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A Multi-Country Cross-Sectional Study of Vaginal Carriage of Group B Streptococci (GBS) and *Escherichia coli* in Resource-Poor Settings: Prevalences and Risk Factors

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Abstract

Background

One million neonates die each year in low- and middle-income countries because of neonatal sepsis; group B *Streptococcus* (GBS) and *Escherichia coli* are the leading causes. In sub-Saharan Africa, epidemiological data on vaginal GBS and *E. coli* carriage, a prerequisite for GBS and *E. coli* neonatal sepsis, respectively, are scarce but necessary to design and implement prevention strategies. Therefore, we assessed vaginal GBS and *E. coli* carriage rates and risk factors and the GBS serotype distribution in three sub-Saharan countries.

Methods

A total of 430 women from Kenya, Rwanda and South Africa were studied cross-sectionally. Vaginal carriage of GBS and *E. coli*, and GBS serotype were assessed using molecular techniques. Risk factors for carriage were identified using multivariable logistic regression analysis.

Results

Vaginal carriage rates in reference groups from Kenya and South Africa were 20.2% (95% CI, 13.7–28.7%) and 23.1% (95% CI, 16.2–31.9%), respectively for GBS; and 25.0% (95% CI, 17.8–33.9%) and 27.1% (95% CI, 19.6–36.2%), respectively for *E. coli*. GBS serotypes Ia (36.8%), V (26.3%) and III (14.0%) were most prevalent. Factors independently

associated with GBS and *E. coli* carriage were *Candida albicans*, an intermediate vaginal microbiome, bacterial vaginosis, recent vaginal intercourse, vaginal washing, cervical ectopy and working as a sex worker. GBS and *E. coli* carriage were positively associated.

Conclusions

Reduced vaginal GBS carriage rates might be accomplished by advocating behavioral changes such as abstinence from sexual intercourse and by avoidance of vaginal washing during late pregnancy. It might be advisable to explore the inclusion of vaginal carriage of *C. albicans*, GBS, *E. coli* and of the presence of cervical ectopy in a risk- and/or screening-based administration of antibiotic prophylaxis. Current phase II GBS vaccines (a trivalent vaccine targeting serotypes Ia, Ib, and III, and a conjugate vaccine targeting serotype III) would not protect the majority of women against carriage in our study population.

Introduction

One million children die each year in low- and middle-income countries in the first 4 weeks of life because of neonatal sepsis [1]. Early-onset neonatal sepsis (EOS), occurring in the first week of life, accounts for approximately 80% of cases, and is caused by bacteria that are transmitted vertically from the genital tract of the mother to infant before or during delivery [2]. Late-onset neonatal sepsis (LOS) occurs between week 1 and month 2 to 3 of life and may be caused by bacteria acquired vertically or horizontally [3]. Because the transfer of a single species from the maternal genitourinary tract to the neonate before or during delivery is a prerequisite for EOS [4], there are unique opportunities for prevention of EOS.

At present, *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) and *Escherichia coli* are the leading causes of EOS worldwide [5]. Furthermore, GBS and *E. coli* are associated with preterm birth, very-low-birth-weight delivery and puerperal sepsis [6, 7], which cause substantial morbidity and mortality in sub-Saharan Africa (SSA) [2, 8, 9].

To prevent EOS, efforts have been focusing mainly on GBS and high-income countries, based on two strategies, namely the screening- or risk-based administration of intrapartum antibiotic prophylaxis (IAP) and the development of vaccines [10].

IAP has been shown to reduce the incidence of GBS EOS from 1.7/1000 to 0.6/1000 in the US [11], but is not effective against *E. coli* EOS, LOS, and adverse perinatal outcomes related to GBS [12, 13]. Furthermore, according to the current universal guidelines (Centers for Disease Control and Prevention, CDC), IAP should be administered to women found positive for GBS at 35–37 weeks of gestation [14]. However, these guidelines are not followed in most health-care facilities in low-income countries. The use of intravaginal washes with chlorhexidine (a wide-spectrum microbicide) during labour and neonatal wipes with chlorhexidine, has been explored in low- and middle-income countries, but is unlikely to prevent vertically acquired neonatal infections in any setting or population [4].

Most GBS vaccines under development aim at eliciting protective antibodies against capsular polysaccharides (CPS), the most important GBS virulence factor of which ten antigenically distinct CPS are known [10], and are attractive as some of the IAP-related problems may be circumvented [10]. However, these vaccines might not be effective in low-income countries because of different serotype distribution [15].

Although SSA has the highest rates of neonatal sepsis mortality worldwide, epidemiological data on vaginal GBS and *E. coli* carriage are very limited but necessary to develop and implement prevention strategies [16, 17]. Therefore, in this multi-country cross-sectional study, we assessed the

Table 1. Study population and vaginal GBS and *E. coli* carriage rates.

City, Country	Group	n	GBS prevalence % (95% CI)	*E. coli* prevalence % (95% CI)
Mombasa, Kenya	Reference group	110	20.2 (13.7–28.7)	25.0 (17.8–33.9)
Mombasa, Kenya	Pregnant women	30	14.3 (5.7–31.5)	14.3 (5.7–31.5)
Mombasa, Kenya	Adolescents	30	3.6 (0.6–17.7)	28.6 (15.3–47.1)
Kigali, Rwanda	FSW	30	20.0 (9.5–37.3)	70.0 (52.1–83.3)
Kigali, Rwanda	HIV+ women	30	0.0 (0.0–11.4)	20.0 (9.5–37.3)
Johannesburg, SA	Reference group	109	23.2 (16.2–31.9)	27.1 (19.6–36.2)
Johannesburg, SA	Pregnant women	30	10.0 (3.5–25.6)	33.3 (19.2–51.2)
Johannesburg, SA	Adolescents	30	0.0 (0.0–11.4)	13.3 (5.3–29.7)
Johannesburg, SA	Vaginal practices	31	25.8 (13.7–43.2)	30.0 (16.7–47.9)

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vaginal GBS and *E. coli* carriage prevalence, risk factors for GBS and *E. coli* carriage, and GBS sero-type distribution in populations from three countries: Kenya, Rwanda and South Africa.

Patients and Methods

Study design and population

In 2010–2011, we conducted a multi-country follow-up study entitled "Characterisation of novel microbicide safety biomarkers in East and South Africa". The main aim of that project was to characterise the vaginal microbiome and the cervicovaginal mucosal immune system in African women and to assess changes of these over time [18–21]. In that study, 430 women were recruited at three study sites, i.e. the International Centre for Reproductive Health Kenya (ICRHK) in Mombasa, Kenya (170 women); the non-governmental organisation Rinda Ubuzima (RU) in Kigali, Rwanda (60 women), and the Wits Reproductive Health and HIV Institute (Wits RHI) in Johannesburg, South Africa (SA) (200 women). The women were recruited into 6 predefined study groups: a reference group of 219 women (adult, non-pregnant, HIV-negative women at average risk of HIV), 60 pregnant women (up to 14 weeks of gestational age as determined by abdominal ultrasound at recruitment), 60 adolescent girls (16–17 years), 31 HIV-negative women engaging in vaginal practices (usage of cloth, lemon juice, or detergents to clean, dry or tighten the vagina on a regular basis), 30 self-acknowledged female sex workers (FSW), and 30 HIV-positive women (on antiretroviral treatment for at least 6 months, asymptomatic and with a CD4 count of more than 350 cells/μl) (Table 1). Participants were eligible for inclusion if they were in good physical and mental health, able and willing to participate in the study as required by the protocol, able and willing to give written informed consent (including written parental or guardian consent for adolescents). Women were excluded if they had never had penetrative vaginal intercourse, if they had a history of hysterectomy or other genital tract surgery in the three months prior to the screening visit, if external and/or internal genital warts were found, if they were enrolled in HIV prevention trials involving investigational products, if they were less than 6 months post-partum at the time of enrolment, if they were HIV-positive (unless for inclusion in the HIV-positive women group), or if they were pregnant (unless for inclusion in the pregnant women group). The study population, followed up for approximately eight months per person over 8 visits, is described in detail by Jespers and coworkers [19].

The current study presents one of the tertiary objectives of the above mentioned study, namely to document the vaginal carriage rates of the main pathogens associated with EOS (GBS and *E. coli*) and the risk factors for their carriage. These analyses are based on the screening visit and the first visit (scheduled soon after the last day (day 9 +/- 2 days) of the menstrual period) from the follow-up study.

Study procedures

At the screening visit, blood, vaginal, endocervical and urine samples were taken for diagnostic testing of HIV, HSV-2, syphilis, *Neisseria gonorrheae* (NG), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV), urinary tract infection (UTI), pregnancy, cervical dysplasia (by Pap smear), bacterial vaginosis (BV) (Amsel criteria), and vaginal candidiasis. Treatment was provided according to national guidelines, voluntary HIV counseling was offered, and condoms were provided free-of-charge.

At visit 1, two sterile Copan flocked® vaginal swabs (Copan Diagnostics, Inc., Murrieta, CA), to be used for the molecular detection of GBS and *E. coli*, were brought into the vaginal vault by the study clinician, rotated against the vaginal wall at the midportion of the vault, gently dipped in the posterior fornix and carefully removed to prevent contamination with the microbiome of the vulva and introitus. The swab heads were collected into two 1.5 ml cryovials, labelled and immediately frozen at -80°C until shipment to the central laboratory at the Institute of Tropical Medicine (ITM, Antwerp, Belgium) using temperature-monitored dry shippers filled with liquid nitrogen. One Amies swab (Copan Diagnostics, Inc.) for culturing was taken in a likewise manner, placed in the Amies tube, and transported at 4°C in a temperature-monitored cooler to the local laboratory, where it was processed immediately. At both visits, women were interviewed during face-to-face interviews about their general and sexual health, vaginal habits and sociodemographic characteristics. A physical examination including speculum and bimanual pelvic examination was carried out by a clinician. At each visit, participants were compensated for their time and transportation.

Diagnosis of genital infections

At the local laboratories, tests for HIV, HSV-2, syphilis, CT and NG were performed. For immediate detection of *Candida* cells and hyphae, TV, and clue cells, wet mount microscopy was used. For the purpose of this study, a commercially available TV InPouch™ system (BioMed Diagnostics, White City, Oregon) was used. For this, a vaginal swab was inoculated according to the manufacturer's instructions and InPouch cultures were monitored on a daily basis. InPouch bags with no growth at the end of five days were considered negative. BV diagnosed according to the Amsel criteria was used for immediate treatment. For research, vaginal smears were made and sent to ITM for Gram staining and Nugent scoring, a scoring system to diagnose BV. Briefly, smears from vaginal swabs were prepared by rolling the swab onto a glass slide. Slides were air-dried and fixed using 70% ethanol. For the Gram-staining at ITM, the fixed smear was covered with crystal violet for 1 minute, washed with water, flooded with Lugol's iodine for 1 minute, washed with water, and then decolorized with acetone-alcohol for 2–3 seconds. The smears were rinsed quickly under running water to stop the decolorisation and then counterstained with safranin for 1 minute. All reagents were from Becton Dickinson (BD). All smears were examined microscopically with the 40X objective to check the staining and the distribution of the material, and then assessed under oil immersion objective (1000x magnification) using the grading system described by Nugent and co-workers [22]. The Nugent score is calculated by assessing for the presence of *Lactobacillus* cell types, small Gram-variable coccobacilli, and curved Gram-variable rods. A score of 0–3 is considered as normal (BV-negative); a score of 4–6 as an intermediate vaginal microbiome; and a score of 7–10 as BV-positive.

DNA extraction

For the molecular detection of GBS and *E. coli*, DNA extraction from the two Copan swabs of each subject was carried out at ITM by thawing the swabs at room temperature for 30 minutes. After adding 1200 μL of diluted PBS, each swab was gently vortexed for 15 seconds, and 1 mL

of each swab suspension was pooled into a final volume of 2 mL. An aliquot of 250 µL was extracted using the Abbott m24sp automated extraction platform (Abbott, Maidenhead, UK), according to the manufacturer's instructions, and 200 µl of eluted DNA—to be used in the quantitative PCR (qPCR) assays—was stored at– 80°C.

For the construction of qPCR standard curves, DNA was extracted from overnight cultures of *S. agalactiae* LMG 14694[T] on TSA + 5% sheep blood, *E. coli* ATCC 25922 grown on TSA + 5% sheep blood, and *C. albicans* ATCC 90028 grown on Sabouraud agar (all BD). All growth was harvested from the plate and resuspended in 1 ml of saline. DNA of this suspension was extracted using the High Pure PCR Template Preparation Kit (Roche Applied Science, Basel, Switzerland) according to the manufacturer's instructions.

For capsular genotyping of GBS, 1 ml of inoculated Lim Broth medium (see Microbiological culturing) was used for DNA extraction using the High Pure PCR Template Preparation Kit (Roche), according to the the manufacturer's instructions.

Streptococcus agalactiae qPCR

To detect *S. agalactiae* in vaginal DNA extracts, a *S. agalactiae* specific qPCR was carried out, using primers previously described [23]. The qPCR reactions for *S. agalactiae* were performed in a final volume of 10 µl, containing 5 µl of LightCycler 480® SYBR Green I Master (Roche), 0.5 µM of both forward primer Sip1 (5'-ATCCTGAGACAACACTGACA-3') and reverse primer Sip2 (5'-TTGCTGGTGTTTCTATTTTCA-3'), 0.3 µM of probe (5'- 6-FAM–ATCAG AAGAGTCATACTGCCACTTC–TAMRA-3') (Eurogentec, Liège, Belgium) and 2 µl of DNA extract or 2 µl of HPLC water (as negative template control). Cycling conditions were as follows: 95°C for 5 min; 40 cycles of 95°C for 10 s, 58°C for 15 s and 72°C for 20 s. For the standard series, DNA concentration of the extract of *S. agalactiae* LMG 14694[T] was determined using the Qubit® Fluorometer (Invitrogen, Auckland, New Zealand) and the genomic concentration was calculated based on the GC% content and genome size of the type strain. A tenfold dilution standard series of *S. agalactiae* LMG 14694[T] DNA was prepared by dilution of the DNA stock in HPLC grade water. All standard tenfold dilution series and samples were run in duplicate. Amplification, detection and quantification were carried out using the LightCycler480® platform and the LightCycler® 480 Software Version 1.5 (Roche).

Escherichia coli qPCR

To detect *E. coli* in vaginal DNA extracts, an *E. coli* specific qPCR was carried out, using primers targeting the β-glucuronidase encoding gene uidA, previously described [24]. The qPCR reactions were performed in a final volume of 10 µl, containing 5 µl of LightCycler 480® SYBR Green I Master (Roche), 0.3 µM of both forward primer EcoliFW (5'-CAACGAACTGA ACTGGCAGA-3') and reverse primer EcoliRV (5'- CATTACGCTGCGATGGAT -3') (Eurogentec) and 2 µl of DNA extract or 2 µl of HPLC water (as negative template control). Cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min; 40 cycles of 95°C for 15 s and 60°C for 1 min. A standard series (using *E. coli* ATCC 25922 grown on TSA + 5% sheep blood (BD)), was constructed as described for *S. agalactiae*.

Candida albicans qPCR

To detect *C. albicans* in vaginal DNA extracts, a *C. albicans* specific qPCR was carried out, using primers targeting the ITS-1 gene (adapted from [25]). The qPCR reactions were performed in a final volume of 10 µl, containing 5 µl of LightCycler 480® SYBR Green I Master (Roche), 0.3 µM of both forward primer CA_FW (5'-CAACGAACTGAACTGGCAGA-3') and reverse primer CA_RV (5'- CATTACGCTGCGATGGAT -3') (Eurogentec) and 2 µl of

DNA extract or 2 μl of HPLC water (as negative template control). Cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min; 40 cycles of 95°C for 15 s and 60°C for 1 min. A standard series (using *C. albicans* ATCC 90028 grown on Sabouraud agar (BD)), was constructed as described for *S. agalactiae*.

Microbiological culturing. At the local laboratories, the Amies swab was inoculated on in-house TMB^plus plates (a medium supporting growth of anaerobes and allowing assessment of hydrogen peroxide production of strains) [26], after which the plates were incubated anaerobically as described previously [27]. After 48–72 h, depending on the growth, all biological material of the culture plate was harvested using sterile cotton swabs and stored in cryovials with 1 ml of tryptic soy broth + 5% glycerol at– 80°C until shipment. After shipment to the ITM, bacteria from the cryovial were inoculated in commercial Lim Broth medium (BD)–a selective enrichment medium for GBS–according to the manufacturer's instructions (5% CO_2 at 35°C for 24 hours). The latter procedure was performed only for women found to be positive for vaginal GBS carriage by means of qPCR. DNA extracts of inoculated Lim Broth medium was used for direct molecular capsular typing of GBS.

S. agalactiae molecular capsular typing

To determine the GBS serotype, we used a flowchart described by [28], based on the multiplex PCRs with primers as described by Poyart and co-workers and Imperi and co-workers [29, 30]. The multiplex PCRs were performed directly on DNA extracted from the inoculated Lim Broth medium. The reactions were performed in a final reaction mixture of 20 μl, containing 10 μl of FastStart PCR Master Mix (Roche), 0.2 μM of each primer, and 2 μl of DNA template. Using a Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA), the following PCR program was run: 94°C for 5 min, 3 cycles of 45 s at 94°C, 2 min at 50°C, 1 min at 72°C, and 30 cycles of 20 s at 94°C, 1 min at 50°C and 1 min at 72°C, with a final extension at 72°C for 7 min. PCR amplification products were visualised under UV light after electrophoresis on 1% agarose gels (30 minutes at 10 V/cm) and staining with ethidiumbromide. Twenty five control strains (covering all GBS serotypes and provided by the Belgian *Streptococcus agalactiae* reference center (Dr. Pierette Melin, University of Liège, Belgium)) were used as a positive control.

Physiological parameters

Vaginal pH was measured during the speculum examination by pressing commercial pH strips (pH Fix 3.6–6.1, Machery-Nagel) against the vaginal wall.

Detection of prostate-specific antigen (PSA), a marker for sexual intercourse within the past 24 hours [31] in vaginal swab fluid was performed using a chromatographic immune assay (the Seratec® PSA SemiQuant Cassette Test, Seratec, Gottingen, Germany) according to the manufacturer's instructions. Pregnancy was assessed by testing urine with a rapid hCG test (QuickVue One-Step hCG Test (Kigali, Johannesburg) or Unimed First Sign hCG test (Mombasa)). Leucocytes and erythrocytes in urine were detected using dipsticks according to the manufacturer's instructions (Siemens Multistix 10 sg in Kigali, Mission® urinalysis strips in Mombasa, and Neotest 4 Urine Dipstick in Johannesburg).

Statistical analysis

Data were analyzed with SPSS software version 22 (SPSS Inc.). Prevalences were reported with their 95% confidence interval. Outcomes for this analysis were vaginal GBS carriage and vaginal *E. coli* carriage, as determined by a positive qPCR.

Independent variables considered were study site, sociodemographic characteristics, reproductive health characteristics, sexual behavioural factors, vaginal practices characteristics,

cervicovaginal signs and symptoms and microbiological characteristics. Variables were analyzed using logistic regression in univariable and multivariable ways, with p-values < 0.05 indicating significance. In order not to overfit our multivariable models, variables were restricted in proportion to the number of cases positive for GBS and *E. coli*, i.e. maximum one degree of freedom per 10 cases [32]. Variables included in the models were selected as follows [33]: firstly, only variables found to be significantly associated with GBS or *E. coli* carriage in univariable analysis were considered for inclusion the multivariate GBS or *E. coli* model, respectively. Subsequently, of correlated variables (e.g. 'having had recent vaginal intercourse' and a positive PSA test), only one was kept for further consideration to avoid collinearity. The final selection of variables was based on literature and clinical expertise/relevance. The multivariable models were controlled for possible confounding variables and were validated with bootstrap analysis.

Ethics statement

Written information and consent forms in the local language were provided to the women or to the Legally Authorized Representatives for their review. After the interview, the participants were asked to express their willingness to participate in the study by signing (or thumb-printing in case they were illiterate) the consent form. In case they were of minor age (age below 18 in Kenya and SA, and below 21 in Rwanda), also the parents or guardians were asked to give consent. The study was approved by the Kenyatta National Hospital Ethical Review Committee, Kenya; the Human Research Ethics Committee (Medical), University of the Witwatersrand, SA; the Rwanda National Ethics Committee, Rwanda; the Institutional Review Boards of the Institute of Tropical Medicine in Antwerp, of Ghent University, and of the University Teaching Hospital in Antwerp, Belgium. In addition, the study was approved by the National Council on Science and Technology in Kenya, and the National AIDS Control Commission in Rwanda. The study is registered at the Trial Registration at the National Health Research Ethics Council South Africa (DOH2709103223) [19].

Results

Vaginal GBS and *E. coli* carriage and GBS serotype distribution

Of the 430 women enrolled in the study, 424 and 421 vaginal swab DNA extracts were analysed for the presence of GBS and *E. coli*, respectively. The vaginal GBS and *E. coli* carriage rates in the different study groups are presented in Table 1.

The GBS serotype distribution is presented in Table 2 and Fig 1. For 12 GBS carriers, the serotype could not be determined because samples were no longer available. Serotype distribution was largely comparable between sites. The most prevalent serotypes were Ia (27.3%), V (27.3%), and III (22.7%) in Kenya; Ia (34.5%), V (31.0%), and IV (13.8%) in SA; and Ia (83.3%) and II (16.7%) in Rwanda.

Univariable and multivariable analyses

Tables 3 and 4 present the univariable associations of the sociodemographics, sexual behavior, vaginal practices, cervicovaginal signs and symptoms, and microbiological characteristics with vaginal GBS and *E. coli* carriage, respectively. Because of the low prevalence, CT, NG, TV, and syphilis were not considered for further analysis.

In our final multivariable GBS model (Table 5), BV by Nugent score remained significantly negatively associated with GBS carriage (AOR, 0.43; 95% CI, 0.21–0.88; p = 0.022), and a positive association was observed for vaginal *Candida albicans* carriage (AOR, 3.25; 95% CI, 1.50–7.06; p = 0.003), vaginal *E. coli* carriage (AOR, 2.01; 95% CI, 1.10–3.80; p = 0.023), recent

Table 2. Studies reporting GBS serotype distribution of (recto)vaginal isolates in SSA.

Country	Year	Population	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX	NT	Reference
The Gambia	1994	P	19		28	6	3	38						[34][$]
Malawi	2011	P, HIV+, HIV-	18.2	6.2	10.3	39.0	0.3	23.9	0.8		0.8		1.5	[35]
SA	2011	P	30.1	6.7	11.3	37.3	3.7	10.2						[36]
SA	2014	P	36.2–41.4	3.5–4.6	7.2–7.5	31.3–34.9	2.0–4.0	10.3–15.6				0.0–3.3		[37]
Kenya	2015	P, NP	27.3			22.7		27.3	13.6	4.5	4.5			This study
Rwanda	2015	NP	83.3		16.7									This study
SA	2015	P, NP	34.5			10.3	13.8	31.0		6.9	3.4			This study
Europe	2010	N/A	18.2	12.4	14.4	28.1	3.7	14.9	0.6	0.6	0.6			[38][£]
US	2010	N/A	26.8	8.1	10.9	24.8	1.0	15.0	0.3	0.0	0.2			[38][£]

P, pregnant; NP, non-pregnant

[$]determined serotypes I-VI (no differentiation between Ia and Ib); N/A, not applicable (review)

[£]data from meta-analysis but excluding isolates from non-sterile sites and from neonates were excluded.

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vaginal intercourse (AOR, 2.63; 95% CI, 1.35–5.15; p = 0.005), and currently washing the vagina (AOR, 2.26; 95% CI, 1.16–4.37; p = 0.016).

In our multivariable *E. coli* model, an intermediate Nugent score remained significantly negatively associated with vaginal *E. coli* carriage (AOR, 2.61; 95% CI, 1.15–5.94; p = 0.023), and a positive association was observed with working as a FSW (AOR, 7.83; 95% CI, 2.88–21.30; p<0.001), vaginal GBS carriage (AOR, 2.05; 95% CI, 1.09–3.83; p = 0.025), and cervical ectopy (AOR, 1.64; 95% CI, 1.01–2.68; p = 0.046) (Table 6).

Discussion

Group B streptococci (GBS) and *E. coli* account for the majority of EOS cases worldwide.

Vaginal carriage of GBS and *E. coli* is considered a prerequisite for GBS or *E. coli* transmission to the neonate in GBS EOS and *E. coli* EOS, respectively. However, epidemiological data of vaginal GBS and *E. coli* carriage, which are essential for the development and implementation of prevention strategies, are very limited in sub-Saharan Africa (SSA) [16, 17].
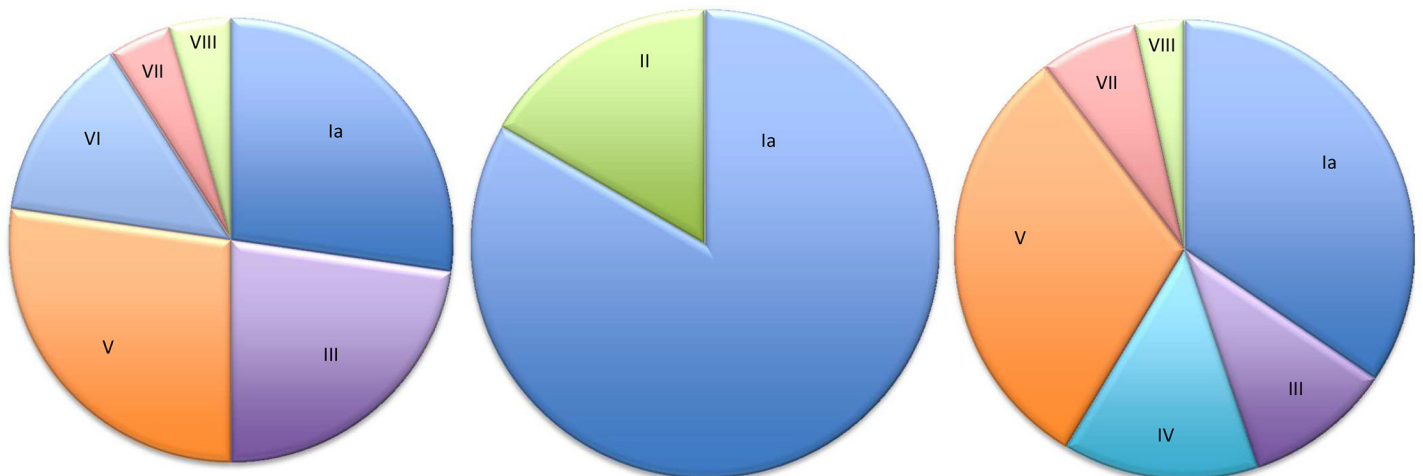

Fig 1. Distribution of GBS capsular serotypes. Left, Kenya (n = 22); middle, Rwanda (n = 6); right, South Africa (n = 29).

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Table 3. Sociodemographic characteristics, reproductive health, sexual behavior, vaginal practices, vaginal signs & symptoms, and microbiological associations with vaginal GBS carriage (univariable analysis).

	n	GBS+ n (%)	Crude OR (95% CI)	p-value*
	424	69 (16.3)		
Sociodemographic characteristics				
City (Country)				
Mombasa (Kenya)	165	27 (16.4)	0.89 (0.51–1.53)	0.665
Kigali (Rwanda)	60	6 (10.0)	0.50 (0.20–1.26)	0.142
Johannesburg (SA)	199	36 (18.1)	1	-
Age (years)				
<18	58	1 (1.7)	0.07 (0.01–0.50)	**0.008**
18–24	148	23 (15.5)	0.71 (0.41–1.23)	0.219
>24	218	45 (20.6)	1	-
Educational level				
Higher educational level£	189	39 (20.6)	1	-
Lower educational level££	235	30 (12.8)	0.56 (0.33–0.95)	**0.030**
Marital status				
Never married	242	34 (14.0)	1	-
Married	148	30 (20.2)	1.55 (0.91–2.67)	0.109
Separated/divorced/widowed	34	5 (14.7)	1.06 (0.38–2.91)	0.918
Socio-economic status#				
Low	106	17 (13.0)	1	-
Medium	163	31 (19.0)	1.23 (0.64–2.36)	0.533
High	155	21 (13.5)	0.82 (0.41–1.64)	0.576
Reproductive health				
Pregnant				
No	366	62 (16.9)	1	-
Yes	58	7 (12.1)	0.67 (0.29–1.55)	0.353
Parity				
0	149	18 (12.1)	1	-
1–2	211	42 (19.9)	1.81 (1.00–3.29)	0.052
>2	64	9 (14.1)	1.19 (0.50–2.81)	0.690
Gravity				
0	118	14 (11.9)	1	-
1–2	210	38 (18.1)	1.64 (0.85–3.17)	0.141
>2	96	17 (17.7)	1.60 (0.74–3.44)	0.230
Regular cycle				
Yes	256	42 (16.4)	1	-
No/unknown	168	27 (16.1)	0.98 (0.58–1.66)	0.927
Menstrual cycle				
No cycle	194	33 (17.0)	1	-
With cycle	230	36 (15.7)	0.91 (0.54–1.51)	0.706
Contraceptive				
None	73	15 (20.5)	1	-
Condom only	108	10 (9.3)	0.40 (0.17–0.94)	**0.035**
Others (hormones/IUD/sterilisation/pregnant)	243	44 (18.1)	0.86 (0.44–1.65)	0.639
Currently breastfeeding				
No	390	64 (16.4)	1	-
Yes	34	5 (14.7)	0.88 (0.33–2.36)	0.796

(*Continued*)

Table 3. (*Continued*)

	n	GBS+ n (%)	Crude OR (95% CI)	p-value*
Sexual behaviour				
Age at first sexual encounter (years)				
<16	80	8 (10.0)	1	-
16–18	197	36 (18.3)	2.01 (0.89–4.5)	0.093
19–21	104	16 (15.4)	1.64 (0.66–4.04)	0.286
>21	43	9 (20.9)	2.38 (0.85–6.71)	0.101
Sexually active (last 3 months)				
No	55	4 (7.3)	1	-
Yes	369	65 (17.6)	2.73 (0.95–7.81)	0.062
Condom use (at last sexual encounter)@				
No	229	47 (20,5)	1.75 (0.94–3.16)	0.050
Yes	140	18 (12.9)	1	-
Lifetime n° of sex partners				
1	112	13 (11.6)	1	-
2–3	186	32 (17.2)	1.58 (0.79–3.16)	0,194
> 3	126	24 (19.0)	1.79 (0.86–3.71)	0,117
N° of sex partners in the last 3 months				
0	25	0 (0.0)	1	-
> = 1	399	69 (17.3)	N/A	**0.010**
Recent vaginal sex%				
No	343	49 (14.3)	1	-
Yes	81	20 (24.7)	2.73 (0.95–7.81)	**0.024**
Sexual risk taking€				
Low	167	25 (15.0)	1	-
Medium	155	30 (19.4)	1.36 (0.76–2.44)	0.297
High	102	14 (13.7)	0.90 (0.45–1.83)	0.779
Estimated frequency of sexual encounters in last 3 months@, &				
0	55	4 (7.3)	1	-
< 10 times	137	18 (13.1)	1.93 (0.62–5.98)	0.255
11–30 times	129	24 (18.6)	2.91 (0.96–8.84)	0.059
> 30 times	98	23 (23.5)	3.91 (1.27–11.98)	**0.017**
HIV status partner@,				
HIV positive	38	3 (7.9)	1	-
HIV negative	250	52 (20.8)	3.06 (0.91–10.36)	0.072
Unknown	79	10 (12.6)	1.69 (0.44–6.54)	0.447
Estimated frequency of unprotected sex in last 3 months				
No sexual contacts	55	4 (7.3)	1	-
Never unprotected	104	12 (11.5)	1.66 (0.51–5.42)	0.399
< 10 times	88	14 (15.9)	2.41 (0.75–7.75)	0.139
> = 10 times	177	39 (22.0)	3.60 (1.23–10.59)	**0.020**
New partner (within 3 months)				
No	378	60 (15.9)	1	-
Yes	46	9 (19.6)	1.29 (0.59–2.81)	0.523
Circumcision status partner@,				
Circumcised	240	38 (15.8)	1	-
No/don't know Not circumcised/don't know	129	27 (20.9)	1.41 (0.81–2.43)	0.222
Female sex worker				

(*Continued*)

Table 3. (*Continued*)

	n	GBS+ n (%)	Crude OR (95% CI)	p-value*
Yes	30	6 (20.0)	1	-
No	394	64 (16.2)	1.03 (0.38–2.97)	0.952
Vaginal practices				
Washing inside the vagina when bathing				
No	176	15 (8.5)	1	-
Yes	248	54 (21.8)	2.99 (1.63–5.49)	**<0.001**
Drying the vagina before sex				
Yes	10	4 (40.0)	1	-
No	414	65 (15.7)	3.58 (0.98–13.04)	0.053
Washed inside the vagina recently (morning or evening before study visit)				
No	231	28 (12.1)	1	-
Yes	193	41 (21.2)	1.96 (1.16–3.30)	**0.012**
Products to wash/clean/dry/tighten the vagina				
None	153	13 (8.5)	1	-
Water/fingers only or water/soap	211	44 (20.9)	2.84 (1.47–5.48)	**0.002**
Cloth	48	9 (18.8)	2.49 (0.99–6.24)	0.053
Lemon juice/detergents	12	3 (25.0)	3.59 (0.86–14.92)	0.079
Cleaning the vagina after sexual intercourse				
No	227	28 (12.3)	1	-
Yes	197	41 (20.8)	1.87 (1.11–3.16)	**0.019**
Cervicovaginal signs and symptoms				
Ectopy¶				
No	226	34 (15.0)	1	-
Yes	197	34 (17.3)	1.18 (0.70–1.98)	0.536
Degree of ectopy¶				
Absent	226	34 (15.0)	1	-
Small	53	8 (15.1)	1.00 (0.44–2.32)	0.993
Moderate	139	24 (17.3)	1.18 (0.67–2.09)	0.573
Large	5	2 (40.0)	3.77 (0.61–23.4)	0.155
Colposcopic findings§,¶				
No	380	59 (15.5)	1	-
Yes	43	10 (23.3)	1.65 (0.77–3.53)	0.197
Cervical mucus				
No	270	39 (14.4)	1	-
Mild to moderate	140	28 (20.0)	1.48 (0.87–2.53)	0.151
Abundant	14	2 (14.3)	0.99 (0.21–4.58)	0.987
Reported abnormal discharge				
No	399	64 (16.0%)	1	-
Yes	25	5 (20%)	1.31 (0.47–3.61)	0.604
Vaginal discharge on speculum				
No	332	52 (15.7)	1	-
Yes	92	17 (18.5)	0.82 (0.45–1.50)	0.518
Vaginal epithelial abnormalities				
No	419	67 (16.0)	1	-
Yes	5	2 (40.0)	3.50 (0.57–21.36)	0.174
Cervical epithelial abnormalities				
No	379	64 (16.9)	1	-

(*Continued*)

Table 3. (*Continued*)

	n	GBS+ n (%)	Crude OR (95% CI)	p-value*
Yes	45	5 (11.1)	0.62 (0.23–1.62)	0.325
Red blood cells in urine				
No	358	58 (16.2)	1	-
Yes	66	11 (16.7)	1.03 (0.51–2.10)	0.925
White blood cells in urine				
No	328	56 (17.1)	1	-
Yes	96	13 (13.5)	0.76 (0.40–1.46)	0.411
Microbiological factors				
BV visit 1 (Amsel criteria)				
No BV	346	61 (17.6)	1	-
BV	78	8 (10.3)	0.53 (0.24–1.17)	0.116
BV (Nugent) ¶				
No BV (Nugent 0–3)	217	37 (17.1)	1	-
Intermediate (Nugent 4–6)	29	6 (20.7)	1.30 (0.60–2.83)	0.507
BV (Nugent 7–10)	137	13 (9.5)	0.29 (0.12–0.70)	**0.006**
Reproductive tract infection (RTI)				
No RTI	352	60 (17.0)	1	-
1 or more RTI	60	8 (13.3)	0.75 (0.34–1.66)	0.475
>1 RTI	12	1 (8.3)	0.44 (0.06–3.50)	0.439
Syphilis				
No	415	69 (16.6)	1	-
Yes	9	0 (0.0)	N/A	**0.001**
Chlamydia trachomatis				
No	382	62	1	-
Yes	42	7	1.03 (0.44–2.43)	0.942
Neisseria gonorrhoeae				
No	415	69 (16.6)	1	-
Yes	9	0 (0.0)	N/A	**0.001**
Trichomonas vaginalis				
No	390	66 (16.9)	1	-
Yes	26	3 (11.5)	0.64 (0.19–2.20)	0.478
Candida albicans (qPCR)				
No	375	50 (13.3)	1	-
Yes	46	17 (37.0)	3.81 (1.95–7.44)	**<0.001**
Escherichia coli (qPCR)				
No	354	89 (25.1)	1	-
Yes	67	29 (43.3)	2.27 (1.33–3.90)	**0.003**
HSV-2 serology				
No	276	42 (15.2%)	1	-
Yes	147	27 (18.4%)	1.25 (0.74–2.13)	0.404
Vaginal pH				
< 4.4	126	15 (11.9)	1	-
4.4–5.3	240	42 (17.5)	1.57 (0.83–2.96)	0.163
5.4 and more	58	12 (20.7)	1.93 (0.84–4.44)	0.122
PSA present				
No	233	31 (13.3)	1	-
Yes	181	38 (21.0)	1.73 (1.03–2.91)	**0.039**

(*Continued*)

Table 3. (*Continued*)

	n	GBS+ n (%)	Crude OR (95% CI)	p-value*
Systemic antibiotics visit 1				
No	341	62 (18.1)	1	-
Yes	83	7 (8.4)	0.41 (0.18–0.94)	**0.036**
Systemic antibiotics screening visit				
No	391	68 (17.4)	1	-
Yes	33	1 (3.0)	0.15 (0.02–1.11)	0.063

*bold: significant at the 5% level

£completed secondary school, or post-secondary school

££Primary school (completed or not), secondary school but not completed

#Socio-economic-status was constructed from total income, type of housing, type of toilet

@with partners within three months prior to enrolment

&missing data for 5

%sex morning or evening before visit &

€low risk: 1 or no partners in last year and did not have any partner (in the last 3 months) with multiple partners and age first sex at least 15 years; medium risk: 2 partners last year or had at least one sexual partner (in the last 3 months) who had multiple partners; high risk: sex worker or at least 3 partners last year or at had at least one sexual partner with HIV in the last 3 months or age first sex less than 15 years; N/A, no odds ratio due to no cases in one category

¶data missing for 1 (ectopy, colposcopic findings, HSV-2 serology), 3 (*C. albicans*, *E. coli*), 8 (*T. vaginalis*), 41 (BV Nugent, unreadable slides)

§petechiae (6 GBS cases/20), abrasion (2 GBS cases/5), erythema (1 GBS case/10), laceration (1 GBS case/4); BV, bacterial vaginosis; IUD, intrauterine device.

In this study, we aimed to present vaginal GBS and *E. coli* carriage rates, GBS serotype distribution and define risk factors for carriage in populations from three SSA countries.

Vaginal GBS and *E. coli* carriage rates

We found a vaginal GBS carriage rate of 20.2% and 23.2% in the Kenyan and South African reference groups (adult, non-pregnant, HIV-negative women at average risk of HIV), respectively. Compared to these reference groups, adolescents in our study were found to have lower GBS carriage rates: 3.6% of the Kenyan and 0% of the SA adolescents carried GBS vaginally. Other studies report conflicting associations between age and vaginal GBS carriage [39–47]. All of these studies (except for [44]) report on pregnant women. Interestingly, when we compare different age groups (< 18 years, 18–24 years, > 24 years) in our Kenyan and SA population, we see no age-group dependent GBS colonization in the pregnant women. However, we do see a statistically significant age-group dependent GBS association in the non-pregnant women, with the lowest and the highest GBS carrier rates in the youngest and the oldest age groups, respectively (Pearson Chi-Square test, data not shown).

The pregnant women in our study population had vaginal GBS carriage rates of 14.3% and 10.0% in Kenya and SA, respectively. This is lower then most other studies reporting (recto) vaginal GBS carriage rates in SSA (see Table 7). Although the CDC recommends rectovaginal sampling for detection of GBS in pregnant women, we only swabbed vaginally, to be able to study the interaction of GBS with the vaginal immune system and vaginal microbiome (to be published). This vaginal sampling may (partly) explain the lower GBS carriage rates found by us, as rectovaginal sampling has been shown to yield higher GBS recovery rates compared to vaginal sampling alone [48, 49]. Furthermore, in contrast to other studies (listed in Table 7) using culturing techniques, we used qPCR without prior enrichment step to detect GBS.

Table 4. Sociodemographic characteristics, reproductive health, sexual behavior, vaginal practices, vaginal signs & symptoms, and microbiological associations with vaginal *E. coli* carriage (univariable analysis).

	n	*E. coli* + n (%)	Crude OR (95% CI)	p-value*
	421	118 (28.0)		
Sociodemographic characteristics				
City (Country)				
Mombasa (Kenya)	164	39 (23.6)	0.87 (0.54–1.41)	0.569
Kigali (Rwanda)	60	27 (45.0)	2.28 (1.25–4.15)	**0.007**
Johannesburg (SA)	197	52 (26.5)	1	-
Age (years)				
<18	58	12 (20.7)	0.73 (0.36–1.47)	0.376
18–24	147	49 (33.3)	1.40 (0.88–2.20)	0.154
>24	216	57 (26.4)	1	-
Educational level				
Higher educational level£	187	56 (29.9)	1	-
Lower educational level££	234	62 (26.5)	0.84 (0.55–1.29)	0.434
Marital status				
Never married	240	71 (29.6)	1	-
Married	147	37 (25.2)	0.80 (0.50–1.27)	0.348
Separated/divorced/widowed	34	10 (29.4)	0.99 (0.45–2.18)	0.984
Socio-economic status#				
Low	106	27 (25.5)	1	-
Medium	163	46 (28.2)	1.15 (0.66–2.00)	0.620
High	152	45 (29.6)	1.23 (0.70–2.15)	0.467
Reproductive health				
Pregnant				
No	363	104 (28.7)	1	-
Yes	58	14 (24.1)	0.79 (0.42–1.51)	0.478
Parity				
0	149	35 (23.5)	1	-
1–2	209	62 (29.7)	1.37 (0.85–2.22)	0.196
>2	63	21 (33.3)	1.63 (0.85–3.11)	0.139
Gravity				
0	118	25 (21.2)	1	-
1–2	208	63 (30.3)	1.62 (0.95–2.75)	0.077
>2	95	30 (31.6)	1.72 (0.93–3.19)	0.087
Regular cycle				
Yes	254	65 (25.6)	1	-
No/unknown	167	54 (33.3)	1.42 (0.92–2.18)	0.111
Menstrual cycle				
No cycle	194	58 (29.9)	1	-
With cycle	227	60 (26.4)	0.84 (0.55–1.29)	0.430
Contraceptive				
None	72	20 (27.8)	1	-
Condom only	106	28 (26.4)	0.93 (0.48–1.83)	0.841
Others (hormones/IUD/sterilisation/pregnant)	243	70 (28.8)	1.05 (0.59–1.89)	0.865
Currently breastfeeding				
No	387	106 (27.4)	1	-
Yes	34	12 (35.3)	1.45 (0.69–3.03)	0.327

(Continued)

Table 4. (*Continued*)

	n	*E. coli* + n (%)	Crude OR (95% CI)	p-value*
Sexual behaviour				
Age at first sexual encounter (years)				
<16	79	29 (36.7)	1	-
16–18	197	52 (26.4)	0.62 (0.35–1.08)	0.090
19–21	103	26 (25.2)	0.58 (0.31–1.10)	0.096
>21	42	11 (26.2)	0.61 (0.27–1.40)	0.224
Sexually active (last 3 months)				
No	55	16 (29.1)	1	-
Yes	366	102 (27.9)	0.94 (0.50–1.76)	0.851
Condom use (at last sexual encounter)				
No	283	68 (24.0)	1.80 (1.16–2.79)	**0.009**
Yes	138	50 (36.2)	1	-
Lifetime n° of sex partners				
1	110	28 (25.5)	1	-
2–3	185	42 (22.7)	0.86 (0.50–1.49)	0.591
> 3	126	48 (38.1)	1.80 (1.03–3.15)	**0.039**
N° of sex partners in the last 3 months				
0	25	6 (24.0)	1	-
> = 1	396	112 (28.3)	1.25 (0.49–3.21)	0.644
Recent vaginal sex[%]				
No	340	91 (26.8)	1	-
Yes	81	27 (33.3)	1.37 (0.81–2.30)	0.238
Sexual risk taking[€]				
Low	167	44 (26.3)	1	-
Medium	152	37 (22.4)	0.90 (0.54–1.49)	0.681
High	102	37 (36.3)	1.59 (0.94–2.71)	0.086
Estimated frequency of sexual encounters in last 3 months[@, &]				
0	55	16 (29.1)	1	-
< 10 times	135	31 (23.0)	0.73 (0.36–1.47)	0.376
11–30 times	128	43 (33.6)	1.23 (0.62–2.45)	0.550
> 30 times	98	27 (27.6)	0.93 (0.45–1.93)	0.839
HIV status partner[@]				
HIV positive	38	11 (28.9)	1	-
HIV negative	247	63 (25.5)	0.84 (0.39–1.79)	0.653
Unknown	79	28 (35.4)	1.35 (0.58–3.12)	0.486
Estimated frequency of unprotected sex in last 3 months				
No sexual contacts	55	16 (29.1)	1	-
Never unprotected	102	34 (33.3)	1.22 (0.60–2.49)	0.586
< 10 times	87	19 (21.8)	0.68 (0.31–1.48)	0.330
> = 10 times	177	49 (27.7)	0.93 (0.48–1.82)	0.839
New partner (within 3 months)				
No	375	96 (25.6)	1	-
Yes	46	22 (47.8)	2,66 (1.43–4.97)	**0.002**
Circumcision status partner[@]				
Circumcised	238	62 (26.1)	1	-
Not circumcised/don't know	128	40 (31.3)	1.29 (0.80–2.07)	0.291
Female sex worker				

(*Continued*)

Table 4. (*Continued*)

	n	*E. coli* + n (%)	Crude OR (95% CI)	p-value*
No	391	97 (24.8)	1	-
Yes	30	21 (70.0)	7.07 (3.13–15.96)	**<0.001**
Vaginal practices				
Washing inside the vagina when bathing				
No	175	44 (25.1)	1	-
Yes	246	74 (30.1)	1.28 (0.83–1.98)	0.267
Drying the vagina before sex				
No	411	114 (27.3)	1	-
Yes	10	4 (40.0)	1.74 (0.48–6.27)	0.399
Washed inside the vagina recently (morning or evening before study visit)				
No	229	64 (27.9)	1	-
Yes	192	54 (28.1)	1.01 (0.66–1.55)	0.968
Products to wash/clean/dry/tighten the vagina				
None	153	40 (26.1)	1	-
Water/fingers only or water/soap	210	59 (28.1)	1.10 (0.69–1.77)	0.680
Cloth	46	16 (34.8)	1.51 (0.74–3.05)	0.255
Lemon juice/detergents	12	3 (25.0)	0.94 (0.24–3.65)	0.931
Cleaning the vagina after sexual intercourse				
No	226	61 (27.0)	1	-
Yes	195	57 (29.2)	1.12 (0.73–1.71)	0.610
Cervicovaginal signs and symptoms				
Ectopy[¶]				
No	224	49 (21.9)	1	-
Yes	196	69 (35.2)	1.94 (1.26–2.99)	**0.003**
Degree of ectopy[¶]				
Absent	224	49 (21.9)	1	-
Small	52	22 (42.3)	2.62 (1.39–4.94)	**0.003**
Moderate	139	46 (33.1)	1.77 (1.10–2.84)	**0.019**
Large	5	1 (20.0)	0.89 (0.10–8.17)	0.920
Colposcopic findings[§, ¶]				
No	377	102 (27.1)	1	-
Yes	43	16 (37.2)	1.60 (0.83–3.09)	0.163
Cervical mucus				
No	269	67 (24.9)	1	-
Mild to moderate	138	47 (34.1)	1.56 (1.00–2.44)	0.052
Abundant	14	4 (28.6)	1.21 (0.37–3.97)	0.758
Reported abnormal discharge				
No	396	112 (28.3)	1	-
Yes	25	6 (24.0)	0.80 (0.31–2.06)	0.644
Vaginal discharge on speculum				
No	331	85 (25.7)	1	-
Yes	90	33 (36.7)	1.68 (1.02–2.75)	**0.041**
Vaginal epithelial abnormalities				
No	416	116 (27.9)	1	-
Yes	5	2 (40.0)	1.72 (0.28–10.45)	0.554
Cervical epithelial abnormalities				
No	377	105 (27.9)	1	-

(*Continued*)

Table 4. (*Continued*)

	n	*E. coli* + n (%)	Crude OR (95% CI)	p-value*
Yes	44	13 (29.5)	1.09 (0.55–2.16)	0.813
Red blood cells in urine				
No	355	101 (28.5)	1	-
Yes	66	17 (25.8)	0.87 (0.48–1.59)	0.655
White blood cells in urine				
No	325	81 (24.9)	1	-
Yes	96	37 (38.5)	1.89 (1.17–3.06)	**0.010**
Microbiological factors				
BV visit 1 (Amsel criteria)				
No BV	344	99 (28.8)	1	-
BV	77	19 (24.7)	0.81 (0.46–1.43)	0.469
BV visit 1 (Nugent) [¶]				
No BV (Nugent 0–3)	217	60 (27.6)	1	-
Intermediate (Nugent 4–6)	29	15 (51.7)	2.80 (1.28–6.16)	**0.010**
BV (Nugent 7–10)	137	33 (24.1)	0.83 (0.51–1.36)	0.459
GBS				
No	354	89 (25.1)	1	-
Yes	67	29 (43.3)	2.27 (1.33–3.90)	**0.003**
Reproductive tract infection (RTI)				
No RTI	349	96 (27.5)	1	-
1 or more RTI	60	19 (31.7)	1.22 (0.68–2.21)	0.508
>1 RTI	12	3 (25.0)	0.88 (0.23–3.31)	0.848
Syphilis				
No	412	117 (28.4)	1	-
Yes	9	1 (11.1)	0.32 (0.04–2.55)	0.279
Chlamydia trachomatis				
No	379	107 (28.2)	1	-
Yes	42	11 (26.2)	0.90 (0.44–1.86)	0.780
Neisseria gonorrhoeae				
No	412	114 (27.7)	1	-
Yes	9	4 (44.4)	2.09 (0.55–7.93)	0.278
Trichomonas vaginalis[¶]				
No	387	105 (27.1)	1	-
Yes	26	11 (42.3)	1.97 (0.88–4.43)	0.101
***Candida albicans* (qPCR)**				
No	375	102 (27.2)	1	-
Yes	46	16 (34.8)	1.43 (0.75–2.73)	0.282
HSV-2 serology[¶]				
No	274	80 (29.2)	1	-
Yes	146	38 (26.0)	0.85 (0.54–1.34)	0.492
Vaginal pH				
< 4.4	125	34 (27.2)	1	-
4.4–5.3	237	65 (27.4)	1.01 (0.62–1.65)	0.963
5.4 and more	58	19 (32.8)	1.30 (0.66–2.56)	0.441
PSA present				
No	231	69 (29.9)	1	-
Yes	180	48 (26.7)	0.85 (0.55–1.32)	0.475

(*Continued*)

Table 4. (*Continued*)

	n	*E. coli* + n (%)	Crude OR (95% CI)	p-value*
Systemic antibiotics visit 1				
No	338	98 (29.0)	1	-
Yes	83	20 (24.0)	0.78 (0.45–2.36)	0.374
Systemic antibiotics screening visit				
No	388	111 (28.6)	1	-
Yes	33	7 (21.2)	0.67 (0.28–1.59)	0.366

*Bold: significant at the 5% level

£completed secondary school, or post-secondary school

££Primary school (completed or not), secondary school but not completed

#Socio-economic-status was constructed from total income, type of housing, type of toilet

@with partners within three months prior to enrolment

&missing data for 5

%sex morning or evening before visit

€low risk: 1 or no partners in last year and did not have any partner (in the last 3 months) with multiple partners and age first sex at least 15 years; medium risk: 2 partners last year or had at least one sexual partner (in the last 3 months) who had multiple partners; high risk: sex worker or at least 3 partners last year or at had at least one sexual partner with HIV in the last 3 months or age first sex less than 15 years; N/A, no odds ratio due to no cases in one category

¶data missing for 1 (ectopy, colposcopic findings, HSV-2 serology), 8 (*T. vaginalis*), 38 (BV Nugent, unreadable slides)

§petechiae (6 *E. coli* cases/20), abrasion (2 *E. coli* cases/5), erythema (3 *E. coli* cases/10), laceration (2 *E. coli* cases/4), ulcer (2 *E. coli* cases/6), ecchymosis (2 *E. coli* cases/6); BV, bacterial vaginosis.

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Table 5. Multivariable associations with vaginal GBS carriage.

	n	GBS+ (%)	adjusted OR (95% CI)	p-value$
	424	69 (16.3)		
Recent vaginal sex%				
No	343	49 (14.3)	1	-
Yes	81	20 (24.7)	2.63 (1.35–5.15)	**0.005**
Washing inside the vagina#				
No	176	15 (8.5)	1	-
Yes	248	54 (21.8)	2.26 (1.16–4.37)	**0.016**
BV (Nugent)¶				
No BV (Nugent 0–3)	217	37 (17.1)	1	-
Intermediate (Nugent 4–6)	29	6 (20.7)	0.93 (0.33–2.64)	0.898
BV (Nugent 7–10)	137	13 (9.5)	0.43 (0.21–0.88)	**0.022**
***Candida albicans* (qPCR)¶**				
No	375	50 (13.3)	1	-
Yes	46	17 (37.0)	3.25 (1.50–7.06)	**0.003**
***Escherichia coli* ¶**				
No	303	38 (12.5)	1	-
Yes	118	29 (24.6)	2.01 (1.10–3.80)	**0.023**

$bold, significant at the 5% level

#when having shower or bath

%morning or evening before study visit

¶data missing for 3 (*C. albicans*, *E. coli*), 41 (BV, unreadable slides).

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Table 6. Multivariable associations with vaginal *E. coli* carriage.

	n	E. coli + (%)	adjusted OR (95% CI)	p-value[$]
	421	118 (25.2)		
Condom use (at last sexual encounter)				
No	283	68 (24.0)	1.53 (0.92–2.56)	0.104
Yes	138	50 (36.2)	1.0	-
Female sex worker				
No	391	97 (24.8)	1	-
Yes	30	21 (70.0)	7.83 (2.88–21.30)	**<0.001**
Ectopy [¶]				
No	224	49 (21.9)	1	-
Yes	196	69 (35.2)	1.64 (1.01–2.68)	**0.046**
Vaginal discharge on speculum				
No	331	85 (25.7)	1	-
Yes	90	33 (36.7)	1.63 (0.92–2.88)	0.095
BV (Nugent)[¶]				
No BV (Nugent 0–3)	217	60 (27.6)	1	-
Intermediate (Nugent 4–6)	29	15 (51.7)	2.61 (1.15–5.94)	**0.023**
BV (Nugent 7–10)	137	33 (24.1)	0.66 (0.38–1.15)	0.140
GBS				
No	354	89 (25.1)	1	-
Yes	67	29 (43.3)	2.05 (1.09–3.83)	**0.025**

[$]bold, significant at the 5% level
[¶]data missing for 1 (ectopy), 38 (BV, unreadable slides).

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Although the CDC allows PCR for the detection of GBS (albeit recommending an enrichment step), this difference with other studies probably does not account (or to a lesser extent) for the lower rates found by us, as PCR (even without an enrichment step) has been shown to be more

Table 7. Studies reporting (recto)vaginal GBS carriage rates in SSA.

Country	Year	n	Population	% GBS	Sample	Detection	Reference
Nigeria	1980	588	P, L	19	V	SB+C	[55]
Nigeria	1983	225	P	20	V	SB+C	[56]
Zimbabwe	1990	89	P	31	V	SB+C	[57]
Togo	1991	106	P	4	V, R	SB+C	[58]
Gambia	1994	136	P	22	V, R	SB+C	[34]
Malawi	2005	97	P	16.5	V, R	SA	[59]
Mozambique	2008	113	P	1.8	V, R	SB+C	[60]
Tanzania	2009	300	P	23.0	V, R	SB+C	[54]
Zimbabwe	2010	780	P	47, 24, 21[#]	V, R	SB+C	[61]
Malawi	2011	1840	P, HIV+ and HIV-	21.2	V, R	SB+C	[35]
South Africa	2014	661, 621, 595, 521[$]	P	33.0, 32.7, 28.7, 28.4[$]	V, R	SA	[37]
DR Congo	2015	509	P	20.2	V	SA	[62]

L, women in labor; NP, non-pregnant women; P, pregnant women; V, vaginal swab; R, rectal swab; SB+C, selective broth and culturing; SA, selective agar
[#]week 20, 26, and delivery, respectively
[$]week 20–25, week 26–30, week 31–35, and week 37+, respectively.

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sensitive than culture [14, 50–53]. A further difference with other studies regards the fact that the pregnant women in our study were up to 14 weeks of gestation, while the other studies listed in Table 7 sampled pregnant women at 35–37 weeks of gestation, which also may account for differences, as some authors have reported on varying GBS rates during pregnancy [45].

In the group of HIV positive women, we did not observe any GBS carriers, which is probably explained by the fact that most of the HIV positive women (26/30) received prophylactic cotrimoxazole, which is largely effective against GBS [54].

Our reference groups from Kenya and SA had vaginal *E. coli* carriage rates of 25.0% and 27.1%, respectively. Compared to other studies from SSA reporting vaginal carriage of *E. coli*, these prevalences are higher than the ones reported by Karou and coworkers (2012) and Ekwempu and coworkers (1981), lower than the ones reported by Schellenberg and coworkers (2011) and Cutland and coworkers (2012), and comparable with the prevalence reported by Sagna and coworkers (2010) (See Table 8)[63–67]. Vaginal *E. coli* carriage rates in Asia, Europe, North and South America appear lower. Different study populations, sampling and detection techniques might account for these differences.

Compared to the reference group in Kenya, pregnant women had a lower prevalence of vaginal *E. coli* carriage; compared to the reference group in SA, adolescent women had a lower prevalence of *E. coli* carriage. The FSW study group in Kigali had a very high prevalence of *E. coli* carriage, i.e. 70%, and will be discussed below (risk factors).

To our knowledge, this is the first study to determine simultaneously the vaginal GBS and *E. coli* carriage rates in SSA populations using qPCR, known to be more sensitive than culture-based techniques. Moreover, vaginal carriage rates of GBS in Kenya and Rwanda and *E. coli* in Rwanda have not yet been described.

Risk factors for vaginal *E. coli* and GBS carriage

The presence of vaginal *C. albicans*, recent vaginal intercourse, working as a FSW, an intermediate vaginal microbiome, BV, washing the vagina and cervical ectopy were independent risk factors for vaginal GBS or *E. coli* colonization.

Women carrying *C. albicans* were 3.6 times more likely also to carry GBS. Three US based studies have shown this same significantly positive association between GBS and *Candida* or yeast [40, 88, 89].

Intravaginal practices, like e.g. cleaning inside the vagina beyond the introitus or insertion of substances into the vagina to dry or tighten the vagina, are common in Africa and are associated with adverse outcomes including increased risk for BV and for sexually transmitted infections [90]. Our model showed that washing inside the vagina was an independent risk factor for vaginal GBS carriage: women were twice as likely to be colonized with GBS compared to women not washing inside the vagina. A study by van de Wijgert and coworkers [91] showed that women using substances other than plain water to finger-clean or wipe inside the vagina had a GBS prevalence of 26.3% (n = 99), whereas women not engaging in these practices had a GBS prevalence of 14.7% (n = 70). However, their findings did not reach significance, most probably because of the smaller sample size (169 women compared to 424 women in our study).

Women who had recent vaginal sex (the morning or evening before the study visit) were more than twice as likely to carry GBS vaginally than women who did not. Accordingly, a positive PSA test was also significantly correlated with GBS carriage. GBS is generally not considered an STI, and the influence of sexual behavior on vaginal GBS carriage or acquisition is a matter of debate [92–94]. Based on published literature and our own data, we hypothesize that sexual activity might lead to a brief temporal GBS colonization of the vagina. This hypothesis is

Table 8. Studies reporting (recto)vaginal *E. coli* carriage rates.

Country	Year	n	Population	% E. coli	Sample	Detection	Reference
Africa (pooled prevalence 36.0% (2846/7912); range 9.1–46.5%)							
Burkina Faso	2010	156	HIV+	28.4	V	C	[67]
Burkina Faso	2012	2000	S	16.7	V	C	[63]
Kenya	2011	44	HIV+, HIV-, HESN	40.1	V	cpn60	[65]
Nigeria	1981	187	L	9.1	C	C	[64]
SA	2012	1347	P, HIV+	42.3	V	C	[66]
SA	2012	3752	P, HIV-	46.5	V	C	[66]
Asia (pooled prevalence 5.3% (163/3072); range 0–25.8%)							
Iraq	2011	90	S, NP	16.2	V	C	[68]
Iraq	2011	20	S, P	25.8	V	C	[68]
Iran	2014	85	S, P	18.0	V	C	[69]
Japan	2002	2575	NP, P	3.4	V	C	[70]
Pakistan	2012	100	HC	28	V	C	[71]
Pakistan	2012	100	H	6	V	C	[71]
Turkey	2007	34	IUD	14.7	V	C	[72]
Turkey	2007	34	HC	2.9	V	C	[72]
Turkey	2007	34	H	0.0	V	C	[72]
Europe (pooled prevalence 13.4% (670/4980); range 3.1–51.2%)							
Croatia	2011	114	IUD	25.5	V	C	[73]
Croatia	2011	122	H	8.2	V	C	[73]
Denmark	2014	668	P	11.7	V	C	[74]
Germany	2007	166	H	16.3, 51.2, 25.9[*]	V	C	[75]
Greece	2008	1632	S	3.1	V	C	[76]
Lithuania	2012	970	P	19.9	V, R	C	[77]
Spain	2002	623	P	27.0	V	C	[78]
Spain	2011	321	P	15	E, V	C	[79]
Spain	2011	327	NP	12	E, V	C	[79]
Sweden	2008	37	H	5.4	V	C	[80]
North America (pooled prevalence 12.7% (430/3373); range 0–29.5%)							
Canada	1983	495	H	12.3	V	C	[81]
US	1997	2646	P	13.0	V	C	[6]
US	2001	44	H	18.2, 9.1, 29.5, 6.8, 6.8[#]	V	C	[82]
US	2005	20	H	0	V	16S	[83]
US	2012	70	P	10	V	C	[84]
US	2012	35	NP	23	V	C	[84]
US	2013	47	P	2.1, 2.2, 5.6, 8.3[£]	V	C	[85]
US	2013	16	NP	6.3, 0, 12.5, 20	V	C	[85]
South America (pooled prevalence 19.7% (135/684); range 14.3–23.0%)							
Argentina	2013	259	P	14.3	V	C	[86]
Chile	2009	425	S	23.0	V	C	[87]

The electronic bibliographic database PubMed was searched for articles using the search terms '(Escherichia) AND (coli) AND (vaginal)' with no date or language restriction. Studies were included if the number of vaginal *E. coli* carriers and the total number of individuals tested were reported; studies were excluded if women were not of childbearing age. 16S, deep sequencing of the 16S rRNA gene; cpn60, deep sequencing of the cpn60 gene; C, conventional culturing and identification; E, endocervical swab; FSW, female sex workers; H, healthy women; HC, women using hormonal contraception; HESN, HIV exposed seronegative women; IUD, women using an intrauterine device as contraception; L, women in labor; NP, non-pregnant women; P, pregnant women; Q, qPCR; R, rectal swab; S, women with vaginal symptoms or clinical diagnosis of infection; V, vaginal swab

[*] pre, mid, post cycle, respectively

[#] visit 1at 1 month before visit 2 and 19–24 days after cycle, 1–2 days before intercourse, 8-12h after intercourse, 3–4 days after intercourse, 5–6 days after intercourse

[£] <14weeks, between 14–28 weeks, >28weeks, postpartum.

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strengthened by a recent longitudinal deep-sequencing study of the vaginal microbiome, where 25 women were sampled on a daily basis over a 10 week period, revealing an average of 0.39 GBS episodes per week and an average GBS episode of 2.8 days (Fig 1 and additional file 4 in [95]), contrasting with earlier studies—where sampling occurred every 3 weeks—that report average GBS episodes of 13.7 weeks [96]. The brief colonization might explain why we and other authors find parameters such as 'age of first sexual intercourse' not to be associated with GBS carriage (they do not cover the recent aspect), while parameters such as 'high frequency of intercourse during last month' (as a consequence, a higher chance of also having had recent intercourse) do correlate. Taken together, GBS should be considered as a potentially pathogenic micro-organism that can be sexually transmitted and whose vaginal presence can be enhanced by sexual activity.

Cervical ectopy was an independent risk factor for vaginal *E. coli* carriage, 21.9% of women without cervical ectopy were *E. coli* carriers as opposed to 35.2% of women with cervical ectopy. Cervical ectopy has been associated with CT [97], HPV [98] and an increased susceptibility to HIV infection [99]. Although we could not determine the cause-effect relation of this association, it seems biologically plausible that a niche is created by the glandular columnar epithelium of women with cervical ectopy that somehow—directly or indirectly—favors the colonization by *E. coli*. Some studies have e.g. related cervical ectopy with a reduced cell-mediated or changed humoral immunity [21, 100].

Working as a FSW was an independent risk factor for vaginal *E. coli* carriage. We could not explain this by any of the sexual behavioural or other parameters presented in Table 4. In our study, none of the participants, including the female sex workers, reported having had anal intercourse during the last 3 months. These percentages probably are underestimates, since stigma associated with anal intercourse often leads to reduced reporting [101]. Furthermore, Ghanem and coworkers [102] showed that regarding anal intercourse, significantly more women reported to engage in these practices when asked by means of computer assisted self interviews compared to face-to-face interviews, which was used in our study. It is not unlikely that FSW engage more in anal intercourse, compared to the general population, and that in our FSW population, vaginal contamination with *E. coli* is higher by transfer of (peri)anal bacteria during anal/vaginal intercourse. Other studies from East-Africa report anal intercourse prevalences in FSW of up to 40.8% [103]. Interestingly, anal intercourse during pregnancy has been reported as a significant risk factor for neonatal *E. coli* colonization [77].

Besides above-mentioned risk factors for GBS or *E. coli* carriage, we show for the first time that colonization with GBS and *E. coli*, the leading causes of EOS, are positively associated.

Abovementioned risk factors can be translated and implemented into strategies that aim to reduce the maternal carriage of GBS and *E. coli*. First, behavioral change by advocating abstinence from sexual intercourse and vaginal washing during late pregnancy, e.g. via counseling in family planning facilities, could help to reduce the risk of maternal GBS and *E. coli* colonization in resource-poor settings. Second, the extension of GBS screening with screening for *C. albicans* and *E. coli*–risk factors for vaginal GBS carriage–should be further investigated. Furthermore, the screening for *E. coli* itself also merits further investigation because of its role as a major EOS causative agent for which currently no prevention measures are taken, nor in low-income, nor in high-income countries [104]. In this context, the presence of cervical ectopy–a risk factor for vaginal *E. coli* carriage–should be further investigated.

GBS serotype distribution

As IAP is not effective against LOS and culture-based screening and administration of costly intravenous antibiotics might not be feasible in most low-income countries, an alternative and

long-term solution lies in the development of effective GBS vaccines, that however would not cover for other micro-organisms causing EOS. As most GBS vaccines under development aim at eliciting protective antibodies against capsular polysaccharides, the principal difficulty in developing globally effective GBS vaccines is the existence of several serotypes with different geographical distributions. In our study, the most prevalent GBS serotypes were Ia (27.3%), V (27.3%) and III (22.7%) in Kenya; Ia (34.5%), V (31.0%), and IV (13.8%) in SA; and Ia (83.3%) and II (16.7%) in Rwanda. Only few other studies have documented vaginal GBS serotype distribution in Africa and these are largely in line with our findings (Table 4 and Fig 1).

Interestingly, compared to the low prevalences in Europe and the US, we found relatively high prevalences of serotypes IV, VI, VII–shown for the first time in sub-Saharan Africa—and VIII in the Kenyan and South African population (VI, 13.6%; VII, 4.5%; VIII 4.5% in Kenya, and IV, 13.8%; VI, 6.9%; VIII, 3.4% in SA) (Table 4). In contrast, we did not detect any serotype Ib, which has, according to a recent meta-analysis, a prevalence of 8.1% in the US and 12.4% in Europe [38].

Differences in serotype distribution between our study and other studies (Table 4) might be explained by differences in study populations, most studies listed in Table 4 typed strains isolated from pregnant women whereas most of our GBS strains were isolated from non-pregnant women. Methodological differences might also contribute. Many GBS capsular polysaccharide typing methods have been described, with the most commonly used method being a serological test, used by all studies listed in Table 4, except our study. We used a molecular capsular typing method, developed and applied by European (reference) laboratories [105, 106]. Brigtsen and coworkers (2015) compared capsular typing of 426 GBS strains by a conventional latex agglutination test with PCR, and found that a substantial proportion of the strains were non-typeable by serotyping, but typeable by genotyping, and that an agreement between serotyping and genotyping was shown in 71.1% (of the isolates that were typeable by both methods) [107]. Moreover, we used a molecular technique directly on DNA extracts from vaginal swabs (and not on DNA extracts from isolates), which could lead to the detection of certain serotypes that would not have been isolated by culture.

Currently, there are two candidate vaccines in phase II clinical trials, i.e. a trivalent vaccine targeting serotypes Ia, Ib, and III, and a conjugate vaccine targeting serotype III (www.clinicaltrials.gov). In theory, the first vaccine could cover for 50.0%, 83.3% and 44.8% of vaginal GBS cases in our Mombasa, Kigali and Johannesburg population, respectively, but only a minority of women would be protected by the conjugate vaccine (22.7%, 0% and 10.3%, respectively).

GBS, *E. coli* and the vaginal microbiome

Our results show that vaginal GBS and *E. coli* carriage were significantly associated with disturbances of the vaginal microbiome: compared to women with a normal vaginal microbiome, women with an intermediate vaginal microbiome were 2.61 times more likely to carry *E. coli*, whereas women with BV were 2.33 less likely to carry GBS. The latter finding is in accordance a study of Hillier and coworkers [108], reporting a significant negative association between GBS carriage and BV by Nugent scoring, studying 7,918 pregnant women. Two other studies did not confirm these findings [94, 109]. In depth analysis of abovementioned interdependencies of GBS, *E. coli*, *C. albicans*, BV and the vaginal microbiome will be published elsewhere.

Our study was limited by the fact that we used vaginal sampling instead of rectovaginal sampling for the detection of GBS (as recommended by the CDC), which has been shown to have higher recovery rates for GBS. Furthermore, we did not use a selective broth enrichment prior to PCR, as recommended by the CDC. In our study, pregnant women were up to 14 weeks of

gestation, and were not sampled at 35–37 weeks' gestation as recommended by the CDC, which could have biased our results. Our study was further limited by the rather small sample size of our Rwanda study population.

In conclusion, vaginal GBS carriage rate and serotype distribution were similar to high-income countries, except for the higher prevalence of serotypes VI, VII and VIII. *E. coli* carriage rate was higher compared to high-income countries. We identified risk factors for GBS or *E. coli* carriage, ie. recent sexual intercourse, vaginal washing, *C. albicans* colonization and presence of cervical ectopy, that can be implemented in strategies to reduce maternal colonization. Immunoprophylaxis with current phase II candidate GBS vaccines would not protect the majority of women against vaginal GBS carriage in our study population. The most important causative agents of EOS, GBS and *E. coli*, both associated with disturbances of the vaginal microbiome, are positively associated.

Acknowledgments

Author Contributions

Conceived and designed the experiments: PC VJ LH TC SD MM GN JVDW MV. Performed the experiments: PC GN SD MM. Analyzed the data: PC. Wrote the paper: PC VJ LH TC SD MM JVDW MV.

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