse, anogenital and particularly orogenital contact enables transmission of yeasts (78, 80, 117, 118). Reed and coworkers (2000) added evidence for this assumption by finding a positive association between recent cunnilungus and vaginal *Candida* carriage in 156 women (OR: 2.22) (119). Based on all these findings, sexual intercourse in his broad meaning could predispose to higher levels of asymptomatic vaginal *Candida* carriage.

During life, *Candida* organisms colonize the vagina predominately from the adjacent perianal area. Consequently, the intestinal reservoir of yeasts is regarded as the source from where re-inoculation occurs (78, 79). In DRC, pit toilets are more frequently used, compared to flush toilets. This type of toilet may implicate less hygienic sanitary conditions, favoring vaginal carriage of *Candida.* This assumption is confirmed by our findings showing that women using pit toilets, were almost 2.50 times more likely to carry *Candida* vaginally. In Cameroon, poor toilet facilities, such as pit toilets, were also found to increase the risk of vaginal *Candida* carriage in pregnant women (112). As unsanitary toilet facilities appear as a risk factor for *Candida* carriage, investments in proper flush toilets could be considered as primary prevention to diminish the manifestation of vaginal *Candida* carriage (120).

In this master thesis, women with an intermediate vaginal microflora (assessed by means of Nugent scoring (59)) were 3.50 times more likely to carry *Candida* vaginally, compared to women with a healthy vaginal microbiome. This finding has been reported in other research. Particularly, *Candida*, was detected in almost half of the women with intermediate microflora, according to Vahidnia and coworkers (121). As a normal lactobacilli-dominated microflora is probably the most important defense mechanism against pathologic microorganisms (78, 79), a dysbiotic microflora may favor *Candida* carriage. Furthermore, bacteriocins and hydrogen peroxide, shed by the lactobacilli, can inhibit further yeast growth (78, 79).

A healthy, protective microflora with lactobacilli is considered as a vital protection against *Candida* carriage (78, 79). Broad-spectrum antibiotics such as tetracyclines, ampicillin and oral cephalosporins, are thought to eliminate the normal, healthy microflora with lactobacilli (78-80). Vaginal *Candida* carriage rates have been shown to increase from 10% to 30% after the use of antibiotics (78). A recent study of Ekuma and coworkers in Nigeria strengthened this positive association between vaginal *Candida* carriage and the administration of antibiotics (122). These findings emphasize the importance of antibiotics as a relevant confounder. However, our multivariate model showed no association between the use of antibiotics and an increased *Candida* carriage.

After stratifying *Candida* for bacterial vaginosis (BV), non-employment was positively associated with vaginal *Candida* carriage. Low socio-economic status (SES) has been established as a risk factor for *Candida* carriage (32, 123). In contrast, three hundred pregnant Nigerian women with a low SES did not carry significantly more *Candida* vaginally (104). In our multivariate model, non-employment was also not retained as an independent risk factor.

4.1.3 Signs and symptoms associated with vaginal *Candida* carriage

The transition from commensalism into a significant vulvovaginal *Candida* infection implicates the occurrence of a large range of clinical manifestations (78-80). Vaginal discharge, vaginal pruritus, vaginal soreness, dysuria and dyspareunia are generally the most typical symptoms associated with vulvovaginal *Candida* carriage (78, 80, 117, 118). Vaginal discharge can variate from a watery substance to a thick, homogenic and cottage-cheese like texture. Vaginal pruritus is the most specific symptom of VVC (vulvovaginal candidiasis - symptomatic vaginal *Candida* carriage), which is probably a consequence of increased host hypersensitivity. Malodor is commonly slight and inoffensive (78).

In our multivariate model, vaginal discharge (AOR: 2.30), thick and heterogeneous vaginal secretions (AOR: 4.62), vaginal itching (AOR: 2.70) and a burning sensation after sexual

intercourse (AOR: 3.06) were independent and significant symptoms for vaginal *Candida* carriage, implicating that our study lines up with the typically known signs and symptoms of VVC.

In Bukavu (DRC), a syndromic approach is maintained to treat vulvovaginal infections (61). This standard of care implicates that women experiencing any vaginal complaints were considered as symptomatic for BV or vaginal infections with *Candida* or *Trichomonas*, and were treated empirically with broad anti-infectious medication (61). As BV and VVC are considered as the two most frequent vulvovaginal infections (80), a demarcation of typical symptoms for BV and VVC can be useful in clinical practice. After stratifying for respectively *Candida* and BV, malodor was typically associated with BV (in *Candida* negative women), while vaginal discharge, abnormal vaginal secretions, vaginal itching and a burning sensation after sexual intercourse were specific for VVC. Based on these findings, the treatment of vulvovaginal infections may be guided by the distinguished symptoms (symptomatic approach). Hence, in contrast with the syndromic approach, more specific treatments could be administered, which would be beneficial to maintain a healthy vaginal microflora (78-80).

4.1.4 Adverse pregnancy outcomes associated with vaginal *Candida* carriage

In our study, vaginal *Candida* carriage was independently associated with PTB (AOR: 7.21) and meconium-stained amniotic fluid (AOR: 2.76).

Preterm birth

In our study population, a PTB rate of 14.85% was found, which is in line with the data presented by Unicef about the PTB rate in DRC (14.90%) (7). Prevention of PTB remains one of the most fundamental challenges in maternity care (20, 30, 31, 34). Lowering the PTB rate is both a medical and health-economic necessity, because of its implications for morbidity and mortality as well as for socio-economic liability (20, 30, 31). Due to the immaturity of multiple organs and the inflammatory status in the uterus, inducing PTB, the neonates face numerous neonatal complications and long-term sequelae (23, 26, 27). The severity of the adverse events is inversely correlated with gestational age (23).

To date, contradictory findings on the role of *Candida* in the mechanism of PTB exist, leaving the impact of *Candida* unclear (28-33, 117, 123-125). In our multivariate model for vaginal *Candida* carriage, PTB was considered as an independent APO, which is in accordance with several studies (28, 30-33). Farr and coworkers (2010) examined vaginal smears by means of microscopy in order to detect vaginal carriage of *Candida.* Afterwards, only symptomatic women with VVC were treated with clotrimazole. A positive association between recurrent *Candida* carriage and

PTB was reported (28). Furthermore, literature shows that treatment for asymptomatic *Candida* carriage early in pregnancy was beneficial to reduce the rates of spontaneous PTB (29-33).

However, screening for asymptomatic *Candida* carriage in pregnancy is not recommended in the guidelines of the Center of Disease and Control (CDC) (126), because large studies of Cotch et al. and McGregor et al. showed no association between moderate to heavy *Candida* carriage and PTB (117, 125, 126). Both studies were carried out, respectively, at 23-26 WGA and 22-30 WGA (117, 125, 126). However, evidence suggests that intrauterine colonization of ascending microorganisms occurs quite early in pregnancy (34). The vaginal microorganisms, causing PTB, are thought to ascend before the expanding membranes seal the endometrial cavity near mid-pregnancy (34). So, it is assumed that screening for vaginal carriage of *Candida* has to be carried out from late in first trimester until 24 WGA, as this is the critical point for vaginal pathogens to ascend (29, 32, 33, 127). The negative association between vaginal *Candida* carriage and PTB could possibly be explained by screening too late in pregnancy.

Briefly, all these findings suggest that screening for vaginal *Candida* carriage could be of value, as several associations with PTB were reported (28, 30-33). Generally, ascending vaginal infections are considered as well-known risk factors for PTB by causing chorioamnionitis (19, 24-26, 34, 36, 37). Nevertheless, *Candida* is seldom identified as a cause of chorioamnionitis (39, 41). Possibly, it could be assumed that *Candida* may distort the vaginal microflora and support the development of bacterial vaginosis or colonization with pathologic bacteria (40).

Microscopy as a tool to detect vaginal Candida carriage

In this master thesis, after stratification for *Candida* concentration, only women carrying high concentrations of *Candida* were more likely (3.50 times) to deliver preterm. As an increasing load of *Candida* may be considered as a pathophysiologic mechanism (40, 78, 79), more APOs, like PTB, could be expected within these pregnant women.

Furthermore, our findings showed that Gram stain and wet mount microscopy were effective to detect moderate and high concentrations of *Candida.* Low concentrations of *Candida* were not identified by microscopy in more than half of the cases (defined by means of qPCR). Generally, qPCR is indeed capable of detecting microorganisms at much lower concentrations compared to microscopy (80). Nonetheless, as a considerable increase of PTB was solely seen in the high concentration group, both Gram stain and wet mount microscopy seem to have potential as diagnostic tool to screen pregnant women for high loads of *Candida* in an effort to reduce PTB by treating these women. Moreover, despite the increased *Candida* carriage in pregnancy, pregnant

women appear to be less symptomatic (39, 40, 78-80). Consequently, microscopy may be implemented as a complementary tool, besides a symptomatic approach, to determine vaginal *Candida* carriage in clinical practice.

Additionally, microscopy is capable of distinguishing between *Candida* blastopores and hyphae. Yeast strains adapt their phenotype to their pathological state (78, 79). Particularly, yeast blastopores represent an asymptomatic carriage in the vagina, whereas germinated yeast switch their phenotype into more virulent mycelia (hyphae) in symptomatic vaginitis (78, 79). Consequently, hyphae are considered as more pathological phenotypes (78, 79) . However, in our multivariate model, the presence of hyphae did not increase APOs, such as PTB. Based on our findings, distinction between blastopores and mycelia on microscopy does not seem relevant for clinical practice.

Generally, a symptomatic approach, combined with Gram stain or wet mount microscopy, could possibly be more appropriate to screen for *Candida* carriage in pregnancy in Bukavu (DRC), instead of the ongoing syndromic approach (61). This hypothesis is reinforced by an RCT (randomized controlled trial) of Kiss and coworkers, who implemented Gram stain microscopy to detect *Candida* carriage. Treatment with clotrimazole was administered when *Candida* carriage was observed, resulting in a significant decrease in PTB compared to the control group. Hence, this study established that Gram stain microscopy could possibly be sufficient to detect asymptomatic vaginal infections in pregnancy (30).

The administration of anti-fungal medication

As asymptomatic carriage of highly concentrated *Candida* early in pregnancy (<24 WGA) could be harmful (29, 32, 33, 127), the need for secondary prevention, such as early detection followed by an adequate anti-fungal treatment, is emphasized. However, despite extended research indicating that screening programs for asymptomatic vaginal carriage of *Candida* could be of value (29-31, 33), therapeutic interventions for *Candida* are only recommended in symptomatic hosts (126). According to the German guidelines, prophylaxis is only recommended during the third trimester of pregnancy to decrease the rates of neonatal candidiasis, especially oral thrush and diaper dermatitis (39, 80, 128).

In our study, Femaclin® (a vaginal ovule containing 200 mg clotrimazole and 100 mg clindamycin) was offered to the pregnant women with symptomatic vaginitis (syndromic approach). Both *Candida albicans* and non-albicans *Candida* vaginal isolates are susceptible for clotrimazole (33, 129). Clotrimazole contributes to the restoration of the normal vaginal microflora by inhibiting the

growth of Gram-positive (except the lactobacilli (123)) and Gram-negative bacteria, anaerobic bacteria, *Trichomonas vaginalis* and yeasts (40, 123). This broad mode of action can possibly be useful as estimates were proposed that not just *Candida,* but the entire disrupted microflora induces PTB (see above). Clindamycin, an antibiotic with activity against anaerobic bacteria, is effective in treating BV (130).

Administration of Femaclin® at V1 was responsible for a decline in vaginal *Candida* carriage rates at V2. However, no decline in PTB was observed after Femaclin® treatment, compared to non-treated women. Possibly, this finding could partially be explained by the fact that 31 (10.61%) pregnant women were included at V1 at 24 WGA or beyond. Screening beyond this time point lost his opportunity to prevent late miscarriage and very PTB, as the infection already exists in the choriodecidual interface, unreachable for topical treatments (25, 29, 32, 34, 79, 127). Consequently, women have to be screened early enough in pregnancy (before 24 WGA), as this is the moment microorganisms are still able to ascend. Treatment later in pregnancy may have a limited effect in preventing PTB, as the inflammatory response is not fully reversible (25, 29, 32, 34, 79, 127). However, as this was not the main focus of the AVEONS project, no well-founded conclusions could be drawn. Future research should focus on the ideal time point to screen and treat for vaginal *Candida* carriage.

Meconium-stained amniotic fluid

The passage of meconium[2] from the fetus into the amniotic fluid is prevented by the lack of intestinal peristaltic. Meconium-stained amniotic fluid reflects a natural phenomenon of a post-term fetus with a mature gastro-intestinal tract with augmented motilin levels. A total of 5% of neonates with meconium-stained amniotic fluid aspirate this meconium. Meconium is toxic to the lungs in many ways, causing an obstruction of the airways, chemical pneumonitis, vasoconstriction of the pulmonary vessels and an inactivation of the surfactant[3] (132).

In our study, after stratifying for *Candida* concentration (low, medium and high), meconium-stained amniotic fluid occurred three times more frequently in the low and moderate concentration group, compared to the high concentration group. As meconium-stained amniotic fluid appears more

[2] A dark greenish mass that accumulates in the bowel during fetal life and is discharged shortly after birth. (131)
[3] A lipoprotein, secreted by alveolar cells (tiny air sacs in the lungs), that decreases the surface tension of the fluid lining the alveoli, permitting expansion. (131)

frequently in post-term neonates, the impact of low concentrations of *Candida* is of minor importance, since only highly concentrated *Candida* is associated with PTB (132).

4.2 *Enterobacter cloacae* and *Klebsiella pneumoniae*: prevalence, risk factors, symptoms and adverse pregnancy outcomes

In Bukavu (DRC), the mortality rate due EONS (DRC: 4.40 deaths per 1000 live births) is substantially higher compared to high resource settings (Belgium: 0.10 deaths per 1000 live births) (2, 17). One of the main reasons for this lingering high mortality rate is the largely unknown etiology of EONS (58). In high-come countries (HIC), Group B *Streptococcus* (GBS, or *Streptococcus agalactiae*) and *Escherichia coli* are the leading cause of EONS (51). In Bukavu (DRC), a previous study in the AVEONS project suggested that *E. cloacae* and *K. pneumoniae,* two Gram-negative gastro-intestinal commensals*,* are the principal microorganisms causing EONS. Problematically, antibiotics administered for the empirical treatment of EONS in DRC are derived from the recommendations of the WHO guidelines, which have been developed based on the etiological pattern of EONS in HIC (58). This possibly contributes to the limited survival rate of EONS in DRC.

It could be assumed that the pathogenesis of EONS, due to other species than GBS, resembles that of GBS EONS. Concerning GBS EONS, vaginal carriage of GBS is a fundamental prerequisite for transmission to the foetus/neonate (51). Consequently, vaginal carriage rates of *E. cloacae* and *K. pneumoniae* were investigated in order to verify, (i) whether these species were prevalent, (ii) (if yes) what the risk factors were (iii) and whether carriage of these species was associated with APOs. If these pathogens indeed colonize the female genital tract and if a link with EONS was established, specific preventive strategies could be considered.

4.2.1 Vaginal carriage rates of *Enterobacter cloacae* and *Klebsiella pneumoniae*

Enterobacter cloacae

The vaginal carriage rate of *E. cloacae* in pregnant women in Bukavu (DRC) was 42.42% (95% CI: 37.21-47.81). In **Table 20**, vaginal/rectal *E. cloacae* carriage rates in pregnant women worldwide are listed as reference (133-136). Both vaginal and rectal swabs were included as vaginal colonization by gastro-intestinal microorganisms is common in pregnancy (115, 135, 137). To our best knowledge, no previous studies investigated the prevalence of vaginal *E. cloacae* carriage in DRC.

Table 20. Prevalence rates of vaginal *Enterobacter cloacae* carriage in pregnant women worldwide.

Country	Year	n	% *E. cloacae*	Timing	Sample	Detection	Reference
DRC	**2019**	**330**	**42.42**	**17-20 WGA**	**V**	**qPCR**	**This master thesis**
France	2008	125	12.80	PD	R	Cu	Gbaguidi-Haore et al. (135)
France	2015	356	1.04	D	R	Cu	Chereau et al. (136)
Italy	2016	600	0.60	28 WGA	V	Cu	Genovese et al. (134)
South-Africa	2016	90	5.55	D	R	Cu	Kaba et al. (133)
Average prevalence			**5.50**				

Based on our findings, the prevalence of vaginal *E. cloacae* carriage of pregnant women in Bukavu (Democratic Republic of the Congo) is 42.42% The mean prevalence of vaginal *E. cloacae* carriage worldwide is 5.50%. This master thesis was excluded to measure the average prevalence, as qPCR is considered as more sensitive compared to culturing techniques (115). Cu: culture. D: delivery. DRC: Democratic Republic of the Congo. *E. cloacae*: *Enterobacter cloacae*. N: number of study participants. PD: post-delivery. qPCR: quantitative polymerase chain reaction. R: rectal swab. V: high vaginal swab. WGA: weeks of gestational age. Year: year of publication of the study.

The (recto)vaginal *E. cloacae* carriage in other studies is substantially lower compared to our findings. The use of qPCR to determine the prevalence of *E. cloacae* carriage, contrary to other existing studies using culturing techniques, may be postulated as a possible explanation for this inconsistency. However, despite the high prevalence of *E. cloacae* carriage in this master thesis, the concentration of *E. cloacae* was low and could not be quantified accurately (i.e., below the limit of quantification (LOQ)). Consequently, lower prevalence rates may be expected, when applying culturing techniques.

Based on research with GBS, it is assumed that neonates acquire EONS, either in utero from ascending vaginal microorganisms after rupture of the membranes, or during passage in the colonized birth canal (43-45, 53, 138, 139). So, assuming that *E. cloacae* EONS follows the same pathogenesis as GBS EONS, colonization of the maternal vaginal tract with *E. cloacae* just before or during delivery is a condition that has to be fulfilled. To our best knowledge, many studies investigating the presence of *Enterobacteriaceae* in the vaginal microflora frequently reported no vaginal *E. cloacae* carriage in pregnancy (140-144). Solely the studies listed above **(Table 20)** determined vaginal/rectal *E. cloacae* carriage rates in low prevalence (133-136). Particularly, Gbaguidi-Haore and coworkers (2008) established relatively higher *E. cloacae* carriage rates compared to other studies, which may be explained by including only mothers of neonates with suspected sepsis. However, only 5.90% of these maternal *E. cloacae* isolates were found in the neonate with documented *E. cloacae* sepsis, by means of genotyping of the maternal and neonatal isolates. Based on these results, Gbaguidi-Haore et al. suggested that maternal-to-neonate transmission of *E. cloacae* contributed in only a minority to the pathogenesis of sepsis (135).

According to the GBS EONS pathogenesis, substantial concentrations of GBS in the maternal genital tract are highly associated with GBS EONS (47). In our study, very low concentrations of *E. cloacae* were found, which is a potential argument against mother-to-neonate transmission of pathogens. However, it may be possible that *E. cloacae* species are more virulent compared to GBS and have no need for a high inoculum to be transmitted to the foetus/neonate.

Based on the available data in this master thesis, the strongest prove of mother-to-neonate transmission would be the association between vaginal *E. cloacae* carriage and the presence of clinical signs of EONS in the neonate. Nevertheless, it is difficult to draw well-funded conclusions, as the sample size was too small (six cases of suspected EONS). However, no associations were found between *E. cloacae* carriage and the presence of EONS signs.

Briefly, *E. cloacae* was found in the maternal genital tract, but concentrations were very low, suggesting that *E. cloacae* does not survive well in the vaginal microbiome. Based on these findings, two possible pathophysiologic mechanisms may be considered. First, *E. cloacae* species could be more virulent than GBS, implicating that even a low concentration could cause EONS. Second, it may be possible that *E. cloacae* originates from the external environment. Potentially, *E. cloacae* could be acquired from the hospital environment during birth. *E. cloacae* is a gastro-intestinal microorganism, but it is also ubiquitous in terrestrial and aquatic environments (e.g. water, sewage and soil), as well as in food (135). Moreover, poor hand hygiene during delivery may be a nosocomial source of infection for neonates (135).

Future research should focus on the correspondence between maternal and neonatal *E. cloacae* strains by means of culturing techniques, qPCR and typing. Furthermore, it may be interesting to take swabs from the environment during delivery, as well as from the hands of healthcare providers to investigate the impact of external *E. cloacae* sources (43).

Klebsiella pneumoniae

The vaginal carriage rate of *K. pneumoniae* in pregnant women in Bukavu (DRC) was 12.12% (95% CI: 9.03-16.09). In **Table 21,** vaginal *K. pneumoniae* carriage rates in pregnant women worldwide are listed as reference (145-151). To our best knowledge, no previous studies investigated the prevalence of vaginal *K. pneumoniae* in DRC.

Table 21. Prevalence rates of vaginal *Klebsiella pneumoniae* carriage in pregnant women worldwide.

Country	Year	n	% *K. pneumoniae*	Timing	Sample	Detection	Reference
Bangladesh	2013	1219	8,50	D	V	Cu	Chan et al. (148)
DRC	**2019**	**330**	**12,12**	**17-20 WGA**	**V**	**qPCR**	**This master thesis**
India	2006	102	4,90	PD	V	Cu	Kerur et al. (149)
India	2014	69	7,30	D	V	Cu	Chaudhary et al. (150)
Saoudi-Arabia	2002	62	12,90	D	C	Cu	Asinidi et al. (145)
Sri Lanka	2018	250	12,40	D	V	Cu	Nanayakkara et al. (151)
Sudan	2019	300	13,40	3th trimester	V	Cu	Gorish et al. (147)
Uganda	2013	53	18,90	PD	V	Cu	Kiwanuka et al. (146)
Average prevalence			**11.20**				

Based on our findings, the prevalence of vaginal *K. pneumoniae* carriage of pregnant women in Bukavu (Democratic Republic of the Congo) is 12.12% The mean prevalence of vaginal *K. pneumoniae* carriage worldwide is 11.20%. This master thesis was excluded to measure the average prevalence, as qPCR is considered as more sensitive compared to culturing techniques (115). C: endocervical swab. Cu: culture. D: delivery. DRC: Democratic Republic of the Congo. *K. pneumoniae*: *Klebsiella pneumoniae*. N: number of study participants. PD: post-delivery. qPCR: quantitative polymerase chain reaction. V: high vaginal swab. WGA: weeks of gestational age. Year: year of publication of the study.

According to the available literature, the mean vaginal *K. pneumoniae* carriage worldwide is 11.20% (151), which is in accordance with our findings. In this master thesis, quite high concentrations of *K. pneumoniae* were determined in some of the positive women. Consequently, contrary to *E. cloacae, K. pneumoniae* seems to survive better in the vaginal microbiome.

Furthermore, maternal-neonatal transmission rates from 8.60% up to 35.70% were identified in studies investigating the role of *Enterobacteriaceae* (mostly *E. coli and K. pneumoniae*) in neonatal sepsis (142-144, 151, 152). Culturing techniques were used in these studies to prove transmission of maternal strains to the neonate (142-144, 151, 152), so probably, the use of qPCR and typing would contribute to an even more precise and sensitive detection.

Moreover, based on the identified adverse pregnancy outcomes (see below), significantly more signs and symptoms of EONS were found in neonates of mothers colonized with *K. pneumoniae*. However, a critical interpretation is indicated as *K. pneumoniae* was not cultured from the blood samples, taken from the neonates, suspected for EONS. Furthermore, the sample size of the suspected EONS cases was too low (N=6) to draw well-founded conclusions.

In order to further elucidate the pathogenesis of *K. pneumoniae* EONS, future research is recommended to investigate the transmission of vaginal *K. pneumoniae* to the neonate more carefully by means of culturing techniques, qPCR and typing.

4.2.2 Risk factors associated with vaginal *Enterobacter cloacae* and *Klebsiella pneumoniae* carriage

Enterobacter cloacae

Pregnant women who had anal sexual intercourse[4] were almost seven times more likely to carry *E. cloacae* vaginally (borderline significance: p=0.054). In previous studies about GBS, the rectum has been suggested as the major source for colonization of the vaginal econiche (115, 137). Several studies found an association between anal sexual intercourse and an increased number of gastrointestinal bacteria in the vaginal microbiome (153, 154). Hence, it could be assumed that gastrointestinal microorganisms spread more easily to the vagina as a consequence of anal sexual intercourse (153, 154).

Klebsiella pneumoniae

Pregnant women with an intermediate microflora were three times more likely to carry *K. pneumoniae* vaginally. To date, little is known about the role of the intermediate vaginal flora (155). Lactobacilli may be absent in the intermediate vaginal microflora (59), favoring colonization by other microorganisms such as rectal *K. pneumoniae*. Particularly, in a healthy microflora, lactobacilli prevent the growth of pathogens by competition for nutrients and by ensuring a steric hindrance for attachment. Furthermore, lactobacilli cause a low pH, found only in the vagina of human beings. Finally, bacteriocins and hydrogen perioxide, produced by the lactobacilli, can inhibit further bacterial growth (78, 79, 156).

4.2.3 Signs and symptoms associated with vaginal *Enterobacter cloacae* and *Klebsiella pneumoniae* carriage

In our study, no typical signs nor symptoms were observed in association with vaginal *E. cloacae* and *K. pneumoniae* carriage. The low concentrations of *E. cloacae* in the vaginal microflora could account for the absence of signs and symptoms.

An elevated maternal heart rate was independently and significantly associated with *K. pneumoniae* carriage (AOR: 4.14). However, no well-grounded conclusions could be drawn as the sample size was too small (N=11).

4.2.4 Adverse pregnancy outcomes associated with vaginal *Enterobacter cloacae* and *Klebsiella pneumoniae* carriage

Enterobacter cloacae

Despite the assumption that *E. cloacae* is one of the most prominent species causing EONS in Bukavu (DRC) (58), no association with signs nor symptoms of neonatal sepsis was observed in

[4] Information about frequency and timing was not known.

this master thesis. A small sample size of neonates suspected for EONS (N=6) could possibly contribute to this finding.

In our study, pregnant women who carried *E. cloacae* vaginally, were 13.50 times more likely to have a history of PTB. However, no significant association between *E. cloacae* carriage and PTB in the current pregnancy was found[5]. Sherman et al. (1997) suggested that Gram-negative enteric rods, including *Enterobacter* spp., were important pathogens responsible for subclinical chorioamnionitis and possibly even for PTB (157). However, in this master thesis, no well-founded conclusions could be drawn as no vaginal *E. cloacae* carriage rates from previous pregnancies were available.

Women who received a treatment for dysuria in the current pregnancy were six times more likely to carry *E. cloacae* vaginally. Pregnant women may be more susceptible to urinary tract infection (UTI), as an increased urinary content of nutrients, such as glucose, amino acids and vitamins, would possibly increase the occurrence of infection (158). Approximately 85-95% of all UTIs are caused by uropathogenic *Enterobacteriaceae*, including *E. cloacae* (159, 160). So probably, it could be suggested that the experienced dysuria was a manifestation of an UTI, possibly caused by *E. cloacae*.

Klebsiella pneumoniae

Despite the small sample size of neonates with suspected EONS (N=6), a significant, independent association was found with *K. pneumoniae* carriage, i.e. four mothers out of six who gave birth to a neonate with suspected sepsis, were colonized with *K. pneumoniae*. Particularly, women carrying *K. pneumoniae* vaginally were 11.76 times more likely to give birth to a neonate with symptoms of generalized sepsis, requiring a blood culture.

Historically, a positive blood culture is considered as the 'golden standard' for the presence of neonatal sepsis. Nevertheless, the clinical presentation of the sick neonate, together with inflammatory biomarkers in blood, would contribute to an even stronger diagnosis (42-48). In this master thesis, *E. cloacae*, nor *K. pneumoniae*, was determined on blood culture[6]. However, clinical signs as respiratory distress (apnea, polypnea), fever and an abnormal and lethargic general state

[5] The sample size of the previous preterm deliveries and the current preterm births is comparable, respectively 20 and 30 cases.

[6] Blood samples were taken from six neonates, showing signs of generalized sepsis. Subsequently, the samples were cultured at the local laboratory at Provincial Referral Hospital of Bukavu (PRBH), establishing two positive cultures with *Citrobacter amalonaticus* and one positive culture with *Group B Streptococcus* (GBS or *Streptococcus agalactiae*). *E. cloacae*, nor *K. pneumoniae*, was determined on blood culture.

were observed in the first week of life. Together with an increased CRP (C-reactive protein) over time, the diagnosis of EONS could probably be suggested.

As *K. pneumoniae* is one of the prominent pathogens causing EONS in Bukavu (DRC) (58), the significant association between neonates with suspected EONS and maternal, vaginal *K. pneumoniae* carriage, reinforces the possible mother-to-neonate transmission, following the GBS EONS pathogenesis. However, a critical interpretation is indicated as *K. pneumoniae* was not defined in the concerning EONS cases. Furthermore, the sample size of the suspected EONS cases was too low (N=6) to draw well-founded conclusions. Future research should broaden the sample size of neonates with EONS to investigate this association more carefully.

Pregnant women who carried *K. pneumoniae* vaginally, were twice as likely to have a history of, mostly spontaneous, abortion. Omwandho and coworkers (2005) suggested that *K. pneumoniae* infection may lead to premature loss of pregnancy. This assumption is mainly based on the establishment of a *K. pneumoniae* infection in one abortive placenta during the post-mortem examination (161). Furthermore, Seliga-Siwecka and coworkers (2012) found that pregnant women who carried *K. pneumoniae* vaginally were more than five times more likely to suffer from chorioamnionitis, which may result in spontaneous abortion (162). Apart from these cases, there is hardly any literature to indicate whether these infections may be responsible for early pregnancy loss (162). Furthermore, no well-founded conclusions could be drawn as no vaginal *K. pneumoniae* carriage rates from previous pregnancies were available.

Finally, women who underwent caesarean section for obstetrical reasons were four times more likely to carry *K. pneumoniae* vaginally. Several studies stated that neonatal sepsis is significantly less prevalent in mothers having an elective caesarean section compared to a vaginal delivery (163, 164). Possibly, it could be suggested that fetal distress, because of emerging neonatal sepsis during labor, resulted in an emergency caesarean section. However, based on current literature, no clear explanation for the association between vaginal *K. pneumoniae* carriage and caesarean section could be found.

4.3 Limitations and future research prospective

To our best knowledge, this master thesis is the first study investigating vaginal *Candida*, *E. cloacae* and *K. pneumoniae* carriage rates in pregnant women in DRC by qPCR.

A first limitation of the AVEONS project is the substantial loss to follow up. Nearly one third of the study participants withdrew from the study cohort due to the worsening socio-political situation in Bukavu (DRC). Furthermore, in this master thesis, a subset of 330 out of 533 pregnant women

was created, based on the presence of CVLs, the vaginal samples used in the molecular tests of this master thesis. Due to this restricted availability, our study may be prone to selection bias.

Furthermore, the sample size was too small to investigate some assumptions in a more precise way, e.g. LBW, EONS…. Regarding the AVEONS project (N=533), only ten (2.97%) neonates showed signs of generalized sepsis, of which in only four cases a pathologic micro-organism was found on blood culture (laboratory confirmed sepsis). Nevertheless, in order to include sufficient cases of EONS, a calculation of the required sample size was set up prior to recruitment. A total of 94 (16.81%) suspected EONS cases were estimated for a sample size of 559 pregnant women. In order to draw more well-grounded conclusions about a potential mother-to-neonate transmission of vaginal *E. cloacae* and *K. pneumoniae* species, the sample size of neonates with EONS should be broadened in future research

Subsequently, future research should focus on sampling during delivery as this is the critical time point for the pathogens to ascend and colonize the neonate (43-47, 53, 139). *E. cloacae* and *K. pneumoniae* were the two most prevalent microorganisms causing EONS in Bukavu (DRC) (58). Both bacteria are part of the gastro-intestinal microflora (57). Consequently, it would be recommended in future research to collect vagino-rectal samples in order to get a broader insight in the carriage of *E. cloacae* and *K. pneumoniae*.

Moreover, new insights on symptoms of vaginal *Candida* carriage were formulated in this master thesis. An implementation study is required to assess the feasibility of this new clinical approach, instead of the ongoing syndromic approach, and to elaborate a clinical protocol (clinical approach complemented with microscopy) to approach pregnant women with vaginal *Candida* carriage. An RCT examining the impact of clotrimazole on (a)symptomatic[7] *Candida* carriage is methodologically the best approach, but ethical reflections have to be considered as it is unethical to not provide adequate care for pregnant women with high concentrations of *Candida*.

Finally, it is recommended to determine maternal-neonatal transmission rates of *E. cloacae* and *K. pneumoniae,* by genotyping isolates from neonates and the maternal recto-genital tract. Regarding *E. cloacae*, apart from mother-to-neonate transmission, transmission from environmental sources should be considered. It may be interesting to take swabs from the

[7] An approach, consisting of the recognition of typical symptoms complemented with microscopy, may include both symptomatic, as well as asymptomatic pregnant women who carry *Candida* vaginally.

environment during delivery and from the hands of healthcare providers to investigate the impact of external *E. cloacae* sources.

4.4 Conclusions

This master thesis provided indications that vaginal *Candida* carriage, early in pregnancy, may be a risk factor for PTB. Primary prevention strategies, such as creating more hygienic toilet facilities, may be effective to diminish vaginal *Candida* carriage. Specific clinical symptoms as vaginal discharge, vaginal itching and a burning sensation after sexual intercourse, complemented by the presence of yeast on Gram stain/wet mount microscopy, may be sufficient to identify the possible virulent *Candida* species causing PTB. Within the scope of secondary prevention for PTB, treatment with exclusively clotrimazole may be opportune to restore the distorted microflora and to eradicate *Candida*, although our study could not confirm this. Future research should focus on identifying the ideal time point to screen and treat for vaginal *Candida* carriage, as well as elaborating a clinical protocol to approach pregnant women with vaginal *Candida* carriage.

Furthermore, this master thesis contributed to the description of the pathogenesis of EONS in Bukavu (DRC). It can be suggested that the two main causative pathogens of EONS in Bukavu (DRC) may have a different mode of action. Whereas *K. pneumoniae* was found in a relative high concentration in the female genital tract, concentrations of *E. cloacae* were rather low. Furthermore, *E. cloacae* was four times more prevalent in the vaginal microflora compared to *K. pneumoniae*. Future research is recommended to elaborate the pathogenesis of EONS more precisely by assessing maternal-neonatal transmission rates by means of culturing techniques, qPCR and typing. Moreover, vaginal *E. cloacae* and *K. pneumoniae* carriage was associated with (a history) of adverse pregnancy outcomes.

Bibliography

1. Grady SC, Frake AN, Zhang Q, Bene M, Jordan DR, Vertalka J, et al. Neonatal mortality in East Africa and West Africa: a geographic analysis of district-level demographic and health survey data. Geospatial health. 2017.
2. Lawn JE, Kerber K, Enweronu-Laryea C, Cousens S, editors. 3.6 million neonatal deaths—what is progressing and what is not? Seminars in perinatology; 2010: Elsevier.
3. Lehtonen L, Gimeno A, Parra-Llorca A, Vento M, editors. Early neonatal death: a challenge worldwide. Seminars in Fetal and Neonatal Medicine; 2017: Elsevier.
4. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. The Lancet. 2016;388(10063):3027-35.
5. Organisation WH. Monitoring health for the Sustainable Development Goals 2020. Available from: file:///H:/Downloads/9789240005105-eng.pdf.
6. Organization WH. Health in 2015: from MDGs, millennium development goals to SDGs, sustainable development goals. 2015.
7. Unicef. Neonatal death report 2018. Available from: https://data.unicef.org/topic/maternal-health/newborn-care/.
8. WHO. Global Health Observatory (GHO) data: trends in child mortality. 2019.
9. Ahmed I, Ali SM, Amenga-Etego S, Ariff S, Bahl R, Baqui AH, et al. Population-based rates, timing, and causes of maternal deaths, stillbirths, and neonatal deaths in south Asia and sub-Saharan Africa: a multi-country prospective cohort study. The Lancet Global Health. 2018;6(12):e1297-e308.
10. Lawn JE. Africa's newborns – couting them and making them count. 2010.
11. Lawn JE, Cousens S, Zupan J, Team LNSS. 4 million neonatal deaths: when? Where? Why? The lancet. 2005;365(9462):891-900.
12. Kinney MV, Kerber KJ, Black RE, Cohen B, Nkrumah F, Coovadia H, et al. Sub-Saharan Africa's mothers, newborns, and children: where and why do they die? PLoS Med. 2010;7(6):e1000294.
13. Lee AC, Blencowe H, Lawn JE. Small babies, big numbers: global estimates of preterm birth. The Lancet Global Health. 2019;7(1):e2-e3.
14. Golding N, Burstein R, Longbottom J, Browne AJ, Fullman N, Osgood-Zimmerman A, et al. Mapping under-5 and neonatal mortality in Africa, 2000–15: a baseline analysis for the Sustainable Development Goals. The Lancet. 2017;390(10108):2171-82.
15. WHO U. Every Newborn: an action plan to end preventable deaths: Executive summary 2014. Available from: http://www.healthynewbornnetwork.org/hnn-content/uploads/Every_Newborn_Action_Plan-EXECUTIVE_SUMMARY-ENGLISH_updated_July2014.pdf.
16. McArthur JW, Rasmussen K, Yamey G. How many lives are at stake? Assessing 2030 sustainable development goal trajectories for maternal and child health. Bmj. 2018;360.
17. Hug L, Alexander M, You D, Alkema L, for Child UI-aG. National, regional, and global levels and trends in neonatal mortality between 1990 and 2017, with scenario-based projections to 2030: a systematic analysis. The Lancet Global Health. 2019;7(6):e710-e20.
18. Ho W. Recommended definition, terminology and format for statistical tables related to the perinatal period. Acta Obstet Gynecol Scand. 1997;56:247-53.
19. Di Renzo GC, Tosto V, Giardina I. The biological basis and prevention of preterm birth. Best Practice & Research Clinical Obstetrics & Gynaecology. 2018;52:13-22.
20. Harrison MS, Goldenberg RL, editors. Global burden of prematurity. Seminars in fetal and neonatal medicine; 2016: Elsevier.
21. Vogel JP, Chawanpaiboon S, Moller A-B, Watananirun K, Bonet M, Lumbiganon P. The global epidemiology of preterm birth. Best Practice & Research Clinical Obstetrics & Gynaecology. 2018;52:3-12.
22. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A-B, et al. Born too soon: the global epidemiology of 15 million preterm births. Reproductive health. 2013;10(S1):S2.
23. Boyle AK, Rinaldi SF, Norman JE, Stock SJ. Preterm birth: Inflammation, fetal injury and treatment strategies. Journal of Reproductive Immunology. 2017;119:62-6.
24. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. The lancet. 2008;371(9606):75-84.
25. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, et al. The preterm parturition syndrome. BJOG: An International Journal of Obstetrics & Gynaecology. 2006;113:17-42.

26. Rubens CE, Sadovsky Y, Muglia L, Gravett MG, Lackritz E, Gravett C. Prevention of preterm birth: harnessing science to address the global epidemic. Science translational medicine. 2014;6(262):262sr5-sr5.
27. Donders GG, Bellen G. Management of abnormal vaginal flora as a risk factor for preterm birth. Preterm Birth—Mother and Child. 2011.
28. Farr A, Kiss H, Holzer I, Husslein P, Hagmann M, Petricevic L. Effect of asymptomatic vaginal colonization with Candida albicans on pregnancy outcome. Acta obstetricia et gynecologica Scandinavica. 2015;94(9):989-96.
29. Holzer I, Farr A, Kiss H, Hagmann M, Petricevic L. The colonization with Candida species is more harmful in the second trimester of pregnancy. Archives of Gynecology and Obstetrics. 2017;295(4):891-5.
30. Kiss H, Petricevic L, Husslein P. Prospective randomised controlled trial of an infection screening programme to reduce the rate of preterm delivery. Bmj. 2004;329(7462):371.
31. Kiss H, Petricevic L, Martina S, Husslein P. Reducing the rate of preterm birth through a simple antenatal screen-and-treat programme: a retrospective cohort study. European journal of obstetrics & gynecology and reproductive biology. 2010;153(1):38-42.
32. Roberts CL, Algert CS, Rickard KL, Morris JM. Treatment of vaginal candidiasis for the prevention of preterm birth: a systematic review and meta-analysis. Systematic reviews. 2015;4(1):31.
33. Roberts CL, Rickard K, Kotsiou G, Morris JM. Treatment of asymptomatic vaginal candidiasis in pregnancy to prevent preterm birth: an open-label pilot randomized controlled trial. BMC pregnancy and childbirth. 2011;11(1):18.
34. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. New England journal of medicine. 2000;342(20):1500-7.
35. Oliver R, Lamont R. Infection and antibiotics in the aetiology, prediction and prevention of preterm birth. Journal of Obstetrics and Gynaecology. 2013;33(8):768-75.
36. Pařízek A, Koucký M, Dušková M. Progesterone, inflammation and preterm labor. The Journal of steroid biochemistry and molecular biology. 2014;139:159-65.
37. Riggs MA, Klebanoff MA. Treatment of vaginal infections to prevent preterm birth: a meta-analysis. Clinical obstetrics and gynecology. 2004;47(4):796-807.
38. Bollopragada S, Youssef R, Jordan F, Greer I, Norman J, Nelson S. Term labor is associated with a core inflammatory response in human fetal membranes, myometrium, and cervix. American journal of obstetrics and gynecology. 2009;200(1):104. e1-. e11.
39. Aguin T, Sobel J. Vulvovaginal candidiasis in pregnancy. Current infectious disease reports. 2015;17(6):30.
40. Hay P, Czeizel AE. Asymptomatic trichomonas and candida colonization and pregnancy outcome. Best Practice & Research Clinical Obstetrics & Gynaecology. 2007;21(3):403-9.
41. Maki Y, Fujisaki M, Sato Y, Sameshima H. Candida chorioamnionitis leads to preterm birth and adverse fetal-neonatal outcome. Infectious Diseases in Obstetrics and Gynecology. 2017;2017.
42. Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal infectious diseases: evaluation of neonatal sepsis. Pediatric Clinics of North America. 2013;60(2):367.
43. Cortese F, Scicchitano P, Gesualdo M, Filaninno A, De Giorgi E, Schettini F, et al. Early and late infections in newborns: where do we stand? A review. Pediatrics & Neonatology. 2016;57(4):265-73.
44. Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. The lancet. 2017;390(10104):1770-80.
45. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. Clinical microbiology reviews. 2014;27(1):21-47.
46. Polin RA. Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics. 2012;129(5):1006-15.
47. Shane AL, Stoll BJ. Neonatal sepsis: progress towards improved outcomes. Journal of Infection. 2014;68:S24-S32.
48. Wynn JL. Defining neonatal sepsis. Current opinion in pediatrics. 2016;28(2):135.
49. Lawn JE, Patterson J, Claeson M, Ayede AI, Stoll B. Opening the black box for etiology of neonatal infections in high burden settings: the contribution of ANISA. The Pediatric infectious disease journal. 2016;35(5):S3-S5.
50. WHO. Global Health Observatory (GHO) data: neonatal mortality 2018. Available from: https://www.who.int/gho/child_health/mortality/neonatal/en/.
51. Cools P, Melin P. Group B Streptococcus and perinatal mortality. Research in microbiology. 2017;168(9-10):793-801.

52. Bunduki GK, Adu-Sarkodie Y. Clinical outcome and isolated pathogens among neonates with sepsis in Democratic Republic of the Congo: a cross-sectional study. BMC research notes. 2019;12(1):303.
53. Chan GJ, Lee AC, Baqui AH, Tan J, Black RE. Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. PLoS Med. 2013;10(8):e1001502.
54. Melin P. Neonatal group B streptococcal disease: from pathogenesis to preventive strategies. Clinical Microbiology and Infection. 2011;17(9):1294-303.
55. Russell NJ, Seale AC, O'Sullivan C, Le Doare K, Heath PT, Lawn JE, et al. Risk of early-onset neonatal group B streptococcal disease with maternal colonization worldwide: systematic review and meta-analyses. Clinical infectious diseases. 2017;65(suppl_2):S152-S9.
56. Schrag SJ, Zell ER, Lynfield R, Roome A, Arnold KE, Craig AS, et al. A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. New England Journal of Medicine. 2002;347(4):233-9.
57. Paauw A, Caspers MP, Schuren FH, Leverstein-van Hall MA, Delétoile A, Montijn RC, et al. Genomic diversity within the Enterobacter cloacae complex. PLoS one. 2008;3(8):e3018.
58. Claeys M. Antibiotic susceptibility of bacterial aetiological agents causing early-onset neonatal sepsis in Bukavu, Democratic Republic of Congo. Clinical Microbiology and Infectious Diseases, 29th European congress, Abstracts. 2019.
59. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. Journal of clinical microbiology. 1991;29(2):297-301.
60. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. The American journal of medicine. 1983;74(1):14-22.
61. M Romorena MV, M Rahmanc, J Sundbyd, E Kloumane, P Hjortdahl. Trichomoniasis and bacterial vaginosis in pregnancy: inadequately managed with the syndromic approach 2007. Available from: https://www.who.int/bulletin/volumes/85/4/06-031922/en/.
62. Tellapragada C, Eshwara VK, Bhat P, Kamath A, Aletty S, Mukhopadhyay C. Screening of vulvovaginal infections during pregnancy in resource constrained settings: Implications on preterm delivery. Journal of Infection and Public Health. 2017;10(4):431-7.
63. De Vulder A. Prevalence, risk factors and associated adverse pregnancy outcome of bacterial vaginosis in pregnant women in Bukavu, Democratic Republic of Congo. Clinical Microbiology and Infectious Diseases, 29th European congress, Abstracts. 2019.
64. Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, et al. Population-level analysis of gut microbiome variation. Science. 2016;352(6285):560-4.
65. Decat E, Van Mechelen E, Saerens B, Vermeulen SJ, Boekhout T, De Blaiser S, et al. Rapid and accurate identification of isolates of Candida species by melting peak and melting curve analysis of the internally transcribed spacer region 2 fragment (ITS2-MCA). Research in microbiology. 2013;164(2):110-7.
66. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. 1990;18(1):315-22.
67. Duyvejonck H, Cools P, Decruyenaere J, Roelens K, Noens L, Vermeulen S, et al. Validation of high resolution melting analysis (HRM) of the amplified ITS2 region for the detection and identification of yeasts from clinical samples: comparison with culture and MALDI-TOF based identification. PLoS One. 2015;10(8):e0132149.
68. Medicine NLo. BLAST 2020. Available from: http://blast.ncbi.nlm.nih.gov/Blast.cgi.
69. sciences Ricoaa. The mfold web server 2020. Available from: http://mfold.rna.albany.edu/?q=mfold.
70. Kim K-P, Klumpp J, Loessner MJ. Enterobacter sakazakii bacteriophages can prevent bacterial growth in reconstituted infant formula. International journal of food microbiology. 2007;115(2):195-203.
71. Multivariable models. 2020.
72. Thorsen P, Vogel I, Olsen J, Jeune B, Westergaard JG, Jacobsson B, et al. Bacterial vaginosis in early pregnancy is associated with low birth weight and small for gestational age, but not with spontaneous preterm birth: a population-based study on Danish women. The Journal of Maternal-Fetal & Neonatal Medicine. 2006;19(1):1-7.
73. Ohad S, Block C, Kravitz V, Farber A, Pilo S, Breuer R, et al. Rapid identification of E nterobacter hormaechei and E nterobacter cloacae genetic cluster III. Journal of applied microbiology. 2014;116(5):1315-21.
74. Hoffmann H, Roggenkamp A. Population genetics of the nomenspecies Enterobacter cloacae. Applied and environmental microbiology. 2003;69(9):5306-18.

75. Trung NT, Van Tong H, Lien TT, Van Son T, Huyen TTT, Quyen DT, et al. Clinical utility of an optimised multiplex real-time PCR assay for the identification of pathogens causing sepsis in Vietnamese patients. International Journal of Infectious Diseases. 2018;67:122-8.
76. Kaushik R, Balasubramanian R. Assessment of bacterial pathogens in fresh rainwater and airborne particulate matter using Real-Time PCR. Atmospheric Environment. 2012;46:131-9.
77. Simhan HN, Caritis SN, Krohn MA, Hillier SL. The vaginal inflammatory milieu and the risk of early premature preterm rupture of membranes. American journal of obstetrics and gynecology. 2005;192(1):213-8.
78. Sobel JD. Vulvovaginal candidosis. The Lancet. 2007;369(9577):1961-71.
79. Sobel JD. Pathogenesis and epidemiology of vulvovaginal candidiasis. Annals of the New York Academy of Sciences. 1988;544:547-57.
80. Chatzivasileiou P, Vyzantiadis TA. Vaginal yeast colonisation: From a potential harmless condition to clinical implications and management approaches—A literature review. Mycoses. 2019;62(8):638-50.
81. Abdelaziz ZA, Ibrahim ME, Bilal NE, Hamid ME. Vaginal infections among pregnant women at Omdurman Maternity Hospital in Khartoum, Sudan. The Journal of Infection in Developing Countries. 2014;8(04):490-7.
82. Aboyeji A, Nwabuisi C. Prevalence of sexually transmitted diseases among pregnant women in Ilorin, Nigeria. Journal of Obstetrics and Gynaecology. 2003;23(6):637-9.
83. Akah P, Nnamani C, Nnamani P. Prevalence and treatment outcome of vulvovaginal candidiasis in pregnancy in a rural community in Enugu State, Nigeria. Journal of Medicine and Medical Sciences. 2010;1(10):447-52.
84. Akerele J, Abhulimen P, Okonofua F. Prevalence of asymptomatic genital infection among pregnant women in Benin City, Nigeria. African journal of reproductive health. 2002:93-7.
85. Apea-Kubi KA, Sakyi B, Yamaguchi S, Ofori-Adjei D. Bacterial vaginosis, Candida albicans and Trichomonas vaginalis infection in antenatal and gynaecological patients in Ghana. Tropical Journal of Obstetrics and Gynaecology. 2005;22:108-12.
86. Bourgeois A, Henzel D, Malonga-Mouelet G, Dibanga G, Tsobou C, Peeters M, et al. Clinical algorithms for the screening of pregnant women for STDs in Libreville, Gabon: which alternatives? Sexually transmitted infections. 1998;74(1):35-9.
87. Blankhart D, Muller O, Gresenguet G, Weis P. Sexually transmitted infections in young pregnant women in Bangui, Central African Republic. International journal of STD & AIDS. 1999;10(9):609-14.
88. Donbraye-Emmanuel O, Donbraye E, Okonko I, Alli J, Ojezele M, Nwanze J. Detection and prevalence of Candida among pregnant women in Ibadan, Nigeria. World Applied Science Journal. 2010;10(9):986-91.
89. Dakey KA. Vaginal Infections in Pregnant Women at the Bè Hospital in Lomé (Togo) from 2008 to 2013. Open Access Library Journal. 2018;5(03):1.
90. Ekwempu C, Lawande R, Egler L. Microbial flora of the lower genital tract of women in labour in Zaria, Nigeria. Journal of clinical pathology. 1981;34(1):82-3.
91. Konadu DG, Owusu-Ofori A, Yidana Z, Boadu F, Iddrisu LF, Adu-Gyasi D, et al. Prevalence of vulvovaginal candidiasis, bacterial vaginosis and trichomoniasis in pregnant women attending antenatal clinic in the middle belt of Ghana. BMC pregnancy and childbirth. 2019;19(1):341.
92. Fonck K, Kidula N, Jaoko W, Estambale B, Claeys P, Ndinya-Achola J, et al. Validity of the vaginal discharge algorithm among pregnant and non-pregnant women in Nairobi, Kenya. Sexually transmitted infections. 2000;76(1):33-8.
93. Jespers V, Crucitti T, Menten J, Verhelst R, Mwaura M, Mandaliya K, et al. Prevalence and correlates of bacterial vaginosis in different sub-populations of women in sub-Saharan Africa: a cross-sectional study. PloS one. 2014;9(10):e109670.
94. Kurewa NE, Mapingure MP, Munjoma MW, Chirenje MZ, Rusakaniko S, Stray-Pedersen B. The burden and risk factors of sexually transmitted infections and reproductive tract infections among pregnant women in Zimbabwe. BMC infectious diseases. 2010;10(1):127.
95. Nelson M, Wanjiru W, Margaret MW. Prevalence of vaginal candidiasis and determination of the occurrence of Candida species in pregnant women attending the antenatal clinic of Thika District Hospital, Kenya. Open Journal of Medical Microbiology. 2013;2013.
96. Meda N, Sangare L, Lankoande S, Sanou PT, Compaore PI, Catraye J, et al. Pattern of sexually transmitted diseases among pregnant women in Burkina Faso, west Africa: potential for a clinical management based on simple approaches. Sexually Transmitted Infections. 1997;73(3):188-93.

97. Mabey DC, Wall R, Bello C. Aetiology of genital ulceration in the Gambia. Sexually Transmitted Infections. 1987;63(5):312-5.
98. Msuya SE, Uriyo J, Hussain A, Mbizvo EM, Jeansson S, Sam NE, et al. Prevalence of sexually transmitted infections among pregnant women with known HIV status in northern Tanzania. Reproductive health. 2009;6:4.
99. Mukasa KJ, Herbert I, Daniel A, Sserunkuma KL, Joel B, Frederick B. Antifungal Susceptibility Patterns of Vulvovaginal Candida species among Women Attending Antenatal Clinic at Mbarara Regional Referral Hospital, South Western Uganda. British microbiology research journal. 2015;5(4):322-31.
100. Mulanga-Kabeya C, Morel E, Patrel D, Delaporte E, Bougoudogo F, Maiga YI, et al. Prevalence and risk assessment for sexually transmitted infections in pregnant women and female sex workers in Mali: is syndromic approach suitable for screening? Sexually transmitted infections. 1999;75(5):358-9.
101. Mulu W, Yimer M, Zenebe Y, Abera B. Common causes of vaginal infections and antibiotic susceptibility of aerobic bacterial isolates in women of reproductive age attending at Felegehiwot Referral Hospital, Ethiopia: a cross sectional study. BMC Womens Health. 2015;15:42.
102. Mumuney KT, Abalaka ME, editors. Comparative Study of Vaginal Candidiasis in Pregnant and Non-Pregnant Women Attending Ibrahim Badamasi Babangida Specialist Hospital and General Hospital, Minna Niger State, Nigeria2019.
103. Nwosu CO, Djieyep NA. Candidiasis and trichomoniasis among pregnant women in a rural community in the semi-arid zone, north-eastern Nigeria. West Afr J Med. 2007;26(1):17-9.
104. Okonkwo NJ, Umeanaeto P. Prevalence of Vaginal Candidiasis among Pregnant Women in Nnewi Town of Anambra State, Nigeria. African Research Review. 2011;4.
105. Olowe OA, Makanjuola OB, Olowe RA, Adekanle DA. Prevalence of vulvovaginal candidiasis, trichomoniasis and bacterial vaginosis among pregnant women receiving antenatal care in Southwestern Nigeria. European journal of microbiology & immunology. 2014;4 4:193-7.
106. O'Farrell N, Hoosen A, Kharsany A, Ende Jvd. Sexually transmitted pathogens in pregnant women in a rural South African community. Genitourinary Medicine. 1989;65:276 - 80.
107. Ortashi OM, Khidir IE, Herieka E. Prevalence of HIV, syphilis, Chlamydia trachomatis, Neisseria gonorrhoea, Trichomonas vaginalis and candidiasis among pregnant women attending an antenatal clinic in Khartoum, Sudan. Journal of Obstetrics and Gynaecology. 2004;24:513 - 5.
108. Nurat AA, Babalola GO, Shittu MO, Tijani MA, Adekola SA. Detection and Epidemiology of Vulvovaginal Candidiasis among Asymptomatic Pregnant Women Attending a Tertiary Hospital in Ogbomoso, Nigeria. International journal of biomedical research. 2015;6:518-23.
109. Sangaré I, Sirima C, Bamba S, Zida A, Cisse M, Bazié WW, et al. Prevalence of vulvovaginal candidiasis in pregnancy at three health centers in Burkina Faso. Journal de mycologie medicale. 2018;28 1:186-92.
110. Sy O, Diongue K, Ahmed CB, Ba O, Moulay F, Lo B, et al. [Vulvovaginal candidiasis in pregnant women in the M?re et Enfant Hospital center in Nouakchott, Mauritania]. Journal de mycologie medicale. 2018;28 2:345-8.
111. Tchelougou D, Karou DS, Kpotsra A, Balaka A, Assih M, Bamoké M, et al. [Vaginal infections in pregnant women at the Regional Hospital of Sokode (Togo) in 2010 and 2011]. Medecine et sante tropicales. 2013;23 1:49-54.
112. Toua V, Djaouda M, Gaké B, Menye DE, Christie EA. Prevalence of Vulvovaginal Candidiasis amongst pregnant women in Maroua (Cameroon) and the sensitivity of Candida albicans to extracts of six locally used antifungal plants. International Research Journal of Microbiology. 2013;4:89-97.
113. Thomas T, Choudhri S, Kariuki CH, Moses SP. Identifying cervical infection among pregnant women in Nairobi, Kenya: limitations of risk assessment and symptom-based approaches. Genitourinary Medicine. 1996;72:334 - 8.
114. Nnadi DC, Singh S. The prevalence of genital Candida species among pregnant women attending antenatal clinic in a tertiary health center in North-west Nigeria. Sahel Medical Journal. 2017;20:33 - 7.
115. El Aila NA, Tency I, Saerens B, De Backer E, Cools P, dos Santos Santiago GL, et al. Strong correspondence in bacterial loads between the vagina and rectum of pregnant women. Research in microbiology. 2011;162(5):506-13.
116. Mukasa KJ, Herbert I, Daniel A, Sserunkuma KL, Joel B, Frederick B. Antifungal susceptibility patterns of vulvovaginal Candida species among women attending antenatal clinic at Mbarara Regional Referral Hospital, South Western Uganda. British microbiology research journal. 2015;5(4):322.

117. Cotch MF, Hillier SL, Gibbs RS, Eschenbach DA, Group PS. Epidemiology and outcomes associated with moderate to heavy Candida colonization during pregnancy. American journal of obstetrics and gynecology. 1998;178(2):374-80.
118. Nwadioha S, Egah D, Alao O, Iheanacho E. Risk factors for vaginal candidiasis among women attending primary health care centers in Jos, Nigeria. Journal of clinical medicine and research. 2010;2(7):111-3.
119. Reed BD, Gorenflo DW, Gillespie BW, Pierson CL, Zazove P. Sexual behaviors and other risk factors for Candida vulvovaginitis. Journal of women's health & gender-based medicine. 2000;9(6):645-55.
120. Iams JD, Romero R, Culhane JF, Goldenberg RL. Primary, secondary, and tertiary interventions to reduce the morbidity and mortality of preterm birth. The lancet. 2008;371(9607):164-75.
121. Vahidnia A, Tuin H, Bliekendaal H, Spaargaren J. Association of sexually transmitted infections, Candida species, gram-positive flora and perianal flora with bacterial vaginosis. New Microbiol. 2015;38(4):559.
122. Ekuma A, Ikenyi C, Moses A. Candida Colonization and Genital Hygiene Habits among Women in Uyo, Nigeria. JOURNAL OF RESEARCH IN BASIC AND CLINICAL SCIENCES. 2019;1(1):17-22.
123. Czeizel AE, Fladung B, Vargha P. Preterm birth reduction after clotrimazole treatment during pregnancy. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2004;116(2):157-63.
124. Czeizel A, Rockenbauer M. A lower rate of preterm birth after clotrimazole therapy during pregnancy. Paediatric and perinatal epidemiology. 1999;13(1):58-64.
125. McGregor JA, French JI, Parker R, Draper D, Patterson E, Jones W, et al. Prevention of premature birth by screening and treatment for common genital tract infections: results of a prospective controlled evaluation. American journal of obstetrics and gynecology. 1995;173(1):157-67.
126. Prevention CfDca. 2015 Sexually Transmitted Diseases Treatment Guidelines 2015. Available from: https://www.cdc.gov/std/tg2015/candidiasis.htm.
127. Peelen MJ, Luef BM, Lamont RF, de Milliano I, Jensen JS, Limpens J, et al. The influence of the vaginal microbiota on preterm birth: A systematic review and recommendations for a minimum dataset for future research. Placenta. 2019;79:30-9.
128. Mendling W, Brasch J. Guideline vulvovaginal candidosis (2010) of the german society for gynecology and obstetrics, the working group for infections and infectimmunology in gynecology and obstetrics, the german society of dermatology, the board of german dermatologists and the german speaking mycological society. Mycoses. 2012;55:1-13.
129. Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. Antifungal susceptibilities of Candida species causing vulvovaginitis and epidemiology of recurrent cases. Journal of clinical microbiology. 2005;43(5):2155-62.
130. Hillier S, Krohn MA, Watts D, Wolner-Hanssen P, Eschenbach D. Microbiologic efficacy of intravaginal clindamycin cream for the treatment of bacterial vaginosis. Obstetrics and gynecology. 1990;76(3 Pt 1):407-13.
131. University C. Dictionary 2020. Available from: https://dictionary.cambridge.org/dictionary/.
132. Walsh MC, Fanaroff JM. Meconium stained fluid: approach to the mother and the baby. Clinics in perinatology. 2007;34(4):653-65.
133. Kaba M, Manenzhe R, Moodley C, Zar H, Nicol M. Epidemiology of extended-spectrum beta-lactamase-and carbapenemase-producing bacteria in stool from apparently healthy children, South Africa. International Journal of Infectious Diseases. 2016;45:96.
134. Genovese C, Corsello S, Nicolosi D, Aidala V, Falcidia E, Tempera G. Alterations of the vaginal microbiota in the third trimester of pregnancy and pPROM. Eur Rev Med Pharmacol Sci. 2016;20(16):3336-43.
135. Gbaguidi-Haore H, Talon D, Thouverez M, Menget A, Bertrand X. Molecular epidemiology of Enterobacter cloacae in a neonatal department: a 2-year surveillance study. European Journal of Clinical Microbiology & Infectious Diseases. 2008;27(8):643.
136. Chereau F, Herindrainy P, Garin B, Huynh B-T, Randrianirina F, Padget M, et al. Colonization of extended-spectrum-β-lactamase-and NDM-1-producing Enterobacteriaceae among pregnant women in the community in a low-income country: a potential reservoir for transmission of multiresistant Enterobacteriaceae to neonates. Antimicrobial agents and chemotherapy. 2015;59(6):3652-5.
137. El Aila NA, Tency I, Claeys G, Verstraelen H, Saerens B, dos Santos Santiago GL, et al. Identification and genotyping of bacteria from paired vaginal and rectal samples from pregnant women indicates similarity between vaginal and rectal microflora. BMC infectious diseases. 2009;9(1):167.

138. Bulabula A, Dramowski A, Mehtar S. Transmission of multidrug-resistant Gram-negative bacteria from colonized mothers to their infants: a systematic review and meta-analysis. Journal of Hospital Infection. 2020;104(1):57-67.
139. Seale AC, Mwaniki M, Newton CR, Berkley JA. Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa. The Lancet infectious diseases. 2009;9(7):428-38.
140. Son K-A, Kim M, Kim YM, Kim SH, Choi S-J, Oh S-y, et al. Prevalence of vaginal microorganisms among pregnant women according to trimester and association with preterm birth. Obstetrics & gynecology science. 2018;61(1):38-47.
141. Villar HE, Aubert V, Baserni MN, Jugo MB. Maternal carriage of extended-spectrum beta-lactamase-producing Escherichia coli isolates in Argentina. Journal of chemotherapy. 2013;25(6):324-7.
142. Danino D, Melamed R, Sterer B, Porat N, Hazan G, Gushanski A, et al. Mother-to-child transmission of extended-spectrum-beta-lactamase-producing Enterobacteriaceae. Journal of Hospital Infection. 2018;100(1):40-6.
143. Jiménez-Rámila C, López-Cerero L, Martín MA, Martín CV, Serrano L, Pascual Á, et al. Vagino-rectal colonization and maternal–neonatal transmission of Enterobacteriaceae producing extended-spectrum β-lactamases or carbapenemases: a cross-sectional study. Journal of Hospital Infection. 2019;101(2):167-74.
144. Rettedal S, Löhr I, Bernhoff E, Natås O, Sundsfjord A, Øymar K. Extended-spectrum β-lactamase-producing Enterobacteriaceae among pregnant women in Norway: prevalence and maternal–neonatal transmission. Journal of Perinatology. 2015;35(11):907-12.
145. Asindi AA, Archibong EI, Mannan NB. Mother-infant colonization and neonatal sepsis in prelabor rupture of membranes. Saudi Med J. 2002;23(10):1270-4.
146. Kiwanuka J, Bazira J, Mwanga J, Tumusiime D, Nyesigire E, Lwanga N, et al. The microbial spectrum of neonatal sepsis in Uganda: recovery of culturable bacteria in mother-infant pairs. PLoS One. 2013;8(8):e72775.
147. Gorish BMT. Phenotypic Detection of Extended-Spectrum B-Lactamase Producing Escherichia coli and Klebsiella pneumoniae from a Vaginal Swab of Pregnant women in Khartoum State. Acta Scientific Women's Health. 2019;1:13-7.
148. Chan GJ, Baqui AH, Modak JK, Murillo-Chaves A, Mahmud AA, Boyd TK, et al. Early-onset neonatal sepsis in Dhaka, Bangladesh: risk associated with maternal bacterial colonisation and chorioamnionitis. Tropical Medicine & International Health. 2013;18(9):1057-64.
149. Kerur BM, Bhat BV, Harish B, Habeebullah S, Kumar CU. Maternal genital bacteria and surface colonization in early neonatal sepsis. The Indian Journal of Pediatrics. 2006;73(1):29-32.
150. Chaudhary B, Nair N. BACTERIOLOGICAL STUDY OF VAGINAL FLORA IN MOTHERS AT TERM. 2014.
151. Nanayakkara D, Liyanapathirana V, Kandauda C, Gihan C, Ekanayake A, Adasooriya D. Maternal vaginal colonization with selected potential pathogens of neonatal sepsis in the era of antimicrobial resistance, a single center experience from Sri Lanka. BMC infectious diseases. 2018;18(1):351.
152. Denkel L, Gastmeier P, Piening B. To screen or not to screen mothers of preterm infants for extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E). Journal of Perinatology. 2015;35(11):893-4.
153. Hong X, Fang S, Huang K, Yin J, Chen J, Xuan Y, et al. Characteristics of the vaginal microbiome in cross-border female sex workers in China: a case-control study. PeerJ. 2019;7:e8131.
154. McBride KR, Fortenberry JD. Heterosexual anal sexuality and anal sex behaviors: a review. Journal of sex research. 2010;47(2-3):123-36.
155. Donders GG. Definition and classification of abnormal vaginal flora. Best Practice & Research Clinical Obstetrics & Gynaecology. 2007;21(3):355-73.
156. Vaneechoutte M. The human vaginal microbial community. Research in microbiology. 2017;168(9-10):811-25.
157. Sherman D, Tovbin J, Lazarovich T, Avrech O, Reif R, Hoffmann S, et al. Chorioamnionitis caused by gram-negative bacteria as an etiologic factor in preterm birth. European Journal of Clinical Microbiology and Infectious Diseases. 1997;16(6):417-23.
158. Manjula N, Math GC, Patil A, Gaddad SM, Shivannavar CT. Incidence of urinary tract infections and its aetiological agents among pregnant women in Karnataka region. Advances in Microbiology. 2013;2013.

159. Gilstrap III LC, Ramin SM. Urinary tract infections during pregnancy. Obstetrics and gynecology clinics of North America. 2001;28(3):581-91.
160. Khatun S, Nessa A, Mahmood A. Urinary tract infections in pregnancy. ORION. 1999;4:15-20.
161. Omwandho CO, Gruessner SE, Tinneberg H-R. Early pregnancy loss and neonatal deaths associated with Klebsiella pneumonia infection: a mini review of possible occupational health risk. Archives of Gynecology and Obstetrics. 2006;273(5):258-60.
162. Seliga-Siwecka JP, Kornacka MK. Neonatal outcome of preterm infants born to mothers with abnormal genital tract colonisation and chorioamnionitis: a cohort study. Early human development. 2013;89(5):271-5.
163. Hook B, Kiwi R, Amini SB, Fanaroff A, Hack M. Neonatal morbidity after elective repeat cesarean section and trial of labor. Pediatrics. 1997;100(3):348-53.
164. Signore C, Klebanoff M. Neonatal morbidity and mortality after elective cesarean delivery. Clinics in perinatology. 2008;35(2):361-71.
165. Liu CF, Shi XP, Chen Y, Jin Y, Zhang B. Rapid diagnosis of sepsis with TaqMan-Based multiplex real-time PCR. Journal of clinical laboratory analysis. 2018;32(2):e22256.
166. Miyoshi-Akiyama T, Hayakawa K, Ohmagari N, Shimojima M, Kirikae T. Multilocus sequence typing (MLST) for characterization of Enterobacter cloacae. PloS one. 2013;8(6):e66358.
167. Pavlovic M, Konrad R, Iwobi AN, Sing A, Busch U, Huber I. A dual approach employing MALDI-TOF MS and real-time PCR for fast species identification within the Enterobacter cloacae complex. FEMS microbiology letters. 2012;328(1):46-53.
168. Silva-Junior WP, Martins AS, Xavier PC, Appel KL, Junior SAO, Palhares DB. Etiological profile of early neonatal bacterial sepsis by multiplex qPCR. The Journal of Infection in Developing Countries. 2016;10(12):1318-24.
169. Anbazhagan D, Mui WS, Mansor M, Yan GOS, Yusof MY, Sekaran SD. Development of conventional and real-time multiplex PCR assays for the detection of nosocomial pathogens. Brazilian Journal of Microbiology. 2011;42(2):448-58.
170. Chen L, Chavda KD, Mediavilla JR, Zhao Y, Fraimow HS, Jenkins SG, et al. Multiplex real-time PCR for detection of an epidemic KPC-producing Klebsiella pneumoniae ST258 clone. Antimicrobial agents and chemotherapy. 2012;56(6):3444-7.
171. Kurupati P, Chow C, Kumarasinghe G, Poh CL. Rapid detection of Klebsiella pneumoniae from blood culture bottles by real-time PCR. Journal of clinical microbiology. 2004;42(3):1337-40.
172. Lee D-Y, Shannon K, Beaudette LA. Detection of bacterial pathogens in municipal wastewater using an oligonucleotide microarray and real-time quantitative PCR. Journal of microbiological methods. 2006;65(3):453-67.

Addendum 1: In silico analysis *Enterobacter cloacae* primers

Addendum Table 1. Results in silico analysis of *Enterobacter cloacae.*

Author	Primer sequence ('5-'3)	Specificity for *E. cloacae* (%)	Amplicon length (bp)	Secondary structure of primer
Hoffmann et al. (74)	F: GGT AGA AGA AGG CGT GGT TGC	5	341	/
	R: ATG CAT TCG GTG GTG ATC ATC AG			
Hoffmann et al.* (74)	F: AAA TCC CTT TGC TGT GCC CTG*	92	657	Not interfering
	R: CCA GGC GTA ATG CGC CTC TTC*			
Hoffmann et al. (74)	F: CGR CGG TTV AGC GGG TTC ATC TG	85	237	Interfering
	R: TGA AYC TBG GCA AGC AGG CBG T			
Liu et al. (165)	F: GCC TTC GGG TTG TAA AGY	0	108	/
	R: CTG CTG GCA CGA AGT TAG C			
Miyoshi-Akiyama et al. (166)	F: AYA ACC CGC TGT TCC TBT ATG GCG GCAC	79	27	Interfering
	R: KGC CAG CGC CAT CGC CAT CTG ACG CGG			
Miyoshi-Akiyama et al. (166)	F: TCG ACG AAG CGC TCG CGG GTC ACT GTA A	67	27	Interfering
	R: GCA GAA CCG CCC GCG GAG TCC CCT TCC AA			
Miyoshi-Akiyama et al. (166)	F: CCG AAC CGT TCC GCG AAC ATC GCG CTG G	0	28	/
	R: CCA GCA GAT CCA GGC TCA GCT CCA TGT T			
Miyoshi-Akiyama et al. (166)	F: GTA AAC CGA CAT CTC CGG GTC GTC GCC A	3	27	/
	R: ACC TTT GGT CTG AAC GCC CCA CGG AGT T			
Miyoshi-Akiyama et al. (166)	F: TCG CGT TCG TTA ACA AAA TGG ACC GTA T	74	27	Interfering
	R: TCG CCA GAC GGC CCA GAG CCA GAC CCA T			
Miyoshi-Akiyama et al. (166)	F: GAT CAR CTS CCG GTK ATC CTG CCG GAA G	85	28	Interfering
	R: ATA GCC GCA ATT GCG GTA TTG AAG GTC T			
Ohad et al.* (73)	F: CTG CGT CAG ATC GTG TCC AA*	91	44	Not interfering
	R: CGT TGT AAC CGT AGT TAC CTT CAC C*			
Ohad et al (73)	F: AAC GCC GGT GAA GAG CC	14	83	/
	R: CGA AGT CGA TCA TGT TGC CGT AT			
Pavlovic et al. (167)	F: AGC GGG TAC GCA GCC ACA AA	0	/	/
	R: GCG TTT CGC CTG GAT TGG			
Silvia-Junio et al. (168)	F: GTC TAT TTC GCA CGT CGT GCT TTG C	82	171	Interfering
	R: CTT CTC AAC TGC GCG GAT GAG ACC			

The listed primer sequences are the result of an extended literature study. The specificity for *E. cloacae* and amplicon length were calculated in BLAST (Basic Local Alignment Search Tool) and the secondary structure of the primer was analyzed in mFOLD. The specificity for *E. cloacae* was determined by considering the amount of *E. cloacae* matches in 100 matches, their query cover and concordance in the primer couple. The primer structure was interpreted as interfering with the qPCR process as hairpins were formed near the 3' end. If a primer showed no relevant relative specificity for *E. cloacae,* the secondary structure of the primer was not determined. Primers with * were purchased for further analysis. These primers had an excellent specificity for *E. cloacae* and their secondary structures were not interfering with their function. Bp: base pairs. *E. cloacae*: *Enterobacter cloacae*. F: forward primer. R: reverse primer.

Addendum 2: In silico analysis *Klebsiella pneumoniae* primers

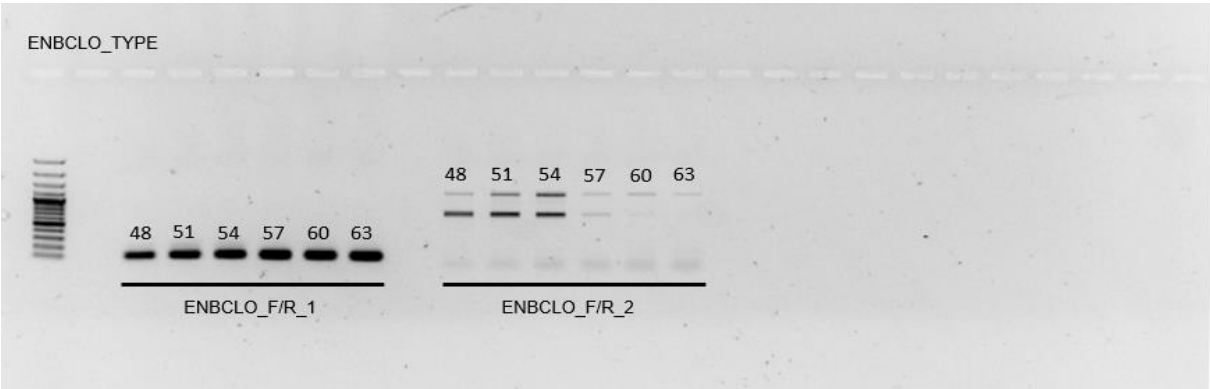
Addendum Table 2. Results in silico analysis of *Klebsiella pneumoniae.*

Author	Primer sequence	Specificity *K. pneumoniae* (%)	Amplicon length (bp)	Secondary structure of primer
Anbazhagan et al. (169)	F: CAT CTC GAT CTG CTG GCC AA	0	/	/
	R: GCG CGG ATC CAG CGA TTG GA			
Chen et al. (170)	F: CGA AAC CGC TCG TAA ACA CA	92	140	Interfering
	R: AGG AAG CGT TGG AAA CGA TG			
Kaushik et al.* (76)	F: GTG CGA TGC GGT CTT TG	93	398	Not interfering
	R: GGG CGA ACT GAA CTG ATG			
Kurupati et al. (171)	F: TGC AAG TCG AGC GGT AGC	6	126	/
	R: GCT AAT ACC GCA TAA CGT CG			
Lee et al. (172)	F: CCT GGA TCT GAC CCT GCA GTA	90	30	Interfering
	R: CCG TCG CCG TTC TGT TTC			
Liu et al. (165)	F: GCC TTC GGG TTG TAA AGY	0	/	/
	R: CTG CTG GCA CGA AGT TAG C			
Silvia-Junio et al. (168)	F: GCA CTG CGT GGT GAT GTC GC	81	82	Interfering
	R: TGT AAC GAC GGG CAA TCT TCA			
Trung et al.* (75)	F: CCG CGG ACT ATC TCG ACT ATA T	86	192	Not interfering
	R: CGA TGG CAT TAT TGG GCG TAA ATT			

The listed primer sequences are the result of an extended literature study. The specificity for *K. pneumoniae* and amplicon length were calculated in BLAST and the secondary structure of the primer was analyzed in mFOLD. The specificity for *K. pneumoniae* was determined by considering the amount of *K. pneumoniae* matches in 100 matches, their query cover and concordance in the primer couple. The primer structure was interpreted as interfering with the qPCR process as hairpins were formed near the 3' end. If a primer showed no relevant relative specificity for *K. pneumoniae,* the secondary structure of the primer was not determined. Primers with * were purchased for further analysis. These primers had an excellent specificity for *K. pneumoniae* and their secondary structures were not interfering with their function. Bp: base pairs. F: forward primer*. K. pneumoniae*: *Klebsiella pneumoniae*. R: reverse primer.

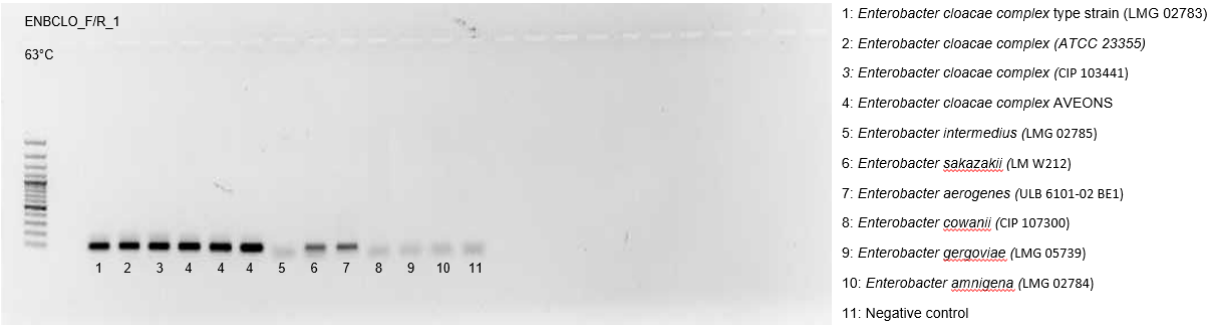
Addendum 3: PCR results

Addendum 3.1: *Enterobacter cloacae* primer selection



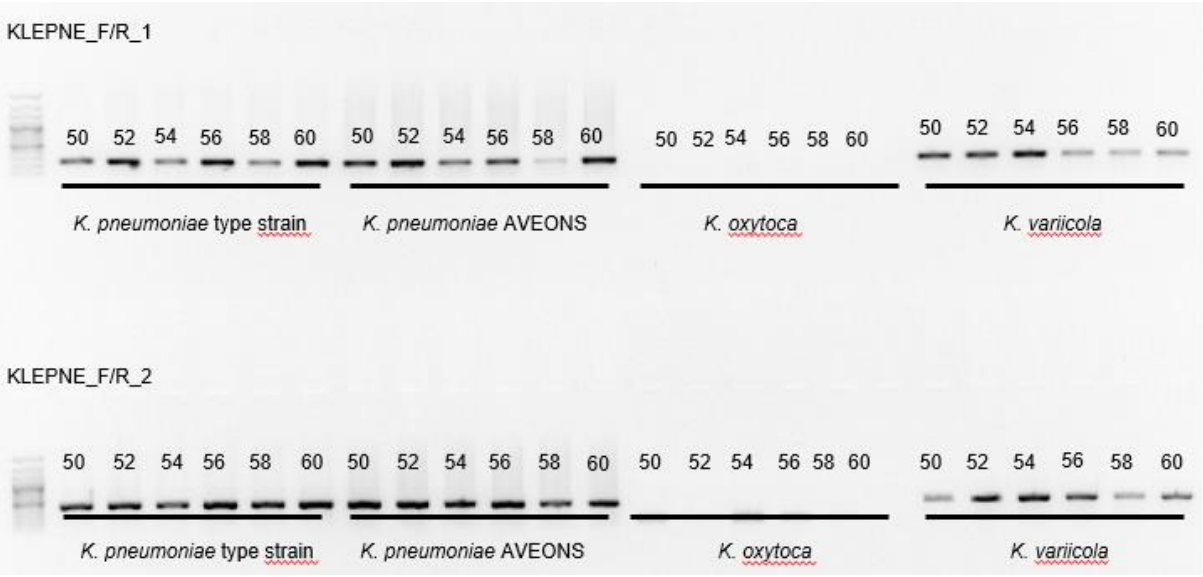
Addendum Figure 3.1. Gradient PCR to identify the most specific *Enterobacter cloacae* primers and their associated annealing temperature. Two primer paires were compared: ENBCLO_F/R_1 and ENBCLO_F/R_2. A gradient PCR was carried out at different annealing temperatures: 48-51-54-57-60-63 °C. As sample, *Enterobacter cloacae* type strain (LMG 02783) was added. ENBCLO_F/R_1 at 63°C was assumed as the most specific primer pair and annealing temperature for Enterobacter cloacae. ENBCLO_F_1: CTG CGT CAG ATC GTG TCC AA. ENBCLO_R_1: CGT TGT AAC CGT AGT TAC CTT CAC C. ENBCLO_F_2: AAA TCC CTT TGC TGT GCC CTG. ENBCLO_R_2: CCA GGC GTA ATG CGC CTC TTC. ENBCLO: *Enterobacter cloacae.* PCR: polymerase chain reaction.

Addendum 3.2: ENBCLO_F/R_1 specificity for *Enterobacter cloacae*

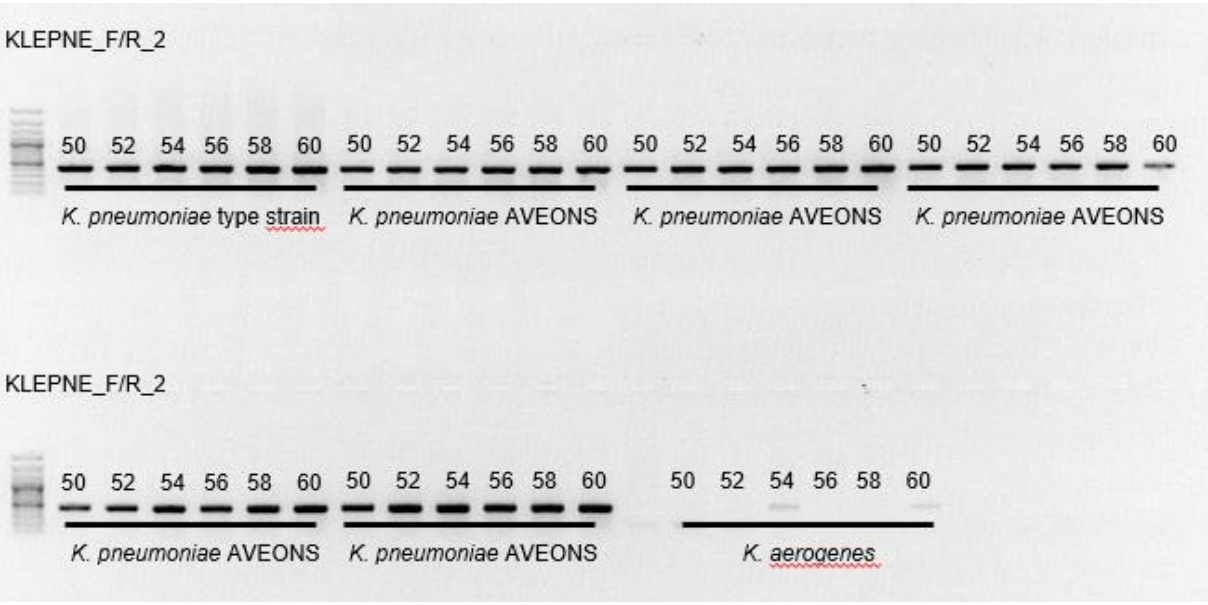


Addendum Figure 3.2. PCR to consider the specificity of the ENBCLO_F/R_1 primers for *Enterobacter cloacae*. A PCR was conducted, using the ENBCLO_F/R_1 primers, at an annealing temperature of 63°C. Several different *Enterobacter* species were added: *Enterobacter aerogenes* (ULB 6101-02 BE1), *Enterobacter amnigena* (LMG 02748), *Enterobacter cowanii* (CIP 107300), *Enterobacter gergoviae* (LMG 05739), *Enterobacter intermedius* (LMG 02785) and *Enterobacter sakazakii* (LM W212). As a control, some *Enterobacter cloacae* strains were added (type strain (LMG 02783), AVEONS strains, *Enterobacter cloacae* (ATCC 23355) and *Enterobacter cloacae* (CP 103441)). Some amplifications were established for *Enterobacter sakazakii* (LM W212) and *Enterobacter aerogenes* (ULB 6101-02 BE1). Other *Enterobacter* species showed no significant amplification. ENBCLO_F_1: CTG CGT CAG ATC GTG TCC AA. ENBCLO_R_1: CGT TGT AAC CGT AGT TAC CTT CAC C. ENBCLO: *Enterobacter cloacae.* PCR: polymerase chain reaction.

Addendum 3.3: *Klebsiella pneumoniae* primer selection and specificity



Addendum Figure 3.3.1. PCR gradient to identify the most specific primer for Klebsiella pneumoniae. Two primer pairs were used: KLEPNE_F/R_1 and KLEPNE_F/R_2. A gradient PCR was carried out at different annealing temperatures: 50-52-54-56-58-60 °C. As samples, *Klebsiella pneumoniae* type strain (ATCC 13883), *Klebsiella oxytoca* (CCUG 29683A), *Klebsiella variicola* (CCUG 47534) and a *Klebsiella pneumoniae* strain from the AVEONS project were added. KLEPNE_F/R_2 was assumed as the most specific primer couple, as a more stable trend was observed. As some temperatures did not amplified as expected, the PCR was re-run (Addendum 3.3.2). Some amplification were noticed for *Klebsiella variicola* (CCUG 47534), but were absent for *Klebsiella oxytoca* (CCUG 29683A). KLEPNE_F_1: CCG CGG ACT ATC TCG ACT ATA T. KLEPNE_R_1: CGA TGG CAT TAT TGG GCG TAA ATT. KLEPNE_F_2: GTG CGA TGC GGT CTT TG. KLEPNE_R_2: GGG CGA ACT GAA CTG ATG. KLEPNE: *Klebsiella pneumoniae*. *K. pneumoniae*: *Klebsiella pneumoniae*. *K. oxytoca*: *Klebsiella oxytoca*. *K. variicola*: *Klebsiella variicola*. PCR: polymerase chain reaction.



Addendum Figure 3.3.2. Gradient PCR to confirm the annealing temperature for KLEPNE_F/R_2. A gradient PCR was conducted, using the KLEPNE_F/R_2 primers at different annealing temperatures: 50-52-54-56-58-60 °C. As samples, the *Klebsiella pneumoniae* type strain (ATCC 13883), some *Klebsiella pneumoniae* strains from the AVEONS project and *Klebsiella aerogenes* (ULB 6101-02 BE1) were added. A clear increasing trend was noticed. An annealing temperature of 60 °C was assumed as the most appropriate annealing temperature. No significant amplifications for *Klebsiella aerogenes* were seen. KLEPNE_F_2: GTG CGA TGC GGT CTT TG. KLEPNE_R_2: GGG CGA ACT GAA CTG ATG. KLEPNE: *Klebsiella pneumoniae*. *K. aerogenes*: *Klebsiella aerogenes*. *K. pneumoniae*: *Klebsiella pneumoniae*. PCR: polymerase chain reaction.

Addendum 4: Univariate analysis of vaginal *Candida* carriage

Addendum 4.1: Univariate analysis of vaginal *Candida* carriage and risk factor

Addendum Table 4.1: Univariate logistic regressions showing the association between vaginal *Candida* carriage and risk factors.

	n	*Candida* + women (%)	Crude OR (95 % CI)	p-value
Sociodemographic factors				
Age of pregnant woman	**329**	**126 (38.3)**		
≤25 years	116	51 (44.0)	1.44 (0.91-2.29)	0.12
>25 years	213	75 (35.2)	Ref.	-
Tribe	**328**	**126 (38.4)**		
Shi	220	84 (38.2)	Ref.	-
Non-Shi[1]	108	42 (38.9)	1.07 (0.37-1.69)	0.77
Religion	**329**	**126 (38.3)**		
Catholic	203	79 (38.9)	1.07 (0.68-1.69)	0.77
Non-Catholic[2]	126	47 (37.3)	Ref.	
Community	**323**	**126 (39.0)**		
Kadatu	112	50 (44.6)	1.12 (0.61-2.07)	0.71
Ibanda	144	48 (33.3)	0.70 (0.38-1.26)	0.23
Bagira	67	28 (41.8)	Ref.	-
Education[3]	**329**	**126 (38.3)**		
Yes	319	122 (38.2)	Ref.	-
No	10	4 (40.0)	1.08 (0.30-3.89)	0.91
Level of education	**319**	**122 (38.2)**		
Primary	33	15 (45.5)	1.56 (0.71-3.43)	0.27
Secondary	177	69 (39.0)	1.19 (0.73- 1.96)	0.48
Tertiary	109	38 (34.9)	Ref.	-
State of marriage	**330**	**126 (38.2)**		
Married	313	118 (37.7)	Ref.	-
Not married	17	8 (47.1)	1.470 (0.55-3.91)	0.44
Age of marriage	**305**	**114 (37.4)**		
≤18 years	72	27 (37.5)	1.01 (0.58-1.74)	0.98
>18 years	233	87 (37.3)	Ref.	-
Duration of life with husband	**314**	**119 (37.9)**		
≤5 years	166	73 (44.0)	1.74 (1.10-2.77)	**0.02**
>5 years	148	46 (31.1)	Ref.	-
Living with husband or alone	**321**	**122 (38.0)**		
Living with husband	314	118 (37.6)	Ref.	-
Not married or not living with husband	7	4 (57.1)	2.22 (0.49-10.07)	0.30
Extramarital affairs[4]	**155**	**60 (38.7)**		
Yes	31	11 (35.5)	Ref.	-
No	124	49 (39.5)	1.19 (0.52-2.70)	0.68

Number of partners of husband	**46**	**15 (32.6)**		
1	42	14 (33.3)	1.50 (0.14-15.77)	0.74
>1	4	1 (25.0)	Ref.	-
Number of partners of pregnant woman during the last 6 months	**326**	**123 (37.7)**		
1	320	120 (37.5)	Ref.	-
>1	6	3 (50.0)	1.67 (0.33-8.39)	0.54
Number of partners of pregnant woman during life	**324**	**123 (38.0)**		
1	183	70 (38.3)	1.03 (0.65-1.62)	0.90
>1	141	53 (37.6)	Ref.	-
Source of income	**327**	**126 (38.5)**		
Non-employed	155	67 (43.2)	1.46 (0.93-2.28)	0.10
Employed	172	59 (34.3)	Ref.	-
Living circumstances				
Electricity and convenience	**330**	**126 (38.2)**		
Electricity	265	29 (8.8)	Ref.	-
No electricity	65	97 (29.4)	1.40 (0.81-2.42)	0.24
Water source	**329**	**126 (38.3)**		
Tap water	169	57 (33.7)	Ref.	-
Other[5]	160	69 (43.1)	1.49 (0.95-2.33)	0.08
Type of pavement	**329**	**125 (38.0)**		
Tiles	64	18 (28.1)	Ref.	-
Others[6]	265	107 (40.4)	1.73 (0.95-3.15)	0.07
Medical history				
BMI before conception[7]	**328**	**125 (38.1)**		
Underweight (BMI < 18.5)	3	3 (100.0)	-	-
Normal range (BMI: 18.5 - 25)	156	75 (48.1)	1.71 (0.91-3.21)	0.09
Overweight (BMI: 25 - 30)	112	27 (24.1)	0.59 (0.29-1.18)	0.13
Obese (BMI ≥ 30)	57	20 (35.1)	Ref.	-
Administration of current medication	**327**	**125 (38.2)**		
Yes	69	24 (34.8)	Ref.	-
No	258	101 (39.1)	1.21 (0.69-2.10)	0.51
Diabetic	**277**	**110 (39.7)**		
Yes	2	0 (0.0)	Ref.	-
No	275	110 (40.0)	-	-
Diabetic in the family	**317**	**123 (38.8)**		
Yes	72	25 (34.7)	Ref.	-
No	245	98 (40.0)	1.25 (0.72-2.17)	0.42
Chronic illness (e.g. cancer, gastritis, anemia, arterial hypertension…)[8]	**317**	**119 (37.5)**		
Yes	45	16 (35.6)	Ref.	-
No	272	103 (37.9)	1.11 (0.57-2.13)	0.77
Notion of constipation	**322**	**123 (38.2)**		
Yes	147	56 (38.1)	Ref.	-
No	175	67 (38.3)	1.01 (0.64-1.58)	0.97

Use of enema for constipation	**144**	**55 (38.2)**		
Yes	83	29 (34.9)	Ref.	-
No	61	26 (42.6)	1.38 (0.70-2.73)	0.35
Circumcised partner	**326**	**125 (38.3)**		
Yes	318	122 (38.4)	1.04 (0.24-4.42)	0.96
No	8	3 (37.5)	Ref.	-
Extension of the labia[9]	**318**	**121 (38.1)**		
Yes	40	16 (40.0)	1.10 (0.56-2.16)	0.79
No	278	105 (37.8)	Ref.	-
Known serological HIV state of pregnant woman	**315**	**122 (38.7)**		
Yes	212	72 (34.0)	Ref.	-
No	103	50 (48.5)	1.83 (1.14-2.96)	**0.01**
Period of last HIV test	**215**	**75 (34.9)**		
Less than 6 months ago	69	23 (33.3)	Ref.	-
More than 6 months ago	146	52 (35.6)	1.11 (0.61-2.03)	0.74
Knowledge of serological HIV state of husband	**240**	**98 (40.8)**		
Yes	100	42 (42.0)	1.09 (0.65-1.83)	0.76
No	140	56 (40.0)	Ref.	-
Realization of HIV test of couple (rapid test)	**321**	**121 (37.7)**		
Yes	96	35 (36.5)	Ref.	-
No	225	86 (38.2)	1.08 (0.66-1.77)	0.77
Treatment for gonorrhea or syphilis[10]	**299**	**114 (38.1)**		
Yes	7	4 (57.1)	2.21 (0.49-10.04)	0.31
No	292	110 (37.7)	Ref.	-
Cold sore on vulva (herpes)	**308**	**119 (38.6)**		
Yes	54	21 (38.9)	1.01 (0.55-1.85)	0.97
No	254	98 (38.6)	Ref.	-
Antibiotic administration in the past 2 weeks	**328**	**124 (37.8)**		
Yes	46	19 (41.3)	1.19 (0.63-2.24)	0.60
No	282	105 (37.2)	Ref.	-
Usus				
Consumption of alcohol during this pregnancy	**318**	**124 (39.0)**		
Yes	112	45 (40.2)	1.08 (0.67-1.73)	0.75
No	206	79 (38.3)	Ref.	-
Type of alcohol	**108**	**44 (40.7)**		
Beer	100	40 (40.0)	Ref.	-
Others[11]	8	4 (50.0)	1.50 (0.36-6.35)	0.58
Last consumption of alcohol	**79**	**32 (40.5)**		
Less than 1 week	43	17 (39.5)	Ref.	-
More than 1 week	36	15 (41.7)	1.09 (0.44-2.69)	0.85
Amount of alcohol	**109**	**44 (40.4)**		
Less than 1 time a day	4	2 (50.0)	1.50 (0.20-11.07)	0.69
1 or more times a day	105	42 (40.0)	Ref.	-

Geophagia[12]	**309**	**126 (40.8)**		
Yes	85	36 (42.4)	1.26 (0.76-2.08)	0.37
No	224	90 (40.2)	Ref.	-
Consumption of coal[13]	**327**	**126 (38.5)**		
Yes	29	15 (51.7)	1.81 (0.84-3.88)	0.13
No	298	111 (37.2)	Ref.	-
Duration of consumption of geophagy and coal	**50**	**21 (42.0)**		
1 week	26	13 (50.0)	2.00 (0.64-6.29)	0.24
More than 1 week	24	8 (33.3)	Ref.	-
Consummation of tobacco	**328**	**125 (38.1)**		
Yes	2	1 (50.0)	1.63 (0.10-26.28)	0.73
No	326	124 (38.0)	Ref.	-
Use of natural excitants (mairungi chanvre) [14]	**329**	**126 (38.3)**		
Yes	1	0 (0.0)	Ref.	-
No	328	126 (38.4)	-	-
Reproductive health				
Gestational age at V1	**324**	**123 (38.0)**		
≤26 weeks	309	115 (37.2)	Ref.	-
>26 weeks	15	8 (53.3)	1.93 (0.68-5.46)	0.22
Number of previous deliveries on term	**330**	**126 (38.2)**		
0	79	29 (36.7)	1.10 (0.62-1.96)	0.75
1-2	115	50 (43.5)	1.46 (0.87-2.43)	0.15
>3	136	47 (34.6)	Ref.	-
Previous premature delivery	**330**	**126 (38.2)**		
Yes	20	7 (35.0)	Ref.	-
No	310	119 (38.4)	1.16 (0.45-2.98)	0.76
Total parity of the women	**330**	**126 (38.2)**		
0	76	27 (35.5)	1.02 (0.57-1.84)	0.94
1-2	114	50 (43.9)	1.45 (0.87-2.41)	0.15
>3	140	49 (35.0)	Ref.	-
Previous abortion[15]	**330**	**126 (38.2)**		
Yes	108	42 (38.9)	1.05 (0.65-1.68)	0.85
No	222	84 (37.8)	Ref.	-
Previous fetal death in utero[16]	**329**	**126 (38.3)**		
Yes	21	8 (38.1)	Ref.	-
No	308	118 (38.3)	1.01 (0.41-2.51)	0.98
Previous caesarean section [17]	**295**	**111 (37.6)**		
Yes	58	19 (32.8)	Ref.	-
No	237	92 (38.8)	1.30 (0.71-2.39)	0.39
Weight of biggest baby from previous pregnancy	**253**	**98 (38.7)**		
<2500 g	4	2 (50.0)	1.65 (0.21-12.80)	0.63
2500-4000 g	204	79 (38.7)	1.04 (0.54-2.03)	0.91
>4000 g	45	17 (37.8)	Ref.	-

Notion of infection of previously born baby in first week of life	**269**	**103 (38.3)**		
Yes	81	32 (39.5)	1.08 (0.63-1.84)	0.79
No	188	71 (37.8)	Ref.	-
Evolution of the previously born baby	**84**	**32 (38.1)**		
Good	65	26 (40.0)	1.44 (0.49-4.28)	0.51
Handicap or death	19	6 (31.6)	Ref.	-
Number of consultations during current pregnancy	**329**	**125 (38.0)**		
0	102	45 (44.1)	1.45 (0.90-2.34)	0.13
≥1	227	80 (35.2)	Ref.	-
Prevention in current pregnancy				
Administration of substances to diminish neonatal infections[18]	**271**	**105 (38.7)**		
Yes	58	21 (36.2)	Ref.	-
No	213	84 (39.4)	1.15 (0.63-2.09)	0.66
Administration of Fansidar® (prophylaxis against malaria)[19]	**318**	**121 (38.1)**		
Yes	63	24 (38.1)	1.00 (0.57-1.77)	0.99
No	255	97 (38.0)	Ref.	-
Administration of Vermox® (prophylaxis against intestinal worms)	**325**	**125 (38.5)**		
Yes	64	21 (32.8)	Ref.	-
No	261	104 (39.8)	1.36 (0.76-2.42)	0.30
Utilization of mosquito net during pregnancy	**324**	**123 (38.0)**		
Yes	287	114 (39.7)	2.05 (0.93-4.51)	0.07
No	37	9 (24.3)	Ref.	-
Sexual behaviour				
Age of first sexual contact	**268**	**98 (36.6)**		
≤18 years	126	48 (38.1)	1.13 (0.69-1.86)	0.63
>18 years	142	50 (35.2)	Ref.	-
Anal sexual intercourse[20]	**329**	**126 (38.3)**		
Yes	32	15 (46.9)	1.48 (0.71-3.08)	0.30
No	297	111 (37.4)	Ref.	-
Last sexual contact during current pregnancy	**291**	**113 (38.8)**		
≤7 days	224	82 (36.6)	Ref.	-
>7days	67	31 (46.3)	1.49 (0.86-2.59)	0.16
Toilet hygiene				
Type of toilet	**330**	**126 (38.2)**		
Toilet with bowl and flush	81	20 (24.7)	Ref.	-
Other types[21]	249	106 (42.6)	2.26 (1.29-3.97)	**0.01**
Use after toilet	**328**	**125 (38.1)**		
Water	227	93 (41.0)	1.50 (0.91-2.46)	0.11
Tissue or other substances	101	32 (31.7)	Ref.	

Vaginal practices				
Normal vaginal toilet	**323**	**124 (38.4)**		
Only water	264	101 (38.3)	Ref.	-
Other substances or none[22]	59	23 (39.0)	1.03 (5.78-1.84)	0.92
Practices to dry vagina	**327**	**126 (38.5)**		
Yes	49	20 (40.8)	1.12 (0.60-2.08)	0.72
No	278	106 (38.1)	Ref.	-
Vaginal practices	**51**	**22 (43.1)**		
Toilet with cold water	4	2 (50.0)	1.35 (0.18-10.42)	0.77
Other practices[23]	47	20 (42.6)	Ref.	-
Number of vaginal toilets	**13**	**7 (53.8)**		
≤2 a day	12	6 (50.0)	Ref.	-
>2 a day	1	1 (100.0)	-	-
Vaginal toilet after each sexual contact	**328**	**125 (38.1)**		
Yes	281	110 (39.1)	1.37 (0.71-2.65)	0.35
No	47	15 (31.9)	Ref.	-
Type of intimate toilet after sexual contact	**270**	**104 (38.5)**		
Water	201	80 (39.8)	1.24 (0.70-2.19)	0.46
Use of tissue or other	69	24 (34.8)	Ref.	-

[1]Rega, Havu, Tumbo, Hunde, Nyganga, Hutu, Nande, Vira, Fuliru, Bembe. [2]Non-catholic: Protestantism, Angilicanism, Kimbanguism, Moslim, Animism. [3]From primary school. [4]Extramarital affairs of man known by the pregnant women. [5]Rain water, water well.[6]Concrete, carpet, no pavement. [7]Weight before the current pregnancy. [8]A diagnosed chronic illness. [9]A cultural tradition. [10]Diagnosed by acknowledge doctor or a clinical officer. [11]Wine, liqueur, local alcoholic drink (Sorgho). [12]Geophagia is the practice of eating earth or soil-like substrates such as clay or chalk to diminish nausea in pregnancy. [13]In case of Pica syndrome .[14]Khat, marijuana.[15]Natural, spontaneous abortion. [16]From 20 weeks of gestational age. [17]Planned and unplanned section.[18]Seeds, herbs,…[19]This prophylaxis is taken by all women at antenatal consultation during pregnancy at 24 WGA. [20]Information about timing and frequency is unknown. [21]Squat latrine, pit latrin. [22]Use of soap, perfume, powder, lemon juice, Dettol, virginity soap, tissue. [23]Use of soap, perfume, powder, lemon juice, antiseptic soap, Dettol, shaving. BMI: Body Mass Index. HIV: human immunodeficiency virus. N: number of samples. OR: odds ratio. V1: visit 1.

Addendum 4.2: Univariate analysis of vaginal *Candida* carriage and symptoms

Addendum Table 4.2. Univariate logistic regressions showing the association between vaginal *Candida* carriage and signs and symptoms.

	n	*Candida* + women (%)	Crude OR (95% CI)	p-value
General signs and symptoms at V1				
Fever	**324**	**124 (38.3)**		
Yes	37	13 (35.1)	Ref.	-
No	287	111 (38.7)	1.16 (0.57-2.38)	0.68
Headache	**326**	**125 (38.3)**		
Yes	159	57 (35.8)	Ref.	-
No	167	68 (40.7)	1.23 (0.79-1.92)	0.37
Cough	**327**	**125 (38.2)**		
Yes	72	20 (27.8)	Ref.	-
No	255	105 (41.2)	1.82 (1.03-3.23)	**0.04**
Uterine contractions	**291**	**113 (38.8)**		
Yes	40	17 (42.5)	1.19 (0.61-2.35)	0.61
No	251	96 (38.2)	Ref.	-
Lumbar pain	**326**	**126 (38.7)**		
Yes	165	61 (37.0)	Ref.	-
No	161	65 (40.4)	1.15 (0.74-1.80)	0.53
Difficulty to swallow	**326**	**126 (38.7)**		
Yes	32	12 (37.5)	Ref.	-
No	294	114 (38.8)	1.06 (0.50-2.24)	0.89
Vaginal signs and symptoms at V1				
Vaginal discharge	**326**	**124 (38.0)**		
Yes	159	83 (52.2)	3.36 (2.10-5.37)	**<0.001**
No	167	41 (24.6)	Ref.	-
Previous treatment for vaginal discharge[1]	**159**	**82 (51.6)**		
Yes	86	44 (51.2)	Ref.	-
No	73	38 (52.1)	1.03 (0.56-1.94)	0.91
Type of previous treatment for vaginal discharge	**326**	**125 (38.0)**		
Gyogynax	15	8 (53.3)	1.91 (0.45-7.98)	0.38
Anitbiotics	36	19 (52.8)	1.83 (0.56-6.22)	0.31
Antibiotics + other	19	11 (57.9)	2.29 (0.59-8.94)	0.23
Other[2]	16	6 (37.5)	Ref.	-
No treatment	240	80 (33.3)	0.84 (0.30-2.40)	0.75
Vaginal itching	**328**	**125 (38.1)**		
Yes	136	79 (58.1)	4.40 (2.74-7.08)	**<0.001**
No	192	46 (24.0)	Ref.	-
Previous treatment for vaginal itching[1]	**137**	**80 (58.4)**		
Yes	61	37 (60.7)	1.18 (0.60-2.35)	0.63
No	76	43 (56.6)	Ref.	-

Type of previous treatment for vaginal itching	**328**	**125 (38.1)**		
Gyogynax	13	8 (61.5)	4.80 (0.39-59.90)	0.22
Anitbiotics	30	19 (63.3)	5.18 (0.48-56.09)	0.18
Antibiotics + other	14	9 (64.3)	5.40 (0.44-66.67)	0.19
Other[3]	4	1 (25.0)	Ref.	-
No treatment	267	88 (33.0)	1.48 (0.15-14.46)	0.73
Dysuria	**324**	**124 (38.3)**		
Yes	86	39 (45.3)	1.49 (0.91-2.46)	0.12
No	238	85 (35.7)	Ref.	-
Previous treatment for dysuria[1]	**89**	**42 (47.2)**		
Yes	34	16 (47.1)	Ref.	-
No	55	26 (47.3)	1.01 (0.43-2.38)	0.98
Type of previous treatment for dysuria	**324**	**124 (38.3)**		
Gyogynax	4	1 (25.0)	0.56 (0.06-5.46)	0.62
Anitbiotics	22	9 (40.9)	1.16 (0.48-2.81)	0.74
Antibiotics + other	7	5 (71.4)	4.21 (0.80-22.04)	0.09
Other[4]	2	1 (50.0)	1.68 (0.10-27.16)	0.71
No treatment	289	108 (37.4)	Ref.	-
Burning sensation after sexual contact[5]	**313**	**118 (37.7)**		
Yes	104	64 (61.5)	4.59 (2.78-7.59)	**<0.001**
No	209	54 (25.8)	Ref.	-
Last episode of burning	**86**	**52 (60.5)**		
Less than 7 days	55	36 (65.5)	1.78 (0.72-4.36)	0.21
More than 7 days	31	16 (51.6)	Ref.	-
Previous treatment for burning[1]	**105**	**64 (61.0)**		
Yes	22	13 (59.1)	Ref.	-
No	83	51 (61.4)	1.10 (0.42-2.88)	0.84
Type of previous treatment for burning	**313**	**118 (37.7)**		
Gyogynax	4	3 (75.0)	3.00 (0.08-107.45)	0.55
Anitbiotics	9	4 (44.4)	0.80 (0.37-17.20)	0.89
Antibiotics + other	7	5 (71.4)	2.50 (0.10-62.61)	0.58
Other[4]	2	1 (50.0)	Ref.	
No treatment	291	105 (36.1)	0.579 (0.036-9.355)	0.70
Sensation of vaginal smell	**297**	**117 (39.4)**		
Yes	77	34 (44.2)	1.31 (0.77-2.21)	0.32
No	220	83 (37.7)	Ref.	-
Last episode of vaginal smell	**48**	**22 (45.8)**		
≤2 days	30	13 (43.3)	Ref.	-
>2 days	18	9 (50.0)	1.31 (0.41-4.23)	0.65
Previous treatment for vaginal smell[1]	**75**	**34 (45.3)**		
Yes	10	4 (40.0)	Ref.	-
No	65	30 (46.2)	1.29 (0.33-4.99)	0.72

Type of previous treatment for vaginal smell	**297**	**117 (39.4)**		
Gyogynax	0	0 (0.0)	-	-
Anitbiotics	4	1 (25.0)	-	-
Antibiotics + other	4	3 (75.0)	-	-
Other[6]	2	0 (0.0)	Ref.	-
No treatment	287	113 (39.4)	-	-
General clinical examination at V1				
Weight evolution during pregnancy[7]	**330**	**126 (38.2)**		
Weight loss	87	38 (43.7)	2.212(1.06-4.65)	**0.04**
Stable weight or ≤5 kg weight gain	189	74 (39.2)	1.84 (0.94-3.61)	0.08
> 5kg weight gain	54	14 (26.0)	Ref.	-
Arm circumference	**328**	**124 (37.9)**		
<22 cm	25	9 (36.0)	1.47 (0.58-3.70)	0.42
22-27.5 cm	202	87 (43.1)	1.97 (1.18-3.31)	**0.01**
>27.5 cm	101	28 (27.8)	Ref.	-
Diastolic blood pressure	**330**	**126 (38.2)**		
<90 mmHg	325	124 (38.5)	Ref.	-
≥90 mmHg	5	2 (40.0)	1.08 (0.18-6.56)	0.93
Systolic blood pressure	**330**	**126 (38.2)**		
<140 mmHg	324	126 (38.9)	-	-
≥140 mmHg	6	0 (0.0)	Ref.	-
Cardiac frequency	**329**	**125 (38)**		
<110 bpm	318	121 (38.1)	1.08 (0.31-3.75)	0.91
≥110 bpm	11	4 (36.4)	Ref.	-
Edema lower legs	**330**	**126 (38.2)**		
Yes	1	1 (100.0)	-	-
No	329	125 (38)	Ref.	-
General physical state	**330**	**126 (38.2)**		
Normal	329	126 (38.3)	-	-
Abnormal[8]	1	0 (0.0)	Ref.	-
Gynaecological examination at V1				
Vulvar state	**328**	**126 (38.5)**		
Normal	322	123 (38.2)	Ref.	-
Abnormal[9]	6	3 (50.0)	1.62 (0.32-8.14)	0.56
Speculum examination	**328**	**126 (38.5)**		
Normal	272	103 (37.9)	Ref.	-
Abnormal[10]	56	23 (41.1)	1.14 (0.64-2.06)	0.65
Vaginal pH	**324**	**96 (29.7)**		
4	5	1 (20.0)	Ref.	-
5-6	261	95 (36.4)	2.29 (0.25-20.78)	0.46
>6	58	27 (46.6)	3.48 (0.37-33.10)	0.28

White blood cells per field on wet mount	**330**	**126 (38.2)**		
0	0	0 (0.0)	-	-
1 -4	178	51 (28.7)	Ref.	-
5-30	132	63 (47.8)	2.274 (1.419-3.643)	**0.00**
30+	20	12 (60.0)	3.735 (1.442-9.676)	**0.01**
Clue cells[11] **on wet mount**	**330**	**126 (38.2)**		
Yes	37	12 (32.5)	Ref.	-
No	293	114 (39.0)	1.33 (0.64-2.75)	0.45
***Trichomonas* on wet mount**	**329**	**126 (38.3)**		
Yes	4	1 (25.0)	Ref.	-
No	325	125 (38.5)	-	-
***Candida* on wet mount**	**329**	**126 (38.3)**		
Yes	91	79 (86.9)	26.75 (13.48-53.13)	**<0.001**
No	238	47 (19.8)	Ref.	-
Epithelial cells per field wet mount	**326**	**126 (38.7)**		
<5	19	9 (47.4)	1.45 (0.54-3.88)	0.47
5-30	208	79 (38.0)	0.98 (0.60-1.61)	0.95
30+	99	38 (38.4)	Ref.	-
Whiff test (KOH)[12]	**330**	**126 (38.2)**		
Positive	32	15 (46.9)	1.49 (0.71-3.09)	0.29
Negative	298	111 (37.3)	Ref.	-
State of vaginal secretions	**330**	**126 (38.2)**		
Normal: fine and homogeneous	297	99 (33.4)	Ref.	-
Abnormal: thick (+heterogeneous)	33	27 (81.9)	9.00 (3.60-22.51)	**<0.001**
BV on gram stain[13]	**326**	**125 (38.4)**		
No BV	176	50 (28.5)	Ref.	-
Intermediate	59	35 (59.4)	3.68 (1.99-6.79)	**<0.001**
BV	91	40 (44.0)	1.98 (1.17-3.35)	**0.011**
Biofilm	**326**	**125 (38.4)**		
Yes	73	34 (46.6)	1.56 (0.92-2.63)	0.1
No	253	91 (36.0)	Ref.	-
Gram + cocci on gram stain	**326**	**125 (38.4)**		
Yes	31	17 (54.9)	2.10 (1.00-4.43)	**0.05**
No	295	108 (36.7)	Ref.	-
Gram - cocci on gram stain	**326**	**125 (38.4)**		
Yes	6	5 (83.4)	8.33 (0.96-72.18)	0.054
No	320	120 (37.5)	Ref.	-
Yeast on gram stain	**326**	**125 (38.4)**		
Yes	88	86 (97.8)	71.67 (29.25-175.60)	**<0.001**
No	238	39 (16.4)	Ref.	-
Hyphae on gram stain[14]	**330**	**126 (38.2)**		
Yes	48	48 (100)	-	-
No	282	78 (27.7)	Ref.	-

***Enterobacter cloacae* in CVL**	**330**	**126 (38.2)**		
Yes	140	53 (37.9)	Ref.	-
No	190	73 (38.5)	1.02 (0.65-1.61)	0.92
***Klebsiella pneumoniae* in CVL**	**330**	**126 (38.2)**		
Yes	40	19 (47.5)	1.55 (0.80-3.01)	0.2
No	290	107 (36.9)	Ref.	-
Clinical diagnosis at V1	**330**	**126 (38.2)**		
Normal	140	16 (11.5)	Ref.	-
Pathological	190	110 (57.9)	10.66 (5.88-19.32)	**<0.001**
Symptomatic treatment for vaginitis (BV and *Candida*) at V1	**329**	**126 (38.3)**		
Femaclin®	109	63 (57.8)	10.44 (5.48-19.91)	**<0.001**
Antibiotic	21	7 (33.4)	3.81 (1.34-10.86)	**0.01**
Femaclin® + Antibiotic	53	38 (71.7)	19.32 (8.74-42.69)	**<0.001**
Other treatment[15]	8	2 (25.0)	2.54 (0.47-13.68)	0.28
No treatment	138	16 (11.6)	Ref.	-
Additional technical examination at V1				
Hemoglobin on Hemocue®	**328**	**125 (38.2)**		
Anemia (<11 Hb)	12	8 (66.7)	3.40 (1.00-11.54)	**0.05**
Normal (≥11 Hb)	316	117 (37.1)	Ref.	-
Rapid test malaria	**330**	**126 (38.2)**		
Positive	1	1 (100.0)	-	-
Negative	329	125 (38.0)	Ref.	-
Rapid test HIV	**330**	**126 (38.2)**		
Positive	1	0 (0.0)	Ref.	-
Negative	329	126 (38.3)	-	-
White blood cells on urine dipstick	**330**	**126 (38.2)**		
Positive	134	69 (51.5)	2.59 (1.64-4.09)	**<0.001**
Negative	196	57 (29.1)	Ref.	-
Nitrite on urine dipstick	**330**	**126 (38.2)**		
Positive	12	5 (41.7)	1.16 (0.36-3.75)	0.80
Negative	318	121 (38.1)	Ref.	-
Glycated keratin	**320**	**125 (39.1)**		
<3.6	181	69 (38.2)	Ref.	-
3.6-10	120	45 (37.5)	0.97 (0.61-1.57)	0.91
>10	19	11 (57.9)	2.23 (0.86-5.82)	0.10
Ultrasound examination at V1				
Estimation of fetal weight centiles[16]	**315**	**120 (38.1)**		
<p10	44	15 (34.1)	Ref.	-
p10 - p90	148	59 (39.9)	1.28 (0.63-2.59)	0.49
>p90	123	46 (37.4)	1.16 (0.56-2.38)	0.70
Fetal sex[17]	**324**	**123 (38.0)**		
Female	162	57 (35.2)	Ref.	-
Male	162	66 (40.8)	1.27 (0.81-1.99)	0.30

Insertion placenta[17]	**326**	**125 (38.4)**		
Normal	310	117 (37.8)	Ref.	-
Low inserted	16	8 (50.0)	1.65 (0.60-4.51)	0.33
Amniotic fluid[17]	**30**	**15 (50.0)**		
Normal	30	15 (50.0)	-	-
Abnormal	0	0 (0.0)	Ref.	-
Length cervix[17]	**330**	**126 (38.2)**		
<25 cm	3	1 (33.4)	Ref.	-
25-30 cm	25	7 (28.0)	0.78 (0.60-10.00)	0.85
>30 cm	302	118 (39.1)	1.28 (0.12-14.30)	0.84
Funnel[17,18]	**329**	**125 (38.0)**		
Present	5	2 (40.0)	1.09 (0.18-6.61)	0.93
Absent	324	123 (37.96)	Ref.	-
Morphological abnormality visible[17]	**326**	**125 (38.3)**		
Yes	6	4 (66.7)	3.29 (0.59-18.23)	0.17
No	320	121 (37.8)	Ref.	-

[1]Treatment in pregnancy. [2]Femaclin®, Gyndodactarin, Nystatin, Tinidazole, Fluomizin. [3]Nystatin, Gynodactarin. [4]Not precised. [5]Burning sensation in the current pregnancy. [6]Femaclin®, Gynodactarin. [7]Weight before pregnancy compared with weight at V1. [8]Deviant compared with healthy pregnant women. [9]Genital wrat, herpetic lesions, chancre, erythema, pustule, abcess (Bartholin's gland), leucorrhoea. [10]Erythema, polyp, ectropion, bleeding, xanthoma, ulcers, leucorrhoea. [11]Clue cells are epithelial cells of the vagina that get their distinctive stippled appearance by being covered with bacteria. It is a typical sign of bacterial vaginosis. [12] A whiff test is performed by adding several drops of 10% potassium hydroxide to a sample of vaginal discharge. A strong fishy odor is indicative of a positive test result. Such a result may suggest either trichomoniasis or bacterial vaginosis. [13]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV). (59) [14]Long, tubular branching structures produced by *Candida*. [15]Tot'hema, gogynax, omnibionta. [16]Based on Percentile table Jeanty. [17]Based on ultrasound examination. [18]Protrusion of the amniotic membranes into the internal os of the cervix. This condition increased the risk on preterm birth. Bpm: beats per minute. CVL: cervicovaginal lavage. Hb: hemoglobin. HIV: human immunodeficiency virus. N: number of samples. OR: odds ratio. P: percentile. V1: visit 1.

Addendum 4.3: Univariate analysis of vaginal *Candida* carriage and adverse pregnancy outcomes

Addendum Table 4.3. Univariate logistic regressions showing the association between vaginal *Candida* carriage and adverse pregnancy outcomes.

	n	*Candida* + women (%)	Crude OR (95% CI)	p-value
Delivery				
Gestational age at labor [1]	**202**	**77 (38.2)**		
28w-32w	2	0 (0.0)	-	-
32w-36w	28	16 (57.2)	2.43 (1.08-5.46)	**0.032**
≥37w	172	61 (35.5)	Ref.	-
Preterm birth	**202**	**77 (38.2)**		
Yes (<37w)	30	16 (53.4)	2.08 (0.95-4.55)	0.067
No (≥37w)	172	61 (35.5)	Ref.	-
Temperature of the mother at labor [2]	**200**	**75 (37.5)**		
<37.2°C	179	63 (35.2)	Ref.	-
≥ 37.2°C	21	12 (57.2)	2.46 (0.98-6.14)	0.055
Development of labor	**199**	**78 (39.2)**		
Spontaneous	182	71 (39.1)	Ref.	-
Induced[3]	17	7 (41.2)	1.09 (0.40-3.01)	0.861
Way of induction of labor	**17**	**7 (41.2)**		
Misoprostol (prostaglandin)	10	5 (50.0)	2.50 (0.32-19.53)	0.382
Foley probe with misoprostol (prostaglandin)	7	2 (28.6)	Ref.	-
Fetal presentation at labor [4]	**204**	**78 (38.3)**		
Cephalic (head)	196	73 (37.3)	Ref.	-
Bottom	5	2 (40.0)	1.12 (0.18-6.88)	0.9
Transversal	3	3 (100.0)	-	-
State of membranes at arrival in hospital (before delivery)	**204**	**78 (38.3)**		
Intact	158	60 (38.0)	Ref.	-
Broken or cracked	46	18 (39.2)	1.05 (0.54-2.06)	0.887
Duration of rupture of membranes	**199**	**78 (39.2)**		
≤6 hours	192	72 (37.5)	Ref.	-
>6 hours	7	6 (85.8)	10.00 (1.18-84.75)	**0.035**
Amniotic fluid type at delivery	**204**	**78 (38.3)**		
Clear	162	54 (33.4)	Ref.	-
Meconium[5] (fresh or old)	42	24 (57.2)	2.67 (1.33-5.33)	**0.006**
Number of vaginal touchers during labor	**204**	**78 (38.3)**		
≤5 times	40	13 (32.5)	Ref.	-
>5 times	164	65 (39.7)	1.36 (0.66-2.84)	0.406
Washing of hands before labor	**188**	**72 (38.3)**		
Yes	188	72 (38.3)	-	-
No	0	0 (0.0)	Ref.	-

Type of labor	**204**	**78 (38.3)**		
Eutocic (with episotomy)[6]	167	64 (38.4)	Ref.	-
Dystocic[7]	1	0 (0.0)	-	-
Caesarean section	36	14 (38.9)	1.02 (0.49-2.15)	0.95
Duration of labor[8]	**195**	**76 (39.0)**		
≤8 hours	139	59 (42.5)	1.69 (0.87-3.28)	0.119
>8 hours	56	17 (30.4)	Ref.	-
Utilization of labor kit[9]	**203**	**78 (38.5)**		
Yes	141	52 (36.9)	Ref.	-
No	62	26 (42.0)	1.24 (0.67-2.27)	0.495
Cord care	**9**	**5 (55.6)**		
No disinfectant	0	0 (0.0)	Ref.	-
Disinfectant[10]	9	5 (55.6)	-	-
APGAR[11] score 5 minutes	**203**	**78 (38.5)**		
<7	2	0 (0.0)	-	-
≥7	201	78 (38.9)	Ref.	-
Sex of the baby	**204**	**78 (38.3)**		
Female	103	35 (34.0)	Ref.	-
Male	101	43 (42.6)	1.44 (0.82-2.54)	0.207
Visible abnormality	**204**	**78 (38.3)**		
Present	4	0 (0.0)	Ref.	-
Absent	200	78 (39.0)	-	-
Disinfectant eye drops[12]	**203**	**78 (38.5)**		
Yes	172	65 (37.8)	Ref.	-
No	31	13 (42.0)	1.19 (0.55-2.59)	0.663
Evolution of neonate	**204**	**78 (38.3)**		
Close to mother	201	78 (38.9)	-	-
Neonatology	3	0 (0.0)	Ref.	-
Neonatal outcome		**()**		
Fever[2]	**203**	**77 (38.0)**		
Yes (>37.2 °C)	3	2 (66.7)	3.33 (0.30-37.39)	0.329
No	200	75 (37.5)	Ref.	-
Temperature neonate	**203**	**77 (38.0)**		
<36.6 °C	117	45 (38.5)	Ref.	-
36.6-37.2 °C	75	26 (34.7)	0.85 (0.46-1.55)	0.595
>37.2 °C	11	6 (54.6)	1.92 (0.55-6.66)	0.304
Hypothermia	**203**	**77 (38.0)**		
Yes (<35 °C)	1	0 (0.0)	Ref.	-
No	202	77 (38.2)	-	-
Lethargy	**203**	**77 (38.0)**		
Yes	3	0 (0.0)	Ref.	-
No	200	77 (38.5)	-	-

Jaundice	**202**	**76 (37.7)**		
Yes	0	0 (0.0)	Ref.	-
No	202	76 (37.7)	-	-
Convulsions	**203**	**77 (38.0)**		
Yes	0	0 (0.0)	Ref.	-
No	203	77 (38.0)	-	-
Apnea	**203**	**77 (38.0)**		
Yes	1	0 (0.0)	Ref.	-
No	202	77 (38.2)	-	-
Hypotonia	**203**	**77 (38.0)**		
Yes	3	0 (0.0)	Ref.	-
No	200	77 (38.5)	-	-
Hypertonia	**203**	**77 (38.0)**		
Yes	0	0 (0.0)	Ref.	-
No	203	77 (38.0)	-	-
Shock	**203**	**77 (38.0)**		
Yes	0	0 (0.0)	Ref.	-
No	203	77 (38.0)	-	-
Dirty umbilicus	**203**	**77 (38.0)**		
Yes	0	0 (0.0)	Ref.	-
No	203	77 (38.0)	-	-
Difficult to suckle	**203**	**77 (38.0)**		
Yes	3	0 (0.0)	Ref.	-
No	200	77 (38.5)	-	-
Alimentation	**202**	**77 (38.2)**		
Maternal milk	200	76 (38.0)	Ref.	-
Bottle milk or combination maternal and bottle milk	2	1 (50.0)	1.63 (0.10-26.47)	0.731
Length of baby	**203**	**77 (38.0)**		
Small: <46 cm	4	1 (25.0)	Ref.	-
Normal: 46 cm – 56 cm	199	76 (38.2)	1.85 (0.19-18.14)	0.596
Large: >56 cm	0	0 (0.0)	-	-
Head circumference	**203**	**77 (38.0)**		
Microcephaly: <32 cm	2	0 (0.0)	-	-
Normal: 32 cm – 37 cm	198	76 (38.4)	-	-
Macrocephaly: >27 cm	3	1 (33.4)	Ref.	-
Weight at birth	**203**	**77 (38.0)**		
<2500 g (low birth weight)	7	1 (14.3)	Ref.	-
≥2500 g	196	76 (38.8)	3.80 (0.45-32.18)	0.221
General physical state	**203**	**77 (38.0)**		
Normal	196	74 (37.8)	Ref.	-
Abnormal (see commentary general state)	7	3 (42.9)	1.24 (0.27-5.68)	0.785

Commentary general state	**7**	**2 (28.6)**		
Fever	4	2 (50.0)	-	-
Prematurity	1	0 (0.0)	-	-
Death	2	0 (0.0)	Ref.	-
Skin	**203**	**77 (38.0)**		
Normal	198	75 (37.9)	Ref.	
Abnormal: erythema	5	2 (40.0)	1.09 (0.18-6.70)	0.923
Mouth	**203**	**77 (38.0)**		
Normal	203	77 (38.0)	-	-
Abnormal	0	0 (0.0)	Ref.	-
ORL	**203**	**77 (38.0)**		
Normal	203	77 (38.0)	-	-
Abnormal	0	0 (0.0)	Ref.	-
Neck	**203**	**77 (38.0)**		
Normal	203	77 (38.0)	-	-
Abnormal	0	0 (0.0)	Ref.	-
Cardiovascular	**203**	**77 (38.0)**		
Normal	199	77 (38.7)	-	-
Abnormal (see commentary cardiovascular)	4	0 (0.0)	Ref.	-
Commentary cardiovascular	**4**	**0 (0.0)**		
Bradycardia	3	0 (0.0)	-	-
Tachycardia	1	0 (0.0)	Ref.	-
Lungs	**203**	**77 (38.0)**		
Normal	197	75 (38.1)	1.23 (0.22-6.88)	0.814
Abnormal (see commentary lungs)	6	2 (33.4)	Ref.	-
Commentary lungs	**7**	**2 (28.6)**		
Apnea	3	0 (0.0)	Ref.	-
Polypnea	4	2 (50.0)	-	-
Abdomen	**203**	**77 (38.0)**		
Normal	203	77 (38.0)	-	-
Abnormal	0	0 (0.0)	Ref.	-
Extremity	**203**	**77 (38.0)**		
Normal	196	76 (38.8)	3.80 (0.45-32.18)	0.221
Abnormal (see commentary extremity)	7	1 (14.3)	Ref.	-
Commentary extremity	**5**	**0 (0.0)**		
Cyanosis	3	0 (0.0)	-	-
Polydactyly	2	0 (0.0)	Ref.	-
Neurological	**203**	**77 (38.0)**		
Normal	198	77 (38.9)	-	-
Abnormal (see commentary neurological)	5	0 (0.0)	Ref.	-
Commentary neurological	**5**	**0 (0.0)**		
Hypotonia	2	0 (0.0)	-	-
Lethargy	2	0 (0.0)	-	-
Hypotonia + lethargy	1	0 (0.0)	Ref.	-

Genito-urinal	**203**	**77 (38.0)**		
Normal	200	77 (38.5)	-	-
Abnormal (see commentary genito-urinal)	3	0 (0.0)	Ref.	-
Commentary genito-urinal	**2**	**0 (0.0)**		
Immaturity	2	0 (0.0)	-	-
Diagnosis in first week of neonatal life	**203**	**77 (38.0)**		
Normal	192	72 (37.5)	Ref.	-
Infection	11	5 (45.5)	1.39 (0.41-4.72)	0.598
Source of infection[13]	**8**	**5 (62.5)**		
Respiratory	1	1 (100.0)	-	-
Cutaneous	1	0 (0.0)	-	-
Generalized sepsis	6	4 (66.7)	Ref.	-
Evolution during first week of neonatal life	**203**	**77 (38.0)**		
Good or status quo	200	77 (38.5)	-	-
Died	3	0 (0.0)	Ref.	-
CRP value at moment of neonatal deterioration[14]	**6**	**3 (50.0)**		
≤5 mg/dL	0	0 (0.0)	-	-
>5 mg/dL	6	3 (50.0)	Ref.	-
Blood culture[15] **during first week of neonatal life**	**203**	**77 (38.0)**		
Done	6	3 (50.0)	1.66 (0.33-8.45)	0.54
Not done	197	74 (37.6)	Ref.	-

[1]Based on last menstruation or ultrasound (before 20 weeks of gestation) if the last menstruation was not known. [2]Measured with thermometer. [3]Induction for obstetrical reasons. [4]Based on physical examination and ultrasound if there was doubt. [5]A dark greenish mass that accumulates in the bowel during fetal life and is discharged shortly after birth. [6]Delivery without medical intervention. Episiotomy: an incision through the area between the vagina and the anus to make the vaginal opening larger for childbirth. [7]Difficult delivery. [8]From arrival in hospital until delivery. [9]A sterile kit with instruments. [10]Chlorhexidine. [11]The Apgar score is determined by evaluating the newborn on five criteria (Appearance, Pulse, Grimace, Activity, Respiration) on a scale from zero to two. Afterwards, a summation of the five values was obtained. [12]Disinfectant against several micro-organisms. [13]WHO protocol was used to detect the source of infection. [14] CRP measured when the general state of the neonate deteriorated. [15]Blood samples were cultured in a BactAlert culture bottle. If bacterial growth was observed, a subculture was made on a blood agar plate. CRP: C-reactive protein. N: number of samples. OR: odds ratio. W: weeks.

Addendum 5: Univariate analysis of vaginal *Enterobacter cloacae* carriage

Addendum 5.1: Univariate analysis of vaginal *Enterobacter cloacae* carriage and risk factors

Addendum Table 5.1. Univariate logistic regressions showing the association between vaginal *Enterobacter cloacae* carriage and risk factors.

	n	*E. cloacae* + women (%)	Crude OR (95% CI)	p-value
Sociodemographic factors				
Age of pregnant woman	**329**	**139 (42.3)**		
≤25 years	116	44 (38.0)	Ref.	-
>25 years	213	95 (44.7)	1.32 (0.83-2.09)	0.242
Tribe	**328**	**140 (42.7)**		
Shi	220	94 (42.8)	1.03 (0.64-1.65)	0.902
Non-Shi[1]	108	46 (42.6)	Ref.	-
Religion	**329**	**140 (42.6)**		
Catholic	203	92 (45.4)	1.35 (0.86-2.12)	0.198
Non-Catholic[2]	126	48 (38.1)	Ref.	-
Community	**323**	**136 (42.2)**		
Kadatu	112	46 (41.1)	Ref.	-
Ibanda	144	62 (43.1)	1.09 (0.66-1.79)	0.750
Bagira	67	28 (41.8)	1.03 (0.56-1.90)	0.925
Education[3]	**329**	**139 (42.3)**		
Yes	319	136 (42.7)	1.73 (0.44-6.83)	0.431
No	10	3 (30.0)	Ref.	-
Level of education	**319**	**136 (42.7)**		
Primary	33	18 (54.6)	1.84 (0.84-4.04)	0.128
Secondary	177	75 (42.4)	1.13 (0.69-1.84)	0.626
Tertiary	109	43 (39.5)	Ref.	
State of marriage	**330**	**140 (42.5)**		
Married	313	135 (43.2)	1.82 (0.63-5.29)	0.271
Not married	17	5 (29.5)	Ref.	-
Age of marriage	**305**	**133 (43.7)**		
≤18 years	72	30 (41.7)	Ref.	-
>18 years	233	103 (44.3)	1.11 (0.65-1.89)	0.704
Duration of life with husband	**314**	**137 (43.7)**		
≤5 years	166	68 (41.0)	Ref.	-
>5 years	148	69 (46.7)	1.26 (0.81-1.97)	0.313
Living with husband or alone	**321**	**140 (43.7)**		
Living with husband	314	136 (43.4)	Ref.	-
Not married or not living with husband	7	4 (57.2)	1.75 (0.38-7.93)	0.471
Extramarital affairs[4]	**155**	**69 (44.6)**		
Yes	31	14 (45.2)	1.03 (0.47-2.28)	0.936
No	124	55 (44.4)	Ref.	-

Number of partners of husband	**46**	**22 (47.9)**		
1	42	20 (47.7)	Ref.	-
>1	4	2 (50.0)	1.10 (0.14-8.56)	0.927
Number of partners of pregnant woman during the last 6 months	**326**	**137 (42.1)**		
1	320	135 (42.2)	1.46 (0.26-8.08)	0.665
>1	6	2 (33.4)	Ref.	-
Number of partners of pregnant woman during life	**324**	**137 (42.3)**		
1	183	82 (44.9)	1.27 (0.81-1.98)	0.295
>1	141	55 (39.1)	Ref.	-
Source of income	**327**	**138 (42.3)**		
Non-employed	155	57 (36.8)	Ref.	-
Employed	172	81 (47.1)	1.53 (0.98-2.38)	0.060
Living circumstances				
Electricity and convenience	**330**	**140 (42.5)**		
Electricity	265	114 (43.0)	1.13 (0.65-1.97)	0.660
No electricity	65	26 (40.0)	Ref.	-
Water source	**329**	**140 (42.6)**		
Tap water	169	74 (43.8)	1.11 (0.72-1.72)	0.642
Other[5]	160	66 (41.3)	Ref.	-
Type of pavement	**329**	**140 (42.6)**		
Tiles	64	25 (39.1)	Ref.	-
Others[6]	265	115 (43.4)	1.20 (0.69-2.09)	0.529
Medical history				
BMI before conception[7]	**328**	**140 (42.7)**		
Underweight (BMI < 18.5)	3	1 (33.4)	Ref.	-
Normal range (BMI: 18.5 - 25)	156	64 (41.1)	1.39 (0.12-15.67)	0.789
Overweight (BMI: 25 - 30)	112	44 (39.3)	1.29 (0.11-14.70)	0.835
Obese (BMI ≥ 30)	57	31 (54.4)	2.39 (0.24-27.81)	0.488
Administration of current medication	**327**	**139 (42.6)**		
Yes	69	29 (42.1)	Ref.	-
No	258	110 (42.7)	1.03 (0.60-1.76)	0.928
Diabetic	**277**	**113 (40.8)**		
Yes	2	0 (0.0)	Ref.	-
No	275	113 (41.1)	-	-
Diabetic in the family	**317**	**135 (42.6)**		
Yes	72	34 (47.3)	1.28 (0.75-2.16)	0.366
No	245	101 (41.3)	Ref.	-
Chronic illness (e.g. cancer, gastritis, anemia, arterial hypertension…)[8]	**317**	**137 (43.3)**		
Yes	45	24 (53.4)	1.61 (0.85-3.03)	0.142
No	272	113 (41.6)	Ref.	-
Notion of constipation	**322**	**139 (43.2)**		
Yes	147	64 (43.6)	1.03 (0.66-1.60)	0.902
No	175	75 (42.9)	Ref.	-

Use of enema for constipation	**144**	**64 (44.5)**		
Yes	83	38 (45.8)	1.14 (0.58-2.21)	0.706
No	61	26 (42.7)	Ref.	-
Circumcised partner	**326**	**138 (42.4)**		
Yes	318	136 (42.8)	2.24 (0.45-11.28)	0.327
No	8	2 (25.0)	Ref.	-
Extension of the labia[9]	**318**	**136 (42.8)**		
Yes	40	23 (57.5)	1.98 (1.10-3.87)	**0.047**
No	278	113 (40.7)	Ref.	-
Known serological HIV state of pregnant woman	**315**	**133 (42.3)**		
Yes	212	89 (42.0)	Ref.	-
No	103	44 (42.8)	1.03 (0.64-1.66)	0.901
Period of last HIV test	**215**	**94 (43.8)**		
Less than 6 months ago	69	29 (42.1)	Ref.	-
More than 6 months ago	146	65 (44.6)	1.11 (0.62-1.98)	0.731
Knowledge of serological HIV state of husband	**240**	**104 (43.4)**		
Yes	100	41 (41.0)	Ref.	-
No	140	63 (45.0)	1.18 (0.70-1.98)	0.538
Realization of HIV test of couple (rapid test)	**321**	**138 (43.0)**		
Yes	96	35 (36.5)	Ref.	-
No	225	103 (45.8)	1.47 (0.90-2.41)	0.123
Treatment for gonorrhea or syphilis[10]	**299**	**124 (41.5)**		
Yes	7	3 (42.9)	1.06 (0.23-4.82)	0.940
No	292	121 (41.5)	Ref.	-
Cold sore on vulva (herpes)	**308**	**133 (43.2)**		
Yes	54	24 (44.5)	1.06 (0.59-1.92)	0.837
No	254	109 (43.0)	Ref.	-
Antibiotic administration in the past 2 weeks	**328**	**139 (42.4)**		
Yes	46	17 (37.0)	Ref.	-
No	282	122 (43.3)	1.30 (0.68-2.48)	0.423
Usus				
Consumption of alcohol during this pregnancy	**318**	**137 (43.1)**		
Yes	112	51 (45.6)	1.17 (0.73-1.86)	0.515
No	206	86 (41.8)	Ref.	-
Type of alcohol	**108**	**51 (47.3)**		
Beer	100	49 (49.0)	2.88 (0.56-14.97)	0.208
Others[11]	8	2 (25.0)	Ref.	
Last consumption of alcohol	**79**	**39 (49.4)**		
Less than 1 week	43	23 (53.5)	1.49 (0.59-3.50)	0.424
More than 1 week	36	16 (44.5)	Ref.	-
Amount of alcohol	**109**	**50 (45.9)**		
Less than 1 time a day	4	3 (75.0)	3.70 (0.37-36.77)	0.264
1 or more times a day	105	47 (44.8)	Ref.	-

Geophagia[12]	**329**	**139 (42.3)**		
Yes	85	38 (44.8)	1.15 (0.70-1.88)	0.595
No	244	101 (41.4)	Ref.	-
Consumption of coal[13]	**327**	**138 (42.3)**		
Yes	29	8 (27.6)	Ref.	-
No	298	130 (43.7)	2.03 (0.87-4.73)	0.101
Duration of consumption of geophagy and coal	**50**	**22 (44.0)**		
1 week	26	13 (50.0)	1.67 (0.54-5.15)	0.375
More than 1 week	24	9 (37.5)	Ref.	-
Consummation of tobacco	**328**	**139 (42.4)**		
Yes	2	0 (0.0)	Ref.	-
No	326	139 (42.7)	-	-
Use of natural excitants (mairungi chanvre) [14]	**329**	**139 (42.3)**		
Yes	1	0 (0.0)	Ref.	-
No	328	139 (42.4)	-	-
Reproductive health				
Gestational age at V1	**324**	**138 (42.6)**		
≤26 weeks	309	129 (41.8)	Ref.	-
>26 weeks	15	9 (60.0)	2.09 (0.73-6.03)	0.171
Number of previous deliveries on term	**330**	**140 (42.5)**		
0	79	29 (36.8)	Ref.	
1-2	115	48 (41.8)	1.26 (0.69-2.23)	0.482
>3	136	63 (46.4)	1.49 (0.84-2.63)	0.170
Previous premature delivery	**330**	**140 (42.5)**		
Yes	20	13 (65.0)	2.68 (1.04-6.94)	**0.041**
No	310	127 (41.0)	Ref.	-
Total parity of the women	**330**	**140 (42.5)**		
0	76	27 (35.6)	Ref.	-
1-2	114	48 (42.2)	1.320 (0.725-2.403)	0.364
>3	140	65 (46.5)	1.573 (0.885-2.796)	0.123
Previous abortion[15]	**330**	**140 (42.5)**		
Yes	222	92 (41.5)	Ref.	-
No	108	48 (44.5)	1.13 (0.71-1.80)	0.605
Previous fetal death in utero[16]	**330**	**140 (42.5)**		
Yes	21	8 (38.1)	Ref.	-
No	308	132 (42.9)	1.22 (0.49-3.03)	0.670
Previous caesarean section [17]	**295**	**129 (43.8)**		
Yes	58	26 (44.9)	1.06 (0.59-1.88)	0.851
No	237	103 (43.5)	Ref.	-

Weight of biggest baby from previous pregnancy	**253**	**112 (44.3)**		
<2500 g	4	3 (75.0)	3.75 (0.36-38.86)	0.268
2500-4000 g	204	89 (43.7)	0.97 (0.51-1.85)	0.920
>4000 g	45	20 (44.5)	Ref.	
Notion of infection of previously born baby in first week of life	**269**	**116 (43.2)**		
Yes	81	35 (43.3)	1.01 (0.59-1.70)	0.985
No	188	81 (43.1)	Ref.	-
Evolution of the previously born baby	**84**	**35 (41.7)**		
Good	65	27 (41.6)	Ref.	-
Handicap or death	19	8 (42.2)	1.02 (0.36-2.88)	0.965
Number of consultations during current pregnancy	**329**	**140 (42.6)**		
0	102	42 (41.2)	Ref.	-
≥1	227	98 (43.2)	1.09 (0.68-1.74)	0.735
Prevention in current pregnancy				
Administration of substances to diminish neonatal infections[18]	**271**	**123 (45.4)**		
Yes	58	27 (46.6)	1.06 (0.59-1.90)	0.841
No	213	96 (45.1)	Ref.	-
Administration of Fansidar® (prophylaxis against malaria)[19]	**318**	**131 (41.2)**		
Yes	63	22 (35.0)	Ref.	-
No	255	109 (42.8)	1.39 (0.78-2.47)	0.260
Administration of Vermox® (prophylaxis against intestinal worms)	**325**	**139 (42.8)**		
Yes	64	24 (37.5)	Ref.	-
No	261	115 (44.1)	1.31 (0.75-2.30)	0.343
Utilization of mosquito net during pregnancy	**324**	**138 (42.6)**		
Yes	287	128 (44.6)	2.17 (1.02-4.66)	**0.046**
No	37	10 (27.1)	Ref.	-
Sexual behaviour				
Age of first sexual contact	**268**	**113 (42.2)**		
≤18 years	126	44 (35.0)	Ref.	-
>18 years	142	69 (48.6)	1.76 (1.08-2.88)	**0.024**
Anal sexual intercourse[20]	**329**	**140 (42.6)**		
Yes	32	22 (68.8)	3.337 (1.526-7.301)	**0.003**
No	297	118 (39.8)	Ref.	-
Last sexual contact during current pregnancy	**291**	**128 (44.0)**		
≤7 days	224	99 (44.2)	1.04 (0.60-1.80)	0.895
>7days	67	29 (43.3)	Ref.	-
Toilet hygiene				
Type of toilet	**330**	**140 (42.5)**		
Toilet with bowl and flush	81	36 (44.5)	1.12 (0.67-1.85)	0.672
Other types[21]	249	104 (41.8)	Ref.	-

Use after toilet	**328**	**139 (42.4)**		
Water	227	97 (42.8)	1.05 (0.65-1.69)	0.846
Tissue or other substances	101	42 (41.6)	Ref.	-
Vaginal practices				
Normal vaginal toilet	**323**	**138 (42.8)**		
Only water	264	119 (45.1)	1.73 (0.95-3.14)	0.073
Other substances or none[22]	59	19 (32.3)	Ref.	-
Practices to dry vagina	**327**	**140 (42.9)**		
Yes	49	21 (42.9)	Ref.	-
No	278	119 (42.9)	1.00 (0.54-1.85)	0.995
Vaginal practices	**51**	**23 (45.1)**		
Toilet with cold water	4	2 (50.0)	1.24 (0.16-9.55)	0.838
Other practices[23]	47	21 (44.7)	Ref.	-
Number of vaginal toilets	**13**	**6 (46.2)**		
≤2 a day	12	5 (41.7)	Ref.	-
>2 a day	1	1 (100.0)	-	-
Vaginal toilet after each sexual contact	**328**	**139 (42.4)**		
Yes	281	119 (42.4)	Ref.	-
No	47	20 (42.6)	1.01 (0.54-1.88)	
Type of intimate toilet after sexual contact	**278**	**118 (42.5)**		
Water	201	84 (41.8)	Ref.	-
Use of tissue or other	77	34 (44.2)	1.277 (0.737-2.211)	0.383

[1]Rega, Havu, Tumbo, Hunde, Nyganga, Hutu, Nande, Vira, Fuliru, Bembe. [2]Non-catholic: Protestantism, Angilicanism, Kimbanguism, Moslim, Animism. [3]From primary school. [4]Extramarital affairs of man known by the pregnant women. [5]Rain water, water well.[6]Concrete, carpet, no pavement. [7]Weight before the current pregnancy. [8]A diagnosed chronic illness. [9]A cultural tradition. [10]Diagnosed by acknowledge doctor or a clinical officer. [11]Wine, liqueur, local alcoholic drink (Sorgho). [12]Geophagia is the practice of eating earth or soil-like substrates such as clay or chalk to diminish nausea in pregnancy. [13]In case of Pica syndrome .[14]Khat, marijuana.[15]Natural, spontaneous abortion. [16]From 20 weeks of gestational age. [17]Planned and unplanned section.[18]Seeds, herbs,…[19]This prophylaxis is taken by all women at antenatal consultation during pregnancy at 24 WGA. [20]Information about timing and frequency is unknown. [21]Squat latrine, pit latrin. [22]Use of soap, perfume, powder, lemon juice, Dettol, virginity soap, tissue. [23]Use of soap, perfume, powder, lemon juice, antiseptic soap, Dettol, shaving. BMI: Body Mass Index. *E. cloacae*: *Enterobacter cloacae*. HIV: human immunodeficiency virus. N: number of samples. OR: odds ratio. V1: visit 1.

Addendum 5.2: Univariate analysis of vaginal *Enterobacter cloacae* carriage and signs and symptoms

Addendum Table 5.2. Univariate logistic regressions showing the association between vaginal *Enterobacter cloacae* carriage and signs and symptoms.

	n	*E. cloacae* + women (%)	Crude OR (95% CI)	p-value
General signs and symptoms at V1				
Fever	**324**	**139 (43.0)**		
Yes	37	16 (43.3)	1.02 (0.51-2.03)	0.964
No	287	123 (42.9)	Ref.	-
Headache	**326**	**137 (42.1)**		
Yes	159	74 (46.6)	1.44 (0.92-2.24)	0.107
No	167	63 (37.8)	Ref.	-
Cough	**327**	**138 (42.3)**		
Yes	72	23 (32.0)	Ref.	-
No	255	115 (45.1)	1.75 (1.01-3.04)	**0.047**
Uterine contractions	**291**	**124 (42.7)**		
Yes	40	17 (42.5)	Ref.	-
No	251	107 (42.7)	1.01 (0.51-1.97)	0.988
Lumbar pain	**326**	**138 (42.4)**		
Yes	165	74 (44.9)	1.23 (0.79-1.091)	0.352
No	161	64 (39.8)	Ref.	-
Difficulty to swallow	**326**	**138 (42.4)**		
Yes	32	11 (34.4)	Ref.	-
No	294	127 (43.2)	1.45 (0.68-3.12)	0.340
Vaginal signs and symptoms at V1				
Vaginal discharge	**326**	**139 (42.7)**		
Yes	159	63 (39.7)	Ref.	-
No	167	76 (45.6)	1.27 (0.82-1.98)	0.283
Previous treatment for vaginal discharge[1]	**159**	**63 (39.7)**		
Yes	86	36 (41.9)	1.23 (0.65-2.33)	0.531
No	73	27 (37.0)	Ref.	-
Type of previous treatment for vaginal discharge	**326**	**139 (42.7)**		
Gyogynax	15	10 (66.7)	2.69 (0.89-8.11)	0.078
Anitbiotics	36	15 (41.7)	0.96 (0.47-1.96)	0.914
Antibiotics + other	19	5 (26.4)	0.48 (0.17-1.38)	0.172
Other[2]	16	6 (37.5)	0.81 (0.29-2.29)	0.688
No treatment	240	103 (43.0)	Ref.	-
Vaginal itching	**328**	**140 (42.7)**		
Yes	136	64 (47.1)	1.36 (0.87-2.12)	0.178
No	192	76 (39.6)	Ref.	-
Previous treatment for vaginal itching[1]	**137**	**66 (48.2)**		
Yes	61	28 (46.0)	Ref.	-
No	76	38 (50.0)	1.18 (0.60-2.32)	0.633

Type of previous treatment for vaginal itching	**328**	**140 (42.7)**		
Gyogynax	13	8 (61.6)	2.24 (0.72-7.04)	0.166
Anitbiotics	30	12 (40.0)	0.94 (0.43-2.02)	0.863
Antibiotics + other	14	6 (42.9)	1.05 (0.36-3.11)	0.928
Other[3]	4	2 (50.0)	1.40 (0.20-10.10)	0.737
No treatment	267	112 (42.0)	Ref.	-
Dysuria	**324**	**137 (42.3)**		
Yes	86	37 (43.1)	1.04 (0.63-1.72)	0.871
No	238	100 (42.1)	Ref.	-
Previous treatment for dysuria[1]	**89**	**41 (46.1)**		
Yes	34	21 (61.8)	2.83 (1.17-6.84)	**0.021**
No	55	20 (36.4)	Ref.	-
Type of previous treatment for dysuria	**324**	**137 (42.3)**		
Gyogynax	4	4 (100.0)	-	-
Anitbiotics	22	15 (68.2)	3.17 (1.25-8.01)	**0.015**
Antibiotics + other	7	2 (28.6)	0.59 (0.11 - 3.10)	0.534
Other[4]	2	0 (0.0)	-	-
No treatment	289	116 (40.2)	Ref.	-
Burning sensation after sexual contact[5]	**313**	**131 (41.9)**		
Yes	104	43 (41.4)	**Ref.**	**-**
No	209	88 (42.2)	1.03 (0.64-1.66)	0.898
Last episode of burning	**86**	**35 (40.7)**		
Less than 7 days	55	22 (40.0)	Ref.	-
More than 7 days	31	13 (42.0)	1.08 (0.44-2.65)	0.861
Previous treatment for burning[1]	**105**	**44 (42.0)**		
Yes	22	10 (45.5)	1.20 (0.47-3.09)	0.704
No	83	34 (41.0)	Ref.	-
Type of previous treatment for burning	**313**	**131 (41.9)**		
Gyogynax	4	2 (50.0)	1.37 (0.19-9.85)	0.755
Anitbiotics	9	5 (55.6)	1.71 (0.45-6.50)	0.430
Antibiotics + other	7	2 (28.6)	0.55 (0.11-2.87)	0.476
Other[4]	2	1 (50.0)	1.37 (0.09-22.09)	0.825
No treatment	291	121 (41.6)	Ref.	-
Sensation of vaginal smell	**297**	**125 (42.1)**		
Yes	77	33 (42.9)	1.04 (0.62-1.76)	0.874
No	220	92 (41.9)	Ref.	-
Last episode of vaginal smell	**48**	**19 (39.6)**		
≤2 days	30	12 (40.0)	1.05 (0.32-3.47)	0.939
>2 days	18	7 (38.9)	Ref.	-
Previous treatment for vaginal smell[1]	**75**	**31 (41.4)**		
Yes	10	3 (30.0)	Ref.	-
No	65	28 (43.1)	1.77 (0.42-7.44)	0.439

Type of previous treatment for vaginal smell	**297**	**125 (42.1)**		
Gyogynax	0	0 (0.0)		
Anitbiotics	4	1 (25.0)	0.45 (0.05-4.43)	0.486
Antibiotics + other	4	1 (25.0)	0.45 (0.05-4.43)	0.486
Other[6]	2	1 (50.0)	1.34 (0.08-21.55)	0.838
No treatment	287	122 (42.6)	Ref.	-
General clinical examination at V1				
Weight evolution during pregnancy[7]	**330**	**140 (42.5)**		
Weight loss	87	40 (46.0)	1.45 (0.72-2.90)	0.297
Stable weight or ≤5 kg weight gain	189	80 (42.4)	1.27 (0.68-2.37)	0.451
> 5kg weight gain	54	20 (37.1)	Ref.	-
Arm circumference	**328**	**140 (42.7)**		
<22 cm	25	10 (40.0)	Ref.	-
22-27.5 cm	202	86 (42.6)	1.11 (0.48-2.60)	0.806
>27.5 cm	101	44 (43.6)	1.16 (0.48-2.82)	0.747
Diastolic blood pressure	**330**	**140 (42.5)**		
<90 mmHg	325	137 (42.2)	Ref.	-
≥90 mmHg	5	3 (60.0)	2.06 (0.34-12.49)	0.430
Systolic blood pressure	**330**	**140 (42.5)**		
<140 mmHg	324	136 (38.2)	Ref.	-
≥140 mmHg	6	4 (66.7)	2.77 (0.50-15.31)	0.244
Cardiac frequency	**329**	**139 (42.3)**		
<110 bpm	318	131 (41.2)	Ref.	-
≥110 bpm	11	8 (72.8)	3.807 (0.991-14.619)	0.052
Edema lower legs		**(0.0)**		
Yes	1	1 (100.0)	-	-
No	329	139 (42.3)	Ref.	-
General physical state	**330**	**140 (42.5)**		
Normal	329	140 (42.6)	-	-
Abnormal[8]	1	0 (0.0)	Ref.	-
Gynaecological examination at V1				
Vulvar state	**328**	**140 (42.7)**		
Normal	322	138 (42.9)	1.50 (0.27-8.31)	0.642
Abnormal[9]	6	2 (33.4)	Ref.	-
Speculum examination	**328**	**140 (42.7)**		
Normal	272	118 (43.4)	1.18 (0.66-2.13)	0.573
Abnormal[10]	56	22 (39.3)	Ref.	-
Vaginal pH	**324**	**139 (43.0)**		
4	5	1 (20.0)	Ref.	-
5-6	261	113 (43.3)	3.05 (0.34-27.70)	0.321
>6	58	25 (43.2)	3.03 (0.32-28.81)	0.335

White blood cells per field on wet mount	**330**	**140 (42.5)**		
0	0	0 (0.0)	-	-
1 -4	178	84 (47.2)	Ref.	-
5-30	132	45 (34.1)	0.58 (0.36-0.92)	**0.021**
30+	20	11 (55.0)	1.39 (0.54-3.46)	0.509
Clue cells[11] **on wet mount**	**330**	**140 (42.5)**		
Yes	37	16 (43.3)	1.04 (0.52-2.07)	0.915
No	293	124 (42.4)	Ref.	-
***Trichomonas* on wet mount**	**329**	**139 (42.3)**		
Yes	4	1 (25.0)	Ref.	-
No	325	138 (42.5)	2.21 (0.23-21.51)	0.493
***Candida* on wet mount**	**329**	**139 (42.3)**		
Yes	91	37 (40.7)	Ref.	-
No	238	102 (42.9)	1.10 (0.67-1.79)	0.718
Epithelial cells per field wet mount	**326**	**138 (42.4)**		
<5	19	11 (57.9)	1.72 (0.64-4.46)	0.285
5-30	208	83 (40.0)	0.83 (0.51-1.35)	0.450
30+	99	44 (44.5)	Ref.	-
Whiff test (KOH)[12]	**330**	**140 (42.5)**		
Positive	32	14 (43.8)	1.06 (0.51-2.22)	0.873
Negative	298	126 (42.3)	Ref.	-
State of vaginal secretions	**330**	**140 (42.5)**		
Normal: fine and homogeneous	297	124 (41.8)	Ref.	-
Abnormal: thick (+heterogeneous)	33	16 (48.5)	1.31 (0.64-2.70)	0.459
BV on gram stain[13]	**326**	**139 (42.6)**		
No BV	176	78 (44.3)	Ref.	-
Intermediate	59	28 (47.5)	1.14 (0.63-2.05)	0.680
BV	91	33 (36.3)	0.72 (0.43-1.20)	0.210
Biofilm	**326**	**139 (42.6)**		
Yes	73	26 (35.6)	Ref.	-
No	253	113 (44.7)	1.46 (0.85-2.50)	0.170
Gram + cocci on gram stain	**326**	**139 (42.6)**		
Yes	31	13 (41.9)	Ref.	-
No	295	126 (42.7)	1.03 (0.49-2.19)	0.930
Gram - cocci on gram stain	**326**	**139 (42.6)**		
Yes	6	3 (50.0)	1.35 (0.27-6.81)	0.710
No	320	136 (42.5)	Ref.	-
Yeast on gram stain	**326**	**139 (42.6)**		
Yes	88	36 (40.9)	Ref.	-
No	238	103 (42.3)	1.22 (0.75-2.00)	0.420
Hyphae on gram stain[14]	**330**	**140 (42.4)**		
Yes	48	21 (43.8)	1.07 (0.58-1.98)	0.840
No	282	119 (42.2)	Ref.	-

***Enterobacter cloacae* in CVL**	**330**	**140 (42.4)**		
Yes	126	53 (42.1)	Ref.	-
No	204	87 (42.7)	1.02 (0.65-1.61)	0.920
***Klebsiella pneumoniae* in CVL**	**330**	**140 (42.4)**		
Yes	40	22 (55.0)	1.78 (0.92-3.47)	0.090
No	290	118 (40.7)	Ref.	-
Clinical diagnosis at V1	**330**	**140 (42.5)**		
Normal	140	60 (42.9)	1.03 (0.66-1.60)	0.891
Pathological	190	80 (42.2)	Ref.	-
Symptomatic treatment for vaginitis (BV and *Candida*) at V1	**329**	**140 (42.6)**		
Femaclin®	109	43 (39.5)	Ref.	-
Antibiotic	21	8 (38.1)	0.95 (0.36-2.47)	0.907
Femaclin® + Antibiotic	53	25 (47.2)	1.37 (0.71-2.66)	0.351
Other treatment[15]	8	5 (62.5)	2.56 (0.58-11.26)	0.214
No treatment	138	59 (42.8)	1.15 (0.69-1.91)	0.601
Additional technical examination at V1				
Hemoglobin on Hemocue®	**328**	**139 (42.4)**		
Anemia (<11 Hb)	12	5 (41.7)	Ref.	-
Normal (≥11 Hb)	316	134 (42.5)	1.03 (0.32-3.32)	0.959
Rapid test malaria	**330**	**140 (42.5)**		
Positive	1	0 (0.0)	Ref.	-
Negative	329	140 (42.6)	-	-
Rapid test HIV	**330**	**140 (42.5)**		
Positive	1	1 (100.0)	Ref.	-
Negative	329	139 (42.3)	-	-
White blood cells on urine dipstick	**330**	**140 (42.5)**		
Positive	134	53 (39.6)	Ref.	-
Negative	196	87 (44.4)	1.22 (0.78-1.91)	0.383
Nitrite on urine dipstick	**330**	**140 (42.5)**		
Positive	12	4 (33.4)	Ref.	-
Negative	318	136 (42.8)	1.50 (0.44-5.07)	0.519
Glycated keratin	**320**	**136 (42.5)**		
<3.6	181	72 (39.8)	Ref.	-
3.6-10	120	53 (44.2)	1.20 (0.75-1.91)	0.450
>10	19	11 (57.9)	2.08 (0.80-5.25)	0.134
Ultrasound examination at V1				
Estimation of fetal weight centiles[16]	**315**	**132 (42.0)**		
<p10	44	16 (36.4)	1.14 (0.56-2.35)	0.716
p10 - p90	148	75 (50.7)	2.06 (1.25-3.37)	**0.004**
>p90	123	41 (33.4)	Ref.	-
Fetal sex[17]	**324**	**137 (42.3)**		
Female	162	71 (43.9)	1.14 (0.73-1.76)	0.574
Male	162	66 (40.8)	Ref.	-

Insertion placenta[17]	**326**	**138 (42.4)**		
Normal	310	129 (41.7)	Ref.	-
Low inserted	16	9 (56.3)	1.80 (0.66-4.97)	0.254
Amniotic fluid[17]	**326**	**138 (42.4)**		
Normal	326	138 (42.4)	-	-
Abnormal	0	0 (0.0)	Ref.	-
Length cervix[17]	**330**	**140 (42.5)**		
<25 cm	3	3 (100.0)	-	-
25-30 cm	25	12 (48.0)	1.31 (0.58-2.96)	0.521
>30 cm	302	125 (41.4)	Ref.	-
Funnel[17.18]	**329**	**140 (42.6)**		
Present	5	4 (80.0)	5.53 (0.61-50.02)	0.128
Absent	324	136 (42.0)	Ref.	-
Morphological abnormality visible[17]	**326**	**139 (42.7)**		
Yes	6	2 (33.4)	Ref.	-
No	320	137 (42.9)	1.50 (0.27-8.29)	0.644

[1]Treatment in pregnancy. [2]Femaclin®, Gyndodactarin, Nystatin, Tinidazole, Fluomizin. [3]Nystatin, Gynodactarin. [4]Not precised. [5]Burning sensation in the current pregnancy. [6]Femaclin®, Gynodactarin. [7]Weight before pregnancy compared with weight at V1. [8]Deviant compared with healthy pregnant women. [9]Genital wrat, herpetic lesions, chancre, erythema, pustule, abcess (Bartholin's gland), leucorrhoea. [10]Erythema, polyp, ectropion, bleeding, xanthoma, ulcers, leucorrhoea. [11]Clue cells are epithelial cells of the vagina that get their distinctive stippled appearance by being covered with bacteria. It is a typical sign of bacterial vaginosis. [12] A whiff test is performed by adding several drops of 10% potassium hydroxide to a sample of vaginal discharge. A strong fishy odor is indicative of a positive test result. Such a result may suggest either trichomoniasis or bacterial vaginosis. [13]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV). (59)[14]Long, tubular branching structures produced by *Candida*. [15]Tot'hema, gogynax, omnibionta. [16]Based on Percentile table Jeanty. [17]Based on ultrasound examination. [18]Protrusion of the amniotic membranes into the internal os of the cervix. This condition increased the risk on preterm birth. Bpm: beats per minute. CVL: cervicovaginal lavage. *E. cloacae*: *Enterobacter cloacae*. Hb: hemoglobin. HIV: human immunodeficiency virus. N: number of samples. OR: odds ratio. P: percentile. V1: visit 1.

Addendum 5.3: Univariate analysis of vaginal *Enterobacter cloacae* carriage and adverse pregnancy outcomes

Addendum Table 5.3. Univariate logistic regressions showing the association between vaginal *Enterobacter cloacae* carriage and adverse pregnancy outcomes.

	n	*E. cloacae* + women (%)	Crude OR (95% CI)	p-value
Delivery				
Gestational age at labor [1]	**202**	**91 (45.1)**		
28w-32w	2	1 (50.0)	1.29 (0.8-21.02)	0.857
32w-36w	28	15 (53.6)	1.49 (0.67-3.33)	0.328
≥37w	172	75 (43.7)	Ref.	-
Preterm birth	**202**	**91 (45.1)**		
Yes (<37w)	30	16 (53.4)	1.78 (0.68-3.22)	0.330
No (≥37w)	172	75 (43.7)	Ref.	-
Temperature of the mother at labor [2]	**200**	**88 (44.0)**		
<37.2°C	179	79 (44.2)	1.05 (0.42-2.63)	0.910
≥ 37.2°C	21	9 (42.9)	Ref.	-
Development of labor	**199**	**88 (44.3)**		
Spontaneous	182	79 (43.5)	Ref.	-
Induced[3]	17	9 (53.0)	1.47 (0.54-3.97)	0.450
Way of induction of labor	**17**	**9 (53.0)**		
Misoprostol (prostaglandin)	10	7 (70.0)	5.83 (0.70-48.87)	0.100
Foley probe with misoprostol (prostaglandin)	7	2 (28.6)	Ref.	-
Fetal presentation at labor [4]	**204**	**91 (44.7)**		
Cephalic (head)	196	88 (44.9)	1.63 (0.15-18.27)	0.692
Bottom	5	2 (40.0)	1.33 (0.7-26.62)	0.851
Transversal	3	1 (33.4)	Ref.	-
State of membranes at arrival in hospital (before delivery)	**204**	**91 (44.7)**		
Intact	158	68 (43.1)	Ref.	-
Broken or cracked	46	23 (50.0)	1.32 (0.69-2.56)	0.400
Duration of rupture of membranes	**199**	**87 (43.8)**		
≤6 hours	192	82 (42.8)	Ref.	-
>6 hours	7	5 (71.5)	3.35 (0.64-17.72)	0.150
Amniotic fluid type at delivery	**204**	**91 (44.7)**		
Clear	162	72 (44.5)	Ref.	-
Meconium[5] (fresh or old)	42	19 (45.3)	1.03 (0.52-2.04)	0.930
Number of vaginal touchers during labor	**204**	**91 (44.7)**		
≤5 times	40	18 (45.0)	1.02 (0.51-2.04)	0.960
>5 times	164	73 (44.6)	Ref.	-
Washing of hands before labor	**188**	**82 (43.7)**		
Yes	188	82 (43.7)	-	-
No	0	0 (0.0)	Ref.	-

Type of labor	**204**	**91 (44.7)**		
Eutocic (with episotomy)[6]	167	78 (46.8)	1.55 (0.74-3.27)	0.250
Dystocic[7]	1	0 (0.0)	-	-
Caesarean section	36	13 (36.2)	Ref.	-
Duration of labor[8]	**195**	**85 (43.6)**		
≤8 hours	139	61 (43.9)	1.04 (0.56-1.95)	0.900
>8 hours	56	24 (42.9)	Ref.	-
Utilization of labor kit[9]	**203**	**91 (44.9)**		
Yes	141	62 (44.0)	Ref.	-
No	62	29 (46.8)	1.12 (0062-2.04)	0.710
Cord care	**9**	**5 (55.6)**		
No disinfectant	0	5 (0.0)	-	-
Disinfectant[10]	9	0 (0.0)	Ref.	-
APGAR[11] score 5 minutes	**203**	**90 (44.4)**		
<7	2	1 (50.0)	1.26 (0.08-20.40)	0.870
≥7	201	89 (44.3)	Ref.	-
Sex of the baby	**204**	**91 (44.7)**		
Female	103	42 (40.8)	Ref.	-
Male	101	49 (48.6)	1.37 (0.79-2.38)	0.270
Visible abnormality	**204**	**91 (44.7)**		
Present	4	1 (25.0)	Ref.	-
Absent	200	90 (45.0)	2.46 (0.25-24.00)	0.440
Disinfectant eye drops[12]	**203**	**90 (44.4)**		
Yes	172	73 (42.5)	Ref.	-
No	31	17 (54.9)	1.65 (0.76-3.55)	0.200
Evolution of neonate	**204**	**91 (44.7)**		
Close to mother	201	90 (44.8)	1.62 (0.15-18.17)	0.700
Neonatology	3	1 (33.4)	Ref.	-
Neonatal outcome				
Fever[2]	**203**	**91 (44.9)**		
Yes (>37.2 °C)	3	1 (33.4)	Ref.	-
No	200	90 (45.0)	1.64 (0.15-28.34)	0.690
Temperature neonate	**203**	**91 (44.9)**		
<36.6 °C	117	54 (46.2)	Ref.	-
36.6-37.2 °C	75	31 (41.4)	0.82 (0.46-1.48)	0.512
>37.2 °C	11	6 (54.6)	1.40 (0.41-4.84)	0.595
Hypothermia	**203**	**91 (44.9)**		
Yes (<35 °C)	1	0 (0.0)	Ref.	-
No	202	91 (45.1)	-	-
Lethargy	**203**	**91 (44.9)**		
Yes	3	1 (33.4)	Ref.	-
No	200	90 (45.0)	1.64 (0.15-18.34)	0.690

Jaundice	**202**	**91 (45.1)**		
Yes	0	0 (0.0)	Ref.	-
No	202	91 (45.1)	-	-
Convulsions	**203**	**91 (44.9)**		
Yes	0	0 (0.0)	Ref.	-
No	203	91 (44.9)	-	-
Apnea	**203**	**91 (44.9)**		
Yes	1	0 (0.0)	Ref.	-
No	202	91 (45.1)	-	-
Hypotonia	**203**	**91 (44.9)**		
Yes	3	1 (33.4)	Ref.	-
No	200	90 (45.0)	1.64 (0.15-18.34)	0.690
Hypertonia	**203**	**91 (44.9)**		
Yes	0	0 (0.0)	Ref.	-
No	203	91 (44.9)	-	-
Shock	**203**	**91 (44.9)**		
Yes	0	0 (0.0)	Ref.	-
No	203	91 (44.9)	-	-
Dirty umbilicus	**203**	**91 (44.9)**		
Yes	0	0 (0.0)	Ref.	-
No	203	91 (44.9)	-	-
Difficult to suckle	**203**	**91 (44.9)**		
Yes	3	1 (33.4)	Ref.	-
No	200	90 (45.0)	1.64 (0.15-18.34)	0.690
Alimentation	**202**	**90 (44.6)**		
Maternal milk	200	89 (44.5)	Ref.	-
Bottle milk or combination maternal and bottle milk	2	1 (50.0)	1.25 (0.08-20.22)	0.880
Length of baby	**203**	**91 (44.9)**		
Small: <46 cm	4	2 (50.0)	1.24 (0.17-8.95)	0.830
Normal: 46 cm – 56 cm	199	89 (44.8)	Ref.	-
Large: >56 cm	0	0 (0.0)	-	-
Head circumference	**203**	**91 (44.9)**		
Microcephaly: <32 cm	2	1 (50.0)	2.00 (0.05-78.25)	0.710
Normal: 32 cm – 37 cm	198	89 (45.0)	1.63 (0.15-18.31)	0.690
Macrocephaly: >27 cm	3	1 (33.4)	Ref.	-
Weight at birth	**203**	**91 (44.9)**		
<2500 g (low birth weight)	7	4 (57.2)	1.67 (0.36-7.66)	0.510
≥2500 g	196	87 (44.4)	Ref.	-
General physical state	**203**	**91 (44.9)**		
Normal	196	87 (44.4)	Ref.	-
Abnormal (see commentary general state)	7	4 (57.2)	1.67 (0.36-7.66)	0.510

Commentary general state	**7**	**3 (42.9)**		
Fever	4	1 (25.0)	Ref.	-
Prematurity	1	1 (100.0)	-	-
Death	2	1 (50.0)	-	-
Skin	**203**	**91 (44.9)**		
Normal	198	88 (44.5)	Ref.	-
Abnormal: erythema	5	3 (60.0)	1.88 (0.31-11.47)	0.500
Mouth	**203**	**91 (44.9)**		
Normal	203	91 (44.9)	-	-
Abnormal	0	0 (0.0)	Ref.	-
ORL	**203**	**91 (44.9)**		
Normal	203	91 (44.9)	-	-
Abnormal	0	0 (0.0)	Ref.	-
Neck	**203**	**91 (44.9)**		
Normal	203	91 (44.9)	-	-
Abnormal	0	0 (0.0)	Ref.	-
Cardiovascular	**203**	**91 (44.9)**		
Normal	199	89 (44.8)	Ref.	-
Abnormal (see commentary cardiovascular)	4	2 (50.0)	1.24 (0.17-8.95)	0.830
Commentary cardiovascular	**4**	**2 (50.0)**		
Bradycardia	3	2 (66.7)	-	-
Tachycardia	1	0 (0.0)	Ref.	-
Lungs	**203**	**91 (44.9)**		
Normal	197	87 (44.2)	Ref.	-
Abnormal (see commentary lungs)	6	4 (66.7)	2.53 (0.45-14.13)	0.290
Commentary lungs	**7**	**4 (57.2)**		
Apnea	3	2 (66.7)	2.00 (0.09-44.35)	0.660
Polypnea	4	2 (50.0)	Ref.	-
Abdomen	**203**	**91 (44.9)**		
Normal	202	91 (45.1)	-	-
Abnormal	1	0 (0.0)	Ref.	-
Extremity	**203**	**91 (44.9)**		
Normal	196	87 (44.4)	Ref.	-
Abnormal (see commentary extremity)	7	4 (57.2)	1.67 (0.36-7.66)	0.510
Commentary extremity	**5**	**2 (40.0)**		
Cyanosis	3	1 (33.4)	Ref.	-
Polydactyly	2	1 (50.0)	2.00 (0.05-78.25)	0.710
Neurological	**203**	**91 (44.9)**		
Normal	198	89 (45.0)	1.23 (0.20-7.49)	0.830
Abnormal (see commentary neurological)	5	2 (40.0)	Ref.	-
Commentary neurological	**5**	**2 (40.0)**		
Hypotonia	2	1 (50.0)	Ref.	-
Lethargy	2	0 (0.0)	-	-
Hypotonia + lethargy	1	1 (100.0)	-	-

Genito-urinal	**203**	**91 (44.9)**		
Normal	200	89 (44.5)	Ref.	-
Abnormal (see commentary genito-urinal)	3	2 (66.7)	2.49 (0.22-27.96)	0.460
Commentary genito-urinal	**2**	**1 (50.0)**		
Immaturity	2	1 (50.0)	-	-
Diagnosis in first week of neonatal life	**203**	**91 (44.9)**		
Normal	192	86 (44.8)	Ref.	-
Infection	11	5 (45.5)	1.03 (0.30-3.48)	0.970
Source of infection[13]	**8**	**5 (62.5)**		
Respiratory	1	1 (100.0)	-	-
Cutaneous	1	0 (0.0)	-	-
Generalized sepsis	6	4 (66.7)	Ref.	-
Evolution during first week of neonatal life	**203**	**91 (44.9)**		
Good or status quo	200	90 (45.0)	1.64 (0.15-18.34)	0.690
Died	3	1 (33.4)	Ref.	-
CRP value at moment of neonatal deterioration[14]	**6**	**3 (50.0)**		
≤5 mg/dL	0	0 (0.0)	Ref.	-
>5 mg/dL	6	3 (50.0)	-	-
Blood culture[15] **during first week of neonatal life**	**203**	**91 (44.9)**		
Done	6	3 (50.0)	1.24 (0.24-6.29)	0.800
Not done	197	88 (44.7)	Ref.	-

[1]Based on last menstruation or ultrasound (before 20 weeks of gestation) if the last menstruation was not known. [2]Measured with thermometer. [3]Induction for obstetrical reasons. [4]Based on physical examination and ultrasound if there was doubt. [5]A dark greenish mass that accumulates in the bowel during fetal life and is discharged shortly after birth. [6]Delivery without medical intervention. Episiotomy: an incision through the area between the vagina and the anus to make the vaginal opening larger for childbirth. [7]Difficult delivery. [8]From arrival in hospital until delivery. [9]A sterile kit with instruments. [10]Chlorhexidine. [11]The Apgar score is determined by evaluating the newborn on five criteria (Appearance, Pulse, Grimace, Activity, Respiration) on a scale from zero to two. Afterwards, a summation of the five values was obtained. [12]Disinfectant against several micro-organisms. [13]WHO protocol was used to detect the source of infection. [14] CRP measured when the general state of the neonate deteriorated. [15]Blood samples were cultured in a BactAlert culture bottle. If bacterial growth was observed, a subculture was made on a blood agar plate. CRP: C-reactive protein. *E. cloacae*: *Enterobacter cloacae*. N: number of samples. OR: odds ratio. W: weeks.

Addendum 6: Univariate analysis of vaginal *Klebsiella pneumoniae* carriage

Addendum 6.1: Univariate analysis of vaginal *Klebsiella pneumoniae* carriage and risk factors

Addendum Table 6.1: Univariate logistic regressions showing the association between vaginal *Klebsiella pneumoniae* carriage and risk factors.

	n	*K. pneumoniae* + n (%)	Crude OR (95 % CI)	p-value
Sociodemographic factors				
Age of pregnant woman	**329**	**40 (12.2)**		
≤25 years	116	12 (10.4)	1.31 (0.64-2.69)	0.46
>25 years	213	18 (8.5)	Ref.	-
Tribe	**328**	**40 (12.2)**		
Shi	220	24 (11.0)	1.42 (0.72-2.80)	0.31
Non-Shi[1]	108	16 (14.9)	Ref.	-
Religion	**329**	**40 (12.2)**		
Catholic	203	28 (13.8)	1.52 (0.74-3.11)	0.25
Non-Catholic[2]	126	12 (9.6)	Ref.	-
Community	**323**	**39 (12.1)**		
Kadatu	112	8 (7.2)	Ref.	-
Ibanda	144	24 (16.7)	2.60 (1.12-6.04)	**0.03**
Bagira	67	7 (10.5)	1.52 (0.52-4.39)	0.44
Education[3]	**329**	**40 (12.2)**		
Yes	319	39 (12.3)	1.25 (0.16-10.17)	0.83
No	10	1 (10.0)	Ref.	-
Level of education	**319**	**39 (12.3)**		
Primary	33	3 (9.1)	Ref.	-
Secondary	177	20 (11.3)	1.27 (0.36-4.56)	0.71
Tertiary	109	16 (14.7)	1.72 (0.47-6.31)	0.41
State of marriage	**330**	**40 (12.2)**		
Married	313	40 (12.8)	-	-
Not married	17	0 (0.0)	Ref.	-
Age of marriage	**305**	**39 (12.8)**		
≤18 years	72	6 (8.4)	Ref.	-
>18 years	233	33 (14.2)	1.82 (0.73-4.52)	0.20
Duration of life with husband	**314**	**40 (12.8)**		
≤5 years	166	25 (15.1)	1.57 (0.79-3.11)	0.19
>5 years	148	15 (10.2)	Ref.	-
Living with husband or alone	**321**	**40 (12.5)**		
Living with husband	314	40 (12.8)	-	-
Not married or not living with husband	7	0 (0.0)	Ref.	-
Extramarital affairs[4]	**155**	**23 (14.9)**		
Yes	31	3 (9.7)	Ref.	-
No	124	20 (16.2)	1.80 (0.50-6.48)	0.37

Number of partners of husband	**46**	**4 (8.7)**		
1	42	4 (9.6)	-	_
>1	4	0 (0.0)	Ref.	_
Number of partners of pregnant woman during the last 6 months	**326**	**39 (12.0)**		
1	320	39 (12.2)	-	_
>1	6	0 (0.0)	Ref.	_
Number of partners of pregnant woman during life	**324**	**37 (11.5)**		
1	183	22 (12.1)	1.15 (0.57-2.30)	0.70
>1	141	15 (10.7)	Ref.	_
Source of income	**327**	**40 (12.3)**		
Non-employed	155	22 (14.2)	1.42 (0.73-2.75)	0.31
Employed	172	18 (10.5)	Ref.	_
Living circumstances				
Electricity and convenience	**330**	**40 (12.2)**		
Electricity	265	33 (12.5)	1.18 (0.50-2.80)	0.71
No electricity	65	7 (10.8)	Ref.	_
Water source	**329**	**40 (12.2)**		
Tap water	169	22 (13.1)	1.18 (0.61-2.29)	0.62
Other[5]	160	18 (11.3)	Ref.	_
Type of pavement	**329**	**40 (12.2)**		
Tiles	64	10 (15.7)	1.45 (0.67-3.15)	0.35
Others[6]	265	30 (11.4)	Ref.	_
Medical history				
BMI before conception[7]	**328**	**40 (12.2)**		
Underweight (BMI < 18.5)	3	1 (33.4)	2.09 (0.17-25.19)	0.56
Normal range (BMI: 18.5 - 25)	156	19 (12.2)	0.58 (0.26-1.31)	0.19
Overweight (BMI: 25 - 30)	112	9 (8.1)	0.37 (0.14-0.94)	**0.04**
Obese (BMI ≥ 30)	57	11 (19.3)	Ref.	_
Administration of current medication	**327**	**40 (12.3)**		
Yes	69	11 (16.0)	1.50 (0.71-3.18)	0.29
No	258	29 (11.3)	Ref.	_
Diabetic	**277**	**34 (12.3)**		
Yes	2	0 (0.0)	Ref.	_
No	275	34 (12.4)	-	_
Diabetic in the family	**317**	**40 (12.7)**		
Yes	72	10 (13.9)	1.16 (0.54-2.50)	0.71
No	245	30 (12.3)	Ref.	_
Chronic illness (e.g. cancer, gastritis, anemia, arterial hypertension…)[8]	**317**	**37 (11.7)**		
Yes	45	2 (4.5)	Ref.	_
No	272	35 (12.9)	3.18 (0.74-13.69)	0.12
Notion of constipation	**322**	**37 (11.5)**		
Yes	147	16 (10.9)	Ref.	_
No	175	21 (12.0)	1.12 (0.56-2.23)	0.76

Use of enema for constipation	**144**	**15 (10.5)**		
Yes	83	9 (10.9)	1.12 (0.38-3.32)	0.85
No	61	6 (9.9)	Ref.	-
Circumcised partner	**326**	**40 (12.3)**		
Yes	318	38 (12.0)	Ref.	-
No	8	2 (25.0)	2.46 (0.48-12.61)	0.28
Extension of the labia[9]	**318**	**38 (12.0)**		
Yes	40	7 (17.5)	1.69 (0.69-4.15)	0.25
No	278	31 (11.2)	Ref.	-
Known serological HIV state of pregnant woman	**315**	**35 (11.2)**		
Yes	212	17 (8.1)	Ref.	-
No	103	18 (17.5)	1.36 (0.63-2.92)	0.44
Period of last HIV test	**215**	**28 (13.1)**		
Less than 6 months ago	69	12 (17.4)	1.71 (0.76-3.85)	0.19
More than 6 months ago	146	16 (11.0)	Ref.	-
Knowledge of serological HIV state of husband	**240**	**37 (15.5)**		
Yes	100	27 (27.0)	1.39 (0.68-2.85)	0.37
No	140	10 (7.2)	Ref.	-
Realization of HIV test of couple (rapid test)	**321**	**40 (12.5)**		
Yes	96	14 (14.6)	1.31 (0.65-2.63)	0.45
No	225	26 (11.6)	Ref.	
Treatment for gonorrhea or syphilis[10]	**299**	**36 (12.1)**		
Yes	7	0 (0.0)	Ref.	-
No	292	36 (12.4)	-	-
Cold sore on vulva (herpes)	**308**	**38 (12.4)**		
Yes	54	7 (13.0)	1.07 (0.45-2.58)	0.88
No	254	31 (12.3)	Ref.	-
Antibiotic administration in the past 2 weeks	**328**	**39 (11.9)**		
Yes	46	6 (13.1)	1.13 (0.45-2.87)	0.79
No	282	33 (11.8)	Ref.	-
Usus				
Consumption of alcohol during this pregnancy	**318**	**36 (11.4)**		
Yes	112	16 (14.3)	1.55 (0.77-3.13)	0.22
No	206	20 (9.8)	Ref.	-
Type of alcohol	**108**	**16 (14.9)**		
Beer	100	16 (16.0)	-	-
Others[11]	8	0 (0.0)	Ref.	-
Last consumption of alcohol	**79**	**12 (15.2)**		
Less than 1 week	43	7 (16.3)	1.21 (0.35-4.18)	-
More than 1 week	36	5 (13.9)	Ref.	-
Amount of alcohol	**109**	**16 (14.7)**		
Less than 1 time a day	4	1 (25.0)	2.00 (0.20-20.52)	0.56
1 or more times a day	105	15 (14.3)	Ref.	-

Geophagia[12]	**309**	**40 (13.0)**		
Yes	85	10 (11.8)	Ref.	-
No	224	30 (13.4)	1.05 (0.49-2.25)	0.90
Consumption of coal[13]	**327**	**40 (12.3)**		
Yes	29	4 (13.8)	1.16 (0.38-3.54)	0.79
No	298	36 (12.1)	Ref.	-
Duration of consumption of geophagy and coal	**50**	**9 (18.0)**		
1 week	26	5 (19.3)	1.19 (0.28-5.08)	0.81
More than 1 week	24	4 (16.7)	Ref.	-
Consummation of tobacco	**328**	**40 (12.2)**		
Yes	2	0 (0.0)	Ref.	-
No	326	40 (12.3)	-	-
Use of natural excitants (mairungi chanvre) [14]	**329**	**40 (12.2)**		
Yes	1	0 (0.0)	Ref.	-
No	328	40 (12.2)	-	-
Reproductive health				
Gestational age at V1	**324**	**39 (12.1)**		
≤26 weeks	309	38 (12.3)	1.96 (0.25-15.36)	0.52
>26 weeks	15	1 (6.7)	Ref.	-
Number of previous deliveries on term	**330**	**40 (12.2)**		
0	79	13 (16.5)	1.29 (0.60-2.80)	0.52
1-2	115	6 (5.3)	0.56 (0.24-1.29)	0.17
>3	136	18 (13.3)	Ref.	-
Previous premature delivery	**330**	**40 (12.2)**		
Yes	20	4 (20.0)	1.90 (0.60-6.01)	0.27
No	310	36 (11.7)	Ref.	-
Total parity of the women	**330**	**40 (12.2)**		
0	76	12 (15.8)	1.19 (0.55-2.61)	0.66
1-2	114	9 (7.9)	0.55 (0.24-1.26)	0.16
>3	140	19 (13.6)	Ref.	-
Previous abortion[15]	**330**	**40 (12.2)**		
Yes	108	19 (17.6)	2.58 (1.32-5.04)	**0.01**
No	222	21 (9.5)	Ref.	-
Previous fetal death in utero[16]	**329**	**40 (12.2)**		
Yes	21	6 (28.6)	3.22 (1.17-8.87)	**0.02**
No	308	34 (11.1)	Ref.	-
Previous caesarean section [17]	**295**	**35 (11.9)**		
Yes	58	8 (13.8)	1.24 (0.53-2.90)	0.61
No	237	27 (11.4)	Ref.	-
Weight of biggest baby from previous pregnancy	**253**	**27 (10.7)**		
<2500 g	4	1 (25.0)	2.67 (0.23-30.80)	0.43
2500-4000 g	204	21 (10.3)	0.92 (0.33-2.58)	0.87
>4000 g	45	5 (11.2)	Ref.	-

Notion of infection of previously born baby in first week of life	**269**	**31 (11.6)**		
Yes	81	11 (13.6)	1.32 (0.60-2.90)	0.49
No	188	20 (10.7)	Ref.	-
Evolution of the previously born baby	**84**	**10 (12.0)**		
Good	65	8 (12.4)	1.19 (0.23-6.16)	0.83
Handicap or death	19	2 (10.6)	Ref.	-
Number of consultations during current pregnancy	**329**	**40 (12.2)**		
0	102	18 (17.7)	2.00 (1.02-3.91)	**0.04**
≥1	227	22 (9.7)	Ref.	-
Prevention in current pregnancy				
Administration of substances to diminish neonatal infections[18]	**271**	**32 (11.9)**		
Yes	58	6 (10.4)	Ref.	-
No	213	26 (12.3)	1.21 (0.47-3.08)	0.70
Administration of Fansidar® (prophylaxis against malaria)[19]	**318**	**318 (100.0)**		
Yes	63	7 (11.2)	Ref.	-
No	255	33 (13.0)	1.19 (0.50-2.83)	0.70
Administration of Vermox® (prophylaxis against intestinal worms)	**325**	**40 (12.4)**		
Yes	64	7 (11.0)	Ref.	-
No	261	33 (12.7)	1.18 (0.50-2.80)	0.71
Utilization of mosquito net during pregnancy	**324**	**40 (12.4)**		
Yes	287	35 (12.2)	Ref.	-
No	37	5 (13.6)	1.13 (0.41-3.08)	0.82
Sexual behaviour				
Age of first sexual contact	**268**	**30 (11.2)**		
≤18 years	126	14 (11.2)	Ref.	-
>18 years	142	16 (11.3)	1.02 (0.48-2.18)	0.97
Anal sexual intercourse[20]	**329**	**40 (12.2)**		
Yes	32	3 (9.4)	Ref.	-
No	297	37 (12.5)	1.38 (0.40-4.47)	0.61
Last sexual contact during current pregnancy	**291**	**33 (11.4)**		
≤7 days	224	28 (12.5)	1.77 (0.66-4.78)	0.26
>7days	67	5 (7.5)	Ref.	-
Toilet hygiene				
Type of toilet	**330**	**40 (12.2)**		
Toilet with bowl and flush	81	13 (16.1)	1.57 (0.77-3.21)	0.22
Other types[21]	249	27 (10.9)	Ref.	-
Use after toilet	**328**	**40 (12.2)**		
Water	227	27 (11.9)	Ref.	-
Tissue or other substances	101	13 (12.9)	1.09 (0.54-2.22)	0.80

Vaginal practices				
Normal vaginal toilet	**323**	**38 (11.8)**		
Only water	264	34 (12.9)	2.03 (0.69-5.97)	0.20
Other substances or none[22]	59	4 (6.8)	Ref.	-
Practices to dry vagina	**327**	**40 (12.3)**		
Yes	49	9 (18.4)	1.79 (0.79-4.05)	0.16
No	278	31 (11.2)	Ref.	-
Vaginal practices	**51**	**11 (21.6)**		
Toilet with cold water	4	1 (25.0)	1.23 (0.12-13.17)	0.86
Other practices[23]	47	10 (21.3)	Ref.	-
Number of vaginal toilets	**13**	**2 (15.4)**		
≤2 a day	12	1 (8.4)	Ref.	-
>2 a day	1	1 (100.0)	-	-
Vaginal toilet after each sexual contact	**328**	**40 (12.2)**		
Yes	281	38 (13.6)	3.52 (0.82-15.11)	0.09
No	47	2 (4.3)	Ref.	-
Type of intimate toilet after sexual contact	**270**	**40 (14.9)**		
Water	201	26 (13.0)	Ref.	-
Use of tissue or other	69	12 (17.4)	1.42 (0.67-2.99)	0.36

[1]Rega, Havu, Tumbo, Hunde, Nyganga, Hutu, Nande, Vira, Fuliru, Bembe. [2]Non-catholic: Protestantism, Angilicanism, Kimbanguism, Moslim, Animism. [3]From primary school. [4]Extramarital affairs of man known by the pregnant women. [5]Rain water, water well.[6]Concrete, carpet, no pavement. [7]Weight before the current pregnancy. [8]A diagnosed chronic illness. [9]A cultural tradition. [10]Diagnosed by acknowledge doctor or a clinical officer. [11]Wine, liqueur, local alcoholic drink (Sorgho). [12]Geophagia is the practice of eating earth or soil-like substrates such as clay or chalk to diminish nausea in pregnancy. [13]In case of Pica syndrome .[14]Khat, marijuana.[15]Natural, spontaneous abortion. [16]From 20 weeks of gestational age. [17]Planned and unplanned section.[18]Seeds, herbs,…[19]This prophylaxis is taken by all women at antenatal consultation during pregnancy at 24 WGA. [20]Information about timing and frequency is unknown. [21]Squat latrine, pit latrin. [22]Use of soap, perfume, powder, lemon juice, Dettol, virginity soap, tissue. [23]Use of soap, perfume, powder, lemon juice, antiseptic soap, Dettol, shaving. BMI: Body Mass Index. *K. pneumoniae*: *Klebsiella pneumoniae*. HIV: human immunodeficiency virus. N: number of samples. OR: odds ratio. V1: visit 1.

Addendum 6.2: Univariate analysis of vaginal *Klebsiella pneumoniae* carriage and symptoms

Addendum Table 6.2. Univariate logistic regressions showing the association between vaginal *Klebsiella pneumoniae* carriage and signs and symptoms

	n	*K. pneumoniae* + n (%)	Crude OR (95% CI)	p-value
General signs and symptoms at V1				
Fever	**324**	**38 (11.8)**		
Yes	37	6 (16.3)	1.54 (0.60-3.98)	0.37
No	287	32 (11.2)	Ref.	-
Headache	**326**	**39 (12.0)**		
Yes	159	17 (10.7)	Ref.	-
No	167	22 (13.2)	1.27 (0.65-2.49)	0.49
Cough	**327**	**39 (12.0)**		
Yes	72	6 (8.4)	Ref.	-
No	255	33 (13.0)	1.64 (0.66-4.07)	0.29
Uterine contractions	**291**	**34 (11.7)**		
Yes	40	4 (10.0)	Ref.	-
No	251	30 (12.0)	1.22 (0.41-3.67)	0.72
Lumbar pain	**326**	**40 (12.3)**		
Yes	165	21 (12.8)	1.09 (0.56-2.11)	0.80
No	161	19 (11.9)	Ref.	-
Difficulty to swallow	**326**	**39 (12.0)**		
Yes	32	4 (12.5)	1.06 (0.35-3.19)	0.92
No	294	35 (12.0)	Ref.	-
Vaginal signs and symptoms at V1				
Vaginal discharge	**326**	**40 (12.3)**		
Yes	159	21 (13.3)	1.19 (0.61-2.30)	0.62
No	167	19 (11.4)	Ref.	-
Previous treatment for vaginal discharge [1]	**159**	**20 (12.6)**		
Yes	86	12 (14.0)	1.32 (0.51-3.42)	0.57
No	73	8 (11.0)	Ref.	-
Type of previous treatment for vaginal discharge [2]	**326**	**40 (12.3)**		
Gyogynax	15	2 (13.4)	1.24 (0.27-5.78)	0.79
Anitbiotics	36	7 (19.5)	1.94 (0.76-4.85)	0.16
Antibiotics + other	19	4 (21.1)	2.14 (0.66-6.93)	0.20
Other	16	0 (0.0)	-	-
No treatment	240	27 (11.3)	Ref.	-
Vaginal itching	**328**	**39 (11.9)**		
Yes	136	15 (11.1)	Ref.	-
No	192	24 (12.5)	1.15 (0.58-2.29)	0.69
Previous treatment for vaginal itching	**137**	**16 (11.7)**		
Yes	61	6 (9.9)	Ref.	-
No	76	10 (13.2)	1.39 (0.48-4.06)	0.55

Type of previous treatment for vaginal itching	**328**	**40 (12.2)**		
Gyogynax	13	1 (7.7)	0.58 (0.07-4.57)	0.60
Anitbiotics	30	2 (6.7)	0.49 (0.11-2.17)	0.35
Antibiotics + other	14	3 (21.5)	1.89 (0.50-7.10)	0.35
Other[3]	4	0 (0.0)	-	-
No treatment	267	34 (12.8)	Ref.	-
Dysuria	**324**	**38 (11.8)**		
Yes	86	11 (12.8)	1.15 (0.54-2.24)	0.72
No	238	27 (11.4)	Ref.	-
Previous treatment for dysuria[1]	**89**	**12 (13.5)**		
Yes	34	6 (17.7)	1.75 (0.52-5.95)	0.37
No	55	6 (11.0)	Ref.	-
Type of previous treatment for dysuria	**324**	**40 (12.4)**		
Gyogynax	4	0 (0.0)	-	-
Anitbiotics	22	4 (18.2)	1.71 (0.55-5.34)	0.36
Antibiotics + other	7	2 (28.6)	3.07 (0.57-16.45)	0.19
Other[4]	2	0 (0.0)	-	-
No treatment	289	34 (11.8)	Ref.	-
Burning sensation after sexual contact[5]	**313**	**39 (12.5)**		
Yes	104	8 (7.7)	Ref.	-
No	209	31 (14.9)	2.09 (0.92-4.73)	0.08
Last episode of burning	**86**	**7 (8.2)**		
Less than 7 days	55	4 (7.3)	Ref.	-
More than 7 days	31	3 (9.7)	1.37 (0.29-6.54)	0.70
Previous treatment for burning[1]	**105**	**7 (6.7)**		
Yes	22	1 (4.6)	Ref.	-
No	83	6 (7.3)	1.64 (0.19-14.35)	0.66
Type of previous treatment for burning	**313**	**40 (12.8)**		
Gyogynax	4	0 (0.0)	-	-
Anitbiotics	9	0 (0.0)	-	-
Antibiotics + other	7	1 (14.3)	1.15 (0.14-9.81)	0.90
Other[4]	2	0 (0.0)	-	-
No treatment	291	39 (13.5)	Ref.	-
Sensation of vaginal smell	**297**	**40 (13.5)**		
Yes	77	6 (7.8)	Ref.	-
No	220	31 (14.1)	1.94 (0.78-4.85)	0.16
Last episode of vaginal smell	**48**	**5 (10.5)**		
≤2 days	30	4 (13.4)	2.62 (0.27-25.44)	0.41
>2 days	18	1 (5.6)	Ref.	-
Previous treatment for vaginal smell[1]	**75**	**6 (8.0)**		
Yes	10	1 (10.0)	1.33 (0.14-12.76)	0.80
No	65	5 (7.7)	Ref.	-

Type of previous treatment for vaginal itching	**297**	**40 (13.5)**		
Gyogynax	0	0 (0.0)	-	-
Anitbiotics	4	0 (0.0)	-	-
Antibiotics + other	4	1 (25)	2.40 (0.24-23.67)	0.45
Other[3]	2	0 (0.0)	-	-
No treatment	287	39 (13.6)	Ref.	-
General clinical examination at V1				
Weight evolution during pregnancy[7]	**330**	**40 (12.2)**		
Weight loss	87	13 (15.0)	2.20 (0.68-7.12)	0.19
Stable weight or ≤5 kg weight gain	189	23 (12.2)	1.75 (0.58-5.31)	0.32
> 5kg weight gain	54	4 (7.5)	Ref.	-
Arm circumference	**328**	**39 (11.9)**		
<22 cm	25	2 (8.0)	Ref.	-
22-27.5 cm	202	24 (11.9)	1.55 (0.34-6.99)	0.57
>27.5 cm	101	13 (12.9)	1.70 (0.36-8.07)	1.70
Diastolic blood pressure	**330**	**40 (12.2)**		
<90 mmHg	325	39 (12.0)	Ref.	-
≥90 mmHg	5	1 (20.0)	1.54 (0.19-12.24)	0.68
Systolic blood pressure	**330**	**40 (12.2)**		
<140 mmHg	324	40 (12.3)	-	-
≥140 mmHg	6	0 (0.0)	Ref.	-
Cardiac frequency	**329**	**40 (12.2)**		
<110 bpm	318	36 (11.4)	Ref.	-
≥110 bpm	11	4 (36.4)	4.48 (1.25-16.04)	**0.02**
Edema lower legs	**330**	**40 (12.2)**		
Yes	1	0 (0.0)	Ref.	-
No	329	40 (12.2)	-	-
General physical state	**330**	**40 (12.2)**		
Normal	329	40 (12.2)	-	-
Abnormal[8]	1	0 (0.0)	Ref.	-
Gynaecological examination at V1				
Vulvar state	**328**	**39 (11.9)**		
Normal	322	37 (11.5)	Ref.	-
Abnormal[9]	6	2 (33.4)	3.85 (0.68-21.76)	0.13
Speculum examination	**328**	**40 (12.2)**		
Normal	272	32 (11.8)	Ref.	-
Abnormal[10]	56	8 (14.3)	1.25 (0.54-2.88)	0.60
Vaginal pH	**324**	**40 (12.4)**		
4	5	1 (20.0)	2.65 (0.25-28.50)	0.42
5-6	261	34 (13.1)	1.59 (0.59-4.25)	0.36
>6	58	5 (8.7)	Ref.	-

White blood cells per field on wet mount	**330**	**40 (12.2)**		
0	0	0 (0.0)	-	-
1 -4	178	19 (10.7)	Ref.	-
5-30	132	17 (12.9)	1.24 (0.62-2.48)	0.55
30+	20	4 (20.0)	2.09 (0.63-6.91)	0.23
Clue cells[11] **on wet mount**	**330**	**40 (12.2)**		
Yes	37	5 (13.6)	1.15 (0.42-3.15)	0.78
No	293	35 (12.0)	Ref.	-
***Trichomonas* on wet mount**	**329**	**40 (12.2)**		
Yes	4	0 (0.0)	Ref.	-
No	325	40 (12.4)	-	-
***Candida* on wet mount**	**329**	**40 (12.2)**		
Yes	91	11 (12.1)	Ref.	-
No	238	29 (12.2)	1.01 (0.48-2.12)	0.98
Epithelial cells per field wet mount	**326**	**40 (12.3)**		
<5	19	2 (10.6)	1.18 (0.23-5.93)	0.84
5-30	208	29 (14.0)	1.62 (0.74-3.57)	0.23
30+	99	9 (9.1)	Ref.	-
Whiff test (KOH)[12]	**330**	**40 (12.2)**		
Positive	32	6 (18.8)	1.79 (0.69-4.67)	0.23
Negative	298	34 (11.5)	Ref.	-
State of vaginal secretions	**330**	**40 (12.2)**		
Normal: fine and homogeneous	297	33 (11.2)	Ref.	-
Abnormal: thick (+heterogeneous)	33	7 (21.3)	2.15 (0.87-5.35)	0.10
BV on gram stain[13]	**326**	**39 (12.0)**		
No BV	176	14 (8.0)	Ref.	-
Intermediate	59	13 (22.1)	3.27 (1.44-7.45)	**0.005**
BV	91	12 (13.2)	1.76 (0.78-3.98)	0.18
Biofilm	**326**	**39 (12.0)**		
Yes	73	7 (9.6)	Ref.	-
No	253	32 (12.7)	1.37 (0.58-3.24)	0.48
Gram + cocci on gram stain	**326**	**39 (12.0)**		
Yes	31	4 (13.0)	1.10 (0.36-3.33)	0.87
No	295	35 (11.9)	Ref.	-
Gram - cocci on gram stain	**326**	**39 (12.0)**		
Yes	6	1 (16.7)	1.48 (0.17-13.05)	0.72
No	320	38 (11.9)	Ref.	-
Yeast on gram stain	**326**	**39 (12.0)**		
Yes	88	11 (12.5)	1.00 (0.48-2.11)	0.99
No	238	28 (11.8)	Ref.	-
Hyphae on gram stain[14]	**330**	**40 (12.2)**		
Yes	48	6 (12.5)	1.04 (0.41-2.63)	0.93
No	282	34 (12.1)	Ref.	-

***Enterobacter cloacae* in CVL**	**330**	**40 (12.2)**		
Yes	126	19 (15.1)	1.55 (0.80-3.00)	0.2
No	204	21 (10.3)	Ref.	-
***Klebsiella pneumoniae* in CVL**	**330**	**40 (12.2)**		
Yes	140	22 (15.8)	1.78 (0.92-3.47)	0.09
No	190	18 (9.5)	Ref.	-
Clinical diagnosis at V1	**330**	**40 (12.2)**		
Normal	140	14 (10.0)	Ref.	-
Pathological	190	26 (13.7)	1.43 (0.72-2.85)	0.31
Symptomatic treatment for vaginitis (BV and *Candida*) at V1	**329**	**39 (11.9)**		
Femaclin®	109	13 (12.0)	1.74 (0.51-5.97)	0.38
Antibiotic	21	4 (19.1)	1.51 (0.60-3.80)	0.38
Femaclin® + Antibiotic	53	9 (17.0)	1.06 (0.12-9.28)	0.96
Other treatment[15]	8	1 (12.5)	0.70 (0.31-1.61)	0.41
No treatment	138	12 (8.7)	Ref.	-
Additional technical examination at V1				
Hemoglobin on Hemocue®	**328**	**40 (12.2)**		
Anemia (<11 Hb)	12	4 (33.4)	3.89 (1.12-13.57)	**0.03**
Normal (≥11 Hb)	316	36 (11.4)	Ref.	-
Rapid test malaria	**330**	**40 (12.2)**		
Positive	1	0 (0.0)	Ref.	-
Negative	329	40 (12.2)	-	-
Rapid test HIV	**330**	**40 (12.2)**		
Positive	1	0 (0.0)	Ref.	-
Negative	329	40 (12.2)	-	-
White blood cells on urine dipstick	**330**	**40 (12.2)**		
Positive	134	16 (12.0)	Ref.	-
Negative	196	24 (12.3)	1.03 (0.52-2.02)	0.93
Nitrite on urine dipstick	**330**	**40 (12.2)**		
Positive	12	1 (8.4)	Ref.	-
Negative	318	39 (12.3)	1.54 (0.19-12.24)	0.68
Glycated keratin	**320**	**39 (12.2)**		
<3.6	181	24 (13.3)	Ref.	-
3.6-10	120	12 (10.0)	0.73 (0.35-1.52)	0.40
>10	19	3 (15.8)	1.23 (0.33-4.53)	0.76
Ultrasound examination at V1				
Estimation of fetal weight centiles[16]	**315**	**37 (11.8)**		
<p10	44	6 (13.7)	1.78 (0.61-5.24)	0.29
p10 - p90	148	21 (14.2)	1.87 (0.84-4.14)	0.12
>p90	123	10 (8.2)	Ref.	-
Fetal sex[17]	**324**	**38 (11.8)**		
Female	162	23 (14.2)	1.62 (0.81-3.33)	0.17
Male	162	15 (9.3)	Ref.	-

Insertion placenta[17]	**326**	**39 (12.0)**		
Normal	310	37 (12.0)	Ref.	-
Low inserted	16	2 (12.5)	1.05 (0.23-4.82)	0.95
Amniotic fluid[17]	**326**	**40 (12.3)**		
Normal	326	40 (12.3)	-	-
Abnormal	0	0 (0.0)	Ref.	-
Length cervix[17]	**330**	**40 (12.2)**		
<25 cm	3	1 (33.4)	3.69 (0.33-41.78)	0.29
25-30 cm	25	3 (12.0)	1.01 (0.28-3.54)	0.99
>30 cm	302	36 (12.0)	Ref.	-
Funnel[17.18]	**329**	**40 (12.2)**		
Present	5	1 (20.0)	1.83 (0.20-16.77)	0.59
Absent	324	39 (12.1)	Ref.	-
Morphological abnormality visible[17]	**326**	**40 (12.3)**		
Yes	6	2 (33.4)	3.82 (0.68-21.61)	0.129
No	320	38 (11.9)	Ref.	-

[1]Treatment in pregnancy. [2]Femaclin®, Gyndodactarin, Nystatin, Tinidazole, Fluomizin. [3]Nystatin, Gynodactarin. [4]Not precised. [5]Burning sensation in the current pregnancy. [6]Femaclin®, Gynodactarin. [7]Weight before pregnancy compared with weight at V1. [8]Deviant compared with healthy pregnant women. [9]Genital wrat, herpetic lesions, chancre, erythema, pustule, abcess (Bartholin's gland), leucorrhoea. [10]Erythema, polyp, ectropion, bleeding, xanthoma, ulcers, leucorrhoea. [11]Clue cells are epithelial cells of the vagina that get their distinctive stippled appearance by being covered with bacteria. It is a typical sign of bacterial vaginosis. [12] A whiff test is performed by adding several drops of 10% potassium hydroxide to a sample of vaginal discharge. A strong fishy odor is indicative of a positive test result. Such a result may suggest either trichomoniasis or bacterial vaginosis. [13]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV). (59) [14]Long, tubular branching structures produced by *Candida*. [15]Tot'hema, gogynax, omnibionta. [16]Based on Percentile table Jeanty. [17]Based on ultrasound examination. [18]Protrusion of the amniotic membranes into the internal os of the cervix. This condition increased the risk on preterm birth. Bpm: beats per minute. CVL: cervicovaginal lavage. *K. pneumoniae*: *Klebsiella pneumoniae.* Hb: hemoglobin. HIV: human immunodeficiency virus. N: number of samples. OR: odds ratio. P: percentile. V1: visit 1.

Addendum 6.3: Univariate analysis of vaginal *Klebsiella pneumoniae* carriage and adverse pregnancy outcomes

Addendum Table 6.3. Univariate logistic regressions showing the association between vaginal *Klebsiella pneumoniae* carriage and adverse pregnancy outcomes.

	n	*K. pneumoniae* + n (%)	Crude OR (95% CI)	p-value
Delivery				
Gestational age at labor [1]	**202**	**28 (13.9)**		
28w-32w	2	1 (50.0)	6.48 (0.39-107.21)	0.19
32w-36w	28	4 (14.3)	1.08 (0.34-3.40)	0.9
≥37w	172	23 (13.4)	Ref.	-
Preterm birth	**202**	**28 (13.9)**		
Yes (<37w)	30	5 (16.7)	Ref.	-
No (≥37w)	172	23 (13.4)	1.30 (0.45-3.72)	0.63
Temperature of the mother at labor [2]	**200**	**27 (13.5)**		
<37.2°C	179	24 (13.5)	Ref.	-
≥ 37.2°C	21	3 (14.3)	1.08 (0.30-3.93)	0.91
Development of labor	**199**	**27 (13.6)**		
Spontaneous	182	25 (13.8)	1.19 (0.26-5.54)	0.82
Induced[3]	17	2 (11.8)	Ref.	-
Way of induction of labor	**17**	**2 (11.8)**		
Misoprostol (prostaglandin)	10	1 (10.0)	Ref.	-
Foley probe with misoprostol (prostaglandin)	7	1 (14.3)	1.50 (0.08-28.89)	0.79
Fetal presentation at labor [4]	**204**	**28 (13.8)**		
Cephalic (head)	196	26 (13.3)	Ref.	-
Bottom	5	1 (20.0)	1.64 (0.18-15.20)	0.67
Transversal	3	1 (33.4)	3.27 (0.29-37.35)	0.34
State of membranes at arrival in hospital (before delivery)	**204**	**28 (13.8)**		
Intact	158	22 (14.0)	1.08 (0.41-2.84)	0.88
Broken or cracked	46	6 (13.1)	Ref.	-
Duration of rupture of membranes	**199**	**28 (14.1)**		
≤6 hours	192	26 (13.6)	Ref.	-
>6 hours	7	2 (28.6)	2.56 (0.47-13.86)	0.28
Amniotic fluid type at delivery	**204**	**28 (13.8)**		
Clear	162	20 (12.4)	Ref.	-
Meconium[5] (fresh or old)	42	8 (19.1)	1.67 (0.68-4.11)	0.26
Number of vaginal touchers during labor	**204**	**28 (13.8)**		
≤5 times	40	10 (25.0)	2.70 (1.14-6.44)	**0.03**
>5 times	164	18 (11.0)	Ref.	-
Washing of hands before labor	**188**	**26 (13.9)**		
Yes	188	26 (13.9)	-	-
No	0	0 (0.0)	Ref.	-

Type of labor	**204**	**18 (8.9)**		
Eutocic (with episotomy)[6]	167	18 (10.8)	Ref.	-
Dystocic[7]	1	0 (0.0)	-	-
Caesarean section	36	10 (27.8)	3.18 (1.32-7.67)	**0.01**
Duration of labor[8]	**195**	**25 (12.8)**		
≤8 hours	139	21 (15.1)	2.31 (0.76-7.08)	0.14
>8 hours	56	4 (7.1)	Ref.	-
Utilization of labor kit[9]	**203**	**28 (13.8)**		
Yes	141	16 (11.4)	Ref.	-
No	62	12 (19.4)	1.88 (0.83-4.25)	0.13
Cord care	**9**	**1 (11.2)**		
No disinfectant	0	0 (0.0)	Ref.	-
Disinfectant[10]	9	1 (11.2)	-	-
APGAR[11] score 5 minutes	**203**	**28 (13.8)**		
<7	2	0 (0.0)	Ref.	-
≥7	201	28 (14.0)	-	-
Sex of the baby	**204**	**28 (13.8)**		
Female	103	14 (13.6)	Ref.	-
Male	101	14 (13.9)	1.02 (0.46-2.27)	0.96
Visible abnormality	**204**	**28 (13.8)**		
Present	4	0 (0.0)	Ref.	-
Absent	200	28 (14.0)	-	-
Disinfectant eye drops[12]	**203**	**28 (13.8)**		
Yes	172	21 (12.3)	Ref.	-
No	31	7 (22.6)	2.10 (0.81-5.57)	0.13
Evolution of neonate	**204**	**28 (13.8)**		
Close to mother	201	27 (13.5)	3.22 (0.28-36.76)	0.35
Neonatology	3	1 (33.4)	Ref.	-
Neonatal outcome				
Fever[2]	**203**	**28 (13.8)**		
Yes (>37.2 °C)	3	2 (66.7)	13.39 (1.17-152.88)	**0.04**
No	200	26 (13.0)	Ref.	-
Temperature neonate	**203**	**28 (13.8)**		
<36.6 °C	117	12 (10.3)	Ref.	-
36.6-37.2 °C	75	11 (14.7)	1.50 (0.63-3.61)	0.361
>37.2 °C	11	5 (45.5)	7.29 (1.93-27.53)	**0.003**
Hypothermia	**203**	**28 (13.8)**		
Yes (<35 °C)	1	0 (0.0)	Ref.	-
No	202	28 (13.9)	-	-
Lethargy	**203**	**28 (13.8)**		
Yes	3	1 (33.4)	3.20 (0.2-36.56)	0.35
No	200	27 (13.5)	Ref.	-

Jaundice	**202**	**28 (13.9)**		
Yes	0	0 (0.0)	Ref.	-
No	202	28 (13.9)	-	-
Convulsions	**203**	**28 (13.8)**		
Yes	0	0 (0.0)	Ref.	-
No	203	28 (13.8)	-	-
Apnea	**203**	**28 (13.8)**		
Yes	1	0 (0.0)	Ref.	-
No	202	28 (13.9)	-	-
Hypotonia	**203**	**28 (13.8)**		
Yes	3	1 (33.4)	3.20 (0.28-36.56)	0.35
No	200	27 (13.5)	Ref.	-
Hypertonia	**203**	**28 (13.8)**		
Yes	0	0 (0.0)	Ref.	-
No	203	28 (13.8)	-	-
Shock	**203**	**28 (13.8)**		
Yes	0	0 (0.0)	Ref.	-
No	203	28 (13.8)	-	-
Dirty umbilicus	**203**	**28 (13.8)**		
Yes	0	0 (0.0)	Ref.	-
No	203	28 (13.8)	-	-
Difficult to suckle	**203**	**28 (13.8)**		
Yes	3	1 (33.4)	3.20 (0.28-36.56)	0.35
No	200	27 (13.5)	Ref.	-
Alimentation	**202**	**28 (13.9)**		
Maternal milk	200	28 (14.0)	Ref.	-
Bottle milk or combination maternal and bottle milk	2	0 (0.0)	-	-
Length of baby	**203**	**28 (13.8)**		
Small: <46	4	2 (50.0)	6.66 (0.90-49.31)	0.06
Normal: 46-56	199	26 (13.1)	Ref.	-
Large: >56	0	(0.0)	-	-
Head circumference	**203**	**28 (13.8)**		
Microcephaly: <32	2	1 (50.0)	2.00 (0.05-78.25)	0.71
Normal: 32-37	198	26 (13.2)	0.30 (0.03-3.45)	0.33
Macrocephaly: >27	3	1 (33.4)	Ref.	
Weight at birth	**203**	**28 (13.8)**		
<2500 g	7	2 (28.6)	2.62 (0.48-14.19)	0.27
≥2500g	196	26 (13.3)	Ref.	-
General physical state	**203**	**28 (13.8)**		
Normal	196	25 (12.8)	Ref.	-
Abnormal	7	3 (42.9)	5.13 (1.08-24.28)	**0.04**

Commentary general state	**7**	**3 (42.9)**		
Fever	4	3 (75.0)	-	-
Prematurity	1	0 (0.0)	-	-
Death	2	0 (0)	Ref.	-
Skin	**203**	**28 (13.8)**		
Normal	198	26 (13.2)	Ref.	-
Abnormal: erythema	5	2 (40.0)	4.41 (0.70-27.66)	0.11
Mouth	**203**	**28 (13.8)**		
Normal	203	28 (13.8)	-	-
Abnormal	0	0 (0.0)	Ref.	-
ORL	**203**	**28 (13.8)**		
Normal	203	28 (13.8)	-	-
Abnormal	0	0 (0.0)	Ref.	-
Neck	**203**	**28 (13.8)**		
Normal	203	28 (13.8)	-	-
Abnormal	0	0 (0.0)	Ref.	-
Cardiovascular	**203**	**28 (13.8)**		
Normal	199	27 (13.6)	Ref.	-
Abnormal (see commentary cardiovascular)	4	1 (25.0)	2.12 (0.21-21.16)	0.52
Commentary cardiovascular	**4**	**1 (25.0)**		
Bradycardia	3	0 (0.0)	Ref.	-
Tachycardia	1	1 (100.0)	-	-
Lungs	**203**	**28 (13.8)**		
Normal	197	25 (12.7)	Ref.	-
Abnormal (see commentary lungs)	6	3 (50.0)	6.88 (1.32-35.98)	**0.02**
Commentary lungs	**7**	**3 (42.9)**		
Apnea	3	0 (0.0)	Ref.	-
Polypnea	4	3 (75.0)	-	-
Abdomen	**203**	**28 (13.8)**		
Normal	202	28 (13.9)	Ref.	-
Abnormal	1	0 (0.0)	-	-
Extremity	**203**	**28 (13.8)**		
Normal	196	26 (13.3)	Ref.	-
Abnormal (see commentary extremity)	7	2 (28.6)	2.62 (0.48-14.19)	0.27
Commentary extremity	**5**	**1 (20.0)**		
Cyanosis	3	0 (0.0)	Ref.	-
Polydactyly	2	1 (50.0)	-	-
Neurological	**203**	**28 (13.8)**		
Normal	198	27 (13.7)	Ref.	
Abnormal (see commentary neurological)	5	1 (20.0)	1.58 (0.17-14.70)	0.69
Commentary neurological	**5**	**1 (20.0)**		
Hypotonia	2	0 (0.0)	-	-
Lethargy	2	1 (50.0)	-	-
Hypotonia + lethargy	1	0 (0.0)	Ref.	-

Genito-urinal	**203**	**28 (13.8)**		
Normal	200	27 (13.5)	Ref.	-
Abnormal (see commentary genito-urinal)	3	1 (33.4)	3.20 (0.28-36.56)	0.35
Commentary genito-urinal	**2**	**1 (50.0)**		
Immaturity	2	1 (50.0)	-	-
Diagnosis in first week of neonatal life	**203**	**28 (13.8)**		
Normal	192	25 (13.1)	Ref.	-
Infection	11	3 (27.3)	2.51 (0.62-10.08)	0.2
Source of infection[13]	**8**	**3 (37.5)**		
Respiratory	1	1 (100.0)	-	-
Cutaneous	1	0 (0.0)	-	-
Generalized sepsis	6	2 (33.4)	Ref.	-
Evolution during first week of neonatal life	**203**	**28 (13.8)**		
Good or status quo	200	27 (13.5)	Ref.	-
Died	3	1 (33.4)	3.20 (0.28-36.56)	0.35
CRP value at moment of neonatal deterioration[14]	**6**	**4 (66.7)**		
≤5 mg/dL	0	0 (0.0)	Ref.	-
>5 mg/dL	6	4 (66.7)	-	-
Blood culture[15] **during first week of neonatal life**	**203**	**28 (13.8)**		
Done	6	4 (66.7)	14.42 (2.51-82.98)	**0.003**
Not done	197	24 (12.2)	Ref.	-

[1]Based on last menstruation or ultrasound (before 20 weeks of gestation) if the last menstruation was not known. [2]Measured with thermometer. [3]Induction for obstetrical reasons. [4]Based on physical examination and ultrasound if there was doubt. [5]A dark greenish mass that accumulates in the bowel during fetal life and is discharged shortly after birth. [6]Delivery without medical intervention. Episiotomy: an incision through the area between the vagina and the anus to make the vaginal opening larger for childbirth. [7]Difficult delivery. [8]From arrival in hospital until delivery. [9]A sterile kit with instruments. [10]Chlorhexidine. [11]The Apgar score is determined by evaluating the newborn on five criteria (Appearance, Pulse, Grimace, Activity, Respiration) on a scale from zero to two. Afterwards, a summation of the five values was obtained. [12]Disinfectant against several micro-organisms. [13]WHO protocol was used to detect the source of infection. [14] CRP measured when the general state of the neonate deteriorated. [15]Blood samples were cultured in a BactAlert culture bottle. If bacterial growth was observed, a subculture was made on a blood agar plate. CRP: C-reactive protein. *K. pneumoniae*: *Klebsiella pneumoniae*. N: number of samples. OR: odds ratio. W: weeks.

Addendum 7: Univariate analysis of vaginal *Candida* carriage, stratified for bacterial vaginosis

Addendum 7.1: Univariate analysis of vaginal *Candida* carriage, stratified for bacterial vaginosis, and risk factors

Addendum Table 7.1: Univariate logistic regressions showing the association between vaginal *Candida* carriage, stratified for bacterial vaginosis, and risk factors.

	No bacterial vaginosis				Bacterial vaginosis			
	n	*Candida* + n (%)	Crude OR (95% CI)	p-value	n	*Candida* + n (%)	Crude OR (95% CI)	p-value
Sociodemographic factors								
Age of pregnant woman	**234**	**85 (36.4)**			**90**	**40 (44.5)**		
≤25 years	78	32 (41.1)	1.35 (0.77-2.37)	0.291	37	19 (51.4)	1.61 (0.69-3.76)	0.27
>25 years	156	53 (34.0)	Ref.	-	53	21 (39.7)	Ref.	-
Tribe	**233**	**85 (36.5)**			**90**	**40 (44.5)**		
Shi	166	60 (36.2)	Ref.	-	52	23 (44.3)	Ref.	-
Non-Shi[1]	67	25 (37.4)	1.05 (0.58-1.89)	0.867	38	17 (44.8)	1.02 (0.44-2.37)	0.962
Religion	**234**	**85 (36.4)**			**90**	**40 (44.5)**		
Catholic	147	54 (36.8)	1.05 (0.60-1.82)	0.865	52	24 (46.2)	1.18 (0.51-2.74)	0.703
Non-Catholic[2]	87	31 (35.7)	Ref.	-	38	16 (42.2)	Ref.	-
Community	**230**	**85 (37.0)**			**88**	**40 (45.5)**		
Kadatu	84	34 (40.5)	Ref.	-	25	15 (60.0)	2.25 (0.73-6.98)	0.16
Ibanda	104	33 (31.8)	0.68 (0.38-1.25)	0.214	38	15 (39.5)	0.98 (0.35-2.74)	0.967
Bagira	42	18 (42.9)	1.10 (0.52-2.34)	0.798	25	10 (40.0)	Ref.	-
Education[3]	**233**	**85 (36.5)**			**91**	**40 (44.0)**		
Yes	224	81 (36.2)	Ref.	-	90	40 (44.5)	-	-
No	9	4 (44.5)	1.41 (0.37-5.41)	0.614	1	0 (0.0)	Ref.	-
Level of education	**224**	**81 (36.2)**			**90**	**40 (44.5)**		
Primary	23	10 (43.5)	1.30 (0.51-3.34)	0.586	10	5 (50.0)	2.22 (0.51-9.65)	0.286
Secondary	123	42 (34.2)	0.88 (0.49-1.58)	0.661	51	26 (51.0)	2.31 (0.89-6.03)	0.087
Tertiary	78	29 (37.2)	Ref.	-	29	9 (31.1)	Ref.	-
State of marriage	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Married	223	80 (35.9)	Ref.	-	85	37 (43.6)	Ref.	-
Not married	11	5 (45.5)	1.49 (0.44-5.04)	0.521	6	3 (50.0)	1.30 (0.25-6.80)	0.758
Age of marriage	**219**	**78 (35.7)**			**82**	**36 (44.0)**		
≤18 years	52	17 (32.7)	Ref.	-	19	10 (52.7)	1.58 (0.56-4.43)	0.384
>18 years	167	61 (36.6)	1.19 (0.61-2.29)	0.614	63	26 (41.3)	Ref.	-
Duration of life with husband	**223**	**81 (36.4)**			**85**	**37 (43.6)**		
≤5 years	120	52 (43.4)	1.98 (1.13-3.46)	**0.017**	45	21 (46.7)	1.31 (0.55-3.11)	0.536
>5 years	103	29 (28.2)	Ref.	-	40	16 (40.0)	Ref.	-
Living with husband or alone	**228**	**82 (36.0)**			**88**	**39 (44.4)**		
Living with husband	224	80 (35.8)	1.80 (0.25-13.02)	0.56	85	37 (43.6)	Ref.	-
Not married or not living with husband	4	2 (50.0)	Ref.	-	3	2 (66.7)	2.60 (0.23-29.72)	0.443

Extramarital affairs[4]	**114**	**43 (37.8)**			**39**	**17 (43.6)**		
Yes	18	8 (44.5)	3.63 (0.32-40.64)	0.296	12	3 (25.0)	Ref.	-
No	96	35 (36.5)	Ref.	-	27	14 (51.9)	3.23 (0.71-14.61)	0.13
Number of partners of husband	**26**	**10 (38.5)**			**19**	**5 (26.4)**		
1	25	10 (40.0)	-	-	16	4 (25.0)	Ref.	-
>1	1	0 (0.0)	Ref.	-	3	1 (33.4)	1.50 (0.11-21.31)	0.765
Number of partners of pregnant woman during the last 6 months	**231**	**83 (36.0)**			**90**	**39 (43.4)**		
1	228	81 (35.6)	Ref.	-	87	38 (43.7)	1.55 (0.14-17.75)	0.724
>1	3	2 (66.7)	3.63 (0.32-40.64)	0.296	3	1 (33.4)	Ref.	-
Number of partners of pregnant woman during life	**231**	**84 (36.4)**			**89**	**38 (42.7)**		
1	135	48 (35.6)	Ref.	-	46	22 (47.9)	1.55 (0.66-3.61)	0.313
>1	96	36 (37.5)	1.087 (0.63-1.87)	0.762	43	16 (37.3)	Ref.	-
Source of income	**232**	**85 (36.7)**			**90**	**40 (44.5)**		
Non-employed	100	45 (45.0)	1.88 (1.10-3.23)	**0.022**	54	22 (40.8)	Ref.	-
Employed	132	40 (30.4)	Ref.	-	36	18 (50.0)	1.46 (0.62-3.40)	0.387
Living circumstances								
Electricity and convenience	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Electricity	188	63 (33.6)	Ref.	-	72	33 (45.9)	1.67 (0.47-6.02)	0.43
No electricity	46	22 (47.9)	1.82 (0.95-3.49)	0.073	19	7 (36.9)	Ref.	-
Water source	**233**	**85 (36.5)**			**91**	**40 (44.0)**		
Tap water	124	42 (33.9)	Ref.	-	41	14 (34.2)	Ref.	-
Other[5]	109	43 (39.5)	1.27 (0.75-2.17)	0.38	50	26 (52.0)	2.09 (0.89-4.89)	0.09
Type of pavement	**234**	**85 (36.4)**			**90**	**39 (43.4)**		
Tiles	42	11 (26.2)	Ref.	-	20	7 (35.0)	Ref.	-
Others[6]	192	74 (38.6)	1.77 (0.84-3.73)	0.135	70	32 (45.8)	1.56 (0.56-4.39)	0.396
Medical history								
BMI before conception[7]	**233**	**84 (36.1)**			**91**	**40 (44.0)**		
Underweight (BMI < 18.5)	2	2 (100.0)	-	-	1	1 (100.0)	-	-
Normal range (BMI: 18.5 - 25)	106	49 (46.3)	1.56 (0.76-3.20)	0.227	48	26 (54.2)	2.48 (0.65-9.37)	0.182
Overweight (BMI: 25 - 30)	80	17 (21.3)	0.49 (0.22-1.10)	0.084	30	9 (30.0)	0.86 (0.21-3.59)	0.833
Obese (BMI ≥ 30)	45	16 (35.6)	Ref.	-	12	4 (33.4)	Ref.	-
Administration of current medication	**231**	**84 (36.4)**			**91**	**40 (44.0)**		
Yes	44	15 (34.1)	Ref.	-	23	9 (39.2)	Ref.	-
No	187	69 (36.9)	1.13 (0.57-2.26)	0.728	68	31 (45.6)	1.30 (0.50-3.42)	0.59
Diabetic	**1977**	**74 (3.8)**			**76**	**35 (46.1)**		
Yes	1	0 (0.0)	Ref.	-	1	0 (0.0)	Ref.	-
No	196	74 (37.8)	-	-	75	35 (46.7)	-	-
Diabetic in the family	**227**	**83 (36.6)**			**85**	**39 (45.9)**		
Yes	53	16 (30.2)	Ref.	-	17	8 (47.1)	1.06 (0.37-3.08)	0.913
No	174	67 (38.6)	1.45 (0.75-2.81)	0.272	68	31 (45.6)	Ref.	-

Chronic illness (e.g. cancer, gastritis, anemia, arterial hypertension…)[8]	**225**	**80 (35.6)**			**88**	**38 (43.2)**		
Yes	34	11 (32.4)	Ref.	-	11	5 (45.5)	1.11 (0.31-3.96)	0.871
No	191	69 (36.2)	1.18 (0.54-2.57)	0.672	77	33 (42.9)	Ref.	-
Notion of constipation	**230**	**83 (36.1)**			**88**	**39 (44.4)**		
Yes	104	38 (36.6)	1.04 (0.60-1.78)	0.897	41	18 (44.0)	Ref.	-
No	126	45 (35.8)	Ref.	-	47	21 (44.7)	1.03 (0.44-2.40)	0.942
Use of enema for constipation	**100**	**37 (37.0)**			**42**	**18 (42.9)**		
Yes	59	21 (35.6)	Ref.	-	22	8 (36.4)	Ref.	-
No	41	16 (39.1)	1.16 (0.51-2.64)	0.727	20	10 (50.0)	1.75 (0.51-6.01)	0.374
Circumcised partner	**232**	**85 (36.7)**			**89**	**39 (43.9)**		
Yes	228	84 (36.9)	1.75 (0.18-17.09)	0.63	85	37 (43.6)	Ref.	-
No	4	1 (25.0)	Ref.	-	4	2 (50.0)	1.30 (0.17-9.65)	0.8
Extension of the labia[9]	**227**	**82 (36.2)**			**86**	**38 (44.2)**		
Yes	26	10 (38.5)	1.12 (0.48-2.60)	0.792	14	6 (42.9)	Ref.	-
No	201	72 (35.9)	Ref.	-	72	32 (44.5)	1.07 (0.34-3.39)	0.913
Known serological HIV state of pregnant woman	**225**	**83 (36.9)**			**85**	**38 (44.8)**		
Yes	151	48 (31.8)	Ref.	-	56	23 (41.1)	Ref.	-
No	74	35 (47.3)	1.93 (1.09-3.41)	**0.024**	29	15 (51.8)	1.54 (0.62-3.79)	0.35
Period of last HIV test	**150**	**48 (32.0)**			**60**	**26 (43.4)**		
Less than 6 months ago	45	13 (28.9)	Ref.	-	22	9 (41.0)	Ref.	-
More than 6 months ago	105	35 (33.4)	1.23 (0.58-2.64)	0.593	38	17 (44.8)	1.17 (0.40-3.39)	0.77
Knowledge of serological HIV state of husband	**170**	**65 (38.3)**			**67**	**32 (47.8)**		
Yes	68	26 (38.3)	Ref.	-	31	16 (51.7)	1.33 (0.51-3.50)	0.558
No	102	39 (38.3)	1.00 (0.53-1.89)	1	36	16 (44.5)	Ref.	-
Realization of HIV test of couple (rapid test)	**229**	**82 (35.9)**			**87**	**38 (43.7)**		
Yes	65	22 (33.9)	Ref.	-	29	13 (44.9)	1.07 (0.44-2.63)	0.879
No	164	60 (36.6)	1.13 (0.62-2.06)	0.697	58	25 (43.2)	Ref.	-
Treatment for gonorrhea or syphilis[10]	**211**	**78 (37.0)**			**83**	**35 (42.2)**		
Yes	4	2 (50.0)	1.72 (0.24-12.49)	0.59	3	2 (66.7)	2.85 (0.29-32.73)	0.401
No	207	76 (36.8)	Ref.	-	80	33 (41.3)	Ref.	-
Cold sore on vulva (herpes)	**218**	**79 (36.3)**			**86**	**39 (45.4)**		
Yes	35	11 (31.5)	Ref.	-	19	10 (52.7)	1.46 (0.52-4.05)	0.471
No	183	68 (37.2)	1.29 (0.60-2.80)	0.519	67	29 (43.3)	Ref.	-
Antibiotic administration in the past 2 weeks	**233**	**84 (36.1)**			**90**	**39 (43.4)**		
Yes	34	12 (35.3)	Ref.	-	10	6 (60.0)	2.14 (0.56-8.17)	0.267
No	199	72 (36.2)	1.04 (0.49-2.22)	0.921	80	33 (41.3)	Ref.	-
Usus								
Consumption of alcohol during this pregnancy	**226**	**84 (37.2)**			**88**	**39 (44.4)**		
Yes	74	30 (40.6)	1.24 (0.70-2.19)	0.464	36	15 (41.7)	Ref.	-
No	152	54 (35.6)	Ref.	-	52	24 (46.2)	1.20 (0.51-2.83)	0.68

Type of alcohol	**73**	**30 (41.1)**			**33**	**14 (42.5)**		
Beer	70	28 (40.0)	Ref.	-	28	12 (42.9)	1.13 (0.16-7.82)	0.905
Others[11]	3	2 (66.7)	3.00 (0.26-34.68)	0.379	5	2 (40.0)	Ref.	-
Last consumption of alcohol	**54**	**24 (44.5)**			**24**	**8 (33.4)**		
Less than 1 week	30	14 (46.7)	1.23 (0.42-3.62)	0.713	12	3 (25.0)	Ref.	-
More than 1 week	24	10 (41.7)	Ref.	-	12	5 (41.7)	2.14 (0.376-12.20)	0.39
Amount of alcohol	**73**	**30 (41.1)**			**35**	**14 (40.0)**		
Less than 1 time a day	3	2 (66.7)	3.00 (0.26-34.68)	0.379	1	0 (0.0)	Ref.	-
1 or more times a day	70	28 (40.0)	Ref.	-	34	14 (41.2)	-	-
Geophagia[12]	**233**	**85 (36.5)**			**91**	**40 (44.0)**		
Yes	58	22 (38.0)	1.09 (0.59-2.01)	0.791	25	13 (52.0)	1.57 (0.62-3.95)	0.343
No	175	63 (36.0)	Ref.	-	66	27 (41.0)	Ref.	-
Consumption of coal[13]	**232**	**85 (36.7)**			**90**	**40 (44.5)**		
Yes	21	10 (47.7)	1.65 (0.67-4.06)	0.277	8	5 (62.5)	2.24 (0.50-10.00)	0.291
No	211	75 (35.6)	Ref.	-	82	35 (42.7)	Ref.	-
Duration of consumption of geophagy and coal	**32**	**12 (37.5)**			**16**	**8 (50.0)**		
1 week	17	8 (47.1)	2.00 (0.45-8.84)	0.361	7	4 (57.2)	1.67 (0.23-12.22)	0.615
More than 1 week	18	4 (22.3)	Ref.	-	9	4 (44.5)	Ref.	-
Consummation of tobacco	**233**	**85 (36.5)**			**90**	**39 (43.4)**		
Yes	0	0 (0.0)	Ref.	-	2	1 (50.0)	1.32 (0.08-21.72)	0.848
No	233	85 (36.5)	-	-	88	38 (43.2)	Ref.	-
Use of natural excitants (mairungi chanvre) [14]	**233**	**85 (36.5)**			**91**	**40 (44.0)**		
Yes	0	0 (0.0)	Ref.	-	1	0 (0.0)	Ref.	-
No	232	85 (36.7)	-	-	90	40 (44.5)	-	-
Reproductive health								
Gestational age at V1	**229**	**83 (36.3)**			**90**	**39 (43.4)**		
≤26 weeks	220	78 (35.5)	Ref.	-	84	36 (42.9)	Ref.	-
>26 weeks	9	5 (55.6)	2.28 (0.59-8.72)	0.23	6	3 (50.0)	1.33 (0.25-7.00)	0.734
Number of previous deliveries on term	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
0	55	18 (32.8)	1.10 (0.53-2.24)	0.805	24	11 (45.9)	1.08 (0.39-2.98)	0.88
1-2	88	39 (44.4)	1.79 (0.97-3.30)	0.062	26	11 (42.4)	0.94 (0.38-2.53)	0.898
>3	91	28 (30.8)	Ref.	-	41	18 (44.0)	Ref.	-
Previous premature delivery	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Yes	15	5 (33.4)	Ref.	-	3	1 (33.4)	Ref.	-
No	219	80 (36.6)	1.15 (0.38-3.49)	0.803	88	39 (44.4)	1.59 (0.14-18.21)	0.708
Total parity of the women	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
0	54	17 (31.5)	0.98 (0.48-2.01)	0.957	22	10 (45.5)	1.11 (0.39-3.14)	0.842
1-2	86	38 (44.2)	1.69 (0.92-3.10)	0.091	27	12 (44.5)	1.07 (0.40-2.83)	0.897
>3	94	30 (32.0)	Ref.	-	42	18 (42.9)	Ref.	
Previous abortion[15]	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Yes	73	28 (38.4)	1.14 (0.64-2.01)	0.66	30	13 (43.4)	Ref.	-
No	161	57 (35.5)	Ref.	-	61	27 (44.3)	1.04 (0.43-2.51)	-

Previous fetal death in utero[16]	**233**	**85 (36.5)**			**91**	**40 (44.0)**		
Yes	16	5 (31.3)	Ref.	-	5	3 (60.0)	1.99 (0.32-12.50)	0.465
No	217	80 (36.9)	1.29 (0.43-3.83)	0.653	86	37 (43.1)	Ref.	-
Previous caesarean section [17]	**209**	**75 (35.9)**			**81**	**35 (43.3)**		
Yes	40	15 (37.5)	1.09 (0.53-2.23)	0.813	14	4 (28.6)	Ref.	-
No	169	60 (35.6)	Ref.	-	67	31 (46.3)	2.15 (0.61-7.55)	0.231
Weight of biggest baby from previous pregnancy	**179**	**67 (37.5)**			**69**	**30 (43.5)**		
<2500 g	1	1 (100.0)	Ref.	-	3	1 (33.4)	Ref.	-
2500-4000 g	151	57 (37.8)	1.21 (0.51-2.88)	0.662	49	21 (42.9)	1.5 (0.13-17.67)	0.747
>4000 g	27	9 (33.4)	Ref.	-	17	8 (47.1)	1.78 (0.13-23.52)	0.662
Notion of infection of previously born baby in first week of life	**192**	**69 (36.0)**			**72**	**33 (45.9)**		
Yes	58	20 (34.5)	Ref.	-	22	12 (54.6)	1.66 (0.60-4.55)	0.327
No	134	49 (36.6)	1.10 (0.57-2.09)	0.782	50	21 (42.0)	Ref.	
Evolution of the previously born baby	**59**	**21 (35.6)**			**24**	**11 (45.9)**		
Good	47	18 (38.3)	1.86 (0.44-7.80)	0.395	17	8 (47.1)	Ref.	-
Handicap or death	12	3 (25.0)	Ref.	-	7	3 (42.9)	1.19 (0.20-6.99)	0.851
Number of consultations during current pregnancy	**234**	**85 (36.4)**			**90**	**39 (43.4)**		
0	75	33 (44.0)	1.62 (0.92-2.84)	0.095	26	12 (46.2)	1.18 (0.47-2.94)	0.731
≥1	159	52 (32.8)	Ref.		64	27 (42.2)	Ref.	-
Prevention in current pregnancy								
Administration of substances to diminish neonatal infections[18]	**198**	**74 (37.4)**			**68**	**30 (44.2)**		
Yes	45	17 (37.8)	1.02 (0.52-2.03)	0.949	12	4 (33.4)	Ref.	-
No	153	57 (37.3)	Ref.	-	56	26 (46.5)	1.73 (0.47-6.43)	0.411
Administration of Fansidar® (prophylaxis against malaria)[19]	**226**	**83 (36.8)**			**87**	**37 (42.6)**		
Yes	42	16 (38.1)	1.08 (0.54-2.15)	0.838	21	8 (38.1)	Ref.	-
No	184	67 (36.5)	Ref.	-	66	29 (44.0)	1.27 (0.47-3.48)	0.637
Administration of Vermox® (prophylaxis against intestinal worms)	**232**	**85 (36.7)**			**88**	**39 (44.4)**		
Yes	48	15 (31.3)	Ref.	-	16	6 (37.5)	Ref.	-
No	184	70 (38.1)	1.35 (0.69-2.66)	0.385	72	33 (45.9)	1.41 (0.46-4.29)	0.545
Utilization of mosquito net during pregnancy	**231**	**86 (37.3)**			**88**	**39 (44.4)**		
Yes	208	77 (37.1)	1.67 (0.63-4.40)	0.304	74	36 (48.7)	3.47 (0.90-13.47)	0.072
No	23	6 (26.1)	Ref.	-	14	3 (21.5)	Ref.	-
Sexual behaviour								
Age of first sexual contact	**191**	**70 (36.7)**			**74**	**28 (37.9)**		
≤18 years	88	33 (37.5)	1.07 (0.59-1.93)	0.822	37	15 (40.6)	1.26 (0.49-3.23)	0.632
>18 years	103	37 (36.0)	Ref.	-	37	13 (35.2)	Ref.	-
Anal sexual intercourse[20]	**233**	**85 (36.5)**			**91**	**40 (44.0)**		
Yes	23	9 (39.2)	1.13 (0.47-2.74)	0.781	7	5 (71.5)	3.50 (0.64-19.09)	0.148
No	210	76 (36.2)	Ref.	-	84	35 (41.7)	Ref.	-

Last sexual contact during current pregnancy	**204**	**73 (35.8)**			**84**	**39 (46.5)**		
≤7 days	152	50 (32.9)	Ref.	-	69	31 (45.0)	Ref.	-
>7days	52	23 (44.3)	1.62 (0.85-3.08)	0.143	15	8 (53.4)	1.40 (0.46-4.29)	0.555
Toilet hygiene								
Type of toilet	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Toilet with bowl and flush	55	14 (25.5)	Ref.	-	24	6 (25.0)	Ref.	-
Other types[21]	179	71 (39.7)	1.93 (0.98-3.79)	0.058	67	34 (50.8)	3.09 (1.09-8.75)	**0.034**
Use after toilet	**233**	**85 (36.5)**			**90**	**39 (43.4)**		
Water	160	65 (40.7)	1.81 (0.99-3.32)	0.053	63	27 (42.9)	Ref.	-
Tissue or other substances	73	20 (27.4)	Ref.	-	27	12 (44.5)	1.07 (0.43-2.65)	0.889
Vaginal practices								
Normal vaginal toilet	**228**	**83 (36.5)**			**90**	**40 (44.5)**		
Only water	190	71 (37.4)	1.93 (0.98-3.79)	0.058	69	29 (42.1)	Ref.	-
Other substances or none[22]	38	12 (31.6)	Ref.	-	21	11 (52.4)	1.52 (0.57-4.04)	0.405
Practices to dry vagina	**233**	**85 (36.5)**			**89**	**40 (45.0)**		
Yes	33	12 (36.4)	Ref.	-	16	8 (50.0)	1.28 (0.43-3.79)	0.654
No	200	73 (36.5)	1.01 (0.47-2.16)	0.988	73	32 (43.9)	Ref.	-
Vaginal practices	**34**	**13 (38.3)**			**17**	**9 (53.0)**		
Toilet with cold water	2	1 (50.0)	1.67 (0.10-29.18)	0.727	2	1 (50.0)	Ref.	-
Other practices[23]	32	12 (37.5)	Ref.	-	15	8 (53.4)	1.14 (0.06-21.87)	0.929
Number of vaginal toilets	**9**	**5 (55.6)**			**4**	**2 (50.0)**		
≤2 a day	8	4 (50.0)	Ref.	-	4	2 (50.0)	-	-
>2 a day	1	1 (100.0)	-	-	0	0 (0.0)	Ref.	-
Vaginal toilet after each sexual contact	**232**	**84 (36.3)**			**91**	**40 (44.0)**		
Yes	202	76 (37.7)	1.66 (0.70-3.91)	0.248	75	34 (45.4)	1.38 (0.46-4.19)	0.57
No	30	8 (26.7)	Ref.	-	16	6 (37.5)	Ref.	-
Type of intimate toilet after sexual contact	**197**	**72 (36.6)**			**69**	**32 (46.4)**		
Water	145	54 (37.3)	1.12 (0.58-2.18)	0.736	53	26 (49.1)	1.61 (0.51-5.05)	0.419
Use of tissue or other	52	18 (34.7)	Ref.	-	16	6 (37.5)	Ref.	-

[1]Rega, Havu, Tumbo, Hunde, Nyganga, Hutu, Nande, Vira, Fuliru, Bembe. [2]Non-catholic: Protestantism, Angilicanism, Kimbanguism, Moslim, Animism. [3]From primary school. [4]Extramarital affairs of man known by the pregnant women. [5]Rain water, water well.[6]Concrete, carpet, no pavement. [7]Weight before the current pregnancy. [8]A diagnosed chronic illness. [9]A cultural tradition. [10]Diagnosed by acknowledge doctor or a clinical officer. [11]Wine, liqueur, local alcoholic drink (Sorgho). [12]Geophagia is the practice of eating earth or soil-like substrates such as clay or chalk to diminish nausea in pregnancy. [13]In case of Pica syndrome .[14]Khat, marijuana.[15]Natural, spontaneous abortion. [16]From 20 weeks of gestational age. [17]Planned and unplanned section.[18]Seeds, herbs,…[19]This prophylaxis is taken by all women at antenatal consultation during pregnancy at 24 WGA. [20]Information about timing and frequency is unknown. [21]Squat latrine, pit latrin. [22]Use of soap, perfume, powder, lemon juice, Dettol, virginity soap, tissue. [23]Use of soap, perfume, powder, lemon juice, antiseptic soap, Dettol, shaving. BMI: Body Mass Index. HIV: human immunodeficiency virus. N: number of samples. OR: odds ratio. V1: visit 1.

Addendum 7.2: Univariate analysis of vaginal *Candida* carriage, stratified for bacterial vaginosis, and symptoms

Addendum Table 7.2. Univariate logistic regressions showing the association between vaginal *Candida* carriage, stratified for bacterial vaginosis and signs and symptoms

	No bacterial vaginosis				Bacterial vaginosis			
	n	*Candida* + n (%)	Crude OR (95% CI)	p-value	n	*Candida* + n (%)	Crude OR (95% CI)	p-value
General signs and symptoms at V1								
Fever	**230**	**83 (36.1)**			**89**	**40 (45.0)**		
Yes	25	11 (44.0)	1.45 (0.63-3.36)	0.385	12	2 (16.7)	Ref.	
No	205	72 (35.2)	Ref.	-	77	38 (49.4)	4.87 (1.01-23.71)	0.051
Headache	**230**	**84 (36.6)**			**91**	**40 (44.0)**		
Yes	116	40 (34.5)	Ref.	-	41	17 (41.5)	Ref.	-
No	114	44 (38.6)	1.19 (0.70-2.04)	0.517	50	23 (46.0)	1.20 (0.52-2.77)	0.665
Cough	**231**	**84 (36.4)**			**92**	**40 (43.5)**		
Yes	52	15 (28.9)	Ref.	-	19	5 (26.4)	Ref.	-
No	179	69 (38.6)	1.55 (0.79-3.03)	0.202	73	35 (48.0)	2.65 (0.86-8.12)	0.09
Uterine contractions	**207**	**78 (37.7)**			**79**	**34 (43.1)**		
Yes	33	14 (42.5)	1.27 (0.60-2.70)	0.54	6	2 (33.4)	Ref.	-
No	174	64 (36.8)	Ref.	-	73	32 (43.9)	1.56 (0.27-9.10)	0.62
Lumbar pain	**230**	**85 (37.0)**			**91**	**40 (44.0)**		
Yes	116	41 (35.4)	Ref.	-	46	19 (41.4)	Ref.	-
No	114	44 (38.6)	1.15 (0.67-1.97)	0.61	45	21 (46.7)	1.24 (0.54-2.85)	0.607
Difficulty to swallow	**232**	**85 (36.7)**			**89**	**40 (45.0)**		
Yes	21	7 (33.4)	Ref.	-	10	5 (50.0)	1.28 (0.34-4.69)	0.733
No	211	78 (37.0)	1.17 (0.45-3.03)	0.742	79	35 (44.4)	Ref.	-
Vaginal signs and symptoms at V1								
Vaginal discharge	**231**	**84 (36.4)**			**90**	**39 (43.4)**		
Yes	108	57 (52.8)	3.97 (2.25-7.03)	**<0.001**	49	25 (51.1)	2.01 (0.86-4.72)	0.11
No	123	27 (22.0)	Ref.		41	14 (34.2)	Ref.	-
Previous treatment for vaginal discharge [1]	**107**	**56 (52.4)**			**50**	**25 (50.0)**		
Yes	60	29 (48.4)	Ref.	-	24	14 (58.4)	1.91 (0.62-5.88)	0.26
No	47	27 (57.5)	1.44 (0.67-3.11)	0.35	26	11 (42.4)	Ref.	-
Type of previous treatment for vaginal discharge [2]	**107**	**56 (52.4)**			**50**	**25 (50.0)**		
Gyogynax	11	5 (45.5)	1.76 (0.51-6.00)	0.369	4	3 (75.0)	4.73 (0.47-47.94)	0.188
Anitbiotics	22	11 (50.0)	2.11 (0.86-5.15)	0.102	13	7 (53.9)	1.84 (0.56-6.08)	0.318
Antibiotics + other	15	8 (53.4)	2.41 (0.83-6.97)	0.105	4	3 (75.0)	4.73 (0.47-47.94)	0.188
Other	12	5 (41.7)	1.51 (0.46-4.95)	0.501	3	1 (33.4)	0.79 (0.07-9.14)	1
No treatment	47	27 (57.5)	Ref.	-	26	11 (42.4)	Ref.	-
Vaginal itching	**232**	**84 (36.3)**			**91**	**40 (44.0)**		
Yes	89	49 (55.1)	3.78 (2.15-6.65)	**<0.001**	46	29 (63.1)	5.27 (2.13-13.05)	**<0.001**
No	143	35 (24.5)	Ref.	-	45	11 (24.5)	Ref.	-

Previous treatment for vaginal itching	**89**	**50 (56.2)**			**47**	**29 (61.8)**		
Yes	40	22 (55.0)	Ref.	-	20	14 (70.0)	1.87 (0.55-6.33)	0.316
No	49	28 (57.2)			27	15 (55.6)	Ref.	-
Type of previous treatment for vaginal itching	**89**	**50 (56.2)**			**47**	**29 (61.8)**		
Gyogynax	8	4 (50.0)	2.00 (0.13-31.98)	0.975	5	4 (80.0)	6.92 (0.73-6.28)	0.091
Anitbiotics	17	9 (53.0)	2.25 (0.17-29.77)	0.624	12	9 (75.0)	5.19 (1.29-20.91)	**0.02**
Antibiotics + other	12	8 (66.7)	4.00 (0.27-58.56)	0.538	2	1 (50.0)	1.73 (0.10-28.85)	0.702
Other[3]	3	1 (33.4)	Ref.	-	1	0 (0.0)	-	-
No treatment	49	28 (57.2)	0.96 (0.09-10.81)	0.975	27	15 (55.6)	Ref.	-
Dysuria	**230**	**83 (36.1)**			**89**	**40 (45.0)**		
Yes	60	24 (40.0)	1.25 (0.69-2.30)	0.463	25	14 (56.0)	1.86 (0.73-4.73)	0.193
No	170	59 (34.8)	Ref.	-	64	26 (40.7)	Ref.	-
Previous treatment for dysuria[1]	**62**	**27 (43.6)**			**26**	**14 (53.9)**		
Yes	22	7 (31.9)	Ref.	-	11	8 (72.8)	4.00((0.74-21.50)	0.106
No	40	20 (50.0)	2.14 (0.72-6.38)	0.171	15	6 (40.0)	Ref.	-
Type of previous treatment for dysuria	**62**	**27 (43.6)**			**26**	**14 (53.9)**		
Gyogynax	4	1 (25.0)	0.57 (0.06-5.56)	0.627	0	0 (0.0)	-	-
Anitbiotics	13	3 (23.1)	0.51 (0.14-1.92)	0.32	8	5 (62.5)	2.50 (0.56-11.20)	0.231
Antibiotics + other	4	2 (50.0)	1.71 (0.24-12.35)	0.597	3	3 (100.0)	-	-
Other[4]	2	1 (50.0)	1.71 (0.11-27.65)	0.707	0	0 (0.0)	-	-
No treatment	40	20 (50.0)	Ref.	-	15	6 (40.0)	Ref.	-
Burning sensation after sexual contact[5]	**221**	**80 (36.2)**			**87**	**37 (42.6)**		
Yes	70	44 (62.9)	5.41(2.93-9.97)	**<0.001**	33	19 (57.6)	2.71 (1.11-6.63)	**0.028**
No	151	36 (23.9)	Ref.	0	54	18 (33.4)	Ref.	-
Last episode of burning	**58**	**35 (60.4)**			**27**	**16 (59.3)**		
Less than 7 days	33	21 (63.7)	1.38 (0.48-3.97)	0.557	21	14 (66.7)	4.00 (0.58-27.41)	0.158
More than 7 days	25	14 (56.0)	Ref.	-	6	2 (33.4)	Ref.	-
Previous treatment for burning[1]	**71**	**44 (62.0)**			**33**	**19 (57.6)**		
Yes	15	8 (53.4)	Ref.	-	7	5 (71.5)	2.14 (0.35-13.12)	0.41
No	56	36 (64.3)	1.58 (0.50-4.99)	0.44	26	14 (53.9)	Ref.	-
Type of previous treatment for burning	**70**	**44 (62.9)**			**33**	**19 (57.6)**		
Gyogynax	1	1 (100.0)	-	-	3	2 (66.7)	2.80 (0.24-32.10)	0.408
Anitbiotics	8	3 (37.5)	0.892 (1.11-0.26)	0.892	1	1 (100.0)	-	-
Antibiotics + other	5	3 (60.0)	2.77	0.271	2	2 (100.0)	-	-
Other[4]	1	1 (100.0)	-	-	1	0 (0.0)	-	-
No treatment	56	36 (64.3)	Ref.	-	26	14 (53.9)	Ref.	-
Sensation of vaginal smell	**208**	**81 (39.0)**			**84**	**35 (41.7)**		
Yes	45	21 (46.7)	1.50 (0.77-2.93)	0.232	29	12 (41.4)	Ref.	-
No	163	60 (36.9)	Ref.	-	55	23 (41.9)	1.02 (0.41-2.54)	0.97
Last episode of vaginal smell	**29**	**12 (41.4)**			**17**	**9 (53.0)**		
≤2 days	18	7 (38.9)	Ref.	-	10	5 (50.0)	Ref.	-
>2 days	11	5 (45.5)	1.31 (0.29-5.98)	0.728	7	4 (57.2)	1.33 (0.19-9.31)	0.772
Previous treatment for vaginal smell[1]	**43**	**21 (48.9)**			**29**	**12 (41.4)**		

Yes	5	1 (20.0)	Ref.	-	4	3 (75.0)	5.33 (0.48-59.14)	0.173
No	38	20 (52.7)	4.44 (0.45-43.54)	0.2	25	9 (36.0)	Ref.	-
Type of previous treatment for vaginal itching	**43**	**20 (46.6)**			**29**	**12 (41.4)**		
Gyogynax	0	0 (0.0)	-	-	0	0 (0.0)	-	-
Anitbiotics	2	0 (0.0)	-	-	2	1 (50.0)	1.73 (0.11-27.96)	0.701
Antibiotics + other	2	1 (50.0)	-	-	2	2 (100.0)	-	-
Other[3]	1	0 (0.0)	-	-	0	0 (0.0)	-	-
No treatment	38	20 (52.7)	Ref.	-	25	9 (36.0)	Ref.	-
General clinical examination at V1								
Weight evolution during pregnancy[7]	**233**	**84 (36.1)**			**90**	**40 (44.5)**		
Weight loss	64	28 (43.8)	3.22 (1.23-8.43)	**0.017**	22	10 (45.5)	1.39 (0.37-5.17)	0.624
Stable weight or ≤5 kg weight gain	133	49 (36.9)	2.42 (0.985-5.93)	0.054	52	24 (46.2)	1.43 (0.45-4.51)	0.543
> 5kg weight gain	36	7 (19.5)	Ref.	-	16	6 (37.5)	Ref.	-
Arm circumference	**232**	**83 (35.8)**			**91**	**40 (44.0)**		
<22 cm	18	6 (33.4)	1.20 (0.40-3.64)	0.748	7	3 (42.9)	2.46 (0.44-13.76)	0.304
22-27.5 cm	146	57 (39.1)	1.54 (0.83-2.85)	0.173	54	30 (55.6)	4.11 (1.51-11.19)	**0.006**
>27.5 cm	68	20 (29.5)	Ref.	-	30	7 (23.4)	Ref.	
Diastolic blood pressure	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
<90 mmHg	230	83 (36.1)	Ref.	-	90	40 (44.5)	-	-
≥90 mmHg	4	2 (50.0)	2.27 (0.59-8.68)	0.233	1	0 (0.0)	Ref.	-
Systolic blood pressure	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
<140 mmHg	228	85 (37.3)	-	-	91	40 (44.0)	-	-
≥140 mmHg	4	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Cardiac frequency	**233**	**84 (36.1)**			**92**	**40 (43.5)**		
<110 bpm	223	80 (35.9)	Ref.	-	91	40 (44.0)	-	-
≥110 bpm	10	4 (40.0)	1.19 (0.33-4.35)	-	1	0 (0.0)	Ref.	-
Edema lower legs	**234**	**85 (36.3)**			**91**	**40 (44.0)**		
Yes	1	1 (100.0)	Ref.	-	0	0 (0.0)	Ref.	-
No	233	84 (36.1)	-	-	91	40 (44.0)	-	-
General physical state	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Normal	234	85 (36.4)	Ref.	-	90	40 (44.5)	Ref.	-
Abnormal[8]	0	0 (0.0)	-	-	1	0 (0.0)	-	-
Gynaecological examination at V1								
Vulvar state	**232**	**85 (36.7)**			**91**	**40 (44.0)**		
Normal	228	82 (36.0)	Ref.	-	89	40 (45.0)	-	-
Abnormal[9]	4	3 (75.0)	5.34 (0.55-52.18)	0.15	2	0 (0.0)	Ref.	-
Speculum examination	**232**	**85 (36.7)**			**91**	**40 (44.0)**		
Normal	189	67 (35.5)	Ref.	-	80	36 (45.0)	1.43 (0.39-5.28)	0.59
Abnormal[10]	43	18 (41.9)	1.31 (0.67-2.58)	0.432	11	4 (36.4)	Ref.	-

Vaginal pH	**228**	**82 (36.0)**			**91**	**40 (44.0)**		
4	5	1 (20.0)	Ref.	-	0	0 (0.0)	-	
5-6	194	66 (34.1)	2.06 (0.23-18.83)	1	62	28 (45.2)	1.17 (0.48-2.85)	0.735
>6	29	15 (51.8)	4.29 (0.43-43.14)	0.217	29	12 (41.4)	Ref.	-
White blood cells per field on wet mount	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
0	0	0 (0.0)	-	-	0	0 (0.0)	-	-
1 -4	135	40 (29.7)	Ref.	-	41	11 (26.9)	Ref.	
5-30	86	37 (43.1)	1.79 (1.02-3.15)	**0.043**	43	25 (58.2)	3.79 (1.51-9.49)	**0.005**
30+	13	8 (61.6)	3.80 (1.71-12.33)	**0.026**	7	4 (57.2)	3.64 (0.70-18.91)	0.125
Clue cells[11] **on wet mount**	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Yes	30	9 (30.0)	Ref.	-	7	3 (42.9)	Ref.	-
No	204	76 (37.3)	1.39 (0.60-3.18)	0.442	84	37 (44.1)	1.05 (0.22-4.98)	0.951
***Trichomonas* on wet mount**	**234**	**85 (36.4)**			**90**	**40 (44.5)**		
Yes	2	1 (50.0)	1.76 (0.11-28.53)	0.69	2	0 (0.0)	Ref.	-
No	232	84 (36.3)	Ref.	-	88	40 (45.5)	-	-
***Candida* on wet mount**	**234**	**85 (36.4)**			**90**	**40 (44.5)**		
Yes	64	55 (86.0)	28.52 (12.72-63.95)	**<0.001**	26	24 (92.4)	36.00 (7.65-169.53)	**<0.001**
No	170	30 (17.7)	Ref.	-	64	16 (25.0)	Ref.	-
Epithelial cells per field wet mount	**231**	**85 (36.8)**			**90**	**40 (44.5)**		
<5	12	5 (41.7)	1.55 (0.45-5.42)	0.49	7	4 (57.2)	1.05 (0.19-5.69)	0.957
5-30	146	57 (39.1)	1.39 (0.77-2.53)	0.276	58	22 (38.0)	0.48 (0.19-1.24)	0.131
30+	73	23 (31.6)	Ref.	-	25	14 (56.0)	Ref.	-
Whiff test (KOH)[12]	**233**	**85 (36.5)**			**91**	**40 (44.0)**		
Positive	14	7 (50.0)	1.82 (0.62-5.38)	0.278	16	7 (43.8)	Ref.	-
Negative	219	78 (35.7)	Ref.	-	75	33 (44.0)	1.01 (0.34-3.00)	0.985
State of vaginal secretions	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Normal: fine and homogeneous	213	68 (32.0)	Ref.	-	80	31 (38.8)	Ref.	-
Abnormal: thick (+heterogeneous)	21	17 (81.0)	9.06 (2.94-27.96)	**<0.001**	11	9 (81.9)	7.11 (1.44-35.12)	**0.016**
BV on gram stain[13]	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
No BV	175	50 (28.6)	Ref.	-	0	0 (0.0)	Ref.	-
Intermediate	59	35 (59.4)	3.65 (1.97-6.74)	**<0.001**	0	0 (0.0)	-	-
BV	0	0 (0.0)	-	-	91	40 (44.0)	-	-
Biofilm	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Yes	16	8 (50.0)	1.83 (0.66-5.07)	0.24	57	26 (45.7)	1.20 (0.51-2.83)	0.68
No	218	77 (35.4)	Ref.	-	34	14 (41.2)	Ref.	-
Gram + cocci on gram stain	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Yes	13	5 (38.5)	1.10 (0.35-3.48)	0.869	18	12 (66.7)	3.21 (1.08-9.54)	**0.035**
No	221	80 (36.2)	Ref.	-	73	28 (38.4)	Ref.	-
Gram - cocci on gram stain	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Yes	4	3 (75.0)	5.42 (0.55-52.90)	0.146	2	2 (100.0)	-	-
No	230	82 (35.7)	Ref.	-	89	38 (42.7)	Ref.	-

Yeast on gram stain	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Yes	64	60 (93.8)	87.00 (29.03-260.71)	**<0.001**	28	26 (92.9)	45.50 (9.60-215.67)	**<0.001**
No	170	25 (14.8)	Ref.	-	63	14 (22.3)	Ref.	-
Hyphae on gram stain[14]	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Yes	34	34 (100.0)	-	-	14	14 (100.0)	-	-
No	200	51 (25.5)	Ref.	-	77	26 (33.8)	Ref.	-
***Enterobacter cloacae* in CVL**	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Yes	106	36 (34.0)	Ref.	-	33	24 (72.8)	1.33 (0.56-3.15)	0.512
No	128	49 (38.3)	1.21 (0.71-2.06)	0.494	58	33 (56.9)	Ref.	-
***Klebsiella pneumoniae* in CVL**	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Yes	26	14 (53.9)	2.25 (0.99-5.13)	0.053	12	5 (41.7)	Ref.	-
No	208	71 (34.2)	Ref.	-	79	35 (44.4)	1.11 (0.33-3.81)	0.864
Clinical diagnosis at V1	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Normal	107	13 (12.2)	Ref.	-	30	3 (10.0)	Ref.	-
Pathological	127	72 (56.7)	9.47 (4.81-18.65)	**<0.001**	61	37 (60.7)	13.88 (3.79-50.85)	**<0.001**
Symptomatic treatment for vaginitis (BV and *Candida*) at V1	**233**	**85 (36.5)**			**91**	**40 (44.0)**		
Femaclin®	79	46 (58.3)	10.08 (4.85-20.96)	**<0.001**	28	16 (57.2)	11.56 (2.82-47.34)	**0.001**
Antibiotic	13	4 (30.8)	3.21 (0.87-11.94)	0.081	8	3 (37.5)	5.20 (0.81-33.56)	0.083
Femaclin® + Antibiotic	31	21 (67.8)	15.19 (5.87-39.28)	**<0.001**	22	17 (77.3)	29.47 (6.21-139.73)	**0.001**
Other treatment[15]	4	1 (25.0)	2.41 (0.23-24.93)	0.461	4	1 (25.0)	2.89 (0.22-37.35)	0.417
No treatment	107	13 (12.2)	Ref.	-	29	3 (10.4)	Ref.	-
Additional technical examination at V1								
Hemoglobin on Hemocue®	**232**	**84 (36.3)**			**91**	**40 (44.0)**		
Anemia (<11 Hb)	3	2 (66.7)	3.59 (0.32-40.14)	0.3	8	6 (75.0)	4.32 (0.82-22.72)	0.084
Normal (≥11 Hb)	229	82 (35.9)	Ref.	-	83	34 (41.0)	Ref.	-
Rapid test malaria	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Positive	1	1 (100.0)	Ref.	-	0	0 (0.0)	Ref.	-
Negative	233	84 (36.1)	-	-	91	40 (44.0)	-	-
Rapid test HIV	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Positive	1	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Negative	233	85 (36.5)	-	-	91	40 (44.0)	-	-
White blood cells on urine dipstick	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Positive	87	44 (50.6)	2.65 (1.52-4.60)	**0.001**	46	25 (54.4)	2.38 (1.02-5.57)	**0.045**
Negative	147	41 (27.9)	Ref.	-	45	15 (33.4)	Ref.	-
Nitrite on urine dipstick	**234**	**85 (36.4)**			**91**	**40 (44.0)**		
Positive	7	3 (42.9)	1.33 (0.29-6.07)	0.716	5	2 (40.0)	Ref.	-
Negative	227	82 (36.2)	Ref.	-	86	38 (44.2)	1.19 (0.19-7.47)	0.86
Glycated keratin	**227**	**84 (37.1)**			**89**	**40 (45.0)**		
<3.6	137	51 (37.3)	Ref.	-	42	17 (40.5)	Ref.	-
3.6-10	77	26 (33.8)	0.86 (0.48-1.54)	0.613	41	19 (46.4)	1.22 (0.51-2.92)	0.66
>10	13	7 (53.9)	1.97 (0.63-6.18)	0.246	6	4 (66.7)	2.82 (0.46-17.21)	0.26

Ultrasound examination at V1								
Estimation of fetal weight centiles[16]	**224**	**81 (36.2)**			**87**	**38 (43.7)**		
<p10	32	11 (34.4)	Ref.	-	12	4 (33.4)	Ref.	-
p10 - p90	106	37 (35.0)	1.02 (0.45-2.35)	0.956	39	21 (53.9)	2.33 (0.60-9.05)	0.22
>p90	86	33 (38.4)	1.19 (0.51-2.78)	0.69	36	13 (36.2)	1.13 (0.29-1.13)	0.862
Fetal sex[17]	**228**	**82 (36.0)**			**91**	**40 (44.0)**		
Female	111	34 (30.7)	Ref.	-	42	18 (42.9)	Ref.	-
Male	117	48 (41.1)	1.58 (0.91-2.72)	0.103	49	22 (44.9)	1.09 (0.47-2.49)	0.845
Insertion placenta[17]	**230**	**84 (36.6)**			**91**	**40 (44.0)**		
Normal	216	77 (35.7)	Ref.	-	89	39 (43.9)	Ref.	-
Low inserted	14	7 (50.0)	1.81 (0.61-5.34)	0.286	2	1 (50.0)	1.28 (0.08-21.15)	0.862
Amniotic fluid[17]	**230**	**83 (36.1)**			**91**	**40 (44.0)**		
Normal	230	229 (99.6)	-	-	91	40 (44.0)	-	-
Abnormal	0	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Length cervix[17]	**234**	**85 (36.4)**			**91**	**39 (42.9)**		
<25 cm	2	0 (0.0)	-	-	1	1 (100.0)	-	-
25-30 cm	16	4 (25.0)	0.56 (0.17-1.78)	0.32	9	3 (33.4)	0.63 (0.15-2.67)	0.526
>30 cm	216	81 (37.5)	Ref.	-	81	36 (44.5)	Ref.	-
Funnel[17.18]	**233**	**84 (36.1)**			**91**	**40 (44.0)**		
Present	4	1 (25.0)	Ref.	-	1	1 (100.0)	-	-
Absent	229	83 (36.3)	1.71 (0.18-16.66)	0.646	90	39 (43.4)	Ref.	-
Morphological abnormality visible[17]	**230**	**84 (36.6)**			**91**	**40 (44.0)**		
Yes	5	3 (60.0)	2.67 (0.44-16.29)	0.288	1	1 (100.0)	-	-
No	225	81 (36.0)	Ref.	-	90	39 (43.4)	Ref.	-

[1]Treatment in pregnancy. [2]Femaclin®, Gyndodactarin, Nystatin, Tinidazole, Fluomizin. [3]Nystatin, Gynodactarin. [4]Not precised. [5]Burning sensation in the current pregnancy. [6]Femaclin®, Gynodactarin. [7]Weight before pregnancy compared with weight at V1. [8]Deviant compared with healthy pregnant women. [9]Genital wrat, herpetic lesions, chancre, erythema, pustule, abcess (Bartholin's gland), leucorrhoea. [10]Erythema, polyp, ectropion, bleeding, xanthoma, ulcers, leucorrhoea. [11]Clue cells are epithelial cells of the vagina that get their distinctive stippled appearance by being covered with bacteria. It is a typical sign of bacterial vaginosis. [12] A whiff test is performed by adding several drops of 10% potassium hydroxide to a sample of vaginal discharge. A strong fishy odor is indicative of a positive test result. Such a result may suggest either trichomoniasis or bacterial vaginosis. [13]Nugent score: 0-3 (no BV), 4-6 (intermediate for BV), 7-10 (BV). (59) [14]Long, tubular branching structures produced by *Candida*. [15]Tot'hema, gogynax, omnibionta. [16]Based on Percentile table Jeanty. [17]Based on ultrasound examination. [18]Protrusion of the amniotic membranes into the internal os of the cervix. This condition increased the risk on preterm birth. Bpm: beats per minute. CVL: cervicovaginal lavage. Hb: hemoglobin. HIV: human immunodeficiency virus. N: number of samples. OR: odds ratio. P: percentile. V1: visit 1.

Addendum 7.3: Univariate analysis of vaginal *Candida* carriage, stratified for bacterial vaginosis, and adverse pregnancy outcomes

Addendum Table 7.3. Univariate logistic regressions showing the association between vaginal *Candida* carriage, stratified for bacterial vaginosis and adverse pregnancy outcomes.

	No bacterial vaginosis				Bacterial vaginosis			
	n	*Candida* + n (%)	Crude OR (95% CI)	p-value	n	*Candida* + n (%)	Crude OR (95% CI)	p-value
Delivery								
Gestational age at labor [1]	**140**	**49 (35.0)**			**58**	**27 (46.6)**		
28w-32w	2	0 (0.0)	-	-	0	0 (0.0)	-	-
32w-36w	19	11 (57.9)	2.93 (1.09-7.88)	**0.033**	8	5 (62.5)	2.12 (0.46-9.86)	0.337
≥37w	119	38 (32.0)	Ref.	-	50	22 (44.0)	Ref.	-
Preterm birth	**140**	**49 (35.0)**			**58**	**27 (46.6)**		
Yes (<37w)	21	11 (52.4)	2.35 (0.92-6.00)	0.075	8	5 (62.5)	2.12 (0.46-9.86)	0.337
No (≥37w)	119	38 (32.0)	Ref.	-	50	22 (44.0)	Ref.	-
Temperature of the mother at labor [2]	**136**	**46 (33.9)**			**60**	**28 (46.7)**		
<37.2°C	120	37 (30.9)	Ref.	-	55	25 (45.5)	Ref.	-
≥ 37.2°C	16	9 (56.3)	2.88 (0.99-8.33)	0.051	5	3 (60.0)	1.80 (0.28-11.64)	0.537
Development of labor	**136**	**49 (36.1)**			**59**	**28 (47.5)**		
Spontaneous	127	46 (36.3)	1.14 (0.27-4.76)	0.862	51	24 (47.1)	Ref.	-
Induced[3]	9	3 (33.4)	Ref.	-	8	4 (50.0)	1.13 (0.25-5.00)	0.877
Way of induction of labor	**9**	**3 (33.4)**			**8**	**4 (50.0)**		
Misoprostol (prostaglandin)	7	3 (42.9)	-	-	3	2 (66.7)	3.00 (0.15-59.89)	0.472
Foley probe with misoprostol (prostaglandin)	2	0 (0.0)	Ref.	-	5	2 (40.0)	Ref.	-
Fetal presentation at labor [4]	**140**	**49 (35.0)**			**60**	**28 (46.7)**		
Cephalic (head)	133	45 (33.9)	Ref.	-	59	27 (45.8)	-	-
Bottom	4	1 (25.0)	0.65 (0.07-6.45)	0.714	1	1 (100.0)	-	-
Transversal	3	3 (100.0)	-	-	0	0 (0.0)	Ref.	-
State of membranes at arrival in hospital (before delivery)	**140**	**49 (35.0)**			**60**	**28 (46.7)**		
Intact	109	38 (34.9)	Ref.	-	46	21 (45.7)	Ref.	-
Broken or cracked	31	11 (35.5)	1.03 (0.45-.37)	0.949	14	7 (50.0)	1.19 (0.36-3.94)	0.775
Duration of rupture of membranes	**135**	**135 (100.0)**			**60**	**28 (46.7)**		
≤6 hours	129	44 (34.2)	Ref.	-	59	27 (45.8)	-	-
>6 hours	6	5 (83.4)	9.66 (1.09-85.25)	**0.041**	1	1 (100.0)	Ref.	-
Amniotic fluid type at delivery	**140**	**49 (35.0)**			**60**	**28 (46.7)**		
Clear	111	31 (28.0)	Ref.	-	49	23 (47.0)	1.06 (.29-3.95)	0.929
Meconium[5] (fresh or old)	29	18 (62.1)	4.22 (1.79-9.95)	**0.001**	11	5 (45.5)	Ref.	-
Number of vaginal touchers during labor	**140**	**49 (35.0)**			**60**	**28 (46.7)**		
≤5 times	26	8 (30.8)	Ref.	-	14	5 (35.8)	Ref.	-
>5 times	114	41 (36.0)	1.26 (0.51-3.16)	0.617	46	23 (50.0)	1.80 (0.52-6.20)	0.35

Washing of hands before labor	**126**	**43 (34.2)**			**58**	**28 (48.3)**		
Yes	126	43 (34.2)	-	-	58	28 (48.3)	-	-
No	0	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Type of labor	**140**	**49 (35.0)**			**60**	**28 (46.7)**		
Eutocic (with episotomy)[6]	114	40 (35.1)	Ref.	-	50	23 (46.0)	Ref.	-
Dystocic[7]	1	0 (0.0)	-	-	0	0 (0.0)	-	-
Caesarean section	25	9 (36.0)	1.04 (0.42-2.57)	0.931	10	5 (50.0)	1.17 (0.30-4.57)	0.817
Duration of labor[8]	**133**	**47 (35.4)**			**58**	**28 (48.3)**		
≤8 hours	93	36 (38.8)	1.67 (0.74-3.74)	0.217	42	22 (52.4)	1.83 (0.56-5.96)	0.314
>8 hours	40	11 (27.5)	Ref.	-	16	6 (37.5)	Ref.	-
Utilization of labor kit[9]	**139**	**49 (35.3)**			**60**	**28 (46.7)**		
Yes	95	31 (32.7)	Ref.	-	43	20 (46.6)	Ref.	-
No	44	18 (41.0)	1.43 (0.68-2.99)	0.343	17	8 (47.1)	1.022 (0.33-3.15)	0.969
Cord care	**8**	**5 (62.5)**			**0**	**0 (0.0)**		
No disinfectant	8	5 (62.5)	-	-	0	0 (0.0)	-	-
Disinfectant[10]	0	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
APGAR[11] score 5 minutes	**140**	**49 (35.0)**			**59**	**28 (47.5)**		
<7	2	0 (0.0)	Ref.	-	0	28 (0.0)	-	-
≥7	138	49 (35.6)	-	-	59	0 (0.0)	Ref.	-
Sex of the baby	**140**	**49 (35.0)**			**60**	**28 (46.7)**		
Female	71	30 (42.3)	1.93 (0.95-3.91)	0.07	28	13 (46.5)	Ref.	-
Male	69	19 (27.6)	Ref.	-	32	15 (46.9)	1.02 (0.37-2.81)	0.972
Visible abnormality	**140**	**49 (35.0)**			**60**	**28 (46.7)**		
Present	4	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Absent	136	49 (36.1)	-	-	60	28 (46.7)	-	-
Disinfectant eye drops[12]	**140**	**49 (35.0)**			**59**	**28 (47.5)**		
Yes	116	38 (32.8)	Ref.	-	53	26 (49.1)	1.93 (0.33-11.42)	0.471
No	24	11 (45.9)	1.74 (0.71-4.24)	0.225	6	2 (33.4)	Ref.	-
Evolution of neonate	**140**	**49 (35.0)**			**60**	**28 (46.7)**		
Close to mother	137	49 (35.8)	-	-	60	28 (46.7)	-	-
Neonatology	3	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Neonatal outcome								
Fever[2]	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Yes (>37.2 °C)	3	2 (66.7)	3.83 (0.34-43.34)	0.278	0	0 (0.0)	Ref.	-
No	137	47 (34.4)	Ref.	-	59	27 (45.8)	-	-
Temperature neonate	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
<36.6 °C	82	30 (36.6)	Ref.	-	32	14 (43.8)	Ref.	-
36.6-37.2 °C	48	14 (29.2)	0.71 (0.33-1.54)	0.389	26	12 (46.2)	1.10 (0.39-3.11)	0.855
>37.2 °C	10	5 (50.0)	1.73 (0.45-6.48)	0.414	1	1 (100.0)	-	-
Hypothermia	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Yes (<35 °C)	1	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
No	139	49 (35.3)	-	-	59	27 (45.8)	-	-

Lethargy	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Yes	2	0 (0.0)	Ref.	-	1	0 (0.0)	Ref.	-
No	138	49 (35.6)	-	-	58	27 (46.6)	-	-
Jaundice	**140**	**49 (35.0)**			**58**	**26 (44.9)**		
Yes	0	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
No	140	49 (35.0)	-	-	58	26 (44.9)	-	-
Convulsions	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Yes	0	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
No	140	49 (35.0)	-	-	59	27 (45.8)	-	-
Apnea	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Yes	1	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
No	139	49 (35.3)	-	-	59	27 (45.8)	-	-
Hypotonia	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Yes	2	0 (0.0)	-	-	1	0 (0.0)	Ref.	-
No	138	49 (35.6)	Ref.	-	58	27 (46.6)	-	-
Hypertonia	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Yes	0	0 (0.0)	-	-	0	0 (0.0)	Ref.	-
No	140	49 (35.0)	Ref.	-	59	27 (45.8)	-	-
Shock	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Yes	0	0 (0.0)	-	-	0	0 (0.0)	Ref.	-
No	140	49 (35.0)	Ref.	-	59	27 (45.8)	-	-
Dirty umbilicus	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Yes	0	0 (0.0)	-	-	0	0 (0.0)	Ref.	-
No	140	49 (35.0)	Ref.	-	59	27 (45.8)	-	-
Difficult to suckle	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Yes	2	0 (0.0)	-	-	1	0 (0.0)	Ref.	-
No	138	49 (35.6)	Ref.	-	58	27 (46.6)	-	-
Alimentation	**140**	**49 (35.0)**			**58**	**27 (46.6)**		
Maternal milk	138	48 (34.8)	Ref.	-	0	0 (0.0)	Ref.	-
Bottle milk or combination maternal and bottle milk	2	1 (50.0)	1.88 (0.12-30.65)	0.659	58	27 (46.6)	-	-
Length of baby	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Small: <46	3	1 (33.4)	Ref.	-	1	0 (0.0)	Ref.	-
Normal: 46-56	137	48 (35.1)	1.08 (0.10-12.20)	0.951	58	27 (46.6)	-	-
Large: >56	0	0 (0.0)	-		0	0 (0.0)	-	-
Head circumference	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Microcephaly: <32	2	0 (0.0)	-	-	0	0 (0.0)	-	-
Normal: 32-37	137	49 (35.8)	-	-	57	26 (45.7)	Ref.	-
Macrocephaly: >27	1	0 (0.0)	Ref.	-	2	1 (50.0)	1.19 (0.07-20.01)	0.903
Weight at birth	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
<2500 g	3	0 (0.0)	Ref.	-	4	1 (25.0)	Ref.	-
≥2500g	137	49 (35.8)	-	-	55	26 (47.3)	2.69 (0.26-27.49)	0.404
General physical state	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	135	47 (34.9)	Ref.	-	57	26 (45.6)	Ref.	-
Abnormal	5	2 (40.0)	1.25 (0.20-7.73)	0.812	2	1 (50.0)	1.19 (0.07-20.01)	0.903

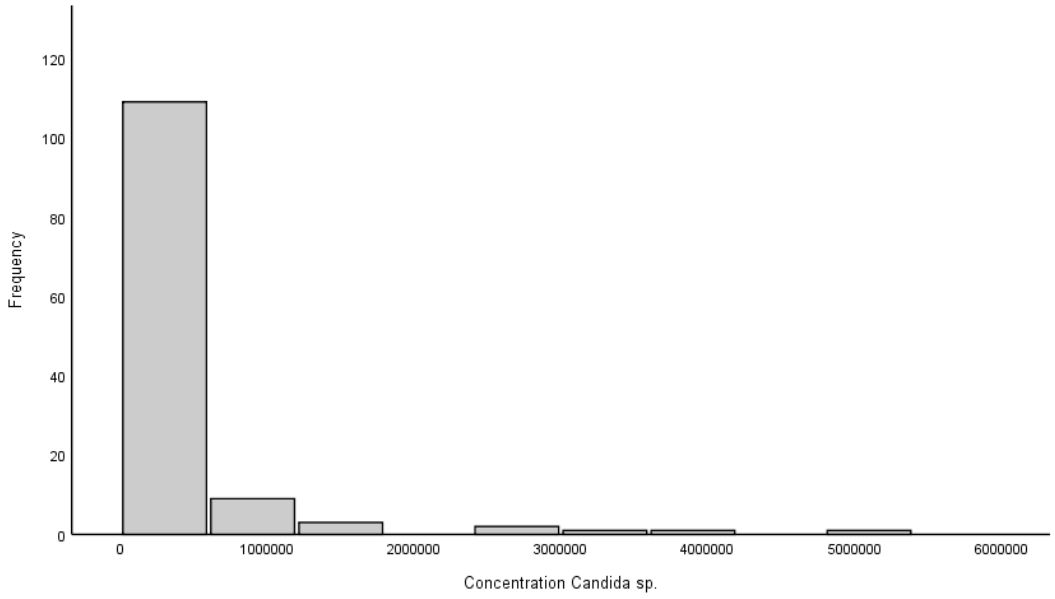
Commentary general state	**5**	**2 (40.0)**			**2**	**0 (0.0)**		
Fever	3	2 (66.7)	-	-	1	0 (0.0)	Ref.	-
Prematurity	1	0 (0.0)	-	-	0	0 (0.0)	-	-
Death	1	0 (0.0)	Ref.	-	1	0 (0.0)	-	-
Skin	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	137	49 (35.8)	-	-	57	25 (43.9)	Ref.	-
Abnormal: erythema	3	0 (0.0)	Ref.	-	2	2 (100.0)	-	-
Mouth	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	140	49 (35.0)	-	-	59	27 (45.8)	-	-
Abnormal	0	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
ORL	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	140	49 (35.0)	-	-	59	27 (45.8)	-	-
Abnormal	0	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Neck	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	140	49 (35.0)	-	-	59	27 (45.8)	-	-
Abnormal	0	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Cardiovascular	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	137	49 (35.8)	-	-	58	27 (46.6)	-	-
Abnormal (see commentary cardiovascular)	3	0 (0.0)	Ref.	-	1	0 (0.0)	Ref.	-
Commentary cardiovascular	**3**	**0 (0.0)**			**1**	**0 (0.0)**		
Bradycardia	2	0 (0.0)	-	-	1	0 (0.0)	-	-
Tachycardia	1	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Lungs	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	136	48 (35.3)	1.64 (0.17-16.16)	0.673	57	26 (45.7)	Ref.	-
Abnormal (see commentary lungs)	4	1 (25.0)	Ref.	-	2	1 (50.0)	1.19 (0.07-20.01)	0.903
Commentary lungs	**4**	**1 (25.0)**			**3**	**1 (33.4)**		
Apnea	2	0 (0.0)	Ref.	-	1	0 (0.0)	Ref.	-
Polypnea	2	1 (50.0)	-	-	2	1 (50.0)	-	-
Abdomen	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	139	49 (35.3)	-	-	59	27 (45.8)	-	-
Abnormal	1	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Extremity	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	134	49 (36.6)	-	-	58	26 (44.9)	Ref.	-
Abnormal (see commentary extremity)	6	0 (0.0)	Ref.	-	1	1 (100.0)	-	-
Commentary extremity	**5**	**5 (100)**			**0**	**0 (0.0)**		
Cyanosis	3	0 (0.0)	-	-	0	0 (0.0)	Ref.	-
Polydactyly	2	0 (0.0)	Ref.	-	0	0 (0.0)	-	-
Neurological	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	136	49 (36.1)	-	-	58	27 (46.6)	-	-
Abnormal (see commentary neurological)	4	0 (0.0)	Ref.	-	1	0 (0.0)	Ref.	-

Commentary neurological	**4**	**0 (0.0)**			**1**	**0 (0.0)**		
Hypotonia	2	0 (0.0)	-	-	0	0 (0.0)	Ref.	-
Lethargy	2	0 (0.0)	-	-	0	0 (0.0)	-	-
Hypotonia + lethargy	0	0 (0.0)	Ref.	-	1	0 (0.0)	-	-
Genito-urinal	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	137	49 (35.8)	-	-	59	27 (45.8)	-	-
Abnormal (see commentary genito-urinal)	3	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
Commentary genito-urinal	**2**	**0 (0.0)**			**0**	**0 (0.0)**		
Immaturity	2	0 (0.0)	-	-	0	0 (0.0)	-	-
Diagnosis in first week of neonatal life	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Normal	130	44 (33.9)	Ref.	-	58	27 (46.6)	-	-
Infection	10	5 (50.0)	1.96 (0.54-7.11)	0.309	1	0 (0.0)	Ref.	-
Source of infection[13]	**7**	**4 (57.2)**			**1**	**1 (100.0)**		
Respiratory	1	1 (100.0)	Ref.	-	0	0 (0.0)	Ref.	-
Cutaneous	1	0 (0.0)	-	-	0	0 (0.0)	-	-
Generalized sepsis	5	3 (60.0)	-	-	1	1 (100.0)	-	-
Evolution during first week of neonatal life	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Good or status quo	137	49 (35.8)	-	-	59	27 (45.8)	-	-
Died	3	0 (0.0)	Ref.	-	0	0 (0.0)	Ref.	-
CRP value at moment of neonatal deterioration[14]	**3**	**1 (33.4)**			**3**	**2 (66.7)**		
≤5 mg/dL	0	0 (0.0)	-	-	0	0 (0.0)	Ref.	-
>5 mg/dL	3	1 (33.4)	Ref.	-	3	2 (66.7)	-	-
Blood culture[15] **during first week of neonatal life**	**140**	**49 (35.0)**			**59**	**27 (45.8)**		
Done	4	2 (50.0)	1.89 (0.26-13.88)	0.53	2	1 (50.0)	1.19 (0.07-20.01)	0.903
Not done	136	47 (34.6)	Ref.	-	57	26 (45.7)	Ref.	-

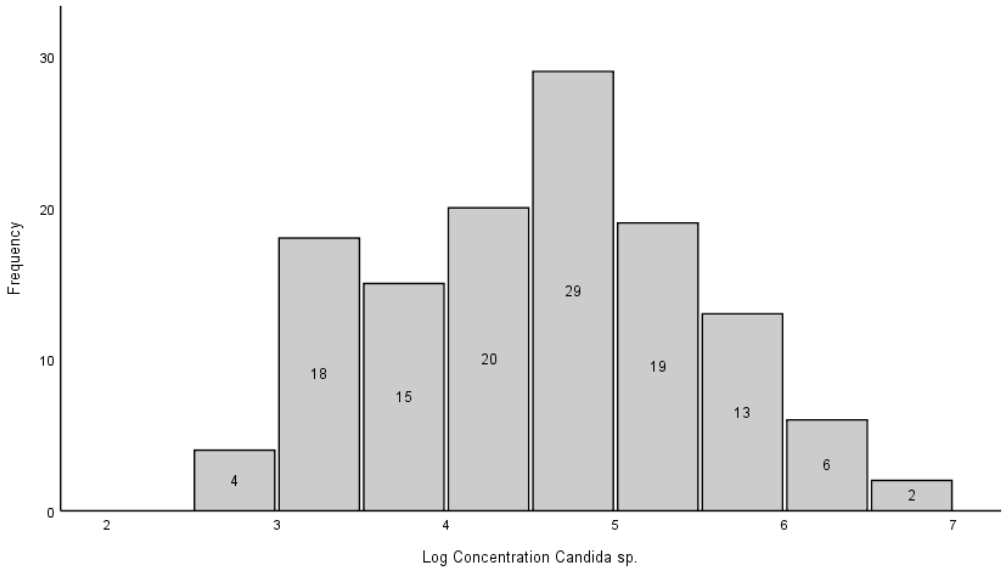
[1]Based on last menstruation or ultrasound (before 20 weeks of gestation) if the last menstruation was not known. [2]Measured with thermometer. [3]Induction for obstetrical reasons. [4]Based on physical examination and ultrasound if there was doubt. [5]A dark greenish mass that accumulates in the bowel during fetal life and is discharged shortly after birth. [6]Delivery without medical intervention. Episiotomy: an incision through the area between the vagina and the anus to make the vaginal opening larger for childbirth. [7]Difficult delivery. [8]From arrival in hospital until delivery. [9]A sterile kit with instruments. [10]Chlorhexidine. [11]The Apgar score is determined by evaluating the newborn on five criteria (Appearance, Pulse, Grimace, Activity, Respiration) on a scale from zero to two. Afterwards, a summation of the five values was obtained. [12]Disinfectant against several micro-organisms. [13]WHO protocol was used to detect the source of infection. [14] CRP measured when the general state of the neonate deteriorated. [15]Blood samples were cultured in a BactAlert culture bottle. If bacterial growth was observed, a subculture was made on a blood agar plate. CRP: C-reactive protein. N: number of samples. OR: odds ratio. W: weeks.

Addendum 8: Comparison between microscopy and *Candida* qPCR

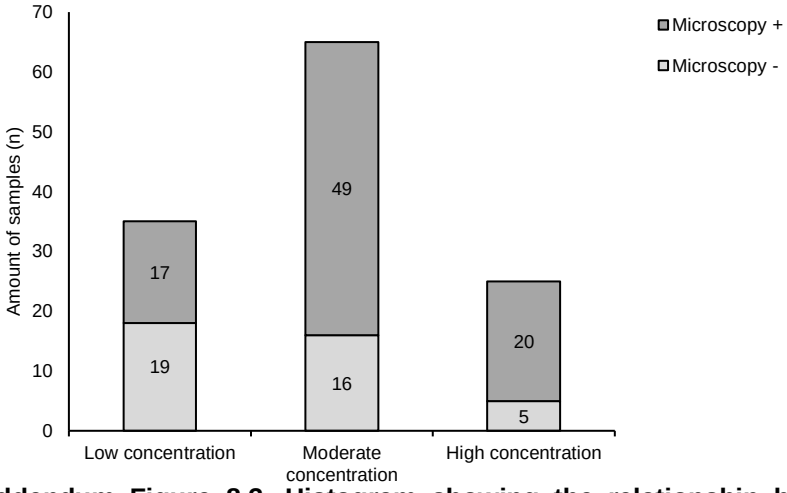
In this master thesis, the presence of *Candida* in the vaginal flora was determined with both microscopy and qPCR. A comparison between these two detection methods was executed. First, a histogram was plotted to determine the distribution of *Candida* concentration, established by qPCR. This histogram showed that the concentration of *Candida* was not normally divided, so a log transformation was carried out. A Shapiro-Wilk test was conducted and indicated that the log concentration of *Candida* was not normally divided (p=0.172). Nevertheless, this log distribution was used to categorize the concentration of *Candida* into three less or more equal groups: low (2.60-3.97 *Candida* cells/ml) - moderate (3.97-5.35 *Candida* cells/ml) - high concentration (5.35-6.72 *Candida* cells/ml). Finally, two histograms were plotted to determine the relationship between the concentration of *Candida*, established on qPCR, and the presence on gram staining/wet mount microscopy. On one side, 48.58 %, 75.39 % and 80.00 % of the samples in respectively the low, moderate and high concentration group, were seen on Gram staining microscopy. On the other hand, 42.11 %, 73.44 % and 66.67 % of the samples in respectively the low, moderate and high concentration group, were seen on wet mount microscopy.



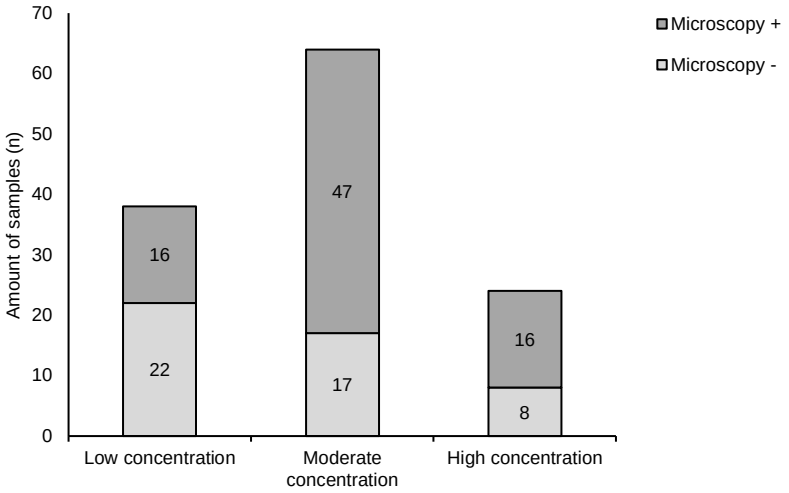
Addendum Figure 8.1. Histogram of the distribution of the *Candida* concentration. This histograms shows that the concentration of *Candida* is not normally divided with substantially more samples in the lower ranges. Concentration is expressed in *Candida* cells per ml.



Addendum Figure 8.2. Histogram of the log transformation of the *Candida* distribution. After log transformation, concentration of *Candida* was almost normally divided and could be used to categorize the concentration of *Candida* in three equal groups: low (2.60-3.97 *Candida* cells/ml) - moderate (3.97-5.35 *Candida* cells/ml) - high concentration (5.35-6.72 *Candida* cells/ml). Concentration is expressed in *Candida* cells per ml.



Addendum Figure 8.3. Histogram showing the relationship between concentration of *Candida* (established by qPCR) and the presence on Gram stain microscopy. The *Candida* positivity on Gram stain microscopy was found to be a function the *Candida* concentration: the higher the *Candida* concentration, the more *Candida* cells were detected on Gram stain microscopy. A total of 48.58 % (17/35), 75.39 % (49/65) and 80.00 % (20/25) of the vaginal smears were found to be positive on Gram stain microscopy in the low, moderate and high concentration group, respectively. Low concentration: 3,95log2 *Candida* cells/ml - 8.68log3 *Candida* cells/ml. Medium concentration: 9.48log3 *Candida* cells/ml – 1.85log5 *Candida* cells/ml. High concentration: 2.24log5 *Candida* cells/ml – 5.25log6 *Candida* cells/ml.



Addendum Figure 8.4. Histogram showing the relationship between concentration of *Candida* (assessed by means of qPCR) and the presence of *Candida* as assessed by wet mount microscopy. The *Candida* positivity on wet mount microscopy was found to be a function the *Candida* concentration: the higher the *Candida* concentration, the more *Candida* cells were detected on wet mount microscopy. A total of 42.11 % (16/38), 73.44 % (47/64) and 66.67 % (16/24) of the vaginal smears were found to be positive on wet mount microscopy in the low, moderate and high concentration group, respectively. Low concentration: 3,95log2 *Candida* cells/ml - 8.68log3 *Candida* cells/ml. Medium concentration: 9.48log3 *Candida* cells/ml – 1.85log5 *Candida* cells/ml. High concentration: 2.24log5 *Candida* cells/ml – 5.25log6 *Candida* cells/ml.