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Maternal carriage of Group B streptococcus and Escherichia coli in a district hospital in Mozambique

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**Full Title:** Maternal carriage of Group B streptococcus and Escherichia coli in a district hospital in Mozambique

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**Manuscript Region of Origin:** MOZAMBIQUE

**Abstract:** Background: In low-income countries, data on prevalence and effects of Group B streptococcus (GBS) and Escherichia coli (E. coli) colonization among pregnant women are scarce, but necessary to formulate prevention strategies. We assessed prevalence of GBS and E. coli colonization and factors associated among pregnant women, its effect in newborns and acceptability regarding the utilized sampling methods in a semirural Mozambican hospital.

Methods: Pregnant women were recruited from June 2014 to January 2015, during routine antenatal clinics at gestational age ≥34 weeks (n=200); or upon delivery.
Maternal risk factors were collected. Vaginal and vagino-rectal samples for GBS and E. coli determination were obtained and characterized in terms of antimicrobial resistance and serotype. Anti-GBS antibodies were also determined. Neonatal follow-up was performed in the first three months after birth. Semi-structured interviews were performed to investigate acceptability of sample collection methods.

Results: 21.3% of women recruited were GBS carriers, while 16.3% were positive for E. coli. Prevalence of HIV was 36.6%. No association was found between being colonized by GBS and E. coli and maternal risk factors. GBS isolates were fully susceptible to penicillin and ampicillin. Serotypes V (32.4%), Ia (14.7%) and III (10.3%) were the most commonly found and 69.2% of the women tested had IgG antibodies against GBS. E. coli isolates showed resistance to ampicillin in 28.9% and trimethoprim/sulfamethoxazole in 61.3% of the cases.

Conclusion: Prevalence of GBS and/or E. coli colonization among pregnant women is high in this semirural community and comparable to those reported in similar settings. Four serotypes accounted for nearly 70% of all isolates of GBS. Population based data on infant GBS infections would enable the design of prevention strategies for GBS disease in Mozambique.

### Suggested Reviewers:

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Dear Editors,

Please find attached a manuscript entitled “Maternal carriage of Group B streptococcus and Escherichia coli in a district hospital in Mozambique”.

This manuscript describes an observational prospective study on carriage of Group B streptococcus (GBS) and Escherichia Coli (E. coli) among pregnant women in a semirural district in southern Mozambique.

GBS and E. coli are leading causes of neonatal sepsis in many industrialized countries, however, in low-income countries (LIC), data on prevalence and effects of these pathogens are scarce. Strategies involving GBS screening during pregnancy, followed by intrapartum antibiotic prophylaxis have managed to significantly wipe GBS early onset disease in those places where they have been implemented. Unfortunately, the fragility of the health system in LIC hinders the applicability of such strategies. A better understanding of the specific risk factors that may favor maternal colonization and transmissibility to the newborn could contribute to the design of preventive strategies for E. coli neonatal disease, and calls for innovative ideas to tackle these vertically transmitted infections, which still account for a significant burden of disease in places like Mozambique. Trying to address this hypothesis we assessed prevalence of GBS and E. coli colonization and factors associated among pregnant women, its effect in newborns and acceptability towards diagnostic methods in a semirural Mozambican hospital.

All the authors have participated in the study and have agreed upon the submitted version of the paper. They concur with the subsequent revisions submitted by the corresponding author.

The material is original and unpublished and has not and will not be simultaneously submitted elsewhere so long as it is under consideration by the Pediatric Infectious Disease Journal.

The authors declare that they have no conflict of interest. No material submitted as part of the manuscripts infringes existing copyrights, or the rights of a third party.
FUNDING
The research leading to these results has received funding from Instituto de Salud Carlos III (ISCIII) through a program Miguel Servet obtained by Quique Bassat (Plan Nacional de I+D+I 2008-2011, grant number: CP11/00269. Sara M. Soto has a fellowship from the program I3, of the ISCIII. During the duration of the study Lola Madrid had a fellowship from the program Rio Hortega of the ISCIII (grant no.: CP13/00260). The CISM receives financial support from the Spanish Agency for International Cooperation (AECID).

FINANCIAL DISCLOSURE
At the time when the study was conducted, Quique Bassat had a fellowship from the program Miguel Servet of the ISCIII (Plan Nacional de I+D+I 2008-2011, grant number: CP11/00269) and Lola Madrid had a fellowship from the program Rio Hortega of the ISCIII (CM13/00260) and Sara M. Soto has a fellowship from the program I3, of the ISCIII.

Yours sincerely,

Lola Madrid

First and corresponding author
RESPONSE TO THE REVIEWERS:

Dear Editors,

Many thanks for the positive feedback for our paper “Maternal carriage of *Group B streptococcus* and *Escherichia coli* in a district hospital in Mozambique”. We also appreciate the careful review and constructive insights provided by the reviewers, which have significantly contributed to improve the manuscript. Below is a point-by-point response to the reviewers’ and Editor’s remaining queries.

We look forward to your response.

Yours sincerely,

Dr Lola Madrid

In preparing your revised manuscript, please pay attention to the following stylistic points that simplify the redactor’s work: (1) List the first and last names and academic degrees of all listed authors on the Title page; (2) Provide "abbreviated title" for cover (max 55 characters); (3) Provide "running head" (max 44 characters); (4) Number all pages of manuscript sequentially, the title page being Page 1; (5) Type legends for figures on a separate page, placed after the references in the manuscript text.

RESPONSE: Dear editors, all these points were already included in the previous submitted version.
Reviewer's comments:

Reviewer #1:

The authors have made good attempts to answer the previous referees' comments. I think the essentials of the paper are as good as they can be, given the difficult circumstances surrounding recruitment. However, the paper is still not written in good English, for example the sentence "We adjusted the multivariate analysis by gestational age as previous studies examining the influence of advancing gestation on GBS colonization have observed (albeit with conflicting results), as colonization rates appear to change over time during pregnancy" is scrambled and I think means "We adjusted the multivariate analysis by gestational age as previous studies examining the influence of advancing gestation on GBS colonization have observed that colonization rates appear to change over time during pregnancy". In fact, my interpretation of previous studies is that the colonisation of individuals varies, not the overall prevalence, which is consistent with their next sentence "However, no associations between gestational age and colonization risk by GBS were found".

RESPONSE: authors have changed the sentence to "We adjusted the multivariate analysis by gestational age as previous studies examining the influence of advancing gestation on GBS colonization have observed that colonization rates appear to change over time during pregnancy" as reviewer has suggested.

The authors refer to 'recto-vaginal swabs' which is unfortunate, as when a single swab is used to sample both areas, it is obviously important to sample the vagina first and then the rectum, not the other way around (as this would be clinically unacceptable). They should be called - 'vagino-rectal swabs'.

RESPONSE: authors have reviewed and modified recto-vaginal swabs to vagino-rectal swabs throughout the full text

This aside, the description "For GBS determination, samples included a lower vaginal swab, and a recto-vaginal sample, consisting on a brief rotation of the swab through the outer sphincter", is not clear. I presume a single swab was used for the vaginal swab. For the vagino-rectal swab, was this two separate swabs or a single swab (both are clinically acceptable)? And were both sampling methods used in all women? (it appears so from comments in the results but this should be clearly stated).

RESPONSE: The reviewer is right, as two swabs were collected in all women. One single swab for vaginal sample and one single swab for vagino-rectal sample: taking first vaginal sample. We have changed these sentences in the Methods section trying to be clearer:

“For GBS determination, samples included a lower vaginal swab (vaginal sample), and a single swab for the vagino-rectal sample, consisting on a sample of the vagina first and then
the rectum obtained performing a brief rotation of the swab through the outer sphincter. Both kinds of swabs were collected in all women in order to compare the prevalence of GBS colonization detected by the two samples.

The authors say "This study shows GBS and E. coli carriage among near term pregnant women is reasonably high in southern Mozambique". What do they mean by 'reasonable'? What would be unreasonable? A value judgement is inappropriate here; a simple statements of the rates would be more appropriate.

RESPONSE: following reviewer's recommendation we have removed the word “reasonable”.

"This study has several limitations. Only women attending the MDH (and no other maternities) were included, and recruitment was not conducted after working hours, being these potential sources of selection bias and limiting the generalization of our results to the whole area" - 'being these' should be 'these being'. And what do they mean by 'the whole area'? They need to be more specific.

RESPONSE: We have modified, according to the reviewer's suggestion 'being these' to 'these being'. The whole area means the entire district. We have changed this sentence too.

The abbreviation for grams is g, not gr.

RESPONSE: authors have reviewed and modified “gr” to “grams” or “g” throughout the full text and tables.

When the authors refer to 'previous abortion', do they mean miscarriage or ToP - or were they unable to tell? In which case, 'previous pregnancies ending before 24 weeks' might be clearer.

RESPONSE: we have included in Definitions, the definition of abortion commonly used in previous studies conducted at CISM which is the definition of The National Center for Health Statistics, the Centers for Disease Control and Prevention (CDC), and the World Health Organization (WHO): Abortion was defined as pregnancy termination prior to 20 weeks' gestation or a foetus born weighting less than 500 g.

The paper would benefit from careful proof reading and correction by a good English writer.

RESPONSE: The manuscript has been reviewed by a native English speaker.
The results in figure two would be much easier to assess if the points were clean scattered, or shown as vertical histograms. 'Area proportional to number of observations' is unclear - it appears to be a lot of circles one on top of another, which does not equate to an area. What is the parameter on the y axis? Is it optical density?

**RESPONSE:** We have reviewed this comment with our statisticians and they do not consider it appropriate to represent this type of distributions as histograms. The scatter plot has the problem of the superposition of points when they fall on the same coordinates, and they do not allow to see well how many there are. To solve this problem, they have created this representation in bubbles, with the size of the bubble proportional to the number of observations overlapping on the same coordinates. In the case of figure 2, most bubbles are the same because they represent a single observation, but if the reviewer observes the figure of serotype II and III, it can be seen that some bubbles are larger than the rest, because they correspond to more observations. Our statisticians have recommended us to keep this figure like it had been originally presented because it is the clearest representation of the data. We have added the meaning of OD in the footnote and clarification on the meaning of 'Area of symbol proportional to number of observations'.

**Editor:**

abstract
suggest: methods "Neonatal follow-up..." results: "had IgG antibodies against GBS."

**RESPONSE:** changes in abstract performed as the Editor has suggested.

P3.

**RESPONSE:** we have updated number of deaths in 2016 as last estimates have been already published.

line 15. remove "a" "...are leading.."

**RESPONSE:** we have removed “a”.

line 24. what's the difference between: preterm birth and very-low-birth-weight delivery?

**RESPONSE:** preterm birth is a live birth <37 weeks of gestational age and very-low-birth-weight is a birth weight <1500gr. I have removed the word “delivery” trying to be clearer.

line 32 remove "currently proposed"

**RESPONSE:** this change has been done.
34. suggest: "Maternal GBS carriage during the period closely related to delivery has consistently been demonstrated to determine the risk of vertical transmission, and thus of ensuing neonatal disease"

RESPONSE: authors have followed Editor’s suggestion.

43 and 48. "Maternal risk factors associated with higher..." and other examples of this later on - change to "associated with" not "associated to"

RESPONSE: we have reviewed the full text and have changed all “associated to” to “associated with”.

P4. line 16. change "essentially decimated" to "significantly reduced"

RESPONSE: change done.

P5. line 1. change "This study aimed to determine the prevalence of pregnant women colonized by GBS and E. coli attending a semi-rural Mozambican hospital"

RESPONSE: authors have followed Editor’s suggestion.

P8. 33. intrapartum antibiotics cant be administered to the newborn - please modify text

RESPONSE: this mistake has been corrected. We have removed “or the newborn”.

P12. 11. "penicillin, ampicillin" review spelling of cotrimoxazole throughout

RESPONSE: authors have reviewed and modified when necessary spelling of cotrimoxazole throughout the full text.

P15. 38 "antibodies against GBS"

RESPONSE: change done.

P16. line 22. "intrauterine infection with subsequent negative cultures" why? did this mum receive antibiotics before cultures were obtained?
RESPONSE: thank you for this comment. This baby was born to an HIV positive mother and was taking co-trimoxazole as prophylaxis. We have included the following comment: “This mother was HIV positive and was taking co-trimoxazole as prophylaxis of opportunistic infections”.

P17. 41. "....working hours, these being potential sources of selection bias and limiting..."

RESPONSE: RESPONSE: authors have followed Editor’s suggestion.

56. "convenience sample"

RESPONSE: change done.
Maternal carriage of *Group B streptococcus* and *Escherichia coli* in a district hospital in Mozambique.

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FUNDING

The research leading to these results has received funding from Instituto de Salud Carlos III (ISCIII) through a program Miguel Servet obtained by Quique Bassat (Plan Nacional de I+D+I 2008-2011, grant number: CP11/00269. Sara M. Soto has a fellowship from the program I3, of the ISCIII. During the duration of the study Lola Madrid had a fellowship from the program Rio Hortega of the ISCIII (grant no.: CP13/00260). The CISM receives financial support from the Spanish Agency for International Cooperation (AECID).

KEYWORDS: Group B streptococcus, Escherichia coli, Recto-vaginal colonization, maternal colonization, maternal risk factors.

Abbreviated title: Maternal colonization by GBS and E. coli in southern Mozambique

Running head title: Maternal colonization by GBS and E. coli

FINANCIAL DISCLOSURE

Quique Bassat had during the duration of the study a fellowship from the program Miguel Servet of the ISCIII (Plan Nacional de I+D+I 2008-2011, grant number: CP11/00269). Lola Madrid had a fellowship from the program Rio Hortega of the ISCIII (CM13/00260) and Sara M. Soto has a fellowship from the program I3, of the ISCIII while the study was conducted. SM, AV, ES, SM, AC, RV, AN, NM, RA, KM, CM, EM and CM have nothing to declare.
ABSTRACT

Background: In low-income countries, data on prevalence and effects of Group B streptococcus (GBS) and Escherichia coli (E. coli) colonization among pregnant women are scarce, but necessary to formulate prevention strategies. We assessed prevalence of GBS and E. coli colonization and factors associated among pregnant women, its effect in newborns and acceptability regarding the utilized sampling methods in a semirural Mozambican hospital.

Methods: Pregnant women were recruited from June 2014 to January 2015, during routine antenatal clinics at gestational age ≥34 weeks (n=200); or upon delivery (n=120). Maternal risk factors were collected. Vaginal and vagino-rectal samples for GBS and E. coli determination were obtained and characterized in terms of antimicrobial resistance and serotype. Anti-GBS antibodies were also determined. Neonatal follow-up was performed in the first three months after birth. Semi-structured interviews were performed to investigate acceptability of sample collection methods.

Results: 21.3% of women recruited were GBS carriers, while 16.3% were positive for E. coli. Prevalence of HIV was 36.6%. No association was found between being colonized by GBS and E. coli and maternal risk factors. GBS isolates were fully susceptible to penicillin and ampicillin. Serotypes V (32.4%), Ia (14.7%) and III (10.3%) were the most commonly found and 69.2% of the women tested had IgG antibodies against GBS. E. coli isolates showed resistance to ampicillin in 28.9% and trimethoprim/sulfamethoxazole in 61.3% of the cases.

Conclusion: Prevalence of GBS and/or E. coli colonization among pregnant women is high in this semirural community and comparable to those reported in similar settings. Four serotypes accounted for nearly 70% of all isolates of GBS. Population based data on infant GBS infections would enable the design of prevention strategies for GBS disease in Mozambique.
Maternal carriage of Group B streptococcus and Escherichia coli in a district hospital in Mozambique
ABSTRACT

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Methods: Pregnant women were recruited from June 2014 to January 2015, during routine antenatal clinics at gestational age ≥34 weeks (n=200); or upon delivery (n=120). Maternal risk factors were collected. Vaginal and *vagina-rectal vaginal* samples for GBS and *E. coli* determination were obtained and characterized in terms of antimicrobial resistance and serotype. Anti-GBS antibodies were also determined. Neonatal follow-up was performed in the first three months after birth. Semi-structured interviews were performed to investigate acceptability of *sample collecting collection methods samples*.

Results: 21.3% of women recruited were GBS carriers, while 16.3% were positive for *E. coli*. Prevalence of HIV was 36.6%. No association was found between being colonized by GBS and *E. coli* and maternal risk factors. GBS isolates were fully susceptible to penicillin and ampicillin. Serotypes V (32.4%), Ia (14.7%) and III (10.3%) were the most commonly found and 69.2% of the women tested had IgG antibodies against *GBS*. *E. coli* isolates showed resistance to ampicillin in 28.9% and trimethoprim/sulfamethoxazole in 61.3% of the cases.

Conclusion: Prevalence of GBS and/or *E. coli* colonization among pregnant women is high in this semi-rural community and comparable to those reported in similar settings. Four serotypes accounted for nearly 70% of all isolates of GBS. Population based data on infant GBS infections would enable the design of prevention strategies for GBS disease in Mozambique.
BACKGROUND

In 2015, 5.96 million children under the age of five died with nearly half of those deaths occurring in the first 28 days of life, the so-called neonatal period (1). Neonatal deaths are disproportionately distributed across the globe, with 95% of them taking place in developing regions and infections remain a major contributor to this preventable mortality (2, 3).

Vertical transmission of bacteria that are normal commensal flora or pathogens of the maternal genitourinary and gastrointestinal tracts, such as Group B streptococcus (GBS) or Escherichia coli (E. coli) are leading determinants of neonatal morbidity and mortality, causing invasive bacterial infections that can manifest as sepsis, pneumonia and meningitis (4, 5). GBS and E. coli are particularly associated with early-onset neonatal disease (EOD, 0-6 days after birth (6)), but can also cause late-onset disease (LOD, 7-89 days (7)), preterm birth and very-low-birth-weight delivery (8, 9), all of which are responsible for substantial morbidity and mortality in sub-Saharan Africa (SSA) (2, 10, 11). The estimated incidence of GBS neonatal disease in SSA countries suggests a burden at least comparable to that found in high-income countries (HIC) before the implementation of the currently proposed preventive strategies (12).

Maternal GBS carriage during the period closely related to the delivery has consistently been demonstrated to determine the risk of vertical transmission, and thus of ensuing neonatal ensuing disease. Prevalence of maternal colonization varies from 6.5 to 36% (13) in Europe and has been reported higher than 20% in Sub-Saharan countries, although precise regional maternal carriage data for this continent are scarce (12, 14, 15). Maternal risk factors associated with higher prevalence of GBS colonization are controversial. Both younger (16) and older maternal ages (17) have been reported as maternal characteristics associated with higher risk of GBS colonization, as well as higher education (17), higher income (18), and high sexual activity (17). The relation between HIV infection and risk for GBS maternal colonization has yet to be fully elucidated. Studies conducted in the United States (19) or in Zimbabwe (15) did not find an increased risk among HIV infected individuals,
whereas researchers from South Africa(20) found a lower colonization prevalence among HIV-infected mothers. Vertical transmission of GBS may significantly increase (up to a 64% higher) among HIV-exposed infants compared with non-HIV exposed ones(12).

The primary intervention to reduce GBS-associated EOD involves the administration of intrapartum antibiotic prophylaxis (IAP) to women identified to either 1) be GBS carriers through microbiological screening (35-37 weeks’ gestation)(21) of samples obtained from their genito-urinary or gastrointestinal lower tract; or 2) fulfil any of the different risk factors associated with neonatal disease(22-24). In HIC, the widespread implementation of the IAP strategy has significantly reduced essentially decimated GBS EOD among those babies born to women in whom it was correctly applied. The IAP strategy has however not demonstrated any impact on GBS-associated LOD, or in the prevention of E. coli neonatal disease of any kind(12, 13). In low and lower-middle income countries (LIC and LMIC), the fragility of the health systems and the generalized lack of microbiology facilities, in the absence of a reliable rapid point of care test for GBS, hinders the applicability of the IAP strategy, therefore jeopardising the prevention of life-threatening GBS neonatal infections(12). Despite SSA having the highest incidence of neonatal sepsis worldwide(12), epidemiological data on GBS and E. coli maternal colonization in this continent are scarce. In Mozambique, as a paradigmatic example, a Pubmed search only provides five results from studies reporting GBS data(12, 25-28), and only two of those related to maternal colonization, describing a prevalence of colonization as low as 1.8%(25) or even lower (1%)(27), difficult to contextualize among much higher prevalence data from neighboring sub-Saharan African countries(12). Additionally, and to our knowledge, no articles reporting E. coli colonization prevalence in pregnant women in Mozambique have been published and only one multicenter study conducted in South Africa, Kenya and Rwanda have determined simultaneously the vaginal GBS and E. coli carriage rates in SSA(29, 30). Such data, however, appear necessary for a better and more evidence-based design of preventive strategies, based on the resources and infrastructures available.
This study aimed to determine the prevalence of pregnant women colonized by GBS and E. coli attending a semi-rural Mozambican hospital, analyze risk factors associated to higher risk of carriage by these pathogens and characterize the isolates in terms of antimicrobial resistance and serotype distribution. As secondary objectives, we determined the neonatal outcomes and assessed the feasibility and acceptability of collecting vaginal and recto-vaginal samples among pregnant women, with the idea of generating locally-relevant data useful to guide national preventive strategies and policies to reduce transmission and the toll of such potentially life-threatening infections in the newborn.

METHODS

Study site

The study was conducted in Manhiça, a semi-rural site in Southern Mozambique. The Manhiça Health Research Center (CISM) runs a Demographic Surveillance System (DSS) in the area and a morbidity surveillance system (MSS) at the Manhiça District Hospital (MDH), across the street. A detailed description of MDH, CISM and the study area can be found elsewhere(31). MDH is the referral hospital for the Manhiça district, covering a population of circa 183,000 inhabitants. The MDH includes adult and paediatric wards, together with a maternity, where between 3500-4000 deliveries take place annually. Institutional delivery rates are around 85-90% in the study area. MDH also includes an outpatient department and an antenatal care (ANC) clinic where pregnant women are routinely followed. As part of the National policy, all pregnant women are invited to attend antenatal consultations during their pregnancy, where HIV testing and other screening of infections and conditions are routinely offered, in addition to intermittent preventive treatment during pregnancy (IPTp) for malaria prevention, a disease highly endemic in the area. Manhiça district has one of the highest prevalence rates of HIV in the world, with HIV prevalence during pregnancy having been estimated at around 29% during antenatal consultations(32). No strategy to prevent neonatal sepsis
is currently implemented in Mozambique. The hospital has recently introduced WHO-recommended Option B+ for the prevention of mother-to-child HIV transmission, which is offered to mothers free of charge.

**Study design and population.**

This observational prospective study was conducted at the ANC and delivery wards of MDH, between June 15 2014 and January 15 2015, running continuously during working hours (8:00–16:00) and working days. We recruited pregnant women at two different time-points during their pregnancy. One group during routine antenatal care with a minimum estimated gestational age ≥34 weeks, as measured by fundal height >=32cms, 2 cms above the midpoint between umbilicus and xiphoid process. A second group of women was recruited upon delivery (regardless of gestational age) if they were not recruited at ANC clinics, in order to understand real life risk for vertical transmission rate of GBS or E. coli to their offspring with no interference of antibiotic treatments. Participants were eligible for inclusion if they lived in the study area, were in good physical and mental health, able and willing to participate in the study and to provide informed consent. All women fulfilling inclusion criteria were eligible to participate in the study, and in order to obtain a more representative sample of the study population, the first two women seen every day were approached for recruitment.

**Definitions**

GBS colonization was considered in the event of a positive vaginal or recto-vaginal-vagino-rectal culture for GBS. E. coli colonization was considered when the positive vaginal culture grew E. coli. E. coli urinary tract infection was diagnosed when E. coli grew (>10^5 colony-forming units/mL) in the urine samples of pregnant women. Abortion was defined as pregnancy termination prior to 20 weeks’ gestation or a foetus born weighing less than 500 g(33). A preterm baby was defined as that with a gestational age at birth <37 weeks and stillbirth as intrauterine deaths occurring after 28 weeks of gestational age. Low-birth weight was defined as weight at birth <2,500 grams.
Study procedures

Sampling procedures

Microbiological swab samples were obtained from each participant (ANC or upon admission in labour at the delivery wards, but always prior to delivery) without the use of antiseptic solution or a speculum. A sample from the lower third of the vagina and a fresh urine sample were taken for \textit{E. coli} determination. For GBS determination, samples included a lower vaginal swab (vaginal sample), and a single swab for the vagino-rectal sample, consisting on a sample of the vagina first and then the rectum obtained performing a brief rotation of the swab through the outer sphincter. Both kinds of swabs were collected in all women in order to compare the prevalence of GBS colonization detected by the two samples. Swabs were immediately placed in Amies transport medium and sent to the laboratory within 24 hours. The vaginal and recto-vaginal vagino-rectal samples for GBS determination were inoculated directly onto Granada medium (Group B Streptococcus Differential Agar, Becton Dickinson, Erembodegem, Belgium) incubated anaerobically at 37°C for 24 hours. Vaginal samples for \textit{E. coli} determination were spread onto MacConkey agar and urine samples were inoculated onto agar Cysteine lactose electrolyte deficient (CLED) and MacConkey agar and incubated at 37°C overnight without CO₂. \textit{E. coli} isolates were identified based on colony appearance, Gram stain, latex agglutination with the Pastorex Strepto kit (Bio-rad Laboratories®, Marnes-la-Coquette, France) and standard biochemical tests for \textit{E. coli} determination. Both, GBS and \textit{E. coli} isolates were confirmed by MALDI-TOF. Resistance profiles were determined via \textit{Kirby-Bauer} disk diffusion method following the Clinical & Laboratory Standards Institute (CLSI) guidelines.

Determination of the GBS capsular type or serotype implied the utilization of a multiplex-PCR using a set of primers described previously(34). DNA of each
isolate was obtained using the High Pure PCR Template Preparation kit (Roche, Spain). Briefly, this procedure consisted in performing three PCR reactions using specific primers for 10 different serotypes. Reaction 1 detects [Ia, Ib, II, III and IV], reaction 2 [V, VI, VII, VIII and IX] and reaction 3 is the amplification control. PCR conditions involved an initial step of 95°C for 3 min, followed by 30 cycles of 95°C for 1 min, 57°C for 1 min and 72°C for 2 min, and a final step of 72°C for 10 min. The PCR products were visualized by electrophoresis using 1% agarose gels. Antibody (AB) determination was identified in blood samples of mothers recruited at delivery. They were performed by ELISA using whole bacteria as antigens. This procedure is a modification based on the protocol proposed by Baker et al. (33), using an optical density to 450 nm with a correction at 620 nm. The cut-off value for positivity was chosen to be ≥ 1 OD units, in order to be more strict than the one proposed by Baker (>0.125).

Maternal HIV infection status was determined and recorded if not previously known. Other screening tests routinely performed at ANC, such as syphilis or hemoglobin determination were also performed and recorded.

Communication of results to mothers and case management

Clinical assessment and management of patients was done following international guidelines for countries with no clear screen-and-treat national rules, both at the ANC and at the maternity. For those women identified as carriers of GBS in recto-vaginal/vagino-rectal swabs collected at the ANC, a field worker delivered to the mother at home a study card detailing the microbiological findings, together with indications of what to do during delivery, so that intrapartum antibiotics could be administered to the mother or the newborn according to the CDC guidelines (35). Urinary tract infections secondary to E. coli were also reported and treated according to national guidelines for pregnant women. All recruited women were encouraged to deliver at hospital and clinical staff was trained to identify them. Any child born to a recruited mother and found to be sick at delivery was assessed by a study clinician, and routine screening for bacterial surveillance (including a
blood culture and a lumbar puncture to obtain cerebrospinal fluid (CSF)) performed and clinical management organized according to MDH guidelines.

Although the aim of this study was not to assess the efficacy of IAP (already known), due to ethical considerations, IAP was started in GBS infected women upon labour initiation, according to CDC guidelines (35). In cases where IAP could not be adequately performed, we followed Spanish recommendations and prophylactic antibiotic treatment (50,000 IU of intramuscular penicillin as a single dose for a newborn weighting >2000 grams, or 25,000 IU if weight <2000 grams) was administered within the first hour after birth to the newborns of mothers with confirmed GBS colonization (36). Such children were observed at hospital for a minimum of 24h. For women recruited at delivery, culture results were not available until at least 24-48h after recruitment. In such cases (and also in cases of women with pending GBS result recruited prior to delivery) we kept the newborns under observation for a minimum of 24 hours after delivery, and provided clear recommendations to mothers regarding the need for a follow up visit should the newborn become sick in the first weeks of life. Babies born to study participant mothers were followed-up during the first three months after birth.

Assessment of the acceptability of vaginal and recto-vaginal-vagino-rectal sampling

The study included a simple socio-behavioural component to evaluate the acceptability of collecting samples (vaginal and recto-vaginal-vagino-rectal swabs) during pregnancy. "Non-participant observations" were conducted, whereby a member of the study team observed the procedures being conducted (excluding genital examinations), and complemented by semi-structured interviews to a small sample of pregnant women not participating in the study but contemporaneously attending the ANC. Finally, semi-structured interviews were conducted among some participants who had accepted to provide vaginal and rectal samples. Questions, themes and probes arising from the non-participant observation, other than stated in the interview guide, were included in the semi-structured interviews.
Statistical analysis

All data were prospectively collected using standardized questionnaires, which were double entered in specific study databases, created using Openclinica® software. Discrepancies were solved after comparison with the original source documents by a senior data clerk, and in close collaboration with the study clinicians. Statistical analyses were performed using StataCorp. 2015. *Stata Statistical Software: Release 14* (College Station, TX: StataCorp LP). Study variables were counted and summarized in frequency tables. Qualitative variables were compared using a Chi-squared test or Fisher’s exact test. Continuous variables were described as mean (standard deviation, SD) or median values (interquartile range, IQR) and were compared using the t test for normal distributions or the Mann Whitney test for skewed data. Logistic regression univariate and multivariate analyses were performed to identify risk factors for GBS or *E. coli* colonization, separately. Variables that were found to be significantly associated with GBS or *E. coli* in the univariate analysis together with those related at a significance level of p<0.10 were entered into a multivariate model. Age and gestational age at recruitment were also included in the multivariate analysis based on previous studies(9, 15-17). A separate univariate and multivariate analysis of risk factor associated to GBS or *E. coli* colonization among HIV pregnant women was also performed.

Ethical considerations

This protocol and all supporting documentation (Informed consent documents, Study questionnaires) were approved by the local bioethics committee of CISM (Comité Institucional de Bioética para Saúde *do CISM* (CIBS-CISM)), and by the National Bioethics Committee of Maputo (CNBS) in Mozambique; and by the Ethics Committee of the Hospital Clinic in Barcelona, Spain. Written information and consent forms in the local language were provided to the women. After the interview, 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. Participation in this study was voluntary, and study-related procedures did not interfere with the pregnant women’s or children’s standard clinical care.
RESULTS

Between June 15 2014 and January 15 2015, 320 pregnant women were recruited at MDH (Study profile in figure 1). Table 1 summarizes the socio-demographic and clinical characteristics of participants. Median age of recruited women was 24 years (Interquartile range, IQR 20-31), with no significant differences according to recruitment place. No major differences could be found in relation with recruitment site, with the exception of a higher frequency of higher education among women recruited upon delivery compared with those recruited at ANC (7.0% vs 26.7%, p<0.001). More than one third of women (117/320, 36.6%) were HIV positive.

Prevalence of GBS and E. coli colonization among pregnant women

More than a fifth (68/320; 21.3%) of all recruited women were colonized by GBS, detected in both samples in 33 women, in 15 in the vaginal one only, and in 20 in the recto-vaginal-vagino-rectal one only. A non-statistically significant higher proportion of GBS were isolated from the recto-vaginal-vagino-rectal sample (16.6%) as compared to the vagina (15.0%, p=0.81). Prevalence of GBS colonization was borderline significantly higher among women recruited upon delivery compared to those recruited at ANC (32/120 (26.7%) vs. 36/200 (18.0%), p=0.07). Fifty-two women had E. coli vaginal colonization (16.3%), being significantly more common among women recruited at delivery (22.5% vs. 12.5%, p=0.019) and 10/320 (3.1%) had a positive E. coli urine culture. Among HIV positive pregnant women recruited, GBS colonization was found in 26/117 (22.2%). E. coli vaginal colonization was determined in 18/117 (15.4%) HIV-positive women.

Anti-group B streptococcus antibodies

Antibodies against GBS were detected in 83/120 (69.1%) women recruited at delivery. Of them, 23/32 (71.9%) were among GBS colonized mothers and 60/88 (68.2%) among non-colonized women (figure 2). Among HIV positive participants, AB anti-GBS were detected in 25/38 (65.8%) of those tested. Forty women had AB against more than one GBS serotype, being the most frequent
AB against serotype Ia (24/120, 20%), against serotype Ib (76/120, 63.3%) and against serotype V (27/120, 22.5%). Correlating presence of antibodies to the homotypic GBS serotype, 2/4 (50%) women colonized by serotype Ia had AB against it, 6/6 (100%) for serotype Ib, 0/3 (0%) for serotype III and 7/14 (50%) of those carriers of Ib serotype had AB against their homotypic serotype.

**Risk factors associated to GBS and E. coli carriage**

Table 2 presents the univariate analyses of associations of the different socio-demographics, clinical and laboratory variables with vaginal GBS and E. coli carriage. In the final multivariate GBS model (Table 3), no risk factors were significantly associated with GBS carriage. Similarly, no risk factors appeared to be independently associated with maternal vaginal E. coli carriage (table 4). The univariate and multivariate analyses performed to identify risk factors of GBS or E. coli colonization but restricted to HIV-infected women showed no differences compared to those including all women (data not shown).

**Antimicrobial susceptibility and serotyping**

One hundred and one specimens were found to be positive for GBS (48 vaginal and 53 recto-vaginal-vagino-rectal). All GBS isolates were fully sensitive to penicillin, ampicillin and ceftriaxone. Thirty-four (32.7%) isolates were resistant to erythromycin and 20 (19.2%) isolates to clindamycin. Seven isolates showed erythromycin-induced resistance to clindamycin. All the E. coli isolates were screened for susceptibility to 18 antimicrobial agents. Susceptibility to all antimicrobial agents tested was seen in 14 isolates (22.6%). E. coli was resistant to ampicillin in 21 (38.9%) cases, ceftriaxone in 2 (3.2%) cases, amoxicillin/clavulanate acid in 12 cases (19.4%), ciprofloxacin in 4 cases (6.5%) and co-trimoxazole in 38 cases (61.3%). Figure 3 summarizes the distribution of antimicrobial resistance (classifying isolates showing intermediate levels of susceptibility as resistant). Details of the resistance profiles of GBS and E. coli isolates are shown in Supplementary material table S1.

The serotype distribution of the GBS isolates is presented in Fig 4 and Fig S1 in the Supplementary material. The most prevalent serotypes were V (32.4%), Ia (14.7%), III and Ib (10.3% and 8.8%, respectively). Sixteen isolates (23.5%) were non-typeable. Twenty-six women had the same serotype detected both in
the vaginal and recto-vaginal vagino-rectal swabs, while in seven cases infections were serotype-discordant.

**Neonatal outcomes**

Three hundred and twenty neonatal outcomes from 316 pregnant women were recorded (98.8%). The Deliveries delivery outcomes of four women in the ANC group were not registered at MDH. Neonatal outcomes included four pair of twins, 290 term babies, 25 preterm and 5 cases of stillbirths. Figure 1 illustrates neonatal outcomes and follow-up in detail. Characteristics of neonates born of mothers participating in the study may be found in table 1 and 2. Thirty-two neonates born of 36 (88.9%) GBS carriers recruited at ANC were born at MDH, and 4 outside of the health system. Due to lack of qualified clinical staff, work saturation and advanced stage of labor, IAP strategy as recommended by CDC(35) was feasible only in two known GBS carriers at time of delivery, we administered a single dose of penicillin to 22 neonates in the first hour after birth. Two hundred and sixty-two infants (81.9%) were followed-up until 90 days of age and 8/262 (3.1%) were admitted in the hospital during this period. Seven infants died among those followed-up until 3 months after birth (2.7%), being five of them HIV-exposed (one clinical sepsis, one perinatal asphyxia and 3 unknown causes). A significantly higher risk for death among those neonates born of mothers recruited at ANC compared to those recruited at delivery (3.6% vs. 1.7%, p<0.001) was found.

**Acceptability of vaginal and recto-vaginal vagino-rectal sampling**

Fifteen study participant women and five non-study pregnant women were recruited for the social component. Acceptability of collecting vaginal and recto-vaginal vagino-rectal samples was 100%. Facilitators for acceptance included: a) Willingness to know whether they had a reproductive tract infection; b) Being interested in understanding the objectives of collecting vaginal and recto-vaginal vagino-rectal samples; and c) Willingness to be treated and accompanied to the hospital in case of reproductive tract infection and avoiding transmitting them to their offsprings. Only a few women felt uncomfortable with sample collection, referring to feeling of burning and/or pain. Although all
participants of the social component accepted sample collection, possible barriers for acceptance of future recto-vaginal sample collection were explored and these included: a) fear in relation to the first time being submitted to this procedure; b) worries regarding being seen at the hospital (stigma); c) lack of privacy at the ANC at time of sample collection.

**DISCUSSION**

To our knowledge, this is the first study presenting data on *GBS* maternal colonization, antibodies against *GBS* and characterization of isolates in a rural area of Mozambique and the first time concomitantly examining *E. coli* colonization in pregnant women in the country. Maternal rate of *GBS* colonization found in this study, 21.3%, was as high as previous work in other countries in Sub-Saharan Africa reported(12). However, two previous studies performed in the capital of Mozambique, Maputo, reported an extremely low prevalence of *GBS* colonization among pregnant women of 1% in 1995(27) and 1.8% in 2008(25). Smaller sample sizes, different study population, and very likely laboratory and microbiology procedures utilized for *GBS* detection, may all contribute to explain the significant increase in terms of overall prevalence found in our study. Our findings are in close agreement with a systematic review on *GBS* disease in sub-Saharan Africa(12), which included 18 studies reporting data on maternal *GBS* colonization, finding an average *GBS* carriage of 21.8% (95% CI: 18.3 - 25.5) among pregnant women across the region. These results are also similar to general prevalence data from other regions, including the United States(9) and Europe(13), or from other neighboring countries in Sub-Saharan Africa such as South Africa (with similar prevalence of HIV(20, 37, 38), Zimbabwe(15) or Malawi(39), supporting the credibility of these data. The yield of recto-vaginal sampling was better for *GBS* colonization than using only vaginal samples as previously reported(40, 41) and recommended(35).

No risk factors independently associated with higher prevalence of *GBS* colonization were found in this study. We adjusted the multivariate analysis by gestational age as previous studies examining the influence of advancing...
gestation on *GBS* colonization have observed *(albeit with conflicting results)*, as that colonization rates appear to change overtime during pregnancy(15, 42, 43).

However, no associations between gestational age and colonization risk by *GBS* were found. Colonization prevalence was similar among age groups, in contrast to what has been described by some studies(16, 17, 44) but in concordance to a recent multicenter study performed in African settings(37), reinforcing the idea that colonization rates are quite stable across a wide variety of African settings. We did not find higher education to be a risk factor for *GBS* colonization as other studies have reported(18), a finding possibly influenced by the homogeneity of lower education backgrounds in our setting. Importantly, this study further contributes to expose the fact that current understanding on maternal risk factors for colonization is incomplete.

No increased risk of *GBS* colonization among HIV-infected women was found in this study, a finding supported by other studies in USA and Zimbabwe(15, 19). However, a South African study reported that maternal *GBS* carriage was lower in HIV-positive women and among those with lower CD4 counts in Malawi(20, 39). This fact could be related to the fact that *GBS* carriage is inversely associated with the use of prophylactic co-trimoxazole among HIV-infected women. Information about co-trimoxazole use in this study was not recorded but due to high prevalence of HIV in our cohort it is likely that a high proportion of participants were routinely taking co-trimoxazole. Although HIV appears not to be a risk factor for maternal colonization during pregnancy, a recent South African study found that incidence of *GBS* neonatal disease may be up to 64% higher among HIV-exposed infants compared with non-HIV exposed ones(12).

As of today, no data are available regarding incidence of neonatal *GBS* invasive disease and HIV co-infection in Mozambique. However, studies conducted in South Africa(20, 45), with a similar HIV prevalence to the one reported in southern Mozambique(32), found an incidence of *GBS* invasive disease among infants higher than that reported in other resource-constrained settings(44, 46).

Hence, it would appear reasonable to expect a high incidence of *GBS* invasive disease in this particularly HIV-struck study area. However, a low incidence of *GBS* invasive cases in neonates born to *GBS* infected women was found in this study. Reasons for this low incidence could be the high prevalence of antibodies against *GBS* found in the studied cohort (69.2%). Maternal
antibody levels have been associated with protection against invasive GBS disease in high (47) and lower-middle income settings (48) and it has been documented that GBS placental transfer is not affected by HIV infection (49). It is difficult to correlate our GBS AB results with what is known regarding GBS maternal colonization and infant disease. The highest proportion of women with anti-GBS AB was against serotype Ib, Ia and V, consistent with predominant serotypes among carriers in our cohort. Although we did not examine antibody correlation between mothers and newborns, the higher prevalence of antibodies in our cohort could also potentially explain this low incidence of GBS invasive disease among our neonate cohort. In addition, prevalence of carriers of serotype III in this population, the known serotype causing more infant invasive disease (50), was lower than reported in other African studies (12), which would be also consistent with a lower incidence among infants. Another reason could be the attempt to implement IAP strategy to those colonized GBS mothers delivering at MDH. None of the neonates who received a single dose of penicillin after birth developed symptoms of sepsis. Understanding that this strategy is not generally recommended on account of the risks of enhancing antimicrobial resistance, and in spite of the small sample, it could be argued that for settings where access to health is problematic, but where GBS maternal carriage can be confirmed, such a strategy could prove effective in decreasing neonatal early morbidity by blocking the infection’s transmissibility at a moment where the baby is still under the surveillance of the health system. The only GBS case in our study was a newborn developing symptoms in the first 24 hours, born to a mother recruited at delivery with negative GBS screening. This mother was HIV positive and was taking co-trimoxazole as prophylaxis of opportunistic infections, suggesting an intravuterine infection with a subsequent negativization.

On the other hand, the prevalence of E. coli found in this study was lower than reported from other authors in different African settings (20, 37) but comparable with the prevalence reported by Karou in Togo (51). No risk factors were found to be independently associated with a higher risk of E. coli vaginal carriage among pregnant women. Some studies have reported specific risks factors for E. coli colonization, including sexual practices such as anal intercourse during
pregnancy(52) or being a sexual worker(37). Such factors were however not explored in our study.

Importantly, GBS continues to be susceptible to penicillin, ampicillin, and ceftriaxone in this setting. Previous studies in Ethiopia(53, 54) and South Africa(18, 51) also reported full susceptibility of GBS strains to penicillin. Rarer cases of decreased susceptibility to penicillin have been reported in Japan and the United States(55). A study in Zimbabwe found almost 100% of isolates sensitive to penicillin, with 2% showing intermediate susceptibility to penicillin. Resistance to erythromycin resistance among invasive GBS isolates in Europe ranges from 3.8% to 21.2%(13) and from 7% to 25% in the USA and Canada(24). Higher levels of resistance to erythromycin (~33%) were found in this study which could be related to mass drug administration (MDA) of azithromycin for trachoma control in sub-Saharan Africa since development of macrolide-resistant pathogens after more than one round of mass treatment has already reported(56, 57). Erythromycin resistance is frequently associated with clindamycin resistance(24). The emergence of non-susceptible GBS strains has important public health implications. GBS is still susceptible to penicillin and ampicillin which are the antibiotics of choice. Erythromycin and clindamycin are the drugs of choice for penicillin-hypersensitive patients and resistance to these antibiotics is emerging.

As other studies have reported(12), serotypes Ia, Ib, II, III and V were predominant. However, the most frequent serotype (V) found in this study differs from those found in the majority of studies conducted in other countries, revealing the need to identify prevalent serotypes in each region, as a prerequisite of establishing the potential coverage, impact and implementation requirements of future anti GBS vaccination strategies.

Characterization of E. coli isolates from this study has been described by Saez et al(58). E. coli isolates showed significant resistance to co-trimoxazole, as a previous study on diarrhoeagenic E. coli(59) conducted in Manhiça already described. Reasons for such high SXT resistance levels may include its extensive use as treatment of community-acquired infections, or as prophylaxis of HIV-related opportunistic infections(60).
This study has several limitations. Only women attending the MDH (and no other maternities) were included, and recruitment was not conducted after working hours, being these potential sources of selection bias and limiting the generalization of our results to the whole entire district area. The first two women fulfilling inclusion criteria every day were invited to participate in the study, leading to only 200 women being recruited at ANC, and 120 additional ones upon delivery, an estimated 10% of all deliveries per year attended at MDH. Pregnant women are less likely to attend ANC at the end of their pregnancies, and some women who attend antenatal care in other maternities do actually choose MDH to deliver. Altogether this justifies our sampling strategy, but it is important to highlight that this convenience sample may not be truly representative of the entire pregnancy cohort in the area. We did not collect information about population not sampled and we were unable to compare it with our population in order to assess such potential selection bias. However, the maternity at MDH is the biggest one in the study area and women seen there come from different places of the district and a sample of women attended at delivery was also recruited, minimizing bias. Other studies have reported association of other sexually transmitted infections such as gonorrhea or bacterial vaginosis(37) or socio-economic status(44) with GBS colonization in pregnant women, but we did not measure these variables. However, an attempt was made to explore the majority of potential risk factors described by other authors. Finally, and albeit this not being an objective of the study, it was impossible to assess the risk of GBS and E. coli transmission in this cohort, due the lack of denominator.

Conclusion

This study shows GBS and E. coli carriage among near term pregnant women is reasonably high in southern Mozambique. HIV infection was not a risk factor for GBS or E. coli colonization. Presence of anti-GBS antibodies, administration of single dose of penicillin to neonates born to colonized mothers or use of prophylactic co-trimoxazole among HIV-infected pregnant women could be reasons to explaining the low incidence of GBS invasive disease among our
cohort of newborns.

Screening mothers near term and providing appropriate antimicrobial prophylaxis could prevent potential adverse neonatal outcomes. Unfortunately, the fragility of the health system in LIC hinders the applicability of such approaches, and calls for innovative ideas to tackle these vertically transmitted infections. Serotype V was the most prevalent in our community and four serotypes cause the majority of cases of GBS colonization. The development and implementation of a conjugate vaccine incorporating the most commonly found serotypes globally, could enhance the transfer of maternal antibodies to the baby and protect their health in those critical first moments for survival.
REFERENCES


LEGENDS FOR FIGURES

Figure 1. Study profile.
ANC: antenatal clinics; IC: informed consent; IAP: intrapartum antibiotic prophylaxis. MDH: Manhiça district hospital. *Microbiologically not confirmed. 'None received any kind of prophylaxis; †neonate died before taking samples. GBS sepsis without meningitis developed in the first 24h of life.

Figure 2. Bubble plot demonstrating antibody antibodies against GBS serotype Ia, Ib, II, III, IV and V in blood samples from women recruited at delivery (n=120).
OD: optical density to 450 nm with a correction at 620 nm. Cut-off value for positivity was ≥ 1 OD units.

Figure 3. Distribution of antimicrobial resistance among GBS and E. coli isolates
PEN, penicillin; AMP, ampicillin; CTX, ceftriaxone; ERY, erythromycin; CD, clindamycin; TET, tetracycline; VA, vancomycin; NAL, nalidixic acid; AMC, amoxicillin/clavulanic acid; CXM, cefuroxime; FOX, cefoxitine; CAZ, ceftazidime; AZT, aztreonam; TZP, piperacillin/tazobactam; ETP, ertapenem; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; GEN, gentamicin; SXT, trimethoprim/ sulfamethoxazole; TOB, tobramycin
Maternal carriage of *Group B streptococcus* and *Escherichia coli* in a district hospital in Mozambique
ABSTRACT

**Background:** In low-income countries, data on prevalence and effects of *Group B streptococcus* (GBS) and *Escherichia coli* (*E. coli*) colonization among pregnant women are scarce, but necessary to formulate prevention strategies. We assessed prevalence of GBS and *E. coli* colonization and factors associated among pregnant women, its effect in newborns and acceptability regarding the utilized sampling methods in a semirural Mozambican hospital.

**Methods:** Pregnant women were recruited from June 2014 to January 2015, during routine antenatal clinics at gestational age ≥34 weeks (n=200); or upon delivery (n=120). Maternal risk factors were collected. Vaginal and vagino-rectal samples for *GBS* and *E. coli* determination were obtained and characterized in terms of antimicrobial resistance and serotype. Anti-GBS antibodies were also determined. Neonatal follow-up was performed in the first three months after birth. Semi-structured interviews were performed to investigate acceptability of sample collection methods.

**Results:** 21.3% of women recruited were GBS carriers, while 16.3% were positive for *E. coli*. Prevalence of HIV was 36.6%. No association was found between being colonized by *GBS* and *E. coli* and maternal risk factors. *GBS* isolates were fully susceptible to penicillin and ampicillin. Serotypes V (32.4%), Ia (14.7%) and III (10.3%) were the most commonly found and 69.2% of the women tested had IgG antibodies against *GBS*. *E. coli* isolates showed resistance to ampicillin in 28.9% and trimethoprim/sulfamethoxazole in 61.3% of the cases.

**Conclusion:** Prevalence of *GBS* and/or *E. coli* colonization among pregnant women is high in this semirural community and comparable to those reported in similar settings. Four serotypes accounted for nearly 70% of all isolates of *GBS*. Population based data on infant *GBS* infections would enable the design of prevention strategies for *GBS* disease in Mozambique.
BACKGROUND

In 2016, 5.6 million children under the age of five died with nearly half of those deaths occurring in the first 28 days of life, the so-called neonatal period (1). Neonatal deaths are disproportionately distributed across the globe, with 95% of them taking place in developing regions and infections remain a major contributor to this preventable mortality(2, 3).

Vertical transmission of bacteria that are normal commensal flora or pathogens of the maternal genitourinary and gastrointestinal tracts, such as Group B streptococcus (GBS) or Escherichia coli (E. coli) are leading determinants of neonatal morbidity and mortality, causing invasive bacterial infections that can manifest as sepsis, pneumonia and meningitis(4, 5). GBS and E. coli are particularly associated with early-onset neonatal disease (EOD, 0-6 days after birth(6)), but can also cause late-onset disease (LOD, 7-89 days(7)), preterm birth and very-low-birth-weight(8, 9), all of which are responsible for substantial morbidity and mortality in sub-Saharan Africa (SSA)(2, 10, 11). The estimated incidence of GBS neonatal disease in SSA countries suggests a burden at least comparable to that found in high-income countries (HIC) before the implementation of the preventive strategies(12).

Maternal GBS carriage during the period closely related to the delivery has consistently been demonstrated to determine the risk of vertical transmission, and thus of ensuing neonatal disease. Prevalence of maternal colonization varies from 6.5 to 36%(13) in Europe and has been reported higher than 20% in Sub-Saharan countries, although precise regional maternal carriage data for this continent are scarce(12, 14, 15). Maternal risk factors associated with higher prevalence of GBS colonization are controversial. Both younger(16) and older maternal ages(17) have been reported as maternal characteristics associated with higher risk of GBS colonization, as well as higher education(17), higher income(18), and high sexual activity(17). The relation between HIV infection and risk for GBS maternal colonization has yet to be fully elucidated. Studies conducted in the United States(19) or in Zimbabwe(15) did not find an increased risk among HIV infected individuals, whereas researchers
from South Africa(20) found a lower colonization prevalence among HIV-infected mothers. Vertical transmission of GBS may significantly increase (up to a 64% higher) among HIV-exposed infants compared with non-HIV exposed ones(12).

The primary intervention to reduce GBS-associated EOD involves the administration of intrapartum antibiotic prophylaxis (IAP) to women identified to either 1) be GBS carriers through microbiological screening (35-37 weeks’ gestation)(21) of samples obtained from their genito-urinary or gastrointestinal lower tract; or 2) fulfil any of the different risk factors associated with neonatal disease(22-24). In HIC, the widespread implementation of the IAP strategy has significantly reduced GBS EOD among those babies born to women in whom it was correctly applied. The IAP strategy has however not demonstrated any impact on GBS-associated LOD, or in the prevention of E. coli neonatal disease of any kind(12, 13). In low and lower-middle income countries (LIC and LMIC), the fragility of the health systems and the generalized lack of microbiology facilities, in the absence of a reliable rapid point of care test for GBS, hinders the applicability of the IAP strategy, therefore jeopardising the prevention of life-threatening GBS neonatal infections(12).

Despite SSA having the highest incidence of neonatal sepsis worldwide(12), epidemiological data on GBS and E. coli maternal colonization in this continent are scarce. In Mozambique, as a paradigmatic example, a Pubmed search only provides five results from studies reporting GBS data(12, 25-28), and only two of those related to maternal colonization, describing a prevalence of colonization as low as 1.8%(25) or even lower (1%)(27), difficult to contextualize among much higher prevalence data from neighboring sub-Saharan African countries(12). Additionally, and to our knowledge, no articles reporting E. coli colonization prevalence in pregnant women in Mozambique have been published and only one multicenter study conducted in South Africa, Kenya and Rwanda have determined simultaneously the vaginal GBS and E. coli carriage rates in SSA(29, 30). Such data, however, appear necessary for a better and more evidence-based design of preventive strategies, based on the resources and infrastructures available.
This study aimed to determine the prevalence of pregnant women colonized by *GBS* and *E. coli* attending a semi-rural Mozambican hospital, analyze risk factors associated to higher risk of carriage by these pathogens and characterize the isolates in terms of antimicrobial resistance and serotype distribution. As secondary objectives, we determined the neonatal outcomes and assessed the feasibility and acceptability of collecting vaginal and vagino-rectal samples among pregnant women, with the idea of generating locally-relevant data useful to guide national preventive strategies and policies to reduce transmission and the toll of such potentially life-threatening infections in the newborn.

**METHODS**

**Study site**

The study was conducted in Manhiça, a semi-rural site in Southern Mozambique. The Manhiça Health Research Center (CISM) runs a Demographic Surveillance System (DSS) in the area and a morbidity surveillance system (MSS) at the Manhiça District Hospital (MDH), across the street. A detailed description of MDH, CISM and the study area can be found elsewhere(31). MDH is the referral hospital for the Manhiça district, covering a population of *circa* 183,000 inhabitants. The MDH includes adult and paediatric wards, together with a maternity, where between 3500-4000 deliveries take place annually. Institutional delivery rates are around 85-90% in the study area. MDH also includes an outpatient department and an antenatal care (ANC) clinic where pregnant women are routinely followed. As part of the National policy, all pregnant women are invited to attend antenatal consultations during their pregnancy, where HIV testing and other screening of infections and conditions are routinely offered, in addition to intermittent preventive treatment during pregnancy (IPTp) for malaria prevention, a disease highly endemic in the area. Manhiça district has one of the highest prevalence rates of HIV in the world, with HIV prevalence during pregnancy having been estimated at around 29% during antenatal consultations(32). No strategy to prevent neonatal sepsis is currently implemented in Mozambique. The hospital has recently introduced
WHO-recommended Option B+ for the prevention of mother-to-child HIV transmission, which is offered to mothers free of charge.

Study design and population.

This observational prospective study was conducted at the ANC and delivery wards of MDH, between June 15 2014 and January 15 2015, running continuously during working hours (8:00–16:00) and working days. We recruited pregnant women at two different time-points during their pregnancy. One group during routine antenatal care with a minimum estimated gestational age ≥34 weeks, as measured by fundal height >=32cm, 2 cm above the midpoint between umbilicus and xiphoid process. A second group of women was recruited upon delivery (regardless of gestational age) if they were not recruited at ANC clinics, in order to understand real life risk for vertical transmission rate of GBS or E. coli to their offspring with no interference of antibiotic treatments. Participants were eligible for inclusion if they lived in the study area, were in good physical and mental health, able and willing to participate in the study and to provide informed consent. All women fulfilling inclusion criteria were eligible to participate in the study, and in order to obtain a more representative sample of the study population, the first two women seen every day were approached for recruitment.

Definitions

GBS colonization was considered in the event of a positive vaginal or vagino-rectal culture for GBS. E. coli colonization was considered when the positive vaginal culture grew E. coli. E. coli urinary tract infection was diagnosed when E. coli grew (>10^5 colony-forming units/mL) in the urine samples of pregnant women. Abortion was defined as pregnancy termination prior to 20 weeks’ gestation or a foetus born weighting less than 500 g(33). A preterm baby was defined as that with a gestational age at birth <37 weeks and stillbirth as intrauterine deaths occurring after 28 weeks of gestational age. Low-birth weight was defined as weight at birth <2,500 grams.

Study procedures
**Sampling procedures**

Microbiological swab samples were obtained from each participant (ANC or upon admission in labour at the delivery wards, but always prior to delivery) without the use of antiseptic solution or a speculum. A sample from the lower third of the vagina and a fresh urine sample were taken for *E. coli* determination. For GBS determination, samples included a lower vaginal swab (vaginal sample), and a single swab for the vagino-rectal sample, consisting on a sample of the vagina first and then the rectum obtained performing a brief rotation of the swab through the outer sphincter. Both kinds of swabs were collected in all women in order to compare the prevalence of GBS colonization detected by the two samples. Swabs were immediately placed in Amies transport medium and sent to the laboratory within 24 hours. The vaginal and vagino-rectal samples for *GBS* determination were inoculated directly onto Granada medium (Group B Streptococcus Differential Agar, Becton Dickinson, Erembodegem, Belgium) incubated anaerobically at 37ºC for 24 hours. Vaginal samples for *E. coli* determination were spread onto MacConkey agar and urine samples were inoculated onto agar Cysteine lactose electrolyte deficient (CLED) and MacConkey agar and incubated at 37ºC overnight without CO₂. *E. coli* isolates were identified based on colony appearance, Gram stain, latex agglutination with the Pastorex Strepto kit (Bio-rad Laboratories®, Marnes-la-Coquette, France) and standard biochemical tests for *E. coli* determination. Both, *GBS* and *E. coli* isolates were confirmed by MALDI-TOF. Resistance profiles were determined via *Kirby-Bauer* disk diffusion method following the Clinical & Laboratory Standards Institute (CLSI) guidelines.

Determination of the *GBS* capsular type or serotype implied the utilization of a multiplex-PCR using a set of primers described previously(34). DNA of each isolate was obtained using the High Pure PCR Template Preparation kit (Roche, Spain). Briefly, this procedure consisted in performing three PCR reactions using specific primers for 10 different serotypes. Reaction 1 detects [Ia, Ib, II, III and IV], reaction 2 [V, VI, VII, VIII and IX] and reaction 3 is the amplification control. PCR conditions involved an initial step of 95ºC for 3 min, followed by 30 cycles of 95º C for 1 min, 57º C for 1 min and 72º C for 2 min, and a final step of 72º C for 10 min The PCR products were visualized by
electrophoresis using 1% agarose gels. Antibody (AB) determination was identified in blood samples of mothers recruited at delivery. They were performed by ELISA using whole bacteria as antigens. This procedure is a modification based on the protocol proposed by Baker et al (33), using an optical density to 450 nm with a correction at 620 nm. The cut-off value for positivity was chosen to be ≥ 1 OD units, in order to be more strict than the one proposed by Baker (>0.125).

Maternal HIV infection status was determined and recorded if not previously known. Other screening tests routinely performed at ANC, such as syphilis or hemoglobin determination were also performed and recorded.

**Communication of results to mothers and case management**

Clinical assessment and management of patients was done following international guidelines for countries with no clear screen-and-treat national rules, both at the ANC and at the maternity. For those women identified as carriers of *GBS* in vagino-rectal swabs collected at the ANC, a field worker delivered to the mother at home a study card detailing the microbiological findings, together with indications of what to do during delivery, so that intrapartum antibiotics could be administered to the mother, following the CDC guidelines(35). Urinary tract infections secondary to *E. coli* were also reported and treated according to national guidelines for pregnant women. All recruited women were encouraged to deliver at hospital and clinical staff was trained to identify them. Any child born to a recruited mother and found to be sick at delivery was assessed by a study clinician, and routine screening for bacterial surveillance (including a blood culture and a lumbar puncture to obtain cerebrospinal fluid (CSF)) performed and clinical management organized according to MDH guidelines.

Although the aim of this study was not to assess the efficacy of IAP (already known), due to ethical considerations, IAP was started in *GBS* infected women upon labour initiation, according to CDC guidelines(35). In cases where IAP could not be adequately performed, we followed Spanish recommendations and prophylactic antibiotic treatment (50,000 IU of intramuscular penicillin as a single dose for a newborn weighting >2000 grams, or 25.000 IU if weight <2000
grams) was administered within the first hour after birth to the newborns of mothers with confirmed *GBS* colonization (36). Such children were observed at hospital for a minimum of 24h. For women recruited at delivery, culture results were not available until at least 24-48h after recruitment. In such cases (and also in cases of women with pending *GBS* result recruited prior to delivery) we kept the newborns under observation for a minimum of 24 hours after delivery, and provided clear recommendations to mothers regarding the need for a follow up visit should the newborn become sick in the first weeks of life. Babies born to study participant mothers were followed-up during the first three months after birth.

**Assessment of the acceptability of vaginal and vagino-rectal sampling**

The study included a simple socio-behavioural component to evaluate the acceptability of collecting samples (vaginal and vagino-rectal swabs) during pregnancy. "Non-participant observations" were conducted, whereby a member of the study team observed the procedures being conducted (excluding genital examinations), and complemented by semi-structured interviews to a small sample of pregnant women not participating in the study but contemporaneously attending the ANC. Finally, semi-structured interviews were conducted among some participants who had accepted to provide vaginal and rectal samples. Questions, themes and probes arising from the non-participant observation, other than stated in the interview guide, were included in the semi-structured interviews.

**Statistical analysis**

All data were prospectively collected using standardized questionnaires, which were double entered in specific study databases, created using Openclinica© software. Discrepancies were solved after comparison with the original source documents by a senior data clerk, and in close collaboration with the study clinicians. Statistical analyses were performed using StataCorp. 2015. *Stata Statistical Software: Release 14* (College Station, TX: StataCorp LP). Study variables were counted and summarized in frequency tables. Qualitative
variables were compared using a Chi-squared test or Fisher’s exact test. Continuous variables were described as mean (standard deviation, SD) or median values (interquartile range, IQR) and were compared using the t test for normal distributions or the Mann Whitney test for skewed data. Logistic regression univariate and multivariate analyses were performed to identify risk factors for GBS or E. coli colonization, separately. Variables that were found to be significantly associated with GBS or E. coli in the univariate analysis together with those related at a significance level of p<0.10 were entered into a multivariate model. Age and gestational age at recruitment were also included in the multivariate analysis based on previous studies (9, 15-17). A separate univariate and multivariate analysis of risk factor associated to GBS or E. coli colonization among HIV pregnant women was also performed.

Ethical considerations

This protocol and all supporting documentation (Informed consent documents, Study questionnaires) were approved by the local bioethics committee of CISM (Comité Institucional de Bioética para Saúde do CISM (CIBS-CISM)), and by the National Bioethics Committee of Maputo (CNBS) in Mozambique; and by the Ethics Committee of the Hospital Clínica in Barcelona, Spain. Written information and consent forms in the local language were provided to the women. After the interview, 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. Participation in this study was voluntary, and study-related procedures did not interfere with the pregnant women’s or children’s standard clinical care.

RESULTS

Between June 15 2014 and January 15 2015, 320 pregnant women were recruited at MDH (Study profile in figure 1). Table 1 summarizes the socio-demographic and clinical characteristics of participants. Median age of recruited women was 24 years (Interquartile range, IQR 20-31), with no significant differences according to recruitment place. No major differences could be found in relation with recruitment site, with the exception of a higher frequency of
higher education among women recruited upon delivery compared with those recruited at ANC (7.0% vs. 26.7%, p<0.001). More than one third of women (117/320, 36.6%) were HIV positive.

**Prevalence of GBS and E. coli colonization among pregnant women**

More than a fifth (68/320; 21.3%) of all recruited women were colonized by *GBS*, detected in both samples in 33 women, in 15 in the vaginal one only, and in 20 in the vagino-rectal one only. A non-statistically significant higher proportion of *GBS* were isolated from the vagino-rectal sample (16.6%) as compared to the vagina (15.0%, p=0.81). Prevalence of *GBS* colonization was borderline significantly higher among women recruited upon delivery compared to those recruited at ANC (32/120 (26.7%) vs. 36/200 (18.0%), p=0.07). Fifty-two women had *E. coli* vaginal colonization (16.3%), being significantly more common among women recruited at delivery (22.5% vs. 12.5%, p=0.019) and 10/320 (3.1%) had a positive *E. coli* urine culture. Among HIV positive pregnant women recruited, *GBS* colonization was found in 26/117 (22.2%). *E. coli* vaginal colonization was determined in 18/117 (15.4%) HIV-positive women.

**Anti-group B streptococcus antibodies**

Antibodies against *GBS* were detected in 83/120 (69.1%) women recruited at delivery. Of them, 23/32 (71.9%) were among *GBS* colonized mothers and 60/88 (68.2%) among non-colonized women (figure 2). Among HIV positive participants, AB anti-*GBS* were detected in 25/38 (65.8%) of those tested. Forty women had AB against more than one *GBS* serotype, being the most frequent AB against serotype Ia (24/120, 20%), against serotype Ib (76/120, 63.3%) and against serotype V (27/120, 22.5%). Correlating presence of antibodies to the homotypic *GBS* serotype, 2/4 (50%) women colonized by serotype Ia had AB against it, 6/6 (100%) for serotype Ib, 0/3 (0%) for serotype III and 7/14 (50%) of those carriers of Ib serotype had AB against their homotypic serotype.

**Risk factors associated to GBS and E. coli carriage**

Table 2 presents the univariate analyses of associations of the different socio-demographics, clinical and laboratory variables with vaginal *GBS* and *E. coli* carriage. In the final multivariate *GBS* model (Table 3), no risk factors were
significantly associated with GBS carriage. Similarly, no risk factors appeared to be independently associated with maternal vaginal E. coli carriage (table 4). The univariate and multivariate analyses performed to identify risk factors of GBS or E. coli colonization but restricted to HIV-infected women showed no differences compared to those including all women (data not shown).

Antimicrobial susceptibility and serotyping

One hundred and one specimens were found to be positive for GBS (48 vaginal and 53 vagino-rectal). All GBS isolates were fully sensitive to penicillin, ampicillin and ceftriaxone. Thirty-four (32.7%) isolates were resistant to erythromycin and 20 (19.2%) isolates to clindamycin. Seven isolates showed erythromycin-induced resistance to clindamycin. All the E. coli isolates were screened for susceptibility to 18 antimicrobial agents. Susceptibility to all antimicrobial agents tested was seen in 14 isolates (22.6%). E. coli was resistant to ampicillin in 21 (38.9%) cases, ceftriaxone in 2 (3.2%) cases, amoxicillin/clavulanate acid in 12 cases (19.4%), ciprofloxacin in 4 cases (6.5%) and co-trimoxazole in 38 cases (61.3%). Figure 3 summarizes the distribution of antimicrobial resistance (classifying isolates showing intermediate levels of susceptibility as resistant). Details of the resistance profiles of GBS and E. coli isolates are shown in Supplementary material table S1.

The serotype distribution of the GBS isolates is presented in Fig 4 and Fig S1 in the Supplementary material. The most prevalent serotypes were V (32.4%), Ia (14.7%), III and Ib (10.3% and 8.8%, respectively). Sixteen isolates (23.5%) were non-typeable. Twenty-six women had the same serotype detected both in the vaginal and vagino-rectal swabs, while in seven cases infections were serotype-discordant.

Neonatal outcomes

Three hundred and twenty neonatal outcomes from 316 pregnant women were recorded (98.8%). The delivery outcomes of four women in the ANC group were not registered at MDH. Neonatal outcomes included four pair of twins, 290 term babies, 25 preterm and 5 cases of stillbirths. Figure 1 illustrates neonatal outcomes and follow-up in detail. Characteristics of neonates born of mothers participating in the study may be found in table 1 and 2. Thirty-two neonates
born of 36 (88.9%) GBS carriers recruited at ANC were born at MDH, and 4 outside of the health system. Due to lack of qualified clinical staff, work saturation and advanced stage of labor, IAP strategy as recommended by CDC(35) was feasible only in two known GBS carriers at time of delivery, we administered a single dose of penicillin to 22 neonates in the first hour after birth. Two hundred and sixty-two infants (81.9%) were followed-up until 90 days of age and 8/262 (3.1%) were admitted in the hospital during this period. Seven infants died among those followed-up until 3 months after birth (2.7%), being five of them HIV-exposed (one clinical sepsis, one perinatal asphyxia and 3 unknown causes). A significantly higher risk for death among those neonates born of mothers recruited at ANC compared to those recruited at delivery (3.6% vs. 1.7%, p<0.001) was found.

Acceptability of vaginal and vagino-rectal sampling

Fifteen study participant women and five non-study pregnant women were recruited for the social component. Acceptability of collecting vaginal and vagino-rectal samples was 100%. Facilitators for acceptance included: a) Willingness to know whether they had a reproductive tract infection; b) Being interested in understanding the objectives of collecting vaginal and vagino-rectal samples; and c) Willingness to be treated and accompanied to the hospital in case of reproductive tract infection and avoiding transmitting them to their offsprings. Only a few women felt uncomfortable with sample collection, referring to feeling of burning and/or pain. Although all participants of the social component accepted sample collection, possible barriers for acceptance of future vagino-rectal sample collection were explored and these included: a) fear in relation to the first time being submitted to this procedure; b) worries regarding being seen at the hospital (stigma); c) lack of privacy at the ANC at time of sample collection.

DISCUSSION

To our knowledge, this is the first study presenting data on GBS maternal
colonization, antibodies against GBS and characterization of isolates in a rural area of Mozambique and the first time concomitantly examining E. coli colonization in pregnant women in the country. Maternal rate of GBS colonization found in this study, 21.3%, was as high as previous work in other countries in Sub-Saharan Africa reported(12). However, two previous studies performed in the capital of Mozambique, Maputo, reported an extremely low prevalence of GBS colonization among pregnant women of 1% in 1995(27) and 1.8% in 2008(25). Smaller sample sizes, different study population, and very likely laboratory and microbiology procedures utilized for GBS detection, may all contribute to explain the significant increase in terms of overall prevalence found in our study. Our findings are in close agreement with a systematic review on GBS disease in sub-Saharan Africa(12), which included 18 studies reporting data on maternal GBS colonization, finding an average GBS carriage of 21.8% (95% CI: 18.3 - 25.5) among pregnant women across the region. These results are also similar to general prevalence data from other regions, including the United States(9) and Europe(13), or from other neighboring countries in Sub-Saharan Africa such as South Africa (with similar prevalence of HIV(20, 37, 38), Zimbabwe(15) or Malawi(39), supporting the credibility of these data. The yield of vagino-rectal sampling was better for GBS colonization than using only vaginal samples as previously reported(40, 41) and recommended(35).

No risk factors independently associated with higher prevalence of GBS colonization were found in this study. We adjusted the multivariate analysis by gestational age as previous studies examining the influence of advancing gestation on GBS colonization have observed that colonization rates appear to change overtime during pregnancy(15, 42, 43). However, no associations between gestational age and colonization risk by GBS were found. Colonization prevalence was similar among age groups, in contrast to what has been described by some studies(16, 17, 44) but in concordance to a recent multicenter study performed in African settings(37), reinforcing the idea that colonization rates are quite stable across a wide variety of African settings. We did not find higher education to be a risk factor for GBS colonization as other studies have reported(18), a finding possibly influenced by the homogeneity of lower education backgrounds in our setting. Importantly, this study further
contributes to expose the fact that current understanding on maternal risk factors for colonization is incomplete.

No increased risk of *GBS* colonization among HIV-infected women was found in this study, a finding supported by other studies in USA and Zimbabwe(15, 19). However, a South African study reported that maternal *GBS* carriage was lower in HIV-positive women and among those with lower CD4 counts in Malawi(20, 39). This fact could be related to the fact that *GBS* carriage is inversely associated with the use of prophylactic co-trimoxazole among HIV-infected women. Information about co-trimoxazole use in this study was not recorded but due to high prevalence of HIV in our cohort it is likely that a high proportion of participants were routinely taking co-trimoxazole. Although HIV appears not to be a risk factor for maternal colonization during pregnancy, a recent South African study found that incidence of *GBS* neonatal disease may be up to 64% higher among HIV-exposed infants compared with non-HIV exposed ones(12).

As of today, no data are available regarding incidence of neonatal *GBS* invasive disease and HIV co-infection in Mozambique. However, studies conducted in South Africa(20, 45), with a similar HIV prevalence to the one reported in southern Mozambique(32), found an incidence of *GBS* invasive disease among infants higher than that reported in other resource-constrained settings(44, 46). Hence, it would appear reasonable to expect a high incidence of *GBS* invasive disease in this particularly HIV-struck study area. However, a low incidence of *GBS* invasive cases in neonates born to *GBS* infected women was found in this study. Reasons for this low incidence could be the high prevalence of antibodies against *GBS* found in the studied cohort (69.2%). Maternal antibody levels have been associated with protection against invasive *GBS* disease in high(47) and lower-middle income settings(48) and it has been documented that *GBS* placental transfer appears not to be affected by HIV infection(49). It is difficult to correlate our *GBS* AB results with what is known regarding *GBS* maternal colonization and infant disease. The highest proportion of women with anti-*GBS* AB was against serotype Ib, Ia and V, consistent with predominant serotypes among carriers in our cohort. Although we did not examine antibody correlation between mothers and newborns, the higher prevalence of antibodies in our cohort could also potentially explain this low incidence of *GBS* invasive disease among our neonate cohort. In addition, prevalence of carriers of
serotype III in this population, the known serotype causing more infant invasive
disease (50), was lower than reported in other African studies (12), which would
be also consistent with a lower incidence among infants. Another reason could
be the attempt to implement IAP strategy to those colonized GBS mothers
delivering at MDH. None of the neonates who received a single dose of
penicillin after birth developed symptoms of sepsis. Understanding that this
strategy is not generally recommended on account of the risks of enhancing
antimicrobial resistance, and in spite of the small sample, it could be argued
that for settings were access to health is problematic, but where GBS maternal
carriage can be confirmed, such a strategy could prove effective in decreasing
neonatal early morbidity by blocking the infection’s transmissibility at a moment
where the baby is still under the surveillance of the health system. The only
GBS case in our study was a newborn developing symptoms in the first 24
hours, born to a mother recruited at delivery with negative GBS screening. This
mother was HIV positive and was taking co-trimoxazole as prophylaxis of
opportunistic infections, suggesting an intrauterine infection with a subsequent
negativization.

On the other hand, the prevalence of E. coli found in this study was lower than
reported from other authors in different African settings (20, 37) but comparable
with the prevalence reported by Karou in Togo (51). No risk factors were found
to be independently associated with a higher risk of E. coli vaginal carriage
among pregnant women. Some studies have reported specific risks factors for
E. coli colonization, including sexual practices such as anal intercourse during
pregnancy (52) or being a sexual worker (37). Such factors were however not
explored in our study.

Importantly, GBS continues to be susceptible to penicillin, ampicillin, and
ceftriaxone in this setting. Previous studies in Ethiopia (53, 54) and South
Africa (18, 51) also reported full susceptibility of GBS strains to penicillin. Rarer
cases of decreased susceptibility to penicillin have been reported in Japan and
the United States (55). A study in Zimbabwe found almost 100 % of isolates
sensitive to penicillin, with 2% showing intermediate susceptibility to penicillin.
Resistance to erythromycin resistance among invasive GBS isolates in Europe
ranges from 3.8% to 21.2%(13) and from 7% to 25% in the USA and Canada(24). Higher levels of resistance to erythromycin (≈33%) were found in this study which could be related to mass drug administration (MDA) of azithromycin for trachoma control in sub-Saharan Africa since development of macrolide-resistant pathogens after more than one round of mass treatment has already reported(56, 57). Erythromycin resistance is frequently associated with clindamycin resistance(24). The emergence of non-susceptible GBS strains has important public health implications. GBS is still susceptible to penicillin and ampicillin which are the antibiotics of choice. Erythromycin and clindamycin are the drugs of choice for penicillin-hypersensitive patients and resistance to these antibiotics is emerging.

As other studies have reported(12), serotypes Ia, Ib, II, III and V were predominant. However, the most frequent serotype (V) found in this study differs from those found in the majority of studies conducted in other countries, revealing the need to identify prevalent serotypes in each region, as a prerequisite of establishing the potential coverage, impact and implementation requirements of future anti GBS vaccination strategies.

Characterization of E. coli isolates from this study has been described by Saez et al(58). E. coli isolates showed significant resistance to co-trimoxazole, as a previous study on diarrhoeagenic E. coli(59) conducted in Manhiça already described. Reasons for such high SXT resistance levels may include its extensive use as treatment of community-acquired infections, or as prophylaxis of HIV-related opportunistic infections(60).

This study has several limitations. Only women attending the MDH (and no other maternities) were included, and recruitment was not conducted after working hours, these being potential sources of selection bias and limiting the generalization of our results to the entire district. The first two women fulfilling inclusion criteria every day were invited to participate in the study, leading to only 200 women being recruited at ANC, and 120 additional ones upon delivery, an estimated 10% of all deliveries per year attended at MDH. Pregnant women are less likely to attend ANC at the end of their pregnancies, and some women who attend antenatal care in other maternities do actually choose MDH to
deliver. Altogether this justifies our sampling strategy, but it is important to highlight that this convenience sample may not be truly representative of the entire pregnancy cohort in the area. We did not collect information about population not sampled and we were unable to compare it with our population in order to assess such potential selection bias. However, the maternity at MDH is the biggest one in the study area and women seen there come from different places of the district and a sample of women attended at delivery was also recruited, minimizing bias. Other studies have reported association of other sexually transmitted infections such as gonorrhea or bacterial vaginosis(37) or socio-economic status(44) with GBS colonization in pregnant women, but we did not measure these variables. However, an attempt was made to explore the majority of potential risk factors described by other authors. Finally, and albeit this not being an objective of the study, it was impossible to assess the risk of GBS and E. coli transmission in this cohort, due the lack of denominator.

Conclusion

This study shows GBS and E. coli carriage among near term pregnant women is high in southern Mozambique. HIV infection was not a risk factor for GBS or E. coli colonization. Presence of anti-GBS antibodies, administration of single dose of penicillin to neonates born to colonized mothers or use of prophylactic co-trimoxazole among HIV-infected pregnant women could be reasons explaining the low incidence of GBS invasive disease among our cohort of newborns.

Screening mothers near term and providing appropriate antimicrobial prophylaxis could prevent potential adverse neonatal outcomes. Unfortunately, the fragility of the health system in LIC hinders the applicability of such approaches, and calls for innovative ideas to tackle these vertically transmitted infections. Serotype V was the most prevalent in our community and four serotypes cause the majority of cases of GBS colonization. The development and implementation of a conjugate vaccine incorporating the most commonly found serotypes globally, could enhance the transfer of maternal antibodies to the baby and protect their health in those critical first moments for survival.
REFERENCES


LEGENDS FOR FIGURES

**Figure 1.** Study profile.

ANC: antenatal clinics; IC: informed consent; IAP: intrapartum antibiotic prophylaxis. MDH: Manhiça district hospital. †Microbiologically not confirmed. ‡None received any kind of prophylaxis; §neonate died before taking samples. ‖GBS sepsis without meningitis developed in the first 24h of life.

**Figure 2.** Bubble plot demonstrating antibodies against GBS serotype Ia, Ib, II, III, IV and V in blood samples from women recruited at delivery (n=120).

OD: optical density to 450 nm with a correction at 620 nm. Cut-off value for positivity was ≥ 1 OD units.

**Figure 3.** Distribution of antimicrobial resistance among GBS and *E. coli* isolates

PEN, penicillin; AMP, ampicillin; CTX, ceftriaxone; ERY, erythromycin; CD, clindamycin; TET, tetracycline; VA, vancomycin; NAL, nalidixic acid; AMC, amoxicillin/clavulanic acid; CXM, cefuroxime; FOX, cefoxitin; CAZ, ceftazidime; AZT, aztreonam; TZP, piperacillin/tazobactam; ETP, ertapenem; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; GEN, gentamicin; SXT, trimethoprim/ sulfamethoxazole; TOB, tobramycin
Table 1. Socio-demographic and clinical characteristics of all pregnant women recruited at antenatal clinics or directly upon delivery.

<table>
<thead>
<tr>
<th></th>
<th>Overall n=320</th>
<th>Antenatal clinics recruitment n=200</th>
<th>Delivery recruitment n=120</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Socio-demographic characteristics</strong></td>
<td></td>
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<tr>
<td>Age in years</td>
<td></td>
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<tr>
<td>&lt; 21</td>
<td>112 (35.0)</td>
<td>61 (30.5)</td>
<td>51 (42.5)</td>
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<td>22 to 29</td>
<td>110 (34.4)</td>
<td>69 (34.5)</td>
<td>41 (34.2)</td>
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<td>≥30</td>
<td>98 (30.6)</td>
<td>70 (35.0)</td>
<td>28 (23.3)</td>
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<td>Second or tertiary education</td>
<td>46 (14.4)</td>
<td>14 (7.0)</td>
<td>32 (26.7)</td>
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<td>Employment</td>
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<td>6 (5.0)</td>
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<td><strong>Obstetric History</strong></td>
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<td>Age of first pregnancy (mean±SD)</td>
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<td>18.6 (±2.9)</td>
<td>18.7 (±2.7)</td>
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<td>Gravidity (mean±SD)</td>
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<td>2.9 (±1.7)</td>
<td>2.7 (±1.9)</td>
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<td>Previous abortion</td>
<td>24 (7.5)</td>
<td>12 (6.0)</td>
<td>12 (10.0)</td>
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<td><strong>History of current pregnancy</strong></td>
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<td>Gestational age in weeks at recruitment (mean±SD)</td>
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<td>36.0 (±1.2)</td>
<td>38.9 (±1.6)</td>
<td>&lt;0.001</td>
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<td>At least 3 antenatal visits during the pregnancy</td>
<td>165 (51.6)</td>
<td>95 (47.5)</td>
<td>70 (58.3)</td>
<td>0.007</td>
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<td>Gestational hypertension</td>
<td>23 (7.2)</td>
<td>16 (8.0)</td>
<td>7 (5.8)</td>
<td>0.51</td>
</tr>
<tr>
<td>Vaginal itching</td>
<td>21 (6.6)</td>
<td>21 (10.5)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>125 (39.1)</td>
<td>123 (61.5)</td>
<td>2 (1.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary symptoms</td>
<td>3 (0.9)</td>
<td>3 (1.5)</td>
<td>0 (0.0)</td>
<td>0.18</td>
</tr>
<tr>
<td>Antibiotic usage*</td>
<td>13 (4.0)</td>
<td>10 (5.0)</td>
<td>3 (2.5)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Investigations during pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syphilis positive</td>
<td>2 (0.6)</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
<td>0.70</td>
</tr>
<tr>
<td>HIV positive</td>
<td>117 (36.6)</td>
<td>79 (39.5)</td>
<td>38 (31.7)</td>
<td>0.16</td>
</tr>
<tr>
<td>HIV positive on HAART*</td>
<td>111 (94.9)</td>
<td>77 (97.5)</td>
<td>34 (89.5)</td>
<td>0.024</td>
</tr>
<tr>
<td>Anemia (&lt;11g/dL)*</td>
<td>206 (64.4)</td>
<td>152 (76.0)</td>
<td>54 (45.0)</td>
<td>0.047</td>
</tr>
<tr>
<td><strong>Neonatal Outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Term newborn</td>
<td>290 (90.6)</td>
<td>177 (88.5)</td>
<td>113 (94.2)</td>
<td>0.09</td>
</tr>
<tr>
<td>Pre term newborn</td>
<td>30 (7.8)</td>
<td>23 (9.5)</td>
<td>7 (5.0)</td>
<td></td>
</tr>
<tr>
<td>Stillbirth</td>
<td>5 (1.6)</td>
<td>4 (2.0)</td>
<td>1 (0.8)</td>
<td>0.26</td>
</tr>
<tr>
<td>Low birth weight (&lt;2500g)</td>
<td>31 (9.7)</td>
<td>9 (4.5)</td>
<td>22 (18.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Death after birth**</td>
<td>7 (2.7)</td>
<td>5 (3.6)</td>
<td>2 (1.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NA: not applicable; *HAART: highly active antiretroviral therapy; p-value was derived from Chi² test for categorical variables and t-test for quantitative variables; *Antibiotic usage two weeks before sample collection. Data available for 259 women.

**Based on data for 262 newborns followed-up 90 days after birth.**
Table 2. Univariate analysis of socio-demographic and clinical variables among women colonized by GBS or E. coli

<table>
<thead>
<tr>
<th>Socio-demographic characteristics</th>
<th>GBS colonized n= 68, n (%)</th>
<th>GBS uncolonized n=252, n (%)</th>
<th>Crude OR (95%CI)</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>GBS colonized n=52, n (%)</th>
<th>GBS uncolonized n=268, n (%)</th>
<th>Crude OR (95%CI)</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>&lt; 21</td>
<td>21 (30.9)</td>
<td>91 (36.1)</td>
<td>1.00</td>
<td>0.70</td>
<td>18 (34.6)</td>
<td>94 (35.1)</td>
<td>1.00</td>
<td>0.70</td>
</tr>
<tr>
<td>22 to 29</td>
<td>24 (35.3)</td>
<td>86 (34.1)</td>
<td>1.21 (0.6 - 2.3)</td>
<td>0.07</td>
<td>18 (34.6)</td>
<td>92 (34.3)</td>
<td>1.02 (0.5 - 2.1)</td>
<td>0.05</td>
</tr>
<tr>
<td>≥30</td>
<td>23 (33.8)</td>
<td>75 (29.8)</td>
<td>1.33 (0.7 - 2.6)</td>
<td>0.38</td>
<td>16 (30.8)</td>
<td>82 (30.6)</td>
<td>1.02 (0.5 - 2.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>Secondary or tertiary education</td>
<td>10 (14.7)</td>
<td>36 (14.3)</td>
<td>1.03 (0.5 - 2.2)</td>
<td>0.93</td>
<td>12 (23.1)</td>
<td>34 (12.7)</td>
<td>2.06 (1.0-4.3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Employment</td>
<td>5 (7.3)</td>
<td>12 (4.8)</td>
<td>1.59 (0.5 - 4.7)</td>
<td>0.40</td>
<td>2 (3.9)</td>
<td>15 (5.6)</td>
<td>0.67 (0.1-3.1)</td>
<td>0.61</td>
</tr>
<tr>
<td>History of current pregnancy</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Place of recruitment</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Antenatal clinic</td>
<td>36 (52.9)</td>
<td>164 (65.1)</td>
<td>1.00</td>
<td>0.07</td>
<td>25 (48.1)</td>
<td>175 (65.3)</td>
<td>1.00</td>
<td>0.02</td>
</tr>
<tr>
<td>At delivery</td>
<td>32 (47.1)</td>
<td>88 (34.9)</td>
<td>1.66 (0.9 - 2.9)</td>
<td>0.07</td>
<td>27 (51.9)</td>
<td>93 (34.7)</td>
<td>2.03 (1.1-2.7)</td>
<td>0.36</td>
</tr>
<tr>
<td>Gestational age at recruitment</td>
<td></td>
<td></td>
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<tr>
<td>(weeks)</td>
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<td></td>
</tr>
<tr>
<td>At least 3 antenatal visits</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>during the pregnancy</td>
<td>37 (54.4)</td>
<td>128 (50.8)</td>
<td>1.39 (0.8-2.5)</td>
<td>0.29</td>
<td>29 (64.4)</td>
<td>136 (57.6)</td>
<td>1.33 (0.7-2.6)</td>
<td>0.40</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vaginal itching</td>
<td></td>
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<tr>
<td>Vaginal discharge</td>
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<tr>
<td>Urinary symptoms</td>
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<td></td>
</tr>
<tr>
<td>Antibiotic usage&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<td></td>
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<tr>
<td>Investigations during pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Syphilis positive</td>
<td>0 (0.0)</td>
<td>2 (0.8)</td>
<td>0 (0.0)</td>
<td>0.46</td>
<td>0 (0.0)</td>
<td>2 (0.8)</td>
<td>0 (0.0)</td>
<td>0.53</td>
</tr>
<tr>
<td>HIV positive</td>
<td>26 (38.2)</td>
<td>91 (36.1)</td>
<td>1.09 (0.6-1.9)</td>
<td>0.75</td>
<td>18 (34.6)</td>
<td>99 (36.9)</td>
<td>0.90 (0.5-1.7)</td>
<td>0.75</td>
</tr>
<tr>
<td>Anemia&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38 (55.9)</td>
<td>168 (66.7)</td>
<td>0.65 (0.3-1.3)</td>
<td>0.21</td>
<td>25 (64.1)</td>
<td>181 (80.4)</td>
<td>0.43 (0.2-0.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Antibodies anti-GBS&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23 (71.9)</td>
<td>60 (68.2)</td>
<td>1.19 (0.5 - 2.9)</td>
<td>0.70</td>
<td>19 (70.4)</td>
<td>64 (68.8)</td>
<td>1.08 (0.4 - 2.8)</td>
<td>0.89</td>
</tr>
<tr>
<td>GBS colonization</td>
<td>NA</td>
<td>NA</td>
<td>1.29 (0.6-2.6)</td>
<td>0.52</td>
<td>NA</td>
<td>NA</td>
<td>1.29 (0.6-2.6)</td>
<td>0.47</td>
</tr>
<tr>
<td>E. coli colonization</td>
<td>13 (19.1)</td>
<td>39 (15.5)</td>
<td>1.29 (0.6-2.6)</td>
<td>0.52</td>
<td>NA</td>
<td>NA</td>
<td>1.29 (0.6-2.6)</td>
<td>0.47</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Term newborn</td>
<td>62 (91.2)</td>
<td>228 (90.5)</td>
<td>1.00</td>
<td>0.86</td>
<td>46 (88.5)</td>
<td>244 (91.0)</td>
<td>1.00</td>
<td>0.56</td>
</tr>
<tr>
<td>Pre term newborn</td>
<td>6 (8.8)</td>
<td>24 (9.5)</td>
<td>0.92 (0.4-2.3)</td>
<td>0.86</td>
<td>6 (11.5)</td>
<td>24 (9.0)</td>
<td>1.33 (0.5-3.4)</td>
<td>0.28</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>1 (1.4)</td>
<td>4 (1.6)</td>
<td>0.86 (0.1-7.9)</td>
<td>0.89</td>
<td>0 (0.0)</td>
<td>5 (2.4)</td>
<td>0 (0.0)</td>
<td>0.28</td>
</tr>
<tr>
<td>Low birth weight (&lt;2.500g)</td>
<td>4 (5.9)</td>
<td>27 (10.7)</td>
<td>0.52 (0.2-1.5)</td>
<td>0.24</td>
<td>7 (13.5)</td>
<td>24 (9.0)</td>
<td>1.58 (0.6-3.9)</td>
<td>0.31</td>
</tr>
<tr>
<td>Infant hospitalized in the first 90 days after birth&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2 (3.4)</td>
<td>6 (3.0)</td>
<td>1.15 (0.2-5.9)</td>
<td>0.86</td>
<td>3 (61)</td>
<td>5 (2.4)</td>
<td>2.7 (0.6-11.9)</td>
<td>0.17</td>
</tr>
<tr>
<td>Death after birth&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1 (1.4)</td>
<td>6 (2.4)</td>
<td>0.57 (0.1-4.8)</td>
<td>0.60</td>
<td>0 (0.0)</td>
<td>7 (3.3)</td>
<td>0 (0.0)</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Gestational age is presented as mean and SD. P-value was derived from Chi² test for categorical variables and t-test for quantitative variables. Data available for 260 women. Antibiotic usage two weeks before sample collection. Data available for 259 women. Data available for 264 women. Antibodies results available for 120 women recruited at delivery; data for 262 newborns 90 days after birth.
### Table 3. Multivariate analysis of socio-demographic and clinical variables of women colonized by GBS.

<table>
<thead>
<tr>
<th>Risk factors for GBS colonization</th>
<th>GBS positive n (%), N=68</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value$^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in years</strong></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td>&lt; 21</td>
<td>21 (30.9)</td>
<td>1.00</td>
<td></td>
<td>0.69</td>
</tr>
<tr>
<td>22 to 29</td>
<td>24 (35.3)</td>
<td>1.26</td>
<td>0.65</td>
<td>2.56</td>
</tr>
<tr>
<td>≥30</td>
<td>23 (33.8)</td>
<td>1.46</td>
<td>0.74</td>
<td>2.89</td>
</tr>
<tr>
<td><strong>Gestational age at recruitment</strong></td>
<td>37.3 (±2.2)</td>
<td>0.98</td>
<td>0.81</td>
<td>1.19</td>
</tr>
<tr>
<td>(mean ±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Place of recruitment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antenatal clinics</td>
<td>200 (62.5)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery ward</td>
<td>120 (37.5)</td>
<td>1.85</td>
<td>0.84</td>
<td>4.08</td>
</tr>
</tbody>
</table>

$^\circ$P-value was derived from likelihood ratio test.
Table 4. Multivariate analysis of socio-demographic and clinical variables of women colonized by *E. coli*.

<table>
<thead>
<tr>
<th>Risk factors for <em>E. coli</em> colonization</th>
<th><em>E. coli</em>, n (%)</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=52</td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td><strong>Age in years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 21</td>
<td>18 (34.6)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 to 29</td>
<td>18 (34.6)</td>
<td>0.99</td>
<td>0.47</td>
<td>2.06</td>
</tr>
<tr>
<td>≥30</td>
<td>16 (30.8)</td>
<td>1.14</td>
<td>0.53</td>
<td>2.47</td>
</tr>
<tr>
<td><strong>Gestational age at recruitment (mean ±SD)</strong></td>
<td>37.3 (±0.3)</td>
<td>0.88</td>
<td>0.71</td>
<td>1.10</td>
</tr>
<tr>
<td><strong>Secondary or tertiary education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>40 (76.9)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>12 (23.1)</td>
<td>1.58</td>
<td>0.71</td>
<td>3.49</td>
</tr>
<tr>
<td><strong>Place of recruitment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antenatal clinic</td>
<td>25 (48.1)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At delivery</td>
<td>27 (51.9)</td>
<td>2.12</td>
<td>0.77</td>
<td>5.84</td>
</tr>
<tr>
<td><strong>Vaginal discharge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>38 (73.1)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14 (26.9)</td>
<td>0.83</td>
<td>0.36</td>
<td>1.95</td>
</tr>
<tr>
<td><strong>Anemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>14 (26.9)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25 (48.1)</td>
<td>0.49</td>
<td>0.23</td>
<td>1.05</td>
</tr>
<tr>
<td>Unknown</td>
<td>13 (25.0)</td>
<td>0.77</td>
<td>0.31</td>
<td>1.91</td>
</tr>
</tbody>
</table>

*P-value was derived from likelihood ratio test.
Antenatal clinics
233 women with gestational age ≥34 weeks screened
(June - September 2014)

Exclusions
Non-emancipated minors: 3
Living out of study area: 30

200 women with gestational age ≥34 weeks recruited

32 newborns born to GBS carriers

2 newborns born to mothers receiving IAP
22 newborns received a single dose of penicillin
8 newborns did not receive prophylaxis

29 newborns followed-up 90 days after birth

0 sepsis

168 newborns born to non-GBS carriers

116 newborns followed-up 90 days after birth

4 admitted at MDH

1 clinical sepsis

Delivery ward
858 deliveries
(September 2014 – January 2015)

Exclusions
Non-emancipated minors: 19
Previously recruited in ANC: 196
Non optimal clinical condition to sign the IC: 22
Refusal to participate: 9
No time to explain study and sign IC before delivery: 56
Delivery out of working hours: 436

120 women regardless of gestational age recruited

32 newborns born to GBS carriers

116 newborns followed-up 90 days after birth

31 newborns followed-up 90 days after birth

1 clinical sepsis

88 newborns born to non-GBS carriers

86 newborns followed-up 90 days after birth

1 GBS sepsis

88 newborns born to non-GBS carriers

88 newborns followed-up 90 days after birth

88 newborns followed-up 90 days after birth

1 GBS sepsis
OD: optical density. GBS: Group B streptococcus. Area of bubbles are proportional to the number of observations overlapping on the same coordinates.
Figure 3

[Bar chart showing prevalence of resistance to various antimicrobial agents for GBS isolates and E. coli isolates.]
Figure 4
Table S1. Resistance profile of group B streptococcal and Escherichia coli isolates.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>GBS isolates, N=104</th>
<th>E.coli isolates, N=62</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recto-vaginal, n(%), N=54</td>
<td>Vaginal, n(%), N=50</td>
</tr>
<tr>
<td><strong>Penicillin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full resistant</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Intermediate resistant</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Ampicillin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full resistant</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Intermediate resistant</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Ceftriaxone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full resistant</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
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<td>4(8.0)</td>
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<tr>
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<td>4 (7.4)</td>
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<tr>
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<tr>
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NA: not applicable
Fig S1. Serotype distribution of vaginal and recto-vaginal GBS isolates

A) Serotype distribution of recto-vaginal isolates (53 samples). B) Serotype distribution of vaginal isolates (48 samples).