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Vorstandsvorsitzender: Prof. Dr. Rolf Horstmann

Group B Streptococci serotype distribution in pregnant women in Ghana: Analysis of risk factors and assessment of potential coverage through future vaccines

Dissertation

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Patrick Brust

aus Sprockhövel

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Group B Streptococci serotype distribution in pregnant women in Ghana: Assessment of potential coverage through future vaccines

C. D. Vinnemeier[1,2,†], P. Brust[2,†], E. Owusu-Dabo[3], N. Sarpong[4], E. Y. Sarfo[5], Y. Bio[6], T. Rolling[1,2], D. Dekker[4], Y. Adu-Sarkodie[7], K. A. Eberhardt[2], J. May[4] and J. P. Cramer[1,2]
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[1]Section Tropical Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[2]Clinical Research Group, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[3]Kumasi Center for Collaborative Research in Tropical Medicine, Kumasi, Ghana
[4]Infectious Diseases Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[5]St. Michael's Hospital, Pramso, Ghana
[6]Campus Hospital, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[7]Faculty of Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[†]Authors contributed equally to the manuscript.

Contents

List of tables

List of figures

List of abbreviations

ANC – antenatal care
ANCC – antenatal care clinic(s)
BNITM – Bernhard-Nocht-Institute for Tropical Medicine
CDC – Centers for Disease Control and Prevention, USA
CI – confidence interval
CLSI – Clinical and Laboratory Standards Institute, USA
CPS – capsular polysaccharides
CRF – Case Report Form
DNA – deoxyribonucleic acid
E. – Enterococcus
e.g. – for example
EOGBS – early-onset GBS disease
GBS – group B streptococcus
GCP – Good Clinical Practice
GDS – group D streptococcus
GH₵ - Ghana Cedi(s)
IAP – intrapartum antibiotic prophylaxis
ICF – Informed Consent Form
Ig – immunoglobulin
IgG – immunoglobulin G
IV – intravenous
JHS – junior high school
KCCR – Kumasi Center for Collaborative Research in Tropical Medicine
KNUST – Kwame Nkrumah University of Science and Technology
LOBGS – late-onset GBS disease
n.t. – non-typable
ORS – oral rehydration solution
PBS – phosphate buffered saline
S. – Streptococcus

SHS – senior high school

spp. – *species*

SSA – Sub-Saharan Africa

1 Introduction

Over 40% of all deaths in children less than five years of age occur in the neonatal period, which is defined as the first 28 days after birth (1). Infections are the largest single cause for neonatal mortality in developing countries. Each year an estimated one million neonates die from invasive bacterial infections, 99% of these deaths occur in low- and middle-income countries (2). Amongst infective agents, *Streptococcus agalactiae* (*S. agalactiae*), or group B streptococcus (GBS), accounts for the majority of neonatal fatalities in large parts of the industrialized world (3, 4). Yet, burden of GBS associated neonatal infections is widely unclear in developing countries, although it is presumed to be significant (5, 6). Knowledge on the etiology of fatal neonatal infections is crucial for advances towards Millennium Development Goal 4, targeting child mortality (7).

1.1 The pathogen

1.1.1 History of GBS disease

In 1933 the endeavor of Rebecca Lancefield allowed the serologic identification of *S. agalactiae* by specific carbohydrate antigens in the cell walls of the bacteria (8). Two years later LANCEFIELD & HARE identified group B streptococcal isolates for the first time in the birth channel of pregnant women, although they associated these bacteria only with minor infections, while severe cases of puerperal sepsis were contributed to group A streptococci (9). In 1938 FRY described the first fatal cases of puerperal sepsis due to GBS infections in women in the post-partum period (10). During the following decades, neonatal sepsis due to GBS was occasionally reported. But only since the 1960s, GBS infections were recognized by clinicians and researchers as major cause of neonatal sepsis in the USA. A decade later GBS became accepted as the clinically most relevant pathogen in newborns` first days of life (4). In 1973 it was described that the disease was bimodal by age of onset, designated as early-onset GBS disease (EOGBS), occurring within the first six days of life, and late-onset GBS disease (LOGBS) with symptoms starting between seven

days and three months after birth (11). BAKER & BARRET discussed the mode of vertical transmission and suggested that infants acquire the infection *in utero* or during their passage through the birth channel (12). In 1974 the same authors described the importance of GBS serotype III for both EOGBS and LOGBS, especially for the manifestation as meningitis (13).

1.1.2 Microbiological characteristics

S. agalactiae is a gram-positive, facultative anaerobic, non-sporeforming, non-motile, catalase negative coccus in chains (14), which usually forms β-hemolytic colonies on blood agar, although non-hemolytic isolates occur (3). According to LANCEFIELD, β-hemolytic streptococci are classified with regard to the C-polysaccharide in their cell walls into groups A-H and K-V, of which *S. agalactiae* features group antigen B. GBS possess an antiphagocytic polysaccharide capsule as a virulence factor (14). By these capsular polysaccharide (CPS) antigens, they are subdivided into ten different serogroups (Ia, Ib, II-IX) (4). Most frequent serotypes in Sub-Saharan Africa (SSA), as well as globally, seem to be Ia, Ib, II, III and V (4, 15-17). GBS possesses various virulence factors, which are further described in chapter **1.4**.

On blood agar plates, colonies usually appear flat, mucoid and gray-white in color with about 1-2 mm in diameter and a narrow zone of β-hemolysis.

1.2 Clinical manifestations

GBS infections are not limited to infants, but the highest burden of disease concerning incidence and case severity is found within the first three months of life. However, disease incidence in adults is increasing (18). As described above, neonatal invasive GBS disease is generally categorized into EOGBS and LOGBS.

EOGBS accounts for approximately two thirds of all GBS infections (4), with a typical time of onset during the first 24 hours after delivery, presenting with sepsis (80%-95%), pneumonia (10%-15%), or meningitis (5-10%) (19). Mortality rates for EOGBS ranged between 4 and 9% in recent years in the USA (3, 18), but have been

reported as high as 50% in the early 1970s. The reduction of the mortality rate in neonates who acquired EOGBS in the last decades is primarily attributed to advances in neonatal care (3). Mortality is nearly eight times higher amongst preterm born infants compared to those born at term (18). To date, little data on GBS case fatalities in developing countries exist, but seem to range between 10 and 60% (median: 20%) (20). This would vaguely be matching with mortality in industrialized countries in the 1980´s (11). GBS serotypes Ia, III, and V are responsible for most cases of EOGBS (21-24).

LOGBS, in contrast, presents more commonly as meningitis and is associated with lower mortality rates of approximately 3% today in the USA, which also decreased significantly in recent decades (11, 19).

About a fifth of children surviving GBS meningitis suffer from severe neurologic sequelae such as hearing impairment, cerebral palsy and delays in cognitive development, while another 30% experience milder neurologic disorders (25). LOGBS is predominantly caused by serotype III (15, 23).

GBS also represents a considerable disease burden for adults, in particular in women during and after pregnancy. Amongst post-partum women, an incidence of 0.5 per 1,000 is reported from the United States (26). The disease usually manifests as infections of the upper genital tract, placenta or amniotic sac or as systemic bacteremia. GBS infection in pregnancy is highly associated with adverse pregnancy outcomes like fetal death, stillbirth and pre-mature delivery (4, 26, 27). The serotypes causing GBS disease in pregnant women appear to be similar to those responsible for invasive disease in infants (28, 29).

Incidences of GBS-related infections in non-pregnant adults have increased over the last years. In the United States the incidence reached 0.26/1,000 in 2005, whereby individuals aging ≥65 years were particularly affected (18). In non-pregnant adults GBS disease commonly presents as skin and soft tissue infection, systemic bacteremia, pneumonia, arthritis or osteomyelitis. Meningitis, endocarditis and streptococcal toxic shock syndrome might also occur (30-32). Among non-pregnant adults, case fatality rates range between 6 and 32%, a markedly higher level compared to those in neonates (31-34). In most adult cases, GBS is associated with

underlying diseases such as diabetes mellitus or malignancies (31, 35). GBS serotypes distribution responsible for adult GBS disease was reported to be similar to those causing infections in infants but, at least in the USA, with a predominance of serotype V (18).

1.3 Epidemiology of neonatal GBS disease

In their national guidelines for the prevention of perinatal GBS disease from 2010, the US Centers for Disease Control and Prevention (CDC) reported EOGBS to be the leading infectious cause of mortality and morbidity amongst infants in the USA with an incidence of 0.36 cases per 1,000 live births. These numbers already represented a steep decline compared to the incidence rate of 1.7 cases per 1,000 live births in the early 1990s when prevention strategies were introduced (3). In European countries, varying EOGBS incidence rates of 0.2-4.0 per 1,000 live births have been reported (36). In many of these countries, implementation of prevention strategies led to a decline of incidence rates (36).

In Sub-Saharan Africa, EOGBS incidence was reported between 0 and 2.1 cases per 1,000 live births (20, 37). Highest numbers were observed in South Africa (37). Studies from other developing countries around the world found EOGBS incidences ranging between 0 and 3.06 cases per 1.000 live births. Large geographical variations of incidence have been describes with lowest incidences repeatedly reported from the Middle East and Asia (20).

However, data on GBS burden in Africa and most other parts of the developing world is scarce, for most countries completely lacking and amongst the existing studies case ascertainment might be underestimated. A possible reason for this might be insufficient infrastructure for microbiological diagnosis (20, 38, 39). The applied methodology for GBS isolation certainly affects the results as well (20). Usage of antibiotics prior to specimen collection might also have affected study outcomes (38). Differences in maternal GBS colonization and serotype distribution might be the primary predictor for EOGBS incidence, but available studies on maternal colonization in developing countries did not find sufficient variations in colonization patterns to support this hypothesis (16, 28, 40-43). Different levels of

protective maternal antibodies might as well be a factor (20). Delivery practices and hygienic conditions that might promote not-vertically acquired infections with gram-negative bacteria, first of all E. coli, in newborns might also contribute to GBS underidentification (39).

The incidence of LOGBS remained unchanged in the last decades with 0.25-0.35 per 1,000 in the USA (3, 11) since *intrapartum* antibiotic prophylaxis (IAP) can only prevent EOGBS (44). Reliable epidemiologic data on LOGBS incidence in developing countries are scarce, reported incidence rates between 0 and 1.0 per 1,000 newborns appear low, which may result from limited diagnostic capacities as described above (20).

For all of West Africa, we could only identify two previous studies, from Gambia in 1994 (76) and Senegal in 2009 (77), that performed the CDC recommended vaginal and rectal specimen collection and tested for serotype distribution.

1.4 Pathogenesis and Transmission

Acquisition of GBS infection leading to EOGBS occurs vertically. The natural reservoir of GBS usually is the gastrointestinal tract, which also represents the most common source for vaginal colonization (3). Colonization of the female genitourinary tract may be transient, intermittent or persistent (45). From the vagina the bacteria might reach the cervix and subsequently the uterus during pregnancy. Thus, after the rupture of membranes GBS can directly infect the fetus, although a penetration of amniotic membranes may also be possible through collagenases (46) and other virulence factors. Swallowing or aspiration of infected amniotic fluids or genital secretions by the newborn enables adhesion of the bacteria to the respiratory or gastrointestinal mucosa through bacterial surface structures such as the alpha C protein (47) and pilus structures (48). GBS beta-hemolysin facilitates epithelial cell invasion and can subsequently lead to pneumonia and bloodstream infections. Meningitis can occur after hematogenous spread to the meninges (11).

To promote survival and intravascular multiplication, the CPS on the bacterial surface mask the organism in order to evade phagocytosis. High levels of maternal

IgG specifically directed against CPS as well as fetal complement factors are needed for opsonization, phagocytosis and elimination of the organisms (11). CPS serotype III seems to be particularly capable of invading brain microvascular endothels (11).

The risk for colonization of a neonate born to a GBS colonized women is approximately 50%. In absence of any intervention, 1-2% of these newborns develop EOGBS (3, 49).

Acquisition of the bacteria in infants developing LOGBS, in contrast to EOGBS, usually occurs horizontally via community sources or nosocomial infection (6, 11). Transmission through maternal breastfeeding has also been discussed (50).

The primary determinant whether an infant born to a GBS-positive mother stays healthy or develops invasive GBS disease appears to be the level of protective maternal IgG and the maturity of the fetal innate immune system (11, 51, 52).

A principal risk factor for EOGBS is the degree of maternal recto-vaginal colonization (3). Heavy vaginal colonization is defined as GBS cultures obtained by direct plating without usage of an enrichment broth, or GBS detection in clean-catch urine specimens (53, 54). Obstetric complications such as premature rupture of membranes >12h, prematurity of less than 37 completed weeks of gestation, intra-amniotic infections and intrapartum temperature of >37.5°C are also associated with an increased risk for EOGBS (55, 56). However, obstetric factors have not been held responsible for LOGBS. Another risk factor discussed for EOGBS is young maternal age (55, 57). Black race seems to be associated with a higher risk for both EOGBS and LOGBS (55, 57).

1.5 Prevention

The current CDC recommendation of IAP administered to GBS positive women under labor represents the current gold standard of EOGBS prevention (3). Through this approach a reduction of 80% in the incidence of EOGBS could be achieved in the USA (4). The effectiveness of IAP to prevent EOGBS in infants born to GBS colonized mothers was found to be close to 90 % (58, 59). Other prevention approaches, such as IAP administered on the basis of obstetric risk factors (59),

antenatal antibiotics (oral or intramuscular) (60, 61), or chlorhexidine vaginal wipes (62) have been shown to be less effective.

Antimicrobial agents of first choice should be intravenous penicillin G or amoxicillin given under delivery with the onset of labor. For women with a history of severe allergic reactions to penicillin, other antibiotics such as cefazolin, erythromycin or clindamycin are recommended, depending on the antibiotic susceptibility of the isolates and allergies of the patient (3).

However, IAP is not capable of eliminating EOGBS since mothers with false negative screening results can give birth to colonized infants and EOGBS can occur despite the administration of IAP (58, 63). Furthermore, certain risks associated with IAP use remain: Severe allergic reactions amongst birth giving women have been reported (64-66). There is also concern about a possible increase in maternal and neonatal yeast infections due to the use of IAP (67) and higher exposure of neonates to ampicillin-resistant gram-negative enterobaceteria (68). In addition to these adverse effects, it is important to highlight that IAP could not reduce the incidence of LOGBS (69).

In settings where scarcity of resources and infrastructure impedes the implementation of routine maternal screening, present international standards of GBS prevention cannot be applied and mothers and infants remain at high risk for invasive GBS disease. The meta-analysis of EDMOND ET AL. could not identify a single study in any low-income country that reported the use of IAP (15). Even in Germany, a country with high standards of medical care, a recent study found adherence to existing GBS prevention guidelines to be low (28).

1.6 Vaccine development

A GBS vaccine could be the most cost-efficient strategy for the prevention of GBS (70). Moreover, it would require minimal patient compliance and could probably be integrated into already existing maternal vaccination schedules. Vaccination of women of childbearing age promises to reduce maternal GBS colonization and subsequent vertical transmission, as well as pregnancy-associated GBS infections in

women (6). Placental transfer of maternal IgG to the fetus could not only prevent EOGBS, but also LOGBS (19). Additionally, maternal immunization could have an important impact on fetal death and stillbirth caused by fetal *in utero* or maternal GBS infection (27).

Implementation of a GBS vaccine could enable mothers and their offspring to achieve immune protection irrespectively of the place of delivery, which in many cases does not occur in a hospital setting (4) – another factor impeding IAP in low-income countries (6). An example for successful maternal immunization in the developing world is the tetanus toxoid vaccine that has saved millions of mothers and infants from death from tetanus (6, 71).

Initial GBS vaccine trials in the seventies and eighties were focusing on pure native GBS CPS as antigens and were found to be non-toxic, safe and - depending on the serotype - immunogenic in 65-95% of non-immune adults (72, 73). However, these vaccines were not able to induce memory B-Cells and increases in CPS antibodies were only moderate (72, 74).

After the success of the type B CPS–protein conjugate vaccine for *Haemophilus influenzae* in the 1990's, attention turned to glycoconjugate vaccines against GBS (74). Clinical trials in pregnant women with mono- or bivalent GBS vaccines comprising CPS types III, or II and III, provided promising results and overcame immunogenic limitations associated with pure CPS vaccines (75). Currently, trivalent conjugate vaccines comprising serotypes Ia, Ib and III are being tested in phase I and II clinical trials (6). A pentavalent vaccine formulation consisting of the five most common serotypes Ia, Ib, II, III and V could theoretically prevent more than 85% of global invasive neonatal GBS disease (15). According to our study and to the limited data of previous African studies, the potential for disease prevention could be even higher in SSA (15).

For GBS vaccine trials in developing countries, antenatal care clinics (ANCC) would represent an ideal location since coverage of the target population and frequent follow-ups of pregnant women is ensured (6).

1.7 Aims of this study

Considering the lack of data in SSA and the severe burden of invasive neonatal GBS disease, more knowledge about GBS epidemiology in this geographical region is urgently needed. The aim of the present study was to provide first data on the burden of GBS colonization in pregnant women in Ghana.

The primary objective was to assess the prevalence of GBS colonization in women in late pregnancy in a rural as well as in an urban setting.

Secondary objectives were to provide data on CPS serotype distribution and antibiotic susceptibility of the isolated GBS strains. Additionally, we endeavored to contribute data for the elucidation of the inconsistent knowledge on risk factors associated with maternal GBS carriage.

As a tertiary objective we aimed at examining the suitability of the study sites chosen for potential future vaccine trials concerning infrastructure of the ANCC, patient compliance and socio-economic profile of the participants.

2 Materials and Methods

2.1 Setting

2.1.1 Geographical setting

This epidemiological cross-sectional study was conducted in Kumasi, the capital of Ghana´s Ashanti Region, and Pramso, a village located in a rural area of the Ashante Akim North District, 22 km south-east of Kumasi. Kumasi is the country´s second biggest city with an estimated population of 2 million people. Kumasi features tropical climate with relatively constant temperatures throughout the year, averaging between 20.4 and 33.5°C. The mean monthly rainfall varies between 15mm in January and 214 mm in July, adding up to 1,509 mm/m² annually (78).

2.1.2 Study sites and facilities

The urban study site was the campus hospital of the Kwame Nkrumah University of Science and Technology (KNUST), located on the university's campus in the south of Kumasi. At the same time, it is directly bordering a part of town that is mainly populated by migrants from the country´s north and surrounding West African countries, and therefore representing an urban setting with patients of different socio-economic background. The pediatric department runs a well-equipped ANCC that is attended by up to 200 pregnant women per week. The rural study site was St. Michaels Hospital in Pramso. It provides care for approximately 100 inpatients and maintains an ANCC attended by approximately 300 pregnant women per week.

Main parts of the laboratory work were performed in the facilities of Kumasi Center for Collaborative Research in Tropical Medicine (KCCR), located on KNUST campus. KCCR is a joint venture between the Bernhard-Nocht-Institute for Tropical Medicine (BNITM), Germany, the Ministry of Health of the Republic of Ghana and KNUST, Ghana.

Enrollment of patients as well as the majority of laboratory work was performed in Kumasi, with the exception of the serotyping of GBS isolates, which took place at the BNITM in Hamburg, Germany.

2.1.3 Study population

The population in the Ashanti Region mainly consists of Ashanti people mixed with migrants from mostly northern Ghanaian tribes and few migrants from surrounding African countries (79). All pregnant women of legal age who participated in the routine antenatal care scheme in one of our two study sites and met all of the inclusion and none of the exclusion criteria were eligible to be enrolled in the study.

2.2 Study procedures

2.2.1 Recruitment

In KNUST Hospital, recruitment took place between June 17th and August 19th 2013 in the ANCC. All pregnant women above 18 years of age, who were pregnant for at least 35 weeks, were asked for potential participation in the study. An informed consent form (ICF) was handed out to any woman who expressed general interest in participation. If the woman was illiterate or not able to comprehend the English version of the ICF, the content was explained to her by a previously trained fieldworker, who also translated from English into Twi, the main local language in the Ashanti Region. In case of illiteracy, an independent witness was also present during the whole consent process. After the eligible person had enough time to ask questions and consider her decision, she was asked to provide her signature or, if she was illiterate, a thumbprint. In the latter case the independent witness had to provide his or her signature as well.

Patient recruitment in Pramso´s St. Michael Hospital proceeded in the same way and took place between May 9th and September 2nd 2013 (see **Figure 1**).

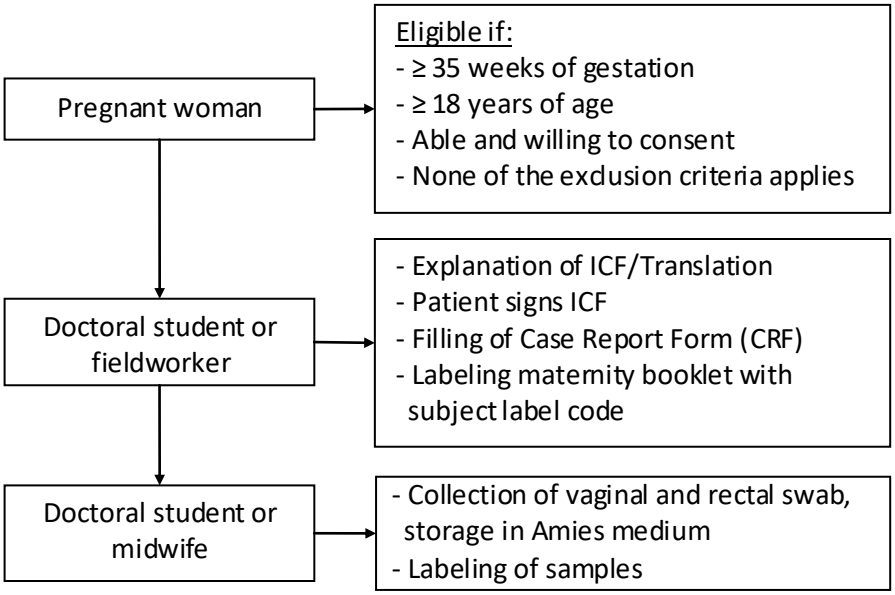

Figure 1: Procedures at the study site

2.2.1.1 Inclusion criteria

- Pregnant women ≥18 years of age who are pregnant for at least 35 weeks
- Participants who are willing to participate in the study
- Participants who are able to comprehend the purpose, background and risks of the study and who confirm their consent by signature or thumbprint on the ICF

2.2.1.2 Exclusion criteria

- Participants who are not willing to provide a vaginal and/or rectal swab
- Participants who are already in labor
- Participants who are not able or not willing to sign or provide their thumbprint for the ICF

2.2.2 Data collection

After signing the ICF, the participants were interviewed by the study team to complete the case report form (CRF). Personal information of the participants was collected. These comprised contact details, as well as data about the current pregnancy, the health condition, the socio-economic background and living situation

of the mother and information about the access to healthcare and healthcare seeking behavior.

We used predefined questionnaires with multiple choice questions and limited free-text options, wherever necessary, to enable the inclusion into descriptive and comparative analyses (see **Appendix**).

2.2.3 Data processing

To ensure anonymisation, every participant was assigned a unique subject code. ICFs and CRFs as well as the collected specimen were labeled with these subject codes. Furthermore, the subject code label was put on the maternal health booklet that the pregnant women carried to every pregnancy medical care examination as well as to the maternity ward when entering into labor. The subject code label indicated enrollment in the study to staff members of the maternity ward, whereupon they had to check the ward book to trace laboratory results and administer IAP if necessary (see **2.2.9**).

Patient related information was always stored in folders separated from any test results. All data was entered into the electronic database (Excel 2007, Microsoft, USA) within a maximum of 24 hours after collection. Thereafter, all confidential paper documents were kept in a locked cabinet only accessible for the local principal investigator. The electronic database was protected by password.

2.2.4 Sample collection

After completion of the CRF, specimen collection was performed in the ANCC. As recommended by current CDC guidelines (3), a vaginal swab from the lower part of the vagina (without speculum) and a rectal swab were collected with two sterile cotton carriers (BBL Venturi Liquid Amies Medium Transport Swabs, Becton Dickinson, USA) from a previously trained member of the study team. The swabs were then placed into sterile Amies medium, which keeps GBS isolates viable for up to four days at room temperature (80, 81).

2.2.5 Sample processing and identification of GBS

2.2.5.1 Selective Enrichment

Samples were given into an enrichment broth in the afternoon of the day of collection. With respect to current CDC guidelines (3), the two swabs that were taken from each participant were pooled and inoculated into 5 ml Lim broth (Becton Dickinson, USA), a selective enrichment media based on Todd-Hewitt broth supplemented with colistin (10µg/ml), nalidixic acid (15µg/ml) and 1% yeast extract. The inoculated Lim broth tubes were then incubated at 35-37°C for 18-24 hours in normal atmosphere.

2.2.5.2 Culturing on blood agar

Columbia blood agar was prepared according to the manufacturer´s instruction using Colombia blood agar base (OXOID, UK) and adding 5% of sterile defibrinated sheep blood.

After one day of incubation, the Lim Broth tubes were gently shaken and the content streaked onto blood agar plates using a 1µl inoculation loop (SARSTEDT, Germany). Hereafter, the agar plates were incubated at 35-37°C in a commercially purchased candle jar (Anaerojar, OXOID, UK) with the addition of a tea light to create an elevated carbon dioxide level. Following 18-24h incubation, culture plates were inspected for β-hemolytic colonies suspicious of GBS. Sub-cultivation was performed until pure growth was obtained. If no β-hemolytic growth was observed after one day incubation, the blood agar plate was reincubated for another 18-24h and examined again (see also **Figure 2**).

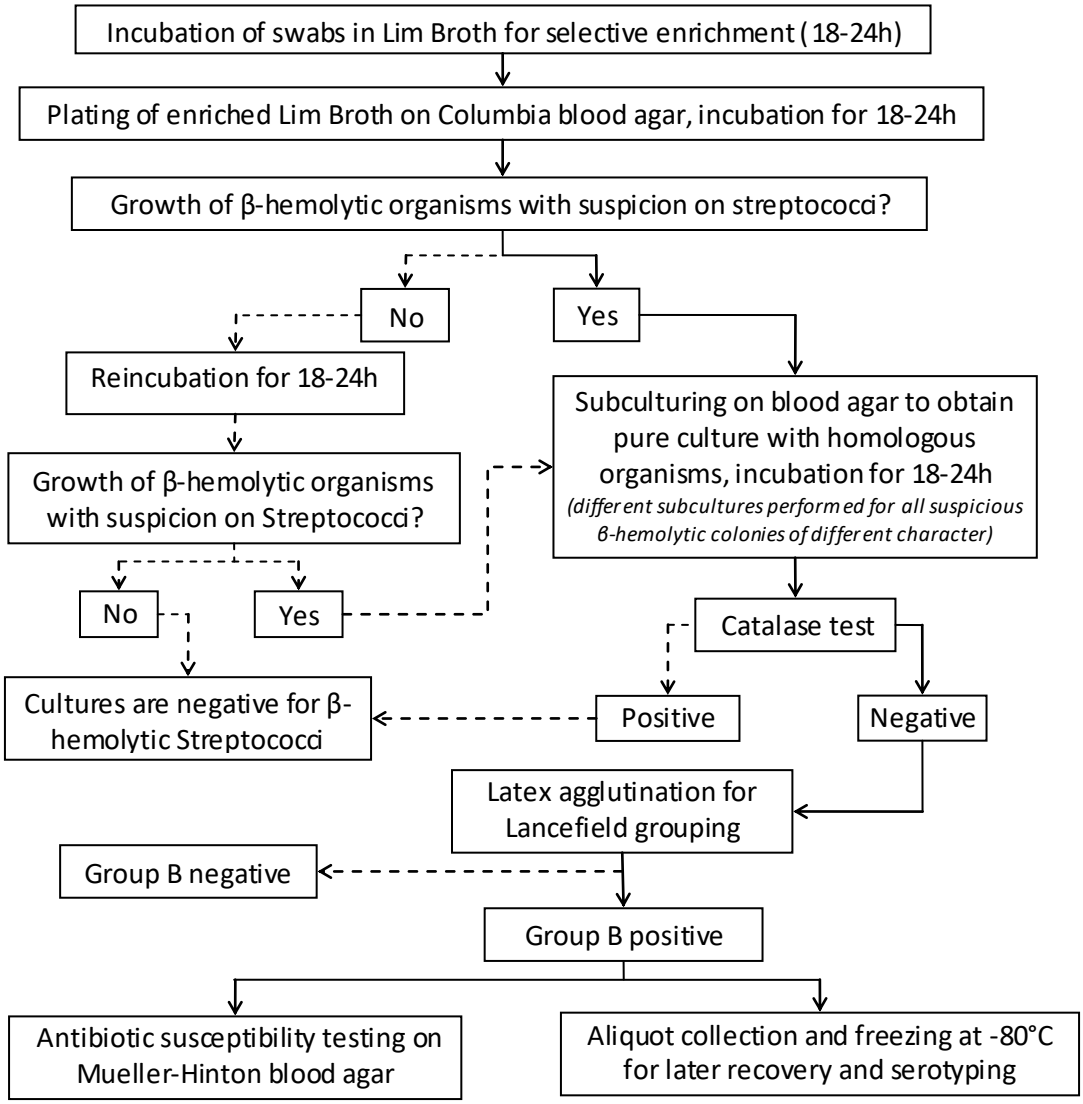

Figure 2: Flowchart of laboratory procedures at KCCR

2.2.5.3 Identification of Group B Streptococci

On pure colonies with suspicion on GBS, a catalase test was performed using 6% hydrogen peroxide. If the test was positive, the isolate was discarded as non-streptococci. If catalase test results were negative, we performed a latex agglutination test (Streptococcal Grouping Kit, OXOID, UK) to determine the Lancefield group of the isolate.

2.2.5.3.1 Materials

- Streptococcal grouping kit containing:
  - Six antisera bottles for streptococci of Lancefield groups A, B, C, D, F, G
  - Dried extraction enzyme for facilitated detection of Lancefield group antigens
  - Disposable reaction cards
  - Polyvalent positive control
  - Mixing sticks
- Test tubes
- Pasteur pipettes
- Water bath

2.2.5.3.2 Preparation of culture

Dried OXOID *Streptococcus* extraction enzyme was reconstituted with the indicated amount of sterile distilled water and brought to room temperature. For each test, 0.4ml of the enzyme preparation was dispensed into a test tube. Homogenous single bacterial colonies equivalent to 2-3mm of growth in diameter were emulsified into the extraction enzyme of each test tube. The test tubes were then incubated for five minutes in a water bath at 37°C, then shaken for 2-3 seconds and incubated for another five minutes. The emulsion was then allowed to cool down to room temperature.

2.2.5.3.3 Test method

The bottles containing the latex reagents were brought to room temperate by hand warming and shaken to achieve full suspension. One drop of each latex reagent was dispensed on the circular rings of the reaction cards. One drop of the bacterial extract was subsequently added, using a Pasteur pipette. With a mixing stick, the mixture was spread over the whole area of the ring. The reaction card was then rocked gently.

Test results were considered positive when a clearly visible agglutination of blue-violet particles appeared within 30 seconds in one of the groups while the others remained in smooth suspension.

Using a polyvalent serum containing all six group antigens, a positive control test was performed on a daily basis to verify performance of latex reagents.

2.2.6 Antibiotic susceptibility testing

For antibiotic susceptibility testing, all isolated GBS strains were tested using the Kirby-Bauer disc diffusion method. In accordance with current Clinical and Laboratory Standards Institute (CLSI) guidelines, the organisms were interpreted as sensitive, intermediate or resistant - depending on measured diameters of inhibition zones. Sensitivity was examined for the antibiotic agents listed in **Table 1** using antimicrobial susceptibility discs (OXOID, UK).

Table 1: Inhibition zone diameters for tested antibiotics according to CLSI

Type of antibiotic agent	Active ingredient, potency	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Penicillin	Penicillin, 10 units	-*	-*	≥ 24
Aminopenicillin	Ampicillin, 10 µg	-*	-*	≥ 24
Aminopenicillin/ Beta-lactamase inhibitor	Ampicillin, 10 µg/ Sulbactam, 10µg	≤ 11	12-14	≥ 15
Macrolide	Erythromycin, 15 µg	≤ 15	16-20	≥ 21
Chloramphenicol	Chloramphenicol, 30 µg	≤ 17	18-20	≥ 21
Lincosamide	Clindamycin, 2 µg	≤ 15	16-18	≥ 19

* No values defined by Clinical and Laboratory Standards Institute (CLSI)

Preparation of Mueller-Hinton blood agar was performed according to manufacturer`s instructions using Mueller-Hinton blood agar base (OXOID, UK) and adding 5% sterile defibrinated sheep blood.

For each isolate, two or three GBS colonies of pure growth were picked and suspended in 0.9% sterile saline to a turbidity of 0.5 McFarland. Using a sterile cotton swab, the suspension was spread evenly to a Mueller-Hinton blood agar plate of 4mm agar depth to create a uniform bacterial lawn. After letting the plate dry for five minutes, the six different antibiotic susceptibility discs were placed onto the agar

plate with an antibiotic disc dispenser. The plates were then incubated for 18-24h at 35-37°C in normal atmosphere and the inhibition zone diameters measured the next day.

2.2.7 Cryopreservation and transport to Germany

Once organisms were confirmed as group B streptococci and subcultured as pure colonies, Microbank cryovials (PRO-LAB DIAGNOSTICS, Canada) were used for storage. Samples were then stored at -80°C.

At the end of the patient recruitment period, the GBS containing cryovials were shipped to BNITM, Germany, using a “dry shipper” (CXR500-Series, Taylor-Wharton, USA).

2.2.8 Serotyping

We performed serotyping for strains Ia, Ib, II, III and V using the IMMULEX^{TM} STREP-B-LATEX agglutination test (STATENS SERUM INSTITUTE, Denmark). The test is based on agglutination of latex particles coated with antibodies to GBS strain-specific capsular polysaccharides (CPS).

2.2.8.1 Materials

- Five bottles, each of which contained one antiserum for CPS Ia, Ib, II, III or V
- Phosphate buffered saline (PBS), pH 7.2 – 7.4
- Reaction cards
- Mixing sticks
- Test tubes
- Pasteur pipettes, 1.5 ml

2.2.8.2 Procedure

For each GBS sample, a bacterial suspension was prepared by suspending colonies of approximately 2-3 mm diameter growth into six drops of PBS in a test tube. The tube was shaken well and one drop of bacterial suspension transferred into one circle of the reaction card. Subsequently, one drop of group specific latex reagent was added. The two drops were then blended with a mixing stick, using a

different stick for each reagent. The reaction card was than rocked gently for up to one minute.

Agglutination showing clearly visible dark blue particles indicated presence of tested capsular polysaccharide and therefore determined a certain serotype. Reaction was negative if the solution remained in homogenous suspension.

2.2.9 Treatment of GBS positive women

Treatment of GBS-positive women was ensured by forwarding the subject label codes of positive women to the study sites. All women who attended the labor ward for delivery were checked for participation in the study. If they were enrolled, the midwife checked the ward book for the test result. All positive women were then treated with 5 million units penicillin G as initial IV dose after the onset of labor, followed by another 2.5 million units every 4 hours prior to delivery. For women with allergy against penicillin, administration of second line antibiotics was foreseen as recommended by CDC guidelines (3).

2.3 Statistical analysis

Statistical evaluation of the collected data was undertaken at BNITM. The program Stata SE 12 (StataCorp, USA) was used for data analysis.

For analysis of demographic, clinical and microbiological parameters Pearson´s Chi-squared test was used for categorical variables. If contingency tables contained values below ten, Fisher´s exact test was applied instead of Pearson´s chi-squared test. The 95% Confidence Interval (CI) was computed to further describe means. Mann-Whitney U test was applied for comparison of medians. *P*-values of <0.05 were regarded as significant.

2.4 Ethical considerations

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and consistent with Good Clinical Practice (GCP). Ethical

approval of the study protocol was obtained from the Committee on Human Research, Publications and Ethics, School of Medical Sciences, KNUST, Kumasi, Ghana. Informed consent was sought and granted by signature or thumbprint.

3 Results

3.1 Study population

A total of 519 women met the inclusion criteria and were enrolled in the study as shown in **Figure 3**. Overall, 107 women were enrolled at KNUST Hospital, Kumasi, and 412 women were recruited in St. Michael´s Hospital, Pramso. Ten women (three at KNUST Hospital, seven in Pramso, respectively) consented and completed the CRF but did not provide a vaginal and rectal swab.

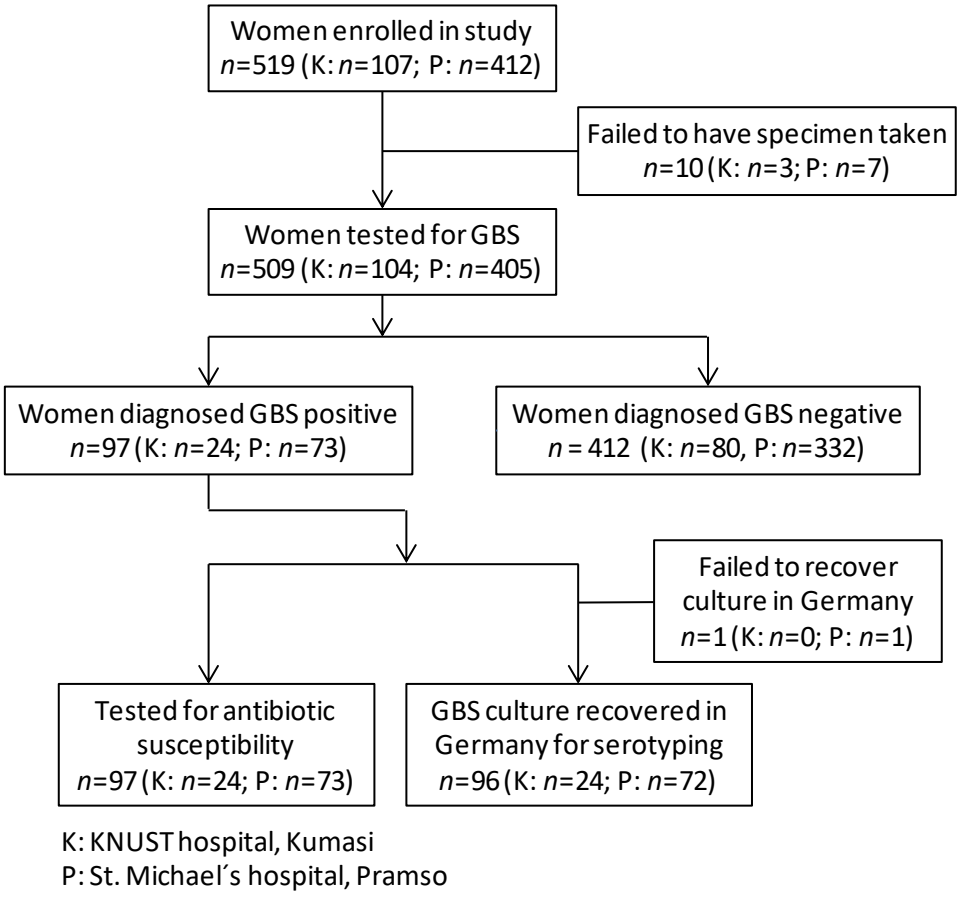

Figure 3: Cohort chart

3.1.1 General socio-economic data

The median age of our participants was 28 years (mean=28.6, 95% CI: 28.1-29.0, means of 28.2 years in KNUST Hospital and 28.6 years in Pramso) with a minimum of 18 and a maximum of 43 years (see **Table 2**).

Tertiary education was completed by 70 women (13.5%), 93 stated senior high school (SHS) as their highest educational level (17.9%). In total, 429 women (82.7%) finished at least junior high school (JHS), 35 (6.7%) said they did not complete primary school and another 36 participants (6.9%) stated that they did not attend school at all. The level of education was significantly (p<0.001) higher at KNUST Hospital. There, the percentage of women naming SHS as their highest educational degree was almost twice as high as in St. Michael's hospital, Pramso. The relative proportion of participants with a tertiary degree was more than three times higher in Kumasi (see **Table 2**).

A total of 72 women (13.9%) said they were illiterate (n=9/107, 8.4% at KNUST Hospital and 15.3% in Pramso, p=0.067). Three-hundred and thirty-seven participants (64.9%) stated to speak English in addition to local languages, whereby a significant difference between the two study sites was found (n=86/107, 80.4% for KNUST Hospital and n=251/412, 60.9% for Pramso, respectively, p<0.001).

Overall, 304 women (58.6%) said they were married, 214 (41.2%) participants stated to have never been married and one woman (0.2%) answered that she was divorced (see **Table 2**). Significantly more women were married amongst the KNUST Hospital´s study population (n=78/107, 72.9%) compared to Pramso (n=226/412, 54.9%) (p=0.001).

As shown in **Table 2**, the median household size was 4 people (mean: 4.2, 95% CI: 4.0-4.4). The minimum was one person, the maximum was 16 persons per household. The median household size was significantly (p<0.001) larger for women attending St. Michaels Hospital, Pramso (median=4, mean=4.4, 95% CI: 4.2-4.7) compared to KNUST Hospitals study cohort (median=3, mean=3.4, 95% CI: 3.1-3.7).

Table 2: Socioeconomic characteristics of study cohorts

Characteristics	KNUST Hospital		St. Michael´s Hospital		Total		*p*
	Number*	%**	Number*	%**	Number*	%**	
Median age (years)	28 (18-41), *n*=107		28 (18-43), *n*=412		28 (18-43), *n*=519		0.740
Highest level of Education							<0.001
None	2/107	1.9	34/412	8.3	36/519	6.9	
Quit primary school	9/107	8.4	26/412	6.3	35/519	6.7	
Primary Education	2/107	1.9	17/412	4.1	19/519	3.7	
Junior High School	32/107	29.9	234/412	56.8	266/519	51.3	
Senior High School	29/107	27.1	64/412	15.5	93/519	17.9	
Tertiary Education	33/107	30.8	37/412	9.0	70/519	13.5	
Literate	98/107	91.6	349/412	84.7	447/519	86.1	0.067
English speaking	86/107	80.4	251/412	60.9	337/519	64.9	<0.001
Marital status							
Never married	29/107	27.1	185/412	44.9	214/519	41.2	
Married	78/107	72.9	226/412	54.9	304/519	58.6	0.001
Divorced	0/107	0.0	1/412	0.2	1/519	0.2	
Median household size	3 (1-10), n=106		4 (1-16), *n*=406		4 (1-16), *n*=512		<0.001
Housing							
Stone/cement house	92/105	87.6	358/405	88.4	450/510	88.2	
Mud house	13/105	12.4	40/405	9.9	53/510	10.4	
Wood house	0/105	0.0	7/405	1.7	7/510	1.4	

*Data are *n*/*N* or median (range)
N-values may vary because of missing values
**May not add up to 100% because of rounding

Of 510 women answering, 450 (88.2%) stated to live in a stone or cement house, 53 (10.4%) said they live in a house build of mud, seven participants (1.4%) replied that they live in a wood house. Overall, the quality of the houses was distributed similarly at both study sites with the exception of wood houses that were not mentioned by participants at the KNUST Hospital.

3.1.2 Health and pregnancy related information

A total of 75 out of 514 answering women (14.6%) were primigravidae, 112 (21.8%) had four pregnancies before or more than that. Women enrolled in the KNUST Hospital were significantly (p=0.004) more often primigravidae. Patients in Pramso were of significantly higher gravidity (p=0.027) (see **Table 3**).

At St. Michael's Hospital, primigravidae tended to be younger (mean=23.7 years; 95% CI: 22.7-24.8) compared to those at the KNUST Hospital (mean=25.3 years; 95% CI: 23.8-26.8). However, the difference was not significant (p=0.08).

The level of education was strongly negatively associated with the frequency of previous pregnancies (p<0.001). Women with tertiary education were primigravidae in 33.3% of all cases (n=23/69) and in 8.7% (n=6/69) had four or more pregnancies before, while women who did not complete primary education were primigravidae in 2.9% of cases (n=2/70) and had four or more previous pregnancies in 45.7% (n=32/70) (data not shown in tables).

Severe complications during the current pregnancy prompting the woman to see a doctor were reported by 98 (19.0%) of the 517 patients who answered. Most commonly mentioned was vaginal discharge, named by 62 women (63.3% of all who mentioned complications), followed by vaginal bleeding in 34 patients (34.7%). Seven women (7.1%) stated to have been diagnosed with vaginal candidiasis and four participants (4.1%) named genital itching as one reasons to consult a doctor. Some women stated several complications. In the KNUST cohort, severe complications were stated slightly more often than in Pramso (n=24/107, 22.4% and n=74/410, 18.1% respectively, p=0.3) (see **Table 3**).

Thirty-five patients (6.8%) replied with "yes" to the question for known diseases or allergies. These women could rarely name a distinct diagnosis. The most common specific answers were HIV and hepatitis B, each of them in four cases (0.8% of all women), and asthma and rheumatism with three cases each (0.6%) (see **Table 3**).

Table 3: Health and pregnancy related data of study cohorts

Characteristics	KNUST Hospital		St. Michael´s Hospital		Total		*p*
	Number*	%**	Number*	%**	Number*	%**	
Gravidity							0.027
Primigravida	25/107	23.4	50/407	12.3	75/514	14.6	
Segundigravida	25/107	23.4	91/407	22.4	116/514	22.6	
Gravida 3-4	38/107	35.5	173/407	42.5	211/514	41.1	
Gravida 5+	19/107	17.8	93/407	22.9	112/514	21.8	
Complications* **	24/107	22.4	74/410	18.1	98/517	19.0	0.303
Vaginal discharge	15/24	62.5	47/74	63.5	62/98	63.3	
Vaginal bleeding	8/24	33.3	26/74	35.1	34/98	34.7	
Others	3/24	12.5	8/74	10.8	11/98	11.2	
Disease[†]	6/107	5.6	29/408	7.1	35/515	6.8	0.672
Mean gestational week	37.0 (35-40), *n*=107		36.7 (35-41), *n*=412		36.8 (35-41), *n*=519		0.063

*Data are *n*/*N* or mean (range)

N-values may vary because of missing values

** May not add up to 100% because of rounding

*** Women who mentioned pregnancy complications prompting her to seek medical help, some women mentioned multiple pregnancy complications

[†]Women who mentioned to have any known diseases or allergies

All women except one (99.8%) stated to take pregnancy medication as routinely prescribed by the ANCC. This scheme consists of three times sulfadoxin/pyrimethamin (1500mg/75mg) as intermittent preventive treatment of malaria in pregnancy at gestational week 20, 24 and 28 and a vitamin/trace elements preparation containing iron III hydroxide polymaltose (dose equivalent to 100mg elemental iron), folic acid (1mg), vitamin C (150mg), zinc (15mg), pyridoxine HCL (1.5mg) and cyanocobalamin (15mg). The latter is taken daily from the beginning of the seventh month of pregnancy onwards. Fifteen women (2.9%) stated to take additional medication on a regular basis. Most frequently mentioned were herbal

medicine (not further specified) in four cases and HIV drugs (not further specified) in three cases (data not shown in tables).

The mean gestational age at the time of sampling was 36.8 weeks (minimum: 35 weeks, maximum: 41 weeks, median: 36 weeks, see **Table 3**).

3.1.3 Information on sanitary facilities

As shown in **Table 4**, of the 518 women who answered, 203 (39.2%) stated to have an own bathroom for their household members, 303 persons (58.5%) shared a bathroom with other households and twelve women (2.3%) used a public bathroom. Women enrolled at KNUST Hospital were non-significantly more likely to have a bathroom for their household than those from Pramso (respectively *n*=50/107, 46.7% and *n*=153/411, 37.2%; *p*=0.07). None of the women at the KNUST Hospital reported the use of public bathrooms.

The same 518 participants replied to the question concerning their toilet facilities. One hundred and fifty-two (29.3%) answered to use a water closet which was shared between 6.6 people on average (95% CI: 5.5-7.8). Use of non-water toilets was reported by 191 women (36.9%). Those were shared between a mean of 11.7 people (95% CI: 10.5-13.0). One hundred and seventy participants (32.8%) said they use a public toilet, shared with an unknown number of people. Three women (0.6%) stated to “go into the bush” and another two (0.4%) said they use a chamber pot. Amongst women at the KNUST Hospital, non-public toilets were significantly (*p*<0.001) more likely to be water closets compared to the study population in Pramso (see **Table 4**).

Table 4: Hygienic facilities of study cohorts

Characteristics	KNUST Hospital		St. Michael´s Hospital		Total		*p*
	Number*	%**	Number*	%**	Number*	%**	
Bathroom facilities							
Own bathroom***	50/107	46.7	153/411	37.2	203/518	39.2	0.073
Shared bathroom†	57/107	53.3	246/411	59.9	303/518	58.5	
Public bathroom	0/107	0.0	12/411	2.9	12/518	2.3	
Toilet facilities							
Water closet	56/107	52.3	96/411	23.4	152/518	29.3	<0.001
Non-water toilet	15/107	14.0	176/411	42.8	191/518	36.9	
Public toilet	35/107	32.7	135/411	32.8	170/518	32.8	
Other (bush or chamber pot)	1/107	0.9	4/411	1.0	5/518	1.0	
Number of people sharing one toilet (mean)							
Water closet	4.9 (1-25), *n*=56		7.7 (1-46), *n*=93		6.6 (1-46), *n*=149		0.008
Non-water toilet	14.1 (5-35), *n*=14		11.5 (2-58), *n*=169		11.7 (2-58), *n*=183		0.165

* Data are *n*/*N or* mean (range)

** May not add up to 100% because of rounding

*** Own bathroom for household members

† Shared with other households

3.2 Microbiological results

3.2.1 GBS prevalence and risk factors

Of 509 tested women, 19.1% (*n*=97; 95% CI: 15.7-22.7) were diagnosed GBS-positive. Stratified by study sites, a GBS prevalence of 23.1% (*n*=24; 95% CI: 15.4-32.4) and 18.0% (*n*=73; 95% CI: 14.4-22.1) were found at KNUST Hospital, Kumasi and in St. Michael´s Hospital, Pramso respectively. The difference was not significant (*p*=0.24).

Women with three or more previous pregnancies showed a significantly (p=0.035) lower GBS colonization rate of 14.3%, compared to 21.9 % in women who had a maximum of two previous pregnancies. In older women (32 years of age and older), who had three or more previous pregnancies (n=139) colonization rates differed even more compared to those with a maximum of two previous pregnancies (11.7% and 30.6%, respectively, p<0.01).

No correlation could be found between GBS carrier status and the women´s highest educational degree (p=0.9), nor literacy (p=0.8), nor the ability to speak English (p=0.9), nor the quality of housing of patients (p=0.6). There was no correlation with the women´s week of gestation (p=0.9).

The correlation between GBS carriage and women´s toilet quality (water/non-water/other), in case they were not using public toilets, also did not reach statistical significance (p=0.12). Women using a public toilet even tended to show a slightly lower GBS prevalence of 17.26% (95% CI: 11.9-23.9; p=0.5).

In patients who mentioned complications during the course of the current pregnancy (n=97), a GBS prevalence of 23.7% (95% CI: 15.7-33.4) was observed, while it was 18.0% (95% CI: 14.4-22.1) in those women without complications (n=411, p=0.20). Comparing only those women who mentioned vaginal discharge as a reason to seek medical attention (n=61, GBS prevalence of 26.2%; 95% CI: 15.8-39.1) to those who did not (n=448, GBS prevalence of 18.1%; 95% CI: 14.6-22.0), this trend was slightly stronger (p=0.13).

In our study population, GBS carriage showed a trend towards younger age. However, this observation was also not significant (p=0.18).

3.2.2 Serotype distribution

All 97 GBS positive aliquots were conserved and shipped to BNITM for serotyping. One GBS sample could not be recovered in Hamburg, thus 96 GBS cultures were serotyped.

As shown in **Figure 4**, capsular polysaccharide serotypes Ia, V and III were the most common serotypes, representing 27 (28.1%), 26 (27.1%) and 21 (21.9%) of 96 positive cultures, respectively. Furthermore, nine samples (9.4%) were typed as CPS

serotype II and eight (8.3%) as serotype Ib. Five (5.2%) samples did not reveal any of the analyzed serotypes.

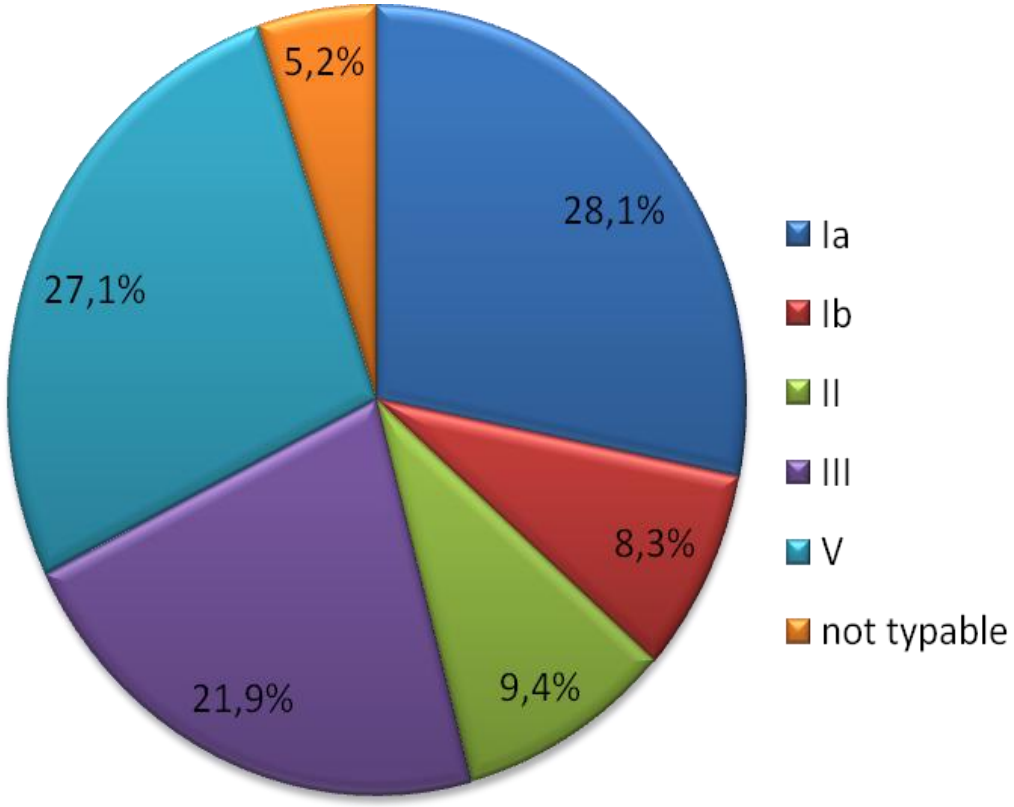

Figure 4: Overall serotype distribution of all 96 typed isolates

Stratified by study site, the serotype distribution was differing especially for serotypes Ia and Ib (see **Table 5**). Serotype Ia occurred notably more often in the population of the rural St. Michael´s Hospital, Pramso, than in the urban population of KNUST Hospital, Kumasi (*n*=25/72, 34.7% and *n*=2/24, 8.3% respectively; *p*=0.017). Serotype Ib, however, represented three out of 72 cases (4.2%) in Pramso and in five of 24 cases (20.8%) at KNUST Hospital (*p*=0.022).

Comparing the five tested capsular polysaccharide antigens with regard to pregnancy complications, it was observed that serotype III showed a trend to a higher relative proportion of pregnant women seeking medical help than any other serotype (*p*=0.08). Among serotype III positive patients (*n*=21), six women (28.6%;

95% CI: 11.3-52.2) sought medical advice because of vaginal discharge, while this proportion varied between 7.4% (in group Ia) and 15.4% (in serotype V) in the other four tested serogroups (data not shown in tables).

Serotype distribution was very similar for older and younger women.

Table 5: Serotype distribution of GBS isolates at the study sites

Serotype	KNUST Hospital		St. Michael´s Hospital		Total		*P*
	Number	%*	Number	%*	Number	%*	
Ia	2	8.3	25	34.7	27	28.1	0.017
Ib	5	20.8	3	4.2	8	8.3	0.022
II	3	12.5	6	8.3	9	9.4	0.686
III	7	29.2	14	19.4	21	21.9	0.393
V	5	20.8	21	29.2	26	27.1	0.597
n.t.	2	8.3	3	4.2	5	5.2	0.596
Total	24	100	72	100	96	100	

* May not add up to 100% because of rounding

3.2.3 Antibiotic susceptibility

Our 97 GBS positive isolates were tested for antibiotic susceptibility using the Kirby-Bauer disc diffusion method in accordance with CLSI guidelines. In total, 15 organisms showed resistance or intermediate resistance to at least one of the tested antibiotics (see **Table 6**).

All isolates were susceptible to penicillin, ampicillin and ampicillin/sulbactam. One strain (1.0%) was resistant to erythromycin, one was intermediately resistant (1.0%), 95 were sensitive (97.9%). Clindamycin resistance was discovered in three cases (3.1%), intermediate resistance to clindamycin in one (1.0%) and sensitivity in 93 cases (95.9%). Most resistances were found to chloramphenicol - in twelve cases (12.4%), while 85 isolates (87.6%) were sensitive.

Two cultures showed resistances to more than one antibiotic agent. One organism resistant to erythromycin was also resistant to chloramphenicol and clindamycin and involved serotype V. One other isolate showing intermediate resistance to erythromycin was also intermediately resistant to clindamycin. It featured CPS serotype Ib.

Table 6: Antibiotic susceptibility of isolated GBS organisms

Antibiotic agent, potency	Sensitive		Intermediately resistent		Resistent	
	Number*	%	Number*	%	Number*	%
Penicillin, 10 units	97	100	0	0.0	0	0.0
Ampicillin, 10 µg	97	100	0	0.0	0	0.0
Ampicillin, 10 µg/Sulbactam, 10 µg	97	100	0	0.0	0	0.0
Erythromycin, 15 µg	95	97.9	1	1.0	1	1.0
Clindamycin, 2 µg	93	95.9	1	1.0	3	3.1
Chloramphenicol, 30 µg	85	87.6	0	0.0	12	12.4

**n*=97 GBS isolates

Stratified by study sites, two out of 24 positive samples originating from KNUST Hospital´s study population (8.3%) and 13 out of 73 (17.8%) from St. Michael´s Hospital´s participants were resistant or intermediately resistant to one or more of the antibiotics. The difference between study sites was not significant (p=0.3).

3.3 Healthcare efforts and healthcare seeking behavior

3.3.1 Transport and time to access healthcare

3.3.1.1 Time expenditure

Of 514 women, 275 (53.4%) stated to have reached the ANCC in less than 30 minutes from their home, 222 (43.1%) needed between 30 and 60 minutes, twelve women (2.3%) said they needed more than one hour. Women attending St. Michael´s Hospital needed significantly ($p<0.001$) more time to reach the hospital than participants visiting KNUST Hospital (see **Figure 5**).

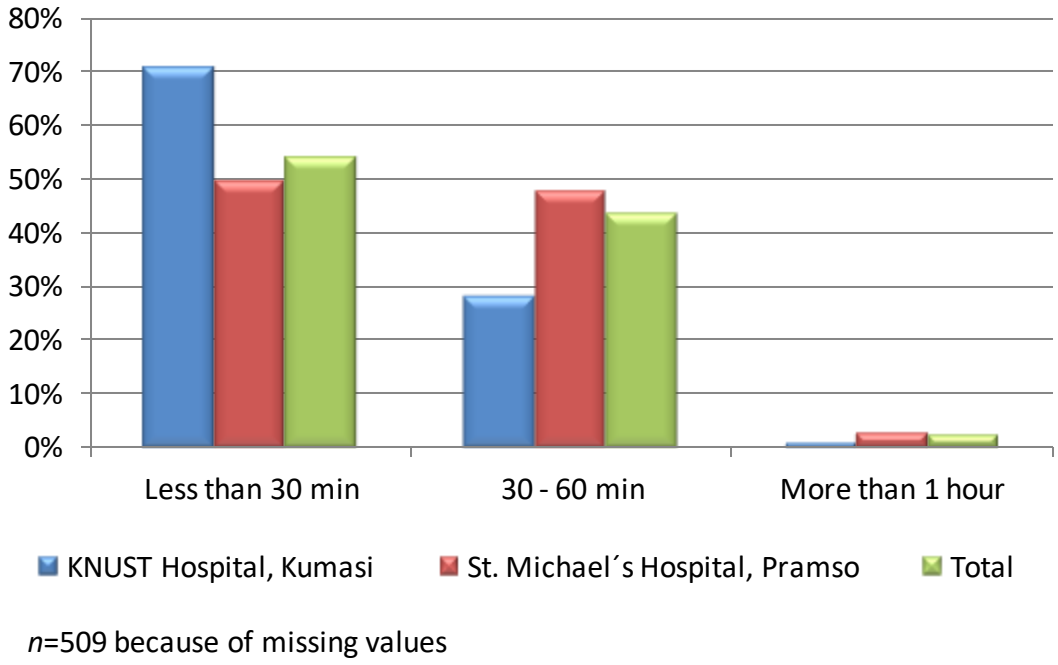

Figure 5: Patient´s time expenditure to reach study hospital

Women were also asked to estimate the time they would need to reach the medical facility closest to their home in case of an emergency and by choosing the fastest kind of transportation. At both study sites, the vast majority of women (*n*=105/106, 99.1% at KNUST vs. *n*=391/408, 95.8% at the St. Michael's Hospital) stated to be able to reach medical help within 30 minutes. None estimated to need more than one hour in an emergency scenario (data not shown in figures).

3.3.1.2 Transport means

The question asking for the chosen means of transport to reach the hospital was answered by 515 women. Of those, 431 participants (83.7%) used a tro-tro (*n*=61/107, 57.0% at KNUST and *n*=370/408, 90.7% in Pramso). Tro-tros are privately owned minibus taxis, usually leaving when full, and are normally the cheapest means of motorized transport. Thirty-five participants (6.8%) stated to have only walked (*n*=19/107, 17.8% and *n*=16/408, 3.9% respectively), 58 women (11.3%) answered to have come by taxi (*n*=19/107, 17.8% and *n*=8/408, 2.0% respectively) and 19 persons (3.7%) said to have used a private car or motorbike (*n*=8/107, 7.5%

and *n*=10/408, 2.5% respectively). Thirty-two women (6.2%) stated to utilize two different means of transportation (*n*=3/107, 2.8% at KNUST and *n*=28/408, 6.9% in Pramso), which in 31 cases was the combination of tro-tro and taxi.

3.3.1.3 Transport costs

A total of 511 participants answered the question for their transport costs to reach the hospital the morning they were enrolled into the study. Of those, 94.3% did not pay more than 1.50 Ghana Cedis (GH₵) for one-way transportation (see **Figure 6**). The median was 0.60 GH₵ and the mean 0.78 GH₵ (95% CI: 0.71-0.86), varying between no costs in 47 cases (9.2%) and a maximum of 10.00 GH₵ in one case. Average costs to reach the ANCC were significantly higher for patients of St. Michael's Hospital ($p<0.001$). The mean costs here being 0.84 GH₵ (95% CI: 0.75-0.92) compared to 0.59 GH₵ (95% CI: 0.46-0.72) for KNUST Hospital´s women. On June 30th, approximately in the middle of the study period, one GH₵ equaled 0.379 € (82).

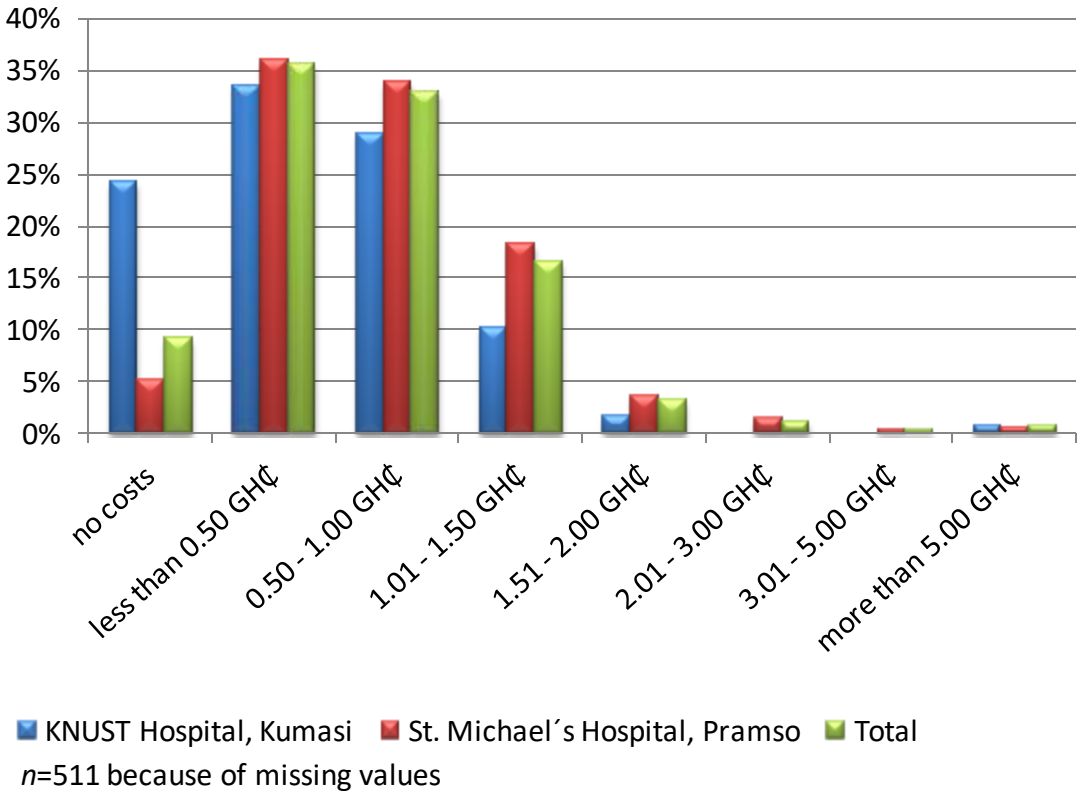

Figure 6: Patient´s one-way transportation costs to reach study hospital

3.3.2 Healthcare seeking behavior

3.3.2.1 Antenatal care adherence

A total of 502 (96.7%) women stated to participate in the hospital´s ANC scheme during the whole pregnancy and 514 (99.0%) participants said they plan to deliver their baby in the same hospital, while three women (0.6%) stated that they plan to deliver at another facility, two women (0.4%) did not answer the question. No women answered to plan to deliver at home.

3.3.2.2 Disease and symptom perception

The open question "What three physical signs/symptoms in your new born baby do you consider as most important danger signs for severe illness?" was asked to 349 study participants (see **Table 7**).

"*Fever*" was the most frequent answer – stated by 275 women (79%). The answer "*crying*" was mentioned second most frequently by 199 (57%).

Different gastrointestinal symptoms were mentioned by 136 women (39%), of which vomiting (*n*=81; 23%) and diarrhea (*n*=64; 18%) were the most commonly stated ones. Inability to suck the mother´s breast was replied in 116 cases (33%). Forty-eight participants (14%) mentioned different respiratory symptoms, first of all cough (*n*=31; 9%). Different descriptions of jaundice (e.g. "*yellow eyes*", *"yellow skin")* as an important danger sign were stated by 33 women (9%).

For other answers see **Table 7**. Ten women answered with "*unknown*" or failed to give an answer to that question.

Table 7: Infant disease symptoms mothers reported as most important danger signs

Symptoms	Frequency of answers (*N*=349*)	%**
Fever	275	79
Crying	199	57
Gastrointestinal symptoms	136	39
Vomiting	81	23
Diarrhea	64	18
Constipation	5	1
Other	1	0
Not breastfeeding	116	33
Respiratory symptoms	48	14
Cough	31	9
Upper respiratory symptoms	16	5
Other	2	1
Jaundice	33	9
Weakness	24	7
Strange crying	20	6
Not sleeping	20	6
Skin rashes	20	6
Not able to cry	19	5
Restlessness	15	4
Eye discharge	7	2
Visible veins appear on abdomen	4	1
Convulsion	3	1
Shaking and shivering	3	1

Women were asked the question:
"What three physical signs/symptoms in your new born baby do you consider as most important danger signs for severe illness?"

* *N*=349 because of missing values

** Does exceed 100% because women mentioned up to three symptoms

3.3.2.3 Adherence to traditional healing

Of the 481 women who answered the question whether there would be any symptoms that would prompt them to seek help of a traditional healer or if they would consult a doctor first in any case, 477 (99.2%) replied that they would always do the latter.

Of the four women (0.8%) who replied to seek the help of a traditional healer, two answered to do so when the baby has a fever, another one replied *"when the baby has a running nose"* and the fourth one answered to do so in case the baby´s fontanels are not closing properly. All four participants were enrolled in St. Michael´s hospital, Pramso.

3.3.2.4 Behavior in case of sickness of the newborn

Study participants were asked for their most likely procedure in the case their baby shows fever or inability to suck their breast.

Four hundred-nineteen of 515 women (81.4%) replied to seek help of a doctor or hospital. No one chose the opportunity of seeing a traditional healer or giving herbal medicine. Eighty-eight women (17.1%) ticked the answer "*Give medicine (e.g. Paracetamol)*". Of those, 71 participants (80.7%) said to give paracetamol, three women (3.4%) replied to give oral rehydration solution (ORS) and thirteen women (14.7%) stated to give ORS and paracetamol. One person (1.8%) replied without further specification to "*give medicine from drugstore*". Specific drugs other than paracetamol and ORS were not mentioned.

A total of 17 of the 515 women (3,3%) ticked the option "*other*". Sixteen of those specified to use water to cool the baby´s body temperature, while one woman answered to see a pharmacist first.

Although the question was supposed to be answered by checking only one answer, nine women checked two answering options, which the responsible fieldworker did not correct. In all of those nine cases the combination was "*Giving medicine, e.g. Paracetamol*" and "*other*", the latter specified as using water to cool the baby's temperature. Those nine double-answers are included in the data described above.

KNUST Hospital´s study population was significantly (p<0.001) more likely than those in Pramso to self medicate their babies in case it shows a fever or inability to suck (n=33/106, 31.1% at KNUST Hospital replied to give medicine compared to n=55/409, 13.5% in Pramso).

The level of education correlated significantly (p<0.001) with the tendency to self-medication.

"*In case of presence of fever or inability to suck, how many days would you wait until you see a doctor/visit a clinic?*" This question was answered by 513 study participants (see **Table 8**). Of those, 328 women (63.9%) checked the option to see a doctor as soon as possible. One hundred and nine patients (21.3%) said to wait for one day, 47 women (9.2%) answered that they would wait for two days and 29 (5.7%) stated to wait for three days before seeking medical help. Behavior was similar for both study site cohorts. No participating woman stated she would wait for more than three days. Furthermore, there seemed to be no considerable association between the waiting behavior and the time or money the women needed to reach the hospital.

Table 8: Waiting behavior in case the infant is sick

Time	KNUST Hospital		St. Michael´s Hospital		Total	
	Number	%*	Number	%*	Number	%*
See doctor as soon as possible	65	60.8	263	64.8	328	63.9
Wait for 1 day	26	24.3	83	20.4	109	21.3
2 days	12	11.2	35	8.6	47	9.2
3 days	4	3.7	25	6.2	29	5.7
Total	**107**	**100**	**406**	**100**	**513**	**100**

Women were asked the following question:
"In case of presence of fever or inability to suck, how many days would you wait until you see a doctor/visit a clinic?"

* May not add up to 100% because of rounding

4 Discussion

4.1 Maternal GBS colonization in the Ashanti Region

GBS carriage in late pregnancy was examined in 509 Ghanaian women enrolled in two study sites through vaginal and rectal swabbing, subsequent culturing and latex agglutination testing. Overall, GBS colonization was found in 19% of all study participants. Most frequent CPS serotypes were Ia, III and V. Resistance to tested antibiotic agents was low. There was no antibiotic resistance against the CDC-recommended penicillin and ampicillin.

Data on GBS carriage in pregnant women in SSA is scarce. Moreover, many of the existing African studies on GBS epidemiology have the drawbacks of low sample sizes and are often lacking the CDC-recommended collection of rectal swab specimens (see below). However, findings of the few more recent studies conducted between 2009 and 2013 performed on maternal GBS colonization in this region are consistent with results of this study as they report prevalence rates of 19-23% in Malawi (83), Senegal (77), Central African Republic (77), Tanzania (84), and Zimbabwe (41). One study from South Africa revealed GBS carriage as high as 36% of pregnant women (85).

Older studies from SSA similarly reported GBS carrier rates of around 20% in the Gambia (76), Nigeria (86), Ivory Coast (87) and Kenya (88). In northern Africa maternal GBS carriage was described with 17% in Tunisia (89) and 25% in Egypt (90).

Data from developed countries, where the epidemiology of *S. agalactiae* is well studied, indicate prevalence of 7-36% in Europe (28, 42, 91, 92) and 19-30% in the USA (43, 93-95). Lower GBS prevalence was found in recent Brazilian and Argentinean studies with 15% and 9% respectively (96, 97). Studies from South-East-Asia, China and Korea described recently prevalence rates of 7-12% (29, 98, 99).

The results of this study are also matching with findings of the analysis conducted by STOLL ET AL. In this extensive meta-analysis, when only studies with adequate

methods were considered, GBS colonization rates in different geographical regions of the developing world was stated as follows: Sub-Saharan Africa 19%; India/Pakistan 12%; Asia/Pacific 19%; Middle East/North Africa 22%; Americas 14% (40).

Concerning specimen collection it should be noted that mentioned studies differed in their techniques of swab taking. In particular in older studies from African countries, only vaginal swabs were analyzed (62, 86-88, 90), while more recent studies took swabs of the vagina and the rectum as recommended by the CDC (3). Swabbing of both sites can increase the sensitivity of GBS detection by 23-37% (100-102). Thus, different methodology in specimen collection might contribute to the divergence of GBS prevalence findings in different studies across the globe.

Besides the differences in sample collection, laboratory procedures for culturing and detection of GBS, such as the utilized enrichment media (e.g. Lim broth, Trans Vag broth or carrot broth), agar materials (e.g. Columbia blood agar vs. chromogenic agars) and the GBS identification methods (e.g. CAMP test, latex agglutination with GBS antisera, peptide nucleic acid fluorescent in situ hybridization or DNA amplification tests such as polymerase chain reaction), vary greatly between published articles on GBS prevalence and may have significant influence on sensitivity of GBS recovery (103-105).

GBS isolates in this study were most commonly of serotype Ia, III or V. Only few isolates were untypable. MADZIVHANDILA ET AL. and MAVENYENGWA ET AL., who probably did the most comprehensive studies on GBS serotype distribution among pregnant women in SSA, reported similar results for South Africa and Zimbabwe, to some extent differing in orders of serotype frequencies. Serotype V was reported less common in South Africa, while serotype III was found to be even more prevalent in South Africa and Zimbabwe (16, 17). Interestingly, the two only other West African studies on GBS serotype distribution in pregnant women from the Gambia and Senegal found a strong predominance of serotype V (76, 77).

The findings of the present study also match well with most literature on GBS serotype distribution in Europe and the USA, where most studies found serotypes Ia,

III and V to be the most prevalent ones, followed by serotypes II and Ib (28, 97, 106-110). Similar findings in serotype distributions were also made in studies from China, Korea and Argentina (97, 98, 111). Very contrasting, a Brazilian study found serotypes Ib and II to be predominant (96), and studies from South-East-Asia and Japan reported serotypes VI and IV, and some studies also Ib and II, to be much more common (29, 112, 113).

We failed to find an explanation for the unequal distribution of serotypes Ia and Ib amongst our two study cohorts. Whether any risk factor in the difference in living conditions between urban and rather rural populations plays a role here remains to be studied.

Concerning the serotypes that are found in invasive disease in infants, EDMOND ET AL. found in their recent meta-analysis that serotype III accounted for 49% of invasive GBS disease in infants around the globe, followed by serotype Ia, accounting for almost a quarter of all cases, and, less frequently V, Ib and II (15). This matches well with most epidemiological findings on GBS carriage in pregnancy (15). Serotype III seems to be more easily transmitted from mother to child and to be more likely to cause invasive disease (16, 28, 114).

Our results suggest that GBS serotype distribution in Ghana does not differ greatly neither from other studies in SSA, nor from most industrialized countries.

All 97 isolated GBS cultures were tested for antibiotic susceptibility to penicillin, ampicillin, ampicillin/sulbactam, clindamycin, erythromycin and chloramphenicol by Kirby-Bauer disc diffusion test according to CLSI guidelines.

We did not observe any case of resistance against penicillin or ampicillin and consequently neither to ampicillin/sulbactam. Reduced sensitivity to penicillin and ampicillin was reported in several studies from different geographical regions around the world (84, 115-118).

In total 4% of our isolates were resistant or intermediately resistant to clindamycin and 2% were resistant to erythromycin. This finding contrasts resistance patterns in the USA, where resistance to clindamycin and erythromycin in GBS isolates increased within the last years and was reported to be 12-20% and 25-32%

respectively (3, 95). A recent study from Germany found resistance to clindamycin in 5% and to erythromycin in 11% of GBS isolates (119). Findings from the Thai-Myanmar border indicated that 92% of GBS isolates were susceptible to both erythromycin and clindamycin (29). On the other hand a study from Zimbabwe found no resistance to clindamycin, while erythromycin resistance was reported to be 14% (118).

The difference between our results concerning erythromycin and clindamycin susceptibility and those from industrialized countries is striking. This might reflect low usage of those antibiotic agents in our study population.

We found 12% of our GBS isolates to be resistant to chloramphenicol. Despite safety concerns, this antibiotic agent has until recently been widely used in Ghana for the treatment of various bacterial infections (120, 121), probably because of its broad antimicrobial-spectrum and low price. Consequently, many organisms with high resistance rates against chloramphenicol have emerged (120, 121). Resistance of different pathogenic bacteria to erythromycin was reported less common (122). In their study in Kumasi, DENNO ET AL. found *S. pneumonia* to be in 13% resistant to chloramphenicol and in 1% intermediately resistant to erythromycin (123), very well reflecting our results for *S. agalactiae*.

Our findings suggest that GBS intrapartum prophylactic treatment with penicillin as first choice antibiotic agent is appropriate in Ghana. For women with a history of severe allergic reactions to penicillin or cephalosporin, clindamycin or erythromycin would be an adequate second-line therapy.

We analyzed GBS carriage with regards to socio-economic and pregnancy related factors, such as urbanity of study population (urban or rural), educational indicators, hygienic facilities, housing and household size, age, previous pregnancies, pregnancy complications and gestational age.

The only significant correlation with GBS colonization was found for the number of previous pregnancies, which was associated with a lower colonization rate. This correlation became stronger when only observing women of at least 32 years of age. We also found a trend for younger women and those attending our urban study site

to be at higher risk for GBS carriage. Moreover, women who were GBS-positive showed a trend to be more likely to report vaginal discharge during their pregnancy.

Matching our results, a Turkish study found younger women and women of lower parity to be more susceptible to GBS colonization (124). Consequently, a study from New Zealand reported infants of younger mothers and those of lower parity to be at higher risk for EOGBS (125). ANTHONY ET AL. found younger women to have lower concentrations of antibodies against capsular polysaccharides of Serotype III (126). CAMPBELL ET AL. made similar observations (43). This might be a possible explanation for the reported increase of risk for GBS colonization in young women in this population. Moreover, these findings match with the promising results of GBS vaccine trials. However, it should be mentioned that recent studies from Sweden and Holland found no association between GBS carriage and socioeconomic factors, parity or the woman´s age (92, 127).

The comprehensive case-control study of STAPLETON ET AL. suggested a significant positive correlation between GBS carriage and higher educational background and income. They could not find an association with the mother´s age or parity (128). GRIMWOOD ET AL. found an independent association between GBS carriage and higher socio-economic status as well as young maternal age (129). REGAN ET AL., in contrast, found GBS carriage to be less common in women with higher education (94). Interestingly, a recent study on GBS colonization from Zimbabwe found pregnant women living in rural areas to be more often colonized than those living in urban regions (41).

Several studies performed on multiethnic study populations in industrialized countries identified black race as a risk factor for maternal GBS colonization as well as for EOGBS (4, 22, 32, 43, 55, 57, 92, 128). Despite this observation, the average GBS carrier rates in the above mentioned African studies, as well as in this study, do not exceed prevalence rates found in high-income countries where study populations mainly consist of Caucasians. One possible explanation might be poorer methodology in laboratory procedures of many studies conducted in SSA, as some of those for example only took vaginal and not rectal swab specimen (40, 62, 86-88, 90). Moreover, studies on GBS epidemiology in industrialized countries are usually

conducted in hospitals and laboratories that are well accustomed to antenatal GBS screening and presumably have a good established routine and expertise in corresponding procedures, while this might be new to some African study sites, where routine GBS-screening in pregnancy is usually not performed. Another explanatory approach might be a decrease in sensitivity of GBS isolation in low-income country settings due to overgrowth by resistant organisms as will be discussed in **4.3**.

On the other hand, most of the cited studies found a correlation rather between higher socioeconomic status and GBS colonization than the opposite, which might lead to speculations about poorer living conditions being associated with raised resistance towards GBS colonization (94, 128, 129). The etiology of risk factors associated to GBS carriage as well as the pathomechansims of GBS colonization are still widely unclear (128), which consequently limits the discussion of epidemiological correlations. Doubtlessly, there is a lack of data on GBS prevalence in SSA settings and further investigations are needed to clarify the in parts conflicting observations regarding risk factors.

4.2 Considerations for future vaccine trials

Complementary to examining the GBS colonization of pregnant women in Ghana, this study schematically characterized the two sites with respect to socio-economic aspects, patient adherence and health care seeking behavior. This data might provide implications for future vaccine trials in Ghana.

Comparing both study sites, findings indicate that the KNUST Hospital´s cohort, representing urban Kumasi, is of significantly higher socio-economic background. This is suggested by higher educational background, smaller household sizes, higher standards of hygienic facilities and higher age of primigravidae. The greater percentage of married women further emphasizes this, as especially life partners with lower financial resources often avoid or postpone marriage due to its high financial expenditure.

Adherence to the study sites seemed to be very high in both locations: Almost all women enrolled in the study (>95%) participated in the hospital´s ANC consultation scheme on a regular basis. The respective visits were recorded in the individual maternal health booklets that every woman always brought to her consultations. Thus, information on adherence to the ANCC in this study is reliable. Furthermor, the majority of women in this study planned to deliver at the hospital where they attend the ANCC, which emphasizes the bonds of our patient cohorts to Western conventional medicine and associated health facilities. Trust in traditional healing - at least regarding ANC - appeared to be low. However, it is questionable whether this fact might have been partly influenced by an interview bias as pregnant women may hesitate to disclose information on traditional healing practices under research conditions.

The moderate estimated travel time to the hospital of less than half an hour for seven out of ten patients at KNUST Hospital and half of the participants in St. Michael´s Hospital, and median one-way travel costs of 0.60 GH₵ (0.23 €) suggests that the study sites are well accessible for most patience of the ANCC. This would facilitate follow-up visits and implies that a potential travel imbursement in trials would be a reasonable expense.

Furthermore, 85% of the study participants stated to seek medical advice as soon as possible or the next day in case their newborns would experience danger signs of illness. Four out of 5 women considered fever as one of the most important danger signs in neonates, which would facilitate early presentation in case of EOGBS and differential diagnoses like neonatal sepsis of other origin and neonatal Malaria.

English language skills of the study population are certainly a point to consider in clinical trial preparations. Sixty-five percent of the study participants stated to speak English. However, our practical experience was very contrasting. Only an estimated fifth of all potential study participants had sufficient English skills to be able to understand the purpose of the study and to complete the interview in English.

Thus, we consider competent bilingual fieldworkers to be indispensable for future clinical trials. Informed consent procedures will have to take place in local languages for a significant proportion of the population.

To estimate the external transferability of the constitution of our study population, we picked the level of education, which we presumed to be one of the best surrogate markers for socio-economic status, and compared the results to the statistics collected by the Ghana Statistical Service for the 2010 Population & Housing Census. The census data for females of ≥15 years of age living in the Ashanti Region state that a percentage of 29% does have no or at most primary school education. This was only 18% amongst study participants. JHS, SHS and tertiary education as highest educational degree was achieved by 41%, 12% and 8 % respectively, according to census data (130), and by 51%, 18% and 13% of women enrolled in our study. This suggests mean educational attainment in our study population to be somewhat above regional average. Yet, reporting bias may have had an effect as well. Nonetheless, it is important to consider that the average level of education in Ghana has risen steeply in the last decades and that the Census data also comprise the older female population beyond their fertile year, amongst which average education might be lower.

4.3 Limitations

Concerning results of socio-economic and other patient data that was collected through interviews, it should be mentioned that most interviews were conducted in Twi, the most widely used local language in Ghana. Linguistical structures vary greatly between Twi and English and for many words and expressions a direct translation does not exist, which might have biased the answers at some points through misunderstandings or categorizations.

Furthermore rigid stigmata exist in many aspects of the Ghanaian society and potential biases through attempts to fulfill the interviewer´s expectations, to hide uncomfortable facts or behavior that is contrary to the routine maternal health education as given by midwifes in the ANCC, cannot be ruled out for some points – e.g. the question regarding consultations of traditional healers. Apart from that, the women's intentions for example on planned delivery in the study site´s maternity

ward should be taken with caution, since infrastructure is not always reliable and unforeseen events might interfere with those plans.

Despite our strict adherence to the CDC recommended protocol, some possible limitations in laboratory procedures should be mentioned.

Specimen storage and transport conditions may influence GBS culture yield. GBS recovery declines during days 1-4 at any temperature, especially at higher temperatures, even if appropriate transport media is used (80, 81, 131). Our swab specimen were stored in Amies media and were kept at ambient temperature but were processed the same day within six hours after collection, which is why we do consider time and transport of swab specimen as unlikely to significantly influence our results.

According to CDC guidelines the addition of 5% sheep blood to commercially purchased enrichment broths might increase GBS recovery (3). We did not include this step into our laboratory procedures, which is why we might have missed a chance to enhance the sensitivity of GBS culture yield slightly.

Between 1-4 % of GBS isolates are commonly nonhemolytic (3, 132). However our methodology was not suitable for the detection of nonhemolytic *S. agalactiae.*

Furthermore there is a controversy about which culturing and identification methods yield highest sensitivity for GBS recovery (85, 103-105).

We experienced a relatively low effectiveness of the employed enrichment media in inhibiting growth of other bacterial fauna, which is why a potential decrease in sensitivity cannot be excluded. Selective enrichment can improve detection of GBS substantially (134, 135). As recommended by CDC (3) we used Lim broth to promote enrichment with streptococci and inhibit growth of gram-negative bacteria of the physiological enteric fauna (134, 135). Still, our blood agar plates that were incubated with the enriched Lim broth often displayed a mixed bacterial growth of very different organisms, gram-positive as well as gram-negative. We commonly found α- or nonhemolytic gram-positive cocci in chains, which our latex agglutination test mostly classified Group D Streptococci (GDS). GDS antisera also agglutinate *E.*

faecalis (manufacturer´s information), which features GDS surface antigens (14) and was formerly classified as part of the group D streptococci system. Different gram-negative bacteria were found frequently, sometimes also swarming bacteria. Those non-GBS organisms sometimes overgrew or overswarmed our blood agar plates, complicating the detection of GBS suspicious β-hemolytic colonies even after repeated pureplating. Quality control of our prepared agar plates was routinely performed and always negative for contamination.

Extensive literature research on the vast amount of articles published on different culture methodology to optimize GBS detection revealed that only very few articles mentioned that problem: PARK ET AL. observed an antagonistic phenomenon between *E. faecalis* and *S. agalactiae* in Lim broth enrichment that can lead to false negative GBS results (136). DUNNE and HOLLAND-STALEY made a similar experience and suggested that heavy *E. faecalis* colonization suppresses GBS in Todd-Hewitt broth with added gentamicin and nalidixic acid (137). GIL ET AL. also reported GBS suppression in Lim broth by vaginal or rectal organisms, primarily attributing this to *E. faecalis* (138). This phenomenon might explain the occasional overgrowth of our blood agar plates with other gram positive cocci, usually identified as GDS. KWATRA ET AL. described in their article from South Africa thoroughly the difficulties concerning overgrowth of their culture plates by different, primarily fecal, but also by resistant vaginal bacteria that masked GBS presence after selective enrichment. Besides our study, this is the only article we could find to also report an occasional overswarming of their plates, rendering them unreadable, which they attribute to resistant *Proteus spp.* (85).

To reduce the loss of sensitivity in GBS detection caused by overgrowth we follow the suggestion of KWATRA ET AL. that for low-income country settings combined processing of vaginal and rectal swab specimen in the same selective enrichment tube might not be adequate. Pooling of specimens from both loci of swab collection might aggravate the loss in sensitivity due to resistant bacterial fauna, since resistant organism from one locus will affect the specimen from the other one as well. CDC guidelines, which explicitly recommend enrichment in selective broth media and combined processing of vaginal and rectal specimen, were developed in high-income

settings, but there is clear lack of available data on the validity of these procedures in low- and middle-income countries, where composition of enteric fauna and antimicrobial resistance differs (3, 85, 139-141).

Clindamycin resistance of GBS isolates was found to be low in this study. However isolates were not tested for inducible clindamycin resistance, which may occur in GBS isolates that appear to be susceptible for clindamycin when no CLSI D-zone test is performed (3, 133).

5 Conclusions

GBS prevalence and serotype distribution in Ghana seems to be similar to findings from Europe and the USA, as well as to results from other countries in SSA. Since all GBS isolates were fully susceptible to first-line antibiotics and resistance to second-line antibiotics was low, we consider the current CDC IAP-scheme as appropriate for this geographical region.

Regarding risk factors, women with a higher number of previous pregnancies seemed to be of lower risk for GBS colonization. Socio-economic status and hygienic conditions did not significantly correlate with higher or lower GBS colonization.

Due to experienced bacterial overgrowth, we suggest awareness for the limitations of the CDC recommended protocol for selective broth culture media and the examination of alternative procedures especially in the setting of low-income countries.

Our two study sites were of mixed socio-economic background. Both study sites were well accessible and we found patient adherence to be high at both sites, which might favor future vaccination trials.

In most SSA settings, a standardized GBS-screening of women in late pregnancy as it is recommended by the CDC and performed in most industrialized countries is not viable due to lacking infrastructure and resources. Vaccinations however, would be cost-efficient, require minimal patient compliance and could be well integrated into established ANC programs. In our study population, a pentavalent vaccine formulation against GBS serotypes Ia, Ib, II, III and V could have the potential of protecting 95% of pregnant women and their offspring against invasive GBS disease.

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6 Abstract

Introduction: Group B streptococcal (GBS) colonization of pregnant women can lead to subsequent infection of the newborn and potentially fatal invasive disease. Data on GBS colonization prevalence and serotype distribution from Africa is scarce, although GBS-related infections are estimated to contribute substantially to infant mortality. In recent years, GBS vaccine candidates provided promising results in clinical trials. We aimed to provide first data on the prevalence and serotype distribution of GBS in Ghana, to examine associated risk factors and to evaluate the suitability of the chosen study sites for potential future vaccine trials.

Methods: This double-centre study was conducted in one urban and one rural hospital in central Ghana. Women in late pregnancy (≥35 weeks of gestation) attending the antenatal care clinic provided a recto-vaginal swab for GBS-testing. GBS isolates were analysed for antibiotic susceptibility and serotype. Colonization was compared with socio-economic data. GBS-positive women were treated with intrapartum antibiotic prophylaxis according to the current guidelines of the Centers for Disease Control and Prevention. Data on socio-economic background and information about the access to healthcare and healthcare seeking behavior was collected using predefined questionnaires with multiple choice questions and limited free-text options to enable descriptive and comparative analyses.

Results: In total, 519 women were recruited at both study sites, recto-vaginal swabs were taken from 509 women. The overall prevalence of GBS was 19.1 % (18.0% and 23.1% in the urban and the rural study site, respectively). Capsular polysaccharide serotype (CPS) Ia was found most frequently (28.1%), followed by serotype V (27.1%) and III (21.9%). No resistance against Penicillin was found, resistances against second line antibiotics clindamycin and erythromycin were 3.1% and 1%, respectively. There was a significant inverse correlation between GBS carriage and the number of previous pregnancies.

Discussion: GBS serotype distribution in Ghana is equal to those worldwide and would be covered by vaccines, currently under clinical development. Antibiotic resistance of GBS strains was low in this study. Both study sites were well accessible

and we found patient adherence to be high at both sites, which might favor future vaccination trials.

Einleitung: Die Besiedlung schwangerer Frauen mit Gruppe B Streptokokken (GBS) kann zu Neugeboreneninfektionen und potenziell tödlicher, invasiver Erkrankung führen. Zur Prävalenz und Serotypenverteilung in Afrika gibt es bisher kaum Daten, obwohl GBS-bedingte Infektionen vermutlich für einen großen Anteil der Neugeborenensterblichkeit verantwortlich sind. In den letzten Jahren gab es vielversprechende Ergebnisse zu GBS-Impfstoffen aus klinischen Studien. Ziel dieser Studie ist es erste Daten zur GBS-Prävalenz und Serotypenverteilung unter schwangeren Frauen in Ghana zu erheben sowie assoziierte Risikofaktoren zu untersuchen und die Tauglichkeit der Studienorte für eventuelle spätere Impfstudien zu evaluieren.

Methoden: Die Studie wurde an zwei Studienzentren, einem urbanen und einem ländlichen Krankenkenhaus, in Zentralghana durchgeführt. Frauen in der Spätschwangerschaft (ab 35. Gestationswoche), die eine der Schwangerschaftsvorsorgeeinrichtungen besuchten, wurde ein rekto-vaginaler Abstrich zur Testung auf GBS entnommen. GBS-Isolate wurden dann auf Antibiotikaresistenzen und ihren Serotyp getestet. Die GBS-Besiedlung wurde mit sozio-ökonomischen Daten verglichen. GBS-positive Frauen erhielten unter Geburt eine Antibiotikaprophylaxe nach den Leitlinien des Centers for Disease Control and Prevention. Zur deskriptiven und vergleichenden Auswertung wurden sozio-ökonomische Daten sowie Informationen über den Zugang zu medizinischen Einrichtungen und das Verhalten bezüglich Inanspruchnahme medizinischer Hilfe mittels vorgefertigter Fragbögen erhoben. Diese enthielten sowohl Multiple-Choice- als auch limitierte Frei-Text-Fragen.

Ergebnisse: In die Studie wurden 519 Frauen eingeschlossen, wobei rekto-vaginale Abstriche von 509 Frauen entnommen wurden. Die GBS-Prävalenz betrug insgesamt 19,1% (18,0% im urbanen und 23,1% im ländlichen Studienzentrum). Kapselpolysaccharidserotyp Ia wurde am häufigsten gefunden (28,1%), gefolgt von Serotyp V (27,1%) und III (21,9%). Es zeigte sich keinerlei Resistenz gegen

Penicillin. Resistenzen gegen die Zweitlinienantibiotika Clindamycin und Erythromycin betrugen 3,1% bzw. 1,0%. Es zeigte sich eine signifikante inverse Korrelation zwischen GBS-Trägerschaft und der Anzahl vorheriger Schwangerschaften.

Diskussion: Die GBS-Serotypenverteilung in Ghana gleicht denen auf anderen Teilen der Welt und würde von zurzeit in Entwicklung befindlichen Impfstoffen gut abgedeckt werden. Es zeigten sich wenige antibiotikaresistente GBS-Stämme in dieser Studie. Beide Studienkrankenhäuser waren gut erreichbar und zeichneten sich durch eine hohe Compliance der Patienten aus. Dies könnte zukünftigen Impfstudien zu Gute kommen.

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8 Appendix – Case Report Form

Subject code label

☐ - ☐☐☐

I. Participant information **Date (dd/mm/yyyy):**_____

_____ _____ ☐☐/☐☐/☐☐☐☐

First name **Last name** **Date of birth (dd/mm/yyyy)**

_____ _____

Address **Age**

_____ _____

City/Town/Village **Phone number**

Literate **No** ☐ **Yes** ☐ **Speaks English** **No** ☐ **Yes** ☐

II. Information about the current pregnancy

1. Gestational age: ☐☐ **weeks** (*To be filled by fieldworker)*

2. Participation in ANC scheme during whole pregnancy? (*To be filled by fieldworker)*

No ☐ **Yes** ☐ **Unknown** ☐ **No answer** ☐

_____

3. How many pregnancies did you have before this pregnancy (including abortions)?

0 ☐ **1** ☐ **2** ☐ **3** ☐ **4** ☐ **More than 4** ☐

4. Have you ever had a spontaneous abortion?

No ☐ **Yes** ☐ **if 'Yes', how many?** ☐ **Unknown** ☐ **No answer** ☐

5. Have you had any severe complications (e.g. bleeding, vaginal discharge) during the current pregnancy, which prompted you to see a doctor?

No ☐ **Yes** ☐ **Unknown** ☐ **No answer** ☐

If 'Yes': _____

III. Information about the mother

1. What is your marital status?

Never married □ **Married** □ **Divorced** □ **Widowed** □ **No answer** □

2. What is your and your husband´s monthly income?

☐☐☐☐☐ **Gh Cedis** **Unknown** □ **No answer** □

3. What is your highest educational degree?

None □ **Quit Primary** □ **Primary** □ **JHS** □ **SHS** □

Tertiary □ **No answer** □

4. Do you have any known diseases/allergies?

No □ **Yes** □ **Unknown** □ **No answer** □

If 'Yes': _____

5. Do you take any regular medication? **No** □ **Yes** □ **Unknown** □ **No answer** □

If 'Yes': **ANC-routine** □

Other □ **:** _____

IV. Information about the living situation of the mother

1. In what kind of house do you live?

Stone/cement house □ **Mud house** □ **Wood house** □ **No answer** □

2. How many people live in your household?

☐☐ **people** **Unknown** □ **No answer** □

3. What kind of bathroom do you use?

Own bathroom for household members □ **Shared bathroom (with other households) □**

Public bathroom □ **No answer □**

4. What kind of toilet do you use?

Water closet □ **Public toilet (KVIP) □**

Private non-water closet □ **Other □, please specify:_____**

No answer □

5. How many people share your toilet?

☐☐ people **Unknown □** **No answer □**

V. Information about access to healthcare and healthcare seeking behavior

1. What was the transportation you took to reach this hospital? *(Check all that applies)*

☐ Bus (e.g. STC/Metromass) **☐ Trotro**

☐ Private car/ Motorbike **☐ Walk**

☐ Taxi **☐ Ambulance**

☐ Other:_____ **☐ No answer**

2. How much time did it take you to reach this hospital?

☐ Less than 30 min. **☐ 30 to 60 min.** **☐ more: ☐☐ hours**

☐ Unknown **☐ No answer**

3. How much money did it cost you to reach this hospital (<u>one-way</u>)?

☐☐.☐☐ Gh Cedis **☐ Unknown** **☐ No anwer**

4. Where do you plan to deliver your baby?

☐ At this hospital **☐ At another facility: _____**

☐ At home **☐ Not yet decided**

☐ No answer

5. Imagine an emergency. How far is the next hospital, medical centre or doctor away from your home, if you chose the quickest kind of transportation available?

☐ **Less than 30 min.** ☐ **30 to 60 min.** ☐ **more:** ☐☐ **hours** ☐ **No answer**

6. What <u>three</u> physical signs/symptoms in your new born baby do you consider as most important danger signs for <u>severe</u> illness? *(Name three:)*

_____

☐ **Unknown** ☐ **No answer**

7. Imagine your child is born. What kind of signs/symptoms in your baby would prompt you to seek the help of a traditional/local healer first (before seeing doctor/hospital)?

_____

_____

☐ **Unknown** ☐ **Always see doctor/hospital first** ☐ **No answer**

8. Imagine your child is born. What kind of signs/symptoms in your baby would prompt you to see a doctor or visit a hospital first (before seeing traditional/local healer)?

_____

_____

☐ **Unknown** ☐ **Always see traditional healer first** ☐ **No answer**

9. What would be your most likely procedure in the case that your baby shows <u>fever or inability to suck</u>? *(Check only one)*

☐ **See local/ traditional healer** ☐ **Give herbal medicine** ☐ **See doctor/ hospital**

☐ **Give Medicine(e.g. Paracetamol). If "Yes": Which?** _____

☐ **Other, please specify:**_____ ☐ **No answer**

10. In case of presence of <u>fever or inability to suck</u>, how many days would you wait until you see a doctor/visit a clinic? *(Check only one)*

☐ **See a doctor as soon as possible**

☐ **Wait for** ☐☐ **days, then see a doctor** ☐ **No answer**

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10 Curriculum vitae

Patrick Brust
Schäferstr. 7, 20357 Hamburg
Tel.: 0177 9364423, patrickbrust@outlook.de

Geburtsdatum und -ort	20. Juli 1986, Wuppertal
Staatsbürgerschaft	Deutsch
Familienstand	Ledig
Fremdsprachen	Englisch, Spanisch und Portugiesisch in Wort und Schrift

Bildung

06/2006	Abitur, Carl-Duisberg-Gymnasium, Wuppertal
10/2008 – 09/2009	Studium der Umweltingenieurwissenschaften, Leuphana Universität Lüneburg
10/2009 – 06/2016	Studium der Humanmedizin, Universität Hamburg

Praktische Tätigkeit

Seit 07/2016	Klinik für Kinder- und Jugendmedizin, Universitätsklinikum Hamburg-Eppendorf

Publikation

Group B Streptococci serotype distribution in pregnant women in Ghana: Assessment of potential coverage through future vaccines.
C. D. Vinnemeier†, P. Brust†, E. Owusu-Dabo, N. Sarpong, E. Y. Sarfo, Y. Bio, T. Rolling, D. Dekker, Y. Adu-Sarkodie, K. A. Eberhardt, J. May, J. P. Cramer
† Authors contributed equally to the manuscript
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11 Eidesstattliche Erklärung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

……

Patrick Brust