Tittle: Prevalence of Group B *Streptococcus* colonization and serotype distribution among pregnant women in South Asian and African countries

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A Research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Masters in Public Health

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Declaration

I, Gaurav Kwatra declare that this research report is my own work. It is being submitted for the degree of Masters of Public Health in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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Date: 18th June 2024

Dedication

I dedicate this work to my Grand-Mother

Late Smt. Amarvati Kwatra

And

My Parents

Harish Kumar Kwatra and Lata Kwatra

And

My Wife and Son (Jai)

Presentations arising from this study

- **Kwatra G**, Izu A , Ali M , Chaka T, Mwakio S, Mwarumba S, Berkley J, Dorji , Sharma R , Tapia M, Keita A, Samba O Sow S, Saha S, Islam M, Ahmed Z, Chakraborty S, Obaro S, Medugu N, Akaba G, Sigaúque B, Mucavele H, Mabombo V , Solomon F, Beck M, Simoes E, Sahni R, Ragupathi N, Santhanam S, Veeraraghavan B , Cutland C , Shabir A Madhi SA. Prevalence of Group B Streptococcus colonization among pregnant women in South Asia and Africa. 2nd International Symposium on Streptococcus agalactiae Disease (ISSAD 2021) 3rd – 5th November 2021, London, UK (Oral presentation).

Publications arising from this study

- Kwatra; G; Izu; A; Cutland; C; Ali, M; Ahmad, Z; Beck, M; Barsosio, H; Berkley, J; Chaka , T; Cosa, A; Chakraborty, S; Dhar,N; Dorji,P; Islam, M; Keita, M; Mwakio S; Mwarumba S; Medugu,N; Mucavele, H; Mabombo V; Obaro, S; Sigaúque, B; Sow, S; Saha, S; Santhanam, S; Sharma, R ; Simoes, E; Sahni, R; Tapia, M; Veeraraghavan, B; Madhi, S. Prevalence of Group B Streptococcus colonization in mother-newborn dyads in low and middle income South Asian and African countries: A prospective, observational cross sectional study (Accepted in Lancet Microbe)

Abstract

Background: Recto-vaginal Group B *Streptococcus* (GBS) colonization in pregnant women at the time of labour is the major risk-factor for developing invasive GBS disease within 7 days of age (early onset disease; EOD). We investigated prevalence of GBS recto-vaginal colonization at the time of labour among pregnant women and vertical transmission to their newborns across six African and two south-Asian countries.

Methods: This multi-country prospective, observational cross-sectional study was undertaken in six African [(Ethiopia (Adama city), Kenya (Kilifi), Mozambique (Manhica), Nigeria (Gwagwalada), Mali (Bamako) and South Africa (Johannesburg) and two Southeast Asian countries (Bangladesh (Mirzapur) and India (Vellore)]. Inclusion criteria included pregnant women 18 to 45 years of age, delivery at ≥37 weeks gestation and documented to be HIV-uninfected prior to study-enrolment. Lower vaginal swabs, rectal swabs and urine were collected from the mothers and separate skin surface swabs of the umbilicus, outer ear and axillary fold; and rectal and throat swabs were obtained from the newborn for GBS culture. Standardized sampling and culture using direct plating and selective broth media for detection of GBS colonization in the mother-newborn dyads was undertaken across the sites. Serotyping of GBS isolates was done in South Africa.

Results: Overall, 6,922 pregnant women were enrolled from January 10th, 2016 to December 11th, 2018. Of 6922 women who were enrolled, 6514 (94.1%; 759 to 892 per site) were included in the analysis. Overall, the prevalence of maternal GBS colonization was 24.1% (95%CI 23.1-25.2; 1572/6514) being highest in Mali (41.1%, 95%CI: 37.7-44.6; 314/764) and lowest in Ethiopia (11.6%, 95%CI:9.5-14.1; 88/759). The overall rate of vertical transmission of GBS was 72.3% (95%CI 70.0-74.4; 1132/1566); being highest in Mozambique (79.2%, 95%CI 73.3-84.2); 168/212) and lowest in Bangladesh (55.8%, 95%CI 47.5-63.8; 77/138). The five

most common GBS colonizing serotypes were Ia (37.3%; 586/1572), V (28.5%; 448/1572), III (25.1%; 394/1572), II (9.2%; 144/1572) and Ib (6.5%; 102/1572). There was geographic variability in serotype proportion distribution. Serotype VII was the third most common serotype in India (8.5%, n=15/176) and serotype VI was mainly identified in Bangladesh (5.8%) and India (5.7%).

Conclusion: Our study reported high prevalence of GBS colonization in most settings, with some geographic variability even within African countries. Our findings suggests that there is likely to be a significant burden of EOD across the study sites. Post-licensure vaccine effectiveness studies should also focus on maternal GBS serotype distribution as non-vaccine serotype replacement could occur due to vaccine immune pressure.

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Contents

List of figures

List of Tables

Abbreviations

GBS: Group B *Streptococcus*

WHO: World Health Organisation

US CDC: United states Centers for Disease Control and Prevention

CPS: Capsular polysaccharide

EOD: Early Onset Disease

LOD: Late Onset Disease

IAP: Intrapartum Antibiotic Prophylaxis

VIDA: Vaccines and Infectious Diseases Analytics Research Unit

SOP: Standard Operating Procedure

STGG: skim milk, tryptone, glucose, and glycerin

DP: Direct Plating

SB: Selective Broth

HREC: Human Research Ethics Committee

Preface

This research report is submitted for examination for a Master's of Public Health degree. It is prepared according to the University of the Witwatersrand School of Public Health guidelines for research reports in submissible format. The report includes a manuscript drafted and submitted to the Lancet Microbe.

Data included in the drafted manuscript and this research report was presented in oral format at 2nd International Symposium on Streptococcus agalactiae Disease (ISSAD 2021) 3rd –5th November 2021, London, UK.

1. Introduction

Invasive bacterial disease and pneumonia, contributes to approximately one-fifth of underlying cause of death in neonates globally, the majority (>90%) of which occur in low and middle income countries (1). Group B *Streptococcus* (GBS) is a leading cause of invasive bacterial disease in infants less than 90 days of age. In 2020, there were an estimated 394,000 cases of invasive GBS disease in infants <90 days of age, which culminated in 90,000 deaths (2). Peri-partum acquisition of GBS by the foetus or newborn from mothers with recto-vaginal colonization, is the major risk factor for developing invasive GBS disease during the first 7 days of life (early onset disease; EOD) (3). The incidence of invasive GBS disease is more variable across World Health Organisation (WHO) regions compared with the heterogeneity reported for maternal GBS colonization, being highest (per 1,000 live births) in Africa (1.12) and lowest in Asia (0.30) (4). The majority (75%-90%) of EOD occurs within 24 hours of birth (5), suggesting in utero acquisition of GBS by the newborn from their colonized mothers.

The use of intrapartum antibiotic prophylaxis (IAP) targeted at women identified to being colonized with GBS beyond 32 weeks of gestational age, reduces the risk of EOD by 80% (6). Nevertheless, screening for GBS recto-vaginal colonization during pregnancy and implementation of IAP is not practiced in most low and middle income countries due to logistical and cost constrains (6). An improved understanding of prevalence of GBS colonization in pregnant women, serotype distribution and dynamics of transmission from women to their new-borns could be informative in indirectly estimating the burden of EOD, with an estimated to occur in 1 to 2% of babies born to women colonized by GBS in the absence of IAP. Furthermore, data on the serotype distribution could inform the development of GBS vaccines for pregnant women, aimed at protecting their offspring during early infancy.

A meta-analysis indicated that the prevalence of GBS colonization in pregnant women range from 0.5% in Mexico to 33.5% in Caribbean region (7). The reported rate of vertical transmission of GBS from colonized pregnant women to their newborns ranged from 7.6% in China to 63.3% in Ethiopia (8-10). The variability in prevalence of GBS colonization in pregnant women between and within regions, as well as differences in vertical transmission, could be due to differences in microbiological culture methods for GBS, sample collection techniques and time of sampling during pregnancy and in the newborn (7, 11). Consequently, standardised methods are required to enable better comparability of the prevalence of maternal GBS colonization and vertical transmission to newborns.

The aim of this study was to investigate the prevalence of GBS recto-vaginal colonization among pregnant women at delivery, GBS serotype distribution and vertical transmission of GBS from the mother to newborn. The study was undertaken using standardized methods for sample collection and bacterial culture across eight low and middle income countries in South Asia and Africa.

2. Literature review

2.1 Burden of Group B Streptococcus infection

Group B *Streptococcus* also known as *Streptococcus agalactiae* is the one of the most common cause of severe bacterial infections in neonates (12). It is a common commensal bacterium colonizing the vagina and rectum of women (12). Group B *Streptococcus* is a gram-positive bacterium and exists as 10 serotypes, Ia, Ib, II-IX based on the structure of the capsular polysaccharide (CPS) (13). Group B *Streptococcus* is a major risk factor for invasive bacterial disease in neonates and young infants (14). Furthermore, maternal infection by GBS, may lead to stillbirths and preterm labour (15). In neonates, 75%-80% of GBS cases occur within the first seven days of life, of which 75%-90% occur within 24 hours of birth, and these are defined

as early-onset disease (EOD). In low-middle income countries where GBS screening and IAP during pregnancy is not standard-of-care, the case fatality mortality rate from invasive GBS disease is higher than in developed countries (10–60% compared to 7–11%) (16). Late onset disease (LOD) occurs from 7 days to 3 months after birth and has a case-fatality risk of 2 to 20% (17-19). Infants who survive GBS infection are often left with neurodevelopmental disorders, hearing or sight loss (16, 20). The global incidence of invasive GBS disease in young infants (<90 days age) is estimated to being 0.49 per 1000 live births, with the highest incidence in Southern Africa (2.0) and lowest in Southeast Asia (0.21). In the Caribbean and Latin America, the incidence of invasive GBS disease is 0.49 (4). Incidence risk also varies by country within Africa, with South Africa having higher incidence (2-3 cases per 1,000 live births) compared with The Gambia (1.33) and Nigeria (0.25) (4, 21).

2.2 Maternal GBS colonization as Risk factor

The first step in the pathogenesis of GBS EOD disease is asymptomatic colonization of the female genital tract and/ or rectum and vertical transmission of GBS from the colonized mother (12). The foetus gets infected *in utero* as the bacteria ascends from the vagina and invades the uterus and placental membranes. Generally, it was estimated that approximately 40-50% of newborns born to GBS colonized women would be colonized at the time of birth, of whom 1-3% would develop GBS EOD (3). These estimates were corroborated by a longitudinal cohort study of 5099 pregnant women in South Africa among whom the prevalence of vaginal colonization at delivery was 21%, with 57% of newborns to colonized women being colonized themselves at birth, and among whom the incidence of EOD was 2% (21). The infection can also be transmitted during birth if the neonate inhales GBS infected amniotic or vaginal fluids (22). The role of maternal GBS as a cause of stillbirths has been investigated in many studies and GBS has been identified as a cause of 4% (23) to 20% (24) of stillbirths, through blood

cultures. The cause of GBS associated LOD is not yet clear, however, it is assumed to be associated with GBS transmitted either vertically or acquired nosocomically or from community or through ingestion of GBS infected breast milk (25, 26).

2.3 Maternal Group B Streptococcus colonization and vertical transmission

The prevalence of maternal GBS recto-vaginal colonization has been observed from various regions of the world. A meta-analysis by *Nick et al*, on 390 studies from 85 countries until January 31st, 2017 reported mean prevalence of GBS colonization of 18% (95% CI,17-19%) (7). Studies have reported significant heterogeneity across all and within all regions with highest prevalence in Caribbean region (33.5%), followed by South African region (25.3%), Australia (23.3%), North America (22.0%), North Europe (20.0%), and lowest in Eastern Asia (9.2%) (7). The site specific prevalence estimates range from 0·5% in Mexico to 43% in Dominican Republic (7, 27). Differences between studies in sample collection techniques, timing of sampling during pregnancy and in the newborn and microbiological culture methods for GBS were identified as potential factors that could have contributed to the variability in prevalence of GBS colonization across the studies. Studies have reported considerable variation within and between geographic regions for vertical transmission of GBS, ranging from 7.6% in China to 63.3% in Ethiopia (8-10). Maternal GBS bacteriuria has been associated with high density of recto-vaginal GBS colonization in the women and increased risk of EOD in their newborns (16, 28). There is paucity of data on the prevalence of GBS associated bacteriuria during pregnancy.

The current US CDC recommendation for the detection of maternal GBS colonization is by isolation of GBS from vaginal and rectal or recto-vaginal swabs by initial growth in a selective broth medium with antibiotics, followed by subculture on selective or blood agar media (3). A meta-analysis on maternal GBS colonization from 1997 to 2014, identified only four studies

from South Asia (which included three from India and one from Bangladesh and four from Africa (including two from Zimbabwe, one from Malawi and one from South Africa that were conducted according to the US CDC recommended or equivalent guidelines (11). The study suggested that comparability between regions or countries could be improved with more standardised sample collection techniques, bacterial culture laboratory methods and reporting of serotype distribution.

We conducted a literature search on PubMed for reports from January 1st, 2016 up to May 31st, 2023 using the keywords ("Group B Streptococcus (Mesh term)" OR "Streptococcus agalactiae" (Mesh term) AND "Colonization" with no restrictions on language and found 470 articles presenting on GBS colonization. We identified 33 observational studies reporting on GBS recto-vaginal colonization among pregnant women which includes 14 and 2 studies from Africa and Southeast Asia, respectively. Of these, only 5 studies reported on GBS vertical transfer rate (range 7.6% to 63.3%) and none reported on GBS bacteriuria. Maternal GBS colonization ranged from 12.3% to 37% in Africa and 14.8% to 14.9% in Southeast Asia. Different culture methods, sample collection techniques, timing of sampling during pregnancy were reported in these studies.

2.4 GBS serotype distribution

There has been global interest to determine the prevalence of maternal colonizing serotypes to guide development of an effective and affordable GBS vaccine which will provide optimal serotypes coverage (29). Collectively, serotypes Ia, Ib, II, III, and V account for 98% of maternal colonizing isolates globally (7, 11).Prevalence of GBS serotypes vary worldwide, with serotype III (25%) and Ia (21%) being the most common serotypes identified followed by serotype V (18%) and II (11%). However, variation exists between and within regions in the reported prevalence of these serotypes. Other GBS serotypes (VI, VII and VIII) attribute to less

than 2% globally and are more frequently reported from Asian region (7). Serotypes IX also been identified as colonizing serotypes in Kenya and South Africa (30, 31). A hexavalent GB6 serotype specific capsular-protein conjugate GBS vaccine which includes serotype Ia, Ib, II, III, IV and V is currently being developed (32). The distribution of GBS serotypes may change over time, hence, ongoing surveillance of circulating serotypes is warranted. Use of standardized culturing and sampling techniques will yield more GBS isolates and will improve data on reporting of GBS serotypes for better comparability across and within geographical regions. There is limited data on the prevalence of multiple colonizing serotypes in pregnant women, particularly in low- and middle-income countries. The reported prevalence of colonization by multiple GBS serotypes vary from 1% to 57% between studies (33).

2.5 Current Interventions

The preventive strategies against GBS infection include intra-partum antibiotic prophylaxis (IAP) and immunization. To date, only IAP has been evaluated with proven efficacy in reducing the burden of early-onset GBS from 1.7 to 0.32 per 1000 live births between 1993 and 2003 in the United States (34). However, the implementation of IAP in low resource settings is handicapped because of logistical issues related to microbiology laboratory testing and health care infrastructure. Furthermore, IAP is not useful in preventing GBS LOD, GBS-related stillbirths or prematurity (19). Maternal vaccination against GBS is a potential alternate strategy in reducing maternal as well as infancy related GBS diseases. Active immunization of pregnant women with GBS polysaccharides-conjugate vaccine and *in utero* transfer of anti-GBS maternal antibody to the fetus could potentially protect young infants from acquiring GBS until 24 weeks of age (35). Therefore, understanding the epidemiology of maternal GBS colonisation, vertical transmission and serotypes distribution could guide development of an effective GBS vaccine.

3 Problem Statement and rationale

Maternal GBS colonization is the primary risk factor for GBS EOD. The true burden of maternal GBS colonization, vertical transmission rate, and serotypes distribution remains largely unclear due to methodological inconsistencies across regions or countries. There have been only a few studies from South Asia and Africa region that were conducted based on standard guidelines as recommended by US CDC. An improved understanding of maternal GBS colonization, vertical transmission rate and serotypes distribution would be valuable for designing an effective GBS vaccine. This study will investigate the prevalence of GBS recto-vaginal colonization among pregnant women at delivery, GBS serotype distribution and rate of maternal vertical transmission of GBS using standardized methods for sample collection and bacterial culture across eight low-middle income South Asian and African countries.

4 Research Question

- What is the prevalence of GBS colonization among pregnant women in India Bangladesh, India, Ethiopia, Kenya, Mozambique, Nigeria, Mali, and South Africa?
- What is the rate of vertical transmission of GBS among pregnant women?
- Are there any specific Group B *Streptococcus* serotypes that are associated with maternal GBS colonization?

5 Aims and Objectives

This study aims to inform the prevalence of GBS colonization among pregnant women in low-middle income countries of South Asia (India and Bangladesh) and Africa (Ethiopia, Kenya, Mozambique, Nigeria, Mali and South Africa). The study will address some of the critical limitations of the current data from low-middle income countries by using standardized

methodologies (swab specimen collection and microbiological culture techniques) for evaluating GBS colonization. The specific objectives of the study are:

Objectives

- To determine the prevalence of GBS colonization in pregnant women at term-delivery in low-middle income countries in South Asia and Africa.
- To determine the rate of vertical transmission of GBS to the newborns in low-middle income countries in South Asia and Africa.
- To determine the serotypes associated with GBS colonization in pregnant women.

6 Methods

6.1 Study Design

This study was a multi-country prospective, observational cross-sectional study in six African (Ethiopia, Kenya, Mozambique, Nigeria, Mali and South Africa) and three Southeast Asian countries (Bangladesh, Bhutan, India) conducted by Vaccines and Infectious Diseases Analytics Research Unit (VIDA) on epidemiology of GBS (Table 1). The study was conducted at eleven maternity and obstetric care facilities in all the countries. Participants were screened for enrolment during the early stages of labour. All eligible pregnant women who presented at the facilities during normal working days were approached for participation in the study, although, the maximum number enrolled per day was determined at the individual site level.

Table 1: Study sites and Population Characteristics

Country	Recruitment Period	Recruitment facility	Population Characteristics
Bangladesh	20 April 2016 -19 May 2017	Kumudini Women's Medical College Hospital, Mirzapur	Rural
Bhutan	02 January 2017- 29 October 2017	Jigme Dorji Wangchuk National Referral Hospital (JDWNRH), Thimphu	Urban and rural both
India	02 May 2017 - 20 November 2017	Christian Medical College Hospital, Vellore	Urban predominance, some women from rural areas
Ethiopia	19 June 2017- 09 October 2018	Adama Hospital Medical College, Adama City	Urban and rural both.
Kenya	10 January 2016 – 24 October 2016	Kilifi County Hospital, Kilifi	Rural
Mali	12 February 2018 - 21November 2018	The community health center (ASACOBA), Bamaku CSREF 1 (Referal Health Center of commune 1), Bamaku	Urban
Mozambique	27 December 2016- 30 January 2018	Manhiça district hospital, Manhica	Rural
Nigeria	05 April 2016 – 28 August 2017	Abuja Teaching Hospital, Gwagwalada	Urban and rural population
South Africa	03 October 2017 – 11 December 2018	Chris Hani Baragwanath Academic Hospital, Soweto Liliyan Ngoyi community clinic, Soweto	Urban

6.2 Inclusion and Exclusion Criteria

Inclusion criteria included pregnant women age 18 to 45 years age, delivery at ≥37 weeks gestation age based on date of the last menstrual period and corroborated by physical exam, or ultrasound examination if available and documented to be HIV-uninfected prior to study-enrolment. Testing of women for HIV-1 was not undertaken in Bangladesh, as the population prevalence of maternal HIV-1 was less than one percent at the time of the study (36). The study exclusion criteria included reported use of antibiotics in the two weeks prior to delivery, any acute illness, symptomatic vaginal discharge and a known or suspected condition in which vaginal examinations and swab collection was contradicted, inability to obtain maternal blood or blood transfusion in the 30 day period before delivery.

6.3 Procedures

Laboratory scientists and clinical staff from all the participating sites received training applicable to their responsibilities in accordance with the Wits Vaccines and Infectious Diseases Analytics Research Unit (Wits-VIDA) laboratory and clinical Standard Operating Procedures (SOP).

Two Laboratory scientists and clinical staff from all the participating sites received training applicable to their responsibilities within 8 to 10 weeks prior to the start of enrolment at the site. Training included methods of specimen collection, data capturing and GBS culture and identification. The training was conducted in phases at Wits-VIDA to ensure that attendees from each study site received close supervision. This was complemented by the study lead undertaking visits to each of the sites immediately prior to enrolment to provide onsite assistance and training to other study-staff members, and evaluate the site readiness (laboratory and clinical) to initiate the study. Furthermore, additional intermittent (2-3 per site) site visits were undertaken by study lead to compliance with the protocol and procedures. External

quality assessment (EQA) was performed prior to the initiation of clinical sample collection for each site.

All laboratory and clinical supplies including culture media required to collect and process specimens were supplied once every two weeks basis to the sites from Wits-vida.

6.4 External Quality Assessment

External Quality assessment (EQA) was performed on GBS culturing, isolation, and identification prior to the initiation of the collection of clinical samples. This was done as follows:

- A materials pack was shipped to each study site, and included:
    - 20 EQA samples which consist of lyophilised mixtures of normal vaginal and rectal flora that are either positive or negative for GBS.
    - All laboratory reagents and culture media required to process these samples.
- Site laboratories were given two weeks to e-mail their GBS isolation and identification results from the EQA samples.
- A pass mark of 90% concordance was considered acceptable.
- Sites which fail to achieve this pass mark were re-evaluated as follows:
    - Problem samples were investigated to determine the cause of the errors. For these purposes, site laboratories were encouraged to take photographs of the plates to assist us in resolving issues.
    - A site laboratory was approved if the discordant results were easily resolved telephonically.

- Site laboratories with low scores that cannot be approved through minor procedural steps were visited by a representative from the VIDA laboratory for further training and approval.

6.5 Specimen collection and processing

Participants were screened for enrolment during the early stages of labor. Consenting and eligible women were allocated a unique identification number, following which demographic and pregnancy related data was collected. All sampling was undertaken by study staff. Separate lower vaginal and rectal swabs, and urine specimens were collected from the women after the onset of labor, and preferably prior to rupture of amniotic sac membranes. Separate skin (surface swabs of the umbilicus, outer ear and axillary fold), rectal and throat swabs were obtained from the baby prior to cleansing or bathing. The swabs were collected using rayon-tipped swabs (MW170, Medicalwire, UK) that were placed into separate Amies transport mediugm without charcoal. Swabs and urine specimens were transported on ice to the site laboratory and processed within 24 hours of collection for GBS detection and isolation.

Swabs were processed onsite laboratories according to wits-Vida SOP for GBS isolation and identification based on the standard Centre for Diseases Control and Prevention (CDC, USA) guidelines (37). Swabs were inoculated onto selective media ((CHROMagar StrepB; CA; Direct Plating (DP)) and into selective broth (SB) (Todd-Hewitt broth supplemented with colistin and nalidixic acid; Lim broth; BD, catalogue number# 296266). The selective broth was further subcultured onto selective media (Selective Broth method, SB). Urine samples (10ul) were directly plated on onto selective media for culturing. A cut-off of ≥10000 CFU/mL was used to define bacteriuria. GBS-like colonies were isolated and confirmed as GBS by

testing for Christie Atkins Munch-Petersen (CAMP) factor, inability to hydrolyze esculin, catalase negativity and group B antigen detection.

For two sites (Ethiopia and Mali) swab specimens were stored in 1.0ml of skim milk, tryptone, glucose, and glycerin (STGG) storage medium and shipped to Wits-VIDA for GBS culturing, due to logistics issues associated with supply of consumables and reagents to the sites. STGG medium containing swab was thoroughly vortexed and 100ul was plated on CA and remaining 900 ul of STGG medium with swab was inoculated into selective broth. Urine samples from Mali and Ethiopia were also shipped frozen and not processed for GBS culture.

6.6 Data Processing, management and storage

Data was collected on study-specific data collection forms and entered into study-specific secure online databases at each study site. Collection forms and databases were uniform across sites. Data was transferred to Wits-VIDA monthly. Internal monitoring and quality assurance was conducted on key variables at each site by the local data teams. On a regular basis the study site data manager, generated a set of data listings that was reviewed by the Wits-VIDA coordinating site. Aggregated data from all the sites was compiled, quality checked, stored and analysed at Vida.

6.7 GBS serotyping

The GBS isolates from the sites were stored in STGG medium at -70°C and shipped to Wits-VIDA for serotyping. Serotyping was performed on all GBS isolates using latex agglutination assay with specific antisera against types Ia, Ib and II to IX CPS antigens (Statens Serum Institute, SSI, Denmark)(38). GBS isolates that tested negative by latex agglutination for all serotypes, were typed by polymerase chain reaction using serotype-specific primer sequences (39).

6.8 Antimicrobial in the urine

The presence of antimicrobials in the urine was tested by disk diffusion method using a sensitive strain of Bacillus (B. subtilis; ATCC 11774) as per Wits-Vida SOP. Urine was added to a sterile disk placed in the centre of the *B. subtilis* inoculated plate. Any zone of inhibition around the disk was considered as evidence of antimicrobial activity.

6.9 Follow up

All the enrolled women were contacted telephonically or in-person around 28 days after birth to determine the health status of infant, including enquiring if the child had been hospitalised for any sepsis episode. The diagnosis of suspected sepsis in a neonate was based on the attending physician discretion as recorded in medical notes where available.

6.10 Sample size

A sample size of 772 was calculated from each study site to detect at least 15% prevalence of colonization and 100 colonization events with ±5% precision (Table 2). The study sample size has been powered to determine GBS vaginal and rectal colonization prevalence in pregnant women at delivery at the different study sites.

Table 2: Sample size to detect maternal GBS colonization prevalence.

Estimated maternal GBS colonization prevalence	Sample size
10%	1161
15%	**772**
20%	576
25%	459

Assuming a colonization prevalence of 15% (n=116), the study had 80% power to detect a 30% difference in the proportion of vertical GBS transmission to newborns compared to an anticipated 50% vertical transmission with α=0.05 (Table 3).

Table 3: Sample size to detect difference in vertical transfer of GBS colonization.

Estimated difference in GBS colonization in newborns	Sample size
10%	1605
15%	**719**
20%	408
25%	262
30%	103

6.11 Bhutan Site Exclusion from analysis

In the interim preliminary analysis (at 50% of the sample size) very low colonization prevalence was reported from Bhutan compared to other sites. To quality control their specimen collection process and microbiological culture methods, duplicate vaginal swab specimens were collected for 46 participants and were shipped to South Africa (SA) for culturing processing. The duplicate vaginal swabs were processed immediately in the laboratory at SA. The GBS positivity rate was highly discordant between Bhutan (4.5%) and South Africa (29.5%) laboratories. In addition, random 48 duplicate swabs specimens (Mix of maternal Rectal, Infant throat, infant rectal and infant skin) were also collected and shipped to South Africa for processing. The GBS positivity results were highly discordant between two laboratories (Bhutan (0%) and South Africa (16.7%), table 4.

Table 4: Comparison of GBS colonization rates for Bhutan swab specimens at Bhutan and South Africa (Wits-Vida) laboratory.

	Vaginal Swabs (n=44)	Swabs (Maternal Rectal, Infant throat, Infant rectal and Infant skin) (n=48)	Overall (n=92)
Bhutan	2 (4.5%)	0 (0%)	2 (2.2%)
South Africa	13 (29.5%)	8 (16.7%)	21 (22.8%)

Retraining and onsite visit was performed at Bhutan site on specimen and culturing process but no improvement in GBS colonization was noted. The results were presented to study scientific advisory committee, and it was decided not to further process swabs at Bhutan site and exclude

Bhutan site from colonization analysis due to sub-optimal processing and GBS culturing process.

6.12 Statistical analysis:

Data was analyzed using R software (version 4.1.1) and Prism Graphpad Prism (version 8.0.2 9). Descriptive statistics were presented using counts with proportions for categorical variables and medians with interquartile ranges (IQRs) for quantitative variables. Sensitivities (detection rates) were calculated by comparing the proportion of positive samples for SB and DP culture method in relation to composite of positive from either SB or DP culture method. For maternal swab specimens, a sample pair of vaginal and rectal swabs was considered negative if no GBS growth was identified on either swab. Conversely, women were categorised as being colonized if GBS was cultured from either the rectal or vaginal swab. Newborns were categorized as being colonized if GBS was cultured from any of the swabs (skin surface, rectal or throat), and not colonized in the absence of GBS from all of these sites. Logistic regression analysis and chi-square test was used to determine the association between GBS colonization and demographic characteristics at enrolment. Multiple logistic regression was used to assess the odds of colonisation and potential risk factors for GBS colonization (country, maternal age, presence of antimicrobial, gravida and mode of delivery). Only complete data was used for the analysis. Multiple logistic regression analysis was an exploratory hypothesis generating analyses and no adjustments were made for multiple testing. A p-value of <0.05 was considered significant.

6.13 Ethics Declaration

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (150214 and M211029), and site-specific ethics approval was obtained from all the participating sites institution Ethics committees. Written informed consent was

obtained from all participating mothers. The trial is registered with South African National Clinical Trials Register, number DOH-27-0418-4989.

7 Publication

This manuscript has been prepared and formatted for submission to the Lancet Microbe (accepted in Lancet Microbe). The role of the authors was as follows: GK, SAM conceptualised the study. GK, CLC, SAM designed the study. GK, SAM oversaw the conduct of the study database design and development, site selection and training, data collection, data cleaning, and interpretation of results. GK and AI did the statistical analysis. GA, MMA, ZA, MMB, HCB, JAB, TEC, AC, SC, ND, PD, MI, AMK, StM, SaM, NM, HM, VM, SO, BS, SOS, SKS, SS, RS, EAFS, RDS, MDT, BV are site principal investigators and enrolled participants, collected data. GK prepared the 1st Draft. GK and AI accessed and verified the data. All authors had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Prevalence of Group B Streptococcus colonization in mother-newborn dyads in low and middle income South Asian and African countries: A prospective, observational cross sectional study.

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Abstract

Background: Recto-vaginal Group B *Streptococcus* (GBS) colonization in pregnant women at the time of labour is the major risk-factor for developing invasive GBS disease within 7 days of age (early onset disease; EOD). We investigated prevalence of GBS recto-vaginal colonization at the time of labour among pregnant women and vertical transmission to their newborns' across six African and two south-Asian countries.

Methods: This multi-country prospective, observational cross-sectional study was undertaken in six African [(Ethiopia (Adama city), Kenya (Kilifi), Mozambique (Manhica), Nigeria (Gwagwalada), Mali (Bamako) and South Africa (Johannesburg) and two Southeast Asian countries (Bangladesh (Mirzapur) and India (Vellore)]. Inclusion criteria included pregnant women age 18 to 45 years age, delivery at ≥37 weeks gestation and documented to be HIV-uninfected prior to study-enrolment. The study was conducted at maternity and obstetric care facilities and participants were screened for enrolment during the early stages of labour. Lower vaginal, rectal swabs and Urine were collected from the mothers and separate skin surface swabs of the umbilicus, outer ear and axillary fold; and rectal and throat swabs were obtained from the newborn for GBS culture. Standardized sampling and culture using direct plating and

selective media broth for detection of GBS colonization in the mother-newborn dyads was undertaken across the sites. Serotyping of GBS isolates was done in South Africa.

Findings: Overall, 6,922 pregnant women were enrolled from January 10th, 2016 to December 11th, 2018. Of 6922 women who were enrolled, 6514 (94.1%; 759 to 892 per site) were included in the analysis. Overall, the prevalence of maternal GBS colonization was 24.1% (95%CI 23.1-25.2; 1572/6514) being highest in Mali (41.1%, 95%CI: 37.7-44.6; 314/764) and lowest in Ethiopia (11.6%, 95%CI:9.5-14.1; 88/759). The overall rate of vertical transmission of GBS was 72.3% (95%CI 70.0-74.4; 1132/1566); being highest in Mozambique (79.2%, 95%CI 73.3-84.2); 168/212) and lowest in Bangladesh (55.8%, 95%CI 47.5-63.8; 77/138). The five most common GBS colonizing serotypes were Ia (37.3%; 586/1572), V (28.5%; 448/1572), III (25.1%; 394/1572), II (9.2%; 144/1572) and Ib (6.5%; 102/1572). There was geographic variability in serotype proportion distribution. Serotype VII was the third most common serotype in India (8.5%, n=15/176) and serotype VI was mainly identified in Bangladesh (5.8%) and India (5.7%).

Interpretation: Our study reported high prevalence of GBS colonization in most settings, with some geographic variability even within African countries., our findings suggest there is likely to be a significant burden of EOD across the study sites. Post-licensure vaccine effectiveness studies should also focus on maternal GBS serotype distribution as non-vaccine serotype replacement could occur due to vaccine immune pressure.

Funding:

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Research in Context

Evidence before this study

Peri-partum acquisition of Group B *Streptococcus* (GBS) by the foetus or newborn from mothers with recto-vaginal colonization, is the major risk factor for developing invasive GBS disease during the first 7 days of life (early onset disease; EOD). A systematic review of all GBS colonization studies until January 31st, 2017 reported the prevalence of GBS recto-vaginal colonization in pregnant women range from 2.0 % in Melanesia to 33.5% in Caribbean region. Differences between studies in sample collection techniques, timing of sampling during pregnancy and in the newborn and microbiological culture methods for GBS were identified as potential factors that could have contributed to the variability in prevalence of GBS colonization and vertical transmission rates across the studies. We conducted a literature search on PubMed for reports from January 1st, 2016 up to May 31st, 2023 using the keywords ("Group B Streptococcus (Mesh term)" OR "Streptococcus agalactiae Mesh term ") AND "Colonization" with no restrictions on language and found 470 articles presenting on GBS colonization. We identified 33 observational studies reporting on GBS recto-vaginal colonization among pregnant women which includes 14 and 2 studies from Africa and Southeast Asia, respectively. Of these, only 5 studies reported on GBS vertical transfer rate (range 7.6% to 63.3%) and none reported on GBS bacteriuria. Maternal GBS colonization ranged from 12.3% to 37% in Africa and 14.8% to 14.9% in Southeast Asia. Different culture methods, sample collection techniques, timing of sampling during pregnancy were reported in these studies.

Added value of this study

Using standardised methods across all the sites, our study provides more robust data than currently exist to enable head-to-head comparability of geographic differences in the

prevalence of GBS colonization in pregnant women and vertical transmission of GBS to their newborns. The overall prevalence of maternal GBS colonization was 24.1% (95%CI 23.1%-25.2%) in our study. There was less variaability in prevalence of GBS colonization in women from Africa (25.8%) compared with Southeast Asian women (19.0%) than reported in past studies. Also, we identified geographic heterogeneity in serotype distribution of colonizing isolates, and particularly serotypes VII and VI being among the top five serotypes in Southeast Asian countries. Our study also identified higher rates of vertical transmission (55.8 -79.2%) of GBS from colonized women to their newborns with maternal GBS bacteriuria being a strong predictor of vertical transmission across all the sites.

Implications of all the available evidence

Our study shows that using a standardised approach to investigate for maternal recto-vaginal GBS colonization and vertical transmission of GBS provided more robust data and enables better inter-country comparison of maternal GBS recto-vaginal colonization and vertical transmission rate to newborns. Based on the estimate that 1-2% of babies born to women with recto-vaginal GBS colonization would develop invasive disease in the absence of intrapartum antibiotic prophylaxis, our findings suggest that newborns are at high risk of invasive GBS disease in our study settings. The current hexavalent GBS polysaccharide-protein conjugate vaccine (GBS6) being evaluated in pregnant women includes six serotypes (Ia, Ib, II, III, IV and V) and accounted for 97.3% of all maternal colonizing isolates in our study, ranging from 100% in Ethiopia and Kenya to 85.8% in India.

Introduction

Invasive bacterial disease and pneumonia contribute to approximately one-fifth of deaths in neonates globally, the majority (>90%) of which occur in low and middle income countries (1). Group B *Streptococcus* (GBS) is a leading cause of invasive bacterial disease in infants less than 90 days of age. In 2020, there were an estimated 394,000 cases of invasive GBS disease in infants <90 days of age, which culminated in 90,000 deaths (2). Peri-partum acquisition of GBS by the foetus or newborn from mothers with recto-vaginal colonization is the major risk factor for developing invasive GBS disease during the first 7 days of life (early onset disease; EOD) (3). In the absence of intrapartum antibiotic prophylaxis (IAP) in pregnant women identified to be colonized with GBS between 32 to 36 weeks gestational age, approximately one to two percent of their newborns would develop EOD (37). Maternal recto-vaginal colonization by GBS may also contribute to illness in the women and may contribute to at least 57,000 stillbirths and 3.5 million preterm labour (15).

A systematic review reported the prevalence of GBS recto-vaginal colonization in pregnant women range from 2.0% in Melanesia to 33.5% in Caribbean region (7). The reported rate of vertical transmission of GBS from colonized pregnant women to their newborns ranged from 7.6% to 63.3% (8-10). Differences between studies in sample collection techniques, timing of sampling during pregnancy and in the newborn and microbiological culture methods for GBS could contribute to the great variability in prevalence of GBS colonization and vertical transmission rates (7, 11). Consequently, standardised methods are required to improve comparison of the prevalence of maternal GBS colonization and vertical transmission to newborns in different settings, which is addressed by our study. Improved understanding of prevalence of GBS colonization in pregnant women, serotype distribution and dynamics of transmission from women to their newborns could also assist in clarifying the variability

reported for incidence of EOD in different settings, which range from 0.71 to 0.32 per 1,000 live births in Africa and Asia, respectively (4, 7).

The aim of this study was to investigate the prevalence of GBS recto-vaginal colonization and serotype distribution among pregnant women at the time of delivery in eight low- and middle-income countries (LMIC) in Southeast Asia and Africa using standardised methods. Furthermore, we evaluated the prevalence of GBS bacteriuria in the women and vertical transmission of GBS to the newborns.

Methods:

Study Design and Participants

We conducted multi-country prospective, observational cross-sectional study in six African (Ethiopia, Kenya, Mozambique, Nigeria, Mali and South Africa) and three Southeast Asian countries (Bangladesh, Bhutan, India).

The study was conducted at eleven maternity and obstetric care facilities in all the countries. Participants were screened for enrolment during the early stages of labour. All eligible pregnant women who presented at the facilities during normal working days were approached for participation in the study, although, the maximum number enrolled per day was determined at the individual site level. Inclusion criteria included pregnant women age 18 to 45 years age, delivery at ≥37 weeks gestation age based on date of the last menstrual period and corroborated by physical exam, or ultrasound examination if available and documented to be HIV-uninfected prior to study-enrolment. Testing of women for HIV-1 was not undertaken in Bangladesh, as the population prevalence of maternal HIV-1 was less than one percent at the time of the study(36). The study exclusion criteria included reported use of antibiotics in the two weeks prior to delivery, any acute illness, symptomatic vaginal discharge and a known or suspected

condition in which vaginal examinations and swab collection was contradicted, inability to obtain maternal blood or blood transfusion in the 30 day period before delivery. Although one of the inclusion criteria was "No Antibiotics treatment in the two weeks prior to delivery", due to the possibility of mis-reporting by the participants, we decided to add an additional layer of screening to ensure that the participant did not have any antimicrobial activity, which could have influenced the sensitivity of identifying GBS by culture. Standardized methods for sample collection, bacterial culture and serotyping were undertaken across all the sites.

Procedures

Laboratory scientists and clinical staff from all the participating sites received training applicable to their responsibilities in accordance with the Wits Vaccines and Infectious Diseases Analytics Research Unit (Wits-VIDA) laboratory and clinical SOP's. All laboratory and clinical supplies including culture media required to collect and process specimens were supplied once every two weeks basis to the sites from Wits-VIDA. Separate lower vaginal and rectal swabs were collected from the women and maternal urine sample were processed for GBS culture and presence of antimicrobials. Separate skin surface swabs of the umbilicus, outer ear and axillary fold; and rectal and throat swabs were obtained from the baby prior to cleansing or bathing. All the swabs were collected by trained study clinical staff and the urine samples were collected by the participants under the supervision of the study staff. Swabs and urine were processed at onsite laboratories according to standard Centre for Diseases Control and Prevention (CDC, USA) guidelines(37). Briefly, swabs were inoculated onto selective media ((CHROMagar StrepB; CA; Direct Plating (DP)) and into selective broth (Todd-Hewitt broth supplemented with colistin and nalidixic acid (SB); Lim broth; BD, catalogue number# 296266). For Ethiopia and Mali, due to logistical issues associated with delivery of consumables and reagents to the sites, swab samples were stored in STGG storage medium and

shipped to Wits-VIDA for GBS culturing. Urine samples from Mali and Ethiopia were also shipped frozen and not processed for GBS culture.

The GBS isolates from the sites were stored in STGG medium at -70°C and shipped to Wits-VIDA for serotyping. Serotyping was performed on all GBS isolates using latex agglutination assay with specific antisera against types Ia, Ib and II to IX CPS antigens (Statens Serum Institute, SSI, Denmark)(38). GBS isolates that tested negative by latex agglutination for all serotypes, were typed by polymerase chain reaction using serotype-specific primer sequences(39). The presence of antimicrobials in the urine was tested by disk diffusion method using a sensitive strain of Bacillus (B. subtilis; ATCC 11774). Briefly, urine was added to a sterile disk placed in the centre of the *B. subtilis* inoculated plate. Any zone of inhibition around the disk was considered as evidence of antimicrobial activity.

All the enrolled women were contacted telephonically or in-person around 28 days after birth to determine the health status of infant, including enquiring if the child had been hospitalised for any sepsis episode. The diagnosis of suspected sepsis in a neonate was based on the attending physician discretion as recorded in medical notes where available.

Outcomes

The primary outcome was GBS recto-vaginal prevalence among pregnant women. Secondary outcomes were vertical transmission rate of GBS from mother to newborn, GBS serotype, and prevalence of GBS from SB and DP methods.

Statistical analysis:

Assuming 15% prevalence for GBS colonization, we targeted enrolling at least 780 pregnant women at each study site to detect at least 100 colonization events with ±5% precision per site. Data was analyzed using R software (version 4.1.1) and Prism Graphpad Prism (version 8.0.2 9). Descriptive statistics were presented using counts with proportions for categorical variables

and medians with interquartile ranges (IQRs) for quantitative variables. Sensitivities (detection rates) were calculated by comparing the proportion of positive samples for SB and DP culture method in relation to composite of positive from either SB or DP culture method. For maternal swab specimens, a sample pair of vaginal and rectal swabs was considered negative if no GBS growth was identified on either swab. Conversely, women were categorised as being colonized if GBS was cultured from either the rectal or vaginal swab. Newborns were categorized as being colonized if GBS was cultured from any of the swabs (skin surface, rectal or throat), and not colonized in the absence of GBS from all of these sites. Logistic regression analysis and chi-square test was used to determine the association between GBS colonization and demographic characteristics at enrolment. Multiple logistic regression was used to assess the odds of colonisation and potential risk factors for GBS colonization (country, maternal age, presence of antimicrobial, gravida and mode of delivery). Only complete data was used for the analysis. Multiple logistic regression analysis was an exploratory hypothesis generating analyses and no adjustments were made for multiple testing. A p-value of <0.05 was considered significant.

Ethics Declaration:

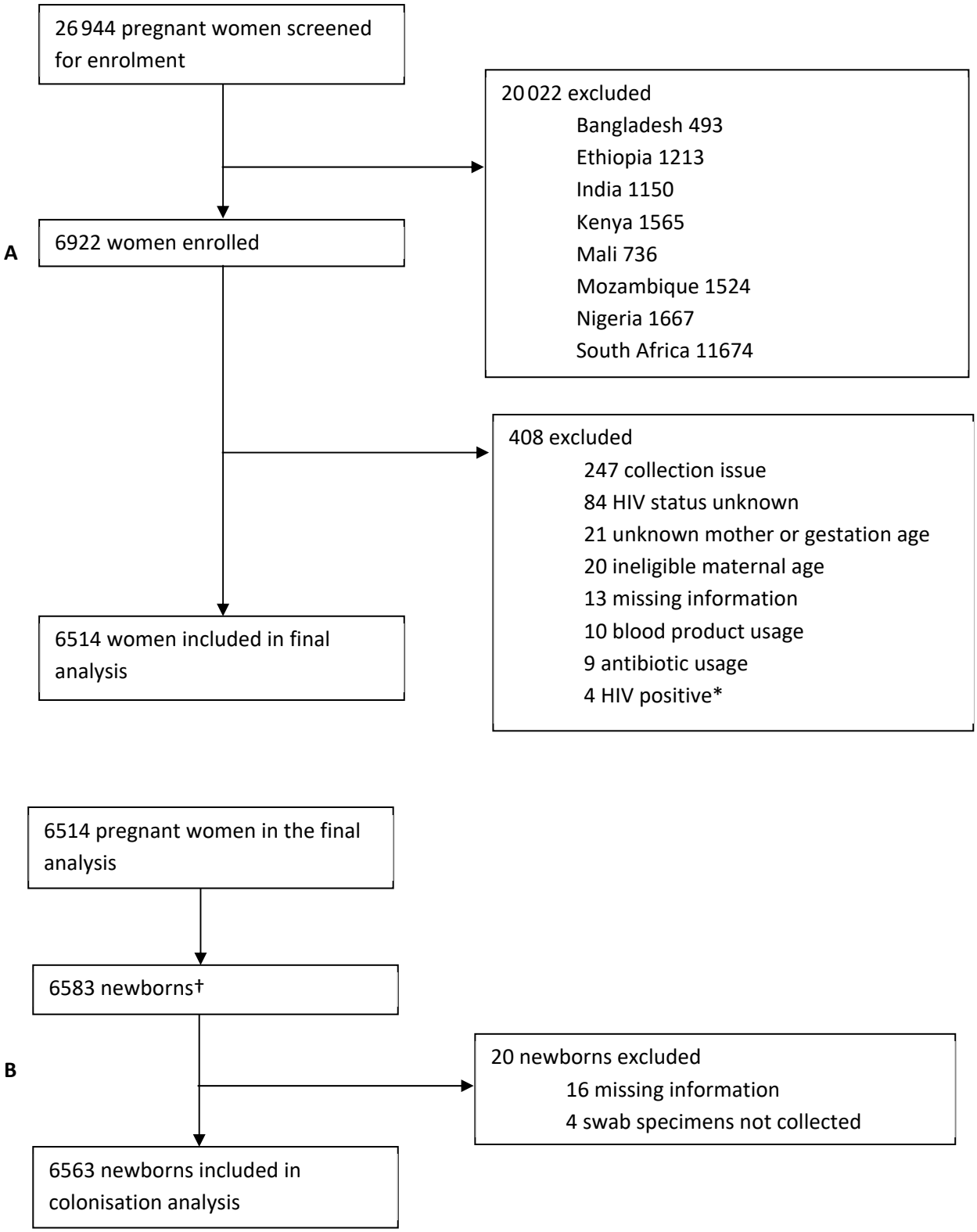
The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (150214), and site-specific ethics approval was obtained from all the participating sites institution Ethics committees. Written informed consent was obtained from all participating mothers. The trial is registered with South African National Clinical Trials Register, number DOH-27-0418-4989.

Funding:

The study was funded by a grant from the Bill and Melinda Gates Foundation. The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Overall, 6,922 pregnant women were enrolled across the eight countries included in the analysis from January 10th, 2016 to December 11th, 2018 (Figure 1). Due to inconsistencies with specimen collection and processing of samples at the Bhutan, the country was excluded from the final analysis. The analysed dataset included 6514 (94.1%) of the enrolled women and 6563 of their newborns. There were 69 twin deliveries, with swabs collected only in 49 sets of twins. The number of participants eligible for analysis ranged from 759 to 892 by site. Reasons for non-inclusion in the final analysis is reported in Table 5 and 6.



(A) Pregnant women participants. (B) Newborn participants. Inclusion and exclusion by country is shown in table 4 and 5. *HIV testing was not done at sites where prevalence of HIV was <1% during the study period and not standard of care (Ie, Bangladesh). †6445 singleton newborns and 69 pairs of twins were born.

Table 5: Maternal Participant enrolled in the study and reasons for non-inclusion in the final analysis.

	Bangladesh	Ethiopia	India	Kenya	Mali	Mozambique	Nigeria	South Africa	Overall
Participant screened	1284	2196	2000	2374	1582	2351	2548	12609	26944
Participant recruited	791	983	850	809	846	827	881	935	6922
Maternal Age ineligibility	3	3		5		3	3	3	20
Sample collection/Process ineligibility		196		1	3	26		21	247
Blood product usage/Missing		4		1		2	2	1	10
Antibiotic usage								9	9
HIV Positive*		2				1	1		4
HIV Unknown		8			74			2	84
Ineligible /Missing gestation age/Missing mother age		5			4	7	1	4	21
Missing multiple information		6			1	3		3	13
Final Eligible participant	788	759	850	802	764	785	874	892	6514

*HIV testing was not done at sites where prevalence of HIV was < 1% at the time study enrolment and not standard of care (Bangladesh).

Table 6: Enrolled newborns and reasons for non-inclusion in the final analysis.

	Bangladesh	Ethiopia	India	Kenya	Mali	Mozambique	Nigeria	South Africa	Overall
Maternal Participant	788	759	850	802	764	785	874	892	6514
Singletons	782	744	848	801	743	779	863	887	6445
Twins	6	15	2	1	21	6	11	7	69
Overall babies born	794	774	852	803	785	791	885	901	6583
Missing multiple information		14	2						16
Swab specimens missed		1		1	2				4
Newborn for colonization analysis	794	759	850	802	783	791	885	901	6563

The median age of the participants across all sites was 25 (IQR: 22-30) years, with median age ranging from 22 (IQR: 20-27) years in Bangladesh to 30 years in Nigeria (IQR: 27-34). All the women were of Asian ethnicity in Bangladesh and India, and 96.5% to 100% of the women at the African sites were black-African; Table 7 and table 9-16. Only complete data was used for the analysis and a summary of the percent missingness is detailed in table 20.

Table 7: Demographics of the study population at time of enrolment

Characteristics		Overall	Colonised	Uncolonised	p-value
Median mother's age (IQR) (n=6512), Median: 25 (22-30)	<20	1238 (19%)[a]	269 (21.7%)[b]	969 (78.3%)[c]	0.01
	20 - <25	2148 (33%)	506 (23.6%)	1642 (76.4%)	
	25 - <30	1835 (28.2%)	436 (23.8%)	1399 (76.2%)	
	30 - <35	851 (13.1%)	234 (27.5%)	617 (72.5%)	
	35 - <40	378 (5.8%)	109 (28.8%)	269 (71.2%)	
	>=40	62 (1%)	18 (29%)	44 (71%)	
Ethnicity (N=6527)	Black	4759 (74%)	1243 (26.1%)	3516 (73.9%)	Ref
	Asian	1640 (25.5%)	312 (19%)	1328 (81%)	<0.0001
	Other	28 (0.4%)	9 (32.1%)	19 (67.9%)	0.47
Gravidity (n=6485) Median (IQR): 1(0-2)	1	2435 (37.5%)	513 (21.1%)	1922 (78.9%)	<0.001
	2	1703 (26.3%)	397 (23.3%)	1306 (76.7%)	
	3	1062 (16.4%)	293 (27.6%)	769 (72.4%)	
	>3	1285 (19.8%)	369 (28.7%)	916 (71.3%)	
Parity, (n=3938) Median (IQR): 1(1-2)	0	343 (8.7%)	80 (23.3%)	263 (76.7%)	0.23
	1	1725 (43.8%)	418 (24.2%)	1307 (75.8%)	
	2	908 (23.1%)	248 (27.3%)	660 (72.7%)	
	3	455 (11.6%)	139 (30.5%)	316 (69.5%)	
	>3	507 (12.9%)	146 (28.8%)	361 (71.2%)	
Previous stillborn (n=3714)[d]	1	217 (5.8%)	61(6.2%)	156 (5.7%)	0.63
	0	3497 (94.2%)	922 (26.4%)	2575 (73.6%)	
Previous spontaneous abortion (n=3910)[d]	1	831 (21.3%)	211(20.6%)	620 (21.5%)	0.57
	0	3079 (78.7%)	814 (26.4%)	2265 (73.6%)	
Median MUAC (IQR)		27 (25-30)	28 (25.5-31)	27 (24.2-30)	<0.001
Chorioamnionitis (n=6489)	1	10 (0.2%)	4 (40%)	6 (60%)	0.27
	0	6479 (99.8%)	1565 (24.2%)	4914 (75.8%)	
Babies born (Maternal n=6514)					
Singleton		6445 (98.9%)	1550 (24%)	4895 (76%)	0.17
Twins		69 (1.1%)	22 (31.9%)	47 (68.1%)	
Antimicrobials in the urine (n=6227)	1	470 (7.5%)	77 (16.4%)	393 (83.6%)	<0.0001
	0	5757 (92.5%)	1429 (24.8%)	4328 (75.2%)	
Bacteriuria (n=4958)	1	435 (8.8%)	405 (93.1%)	30 (6.9%)	
	0	4523 (91.2%)	757 (16.7%)	3766 (83.3%)	<0.0001
Mode of Delivery (n=6562)					

Normal vaginal delivery (NVD)		5599 (85.3%)	1372 (24.5%)	4227 (75.5%)	Ref
caesarean section (C/S) n=963		963 (14.7%)	219 (22.7%)	744 (77.3%)	0.25
Emergency C/S		842 (12.8%)	192 (22.8%)	650 (77.2%)	0.28
Elective C/S		121 (1.8%)	27 (22.3%)	94 (77.7%)	0.58
Median birth weight (IQR)		3100 (2800-3400)	3100 (2800-3400)	3100 (2800-3400)	0.84

a,b,c are row percentages of overall; d did not differ when stratified on number of previous stillbirths; IQR: Inter quartile range; p-value using univariate logistic regression analysis or Chi-square test

Maternal GBS colonization prevalence

The sensitivity of GBS culture from vaginal and rectal swabs using the SB and DP method relative to composite culture positivity by either method is detailed in extended results and supplementary information. Overall, GBS culture positivity was higher in vaginal (21.0%; 95%CI: 20.0-22.0; 1365/6514) compared with rectal swabs (17.1%; 95%CI:16.2-18.1; p<0.0001; 1115/6514); including in country specific analysis except for Bangladesh (12.6% vs 12.9%) and Ethiopia (10.0 vs 9.0%) where the GBS isolation from both vaginal and rectal swabs were similar.

Overall, the prevalence of maternal GBS colonization, irrespective of culture method or colonizing site, was 24.1% (95%CI 23.1%-25.2%; 1572/6514) (Figure 2). The prevalence of maternal GBS colonization was highest in Mali (41.1%, 95%CI:37.7-44.6; 314/764) and lowest in Ethiopia (11.6%, 95%CI:9.5-14.1; 88/759). The prevalence of GBS colonization in the other African countries were 21.2% in Kenya (95%CI:18.5-24.2; 170/802), 23.0% in Nigeria (95%CI:20.3-25.9; 201/874), 27.0% in Mozambique (95%CI:24.0-30.2; 212/785) and 30.8% in South Africa (95%CI:27.9-33.9; 275/892). The prevalence of maternal GBS colonization were 17.5% (95%CI:15-20.3; 138/788) in Bangladesh and 20.5% (95%CI: 17.9-23.3; 174/850) in India; Figure 2.

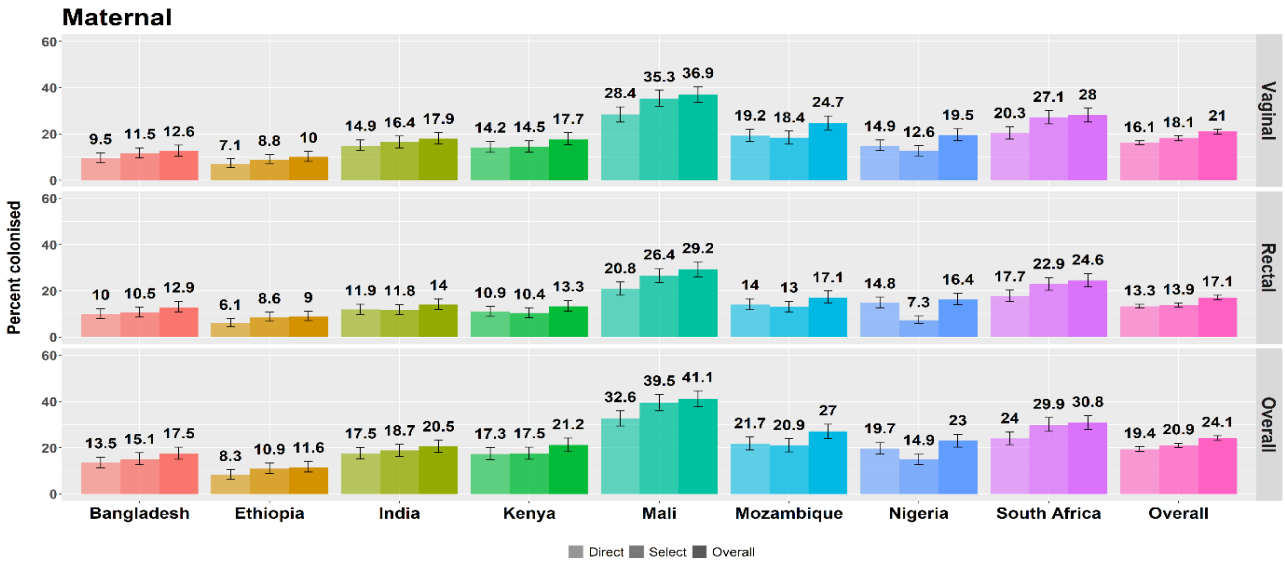
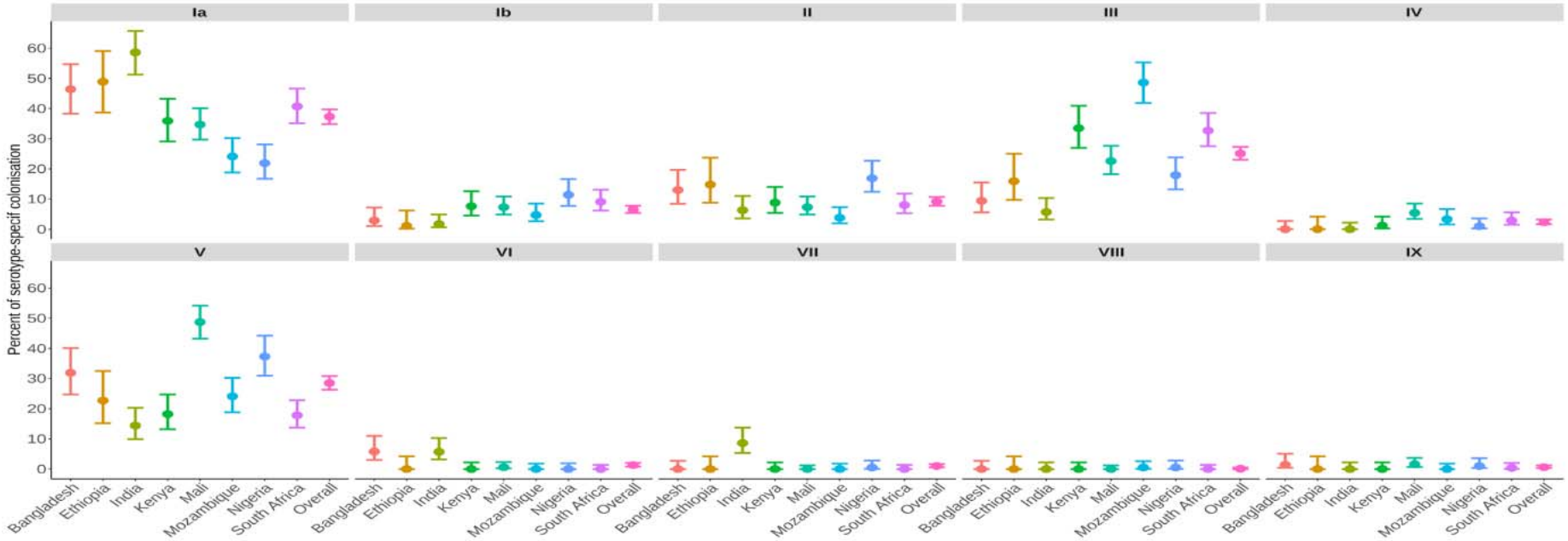


Figure 2: Prevalence of maternal GBS colonization overall, stratified by culture plating method and by specific sampling site.

Eleven percent (95%CI:9.6-12.6; 173/1572) of women were colonized with more than one serotype, including 10.1% (95%CI: 8.6-11.5; 158/1572) and 1.0% (95%CI:0.5-1.4, 15/1572) by two and three serotypes from different sites which includes isolates from DP and SB method, respectively; Table 18. The concordance of serotypes in women with rectal and vaginal colonization was 88.9% (95%CI:87.2-90.4; 1397/1572). All individual serotypes were included in analysis of serotype distribution in colonizing isolates.

Overall, the five most common colonizing serotypes were Ia (37.3%; 95%CI: 34.9-39.7; 586/1572), V (28.5%; 95%CI:26.3-30.8; 448/1572), III (25.1%; 95%CI:23.0-27.3; 394/1572;), II (9.2%; 95%CI: 7.8-10.7; 144/1572) and Ib (6.5%; 95%CI: 5.4-7.8; 102/1572). Geographic variability was observed in the relative serotype distribution. The five most common colonizing serotypes (Ia, Ib, II, III and V) contributed to 100% (91/91) of colonizing isolates in Ethiopia to 85.8% (151/176) in India. The most common serotypes by country were Ia in Bangladesh, India, Ethiopia, Kenya and South Africa (range: 35.9% to 56.8%), serotype V in Mali (48.7%, 95%CI: 43.2-54.2; 153/314) and Nigeria (37.3%, 95%CI: 30.9-44.2, 75/201) and serotype III

in Mozambique (48.6%,95%CI: 41.9-55.3; 103/212), Figure 3. Serotype VII contributed to 8.6% (95%CI:5.3-13.7;15/174) of all the isolates in India, where it was the third most common serotype; but was largely absent at other sites. Serotype VI was identified almost exclusively in Bangladesh (5.6%;95CI%: 3.0-11.0; 8/138) and India (5.7%,95%CI:3.2-10.3; 10/174).



rank	Bangladesh	Ethiopia	India	Kenya	Mali	Mozambique	Nigeria	South Africa	Overall
1	Ia (46.4%; 64/138)	Ia (48.9%; 43/88)	Ia (58.6%; 102/174)	Ia (35.9%; 61/170)	V (48.7%; 153/314)	III (48.6%; 103/212)	V (37.3%; 75/201)	Ia (40.7%; 112/275)	Ia (37.3%;
2	V (31.9%; 44/138)	V (22.7%; 20/88)	V (14.4%; 25/174)	III (33.5%; 57/170)	Ia (34.7%; 109/314)	Ia (24.1%; 51/212)	Ia (21.9%; 44/201)	III (32.7%; 90/275)	V (28.5%;
3	II (13.0%; 18/138)	III (15.9%; 14/88)	VII (8.6%; 15/174)	V (18.2%; 31/170)	III (22.6%; 71/314)	V (24.1%; 51/212)	III (17.9%; 36/201)	V (17.8%; 49/275)	III (25.1%;
4	III (9.4%; 13/138)	II (14.8%; 13/88)	II (6.3%; 11/174)	II (8.8%; 15/170)	Ib (7.3%; 23/314)	Ib (4.7%; 10/212)	II (16.9%; 34/201)	Ib (9.1%; 25/275)	II (9.2%; 144/1572)
5	VI (5.8%; 8/138)	Ib (1.1%; 1/88)	III (5.7%; 10/174)	Ib (7.6%; 13/170)	II (7.3%; 23/314)	II (3.8%; 8/212)	Ib (11.4%; 23/201)	II (8%; 22/275)	Ib (6.5%; 102/1572)
6	Ib (2.9%; 4/138)	ND	VI (5.7%; 10/174)	IV (1.2%; 2/170)	IV (5.4%; 17/314)	IV (3.3%; 7/212)	IV (1.0%; 2/201)	IV (2.9%; 8/275)	IV (2.3%; 36/1572)
7	IX (1.4%; 2/138)	ND	Ib (1.7%; 3/174)	ND	IX (1.6%; 5/314)	VIII (0.5%; 1/212)	IX (1.0%; 2/201)	IX (0.4%; 1/275)	VI (1.3%; 20/1572)
8	ND	ND	ND	ND	VI (0.6%; 2/314)	ND	VII (0.5%; 1/201)	ND	VII (1.0%; 16/1572)
9	ND	ND	ND	ND	ND	ND	VIII (0.5%; 1/201)	ND	IX (0.6%; 10/1572)
10	ND	ND	ND	ND	ND	ND	ND	ND	VIII (0.1%; 2/1572)

Ranks correspond to the site-specific rank from the top ten GBS serotypes of each study site; ND: Not Detected

Figure 3: Maternal Group B Streptococcus site-specific serotype distribution and rank in women with recto-vaginal colonization.

The overall prevalence of GBS bacteriuria was 8.8% (95%CI:8.0%-9.6%; 435/4958), ranging from 14.1% (95%CI11.9-16.5; 123/875) in South Africa to 3.9% (95%CI2.8-5.5; 31/788) in Bangladesh, table 19. Ninety-three percent (405/435) of the women with GBS bacteriuria, also had GBS cultured on either vaginal or rectal swabs. There was 94.1% (381/405) concordance in serotype of GBS isolates from urine and colonizing isolates, table 19.

We detected antimicrobials in urine of 7.5% (470/6227) of the women despite no reporting or record of antibiotic in their ante-natal card within the two-week period prior to onset of labour. Presence of antimicrobials in the urine was associated with lower odds of GBS colonization in the pregnant women (16.4% vs 24.8% overall; OR: 0.59, 95%CI: 0.46-0.76; p<0.0001), table 2; with site specific data in table 9-16.

Newborn GBS colonization

The culture of GBS was higher on SB compared with DP for all sites in the newborn; Figure 4. The culture of GBS was more common in skin surface samples (17.9%, 95%CI:17.0-18.8; 1172/6562), followed by throat (13.7%, 95%CI:12.9-14.5; 898/6562) and rectal swabs (12.8%, 95%CI:12.0-13.6; 839/6563). The overall prevalence of GBS colonization from any site in the newborn was 21.4% (95%CI 20.5-22.4; 1407/6563). The percentage of newborns with colonization of at least one site ranged from 12.3% (95%CI:10.1-14.8; 93/759) in Ethiopia to 38.3% (95%CI:35-41.8; 300/783) in Mali. The overall rate of vertical GBS colonization from the women with recto-vaginal GBS colonization to their newborn was 72.3% (95%CI 70.0-74.4; 1132/1566). The GBS vertical transmission rates were similar (range: 75.7% to 79.2%) in Ethiopia, India, Kenya, Mali and Mozambique; which was higher than in Nigeria (62.2%95%CI 55.3-68.6; 125/201) and South Africa (68.6%,; 95%CI 62.9-73.9; 186/271), whilst being lowest in Bangladesh (55.8%, 95%CI:47.5-63.8; 77/138); Table 8. Group B *Streptococcus* was also cultured from at least one site in 5.2% (95%CI:4.6-5.9; 257/4929) of

newborns' whose mothers were not identified to being colonized. There was a higher prevalence of GBS colonization in newborns of women with GBS bacteriuria (82.4%, 95%CI: 78.6-85.7; 357/433) compared with women colonized by GBS but without bacteriuria (61.1%, 95%CI: 57.6-64.6; 461/754 p<0.0001); Table 19.

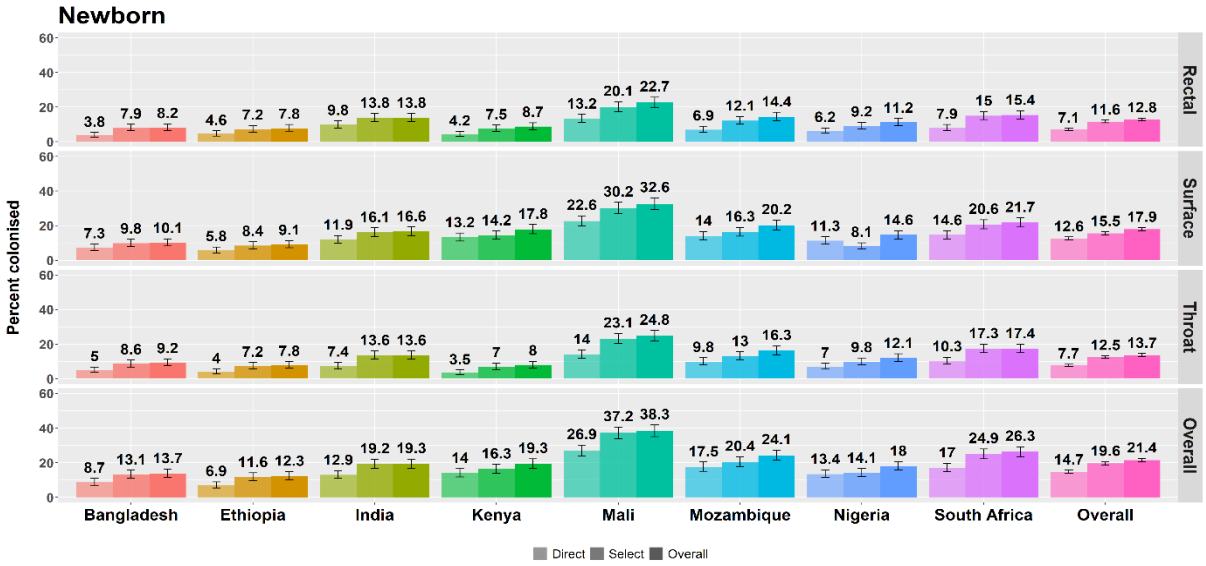


Figure 4: Newborn Group B streptococcus colonization overall, stratified by culture plating method and by specific sampling site.

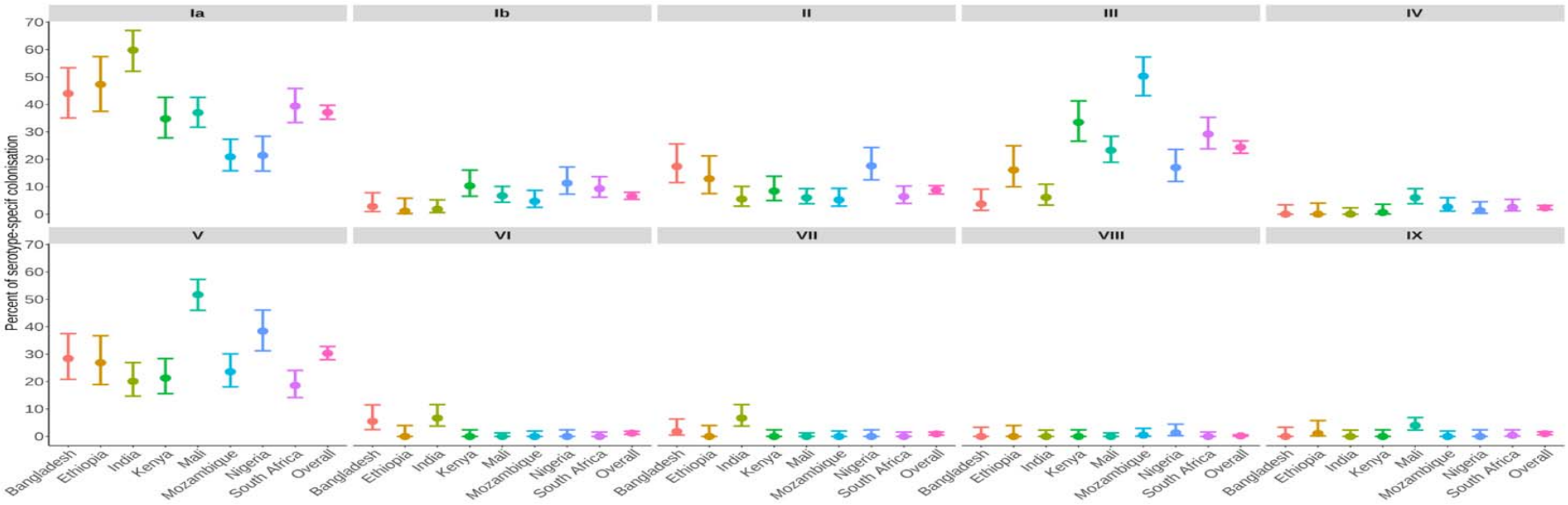
*Swab specimens were not collected for the following newborns
Skin: 4 (Mali: 1, Moz: 1, Nigeria:2) and 6 swabs not processed for selective broth
Rectal: 10 (Bangladesh:1, India;1, Ethiopia 1, Kenya:1, Mali;2, Moz;1, Nigeria 2, SA 1) and 35 swabs not processed for selective broth
Throat: 9 (Bangladesh:1, Ethiopia 1, Kenya:1, Moz;1, Nigeria 4, SA 1) and 32 swabs not processed for selective broth

Table 8: Vertical transfer of Group B streptococcus from the colonized mother to newborn at delivery.

Site	GBS colonization in newborns of women with GBS recto-vaginal colonization	GBS colonization in newborns of women with no GBS recto-vaginal colonization
Bangladesh	55.8% (95CI 47.5-63.8) [n=77/138]	4.9% (95% CI 3.5-6.9) [n=32/650]
Ethiopia	77% (95 CI 67.1-84.6) [n=67/87]	3.7% (95 CI 2.6-5.5) [n=25/667]
India	77% (95 CI 70.2-82.6) [n=134/174]	4.4% (95 CI 3.1-6.3) [n=30/67]
Kenya	75.7% (95 CI 68.8-81.6) [n=128/169]	4.1% (95 CI 2.8-6) [n=26/630]
Mali	78.7% (95 CI 73.8-82.8) [n=247/314]	10.2% (95 CI 7.8-13.4) [n=46/450]
Mozambique	79.2% (95 CI 73.3-84.2) [n=168/212]	3.5% (95 CI 2.3-5.3) [n=20/573]
Nigeria	62.2% (95 CI 55.3-68.6) [n=125/201]	4.6% (95 CI 3.3-6.5) [n=31/673]
South Africa	68.6% (95 CI 62.9-73.9) [n=186/271]	7.7% (95 CI 5.8-10.1) [n=47/610]
Overall	72.3% (95 CI 70.0-74.4) [n=1132/1566]	5.2% (95 CI 4.6-5.9) [n=257/4929]

95CI: 95% Confidence interval

Of the newborns with GBS colonization, 11.2% (95%CI:9.7-13.0; 158/1405) were colonized with two or more serotypes. The highest prevalence of colonization by more than one GBS serotype among newborns colonized with GBS was in Mali (28.0%, 95%CI:23.1-33.5; 84/300), whilst colonization with more than one serotype was lowest in Bangladesh (3.7%; 95%CI: 1.2-9.7; 4/109); Table 18. The GBS serotype distribution in the newborns, expectantly, was similar to their mothers; Figure 5. Among 49 twin sets, both babies were colonized in thirteen (26.5%) sets, whilst only the first and second born twin were colonized in four (8.2%) and three (6.1%) sets, respectively. Among the 13 sets of twins in whom both were colonized, different GBS serotypes were detected in four (30.1%) pairs.



rank	Bangladesh	Ethiopia	India	Kenya	Mali	Mozambique	Nigeria	South Africa	Overall
1	Ia (44%; 48/109)	Ia (47.3%; 44/93)	Ia (59.8%; 98/164)	Ia (34.8%; 54/155)	V (51.7%; 155/300)	III (50.3%; 96/191)	V (38.4%; 61/159)	Ia (39.4%; 93/236)	Ia (37.1%; 522/1407)
2	V (28.4%; 31/109)	V (26.9%; 25/93)	V (20.1%; 33/164)	III (33.5%; 52/155)	Ia (37%; 111/300)	V (23.6%; 45/191)	Ia (21.4%; 34/159)	III (29.2%; 69/236)	V (30.3%; 427/1407)
3	II (17.4%; 19/109)	III (16.1%; 15/93)	VI (6.7%; 11/164)	V (21.3%; 33/155)	III (23.3%; 70/300)	Ia (20.9%; 40/191)	II (17.6%; 28/159)	V (18.6%; 44/236)	III (24.4%; 343/1407)
4	VI (5.5%; 6/109)	II (12.9%; 12/93)	VII (6.7%; 11/164)	Ib (10.3%; 16/155)	Ib (6.7%; 20/300)	II (5.2%; 10/191)	III (17.0%; 27/159)	Ib (9.3%; 22/236)	II (8.8%; 124/1407)
5	III (3.7%; 4/109)	Ib (1.1%; 1/93)	III (6.1%; 10/164)	II (8.4%; 13/155)	II (6.0%; 18/300)	Ib (4.7%; 9/191)	Ib (11.3%; 18/159)	II (6.4%; 15/236)	Ib (6.5%; 92/1407)
6	Ib (2.8%; 3/109)	IX (1.1%; 1/93)	II (5.5%; 9/164)	IV (0.6%; 1/155)	IV (6.0%; 18/300)	IV (2.6%; 5/191)	IV (1.3%; 2/159)	IV (2.5%; 6/236)	IV (2.3%; 32/1407)
7	VII (1.8%; 2/109)	ND	Ib (1.8%; 3/164)	ND	IX (4.0%; 12/300)	VIII (0.5%; 1/191)	VIII (1.3%; 2/159)	IX (0.4%; 1/236)	VI (1.2%; 17/1407)
8	ND	ND	ND	ND	ND	ND	ND	ND	IX (1.0%; 14/1407)
9	ND	ND	ND	ND	ND	ND	ND	ND	VII (0.9%; 13/1407)
10	ND	ND	ND	ND	ND	ND	ND	ND	VIII (0.2%; 3/1407)

Figure 5 Newborn Group B Streptococcus site-specific serotype distribution and rank in newborn with recto-vaginal colonization.

Birth outcomes and deaths after delivery

Twenty women had a stillbirth, of whom six (30%; 95%CI:12.8 -54.3) were colonized with GBS. There were 19 newborn deaths across all the sites (three in Ethiopia, five each from Mali and Nigeria and six in Mozambique); of whom 31.6% (6/19) were colonized by GBS. Overall, 86 cases of attending physician-diagnosed suspected neonatal sepsis were recorded, including three each in India and Mozambique, 13 in South Africa and 67 episodes in Kenya. There were three confirmed cases of invasive GBS including one case each of EOD from Kenya (day 1 of age where neither the newborn or mother had documented GBS colonization) and two cases of LOD in Mozambique (no serotyping available), neither of whose mothers were colonised at delivery but one newborn was colonized by serotype III.

Discussion:

Our study findings demonstrated variability in prevalence of GBS recto-vaginal colonization in pregnant women across different African and Southeast Asian countries. Nevertheless, the differences in prevalence of GBS colonization observed in our study is of a lower magnitude compared with reports from systematic reviews (7), which recognised differences in sampling and culture methodology of studies as being limitations in the comparability of inter-study prevalence. By using standardised methodological approach across all countries our study enables more robust inter-country comparison on prevalence of maternal GBS recto-vaginal colonization and vertical transmission rate to newborns in across different African and Southeast Asian countries. Our findings of higher sensitivity of SB compared with DP GBS culture method for maternal vaginal and newborn skin surface, throat and rectal samples, illustrate how differences in culture methods *inter alia* other variables, could affect evaluating the prevalence of GBS colonization. Using combination of DP and SB should be used to

characterize the prevalence of maternal vaginal colonization, including when screening women ante-natally to decide on IAP.

The overall prevalence of maternal GBS colonization in our study (24.1%, 95%CI: 23.1%-25.2%) is higher than reported from a global meta-analysis (18%, 95%CI:17%–19%) (7). Furthermore, the variability of GBS colonization prevalence in women from African (25.8%) compared with South-east Asian sites (19.0%) was also less than reported in systematic reviews (18.2% vs. 12.0%) (7). At an individual country site-level, we observed a higher prevalence of maternal GBS colonization compared with previous studies in India (20.5% vs 7.6%)(40) and similarly so in Kenya (21.2% vs 11.1%)(41); however, similar prevalence of GBS colonization was observed in our study compared with earlier studies for Mozambique (42), South Africa (43), Bangladesh (10) and Ethiopia (44). An outlier was Nigeria, where prevalence of GBS colonization in our study (23.0%) was lower than reported from the same region but in a different study setting (34.2%) (45). Another notable finding of our study was the extraordinarily high prevalence of maternal GBS recto-vaginal colonization (41.1%) in Mali, for which there are no previous published studies. We believe that the higher overall prevalence of GBS colonization in our study compared with previous reports, is likely due to a more standardised approach been used to investigate for GBS colonization, including separate sampling of rectal and vaginal swabs and the use of SB medium.

The use of IAP targeted at women colonized with GBS beyond 32 weeks of gestational age, reduces the risk of EOD by 80% (6). Nevertheless, screening for GBS recto-vaginal colonization during pregnancy and implementation of IAP is not practiced in most LMIC countries due to logistical and cost constrains (6). Therefore, maternal vaccination against GBS is likely to be a more feasible option for reducing invasive GBS disease. Determining the serotype distribution as done in our study, could guide development of GBS vaccine to provide optimal serotype coverage at least in relation to EOD(46). Our findings on the dominant GBS

serotypes across different settings were comparable with serotype distribution data of maternal colonizing isolates reported in a meta-analysis (7). Serotype Ia, Ib, II, III and V were the most common across all the African sites, whilst serotypes VII and VI were among the top 5 serotypes sites only in the Southeast Asian sites. A recent study from Sri Lanka, also reported serotype VI as a common maternal colonizing serotype (17.9% of all isolates)(47). Furthermore, serotype VI (8.62%) and VII (6.9%) have also been implicated in EOD in India (48), indicating the clinical relevance of identifying these serotypes in colonized pregnant women. The reasons for geographic variability in serotype distribution among African and Southeast Asian women warrants further investigation.

The current hexavalent (serotypes Ia, Ib, II, III, IV and V) GBS polysaccharide-protein conjugate vaccine (GBS6) being evaluated in pregnant women (46), includes 97.3% of all maternal colonizing isolates in our study, ranging from 100% in Ethiopia and Kenya to 85.8% in India. It has been demonstrated that higher serotype-specific immunoglobulin G (IgG) in newborns, which had been acquired through transplacental transfer, is associated with reduced risk for invasive GBS disease and lower risk of acquisition of recto-vaginal colonization of GBS in the women during pregnancy (49, 50). Consequently, although vaccination of pregnant women with a GBS6 vaccine could reduce colonization by vaccine serotypes, there could be replacement colonization by non-vaccine serotypes such as serotype VI and VII, especially where those serotypes already prevail.

Our study also identified a high vertical transmission rate (55.8% to 79.2%) of GBS from women colonized by GBS to their newborns across all the sites. Earlier studies have reported considerable variation within and between geographic regions for vertical transmission of GBS, ranging from 7.6% in China to 63.3% in Ethiopia (8-10, 45). The high vertical transmission of GBS to newborns observed in our study could be due to greater sensitivity to detect GBS by sampling three different sites, as well as the culture method we used. An oddity

in our findings from Ethiopia, was the similar prevalence of newborn GBS colonization (12.3%) compared with the prevalence of recto-vaginal colonization in their mothers (11.6%). The findings from Ethiopia (and Bhutan which was excluded from the analysis) suggest that the maternal recto-vaginal swabbing methods (all culture for the Ethiopia site was done in South Africa) may have been sub-optimal, despite use of standardised operating procedures and training; and repeat attempts at quality assurance which were implemented across all the study sites. Consequently, we may have inadvertently under-estimated the prevalence of maternal GBS colonization in Ethiopia. We also observed that 5.2% of newborns colonized with GBS were born to women in whom no GBS colonization was identified, which was evident across all sites. Consequently, there may have been some under-ascertainment of GBS recto-vaginal colonization in the women, if we assume that the mothers are the sole source of GBS transmission to the newborn at the time of birth. As collection of recto-vaginal swabs is subject to operator competency, even when training is provided and there are standard operating procedures as was the case in our study, there might be inadvertent under-estimation of GBS colonization in women.

We identified GBS bacteriuria in the women to being strongly associated with of vertical transmission of GBS to the newborns. Maternal GBS bacteriuria has been associated with high density of recto-vaginal GBS colonization in the women and increased risk of EOD in their newborns (16, 28, 51). Limited diagnostic facilities for blood culture remains a major challenge in delineating the causes of bacterial sepsis in low-income countries. Consequently, the burden of invasive GBS disease is likely under-estimated and difficult to ascertain in low resource settings. We identified only one case of GBS EOD, yielding an incidence of 1.25 per 1000 livebirths in Kenya; albeit investigation for invasive GBS disease not having been undertaken systematically as part of the study and being dependent on standard of care practices at the site. Evaluating the vaccine efficacy of GBS6 against serotype-specific bacteriuria in pregnant

women should be considered as a potential exploratory objective aimed at imputing on possible efficacy of GBS6 against EOD.

Limitations of our study include that it was designed to determine prevalence of GBS colonization and did not systematically investigate for invasive GBS disease. Participants were enrolled from single study centre in each country, consequently we unable to generalize the data to the general population in a country. Another limitation was that the culture of GBS was reported qualitatively, which precluded a quantitative assessment of the density of GBS recto-vaginal colonization and association with bacteriuria in the women or vertical transmission rates to the newborn. Furthermore, as only a single colony was used for serotyping per sample, we likely under-estimated the frequency of colonization by multiple GBS serotypes. For the Ethiopian and Malian sites, swab specimens were shipped in STGG and processed centrally at south Africa which could have introduced bias with respect site specific prevalence, however, considering that these were the African countries with the lowest and highest prevalence of maternal colonization, there is no suggestion of the potential bias being in a single direction. The method of assessing the health status of infants varied among sites, with telephonic interviews in some and accessing hospital records in others and may have introduce variations in data collection and reporting, potentially affecting the accuracy of health status assessments. Although there were multiple comparisons undertaken in the analyses, no adjustment to p-values were made for this observational study, warranting caution in interpretation of the comparative analysis. Nevertheless, in our analyses, all statistically significant p-values were <0.001 indicating the strong likelihood presence of an association.

In conclusion, using standardised approach in sampling and methods for GBS culture, our study reported high prevalence of GBS colonization in most settings, with some geographic variability within African countries. Post-licensure vaccine effectiveness studies should also

focus on maternal GBS serotype distribution as non-vaccine serotype replacement could occur due to vaccine immune pressure. High prevalence of maternal GBS colonization in our study settings indicates early-onset disease is likely to be prevalent in the study settings, particularly in the absence of any screening for GBS colonization in women during pregnancy and no systematic use of IAP.

Contributors

GK, SAM conceptualised the study. GK, CLC, SAM designed the study. GK, SAM oversaw the conduct of the study database design and development, site selection and training, data collection, data cleaning, and interpretation of results. GK and AI did the statistical analysis. GA, MMA, ZA, MMB, HCB, JAB, TEC, AC, SC, ND, PD, MI, AMK, StM, SaM, NM, HM, VM, SO, BS, SOS, SKS, SS, RS, EAFS, RDS, MDT, BV are site principal investigators and enrolled participants, collected data. GK prepared the 1st Draft. GK and AI accessed and verified the data. All authors had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Conflict of Interest: The study was funded by The Bill and Melinda Gates Foundation. SAM institution has received grant funding on GBS, separate from this study, from Pfizer, GlaxoSmithKline and Minervax.

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helping with the development of EQA panel. The study was funded by a grant from the Bill and Melinda Gates Foundation (Grant number: INV-008687).

Data sharing

Deidentified individual-level participant data and data dictionary will be available for sharing after approval of a proposal by the University of the Witwatersrand Human Ethics Research Committee and following a signed data access agreement. Requests for data sharing can be directed to the corresponding author.

8 Extended results and Supplementary information for the Lancet Microbe journal

Supplementary information for "Prevalence of Group B Streptococcus colonization in mother-newborn dyads in low and middle income South Asian and African countries: A prospective, observational cross sectional study."

Comparison of GBS culture by direct plating and selective broth

In pregnant women:

The culture of GBS from the vaginal swabs was higher using the SB (18.1%; 1177/6504; 95%CI:17.2-19.1), than the DP (16.1%; 1048/6510; 95%CI:15.2-17.0 p=0.003) culture method. Relative to the composite of culture positivity by either method, the sensitivity for culture of GBS was 86.2% (95%CI: 84.3-88.0) and 76.8% (95%CI:74.5-78.9) for the SP and DP methods, respectively. There was no difference in GBS culture positivity between the SB (13.9%; 901/6502; 95%CI: 13.0-14.7) and DP (13.3%; 867/6505; 95%CI 75.2-80.1; p=0.384) methods for rectal swabs. For rectal swabs, the sensitivity of SB and DP were 80.8% (95%CI: 78.4-83.0) and 77.8% (95%CI: 75.2-80.1), respectively, relative to culture positivity by either method, Figure 2.

In newborns:

Overall, the culture of GBS in newborns was higher on SB compared with DP plating, including for skin surface [(15.5%, 95%CI 14.6-16.4; 1014/6552 vs. 12.6%, (95%CI 11.8-13.4; 827/6558); p<0.001)], rectal [(11.6%, 95%CI 10.9-12.4; 758/6516) vs. 7.1%, 95%CI 6.5-7.7; 465/6551; p<0.001)] and throat [(12.5%, 95%CI 11.7-13.3; 815/6523) vs. 7.7%, 95%CI 7.1-8.3; 503/6555; p<0.001)] samples . Similar observations were evident for all sites except in Nigeria, where skin swabs sampled by SB (8.1%, 72/885; 95%CI: 6.5-10.1) had a lower yield of isolating GBS compared with DP method (11.3%, 101/886; 95%CI: 9.4-13.6; p=0.021). Compared with the composite of positivity from either SB or DP, the sensitivity of SB was 91.4% (1293/1414; 95%CI: 90.1-92.8) and 68.4% (968/1414; 95%CI: 66.0-70.8) for DP; Figure 3.

Table 9: Demographics of the study population at Bangladesh at the time of enrolment.

Characteristics		Overall (n=788)	GBS Colonised	GBS Uncolonised	P-value
Mother's age (n=787), Median 22 (20-27)	<20	303 (38.5%) [a]	46 (15.2%) [b]	257 (84.8%)[c]	0.15
	20 - <25	246 (31.3%)	46 (18.7%)	200 (81.3%)	
	25 - <30	192 (24.4%)	36 (18.8%)	156 (81.2%)	
	30 - <35	38 (4.8%)	8 (21.1%)	30 (78.9%)	
	35 - <40	8 (1%)	2 (25%)	6 (75%)	
	>=40	0 (0%)	0 (0%)	0 (0%)	
Ethnicity (n=787)	Asian	787 (100%)	138 (17.5%)	649 (82.5%)	N/A
Gravidity (n=788), Median (IQR): 1(0-1)	1	379 (48.1%)	61 (16.1%)	318 (83.9%)	0.06
	2	231 (29.3%)	43 (18.6%)	188 (81.4%)	
	3	127 (16.1%)	30 (23.6%)	97 (76.4%)	
	>3	51 (6.5%)	4 (7.8%)	47 (92.2%)	
Parity (n=409), Median (IQR): 1(1-2)	0	54 (13.2%)	9 (16.7%)	45 (83.3%)	0.96
	1	233 (57%)	47 (20.2%)	186 (79.8%)	
	2	100 (24.4%)	18 (18%)	82 (82%)	
	3	20 (4.9%)	3 (15%)	17 (85%)	
	>3	2 (0.5%)	0 (0%)	2 (100%)	
Previous stillborns (n=409) [d]	1	29 (7.1%)	8 (27.6%)	21 (72.4%)	0.31
	0	380 (92.9%)	69 (18.2%)	311 (81.8%)	
Previous spontaneous abortion (n=409) [d]	1	78 (19.1%)	11 (14.1%)	67 (85.9%)	0.30
	0	331 (80.9%)	66 (19.9%)	265 (80.1%)	
Median MUAC (IQR)		27 (24-30)	28 (25-30)	27 (24-30)	0.38
Babies born (Maternal n=788)					
Singleton		782 (99.2%)	137 (17.5%)	645 (82.5%)	>0.99
Twins		6 (0.8%)	1 (16.7%)	5 (83.3%)	
Antimicrobial in the urine (n=788)	1	62 (7.9%)	7 (11.3%)	55 (88.7%)	0.24
	0	726 (92.1%)	131 (18%)	595 (82%)	
Bacteriuria (n=788)	1	31 (3.9%)	31 (100%)	0 (0%)	
	0	757 (96.1%)	107 (14.1%)	650 (85.9%)	<0.001
Mode of Delivery (n=794)					
Normal vaginal delivery		589 (74.2%)	98 (16.6%)	491 (83.4%)	Ref
Caesarean section (C/s)		205 (25.8%)	41 (20%)	164 (80%)	0.32
Birth weight Median (IQR)		2800 (2500-3100)	2800 (2500-3100)	2800 (2500-3100)	0.82

[a,b,c] are row percentages of overall; [d] did not differ when stratified on number of previous stillbirths; IQR: Inter quartile range; p-value using univariate logistic regression analysis or Chi-square test. All C/s deliveries were Emergency C/s

Table 10: Demographics of the study population at Ethiopia at time of enrolment.

Characteristics		Overall	Colonised	Uncolonised	P-value
Mother's age N=758, Median: 25 (22-28)	<20	137 (18.1%) [a]	22 (16.1%) [b]	115 (83.9%) [c]	0.11
	20 - <25	320 (42.2%)	37 (11.6%)	283 (88.4%)	
	25 - <30	230 (30.3%)	21 (9.1%)	209 (90.9%)	
	30 - <35	52 (6.9%)	8 (15.4%)	44 (84.6%)	
	35 - <40	16 (2.1%)	0 (0%)	16 (100%)	
	>=40	3 (0.4%)	0 (0%)	3 (100%)	
Ethnicity=678	Black	678 (100%)	81 (11.9%)	597 (88.1%)	N/A
Gravidity (n=737),Median (IQR): 0(0-1)	1	417 (56.6%)	52 (12.5%)	365 (87.5%)	0.87
	2	162 (22%)	18 (11.1%)	144 (88.9%)	
	3	91 (12.3%)	9 (9.9%)	82 (90.1%)	
	>3	67 (9.1%)	9 (13.4%)	58 (86.6%)	
Parity (n=334), Median (IQR): 1(1-2)	0	48 (14.4%)	6 (12.5%)	42 (87.5%)	0.43
	1	160 (47.9%)	15 (9.4%)	145 (90.6%)	
	2	78 (23.4%)	8 (10.3%)	70 (89.7%)	
	3	26 (7.8%)	2 (7.7%)	24 (92.3%)	
	>3	22 (6.6%)	5 (22.7%)	17 (77.3%)	
Previous stillborns (n=323)[d]	1	9 (2.8%)	1(11.1%)	8(88.9%)	>0.999
	0	314 (97.2%)	35 (11.1%)	279 (88.9%)	
Previous spontaneous abortion (n=323)[d]	1	29 (9%)	2(6.9%)	27 (93.1%)	0.75
	0	294 (91%)	34 (11.6%)	260 (88.4%)	
Median MUAC (IQR)		24 (22-25)	24.35 (22-25.075)	24 (22-25)	0.38
Babies born (Maternal n=759)					
Singleton		744 (98%)	86 (11.6%)	658 (88.4%)	0.69
Twins		15 (2%)	2 (13.3%)	13 (86.7%)	
Antimicrobial in the urine(n=748)	1	109 (14.6%)	15(13.8%)	94 (86.2%)	
	0	639 (85.4%)	70 (11%)	569 (89%)	0.49
Mode of Delivery (n=755)					
Normal vaginal delivery		657 (87%)	77 (11.7%)	580 (88.3%)	Ref
Caesarian section (C/s)		98 (13%)	10 (10.2%)	88 (89.8%)	0.79
Birth weight Median (IQR)		3300 (3000-3500)	3300 (3000-3500)	3200 (3000-3500)	0.63

[a,b,c] are row percentages of overall; [d] did not differ when stratified on number of previous stillbirths; IQR: Inter quartile range; p-value using univariate logistic regression analysis or Chi-square test. urine samples were not tested for Bacteriuria

Table 11: Demographics of the study population at India at time of enrolment.

Characteristics		Overall [a]	Colonised [b]	Uncolonised [c]	P-value
Mother's age N=850, Median: 25 (23-28)	<20	69 (8.1%)	13 (18.8%)	56 (81.2%)	0.94
	20 - <25	363 (42.7%)	79 (21.8%)	284 (78.2%)	
	25 - <30	313 (36.8%)	60 (19.2%)	253 (80.8%)	
	30 - <35	97 (11.4%)	19 (19.6%)	78 (80.4%)	
	35 - <40	8 (0.9%)	3 (37.5%)	5 (62.5%)	
	>=40	0 (0%)	0 (0%)	0 (0%)	
Ethnicity (n=850)	Asian	850 (100%)	174 (20.5%)	676 (79.5%)	
Gravidity (n=848),Median (IQR): 0(0-1)	1	465 (54.8%)	97 (20.9%)	368 (79.1%)	0.38
	2	229 (27%)	40 (17.5%)	189 (82.5%)	
	3	107 (12.6%)	24 (22.4%)	83 (77.6%)	
	>3	47 (5.5%)	13 (27.7%)	34 (72.3%)	
Parity (n=319), Median (IQR): 1(1-1)	0	0 (0%)	0 (0%)	0 (0%)	0.05
	1	269 (84.3%)	50 (18.6%)	219 (81.4%)	
	2	47 (14.7%)	13 (27.7%)	34 (72.3%)	
	3	3 (0.9%)	2 (66.7%)	1 (33.3%)	
	>3	0 (0%)	0 (0%)	0 (0%)	
Previous stillborns (n=228)[d]	1	17 (7.5%)	5(29.4%)	12(70.6%)	0.60
	0	211 (92.5%)	44 (20.9%)	167 (79.1%)	
Previous spontaneous abortion (n=314)[d]	1	104(33.1%)	15	89	0.21
	0	210 (66.9%)	44 (21%)	166 (79%)	
Median MUAC (IQR)		29 (26-32)	29 (26.5-32)	28 (26-32)	0.04
Babies born (Maternal n=850)					
Singleton		848 (99.8%)	174 (20.5%)	674 (79.5%)	>0.999
Twins		2 (0.2%)	0 (0%)	2 (100%)	
Antimicrobial in the urine (n=769)	1	56 (7.3%)	7 (12.5%)	49 (87.5%)	0.18
	0	713 (92.7%)	150 (21%)	563 (79%)	
Bacteriuria (n=841)		38 (4.5%)	34 (89.5%)	4 (10.5%)	
		803 (95.5%)	137 (17.1%)	666 (82.9%)	<0.001
Mode of Delivery					
Normal vaginal delivery		850 (100%)	174 (20.5%)	676 (79.5%)	Ref
Caesarian section (C/s)		0 (0%)			N/A
Birth weight median (IQR)		3000 (2740-3280)	3005 (2730-3277.5)	2992 (2740-3279)	0.62

[a,b,c] are row percentages of overall. [d] did not differ when stratified on number of previous stillbirths; IQR: Inter quartile range; p-value using univariate logistic regression analysis or Chi-square test.

Table 12: Demographics of the study population at Kenya at time of enrolment.

Characteristics		Overall	Colonised	Uncolonised	P-value
Mother's age N=802, Median: 25 (22-29)	<20	155 (19.3%) [a]	28 (18.1%) [b]	127 (81.9%) [c]	0.82
	20 - <25	289 (36%)	68 (23.5%)	221 (76.5%)	
	25 - <30	213 (26.6%)	44 (20.7%)	169 (79.3%)	
	30 - <35	98 (12.2%)	20 (20.4%)	78 (79.6%)	
	35 - <40	38 (4.7%)	9 (23.7%)	29 (76.3%)	
	>=40	9 (1.1%)	1 (11.1%)	8 (88.9%)	
Ethnicity (n=802)	Black	801 (99.9%)	170 (21.2%)	631 (78.8%)	Ref
	Asian	1 (0.1%)	0 (0%)	1 (100%)	>0.99
Gravidity (n=8-2),Median (IQR): 1(0-3)	1	273 (34%)	62 (22.7%)	211 (77.3%)	0.83
	2	200 (24.9%)	43 (21.5%)	157 (78.5%)	
	3	126 (15.7%)	26 (20.6%)	100 (79.4%)	
	>3	203 (25.3%)	39 (19.2%)	164 (80.8%)	
Parity (n=529), Median (IQR): 2(1-3)	0	36 (6.8%)	8 (22.2%)	28 (77.8%)	0.79
	1	195 (36.9%)	39 (20%)	156 (80%)	
	2	119 (22.5%)	26 (21.8%)	93 (78.2%)	
	3	76 (14.4%)	18 (23.7%)	58 (76.3%)	
	>3	103 (19.5%)	17 (16.5%)	86 (83.5%)	
Previous stillborns (n=529)[d]	1	25 (4.7%)	2 (8%)	23(92%)	0.13
	0	504 (95.3%)	106 (21%)	398 (79%)	
Previous spontaneous abortion (n=529)[d]	1	92 (17.4%)	18 (19.6%)	74 (80.4%)	0.94
	0	437 (82.6%)	90 (20.6%)	347 (79.4%)	
Median MUAC (IQR)		25 (24-28)	26 (24-28)	25 (24-28)	0.31
Babies born (Maternal n=802)					
Singleton		801 (99.9%) N=802	170 (21.2%)	631 (78.8%)	>0.99
Twins		1 (0.1%) N=802	0 (0%)	1 (100%)	
Antimicrobial in the urine (n=777)	1	21 (2.7%)	5 (23.8%)	16 (76.2%)	
	0	756 (97.3%)	165 (21.8%)	591 (78.2%)	>0.99
Bacteriuria (n=802)	1	72 (9%)	71 (98.6%)	1 (1.4%)	
	0	730 (91%)	99 (13.6%)	631 (86.4%)	<0.001
Mode of Delivery (n=803)					
Normal vaginal delivery		716 (89.2%)	156 (21.8%)	560 (78.2%)	Ref
Caesarean section (C/s)		87 (10.8%)	14 (16.1%)	73 (83.9%)	0.28
Emergency C/s		85 (10.6%)	13 (15.3%)	72 (84.7%)	0.22
Elective C/s		2 (0.2%)	1 (50%)	1 (50%)	0.33
Birth weight Median (IQR)		3070 (2800-3350)	3022.5 (2767.5-3240)	3075 (2818.75-3380)	0.11

[a,b,c] are row percentages of overall, [d] did not differ when stratified on number of previous stillbirths; IQR: Inter quartile range; p-value using univariate logistic regression analysis or Chi-square test.

Table 13: Demographics of the study population at Mali at time of enrolment.

Characteristics		Overall	Colonised	Uncolonised	P-value
Mother's age N=764, Median: 25 (21-30)	<20	180 (23.6%) [a]	65 (36.1%) [b]	115 (63.9%) [c]	0.12
	20 - <25	234 (30.6%)	92 (39.3%)	142 (60.7%)	
	25 - <30	193 (25.3%)	90 (46.6%)	103 (53.4%)	
	30 - <35	92 (12%)	35 (38%)	57 (62%)	
	35 - <40	59 (7.7%)	28 (47.5%)	31 (52.5%)	
	>=40	6 (0.8%)	4 (66.7%)	2 (33.3%)	
Ethnicity (n=764)	Black	764 (100%)	314 (41.1%)	450 (58.9%)	
Gravidity (n=764),Median (IQR): 2(1-4)	1	155 (20.3%)	58 (37.4%)	97 (62.6%)	0.62
	2	170 (22.3%)	67 (39.4%)	103 (60.6%)	
	3	124 (16.2%)	54 (43.5%)	70 (56.5%)	
	>3	315 (41.2%)	135 (42.9%)	180 (57.1%)	
Parity (609), Median (IQR): 2(1-4)	0	17 (2.8%)	6 (35.3%)	11 (64.7%)	0.92
	1	172 (28.2%)	69 (40.1%)	103 (59.9%)	
	2	126 (20.7%)	54 (42.9%)	72 (57.1%)	
	3	96 (15.8%)	43 (44.8%)	53 (55.2%)	
	>3	198 (32.5%)	84 (42.4%)	114 (57.6%)	
Previous stillborns (n=609)[d]	1	45 (7.4%)	19 (42.2%)	26 (57.8%)	>0.99
	0	564 (92.6%)	237 (42%)	327 (58%)	
Previous spontaneous abortion (609)[d]	1	77 (12.6%)	30 (11.7%)	47 (13.3%)	0.64
	0	532 (87.4%)	226 (42.5%)	306 (57.5%)	
Median MUAC (IQR)		29 (26-32)	30 (26-32)	29 (26-31)	0.17
Babies born (Maternal n=764)					
Singleton		743 (97.3%)	302 (40.6%)	441 (59.4%)	0.20
Twins		21 (2.7%)	12 (57.1%)	9 (42.9%)	
Antimicrobial in the urine, n=762	1	41 (5.4%)	14 (34.1%)	27 (65.9%)	0.44
	0	721 (94.6%)	299 (41.5%)	422 (58.5%)	
Mode of Delivery					
Normal vaginal delivery (n=785)		754 (96.1%)	310 (41.1%)	444 (58.9%)	Ref
Caesarian section (C/s) (n=31)		31 (3.9%)	16 (51.6%)	15 (48.4%)	0.33
Emergency C/s		31 (100%)	16 (51.6%)	15 (48.4%)	
Birth weight Median (IQR)		3000 (2700-3400)	3002.5 (2700-3400)	3000 (2700-3350)	0.31

[a,b,c] are row percentages of overall; [d] did not differ when stratified on number of previous stillbirths; IQR: Inter quartile range; p-value using univariate logistic regression analysis or Chi-square test. urine samples were not tested for Bacteriuria

Table 14: Demographics of the study population at Mozambique at time of enrolment.

Characteristics		Overall	Colonised	Uncolonised	P-value
Mother's age N=785, Median: 23 (20-28)	<20	231 (29.4%) [a]	53 (22.9%) [b]	178 (77.1%) [c]	0.19
	20 - <25	273 (34.8%)	80 (29.3%)	193 (70.7%)	
	25 - <30	129 (16.4%)	40 (31%)	89 (69%)	
	30 - <35	80 (10.2%)	20 (25%)	60 (75%)	
	35 - <40	58 (7.4%)	18 (31%)	40 (69%)	
	>=40	14 (1.8%)	1 (7.1%)	13 (92.9%)	
Ethnicity (n=785)	Black	784 (99.9%)	212 (27%)	572 (73%)	Ref >0.99
	Other	1 (0.1%)	0 (0%)	1 (100%)	
Gravidity (n=784)Median (IQR): 1(0-3)	1	204 (26%)	51 (25%)	153 (75%)	0.81
	2	217 (27.7%)	57 (26.3%)	160 (73.7%)	
	3	135 (17.2%)	39 (28.9%)	96 (71.1%)	
	>3	228 (29.1%)	65 (28.5%)	163 (71.5%)	
Parity (n=580), Median (IQR): 2(1-3)	0	60 (10.3%)	14 (23.3%)	46 (76.7%)	0.54
	1	203 (35%)	62 (30.5%)	141 (69.5%)	
	2	131 (22.6%)	37 (28.2%)	94 (71.8%)	
	3	87 (15%)	26 (29.9%)	61 (70.1%)	
	>3	99 (17.1%)	22 (22.2%)	77 (77.8%)	
Previous stillborns (n=578) [d]	1	22 (3.8%)	8 (36.4%)	14 (63.6%)	0.49
	0	556 (96.2%)	152 (27.3%)	404 (72.7%)	
Previous spontaneous abortion (n=578) [d]	1	95 (16.4%)	38 (40%)	57 (60%)	0.01
	0	483 (83.6%)	123 (25.5%)	360 (74.5%)	
Median MUAC (IQR)		27 (26-29)	27 (25-29)	27 (26-29)	0.73
Babies born (Maternal n=785)					
Singleton		779 (99.2%)	208 (26.7%)	571 (73.3%)	0.05
Twins		6 (0.8%)	4 (66.7%)	2 (33.3%)	
Antimicrobial in the urine, n=750	1	46 (6.1%)	7 (15.2%)	39 (84.8%)	0.08
	0	704 (93.9%)	198 (28.1%)	506 (71.9%)	
Bacteriuria (n=778)	1	89 (11.4%)	84 (94.4%)	5 (5.6%)	
	0	689 (88.6%)	127 (18.4%)	562 (81.6%)	<0.001
Mode of Delivery (n=791)					
Normal vaginal delivery		791 (100%)	216 (27.3%)	575 (72.7%)	Ref
Caesarian section (C/s)		0 (0%)	0 (0%)	0 (0%)	N/A
Birth weight median (IQR)		3200 (2900-3400)	3200 (2900-3400)	3200 (2900-3400)	0.64

[a,b,c] are row percentages of overall; [d] did not differ when stratified on number of previous stillbirths; IQR: Inter quartile range; p-value using univariate logistic regression analysis or Chi-square test.

Table 15: Demographics of the study population at Nigeria at time of enrolment.

Characteristics		Overall	Colonised	Uncolonised	P-value
Mother's age N=874, Median: 30 (27-34)	<20	16 (1.8%) [a]	3 (18.8%) [b]	13 (81.2%) [c]	0.35
	20 - <25	117 (13.4%)	20 (17.1%)	97 (82.9%)	
	25 - <30	337 (38.6%)	77 (22.8%)	260 (77.2%)	
	30 - <35	266 (30.4%)	72 (27.1%)	194 (72.9%)	
	35 - <40	127 (14.5%)	26 (20.5%)	101 (79.5%)	
	>=40	11 (1.3%)	3 (27.3%)	8 (72.7%)	
Ethnicity (n=872)	Black	872 (100%)	201 (23.1%)	671 (76.9%)	
Gravidity (n=871),Median (IQR): 2(1-3)	1	185 (21.2%)	37 (20%)	148 (80%)	0.41
	2	217 (24.9%)	46 (21.2%)	171 (78.8%)	
	3	189 (21.7%)	50 (26.5%)	139 (73.5%)	
	>3	280 (32.1%)	68 (24.3%)	212 (75.7%)	
Parity (n=624), Median (IQR): 2(1-3)	0	52 (8.3%)	15 (28.8%)	37 (71.2%)	0.57
	1	239 (38.3%)	53 (22.2%)	186 (77.8%)	
	2	162 (26%)	41 (25.3%)	121 (74.7%)	
	3	108 (17.3%)	28 (25.9%)	80 (74.1%)	
	>3	63 (10.1%)	11 (17.5%)	52 (82.5%)	
Previous stillborns (n=516) [d]	1	50 (9.7%)	11 (22%)	39 (78%)	0.94
	0	466 (90.3%)	110 (23.6%)	356 (76.4%)	
Previous spontaneous abortion (617) [d]	1	239 (38.7%)	57 (23.8%)	182 (76.2%)	0.91
	0	378 (61.3%)	93 (24.6%)	285 (75.4%)	
Median MUAC (IQR)		30 (28-32)	30 (28-33)	30 (28-32)	0.04
Babies born (Maternal=874)					
Singleton		863 (98.7%)	198 (22.9%)	665 (77.1%)	0.72
Twins		11 (1.3%)	3 (27.3%)	8 (72.7%)	
Antimicrobial in the urine, n=805	1	67 (8.3%)	9 (13.4%)	58 (86.6%)	0.08
	0	738 (91.7%)	173 (23.4%)	565 (76.6%)	
Bacteriuria (n=874)	1	82 (9.4%)	71 (86.6%)	11 (13.4%)	
	0	792 (90.6%)	130 (16.4%)	662 (83.6%)	<0.001
Mode of Delivery (n=885)					
Normal vaginal delivery		600 (67.8%)	149 (24.8%)	451 (75.2%)	Ref
Caesarian section (C/s)		285 (32.2%)	55 (19.3%)	230 (80.7%)	0.08
Emergency C/s		177 (20%)	33 (18.6%)	144 (81.4%)	0.09
Elective C/s		108 (12.2%)	22 (20.4%)	86 (79.6%)	0.32
Birth weight Median (IQR)		3200 (2900-3500)	3175 (2850-3500)	3200 (2900-3500)	0.28

[a,b,c] are row percentages of overall. [d] did not differ when stratified on number of previous stillbirths; IQR: Inter quartile range; p-value using univariate logistic regression analysis or Chi-square test.

Table 16: Demographics of the study population at South Africa at time of enrolment.

Characteristics		Overall [a]	Colonised [b]	Uncolonised [c]	P-value
Mother's age N=892, Median: 25 (22-30)	<20	147 (16.5%)	39 (26.5%)	108 (73.5%)	0.01
	20 - <25	306 (34.3%)	84 (27.5%)	222 (72.5%)	
	25 - <30	228 (25.6%)	68 (29.8%)	160 (70.2%)	
	30 - <35	128 (14.3%)	52 (40.6%)	76 (59.4%)	
	35 - <40	64 (7.2%)	23 (35.9%)	41 (64.1%)	
	>=40	19 (2.1%)	9 (47.4%)	10 (52.6%)	
Ethnicity (n=889)	Black	860 (96.7%)	265 (30.8%)	595 (69.2%)	Ref
	Asian	2 (0.2%)	0 (0%)	2 (100%)	>0.999
	Other	27 (3%)	9 (33.3%)	18 (66.7%)	0.78
Gravidity (n=891),Median (IQR): 1(0-2)	1	357 (40.1%)	95 (26.6%)	262 (73.4%)	0.03
	2	277 (31.1%)	83 (30%)	194 (70%)	
	3	163 (18.3%)	61 (37.4%)	102 (62.6%)	
	>3	94 (10.5%)	36 (38.3%)	58 (61.7%)	
Parity (n=534), Median (IQR): 1(1-2)	0	76 (14.2%)	22 (28.9%)	54 (71.1%)	0.60
	1	254 (47.6%)	83 (32.7%)	171 (67.3%)	
	2	145 (27.2%)	51 (35.2%)	94 (64.8%)	
	3	39 (7.3%)	17 (43.6%)	22 (56.4%)	
	>3	20 (3.7%)	7 (35%)	13 (65%)	
Previous stillborns (n=522)[d]	1	20 (3.8%)	7 (35%)	13 (65%)	>0.99
	0	502 (96.2%)	169 (33.7%)	333 (66.3%)	
Previous spontaneous abortion (n=531)[d]	1	117 (22%)	40 (34.2%)	77 (65.8%)	0.95
	0	414 (78%)	138 (33.3%)	276 (66.7%)	
Median MUAC (IQR)		29 (26-32)	29 (27-32)	28 (26-32)	0.01
Babies born (Maternal n=892)					
Singleton		885 (99.2%)	275 (31.1%)	610 (68.9%)	0.10
Twins		7 (0.8%)	0 (0%)	7 (100%)	
Antimicrobial in the urine (n=828)	1	68 (8.2%)	13 (19.1%)	55 (80.9%)	0.04
	0	760 (91.8%)	243 (32%)	517 (68%)	
Bacteriuria (n=875)	1	123 (14.1%)	114 (92.7%)	9 (7.3%)	
	0	752 (85.9%)	157 (20.9%)	595 (79.1%)	<0.001
Mode of Delivery (n=889)					
Normal vaginal delivery		642 (71.4%)	192 (29.9%)	450 (70.1%)	Ref
Caesarian section (C/s)		257 (28.9%)	83 (32.3%)	174 (67.7%)	0.53
Emergency C/s		246 (27.7%)	79 (32.1%)	167 (67.9%)	0.52
Elective C/s		11 (1.2%)	4 (36.4%)	7 (63.6%)	0.74
Birth weight Median (IQR)		3205 (2915-3507.5)	3200 (2910-3515)	3207.5 (2920-3503.75)	0.82

[a,b,c] are row percentages of overall; [d] did not differ when stratified on number of previous stillbirths; IQR: Inter quartile range; p-value using univariate logistic regression analysis or Chi-square test.

Table 17: Association between GBS colonization and demographic characteristics at enrolment.

Characteristics		Overall n (%)	Not colonised n (%)	Colonised n (%)	aOR (95% CI)	P value
Study site	South Africa	892 (13.7%) N=6514	617 (12.5%) N=4942	275 (17.5%) N=1572	Reference	Reference
	Bangladesh	788 (12.1%) N=6514	650 (13.2%) N=4942	138 (8.8%) N=1572	0.50 (0.39-0.64)	<0.001
	Ethiopia	759 (11.7%) N=6514	671 (13.6%) N=4942	88 (5.6%) N=1572	0.31 (0.24-0.41)	<0.001
	India	850 (13%) N=6514	676 (13.7%) N=4942	174 (11.1%) N=1572	0.59 (0.47-0.75)	<0.001
	Kenya	802 (12.3%) N=6514	632 (12.8%) N=4942	170 (10.8%) N=1572	0.62 (0.49-0.78)	<0.001
	Mali	764 (11.7%) N=6514	450 (9.1%) N=4942	314 (20%) N=1572	1.57 (1.26-1.96)	<0.001
	Mozambique	785 (12.1%) N=6514	573 (11.6%) N=4942	212 (13.5%) N=1572	0.86 (0.68-1.09)	0.21
	Nigeria	874 (13.4%) N=6514	673 (13.6%) N=4942	201 (12.8%) N=1572	0.62 (0.49-0.78)	<0.001
Age	<20	1238 (19%) N=6512	969 (19.6%) N=4940	969 (19.6%) N=4940	Ref	Ref
	20 - <25	2148 (33%) N=6512	1642 (33.2%) N=4940	1642 (33.2%) N=4940	1.15 (0.95-1.38)	0.15
	25 - <30	1835 (28.2%) N=6512	1399 (28.3%) N=4940	1399 (28.3%) N=4940	1.15 (0.93-1.41)	0.21
	30 - <35	851 (13.1%) N=6512	617 (12.5%) N=4940	617 (12.5%) N=4940	1.28 (0.98-1.65)	0.07
	35 - <40	378 (5.8%) N=6512	269 (5.4%) N=4940	269 (5.4%) N=4940	1.26 (0.91-1.73)	0.16
	>=40	62 (1%) N=6512	44 (0.9%) N=4940	44 (0.9%) N=4940	1.41 (0.77-2.6)	0.27
Antimicrobials in the urine	No	5757 (92.5%) N=6227	4328 (91.7%) N=4721	4328 (91.7%) N=4721	Ref	Ref
	Yes	470 (7.5%) N=6227	393 (8.3%) N=4721	393 (8.3%) N=4721	0.64 (0.49-0.83)	0.001
Gravida	1	2435 (37.5%) N=6485	1922 (39.1%) N=4913	513 (32.6%) N=1572	Ref	Ref
	2	1703 (26.3%) N=6485	1306 (26.6%) N=4913	397 (25.3%) N=1572	1.03 (0.87-1.21)	0.77

	3	1062 (16.4%) N=6485	769 (15.7%) N=4913	293 (18.6%) N=1572	1.17 (0.97-1.43)	0.11
	>3	1285 (19.8%) N=6485	916 (18.6%) N=4913	369 (23.5%) N=1572	1.05 (0.85-1.31)	0.63
Mode of delivery	NVD	5562 (85.5%) N=6509	4207 (85.2%) N=4938	1355 (86.3%) N=1571	Ref	Ref
	Emergency CS	828 (12.7%) N=6509	639 (12.9%) N=4938	189 (12%) N=1571	1.07 (0.88-1.3)	0.51
	Elective CS	119 (1.8%) N=6509	92 (1.9%) N=4938	27 (1.7%) N=1571	1.1 (0.68-1.79)	0.69

aOR: Adjusted Odd's Ratio

95% CI: 95% Confidence Interval

Table 18: Frequency of Group B streptococcus colonization by a single or multiple serotypes.

	Colonized with GBS serotypes in women			Colonized with GBS serotypes in newborns			
Study Site	1	2	3	1	2	3	4
Bangladesh	90.6% (125/138)	8% (11/138)	1.4% (2/138)	96.3% (105/109)	3.7% (4/109)	0% (0/109)	0% (0/109)
Ethiopia	96.6% (85/88)	3.4% (3/88)	0% (0/88)	95.7% (89/93)	3.2% (3/93)	1.1% (1/93)	0% (0/93)
India	97.7% (170/174)	2.3% (4/174)	0% (0/174)	92% (149/162)	8% (13/162)	0% (0/162)	0% (0/162)
Kenya	94.7% (161/170)	5.3% (9/170)	0% (0/170)	91.6% (142/155)	7.7% (12/155)	0.6% (1/155)	0% (0/155)
Mali	75.8% (238/314)	20.1% (63/314)	4.1% (13/314)	72% (216/300)	22% (66/300)	5.3% (16/300)	0.7% (2/300)
Mozambique	91% (193/212)	9% (19/212)	0% (0/212)	92.1% (176/191)	7.9% (15/191)	0% (0/191)	0% (0/191)
Nigeria	91.5% (184/201)	8.5% (17/201)	0% (0/201)	93.1% (148/159)	5.7% (9/159)	1.3% (2/159)	0% (0/159)
South Africa	88.4% (243/275)	11.6% (32/275)	0% (0/275)	94.1% (222/236)	5.9% (14/236)	0% (0/236)	0% (0/236)
Overall	89.0% (1399/1572)	10.1% (158/1572)	1.0% (15/1572)	88.8% (1247/1405)	9.7% (136/1405)	1.4% (20/1405)	0.1% (2/1405)

Table 19: Group B Streptococcus bacteriuria among mothers.

Site	GBS bacteriuria	Vertical transfer of Group B streptococcus to newborn at delivery.	Same serotype detected from Urine and vaginal/rectal swabs
Bangladesh	3.9% (2.8-5.5); [31/788]	93.5% (79.3-98.2) [29/31]	30/31 (96.8%)
India	4.5% (3.3-6.1); [38/841]	92.1% (79.2-97.3) [35/38]	31/34 (91.2%)
Kenya	9% (7.2-11.2); [72/802]	84.7% (74.7-91.2) [61/72]	68/71 (95.8%)
Mozambique	11.4% (9.4-13.9); [89/778]	91% (83.3-95.4) [81/89]	81/84 (96.4%)
Nigeria	9.4% (7.6-11.5); [82/874]	70.7% (60.1-79.5) [58/82]	62/71 (87.3%)
South Africa	14.1%(11.9-16.5); [123/875]	76.9% (68.6-83.5) [93/121]	109/114 (95.6%)
Overall	8.8% (8-9.6); [435/4958]	82.4% (78.6-85.7)[357/433]	381/405 (94.1%)

Table 20: Frequencies and percent of missingness for each variable included in the multiple logistic regression model overall and by site.

Site	Maternal colonisation	Age	Antimicrobials in the urine	Gravida	Mode of delivery	Excluded from complete case analysis
South Africa	0 (0)	0 (0)	64 (7.2)	1 (0.1)	0 (0)	65 (7.3)
Bangladesh	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	1 (0.1)
Ethiopia	0 (0)	1 (0.1)	11 (1.4)	22 (2.9)	5 (0.7)	39 (5.1)
India	0 (0)	0 (0)	81 (9.5)	2 (0.2)	0 (0)	83 (9.8)
Kenya	0 (0)	0 (0)	25 (3.1)	0 (0)	0 (0)	25 (3.1)
Mali	0 (0)	0 (0)	2 (0.3)	0 (0)	0 (0)	2 (0.3)
Mozambique	0 (0)	0 (0)	35 (4.5)	1 (0.1)	0 (0)	36 (4.6)
Nigeria	0 (0)	0 (0)	69 (7.9)	3 (0.3)	0 (0)	72 (8.2)
Overall	0 (0)	2 (0)	287 (4.4)	29 (0.4)	5 (0.1)	323 (5)

9 Conclusion

Using a standardised approach to investigate for maternal recto-vaginal GBS colonization and vertical transmission of GBS provided more robust data and enabled better inter-country comparison of maternal GBS recto-vaginal colonization and vertical transmission rate to newborns. Our study reported high prevalence of GBS colonization in most settings, with some geographic variability within African countries. Based on the estimate that 1-2% of babies born to women with recto-vaginal GBS colonization would develop invasive disease in the absence of IAP, our findings suggest that newborns are at high risk of invasive GBS disease in our study settings. The current hexavalent GBS polysaccharide-protein conjugate vaccine (GBS6) being evaluated in pregnant women includes six serotypes (Ia, Ib, II, III, IV and V) and post-licensure vaccine effectiveness studies should also focus on maternal GBS serotype distribution as non-vaccine serotype replacement could occur due to vaccine immune pressure.

10 Future Research Work

We are currently evaluating GBS serotype-specific transplacental acquired immunoglobulin G in the newborns and sequencing the GBS isolates to determine the prevalence of more virulent sequence type (e.g. ST17) and clonal complexes (e.g. CC17), which together with the current data could be used to model the expected burden of EOD in the study sites.

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12. Appendices

Appendix 1: Ethics clearance certificate.

Appendix 2: Letter stating contribution of candidate to the manuscript.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Gaurav Kwatra

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M211029

NAME: (Principal Investigator)	Dr Gaurav Kwatra
DEPARTMENT:	Vaccines & Infectious Diseases Analytics Research Unit (Wits-VIDA)
PROJECT TITLE:	Prevalence of Group B Streptococcus colonization and serotype distribution among pregnant women in South Asian and African countries
DATE CONSIDERED:	29/10/2021
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof S. Madhi
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	25/11/2021

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **October** and will therefore be due in the month of **October** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature
(GAURAV KWATRA)

Date 25/Nov/2021

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

To Whom it may Concern

This letter serves to confirm that Gaurav Kwatra (student number 416220) will be submitting a drafted manuscript entited “Prevalence of Group B Streptococcus colonization in mother-newborn dyads in low and middle income South Asian and African countries: A prospective, observational study”, as part of hisresearch report for a Master’s in Public Health at the University of the Witwatersrand, Johannesburg, South Africa.

This manuscript is derived from work done on the GBS multicentre study (HREC: 150214; M211029) conducted by Vaccines and Infectious Diseases Analytics Research Unit (VIDA) from 2015-2020 with the support from Bill and Melinda gates foundation. Gaurav Kwatra was Co-Principal investigator of the study and designed and implemented the study.Under the guidance of his Supervisor Prof.Shabir Madhi, Gaurav Kwatra wrote the initial draft of the manuscript in its entirety.

Your Sincerely

Name: Phurb Dorji Signature: 

To Whom it may Concern

This letter serves to confirm that Gaurav Kwatra (student number 416220) will be submitting a drafted manuscript entited "Prevalence of Group B Streptococcus colonization in mother-newborn dyads in low and middle income South Asian and African countries: A prospective, observational study", as part of his research report for a Master's in Public Health at the University of the Witwatersrand, Johannesburg, South Africa.

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Your Sincerely

Name: Alane Izu Signature: _____

Name: Clare Cutland Signature: _____

Name: Godwin Akaba Signature: _____

Name: Musa Mohammed Ali Signature: _____

Name: Zabed Ahmed Signature: _____

Name: Manisha Madhai Beck Signature: _____

Name: Hellen Cherono Barsosio Signature: _____

Name: James A Berkley Signature: _____

Name: Tolossa E Chaka Signature: _____

Name: Anelsio Cosa Signature: _____

Name: Sowmitra Chakraborty Signature: _____

Name: Nisha Dhar Signature: _____

Name: Phurb Dorji Signature: _____

Name: Maksuda Islam Signature: _____

Name: Adama Mamby Keita Signature: _____

Name: Stella Mwakio Signature: _____

Name: Salim Mwarumba Signature: _____

Nubwa Medugu Signature: _____

Name: Helio Mucavele Signature: _____

Name: Stephen Obaro Signature: _____

Name: Betuel Sigaúque Signature: _____

Name: Samba O Sow Signature: _____

Name: Samir K Saha Signature: _____

Name: Sridhar Santhanam Signature: _____

Name: Ragunath Sharma Signature: _____

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Name: Milagritos D Tapia Signature: _____

Name: Balaji Veeraraghavan Signature: V-Balaji

Name: Shabir A Madhi Signature: _____

Name: Phurb Dorji Signature: _____

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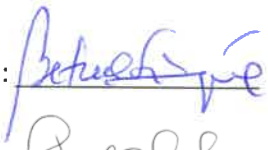
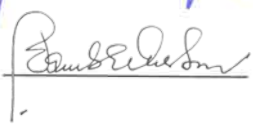
Name: Betuel Sigaúque Signature: 

Name: Samba O Sow Signature: 

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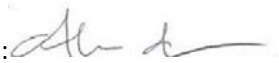
Name: Alane Izu Signature: 

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Name: Anélsio Carlos Alfredo Cossa Signature: