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

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Abstract:	<p>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.</p> <p>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</p>

	<p>(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.</p> <p>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.</p>	
<p>Suggested Reviewers:</p>	<p>Shabir Madhi shabirm@nicd.ac.za Expertise in GBS invasive disease and other paediatric infectious diseases in sub-Saharan Africa</p> <hr/> <p>Anna Seale anna.seale@lshtm.ac.uk Expert in GBS disease</p> <hr/> <p>Paul Heath pheath@sgul.ac.uk Reknown expert in GBS invasive disease among infants</p>	
<p>Funding Information:</p>	<p>ISCI (CP11/00269)</p>	<p>Quique Bassat</p>
	<p>ISCI (CP13/00260)</p>	<p>Mrs Lola Madrid</p>

Manhiça, Spain, 11th of July 2017

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 Río 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 Río Hortega of the ISCIII (CM13/00260) and Sara M. Soto has a fellowship from the program I3, of the ISCIII

Yours sincerely,

A handwritten signature in blue ink, consisting of several overlapping loops and a horizontal stroke at the bottom.

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 overtime 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. whats 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.

Lola Madrid*^{1,2} MD, MSc; **Sónia Amós Maculuve**¹ MD; **Alba Vilajeliu**³ MD, MSc; **Emma Sáez**² MD, MSc, PhD; **Sergio Massora**¹ MSc; **Anelsio Cossa**¹ Biologist; **Rosauro Varo**^{1,2} MD, MSc; **Antonio Siteo**¹ MD; **Noraida Mosqueda**² Biologist; **Rui Anselmo**¹ Sociologist; **Khatia Munguambe**¹ MSc, PhD; **Sara M Soto**² PhD; **Cinta Moraleda**^{1,4} MD, MSc, PhD; **Eusebio Macete**¹ MD, PhD; **Clara Menéndez**^{1,2,5} MD, MSc, PhD; **Quique Bassat**^{1,2,6,7} MD, MSc, PhD.

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FUNDING

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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

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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.

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

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7 **ABSTRACT**

8 **Background:** In low-income countries, data on prevalence and effects of *Group*
9 *B streptococcus* (*GBS*) and *Escherichia coli* (*E. coli*) colonization among
10 pregnant women are scarce, but necessary to formulate prevention strategies.
11 We assessed prevalence of *GBS* and *E. coli* colonization and factors
12 associated among pregnant women, its effect in newborns and acceptability
13 ~~towards regarding the utilized diagnostic sampling~~ methods in a semirural
14 Mozambican hospital.
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19 **Methods:** Pregnant women were recruited from June 2014 to January 2015,
20 during routine antenatal clinics at gestational age ≥ 34 weeks (n=200); or upon
21 delivery (n=120). Maternal risk factors were collected. Vaginal and ~~vagino-~~
22 ~~rectale-vaginal~~ samples for *GBS* and *E. coli* determination were obtained and
23 characterized in terms of antimicrobial resistance and serotype. Anti-*GBS*
24 antibodies were also determined. Neonatal follow-up was performed in the first
25 three months after birth. Semi-structured interviews were performed to ~~know~~
26 ~~investigate~~ acceptability of ~~sample collecting collection methods~~ samples.
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30 **Results:** 21.3% of women recruited were *GBS* carriers, while 16.3% were
31 positive for *E. coli*. Prevalence of HIV was 36.6%. No association was found
32 between being colonized by *GBS* and *E. coli* and maternal risk factors. *GBS*
33 isolates were fully susceptible to penicillin and ampicillin. Serotypes V (32.4%),
34 Ia (14.7%) and III (10.3%) were the most commonly found and 69.2% of the
35 women tested had IgG antibodies ~~against anti-~~ *GBS*. *E. coli* isolates showed
36 resistance to ampicillin in 28.9% and trimethoprim/sulfamethoxazole in 61.3% of
37 ~~the~~ cases.
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41 **Conclusion:** Prevalence of *GBS* and/or *E. coli* colonization among pregnant
42 women is high in this semirural community and comparable to those reported in
43 similar settings. Four serotypes accounted for nearly 70% of all isolates of *GBS*.
44 Population based data on infant *GBS* infections, would enable the design of
45 prevention strategies for *GBS* disease in Mozambique.
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7 **BACKGROUND**
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9 In 2015, 5.96 million children under the age of five died with nearly half of
10 those deaths occurring in the first 28 days of life, the so-called neonatal period
11 (1)(2). Neonatal deaths are disproportionately distributed across the globe,
12 with 95% of them taking place in developing regions and infections remain a
13 major contributor to this preventable mortality(2, 3).
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17 Vertical transmission of bacteria that are normal commensal flora or pathogens
18 of the maternal genitourinary and gastrointestinal tracts, such as *Group B*
19 *streptococcus* (*GBS*) or *Escherichia coli* (*E. coli*) are a leading determinants of
20 neonatal morbidity and mortality, causing invasive bacterial infections that can
21 manifest as sepsis, pneumonia and meningitis(4, 5). *GBS* and *E. coli* are
22 particularly associated with early-onset neonatal disease (EOD, 0-6 days after
23 birth(6)), but can also cause late-onset disease (LOD, 7-89 days(7)), preterm
24 birth and very-low-birth-weight-delivery(8, 9), all of which are responsible for
25 substantial morbidity and mortality in sub-Saharan Africa (SSA)(2, 10, 11).The
26 estimated incidence of *GBS* neonatal disease in SSA countries suggests a
27 burden at least comparable to that found in high-income countries (HIC) before
28 the implementation of the currently proposed preventive strategies(12).
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36 Maternal *GBS* carriage during the period closely related to the delivery has
37 consistently been demonstrated to determine the risk of vertical transmission,
38 and thus of ensuing neonatal disease. Prevalence of maternal
39 colonization varies from 6.5 to 36%(13) in Europe and has been reported higher
40 than 20% in Sub-Saharan countries, although precise regional maternal
41 carriage data for this continent are scarce(12, 14, 15). Maternal risk factors
42 associated with higher prevalence of *GBS* colonization are controversial. Both
43 younger(16) and older maternal ages(17) have been reported as maternal
44 characteristics associated with higher risk of *GBS* colonization, as well as
45 higher education(17), higher income(18), and high sexual activity(17). The
46 relation between HIV infection and risk for *GBS* maternal colonization has yet to
47 be fully elucidated. Studies conducted in the United States(19) or in
48 Zimbabwe(15) did not find an increased risk among HIV infected individuals,
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7 whereas researchers from South Africa(20) found a lower colonization
8 prevalence among HIV-infected mothers. Vertical transmission of *GBS* may
9 significantly increase (up to a 64% higher) among HIV-exposed infants
10 compared with non-HIV exposed ones(12).
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14 The primary intervention to reduce *GBS*-associated EOD involves the
15 administration of intrapartum antibiotic prophylaxis (IAP) to women identified to
16 either 1) be *GBS* carriers through microbiological screening (35-37 weeks'
17 gestation)(21) of samples obtained from their genito-urinary or gastrointestinal
18 lower tract; or 2) fulfil any of the different risk factors associated ~~te~~with neonatal
19 disease(22-24). In HIC, the widespread implementation of the IAP strategy has
20 ~~significantly reduced essentially decimated~~ *GBS* EOD among those babies born
21 to women in whom it was correctly applied. The IAP strategy has however not
22 demonstrated any impact on *GBS*-associated LOD, or in the prevention of *E.*
23 *coli* neonatal disease of any kind(12, 13). In low and lower-middle income
24 countries (LIC and LMIC), the fragility of the health systems and the generalized
25 lack of microbiology facilities, in the absence of a reliable rapid point of care test
26 for *GBS*, hinders the applicability of the IAP strategy, therefore jeopardising the
27 prevention of life-threatening *GBS* neonatal infections(12).
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31 Despite SSA having the highest incidence of neonatal sepsis worldwide(12),
32 epidemiological data on *GBS* and *E. coli* maternal colonization in this continent
33 are scarce. In Mozambique, as a paradigmatic example, a Pubmed search only
34 provides five results from studies reporting *GBS* data(12, 25-28), and only two
35 of those related to maternal colonization, describing a prevalence of
36 colonization as low as 1.8%(25) or even lower (1%)(27), difficult to contextualize
37 among much higher prevalence data from neighboring sub-Saharan African
38 countries(12). Additionally, and to our knowledge, no articles reporting *E. coli*
39 colonization prevalence in pregnant women in Mozambique have been
40 published and only one multicenter study conducted in South Africa, Kenya and
41 Rwanda have determined simultaneously the vaginal *GBS* and *E. coli* carriage
42 rates in SSA(29, 30). Such data, however, appear necessary for a better and
43 more evidence-based design of preventive strategies, based on the resources
44 and infrastructures available.
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7 This study aimed to determine the prevalence of pregnant women colonized
8 pregnant women by *GBS* and *E. coli* attending in a semi-rural Mozambican
9 hospital, analyze risk factors associated to higher risk of carriage by these
10 pathogens and characterize the isolates in terms of antimicrobial resistance and
11 serotype distribution. As secondary objectives, we determined the neonatal
12 outcomes and assessed the feasibility and acceptability of collecting vaginal
13 and recto-vaginalvagino-rectal samples among pregnant women, with the idea
14 of generating locally relevant data evidence useful to guide national preventive
15 strategies and policies to reduce transmission and the toll of such potentially
16 life-threatening infections in the newborn.
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22 23 24 **METHODS**

25 26 **Study site**

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28 The study was conducted in Manhiça, a semi-rural site in Southern
29 Mozambique. The Manhiça Health Research Center (CISM) runs a
30 Demographic Surveillance System (DSS) in the area and a morbidity
31 surveillance system (MSS) at the Manhiça District Hospital (MDH), across the
32 street. A detailed description of MDH, CISM and the study area can be found
33 elsewhere(31). MDH is the referral hospital for the Manhiça district, covering a
34 population of *circa* 183,000 inhabitants. The MDH includes adult and paediatric
35 wards, together with a maternity, where between 3500-4000 deliveries take
36 place annually. Institutional delivery rates is-are around 85-90% in the study
37 area. ~~#-MDH~~ also includes an outpatient department and an antenatal care
38 (ANC) clinic where pregnant women are routinely followed. As part of the
39 National policy, all pregnant women are invited to attend antenatal consultations
40 during their pregnancy, where HIV testing and other screening of infections and
41 conditions are routinely offered, in addition to intermittent preventive treatment
42 during pregnancy (IPTp) for malaria prevention, a disease highly endemic in the
43 area. Manhiça district has one of the highest prevalence rates of HIV in the
44 world, with HIV prevalence during pregnancy having been estimated at around
45 29% during antenatal consultations(32). No strategy to prevent neonatal sepsis
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7 is currently implemented in Mozambique. The hospital has recently introduced
8 WHO-recommended Option B+ for the prevention of mother-to-child HIV
9 transmission, which is offered to mothers free of charge.
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11 **Study design and population.**

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

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

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9 Sampling procedures

10 Microbiological swab samples were obtained from each participant (ANC or
11 upon admission in labour at the delivery wards, but always prior to delivery)
12 without the use of antiseptic solution or a speculum. A sample from the lower
13 third of the vagina and a fresh urine sample were taken for *E. coli*
14 determination. ~~For GBS determination, samples included a lower vaginal swab~~
15 ~~(vaginal sample), and a single swab for the vagino-rectal sample, consisting on~~
16 ~~a sample of the vagina first and then the rectum obtained performing a brief~~
17 ~~rotation of the swab through the outer sphincter. Both kinds of swabs were~~
18 ~~collected in all women in order to compare the prevalence of GBS colonization~~
19 ~~detected by the two samples~~ ~~For GBS determination, samples included a lower~~
20 ~~vaginal swab (vaginal sample), and a single swab for recto-vaginal/vagino-rectal~~
21 ~~sample, consisting on a sample of the vagina first and then the rectum through~~
22 ~~a brief rotation of the swab through the outer sphincter. Both kinds of swabs~~
23 ~~were collected in all women in order to compare the prevalence of GBS~~
24 ~~colonization detected by the two samples.~~ Swabs were immediately placed in
25 Amies transport medium and sent to the laboratory within 24 hours. The vaginal
26 and ~~recto-vaginal/vagino-rectal~~ samples for GBS determination were inoculated
27 directly onto Granada medium (Group B Streptococcus Differential Agar,
28 Becton Dickinson, Erembodegem, Belgium) incubated anaerobically at 37°C for
29 24 hours. Vaginal samples for *E. coli* determination were spread onto
30 MacConkey agar and urine samples were inoculated onto agar Cysteine lactose
31 electrolyte deficient (CLED) and MacConkey agar and incubated at 37°C
32 overnight without CO₂. *E. coli* isolates were identified based on colony
33 appearance, Gram stain, latex agglutination with the Pastorex Strepto kit (Bio-
34 rad Laboratories®, Marnes-la-Coquette, France) and standard biochemical
35 tests for *E. coli* determination. Both, GBS and *E. coli* isolates were confirmed by
36 MALDI-TOF. Resistance profiles were determined via Kirby-Bauer disk diffusion
37 method following the Clinical & Laboratory Standards Institute (CLSI)
38 guidelines.

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

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26 Maternal HIV infection status was determined and recorded if not previously
27 known. Other screening tests routinely performed at ANC, such as syphilis or
28 hemoglobin determination were also performed and recorded.
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31 32 Communication of results to mothers and case management

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

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10 Although the aim of this study was not to assess the efficacy of IAP (already
11 known), due to ethical considerations, IAP was started in *GBS* infected women
12 upon labour initiation, according to CDC guidelines(35). In cases where IAP
13 could not be adequately performed, we followed Spanish recommendations and
14 prophylactic antibiotic treatment (50,000 IU of intramuscular penicillin as a
15 single dose for a newborn weighting >2000_grams, or 25.000 IU if weight <2000
16 grams) was administered within the first hour after birth to the newborns of
17 mothers with confirmed *GBS* colonization(36). Such children were observed at
18 hospital for a minimum of 24h. For women recruited at delivery, culture results
19 were not available until at least 24-48h after recruitment. In such cases (and
20 also in cases of women with pending *GBS* result recruited prior to delivery) we
21 kept the newborns under observation for a minimum of 24 hours after delivery,
22 and provided clear recommendations to mothers regarding the need for a follow
23 up visit should the newborn become sick in the first weeks of life. Babies born to
24 study participant mothers were followed-up during the first three months after
25 birth.
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32 33 34 **Assessment of the acceptability of vaginal and ~~recto-vaginal~~vagino-rectal** 35 **sampling** 36

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

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

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38 This protocol and all supporting documentation (Informed consent documents,
39 Study questionnaires) were approved by the local bioethics committee of CISM
40 (Comité Institucional de Bioética para Saúde [do CISM](#) (CIBS-CISM)), and by
41 the National Bioethics Committee of Maputo (CNBS) in Mozambique; and by
42 the Ethics Committee of the Hospital Clínic in Barcelona, Spain. Written
43 information and consent forms in the local language were provided to the
44 women. After the interview, participants were asked to express their willingness
45 to participate in the study by signing (or thumb-printing in case they were
46 illiterate) the consent form. Participation in this study was voluntary, and study-
47 related procedures did not interfere with the pregnant women's or children's
48 standard clinical care.
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8 **RESULTS**
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10 Between June 15 2014 and January 15 2015, 320 pregnant women were
11 recruited at MDH (Study profile in figure 1). Table 1 summarizes the socio-
12 demographic and clinical characteristics of participants. Median age of recruited
13 women was 24 years (Interquartile range, IQR 20-31), with no significant
14 differences according to recruitment place. No major differences could be found
15 in relation with recruitment site, with the exception of a higher frequency of
16 higher education among women recruited upon delivery compared with those
17 recruited at ANC (7.0% vs. 26.7%, $p < 0.001$). More than one third of women
18 (117/320, 36.6%) were HIV positive.
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23 *Prevalence of GBS and E. coli colonization among pregnant women*
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25 More than a fifth (68/320; 21.3%) of all recruited women ~~was~~-were colonized by
26 *GBS*, detected in both samples in 33 women, in 15 in the vaginal one only, and
27 in 20 in the ~~recto-vaginal~~vagino-rectal one only. A non-statistically significant
28 higher proportion of *GBS* were isolated from the ~~recto-vaginal~~vagino-rectal
29 sample (16.6%) as compared to the vagina (15.0%, $p = 0.81$). Prevalence of
30 *GBS* colonization was borderline significantly higher among women recruited
31 upon delivery compared to those recruited at ANC (32/120 (26.7%) vs. 36/200
32 (18.0%), $p = 0.07$). Fifty-two women had *E. coli* vaginal colonization (16.3%),
33 being significantly more common among women recruited at delivery (22.5% vs.
34 12.5%, $p = 0.019$) and 10/320 (3.1%) had a positive *E. coli* urine culture. Among
35 HIV positive pregnant women recruited, *GBS* colonization was found in 26/117
36 (22.2%). *E. coli* vaginal colonization was determined in 18/117 (15.4%) HIV-
37 positive women.
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44 *Anti-group B streptococcus antibodies*
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46 Antibodies against *GBS* were detected in 83/120 (69.1%) women recruited at
47 delivery. Of them, 23/32 (71.9%) were among *GBS* colonized mothers and
48 60/88 (68.2%) among non-colonized women (figure 2). Among HIV positive
49 participants, AB anti-*GBS* were detected in 25/38 (65.8%) of those tested. Forty
50 women had AB against more than one *GBS* serotype, being the most frequent
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7 AB against serotype Ia (24/120, 20%), against serotype Ib (76/120, 63.3%) and
8 against serotype V (27/120, 22.5%). Correlating presence of antibodies to the
9 homotypic GBS serotype, 2/4 (50%) women colonized by serotype Ia had AB
10 against it, 6/6 (100%) for serotype Ib, 0/3 (0%) for serotype III and 7/14 (50%) of
11 those carriers of Ib serotype had AB against their homotypic serotype.
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14 Risk factors associated to GBS and E. coli carriage

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

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30 One hundred and one specimens were found to be positive for GBS (48 vaginal
31 and 53 ~~recto-vaginal~~vagino-rectal). All GBS isolates were fully sensitive to
32 penicillin, ampicillin and ceftriaxone. Thirty-four (32.7%) isolates were resistant
33 to erythromycin and 20 (19.2%) isolates to clindamycin. Seven isolates showed
34 erythromycin-induced resistance to clindamycin. –All the E. coli isolates were
35 screened for susceptibility to 18 antimicrobial agents. Susceptibility to all
36 antimicrobial agents tested was seen in 14 isolates (22.6%).– E. coli was
37 resistant to ampicillin in 21 (38.9%) cases, ceftriaxone in 2 (3.2%) cases,
38 amoxicillin/clavulanate acid in 12 cases (19.4%), ciprofloxacin in 4 cases (6.5%)
39 and co-trimoxazole in 38 cases (61.3%). Figure 3 summarizes the distribution of
40 antimicrobial resistance (classifying isolates showing intermediate levels of
41 susceptibility as resistant). Details of the resistance profiles of GBS and E. coli
42 isolates are shown in Supplementary material table S1.
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49 The serotype distribution of the GBS isolates is presented in Fig 4 and Fig S1 in
50 the Supplementary material. The most prevalent serotypes were V (32.4%), Ia
51 (14.7%), III and Ib (10.3% and 8.8%, respectively). Sixteen isolates (23.5%)
52 were non-typeable. Twenty-six women had the same serotype detected both in
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7 the vaginal and ~~recto-vaginal~~[vagino-rectal](#) swabs, while in seven cases
8 infections were serotype-discordant.
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10 Neonatal outcomes

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12 Three hundred and twenty neonatal outcomes from 316 pregnant women were
13 recorded (98.8%). ~~The Deliveries-delivery outcomes~~ of four women in the ANC
14 group were not registered at MDH. Neonatal outcomes included four pair of
15 twins, 290 term babies, 25 preterm and 5 cases of stillbirths. Figure 1 illustrates
16 neonatal outcomes and follow-up in detail. Characteristics of neonates born of
17 mothers participating in the study may be found in table 1 and 2. Thirty-two
18 neonates born of 36 (88.9%) *GBS* carriers recruited at ANC were born at MDH,
19 and 4 outside of the health system. Due to lack of qualified clinical staff, work
20 saturation and advanced stage of labor, IAP strategy as recommended by
21 CDC(35) was feasible only in two known *GBS* carriers at time of delivery, we
22 administered a single dose of penicillin to 22 neonates in the first hour after
23 birth. Two hundred and sixty-two infants (81.9%) were followed-up until 90
24 days of age and 8/262 (3.1%) were admitted in the hospital during this period.
25 Seven infants died among those followed-up until 3 months after birth (2.7%),
26 being five of them HIV-exposed (one clinical sepsis, one perinatal asphyxia and
27 3 unknown causes). A significantly higher risk for death among those neonates
28 born of mothers recruited at ANC compared to those recruited at delivery (3.6%
29 vs. 1.7%, $p<0.001$) was found.
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40 Acceptability of vaginal and ~~recto-vaginal~~[vagino-rectal](#) sampling

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

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18 To our knowledge, this is the first study presenting data on *GBS* maternal
19 colonization, antibodies ~~against~~against *GBS* and characterization of isolates in a
20 rural area of Mozambique and the first time concomitantly examining *E. coli*
21 colonization in pregnant women in the country. Maternal rate of *GBS*
22 colonization found in this study, 21.3%, was as high as previous work in other
23 countries in Sub-Saharan Africa reported(12). However, two previous studies
24 performed in the capital of Mozambique, Maputo, reported an extremely low
25 prevalence of *GBS* colonization among pregnant women of 1% in 1995(27) and
26 1.8% in 2008(25). Smaller sample sizes, different study population, and very
27 likely laboratory and microbiology procedures utilized for *GBS* detection, may all
28 contribute to explain the significant increase in terms of overall prevalence
29 found in our study. Our findings are in close agreement with a systematic review
30 on *GBS* disease in sub-Saharan Africa(12), which included 18 studies reporting
31 data on maternal *GBS* colonization, finding an average *GBS* carriage of 21.8%
32 (95% CI: 18.3 - 25.5) among pregnant women across the region. These results
33 are also similar to general prevalence data from other regions, including the
34 United States(9) and Europe(13), or from other neighboring countries in Sub-
35 Saharan Africa such as South Africa (with similar prevalence of HIV(20, 37, 38),
36 Zimbabwe(15) or Malawi(39), supporting the credibility of these data. The yield
37 of ~~recto-vaginal~~vagino-rectal sampling was better for *GBS* colonization than
38 using only vaginal samples as previously reported(40, 41) and
39 recommended(35).
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50 No risk factors independently associated with higher prevalence of *GBS*
51 colonization were found in this study. We adjusted the multivariate analysis by
52 gestational age as previous studies examining the influence of advancing
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7 gestation on *GBS* colonization have observed ~~(albeit with conflicting results)~~, as
8 [that](#) colonization rates appear to change overtime during pregnancy(15, 42, 43).

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

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21 No increased risk of *GBS* colonization among HIV-infected women was found in
22 this study, a finding supported by other studies in USA and Zimbabwe(15, 19).
23 However, a South African study reported that maternal *GBS* carriage was lower
24 in HIV-positive women and among those with lower CD4 counts in Malawi(20,
25 39). This fact could be related to the fact that *GBS* carriage is inversely
26 associated with the use of prophylactic co-trimoxazole among HIV-infected
27 women. Information about co-trimoxazole use in this study was not recorded but
28 due to high prevalence of HIV in our cohort it is likely that a high proportion of
29 participants were routinely taking co-trimoxazole. Although HIV appears not to
30 be a risk factor for maternal colonization during pregnancy, a recent South
31 African study found that incidence of *GBS* neonatal disease may be up to 64%
32 higher among HIV-exposed infants compared with non-HIV exposed ones(12).
33 As of today, no data are available regarding incidence of neonatal *GBS* invasive
34 disease and HIV co-infection in Mozambique. However, studies conducted in
35 South Africa(20, 45), with a similar HIV prevalence to the one reported in
36 southern Mozambique(32), found an incidence of *GBS* invasive disease among
37 infants higher than that reported in other resource-constrained settings(44, 46).
38 Hence, it would appear reasonable to expect a high incidence of *GBS* invasive
39 disease in this particularly HIV-struck study area. However, a low incidence of
40 *GBS* invasive cases in neonates born to *GBS* infected women was found in this
41 study. Reasons for this low incidence could be the high prevalence of
42 antibodies ~~against~~ *-GBS* found in the studied cohort (69.2%). Maternal
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7 antibody levels have been associated with protection against invasive *GBS*
8 disease in high(47) and lower-middle income settings(48) and it has been
9 documented that ~~against anti-*GBS* placental transfer is appears not to be not~~
10 affected by HIV infection(49). It is difficult to correlate our ~~against anti-*GBS* AB~~
11 results with what is known regarding *GBS* maternal colonization and infant
12 disease. The highest proportion of women with anti-*GBS* AB ~~was were~~
13 serotype Ib, Ia and V, consistent with predominant serotypes among carriers in
14 our cohort. Although we did not examine antibody correlation between mothers
15 and newborns, the higher prevalence of antibodies in our cohort could also
16 potentially explain this low incidence of *GBS* invasive disease among our
17 neonate cohort. In addition, prevalence of carriers of serotype III in this
18 population, the known serotype causing more infant invasive disease(50), was
19 lower than reported in other African studies(12), which would be also consistent
20 with a lower incidence among infants. Another reason could be the attempt to
21 implement IAP strategy to those colonized *GBS* mothers delivering at MDH.
22 None of the neonates who received a single dose of penicillin after birth
23 developed symptoms of sepsis. Understanding that this strategy is not generally
24 recommended on account of the risks of enhancing antimicrobial resistance,
25 and in spite of the small sample, it could be argued that for settings where
26 access to health is problematic, but where *GBS* maternal carriage can be
27 confirmed, such a strategy could prove effective in decreasing neonatal early
28 morbidity by blocking the infection's transmissibility at a moment where the
29 baby is still under the surveillance of the health system. The only *GBS* case in
30 our study was a newborn developing symptoms in the first 24 hours, born to a
31 mother recruited at delivery with negative *GBS* screening. This mother was HIV
32 positive and was taking co-trimoxazole as prophylaxis of opportunistic
33 infections, suggesting an intrauterine infection with a subsequent negativization.
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46 On the other hand, the prevalence of *E. coli* found in this study was lower than
47 reported from other authors in different African settings(20, 37) but comparable
48 with the prevalence reported by Karou in Togo(51). No risk factors were found
49 to be independently associated with a higher risk of *E. coli* vaginal carriage
50 among pregnant women. Some studies have reported specific risks factors for
51 *E. coli* colonization, including sexual practices such as anal intercourse during
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7 pregnancy(52) or being a sexual worker(37). Such factors were however not
8 explored in our study.
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10 Importantly, *GBS* continues to be susceptible to penicillin, ampicillin, and
11 ceftriaxone in this setting. Previous studies in Ethiopia(53, 54) and South
12 Africa(18, 51) also reported full susceptibility of *GBS* strains to penicillin. Rarer
13 cases of decreased susceptibility to penicillin have been reported in Japan and
14 the United States(55). A study in Zimbabwe found almost 100 % of isolates
15 sensitive to penicillin, with 2% showing intermediate susceptibility to penicillin.
16 Resistance to erythromycin resistance among invasive *GBS* isolates in Europe
17 ranges from 3.8% to 21.2%(13) and from 7% to 25% in the USA and
18 Canada(24). Higher levels of resistance to erythromycin (~33%) were found in
19 this study which could be related to mass drug administration (MDA) of
20 azithromycin for trachoma control in sub-Saharan Africa since development of
21 macrolide-resistant pathogens after more than one round of mass treatment has
22 already reported(56, 57). Erythromycin resistance is frequently associated with
23 clindamycin resistance(24). The emergence of non-susceptible *GBS* strains has
24 important public health implications. *GBS* is still susceptible to penicillin and
25 ampicillin which are the antibiotics of choice. Erythromycin and clindamycin are
26 the drugs of choice for penicillin-hypersensitive patients and resistance to these
27 antibiotics is emerging.
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36 As other studies have reported(12), serotypes Ia, Ib, II, III and V were
37 predominant. However, the most frequent serotype (V) found in this study
38 differs from those found in the majority of studies conducted in other countries,
39 revealing the need to identify prevalent serotypes in each region, as a
40 prerequisite of establishing the potential coverage, impact and implementation
41 requirements of future anti *GBS* vaccination strategies.
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45 Characterization of *E. coli* isolates from this study has been described by Saez
46 et al(58). *E. coli* isolates showed significant resistance to co-trimoxazole, as a
47 previous study on diarrhoeagenic *E. coli*(59) conducted in Manhiça already
48 described. Reasons for such high SXT resistance levels may include its
49 extensive use as treatment of community-acquired infections, or as prophylaxis
50 of HIV-related opportunistic infections(60).
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7 This study has several limitations. Only women attending the MDH (and no
8 other maternities) were included, and recruitment was not conducted after
9 working hours, ~~being~~ these ~~being~~ potential sources of selection bias and
10 limiting the generalization of our results to the ~~whole-entire district~~ area. The first
11 two women fulfilling inclusion criteria every day were invited to participate in the
12 study, leading to only 200 women being recruited at ANC, and 120 additional
13 ones upon delivery, an estimated 10% of all deliveries per year attended at
14 MDH. Pregnant women are less likely to attend ANC at the end of their
15 pregnancies, and some women who attend antenatal care in other maternities
16 do actually choose MDH to deliver. Altogether this justifies our sampling
17 strategy, but it is important to highlight that this ~~convenience~~ ~~convenient~~ sample
18 may not be truly representative of the entire pregnancy cohort in the area. We
19 did not collect information about population not sampled and we were unable to
20 compare it with our population in order to assess such potential selection bias.
21 However, the maternity at MDH is the biggest one in the study area and women
22 seen there come from different places of the district and a sample of women
23 attended at delivery was also recruited, minimizing bias. Other studies have
24 reported association of other sexually transmitted infections such as gonorrhoea
25 or bacterial vaginosis(37) or socio-economic status(44) with *GBS* colonization in
26 pregnant women, but we did not measure these variables. However, an attempt
27 was made to explore the majority of potential risk factors described by other
28 authors. Finally, and albeit this not being an objective of the study, it was
29 impossible to assess the risk of *GBS* and *E. coli* transmission in this cohort, due
30 the lack of denominator.
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43 Conclusion

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

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7 **LEGENDS FOR FIGURES**
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9 **Figure 1.** Study profile.

10 ANC: antenatal clinics; IC: informed consent; IAP: intrapartum antibiotic
11 prophylaxis. MDH: Manhiça district hospital. †Microbiologically not confirmed.
12 ‡None received any kind of prophylaxis; ‡neonate died before taking samples.‡
13 GBS sepsis without meningitis developed in the first 24h of life.
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17 **Figure 2.** Bubble plot demonstrating ~~antibody-antibodies~~ against GBS serotype
18 Ia, Ib, II, III, IV and V in blood samples from women recruited at delivery
19 (n=120).
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21 OD: optical density to 450 nm with a correction at 620 nm. Cut-off value for
22 positivity was ≥ 1 OD units.
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26 **Figure 3.** Distribution of antimicrobial resistance among GBS and *E. coli*
27 isolates

28 PEN, penicillin; AMP, ampicillin; CTX, ceftriaxone; ERY: erythromycin; CD,
29 clindamycin; TET, tetracycline; - VA, vancomycin; NAL, nalidixic acid; AMC,
30 amoxicillin/clavulanic acid; CXM, cefuroxime,; FOX, ceftazidime; CAZ,
31 ceftazidime; AZT, aztreonam; TZP, piperacillin/tazobactam; ETP, ertapenem;
32 IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; GEN, gentamicin; SXT,
33 trimethoprim/ sulfamethoxazole; TOB, tobramycin
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Maternal carriage of *Group B streptococcus* and *Escherichia coli* in a district hospital in Mozambique

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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

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3 In 2016, 5.6 million children under the age of five died with nearly half of those
4 deaths occurring in the first 28 days of life, the so-called neonatal period (1).
5 Neonatal deaths are disproportionately distributed across the globe, with 95% of
6 them taking place in developing regions and infections remain a major
7 contributor to this preventable mortality(2, 3).
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13 Vertical transmission of bacteria that are normal commensal flora or pathogens
14 of the maternal genitourinary and gastrointestinal tracts, such as *Group B*
15 *streptococcus (GBS)* or *Escherichia coli (E. coli)* are leading determinants of
16 neonatal morbidity and mortality, causing invasive bacterial infections that can
17 manifest as sepsis, pneumonia and meningitis(4, 5). *GBS* and *E. coli* are
18 particularly associated with early-onset neonatal disease (EOD, 0-6 days after
19 birth(6)), but can also cause late-onset disease (LOD, 7-89 days(7)), preterm
20 birth and very-low-birth-weight(8, 9), all of which are responsible for substantial
21 morbidity and mortality in sub-Saharan Africa (SSA)(2, 10, 11).The estimated
22 incidence of *GBS* neonatal disease in SSA countries suggests a burden at least
23 comparable to that found in high-income countries (HIC) before the
24 implementation of the preventive strategies(12).
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37 Maternal *GBS* carriage during the period closely related to the delivery has
38 consistently been demonstrated to determine the risk of vertical transmission,
39 and thus of ensuing neonatal disease. Prevalence of maternal colonization
40 varies from 6.5 to 36%(13) in Europe and has been reported higher than 20% in
41 Sub-Saharan countries, although precise regional maternal carriage data for
42 this continent are scarce(12, 14, 15). Maternal risk factors associated with
43 higher prevalence of *GBS* colonization are controversial. Both younger(16) and
44 older maternal ages(17) have been reported as maternal characteristics
45 associated with higher risk of *GBS* colonization, as well as higher
46 education(17), higher income(18), and high sexual activity(17). The relation
47 between HIV infection and risk for *GBS* maternal colonization has yet to be fully
48 elucidated. Studies conducted in the United States(19) or in Zimbabwe(15) did
49 not find an increased risk among HIV infected individuals, whereas researchers
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1 from South Africa(20) found a lower colonization prevalence among HIV-
2 infected mothers. Vertical transmission of *GBS* may significantly increase (up to
3 a 64% higher) among HIV-exposed infants compared with non-HIV exposed
4 ones(12).
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9 The primary intervention to reduce *GBS*-associated EOD involves the
10 administration of intrapartum antibiotic prophylaxis (IAP) to women identified to
11 either 1) be *GBS* carriers through microbiological screening (35-37 weeks'
12 gestation)(21) of samples obtained from their genito-urinary or gastrointestinal
13 lower tract; or 2) fulfil any of the different risk factors associated with neonatal
14 disease(22-24). In HIC, the widespread implementation of the IAP strategy has
15 significantly reduced *GBS* EOD among those babies born to women in whom it
16 was correctly applied. The IAP strategy has however not demonstrated any
17 impact on *GBS*-associated LOD, or in the prevention of *E. coli* neonatal disease
18 of any kind(12, 13). In low and lower-middle income countries (LIC and LMIC),
19 the fragility of the health systems and the generalized lack of microbiology
20 facilities, in the absence of a reliable rapid point of care test for *GBS*, hinders
21 the applicability of the IAP strategy, therefore jeopardising the prevention of life-
22 threatening *GBS* neonatal infections(12).
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34 Despite SSA having the highest incidence of neonatal sepsis worldwide(12),
35 epidemiological data on *GBS* and *E. coli* maternal colonization in this continent
36 are scarce. In Mozambique, as a paradigmatic example, a Pubmed search only
37 provides five results from studies reporting *GBS* data(12, 25-28), and only two
38 of those related to maternal colonization, describing a prevalence of
39 colonization as low as 1.8%(25) or even lower (1%)(27), difficult to contextualize
40 among much higher prevalence data from neighboring sub-Saharan African
41 countries(12). Additionally, and to our knowledge, no articles reporting *E. coli*
42 colonization prevalence in pregnant women in Mozambique have been
43 published and only one multicenter study conducted in South Africa, Kenya
44 and Rwanda have determined simultaneously the vaginal *GBS* and *E. coli* carriage
45 rates in SSA(29, 30). Such data, however, appear necessary for a better and
46 more evidence-based design of preventive strategies, based on the resources
47 and infrastructures available.
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1 This study aimed to determine the prevalence of pregnant women colonized by
2 *GBS* and *E. coli* attending a semi-rural Mozambican hospital, analyze risk
3 factors associated to higher risk of carriage by these pathogens and
4 characterize the isolates in terms of antimicrobial resistance and serotype
5 distribution. As secondary objectives, we determined the neonatal outcomes
6 and assessed the feasibility and acceptability of collecting vaginal and vagino-
7 rectal samples among pregnant women, with the idea of generating locally-
8 relevant data useful to guide national preventive strategies and policies to
9 reduce transmission and the toll of such potentially life-threatening infections in
10 the newborn.
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20 **METHODS**

21 **Study site**

22 The study was conducted in Manhiça, a semi-rural site in Southern
23 Mozambique. The Manhiça Health Research Center (CISM) runs a
24 Demographic Surveillance System (DSS) in the area and a morbidity
25 surveillance system (MSS) at the Manhiça District Hospital (MDH), across the
26 street. A detailed description of MDH, CISM and the study area can be found
27 elsewhere(31). MDH is the referral hospital for the Manhiça district, covering a
28 population of *circa* 183,000 inhabitants. The MDH includes adult and paediatric
29 wards, together with a maternity, where between 3500-4000 deliveries take
30 place annually. Institutional delivery rates are around 85-90% in the study area.
31 MDH also includes an outpatient department and an antenatal care (ANC) clinic
32 where pregnant women are routinely followed. As part of the National policy, all
33 pregnant women are invited to attend antenatal consultations during their
34 pregnancy, where HIV testing and other screening of infections and conditions
35 are routinely offered, in addition to intermittent preventive treatment during
36 pregnancy (IPTp) for malaria prevention, a disease highly endemic in the area.
37 Manhiça district has one of the highest prevalence rates of HIV in the world,
38 with HIV prevalence during pregnancy having been estimated at around 29%
39 during antenatal consultations(32). No strategy to prevent neonatal sepsis is
40 currently implemented in Mozambique. The hospital has recently introduced
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1 WHO-recommended Option B+ for the prevention of mother-to-child HIV
2 transmission, which is offered to mothers free of charge.
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5 **Study design and population.** 6

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8 This observational prospective study was conducted at the ANC and delivery
9 wards of MDH, between June 15 2014 and January 15 2015, running
10 continuously during working hours (8:00–16:00) and working days. We recruited
11 pregnant women at two different time-points during their pregnancy. One group
12 during routine antenatal care with a minimum estimated gestational age ≥ 34
13 weeks, as measured by fundal height ≥ 32 cm, 2 cm above the midpoint
14 between umbilicus and xiphoid process. A second group of women was
15 recruited upon delivery (regardless of gestational age) if they were not recruited
16 at ANC clinics, in order to understand real life risk for vertical transmission rate
17 of *GBS* or *E. coli* to their offspring with no interference of antibiotic treatments.
18 Participants were eligible for inclusion if they lived in the study area, were in
19 good physical and mental health, able and willing to participate in the study and
20 to provide informed consent. All women fulfilling inclusion criteria were eligible
21 to participate in the study, and in order to obtain a more representative sample
22 of the study population, the first two women seen every day were approached
23 for recruitment.
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40 **Definitions** 41

42 *GBS* colonization was considered in the event of a positive vaginal or vagino-
43 rectal culture for *GBS*. *E. coli* colonization was considered when the positive
44 vaginal culture grew *E. coli*. *E. coli* urinary tract infection was diagnosed when
45 *E. coli* grew ($>10^5$ colony-forming units/mL) in the urine samples of pregnant
46 women. Abortion was defined as pregnancy termination prior to 20 weeks'
47 gestation or a foetus born weighting less than 500 g(33). A preterm baby was
48 defined as that with a gestational age at birth <37 weeks and stillbirth as
49 intrauterine deaths occurring after 28 weeks of gestational age. Low-birth
50 weight was defined as weight at birth $<2,500$ grams.
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60 **Study procedures** 61 62 63 64 65

Sampling procedures

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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 37°C 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 37°C 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 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

1 electrophoresis using 1% agarose gels. Antibody (AB) determination was
2 identified in blood samples of mothers recruited at delivery. They were
3 performed by ELISA using whole bacteria as antigens. This procedure is a
4 modification based on the protocol proposed by Baker et al (33), using an
5 optical density to 450 nm with a correction at 620 nm. The cut-off value for
6 positivity was chosen to be ≥ 1 OD units, in order to be more strict than the one
7 proposed by Baker (>0.125).

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9 Maternal HIV infection status was determined and recorded if not previously
10 known. Other screening tests routinely performed at ANC, such as syphilis or
11 hemoglobin determination were also performed and recorded.
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13 Communication of results to mothers and case management

14 Clinical assessment and management of patients was done following
15 international guidelines for countries with no clear screen-and-treat national
16 rules, both at the ANC and at the maternity. For those women identified as
17 carriers of *GBS* in vagino-rectal swabs collected at the ANC, a field worker
18 delivered to the mother at home a study card detailing the microbiological
19 findings, together with indications of what to do during delivery, so that
20 intrapartum antibiotics could be administered to the mother, following the CDC
21 guidelines(35). Urinary tract infections secondary to *E. coli* were also reported
22 and treated according to national guidelines for pregnant women. All recruited
23 women were encouraged to deliver at hospital and clinical staff was trained to
24 identify them. Any child born to a recruited mother and found to be sick at
25 delivery was assessed by a study clinician, and routine screening for bacterial
26 surveillance (including a blood culture and a lumbar puncture to obtain
27 cerebrospinal fluid (CSF)) performed and clinical management organized
28 according to MDH guidelines.
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30 Although the aim of this study was not to assess the efficacy of IAP (already
31 known), due to ethical considerations, IAP was started in *GBS* infected women
32 upon labour initiation, according to CDC guidelines(35). In cases where IAP
33 could not be adequately performed, we followed Spanish recommendations and
34 prophylactic antibiotic treatment (50,000 IU of intramuscular penicillin as a
35 single dose for a newborn weighting >2000 grams, or 25.000 IU if weight <2000
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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

1 variables were compared using a Chi-squared test or Fisher's exact test.
2 Continuous variables were described as mean (standard deviation, SD) or
3 median values (interquartile range, IQR) and were compared using the t test for
4 normal distributions or the Mann Whitney test for skewed data. Logistic
5 regression univariate and multivariate analyses were performed to identify risk
6 factors for *GBS* or *E. coli* colonization, separately. Variables that were found to
7 be significantly associated with *GBS* or *E. coli* in the univariate analysis together
8 with those related at a significance level of $p<0.10$ were entered into a
9 multivariate model. Age and gestational age at recruitment were also included
10 in the multivariate analysis based on previous studies(9, 15-17). A separate
11 univariate and multivariate analysis of risk factor associated to *GBS* or *E. coli*
12 colonization among HIV pregnant women was also performed.
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22 **Ethical considerations**

23 This protocol and all supporting documentation (Informed consent documents,
24 Study questionnaires) were approved by the local bioethics committee of CISM
25 (Comité Institucional de Bioética para Saúde do CISM (CIBS-CISM)), and by
26 the National Bioethics Committee of Maputo (CNBS) in Mozambique; and by
27 the Ethics Committee of the Hospital Clínic in Barcelona, Spain. Written
28 information and consent forms in the local language were provided to the
29 women. After the interview, participants were asked to express their willingness
30 to participate in the study by signing (or thumb-printing in case they were
31 illiterate) the consent form. Participation in this study was voluntary, and study-
32 related procedures did not interfere with the pregnant women's or children's
33 standard clinical care.
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48 **RESULTS**

49 Between June 15 2014 and January 15 2015, 320 pregnant women were
50 recruited at MDH (Study profile in figure 1). Table 1 summarizes the socio-
51 demographic and clinical characteristics of participants. Median age of recruited
52 women was 24 years (Interquartile range, IQR 20-31), with no significant
53 differences according to recruitment place. No major differences could be found
54 in relation with recruitment site, with the exception of a higher frequency of
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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

1 significantly associated with *GBS* carriage. Similarly, no risk factors appeared to
2 be independently associated with maternal vaginal *E. coli* carriage (table 4).
3 The univariate and multivariate analyses performed to identify risk factors of
4 *GBS* or *E. coli* colonization but restricted to HIV-infected women showed no
5 differences compared to those including all women (data not shown).
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10 Antimicrobial susceptibility and serotyping

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12 One hundred and one specimens were found to be positive for *GBS* (48 vaginal
13 and 53 vagino-rectal). All *GBS* isolates were fully sensitive to penicillin,
14 ampicillin and ceftriaxone. Thirty-four (32.7%) isolates were resistant to
15 erythromycin and 20 (19.2%) isolates to clindamycin. Seven isolates showed
16 erythromycin-induced resistance to clindamycin. All the *E. coli* isolates were
17 screened for susceptibility to 18 antimicrobial agents. Susceptibility to all
18 antimicrobial agents tested was seen in 14 isolates (22.6%). *E. coli* was
19 resistant to ampicillin in 21 (38.9%) cases, ceftriaxone in 2 (3.2%) cases,
20 amoxicillin/clavulanate acid in 12 cases (19.4%), ciprofloxacin in 4 cases (6.5%)
21 and co-trimoxazole in 38 cases (61.3%). Figure 3 summarizes the distribution of
22 antimicrobial resistance (classifying isolates showing intermediate levels of
23 susceptibility as resistant). Details of the resistance profiles of *GBS* and *E. coli*
24 isolates are shown in Supplementary material table S1.
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36 The serotype distribution of the *GBS* isolates is presented in Fig 4 and Fig S1 in
37 the Supplementary material. The most prevalent serotypes were V (32.4%), Ia
38 (14.7%), III and Ib (10.3% and 8.8%, respectively). Sixteen isolates (23.5%)
39 were non-typeable. Twenty-six women had the same serotype detected both in
40 the vaginal and vagino-rectal swabs, while in seven cases infections were
41 serotype-discordant.
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47 Neonatal outcomes

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

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

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59 To our knowledge, this is the first study presenting data on *GBS* maternal
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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

1 contributes to expose the fact that current understanding on maternal risk
2 factors for colonization is incomplete.

3 No increased risk of *GBS* colonization among HIV-infected women was found in
4 this study, a finding supported by other studies in USA and Zimbabwe(15, 19).
5 However, a South African study reported that maternal *GBS* carriage was lower
6 in HIV-positive women and among those with lower CD4 counts in Malawi(20,
7 39). This fact could be related to the fact that *GBS* carriage is inversely
8 associated with the use of prophylactic co-trimoxazole among HIV-infected
9 women. Information about co-trimoxazole use in this study was not recorded but
10 due to high prevalence of HIV in our cohort it is likely that a high proportion of
11 participants were routinely taking co-trimoxazole. Although HIV appears not to
12 be a risk factor for maternal colonization during pregnancy, a recent South
13 African study found that incidence of *GBS* neonatal disease may be up to 64%
14 higher among HIV-exposed infants compared with non-HIV exposed ones(12).
15 As of today, no data are available regarding incidence of neonatal *GBS* invasive
16 disease and HIV co-infection in Mozambique. However, studies conducted in
17 South Africa(20, 45), with a similar HIV prevalence to the one reported in
18 southern Mozambique(32), found an incidence of *GBS* invasive disease among
19 infants higher than that reported in other resource-constrained settings(44, 46).
20 Hence, it would appear reasonable to expect a high incidence of *GBS* invasive
21 disease in this particularly HIV-struck study area. However, a low incidence of
22 *GBS* invasive cases in neonates born to *GBS* infected women was found in this
23 study. Reasons for this low incidence could be the high prevalence of
24 antibodies against *GBS* found in the studied cohort (69.2%). Maternal antibody
25 levels have been associated with protection against invasive *GBS* disease in
26 high(47) and lower-middle income settings(48) and it has been documented that
27 *GBS* placental transfer appears not to be affected by HIV infection(49). It is
28 difficult to correlate our *GBS* AB results with what is known regarding *GBS*
29 maternal colonization and infant disease. The highest proportion of women with
30 anti-*GBS* AB was against serotype Ib, Ia and V, consistent with predominant
31 serotypes among carriers in our cohort. Although we did not examine antibody
32 correlation between mothers and newborns, the higher prevalence of antibodies
33 in our cohort could also potentially explain this low incidence of *GBS* invasive
34 disease among our neonate cohort. In addition, prevalence of carriers of

1 serotype III in this population, the known serotype causing more infant invasive
2 disease(50), was lower than reported in other African studies(12), which would
3 be also consistent with a lower incidence among infants. Another reason could
4 be the attempt to implement IAP strategy to those colonized *GBS* mothers
5 delivering at MDH. None of the neonates who received a single dose of
6 penicillin after birth developed symptoms of sepsis. Understanding that this
7 strategy is not generally recommended on account of the risks of enhancing
8 antimicrobial resistance, and in spite of the small sample, it could be argued
9 that for settings where access to health is problematic, but where *GBS* maternal
10 carriage can be confirmed, such a strategy could prove effective in decreasing
11 neonatal early morbidity by blocking the infection's transmissibility at a moment
12 where the baby is still under the surveillance of the health system. The only
13 *GBS* case in our study was a newborn developing symptoms in the first 24
14 hours, born to a mother recruited at delivery with negative *GBS* screening. This
15 mother was HIV positive and was taking co-trimoxazole as prophylaxis of
16 opportunistic infections, suggesting an intrauterine infection with a subsequent
17 negativization.
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32 On the other hand, the prevalence of *E. coli* found in this study was lower than
33 reported from other authors in different African settings(20, 37) but comparable
34 with the prevalence reported by Karou in Togo(51). No risk factors were found
35 to be independently associated with a higher risk of *E. coli* vaginal carriage
36 among pregnant women. Some studies have reported specific risks factors for
37 *E. coli* colonization, including sexual practices such as anal intercourse during
38 pregnancy(52) or being a sexual worker(37). Such factors were however not
39 explored in our study.
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48 Importantly, *GBS* continues to be susceptible to penicillin, ampicillin, and
49 ceftriaxone in this setting. Previous studies in Ethiopia(53, 54) and South
50 Africa(18, 51) also reported full susceptibility of *GBS* strains to penicillin. Rarer
51 cases of decreased susceptibility to penicillin have been reported in Japan and
52 the United States(55). A study in Zimbabwe found almost 100 % of isolates
53 sensitive to penicillin, with 2% showing intermediate susceptibility to penicillin.
54 Resistance to erythromycin resistance among invasive *GBS* isolates in Europe
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1 ranges from 3.8% to 21.2%(13) and from 7% to 25% in the USA and
2 Canada(24). Higher levels of resistance to erythromycin (~33%) were found in
3 this study which could be related to mass drug administration (MDA) of
4 azithromycin for trachoma control in sub-Saharan Africa since development of
5 macrolide-resistant pathogens after more than one round of mass treatment has
6 already reported(56, 57). Erythromycin resistance is frequently associated with
7 clindamycin resistance(24). The emergence of non-susceptible *GBS* strains has
8 important public health implications. *GBS* is still susceptible to penicillin and
9 ampicillin which are the antibiotics of choice. Erythromycin and clindamycin are
10 the drugs of choice for penicillin-hypersensitive patients and resistance to these
11 antibiotics is emerging.
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21 As other studies have reported(12), serotypes Ia, Ib, II, III and V were
22 predominant. However, the most frequent serotype (V) found in this study
23 differs from those found in the majority of studies conducted in other countries,
24 revealing the need to identify prevalent serotypes in each region, as a
25 prerequisite of establishing the potential coverage, impact and implementation
26 requirements of future anti *GBS* vaccination strategies.
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32 Characterization of *E. coli* isolates from this study has been described by Saez
33 et al(58). *E. coli* isolates showed significant resistance to co-trimoxazole, as a
34 previous study on diarrhoeagenic *E. coli*(59) conducted in Manhiça already
35 described. Reasons for such high SXT resistance levels may include its
36 extensive use as treatment of community-acquired infections, or as prophylaxis
37 of HIV-related opportunistic infections(60).
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44 This study has several limitations. Only women attending the MDH (and no
45 other maternities) were included, and recruitment was not conducted after
46 working hours, these being potential sources of selection bias and limiting the
47 generalization of our results to the entire district. The first two women fulfilling
48 inclusion criteria every day were invited to participate in the study, leading to
49 only 200 women being recruited at ANC, and 120 additional ones upon delivery,
50 an estimated 10% of all deliveries per year attended at MDH. Pregnant women
51 are less likely to attend ANC at the end of their pregnancies, and some women
52 who attend antenatal care in other maternities do actually choose MDH to
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1 deliver. Altogether this justifies our sampling strategy, but it is important to
2 highlight that this convenience sample may not be truly representative of the
3 entire pregnancy cohort in the area. We did not collect information about
4 population not sampled and we were unable to compare it with our population in
5 order to assess such potential selection bias. However, the maternity at MDH is
6 the biggest one in the study area and women seen there come from different
7 places of the district and a sample of women attended at delivery was also
8 recruited, minimizing bias. Other studies have reported association of other
9 sexually transmitted infections such as gonorrhoea or bacterial vaginosis(37) or
10 socio-economic status(44) with *GBS* colonization in pregnant women, but we
11 did not measure these variables. However, an attempt was made to explore the
12 majority of potential risk factors described by other authors. Finally, and albeit
13 this not being an objective of the study, it was impossible to assess the risk of
14 *GBS* and *E. coli* transmission in this cohort, due the lack of denominator.
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29 **Conclusion**

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31 This study shows *GBS* and *E. coli* carriage among near term pregnant women
32 is high in southern Mozambique. HIV infection was not a risk factor for *GBS* or
33 *E. coli* colonization. Presence of anti-*GBS* antibodies, administration of single
34 dose of penicillin to neonates born to colonized mothers or use of prophylactic
35 co-trimoxazole among HIV-infected pregnant women could be reasons
36 explaining the low incidence of *GBS* invasive disease among our cohort of
37 newborns.
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45 Screening mothers near term and providing appropriate antimicrobial
46 prophylaxis could prevent potential adverse neonatal outcomes. Unfortunately,
47 the fragility of the health system in LIC hinders the applicability of such
48 approaches, and calls for innovative ideas to tackle these vertically transmitted
49 infections. Serotype V was the most prevalent in our community and four
50 serotypes cause the majority of cases of *GBS* colonization. The development
51 and implementation of a conjugate vaccine incorporating the most commonly
52 found serotypes globally, could enhance the transfer of maternal antibodies to
53 the baby and protect their health in those critical first moments for survival.
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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, cefoxitine; 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.

	Overall n=320 n (%)	Antenatal clinics recruitment n=200 n (%)	Delivery recruitment n=120 n (%)	p value [§]
Socio-demographic characteristics				
Age in years				0.040
< 21	112 (35.0)	61 (30.5)	51 (42.5)	
22 to 29	110 (34.4)	69 (34.5)	41 (34.2)	
≥30	98 (30.6)	70 (35.0)	28 (23.3)	
Secondary or tertiary education	46 (14.4)	14 (7.0)	32 (26.7)	<0.001
Employment	17(5.3)	11 (5.5)	6 (5.0)	0.85
Obstetric History				
Age of first pregnancy (mean±SD)	18.6 (±2.6)	18.6 (±2.9)	18.7 (±2.7)	0.67
Gravidity (mean±SD)	2.8 (±1.8)	2.9 (±1.7)	2.7 (±1.9)	0.43
Previous abortion	24 (7.5)	12 (6.0)	12 (10.0)	0.19
History of current pregnancy				
Gestational age in weeks at recruitment (mean±SD)	37.1 (±2.0)	36.0 (±1.2)	38.9 (±1.6)	<0.001
At least 3 antenatal visits during the pregnancy	165 (51.6)	95 (47.5)	70 (58.3)	0.007
Gestational hypertension	23 (7.2)	16 (8.0)	7 (5.8)	0.51
Vaginal itching	21 (6.6)	21 (10.5)	0 (0)	<0.001
Vaginal discharge	125 (39.1)	123 (61.5)	2 (1.7)	<0.001
Urinary symptoms	3 (0.9)	3 (1.5)	0 (0.0)	0.18
Antibiotic usage[‡]	13 (4.0)	10 (5.0)	3 (2.5)	0.10
Investigations during pregnancy				
Syphilis positive	2 (0.6)	1 (0.5)	1 (0.5)	0.70
HIV positive	117 (36.6)	79 (39.5)	38 (31.7)	0.16
HIV positive on HAART^ψ	111 (94.9)	77 (97.5)	34 (89.5)	0.024
Anemia (<11g/dL)^h	206 (64.4)	152 (76.0)	54 (45.0)	0.047
Neonatal Outcome				
Gestational age at birth				
Term newborn	290 (90.6)	177 (88.5)	113 (94.2)	0.09
Pre term newborn	30 (7.8)	23 (9.5)	7 (5.0)	
Stillbirth	5 (1.6)	4 (2.0)	1 (0.8)	0.26
Low birth weight (<2500g)	31 (9.7)	9 (4.5)	22 (18.3)	<0.001
Death after birth[™]	7 (2.7)	5 (3.6)	2 (1.7)	<0.001

NA: not applicable; ^ψHAART: 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*

	GBS colonized n= 68, n (%)	GBS uncolonized n=252, n (%)	Crude OR (95%CI)	p value [§]	<i>E. coli</i> colonized n= 52, n (%)	<i>E. coli</i> uncolonized n=268, n (%)	Crude OR (95%CI)	p value [§]
Socio-demographic characteristics								
Age in years				0.70				0.70
< 21	21 (30.9)	91 (36.1)	1.00		18 (34.6)	94 (35.1)	1.00	
22 to 29	24 (35.3)	86 (34.1)	1.21 (0.6 - 2.3)		18 (34.6)	92 (34.3)	1.02 (0.5- 2.1)	
≥30	23 (33.8)	75 (29.8)	1.33 (0.7 - 2.6)		16 (30.8)	82 (30.6)	1.02 (0.5 - 2.1)	
Secondary or tertiary education	10 (14.7)	36 (14.3)	1.03 (0.5-2.2)	0.93	12 (23.1)	34 (12.7)	2.06 (1.0-4.3)	0.05
Employment	5 (7.3)	12 (4.8)	1.59 (0.5-4.7)	0.40	2 (3.9)	15 (5.6)	0.67 (0.1-3.1)	0.61
History of current pregnancy								
Place of recruitment								
Antenatal clinic	36 (52.9)	164 (65.1)	1.00		25 (48.1)	175 (65.3)	1.00	0.02
At delivery	32 (47.1)	88 (34.9)	1.66 (0.9 - 2.9)	0.07	27 (51.9)	93 (34.7)	2.03 (1.1-2.7)	
Gestational age at recruitment (weeks) (mean±SD)[¶]	37.3 (±2.2)	37.0 (±1.9)	1.08 (0.9-1.2)	0.38	37.3 (±0.3)	37.0 (±0.1)	1.07 (0.9-1.2)	0.36
At least 3 antenatal visits during the pregnancy	37 (54.4)	128 (50.8)	1.39 (0.8-2.5)	0.29	29 (64.4)	136 (57.6)	1.33 (0.7-2.6)	0.40
Gestational hypertension	4 (5.9)	19 (7.5)	0.75 (0.2-2.3)	0.26	4 (8.0)	19 (7.2)	1.13 (0.4-3.5)	0.84
Vaginal itching	2 (2.9)	19 (7.5)	0.37 (0.1 – 1.6)	0.17	4 (7.7)	17 (6.3)	1.23 (0.4 – 3.8)	0.13
Vaginal discharge	22 (32.4)	103 (40.9)	0.69 (0.4-1.2)	0.20	14 (26.9)	111 (41.4)	0.52 (0.3-1.0)	0.05
Urinary symptoms	0 (0.0)	3 (1.2)	0 (0.0)	0.37	1 (1.9)	2 (0.8)	2.61 (0.2-29.5)	0.42
Antibiotic usage[‡]	3 (4.4)	10 (4.0)	1.07 (0.3 -4.0)	0.92	2 (3.8)	11 (4.1)	0.78 (0.2-2.9)	0.69
Investigations during pregnancy								
Syphilis positive	0 (0.0)	2 (0.8)	0 (0.0)	0.46	0 (0.0)	2 (0.8)	0 (0.0)	0.53
HIV positive	26 (38.2)	91 (36.1)	1.09 (0.6-1.9)	0.75	18 (34.6)	99 (36.9)	0.90 (0.5-1.7)	0.75
Anemia^h	38 (55.9)	168 (66.7)	0.65 (0.3-1.3)	0.21	25 (64.1)	181 (80.4)	0.43 (0.2-0.9)	0.02
Antibodies anti-GBS^g	23 (71.9)	60 (68.2)	1.19 (0.5 - 2.9)	0.70	19 (70.4)	64 (68.8)	1.08 (0.4 - 2.8)	0.89
GBS colonization	NA	NA	NA		13 (25)	55 (20.5)	1.29 (0.6-2.6)	0.47
<i>E. coli</i> colonization	13 (19.1)	39 (15.5)	1.29 (0.6-2.6)	0.52	NA	NA	NA	
Outcome								
Gestational age at birth								
Term newborn	62 (91.2)	228 (90.5)	1.00		46 (88.5)	244 (91.0)	1.00	0.56
Pre term newborn	6 (8.8)	24 (9.5)	0.92 (0.4-2.3)	0.86	6 (11.5)	24 (9.0)	1.33 (0.5-3.4)	
Stillbirth	1 (1.4)	4 (1.6)	0.86 (0.1-7.9)	0.89	0 (0.0)	5 (2.4)	0 (0.0)	0.28
Low birth weight (<2500g)	4 (5.9)	27 (10.7)	0.52 (0.2-1.5)	0.24	7 (13.5)	24 (9.0)	1.58 (0.6-3.9)	0.31
Infant hospitalized in the first 90 days after birth^m	2 (3.4)	6 (3.0)	1.15 (0.2-5.9)	0.86	3 (6.1)	5 (2.4)	2.7 (0.6-11.9)	0.17
Death after birth^m	1 (1.4)	6 (2.4)	0.57 (0.1-4.8)	0.60	0 (0.0)	7 (3.3)	0 (0.0)	0.19

[†]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*.

Risk factors for <i>GBS</i> colonization	<i>GBS</i> positive n (%), N=68	Adjusted OR	95% CI		p-value ^a
			Lower	Upper	
Age in years					
< 21	21 (30.9)	1.00			
22 to 29	24 (35.3)	1.26	0.65	2.56	0.69
≥30	23 (33.8)	1.46	0.74	2.89	
Gestational age at recruitment (mean ±SD)					
	37.3 (±2.2)	0.98	0.81	1.19	0.82
Place of recruitment					
Antenatal clinics	200 (62.5)	1.00			
Delivery ward	120 (37.5)	1.85	0.84	4.08	0.125

^aP-value was derived from likelihood ratio test.

Table 4. Multivariate analysis of socio-demographic and clinical variables of women colonized by *E. coli*.

Risk factors for <i>E. coli</i> colonization	<i>E. coli</i> , n (%), N=52	Adjusted OR	95% CI		p-value ⁶
			Lower	Upper	
Age in years					
< 21	18 (34.6)	1.00			
22 to 29	18 (34.6)	0.99	0.47	2.06	0.97
≥30	16 (30.8)	1.14	0.53	2.47	
Gestational age at recruitment (mean ±SD)					
	37.3 (±0.3)	0.88	0.71	1.10	0.27
Secondary or tertiary education					
Negative	40 (76.9)	1.00			
Positive	12 (23.1)	1.58	0.71	3.49	0.26
Place of recruitment					
Antenatal clinic	25 (48.1)	1.00			
At delivery	27 (51.9)	2.12	0.77	5.84	0.15
Vaginal discharge					
No	38 (73.1)	1.00			
Yes	14 (26.9)	0.83	0.36	1.95	0.67
Anemia					
No	14 (26.9)	1.00			
Yes	25 (48.1)	0.49	0.23	1.05	0.18
Unknown	13 (25.0)	0.77	0.31	1.91	

⁶P-value was derived from likelihood ratio test.

Figure 1

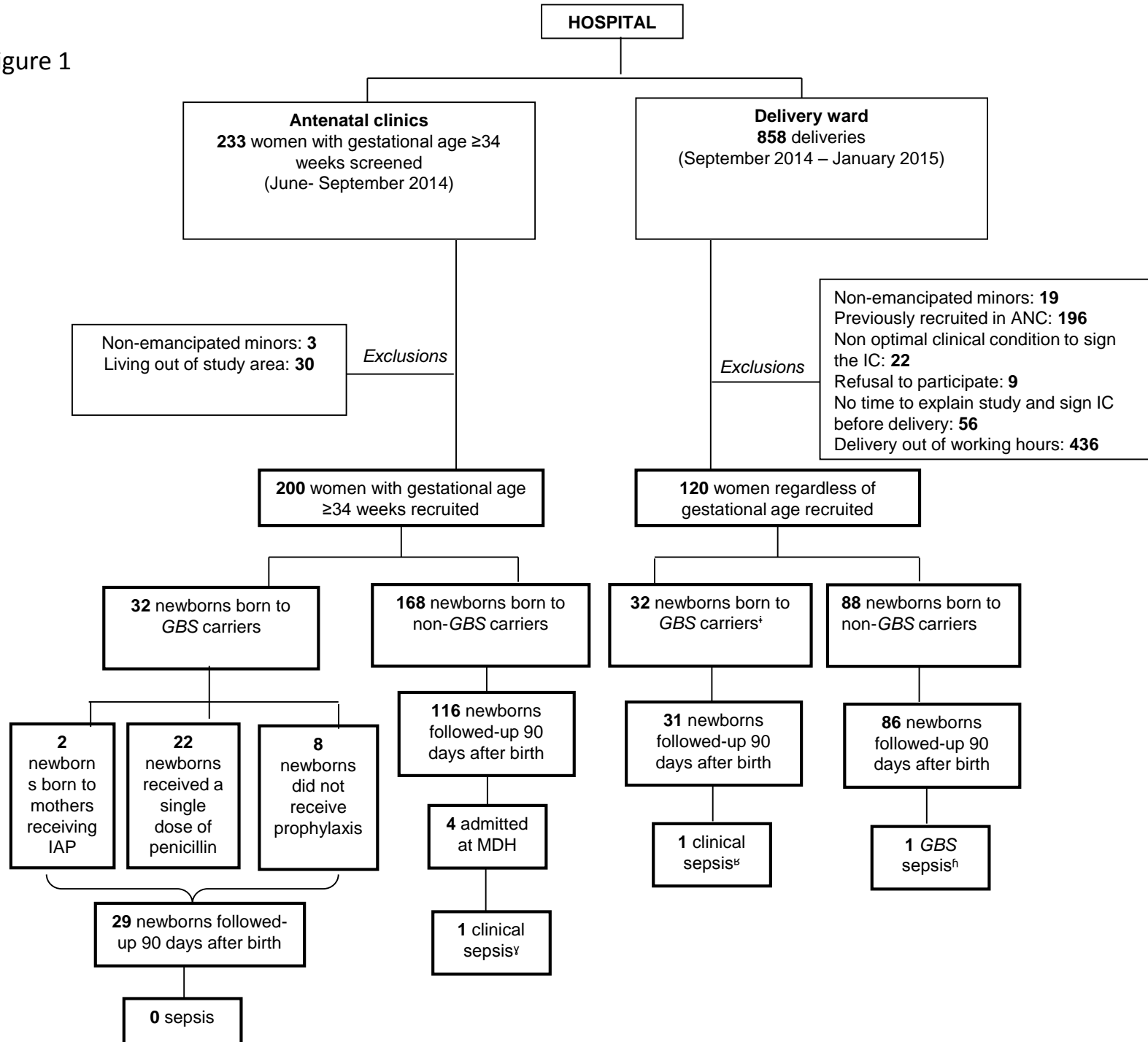
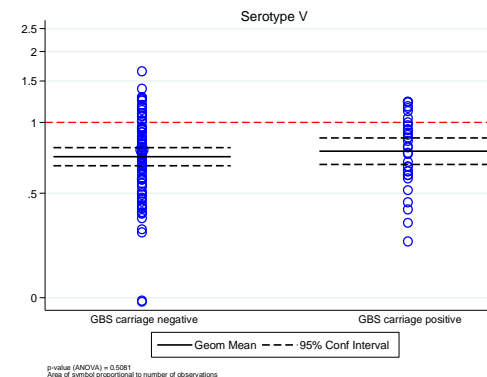
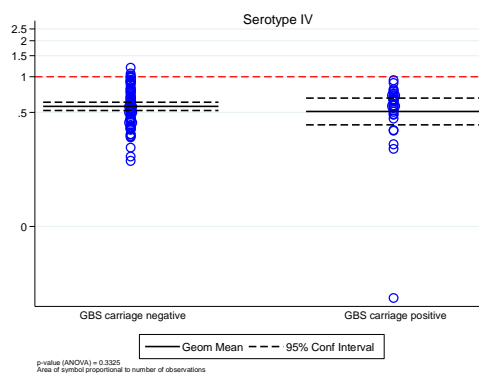
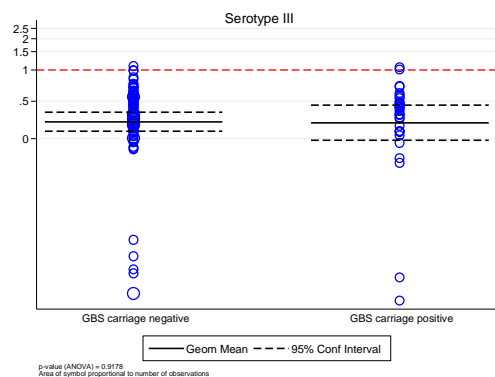
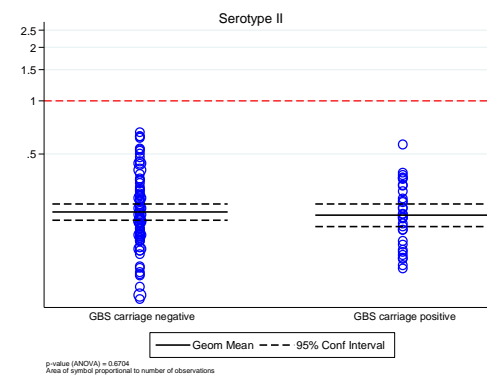
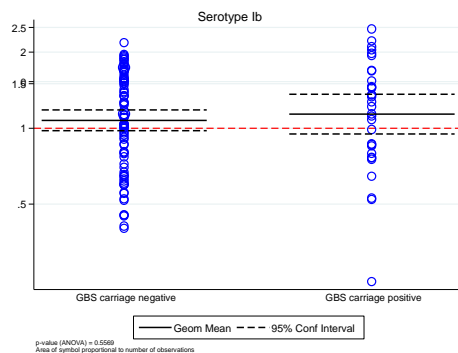
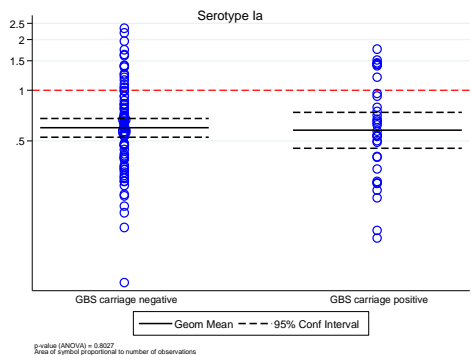


Figure 2



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

Figure 3

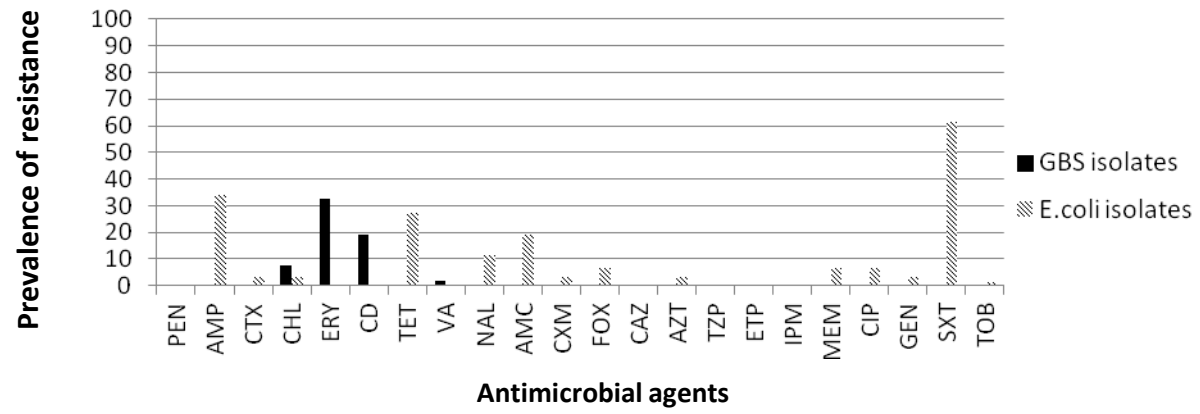
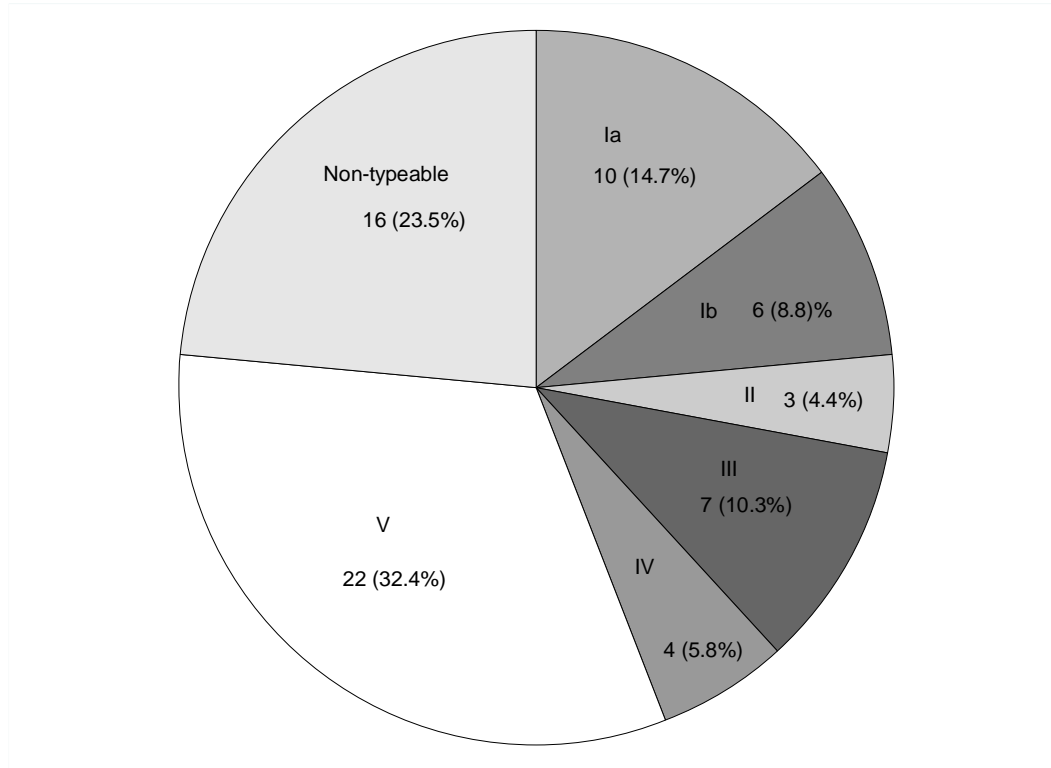


Figure 4



SUPPLEMENTARY MATERIAL

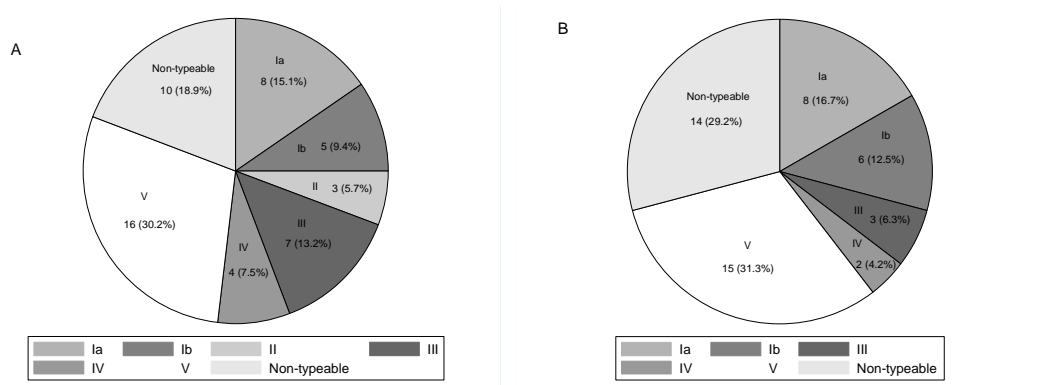
Table S1. Resistance profile of group B streptococcal and Escherichia coli isolates.

Antimicrobial agents	<i>GBS isolates, N=104</i>		<i>E.coli isolates, N=62</i>	
	Recto-vaginal, n(%), N=54	Vaginal, n(%), N=50	Vaginal, n(%), N=52	Urine, n(%), N=10
Penicillin				
Full resistant	0 (0)	0 (0)	NA	NA
Intermediate resistant	0 (0)	0 (0)	NA	NA
Ampicillin				
Full resistant	0 (0)	0 (0)	17 (32.7)	4 (40.0)
Intermediate resistant	0 (0)	0 (0)	0 (0)	0 (0)
Ceftriaxone				
Full resistant	0 (0)	0 (0)	0 (0)	0 (0)
Intermediate resistant	0 (0)	0 (0)	1 (1.9)	1 (10.0)
Chloramphenicol				
Full resistant	1 (1.9)	0 (0)	2 (3.9)	0 (0)
Intermediate resistant	3 (5.6)	4(8.0)	0 (0)	0 (0)
Erythromycin				
Full resistant	14 (25.9)	10(20.0)	NA	NA
Intermediate resistant	4 (7.4)	6 (12.0)	NA	NA
Clindamycin				
Full resistant	10 (18.5)	7 (14.0)	NA	NA
Intermediate resistant	1 (1.9)	2 (4.0)	NA	NA
Tetracycline				
Full resistant	NA	NA	15 (28.9)	2 (20.0)
Intermediate resistant	NA	NA	0.50	2.16
Vancomycine				
Full resistant	0 (0)	0 (0)	NA	NA
Intermediate resistant	2 (3.7)	0 (0)	NA	NA
Nalidixic acid				
Full resistant	NA	NA	3 (5.8)	3 (30.0)
Intermediate resistant	NA	NA	1 (1.9)	0 (0.0)
Amoxicillin/clavulanic acid				
Full resistant	NA	NA	11 (21.2)	1 (10.0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Cefuroxime				
Full resistant	NA	NA	1 (1.9)	1 (10.0)
Intermediate resistant	NA	NA	0.50	2.16
Cefoxitine				
Full resistant	NA	NA	0 (0)	0 (0)

Intermediate resistant	NA	NA	3 (5.8)	1 (10.0)
Ceftazidime				
Full resistant	NA	NA	0 (0)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Aztreonam				
Full resistant	NA	NA	1 (1.9)	1 (10.0)
Intermediate resistant	NA	NA	0.50	2.16
Piperacillin-Tazobactam				
Full resistant	NA	NA	0 (0)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Ertapenem				
Full resistant	NA	NA	0 (0)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Imipenem				
Full resistant	NA	NA	0 (0)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Meropenem				
Full resistant	NA	NA	0 (0)	0 (0)
Intermediate resistant	NA	NA	4 (7.7)	0 (0)
Ciprofloxacin				
Full resistant	NA	NA	1(1.9)	2 (20.0)
Intermediate resistant	NA	NA	1(1.9)	0 (0)
Gentamicin				
Full resistant	NA	NA	2 (3.9)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Trimethoprim/ sulfamethoxazole				
Full resistant	NA	NA	34 (65.4)	4 (40.0)
Intermediate resistant	NA	NA	0.50	2.16
Trobramicin				
Full resistant	NA	NA	1(1.9)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)

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).