The clinical and molecular epidemiology of *Streptococcus agalactiae* in Kenya: Maternal colonisation and perinatal outcomes.

Thesis presented for the degree of Doctor of Philosophy

Michaelmas 2014

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Abstract

Robust data on *Streptococcus agalactiae*, Group B Streptococcus (GBS) maternal colonisation and perinatal disease in sub-Saharan Africa are lacking, and essential to inform prevention. A cohort study was undertaken to examine risk factors for maternal GBS colonisation, transmission and association with adverse perinatal outcomes. Invasive GBS disease was further examined in stillbirths (case-control study) and neonates (clinical surveillance). GBS bacterial isolates were whole genome sequenced and types defined.

There were 7967 mothers in the cohort study; 934/7967 (11.7%, 95%CI 11.0-12.5) were GBS colonised at delivery. Higher socio-economic status (OR 1.19 (1.08-1.30)) and handling cattle (1.29(1.16-1.43)) were associated with increased maternal GBS colonisation; lower nutritional status (0.73 (0.59-0.89), and Mijikenda ethnicity (indigenous) (0.73 (0.60-0.94)) were associated with decreased maternal GBS colonisation. Maternal colonising serotypes (N=910) were III (39%), 1a (21%), V (17%), Ib (12%), II (9%), IV (2%) and VII (0.3%).

Maternal GBS colonisation was associated with invasive perinatal GBS disease (OR 12.6 (2.9–58.9)), but no other adverse perinatal outcomes, outside of possible effect modifiers. Vertical transmission, evidenced through genetic comparison, resulted in neonatal surface colonisation and perinatal disease. GBS was isolated from stillbirths (0.91 (0.25-2.3)/1000 hospital births, and neonates (0.76 (0.25-1.77)/1000 live hospital births; 0.35 (0.25-0.47)/1000 live births). In days 0-6 of life (n=31) invasive GBS serotypes were; Ia (23%), Ib (10%), II (3%), III (61%), IV (3%). For days 7-27 (n=39), GBS serotype III (almost all clonal complex 17), caused 35/39 (90%) of all cases.

Group B streptococcus is an important cause of perinatal disease. Maternal GBS colonisation increased with improved socio-demographic factors and further development may result in higher disease burden. Most GBS disease was caused by the most virulent clonal complex (CC-17), especially after the first few days of life. Over 90% of GBS disease would be covered by the most advanced trivalent vaccine in development. Prevention is essential and maternal vaccination is likely the most feasible and effective strategy.

Declaration and attributions

I, Anna Seale, conceived and designed these studies, sought ethical approval, oversaw the conduct of the studies, undertook the analyses and wrote this thesis. The DPhil was supervised by Professor James Berkley and Professor Derrick Crook. A detailed description of work undertaken and assistance obtained is set out below.

Clinical data collection

I designed the studies and materials (admission records, databases) to collect clinical data, undertook public engagement work, and trained a team of medical and clinical officers (Dr Angela Koech, Dr Hellen Barsosio; Joyce Langat, Emily Anyango, Stella Mwakio), study fieldworkers and nurses (on locum shifts in their normal place of work) on study procedures in the four study sites in Kenya. I wrote the consent forms in English, which were translated by members of the study team into Kiswahili and Kigiriama. I oversaw the study team during recruitment; the study team requested consent, collected clinical admission data and undertook data entry.

Sample collection

I determined sampling procedures and transportation of samples. I revised the microbiology standard operating procedures to cover the study's requirements, with advice and in conjunction with Dr Susan Morpeth (Microbiology, KEMRI-Wellcome Trust). Microbiological testing was undertaken by the Microbiology laboratory staff in KEMRI-Wellcome Trust Research Laboratories.

Molecular microbiology

I was shown how to extract and prepare DNA according to established laboratory protocols by Alison Vaughan. I undertook DNA extraction and preparation for whole genome

sequencing of the GBS isolates, which were then processed at the Wellcome Trust Centre for Human Genetics. Initial pre-processing of raw sequence data was undertaken using a data processing pipeline developed by the Department of Statistics, University of Oxford. Quality assessment of DNA was undertaken by Dr Anna Sheppard (Department of Bioinformatics, University of Oxford), and she developed the GBS sequetyping method described and used. Adam Giess (Department of Bioinformatics, University of Oxford) assigned MLST types based on the presence of 7 housekeeping genes. I designed and undertook the analyses of WGS data by adapting and running existing python scripts. Anna Sheppard designed an additional script for illustration of these data using colour.

Statistics

I undertook all statistical analyses using Stata version 13. I received advice on these from Dr Greg Fegan (Statistics, KEMRI-Wellcome Trust) and on multiple imputation models from Professor Sarah Walker (Nuffield Department of Medicine, University of Oxford).

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Definitions and acronyms

Acronym	Term	Definition or description
	Child death	Death in the first 5 years of life after live birth
CPGH	Coast Provincial General Hospital	Provincial Hospital in Mombasa, Kenya
EOS	Early onset sepsis	Sepsis in days 0-6 of life
EOD	Early onset disease	Invasive bacterial disease in days 0-6 of life
ERC	Ethical Review Committee	Kenya national ethical review committee (Nairobi) of KEMRI (see below)
	Gravidity	Number of times a woman has been pregnant
GBS	Group B Streptococcus *(Streptococcus agalactiae)*	Beta-haemolytic Gram positive *Streptococcus*[1]
IAP	Intrapartum chemoprophylaxis	Administration of intravenous antibiotics to prevent early onset GBS disease.
KEMRI	Kenyan Medical Research Institute	The national medical research institute in Kenya; a parastatal organisation (http://www.kemri.org/)
KDH	Kilifi District Hospital	A rural district hospital on the coast of Kenya
KHDSS	Kilifi Health and Demographic Surveillance Site	Area of demographic and health surveillance around Kilifi District Hospital, in coastal Kenya[2]
KIPMAT	Kilifi Perinatal and Maternal Health Research Programme	Research programme of the KEMRI-Wellcome Trust in Kilifi, Kenya, studying maternal and newborn health (http://clinicaltrials.gov/show/NCT01757028)
KWTRP	KEMRI-Wellcome Trust Research Programme	Research programme of the KEMRI-Wellcome Trust in Kilifi, Kenya (http://www.kemri-wellcome.org/)
LOS	Late onset sepsis	Sepsis from days 7-27 days of life
LRT	Likelihood ratio test	Test to assess overall effect of a variable within a model.
MAR	Missing at Random	The probability that the data are missing depends on the values of the observed data, but does not depend on the values of the missing data.
MAR	Maternal Admission Record	Standardised clinical admission forms

MIC	Minimum Inhibitory Concentration	The lowest concentration of an antimicrobial required to inhibit growth of an organism in culture (usually ~12h)
MLST	Multi-locus sequence typing	Method of typing organisms based on gene loci of house-keeper genes
MUAC	Mid-upper arm circumference	Measurement taken around circumference of upper arm, at the mid-point between the acromion and olecranon.
	Neonatal	0-27 days of life
NDI	Neurodevelopmental Impairment	Functional impairment from an insult, in this thesis arising from infection in the perinatal and/or neonatal period
NMR	Neonatal mortality rate	Neonatal deaths per 1000 live births
OXTREC	Oxford Tropical Research and Ethics Committee	Ethical review committee associated with University of Oxford, UK (http://www.tropicalmedicine.ox.ac.uk/oxtrec)
	Parity	The number of previous pregnancies carried to a viable gestation ($\geq$28 weeks).
PNSGBS	Penicillin non-susceptible Group B Streptococci	Group B Streptococci which are not susceptible to penicillin
PCA	Principal Components Analysis	Transformation of a set of correlated variables to linear uncorrelated variables
PMR	Perinatal mortality rate	Stillbirths and deaths in the first 7 days of life per 1000 births
pSBI	Possible Severe Bacterial Infection	Presence of any one of: a history of difficulty feeding, history of convulsions, movement only when stimulated, respiratory rate of 60 breaths per minute or more, severe chest in-drawing, temperature of 37·5 ^{0}C or more, or below 35·5 ^{0}C.[1]
PMTCT	Prevention of Mother to Child Transmission (of HIV)	Methods which prevent the mother passing HIV infection to her baby.
Primip	Primiparous	Women in her first pregnancy
RNA	Ribonucleic acid	A single stranded polymer of nucleotides made through the process of transcription.

RSV	Respiratory Syncytial Virus	A respiratory virus causing neonate, infant and child respiratory tract infection.
SES	Socio-economic status	Measure of household or individual prosperity
SNP	Single Nucleotide Polymorphism	Genetic variation in a DNA sequence that occurs when a single nucleotide in a genome is altered
SSC	Scientific Steering Committee	Scientific committee of the Kenyan Medical Research Institute (http://www.kemri.org/)
	Stillbirth; ante-partum and post-partum	Born over 28 weeks gestation with no signs of life; may be separated into ante-partum (skin broken) and intra-partum (skin intact).[3]
sSA	Sub-Saharan Africa	Includes Global Burden of Disease (GBD) regions sub-Saharan Africa central, sub-Saharan Africa east, sub-Saharan Africa west and sub-Saharan Africa southern. [4]
UPGMA	Unweighted Pair Group Method with Arithmetic Mean	UPGMA trees reflects the structure present in a pairwise similarity matrix and assume a constant rate of evolution (which may not hold true, particularly with high recombination)
YICSS	Young Infants Clinical Signs and Symptoms	WHO Study group conducting large multi-site studies of possible severe bacterial infections in young infants.[5]
WHO	World Health Organisation	An agency of the United Nations specifically concerned with global health
WGS (or NGS)	Whole Genome Sequencing (or Next Generation Sequencing)	Next Generation Sequencing platforms,[6] which allow entire genome sequencing, as opposed to MLST typing, for example, where only a part of the genome sequence data are sequenced.

Chapter 1: Introduction

1.1 Burden

1.1.1 Burden of child mortality

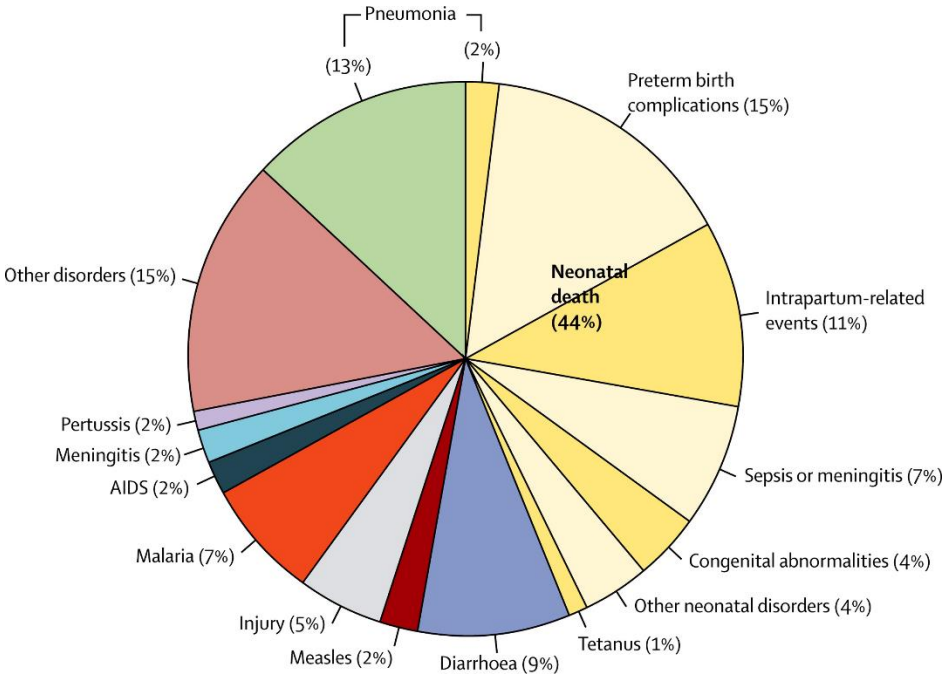
Worldwide child mortality (deaths in children aged under 5 years of age) has decreased, moving towards the aim of Millennium Development Goal 4 (1990-2015), to decrease child mortality by two thirds. The reduction is largely attributed to fewer deaths from pneumonia, measles, and diarrhoea.[7] However, whilst there has been progress in reducing overall child mortality, mostly in children between 1 and 59 months of life, there has been far less progress in reducing neonatal mortality (death in days 0-27 of life), and neonatal deaths now account for 44% of all child deaths (Figure 1).

In sub-Saharan Africa, progress reducing child mortality has been slower, accounting for around a half of all child deaths (3.1 million child deaths out of the 6.3 million worldwide in 2013).[8] Furthermore, there is evidence that as sub-Saharan Africa undergoes an epidemiological transition,[9] with an urban population expected to double from 1010 million in 2009 to 1998 million in 2050,[10] it faces a "triple burden" of communicable disease, non-communicable disease and socio-behavioural illness.

In sub-Saharan Africa around a third of all child deaths are in neonates,[7] but this burden is concentrated in the first seven days of life, when three quarters of all neonatal deaths occur.[11] In addition, there is the often unrecognised burden of stillbirth, foetal deaths after 28 weeks' gestation, which are estimated to equal neonatal deaths.[12] It is therefore the perinatal period, which includes stillbirths and neonates in days 0-6 of life, where reductions in child mortality must be made. Recently published targets recognise this, aiming for "no more than 10 stillbirths per 1000 total births, and no more than 10 neonatal deaths per 1000

live births" by 2035.[13] In order for these targets to be met, and further reductions in child mortality achieved, it is essential to improve our understanding of the causes of morbidity and mortality in the perinatal period, enabling assessment of potential methods of reducing this burden in sub-Saharan Africa.

Figure 1 Global causes of child deaths in 2013[8]*



*Reprinted from The Lancet, 385, Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J. E., Cousens, S., Mathers, C., Black, R. E.. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. P430-44, Copyright (2015), with permission from Elsevier.

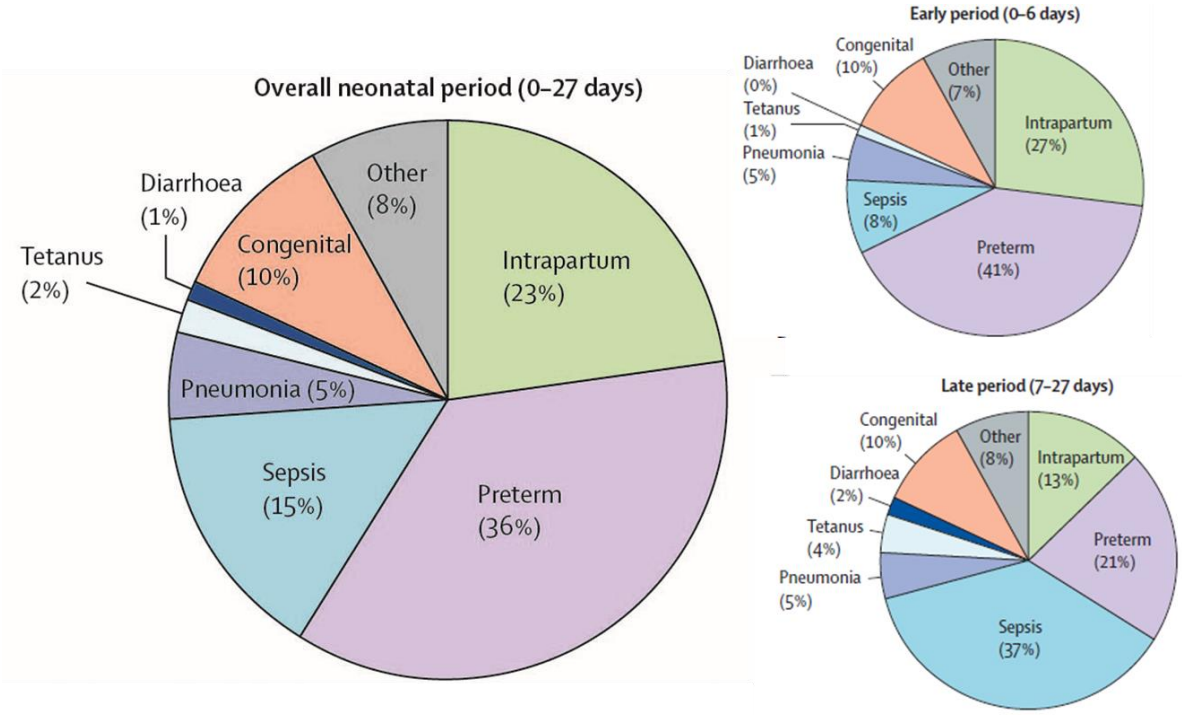
1.1.2 Burden of infection

Mortality burden

There are three main causes of neonatal deaths: infection, complications relating to preterm delivery (<37 weeks gestation) and intra-partum complications,[14] (Figure 2), with considerable overlap between causes.[15] Infection is a direct cause of death, but it is also often the final common pathway for neonates with intra-partum complications, and neonates who are born preterm; both very common complications of death in the first few days of life.[13] The burden of infection is much higher in resource-poor settings; neonatal deaths associated with infection in high burden countries (NMR $\geq$ 30) are over thirty times those in low-burden

countries (NMR <5/1000).[13] For stillbirths, data are extremely limited, but infection is estimated to account for around a half of stillbirths in high burden regions.[16]

Figure 2 Cause of neonatal death by time period [13]*



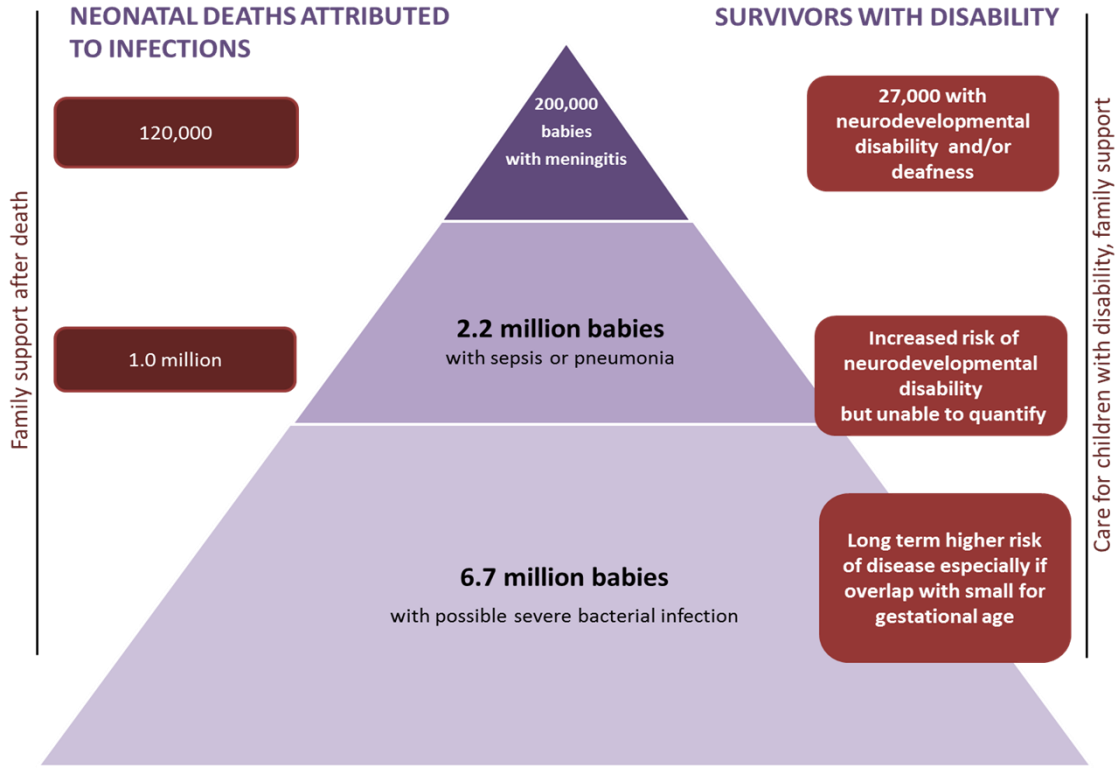
*Reprinted from The Lancet, 384, Lawn, J. E., Blencowe, H., Oza, S., You, D., Lee, A. C., Waiswa, P., Lalli, M., Bhutta, Z., Barros, A. J., Christian, P., Mathers, C., Cousens, S. N., for The Lancet Every Newborn Study Group. Every Newborn: progress, priorities, and potential beyond survival. P189-205, Copyright (2014), with permission from Elsevier.

Morbidity burden

In 2012 it was estimated there were 2.6 million (uncertainty range 2.1 million-3.1 million) neonatal cases of possible severe bacterial infection (pSBI) in sub-Saharan Africa.[17] This was the estimated number of neonates requiring treatment who had at least one clinical sign raising suspicion of possible severe bacterial infection. The estimate reflects the substantial burden of neonatal infection on health care services and staff, as well as families seeking care. Difficulties in seeking care, both practical and financial, mean that care seeking for neonates in resource-poor settings varies from 10% to 100%.[18]

For those neonates who survive invasive bacterial infections (sepsis, pneumonia, meningitis), there may be long term neurodevelopmental impairment (NDI), particularly in meningitis cases where moderate to severe impairment occurs in 23% (95%CI 19-26%) of those who survive.[19] Data are lacking for estimates of NDI associated with neonatal sepsis and pneumonia, which is an important omission, because although NDI is likely to follow these infections less frequently, neonatal sepsis and pneumonia account for a much greater proportion of neonatal invasive bacterial infections, as illustrated in Figure 3.[19]

Figure 3 Estimates of impairment in survivors of neonatal infections for 2010 in sub-Saharan Africa, South Asia and Latin America[19]*



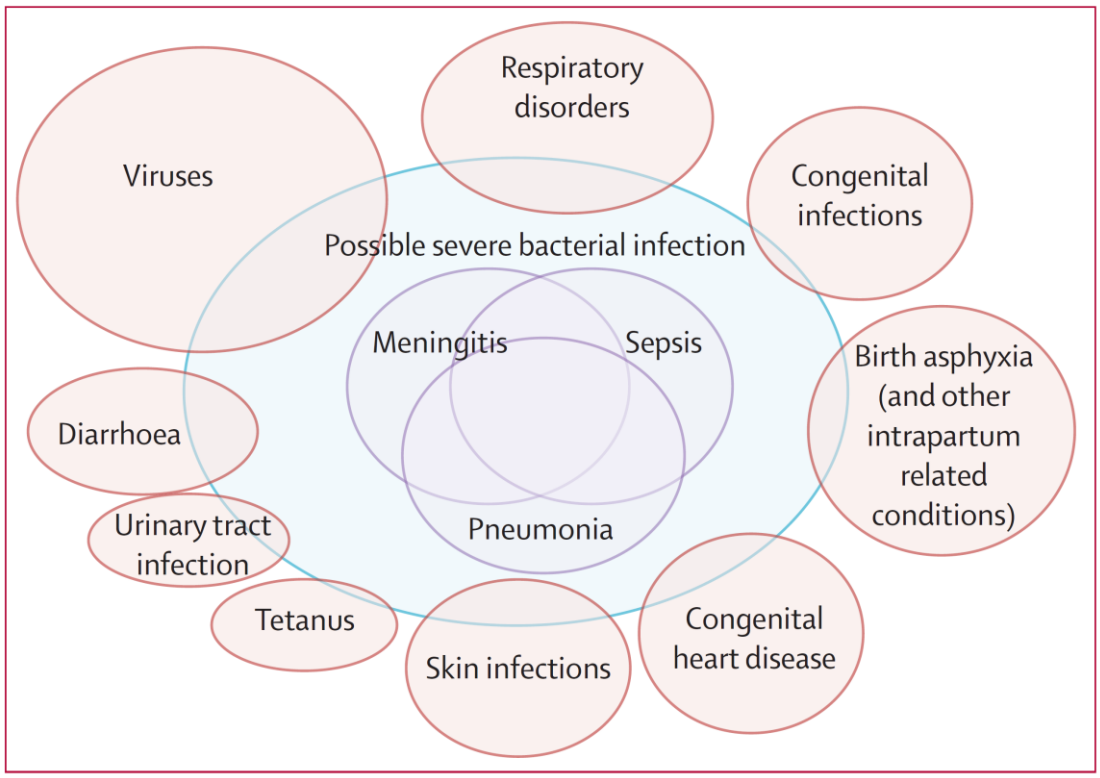
* Reprinted from Paediatric Research, 17 (Suppl 1), Seale, A. C., Blencowe, H., Zaidi, A., Ganatra, H., Syed, S., Engmann, C., Newton, C. R., Vergnano, S., Stoll, B. J., Cousens, S. N., Lawn, J. E. Neonatal severe bacterial infection impairment estimates in South Asia, sub-Saharan Africa, and Latin America for 2010. P73-85.

Diagnosis

Diagnosing severe bacterial infection in neonates is challenging in all settings as clinical signs are non-specific and subtle, and no simple diagnostic test exists.[20] However, whereas in

resource-rich settings diagnosis is supported by experienced paediatricians and a full sepsis screen (cultures of blood, cerebrospinal fluid and urine), in high burden regions, such as sub-Saharan Africa, diagnosis is usually by first line health care workers with no or limited access to laboratory investigations. The WHO guidelines (Integrated Management of Childhood Illness) promote the identification of pSBI through the use of clinical algorithms,[5] where cases can be identified by primary health care workers based on the presence of specified clinical signs. These clinical signs were based on the findings of the WHO Young Infant Clinical Signs and Symptoms (YICSS) multi-site study.[5] In terms of case management, specificity has to be sacrificed for sensitivity, as it is important that all neonates who do have severe bacterial infection are treated, because of the high case fatality risk associated with severe bacterial infection. There is therefore some over-diagnosis, with conditions such as respiratory distress syndrome and viral infections, as illustrated in Figure 4, treated as pSBI and therefore antibiotics prescribed.[17] Confirmation through microbiological investigation is usually not possible as high quality microbiological laboratories are limited to research settings in sub-Saharan Africa. This limits the information available for patient care, and restricts our knowledge of the aetiology of neonatal infection and antimicrobial susceptibilities of pathogens in this setting. In addition, there is an extraordinary lack of data on the infectious aetiology of stillbirth, given that in resource-poor settings infection it is, in expert opinion, estimated to be the cause of half of all stillbirths.[16]

Figure 4 Aetiologies of possible severe bacterial infection in neonates [17]*



* Reprinted from Lancet Infectious Diseases, 14(8), Seale, A. C., Blencowe, H., Manu, A. A., Nair, H., Bahl, R., Qazi, S. A., Zaidi, A. K., Berkley, J. A., Cousens, S. N., Lawn, J. E. for the PSBI Investigator Group. Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, south Asia, and Latin America for 2012: a systematic review and meta-analysis. P731-41. 2014.

Risk factors

Risk factors for neonatal sepsis in sub-Saharan Africa include preterm birth and low birth weight (although differentiating between preterm deliveries and those which are small for gestational age is difficult due to uncertainty in gestational age, particularly as early antenatal ultrasound scanning is not routine). Other risk factors include intra-partum complications and associated birth asphyxia, as well as prolonged rupture of membranes (≥18 hours), and maternal pyrexia (≥38.0°C).[21] These risk factors are consistent with those identified in resource-rich settings, which also include a history of *Streptococcus agalactiae* infection in a previous baby or a urinary tract infection caused by *Streptococcus agalactiae* this pregnancy, and are used to guide intrapartum antibiotic prophylaxis and/or early treatment of the neonate once born. Due to the lack of culture facilities in sub-Saharan Africa, *Streptococcus agalactiae* infections are unlikely to be identified as causing urinary tract infections or even

neonatal sepsis, so assessment of these risk factors has not been undertaken. There are other potential risk factors which are more common in sub-Saharan Africa than other regions, for example HIV infection, which has been associated with adverse perinatal outcomes.[22] There is also some evidence that HIV infection is associated with increased incidence of neonatal sepsis, although data are limited.[23]

Prevention

Prevention strategies in sub-Saharan Africa need to be pragmatic, considering that the majority of women deliver at home,[24] and resources are limited. WHO guidelines for reducing neonatal sepsis include general anti-sepsis measures such as hand washing, as well as exclusive breastfeeding, and Kangaroo Mother Care.[25] WHO paediatric guidelines also advise that neonates with documented risk factors such as prolonged rupture of membranes ($\geq$18h) or maternal fever ($\geq 38^0$C) or foul-smelling amniotic fluid, receive intravenous ampicillin and gentamicin for 48h, after which antibiotics can be stopped assuming no signs of sepsis (or positive blood culture, if available).[25] Research studies in sub-Saharan Africa have explored other methods of preventing neonatal infection. Cord-care with chlorhexidine after delivery has been shown to be effective in reducing all-cause neonatal mortality in randomised controlled trials.[26,27] However, antisepsis at delivery, although early results showed benefit,[28,29] has been shown to be ineffective in a large randomised trial.[30] Furthermore targeted intra-partum antibiotics in high risk groups, such as HIV-1 infected mothers, have shown inconclusive results.[31]

Maternal vaccination is an attractive strategy to reduce neonatal infection: it can be given at ante-natal clinic, which WHO recommends mothers attend four times during the course of their pregnancy. Vaccinations may be more effective than antisepsis in preventing infections and may confer immunity to both the mother and neonate for a longer duration (ante-partum and post-partum through the neonatal period). There are however, barriers to

maternal vaccinations, and safety in pregnancy must be established. There is some precedent to maternal vaccination, however, as maternal tetanus vaccination has been successfully used to reduce neonatal tetanus in sub-Saharan Africa.[32] More recently, maternal vaccines against influenza and pertussis have been safely and successfully implemented in the USA and UK. There is now interest in other potential vaccines which are in development, which could in the future be given to mothers to prevent neonatal disease, for both respiratory syncytial virus (RSV) and *Streptococcus agalactiae*.[33]

1.1.3 Bacterial infection in the perinatal and neonatal period

Bacterial infections can cause disease throughout the perinatal period leading to ante-partum stillbirth (death in-utero, usually predating delivery to the extent that the skin of the foetus is broken at delivery), intra-partum stillbirth (less commonly associated with infection, more likely due to obstetric complications, skin of foetus is unbroken), early onset neonatal infection (definitions vary and may include clinical signs of infection up to 48h of life, or include 0-6 days of life)[21,34] and late onset infection in the remainder of the neonatal period (days 7-27). Bacterial infection may also result in other perinatal complications, such as preterm delivery and low birth weight.[35]

Infectious aetiology of stillbirth

There are extremely limited data on the infectious aetiology of stillbirth in sub-Saharan Africa. Pathogens reported to be associated with stillbirth in sub-Saharan Africa through transplacental infection include syphilis (*Treponema pallidum*)[36] and other spirochaetal infections such as *Borrelia* sp..[37] Other bacterial infections able to cause trans-placental infection include *Mycobacterium tuberculosis, Haemophilus influenzae*, *Salmonella typhi*, and *Listeria monocytogenes* (very rare in sub-Saharan Africa as a cause of neonatal sepsis).[16] In addition, organisms such as *Escherichia coli*, *Streptococcus agalactiae* (Group B Streptococcus, GBS) and *Klebsiella pneumoniae* can cause ascending infection from the

maternal genito-urinary tract, crossing intact amniotic membranes,[38] or entering after rupture of amniotic membranes. These organisms may cause congenital pneumonia from aspiration of contaminated fluid.[16]

Infectious aetiology of early and late onset neonatal sepsis

Organisms which ascend from the maternal genito-urinary tract, are often associated with early-onset sepsis, [39] where neonatal infection is likely a continuum between ascending infection and infection during delivery. Late onset neonatal sepsis is more likely to result from other environmental sources.

Data on the aetiology of neonatal infection are limited; there have been no large community cohorts studying the aetiology of disease in sub-Saharan Africa, (unlike South Asia);[40,41] and published studies are health facility-based. In addition, variations in methodologies in definitions for diagnosis, sampling strategies and laboratory microbiology methods make comparison between facility studies difficult, as illustrated in Table 1. Some studies only include neonates born in the hospital (in-borns), others include a mixture, and some studies focus on neonates born out of hospital (out-borns), which each bring different biases. Studies focussing on in-borns reflect the population able to access health care (which may be less than half the population),[18] and are less likely to include cases of late-onset neonatal disease; studies focussing on out-borns depend on care being sought for a sick neonate, underrepresenting total cases and resulting in selection bias, with likely fewer cases of early onset neonatal disease, as well as excluding cases which were rapidly fatal.

The first large multisite aetiology study of bacterial infection in young infants (under 60 days) was conducted by WHO,[42] with sites in The Gambia,[43] Ethiopia,[44] the Philippines,[45] and Papua New Guinea. The study highlighted the important role of *Streptococcus pneumoniae* in young infants (under 60 days), as well as *Staphylococcus aureus* and Salmonella sp.,[43] but,

surprisingly reported “strikingly” little *Streptococcus agalactiae* (Group B Streptococcus, GBS), suggesting it may not be an important pathogen in sub-Saharan Africa.[46] In contrast, subsequent studies from sub-Saharan Africa, including Kenya,[47] Malawi[48] and South Africa,[49] have identified GBS as an important pathogen in early onset sepsis. Some of the differences may be explained by the methodologies used, in particular the focus on out-patients in the WHO study,[46] and lack of recruitment of neonates in the first 24h of life, when most GBS infections are reported to occur in resource-rich regions.[50] However, the data may also reflect true emergence of disease, following the emergence of GBS in the USA and Europe from the 1960s to 1990s.[34,51,52]

There are other differences in reported aetiologies of neonatal infection when comparing resource-rich and resource-poor settings. In resource-rich settings intensive care facilities support very preterm neonates (from 23 weeks’ gestation) whereas in most resource-poor settings facilities are usually insufficient to support care in neonates under 28 weeks’ gestation and mortality in preterm neonates even over this gestation is still extremely high.[53] This can lead to differences in aetiologies of neonatal disease. For example, in the United Kingdom, Coagulase Negative Staphylococcus (CoNS) is the leading neonatal pathogen;[54] CoNS has a particular ability to form biofilms and colonise indwelling devices, such as neonatal long lines, which are often used in intensive-care settings in preterm neonates in resource-rich settings.[55] In resource-poor settings it is unclear whether CoNS is a contaminant of cultures (as a common skin commensal) or can act as a pathogen. In contrast to resource-rich settings, and due to limitations of resources for health care, indwelling devices are rarely used.

Despite the limited data, from the facility studies available, important pathogens have been identified and include Gram negatives such as *Klebsiella pneumoniae,* and *Escherichia Coli. Streptococcus agalactiae* (Group B Streptococcus, GBS) has recently been identified as a

common pathogen in early onset neonatal sepsis in sub-Saharan Africa; *Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus pneumoniae* are also common, particularly in late onset neonatal sepsis.[21] More data on the aetiology of neonatal sepsis are needed, especially with important concerns emerging in terms of increasing nosocomial infections and antibiotic resistance, especially to gram negative infections such as *Klebsiella pneumoniae*.[56,57]

Streptococcus agalactiae, or Group B Streptococcus (GBS) is of particular interest in sub-Saharan Africa because it is a perinatal pathogen, able to cause disease through ascending infection in-utero,[38] and in the perinatal period, through to young infancy; the times when the burden of mortality in sub-Saharan Africa is highest. Our understanding of the emergence of GBS is limited, and the extent to which it is causing disease in sub-Saharan Africa particularly important, because there are potential methods of prevention. Group B Streptococcus in coastal Kenya is the focus of this thesis, and is now described in more detail, including what is known to date about GBS in sub-Saharan Africa.

Table 1 Summary of studies including microbiological diagnosis of early onset neonatal infection in sub-Saharan Africa*

	Location	Study type	Population	Definition of sepsis	Results (EOS if available)	Organisms (EOS if available)	Limitations
Gray et al[48]	Queen Elizabeth Hospital, Malawi	Prospective descriptive study, referral hospital, EOS specified	3159 in-born and out-born neonatal hospital admissions, 681 investigated with blood cultures	Culture of GBS (blood or CSF); investigation based on clinical suspicion	EOS: GBS isolated in 29 of 681 cases (blood or CSF), rate 0·92 per 1000 live births	GBS 29	Single-site referral hospital; rate may be underestimate due to lack of presentation to health care facility
Berkley et al[47]	Kilifi District Hospital, Kenya	Prospective surveillance study, district hospital, EOS specified	867 in-born and out-born neonatal hospital admissions under 7 days	Positive blood culture, investigation on the basis of clinical suspicion	EOS: 117 positive blood cultures, 5·46 per 1000 live births had neonatal bacteraemia	*Escherichia coli* 25 (19%), *Acinetobacter* spp 16 (12%), *Klebsiella* spp 13 (10%) GBS 11 (9%), *Staphylococcus aureus* 7 (5%), *Pseudomonas* spp 6 (5%), *Streptococcus pneumoniae* 5 (4%), *Streptococcus pyogenes* 3 (2%)	Single site; rate might be underestimated through lack of presentation to health care facility
Aired e[58]	Jos University Teaching Hospital, Nigeria	Prospective descriptive study, referral hospital, EOS not specified	In-born (76%) and out-born (24%) neonatal admissions with suspected sepsis	Positive blood culture; investigation based on clinical suspicion	99 positive blood cultures, neonatal sepsis 6·5 per 1000 live births (in-borns)	*Klebsiella* spp 27 (37%), *S aureus* 27 (37%), others (*E coli*, *Alcaligenes faecalis*, *Citrobacter difficile*)	Single site, in-born neonates only for rate, EOS not specified
Nathoo et al[59]	Harare Hospital, Zimbabwe	Prospective descriptive study, referral hospital, EOS (defined as less than 48 h) specified	In-born (89%) and out-born (11%) neonatal admissions: 161 hospital admissions with positive blood cultures	Positive blood cultures investigation on the basis of clinical suspicion	Neonatal sepsis 21 per 1000 live births (in-borns)	EOS <48 h; 110 +ve cultures: *S aureus* 34 (31%), non-lactose fermenting coliforms 15 (14 GBS 13 (12%), other *Streptococcus* spp 13 (12%), *Staphylococcus epidermidis* 10 (9%), *Klebsiella* spp 9 (8%), lactose-fermenting coliforms 6 (5%), *E coli* 5 (5%), others 5 (5%)	Single-site referral centre, investigation of blood based on clinical suspicion, high-risk maternal deliveries
Mulhollan d et al[43]	Fajara and Royal Victoria Hospital, Banjul, The Gambia	Prospective descriptive study, outpatient department and referral hospital, EOS not specified	Out-born neonatal admissions, 497 enrolled and 239 investigated	Positive blood cultures or CSF in young infants (younger than 91 days), investigation on the basis of clinical suspicion	53 cases of young infant sepsis, 4·42 per 1000 live births (extrapolated from given data)	38 +ve blood cultures (without meningitis): *S aureus* (17), *S pneumoniae* (3), *Salmonella* spp (5), *E coli* (3), other enterobacteriaceae (4), *S pyogenes* (3), GBS (1), *Moraxella* spp (1), group G streptococci (1)	EOS numbers low in study based on out-patients or referrals
Muhe et al[44]	Ethio-Swedish Children's Hospital, Addis Ababa, Ethiopia	Prospective descriptive study, referral hospital, EOS not specified	405 infant admissions (under 3 months)	Positive blood (or CSF culture), investigation based on clinical suspicion	41 isolates (blood) from 40 infants, 15 isolates from CSF	*S pneumoniae* 10, *S pyogenes* 9, *Salmonella* spp 5, positive CSF cultures predominantly *S pneumoniae* 7	Out-patient referrals, likely to miss rapidly fatal early onset infections

Milledge et al[60]	Queen Elizabeth Hospital, Blantyre, Malawi	Prospective descriptive study, referral hospital, EOS specified	In-born neonates (94%)	Positive blood or CSF culture; investigation on the basis of clinical suspicion	EOS: 380 (48%) isolates	*EOS:* GBS *61 (16%), S aureus 57 (15%), E coli 41 (11%), Klebsiella spp 41 (11%)*	Single-site referral hospital
Laving et al[61]	Kenyatta National Hospital, Nairobi, Kenya	Prospective descriptive study, referral hospital, EOS not specified	In-born neonates	Positive CSF culture, investigation based on clinical suspicion	84 patients investigated, 15 positive cultures	*E coli 7 (47%),* GBS *4 (27%), Klebsiella pneumoniae 2 (13%)*	Single-site referral hospital
Madhi et al[49]	Chris Hani Baragwanath Hospital, Soweto, South Africa	Retrospective review of culture-positive cases S agalactiae, provincial hospital, EOS specified	Paediatric admissions	Positive blood or CSF culture for GBS investigation based on clinical suspicion	208 of 220 paediatric admissions with S. agalactiae sepsis, 63% EOS	*EOS from* GBS*: 2·06 per 1000 births*	Single-site referral hospital
English et al[62]	Kilifi District Hospital, Kenya	Prospective study infant admissions, district hospital, EOS specified	In-born and out-born neonates	Positive blood or CSF culture	EOS: 41 of 432 positive cultures	*EOS: Klebsiella spp (10), E coli (8), S agalactiae (6)*	Single site, small numbers for EOS (41 positive isolates)
Udo et al[63]	University of Calabar Hospital, Nigeria	Retrospective review of infant admissions, EOS not specified	In-born and out-born neonatal admissions	Positive blood culture, investigation on clinical suspicion	178 positive blood cultures	*S aureus 109 (61·2%), coliforms 39 (21·9%), Strep. spp 15 (8·5%), Enterobacteriaceae 5 (2·8%)*	Single site, retrospective study
Ghiorgis[64]	Ethio-Swedish Children's Hospital, Addis Ababa, Ethiopia	Retrospective review of neonatal sepsis admissions, referral hospital, EOS not specified	In-born and out-born neonatal admissions (542)	Positive blood culture, investigation based on clinical suspicion	Neonatal sepsis rate 11 per 1000 live births (in-borns), 151 of 542 positive cultures	*Klebsiella spp 34 (38%), E coli 9 (11%), Pseudomonas spp 5 (7%), S epidermidis 13 (25%)*	Single site, retrospective review of admissions
Adejuyigbe et al[65]	Outpatient department, Ile-Ife, Nigeria	Prospective descriptive study bacterial isolates from young infants, EOS not specified	Infants attending outpatients (7–55 days old)	Positive blood culture, investigation on the basis of clinical suspicion	54 of 124 positive blood cultures	*S aureus 28, Staphylococcus spp 17, Proteus vulgaris 3*	Single site, out-born infants only, no EOS
Ojukwu et al[66]	Ebonyi State University Teaching Hospital, Abakaliki, Nigeria	Prospective descriptive study, referral hospital, EOS specified	In-born and out-born neonatal admissions	Positive blood culture; investigation based on clinical suspicion	Neonatal sepsis risk 7·98 per 1000 live births (in-borns), 33 positive blood cultures of 138 (19 of 92 in-born and 14 of 46 out-borns), 20 from EOS	*EOS: S aureus 7 (35%), E coli 4 (20%), Streptococcus spp 3 (15%), Klebsiella spp 2 (10%)*	Single-site referral centre

* Reprinted from Lancet Infectious Diseases, 9(7), Seale, A. C., Mwaniki, M., Newton, C. R., Berkley, J. A. Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa. P428-38. 2009, with permission from Elsevier.[21]

1.2 *Streptococcus agalactiae* (Group B Streptococcus (GBS)): Background

Streptococcus agalactiae (GBS), like other Streptococci, can be part of the normal human and animal micro-flora. GBS was initially thought only to act as a pathogen in animals, causing bovine mastitis, first described in 1887.[67] However, GBS was subsequently described as a cause of puerperal sepsis, in London, UK in 1938.[68] GBS then emerged as the leading cause of neonatal septicaemia and meningitis in the United States, with cases increasing from the 1960s,[69,70] followed by increases in the United Kingdom by the 1980s.[71] The reasons for the emergence of GBS are unclear; theories have included the mechanisation of dairy farming increasing the spread of GBS,[72] a species jump from bovines,[73] and/or the spread of a virulent GBS clone,[74,75] possibly related to the development of tetracycline resistance, with its widespread use. [76]

Although GBS is primarily a neonatal infection, with early infection usually acquired from gastro-intestinal colonisation (its reservoir)[77] and maternal vaginal colonisation, GBS is now also recognised as an increasingly important pathogen in resource-rich regions in immunosuppressed and elderly individuals.[78,79] Preventative methods have focussed on reducing neonatal disease, by administration of intra-partum antibiotics to mothers identified through screening to be colonised with GBS, or to mothers with risk factors. Although screening has been shown to be very effective in the United States in reducing early onset neonatal disease,[80] in the future prevention of GBS disease in mothers and neonates, as well as other high-risk groups, may be possible through vaccination, with the first putative conjugate vaccine about to start phase 3 trials.[81]

1.3 Streptococcus agalactiae (Group B Streptococcus (GBS)): Microbiology

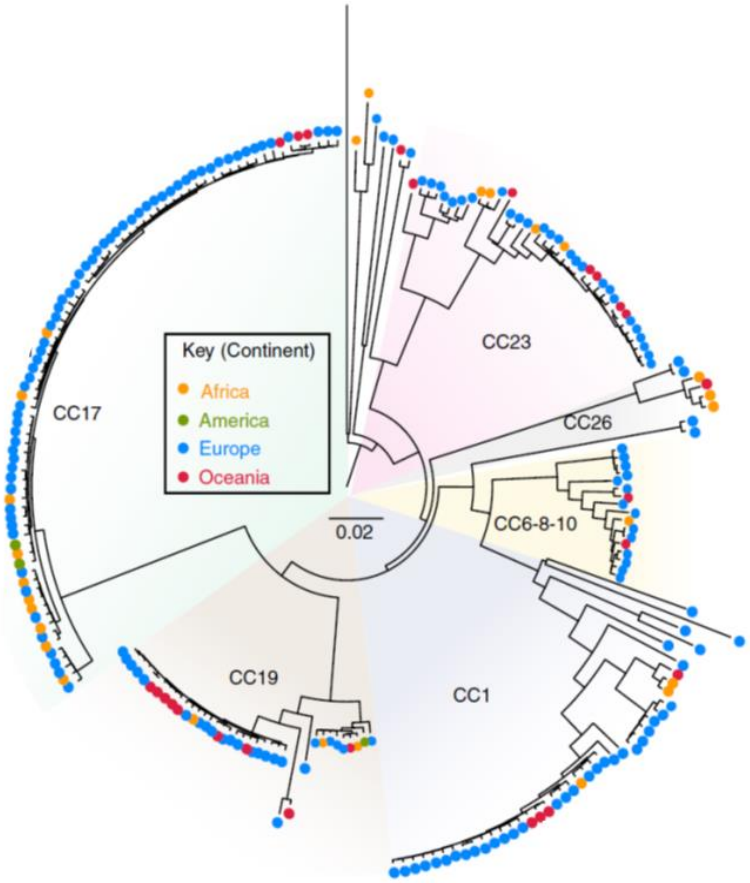
Streptococci are a heterogeneous group of bacteria, included within the group of lactic acid forming bacteria. They are Gram-positive, catalase-negative cocci that occur in pairs or chains and are characterised by the type of haemolysis with which they are associated; alpha, beta or gamma. Partial haemolysis is seen with α-haemolytic Streptococci, complete rupture of erythrocytes can be seen with ß-haemolytic Streptococci and no haemolysis is seen with γ-haemolytic Streptococci.

The ß-haemolytic Streptococci are grouped by their Lancefield antigen, into sero-groups A, B, C, D, and E, based on a serological assay for detection of specific carbohydrate in the polysaccharide cell wall, developed by Rebecca Lancefield in the 1930s.[82] *Streptococcus agalactiae* is characterised by the presence of Group B Lancefield antigen,[82] hence Group B Streptococcus (GBS). Clinical identification of GBS is presumptive, through recognition of its morphology and observation of haemolysis on colonies cultured on sheep blood agar, as well as a positive Christie, Atkins, and Munch-Peterson (CAMP) test. Definitive identification is through sero-grouping with commercially available agglutination assays. [82]

There are different serotypes of GBS; first described by Rebecca Lancefield, using capillary precipitation tests, identifying serotypes I, II, and III.[83] A latex agglutination assay was later developed, and the number of serotypes expanded to include Ia, Ib, II, III, IV, V, VI, VII, and VIII,[84] with serotype IX reported in 2007.[85] Capsular gene typing has been developed using PCR-based methods,[86] and other discriminatory molecular analyses, such as multi-locus sequence typing (MLST), have been developed. The combination of alleles at seven loci (housekeeping genes) provides a sequence type (ST) for each strain.[87] These molecular methods have allowed further genetic analyses, grouping GBS into five major clonal complexes (CC-1, CC-10, CC-19, CC-17 and CC-23), (http://pubmlst.org/sagalactiae/).

Whole gene sequencing technology has recently become possible for pathogens and the invasive serotype III GBS isolate, NEM316, was first sequenced in 2002.[88] This enabled detailed examination of the GBS genome, a circular chromosome, around 2,211,485 base pairs long with 2118 protein coding genes. With very recent advances in whole genome sequencing technology it is now possible to whole genome sequence (WGS) pathogens on a much larger scale, allowing detailed phylogenetic examination of multiple pathogen genomes and their evolution. The population structure of GBS is illustrated in Figure 5, based on 229 sequenced GBS isolates. The branches correspond to the five major clonal complexes previously identified, with the addition of clonal complex 26.[76]

Figure 5: Whole-genome-based phylogeny and major clonal complexes of GBS* # [76]



* Whole-genome-based phylogeny of 229 sequenced GBS isolates and strain SS1219 isolated from fish. Maximum Likelihood (ML) using MEGA was used to infer phylogenetic relationships. The major clonal complexes (CC) 1, 10, 17, 19, 23 and 26 as defined on the GBS MLST web site (http://pubmlst.org/sagalactiae/) correspond towell-defined branches. Isolates are indicated by dots coloured according to their geographical origin.

Reprinted from Nature Communications, 5, Da Cunha, V., Davies, M. R., Douarre, P. E., Rosinski-Chupin, I., Margarit, I., Spinali, S., Perkins, T., Lechat, P., Dmytruk, N., Sauvage, E., Ma, L., Romi, B., Tichit, M., Lopez-Sanchez, M. J., Descorps-Declere, S., Souche, E., Buchrieser, C., Trieu-Cuot, P., Moszer, I., Clermont, D., Maione, D., Bouchier, C., McMillan, D. J., Parkhill, J., Telford, J. L., Dougan, G., Walker, M. J., Devani Consortium, Holden, M. T., Poyart, C., Glaser, P., Devani Consortium. Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, south Asia, and Latin America for 2012: a systematic review and meta-analysis. P731-41.

1.4 *Streptococcus agalactiae* (Group B Streptococcus (GBS)): Clinical

1.4.1 Maternal colonisation in sub-Saharan Africa

The reported prevalence of maternal GBS colonisation in sub-Saharan Africa ranges from 9% to 47% (studies summarised in Table 2).[89-96] This is comparable to the range of maternal GBS colonisation in a review of European studies; (7% to 36%),[97] and the range reported in studies from the USA (5% to 40%).[98]

Maternal GBS colonisation is intermittent,[99] and the timing of sample-taking may therefore play a role in the heterogeneity in prevalence of maternal GBS reported. However, there are inconsistencies in the literature as to whether maternal GBS colonisation is more likely to decrease,[100] or increase,[101,102] towards term. The only study from sub-Saharan Africa concerning changes in GBS colonisation through pregnancy was from Zimbabwe, published in 2010, and it reported that maternal GBS colonisation decreased towards term. Maternal GBS colonisation prevalence at 20, 26 weeks' gestation and delivery was 47%, 24% and 21% respectively.[94] Factors at delivery, such as rupture of membranes and digital vaginal examinations,[103] have also been shown to reduce the probability of detecting GBS, presumably through washing away or mechanical removal of GBS.

The site the swab sample is taken is also important for sensitivity of GBS detection. GBS can be isolated from the rectum, vagina, cervix, urethra (as well as skin and pharynx). However, GBS is more consistently isolated from the lower gastrointestinal tract,[99] suggesting this is the main human reservoir (in women and men), and taking recto-vaginal swabs for diagnostics has become established as the most sensitive, and recommended procedure (compared to vaginal swabs alone) for detecting maternal GBS colonisation.[104-106]

The time taken between swab sampling and laboratory culture is also important, as well as the use of appropriate transport media and environmental conditions. The CDC guidelines

(2010), suggest Amies or Stuart transport media are used and that viability can be maintained up to four days.[105] This is supported by more recent work, but there was also a significant decrease in viability at temperatures over 30 ^{o}C.[107]

Culture methods are also important. The use of selective broth medium (containing nalidixic acid plus gentamicin or colistin) is more sensitive for culture of GBS than inoculation directly onto blood agar plates, and this step can again influence the detection of GBS and reported prevalence.[108]

Table 2 Maternal GBS colonisation studies in sub-Saharan Africa

Author	**Country**	**N**	**Time of sample**	**Site of sample**	**GBS prevalence**
Cutland[30]	South Africa	3964	Ante-natal or labour	Lower vagina swab	21%
Mavenyengwa[94]	Zimbabwe	1037	20 weeks', 26 weeks' and delivery	Vaginal and rectal swabs	47% 24% 21%
Joachim[109]	Tanzania	300	>37 weeks'	High vaginal and rectal swabs	23%
Suara[110]	The Gambia	196	Delivery	Vaginal and rectal swabs	22%
Uhiara[111]	Nigeria	100	Delivery	High vaginal and perineal swabs	14%
Dawodu[112]	Nigeria	225	Delivery	Lower vaginal swabs	20%
Onile[113]	Nigeria:	388	N/A	Post and lat vaginal	19%
Schmidt[114]	Ethiopia	200	Post-partum	Vaginal and rectal swabs	9%
De Steenwinkel[115]	Mozambique	113	35-37 weeks'	Rectovaginal	2%

1.4.2 Maternal Characteristics as Risk factors for GBS Colonisation

Risk factors for maternal GBS colonisation relate to demographics, behaviour and co-morbidities; the risk factors that have been examined are summarised in Table 3. It is important to differentiate risk factors for maternal GBS colonisation from those factors associated with early onset neonatal GBS disease, for which maternal characteristics have been more consistently described, relating to ethnicity (higher risk in African-American ethnicity) and maternal age (higher risk in younger mothers),[51] and discussed further in the section on adverse perinatal outcomes (page 43).

Demographics and socio-economics

There are inconsistent study findings for risk factors for maternal GBS colonisation. Some studies have reported higher maternal GBS colonisation in younger age groups,[116,117] others describe an increase with maternal age, after adjusting for parity.[118] In contrast, the largest study (124747 women), a retrospective case-control study from health care centres across the USA published in 2005, did not find any association with age after adjustment.[119] Differences in findings could be due to chance, particularly in the smaller studies, as well as study design (cohort or case control) and differing analyses (univariable or multivariable). Multivariable analyses account for potential confounding of age by parity, as lower parity has been reported to be associated with increased maternal GBS colonisation.[117,118]

Ethnicity has been reported as a risk factor for maternal GBS colonisation, as well as neonatal early onset GBS disease. Studies from the United States have considered this in the most detail, and report that African-American ethnicity is associated with increased maternal GBS colonisation compared to Caucasian ethnicity, which in turn is associated with higher maternal GBS colonisation than seen in Hispanic women.[116 118 120] The differences are not straightforward, however, as the largest study from the USA differentiated between New-York Hispanics (mainly Caribbean origin) and non-New York Hispanics (mainly Mexican

origin); and reported that the former had the highest odds of maternal GBS colonisation, the latter had the lowest odds of maternal GBS colonisation.[118] Whilst genetic associations are therefore possible, it is also plausible that ethnicity is confounded by another exposure.

Occupation has been considered as a risk factor for maternal GBS colonisation, in the context of health care workers, on the basis that they may have higher exposure to GBS from patient contact. The largest, but retrospective, study from the USA found that the odds of maternal GBS colonisation were increased in health care professionals compared to non-health care professionals,[119] although one limitation of this study was the inability to distinguish between mothers who were not colonised, and mothers who were not screened; it could be that health care workers were more likely to be screened, and were therefore over-represented in terms of maternal GBS colonisation.

Geographic variation in maternal GBS colonisation has been reported in many studies. The prevalence reported between sites in a study in Zimbabwe varied from rural (67%) compared to urban (48%) areas.[94] In studies from the USA[119] and Korea maternal GBS colonisation prevalence differs by health facility.[117] Socio-economic factors could explain some of this variation; in the two largest studies from the USA, higher education was associated with increased odds of GBS maternal colonisation.[118,119] In another two studies, however, education was not associated with GBS maternal colonisation.[94,117] These studies were both smaller, and may have been underpowered to find this association, or had a more homogeneous population in terms of educational standard; they were also from very different regions, in Korea and Zimbabwe.[117] [94] Other socio-economic risk factors that have been studied include income; this was considered by two studies, the large USA study,[119] and the study from Zimbabwe.[94] The USA study reported that the highest income quintile had the highest odds for maternal GBS colonisation (OR 1.29, 95%CI 1.20-1.39).[119] In contrast the study from Zimbabwe,[94] reported that women with an employed husband were less likely to

be colonised than a woman with an unemployed husband, but 95% confidence intervals were overlapping, and this could have been due to chance.[94]

Behaviours

Behaviours have also been considered as risk factors for maternal GBS colonisation, including frequency of sexual intercourse, [121] smoking and alcohol consumption. Alcohol has not been reported to be associated with maternal GBS colonisation,[117-120] but smoking has been associated with around 10% lower odds of maternal GBS colonisation,[118,119] although not in every study.[117] For sexual intercourse frequency, there is only increased risk of maternal GBS colonisation with extreme increases in sexual activity, both in terms of frequent intercourse and multiple partners.[118]

Co-morbidities

Risk factors for maternal GBS colonisation may also include co-morbidities; maternal diabetes was reported to be associated with increased maternal GBS colonisation,[122] but this finding has not been reproduced by others.[119,120,123] In contrast most studies which have looked for an association with body mass index (BMI) or obesity have reported higher maternal BMI to be associated with increased maternal GBS colonisation. This finding has been reported in studies from the United States,[119,120] and also in a study based in the Reunion Islands in the Indian Ocean.[123]

HIV is an important co-morbidity in sub-Saharan Africa and a potentially important risk factor for maternal GBS colonisation. One study from Malawi specifically addressed the relationship between HIV infection and maternal GBS colonisation, recruiting 1857 pregnant women (21.7% HIV positive) at a tertiary referral hospital.[124] Maternal GBS colonisation did not differ by HIV status, but there was some evidence that GBS colonisation was increased among HIV-positive women with higher CD4 counts, and lower in HIV positive women with lower CD4

counts.[124] A post-hoc analysis separating women recently diagnosed with HIV (used as a proxy for not being on co-trimoxazole prophylaxis) from those previously diagnosed found the same trend in both groups. However, in studies from Zimbabwe,[94] and the USA,[120] no association between maternal GBS colonisation and HIV status was detected, and in a study of HIV infected mothers in Brazil there was no association between maternal GBS colonisation status and HIV viral load.[125] A study from South Africa reported in fact lower prevalence of maternal GBS colonisation in HIV infected mothers compared to HIV uninfected mothers (17% vs 23%; P = 0.0002).[30] The reasons for the conflicting findings are unclear, but could be due to differing therapies in different regions; more women may be on effective anti-retrovirals in some settings, reducing the need for antibiotics to treat concurrent infections, or for co-trimoxazole prophylaxis, which could reduce GBS maternal colonisation in HIV infected mothers in some settings.

Other exposures

Maternal GBS colonisation could also be associated with proximity to cattle,[126] and/or gutting fish, since GBS has been isolated from both of these sources.[127] This would, however, assume ongoing transmission of GBS between species. There are case reports showing GBS transmission between humans and cattle,[126] but it is difficult to know whether this is an important component of transmission; a study from Korea considered whether consumption of either raw or cooked fish, and milk, was associated with maternal GBS colonisation, but did not find any association.[117]

Vaginal microbiome

Differences in maternal characteristics and exposures and the incidence risk of GBS are thought to relate to differences in the vaginal microbiome. Briefly, vaginal acidity is important for the maintenance of vaginal health; a more acid environment reduces the likelihood of infection from pathogenic bacteria such as *Escherichia coli* (optimum pH is 6.5

but grows between pH 4.4 and 7.8), *Gardnerella vaginitis*, fungi (Candida sp.) and viruses (such as HIV). The maintenance of a pH of 3.5-4.5 is attributed largely to fermentation taking place between the glycogen-containing epithelial cells and the cervical mucus,[128] by Lactobacilli sp., first described in 1892.[129-131]

Since then our understanding of the vaginal microbiome has increased, most recently with the advance of molecular techniques. Although Lactobacilli are not essential for a healthy vaginal microbiome,[132] and may commonly coexist with yeasts,[133] patterns of the microbiome that have been identified tend to differentiate between "normal flora" and "bacterial vaginosus (BV) type flora", which is associated with increased discharge, higher pH (~pH5.5) and pathogenic bacteria; although this is increasingly being challenged. A study of pregnant women from the USA in 1993 reported the most commonly isolated organisms associated with BV were: *Gardnerella vaginalis*, *Prevotella bivia/disiens, Bacteroides ureolyticus, Prevotella corporis/Bacteroides levii, Fusobacterium nucleatum,* Mobiluncus sp., *Peptostreptococcus prevotii, Peptostreptococcus tetradius, Peptostreptococcus anaerobius, viridans streptococci, Ureaplasma urealyticum*, and *Mycoplasma hominis* ($p<0.05$ for each).[134]

A more recent study (2011), of 396 asymptomatic North American women, used 16S RNA techniques to identify bacterial species in the vaginal microbiome and identified five community states. Community state I, II, III and V were all predominantly Lactobacilli sp. (*L. crispatus* predominant in community state I*, L. gasseri* in community state II, *Lactobacillus iners* in community state III, or *L. jensenii* in community state V), but community state IV had a lower proportion of lactic acid producing bacteria and higher proportions of strictly anaerobic bacteria. These findings are supported by others, [135] [136,137] with the sub-division of type IV into two divisions; IV-A and IV-B.[138] This study also reported that the incidence of vaginal communities in which Lactobacilli were not dominant was higher in African-American

women (33%) compared to Caucasian women (7%).[139] The authors suggested that these differences were important clinically; there is no one core, healthy microbiome, but several different vaginal microbiomes, which may reflect positive health states in different ethnicities. [139,140]

Studies of the vaginal microbiome have largely been undertaken on healthy, non-pregnant women. However, one recent study has used molecular techniques to study the vaginal microbiome in pregnant women without complications (n=22), compared to non-pregnant women (n=32). This longitudinal study found that the pregnant women had more stable microbiomes compared to non-pregnant women, and community state IV was less common in pregnancy, with more *Lactobacillus vaginalis, L. crispatus, L. gasseri* and *L. jensenii*.[138] The authors suggested that this stability and predominance of *Lactobacilli sp.* was an adaptation of the host and microbial population to maximise reproductive fitness. This may be true, but the study only included women with no medical, surgical or obstetric complications, who delivered at term, which may have introduced selection bias by excluding women who are more likely to have a more diverse microbiome (community state IV).

The vaginal microbiome community state with which *Streptococcus agalactiae* is most commonly identified is not completely clear. In older studies, GBS was not found to differ in frequency in women with BV or women without.[134] In more recent studies, *Streptococcus* sp. appears to be present both in community states where there are otherwise predominantly lactobacilli,[136] which may be able to out-compete GBS in binding to host epithelial receptors,[141] and, perhaps more frequently, in more diverse community states (community state IV).[139] This would concur with the more frequent isolation of GBS from African-American women.

Summary of risk factors for maternal GBS colonisation

The exposures associated with maternal GBS colonisation at delivery are clearly complex, and result in ranges in the prevalence of maternal GBS colonisation reported in the literature. Conflicts in terms of risk factors for maternal GBS colonisation may relate to methodologies, chance, selection bias or residual confounding, as well as true variation in populations.

1.4.3 Maternal GBS Colonising Serotypes

The GBS serotypes colonising mothers were recently reviewed worldwide; in Europe, the Middle East, Africa, Australia and Asia, GBS serotype III predominated whilst in the Americas serotype Ia was predominant.[142] More data have been published since then, including a study of 551 GBS isolates in South Africa which reported that serotype III was the most common (37%), then Ia (30%), with other serotypes II (11%), V (10%), Ib (7%) and IV (4%) also present.[143] In contrast, a recent study from the Thai-Burmese border, a region from where data on GBS colonisation and disease are extremely limited, reported that serotype II was the most commonly isolated GBS serotype, and serotype VI, rarely found elsewhere, was the third most commonly isolated GBS serotype.[144]

The variation in maternal colonising GBS serotypes worldwide has been suggested as one reason for variation in neonatal GBS disease incidence, and an explanation for the strikingly little GBS disease in the WHO multi-site study of invasive disease in infants in the 1990s,[46] particularly as there was evidence of maternal GBS colonisation in The Gambia at that time. Although relatively small, this study of maternal GBS colonisation in The Gambia reported serotype V as the most frequently GBS serotype isolated (12/30, 38%), then serotype II, (8/30, 28%), serotype I (6/30, 19%), serotype III (2/30, 6%), and serotype IV (1/30, 3%).[110] This differed to the GBS serotype distribution colonising mothers in the United States at that time, where Serotypes Ia and III, were most commonly being reported.[142]

The data on MLST types are far more limited in maternal GBS colonisation studies, particularly with respect to clonal complexes, which have relatively recently been defined.[76,145] Capsular serotypes for GBS are not usually specific to particular clonal complexes, and there is considerable recombination within GBS strains. However, clonal complex 17 is less diverse than other clonal complexes, (CC-1, CC-10, CC-19, CC-23 and CC-26), with far less recombination,[76] and has been consistently reported as serotype III.

Table 3 Summary of studies looking at risk factors for increased maternal GBS colonisation

Author	**Location**	**Year**	**Sample**	**Age**	Parity	**Ethnicity**	**Socio-economic (education, income, social)**	**Behaviours (smoking, sex)**	**Co-morbidities (BMI, diabetes, HIV)**
Anthony[116]	3 clinics, California, USA	1978	382 pregnant women	Highest in 13 to 19 year age group ($p<0.05$)	Gravida $\geq$ 4 > than gravida 1-3 (chi2 <0.05)	Caucasian > Black > other non-Caucasian > Mexican Hispanic	N/A	N/A	N/A
Regan[118]	7 medical centres across USA	1991	7742	Higher over 20 years ($p <0.001$)	Low parity > high parity after adjusting for age	New York Hispanics (OR 1.18) > blacks (OR 1.00)> whites (OR 0.69)> non New York Hispanics (OR 0.46), $p<0.001$	OR 0.70 for eight additional years schooling, $p = 0.019$; OR 0.87 for additional 10 cigs/day, $p = 0.015$; OR 1.38 for living alone $p=0.01$	OR 0.83 for 5 yrs additional sexual experience $p = 0.005$	N/A
Shah[120]	Urban public hospital, California, USA	2011	90 HIV +ve and 1947 HIV-ve pregnant women	No association	N/A	Hispanic (OR 1); Asian (OR 1.11 (0.83-1.48) White (OR 1.89, 95% CI 1.30-2.75), and Black (OR 1.78, 95% CI 1.32-2.41)	N/A	No association	For obesity: OR 1.53 (95% CI1.13-2.07); no association with diabetes or HIV
Ramos[122]	Jacksonville, USA	1997	405 pregnant women	N/A	N/A	N/A	N/A	N/A	Diabetes: OR 3.1 (1.8- 5.2)
Stapleton[119]	Health care facilities offering screening in USA	2005	124727 pregnant women; 40459 colonised 84268 not colonised	No association	No association	Black (OR 1.54, 1.36-1.74), Hispanic women (OR 0.88, 0.80-0.96)	Some high school education (OR 1.21, 1.05-1.40), high school (OR 1.35, 1.16-1.56); highest income quintile (OR 1.29, 1.20-1.39); No association with marital status	10 cigarettes per day (OR 0.90, 0.83-0.97)	Overweight (OR 1.07, 1.01-1.12), obesity (OR 1.20, 1.13-1.28), severe obesity (OR 1.45, 1.28-1.63); no association with diabetes

Mavenyengwa[94]	Zimbabwe	2010	1037 pregnant women	No association	No association	N/A	unemployed > employed husband (p=0.05); unmarried > married (p = 0.06) Education no association	N/A	No association with HIV
Najmi[146]	Pakistan	2013	405 pregnant women						Increasing BMI OR 0.91; 95% CI: 0.08-1.00
Cutland[23]	South Africa	2012	1346 HIV +ve 1346 HIV-ve	N/A	N/A	N/A	N/A	N/A	HIV+ ve 17% vs HIV –ve 23%; *P* = 0.0002)
Gray[124]	Malawi	2012	1857 pregnant women	N/A	N/A	N/A	N/A	N/A	HIV+ve 19.4% vs 21.7% p=0.32
Linhares[147]	Ceará, Brazil; tertiary referral	2011	213 pregnant women	N/A	N/A	White skin colour associated with lower GBS colonisation (0.45>OR>0.94, p=0.01)	N/A	N/A	N/A
Kim, E. J.[117]	4 hospitals, Korea	2011	2644 pregnant women	<25yrs OR 1.83; 25-29 yrs OR 0.58; 30-34 yrs OR 0.76; 35+ yrs OR 1	first pregnancy (OR, 2.32; 95% CI, 1.12-4.81)	No association	No association with education, or income	No association with smoking	N/A
Dahan Saal[123]	Southern Reunion maternity	2011	17430 pregnant women	N/A	No association	Non- Reunion (OR: 1.29: 1.05–1.57; mainland France (OR: 0.82, 0.69–0.97)	N/A	N/A	Obese (OR: 1.19, 1.03–1.18); underweight (OR:0.81, 0.69–0. 95); no association with diabetes
Hakansson[148]	Sweden; national cohort	2008	1569 pregnant women	No association	No association	N/A	N/A	No association	N/A

** N/A = non-applicable (not investigated in the study)

1.4.4 GBS Transmission

Invasive early neonatal GBS disease (isolation of the organism from a usually sterile site, primarily blood or cerebrospinal fluid) is thought to arise from ascending infection in utero, acquisition during passage in the maternal birth canal. There is evidence that GBS can pass through intact amniotic membranes,[38,149] while haematogenous spread (from mother to foetus) has been less commonly reported.[150] Late-onset neonatal sepsis has been hypothesised to have a more varied environmental origin. In three studies in different countries in sub-Saharan Africa, the prevalence of GBS neonatal surface colonisation after birth (born to mothers colonised with GBS) was 62% in Zimbabwe,[94] 55% in South Africa,[30] and 39% in Tanzania.[109] Risk factors for neonatal colonisation are difficult to determine as most studies are not powered to look for these associations, as they would require a very large study. However, a study in Tanzania suggested that prolonged duration of labour (>12hrs) increased neonatal surface GBS colonisation.[151]

Bacterial transmission events can now be studied using whole genome sequencing (WGS). This can be undertaken using different methods, including description of differences in single nucleotide polymorphisms (SNPs) determined after mapping to a reference genome. This has been used, for example, to show that most cases of *Clostridium difficile* infection are acquired in the community, rather than as nosocomial infections.[152] However, these methods have not previously been used to examine vertical transmission of GBS from mothers to neonates, either in terms of surface colonisation, or invasive disease.

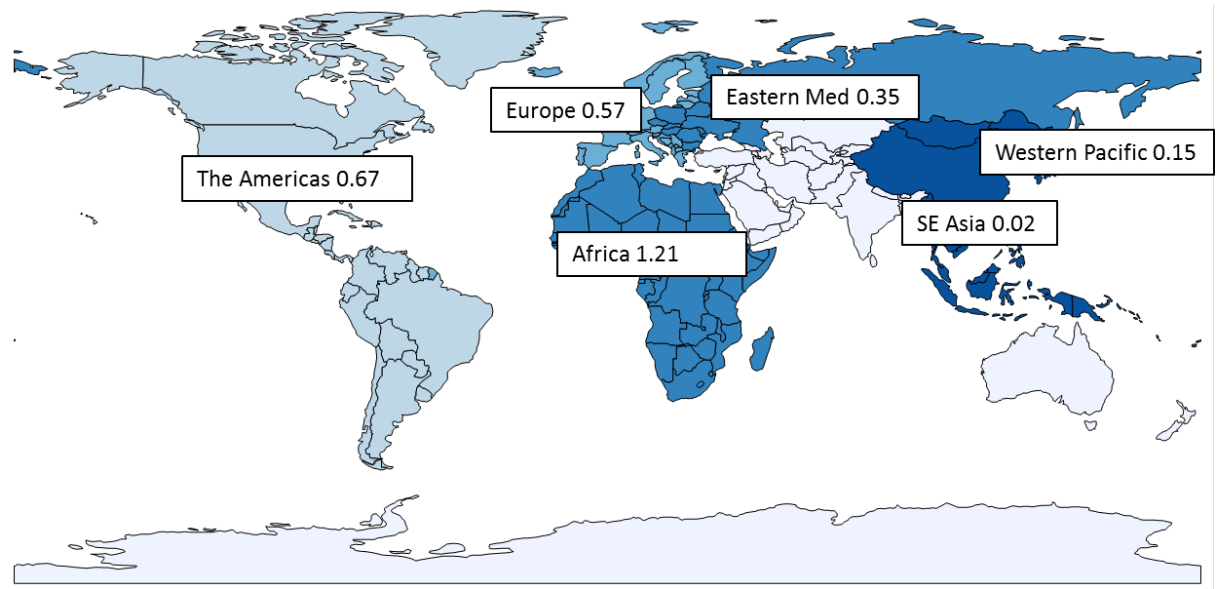
1.4.5 Adverse perinatal outcomes

Neonatal sepsis and meningitis

The incidence of GBS disease in young infants (to 90 days) in a recent systematic review and meta-analysis was highest in Africa, and lowest in Asia (Figure 6).[153] The pooled incidence risk

of GBS disease in infants <90 days worldwide, was 0·53/1000 live births (95% CI 0·44 to 0·62).[153] Data meeting the inclusion criteria from Africa were limited,[30,47,48,154] and heterogeneous (I^2=82·9%) with an overall estimated of incidence of 1·21/1000 live births (95%CI 0·50 to 1·91), considerably higher than any other region, although with considerable uncertainty.[153] In the same study, mortality associated with GBS disease was 0.10/1000 live births (95% CI 0·07 to 0·12).[153] Data from Africa were again limited (three studies),[48,60,155] but mortality was over twice as high, at 0.22/1000 live births (95%CI 0.12-0.32).[153] Mortality associated with GBS disease is also higher in preterm neonates.[156,157] For survivors, in both term and preterm neonates, subsequent neurodevelopmental impairment is high; moderate to severe neurodevelopmental impairment occurs in around a half of those surviving GBS meningitis.[158]

Figure 6 Estimated GBS incidence risk in infants <3 months per 1000 births by region[153]



In resource rich settings it is well established that the majority of GBS disease occurs early in the neonatal period, when sepsis is more common, with more cases of meningitis later in the neonatal period (around one third).[34,98] Although early onset disease is described as including days 0-6 days of life, most early onset GBS disease occurs in the first 24 hours;[98] 90% of infections were reported to occur in the first 12h of life in a study from the UK and Ireland.[159]

In contrast, studies in sub-Saharan Africa report that GBS disease is more equally divided between the early and late neonatal periods.[48,49] The difference is important, because of the differing modes of infection (from maternal colonisation or other sources), and the potential for prevention of early onset disease through intra-partum antibiotic prophylaxis (see prevention of neonatal disease, page 49). However, in facility based studies in resource-poor settings there is greater potential for selection bias, as accessing care may be difficult, or too expensive,[18] or travel time may mean sick neonates die before arrival. This makes it more difficult to know if there are fewer neonates with GBS disease in the first few days of life accessing care, or if there are true regional differences. If there are true differences, these could be due to increased host vulnerability in preterm neonates in sub-Saharan Africa, increasing the proportion at risk of late-onset disease, or to differences in intravenous antibiotic use at delivery, which would reduce detection of early onset GBS disease in sub-Saharan Africa.

The risk of neonatal GBS disease depends on exposure to GBS, for early GBS neonatal disease this is exposure to maternal GBS colonisation.[78] Risk factors for neonatal GBS disease could therefore reflect risk factors for maternal GBS colonisation. However, the increased risk of neonatal GBS disease in neonates born to African-American mothers in the United States may not only be accounted for by increased prevalence of maternal GBS colonisation, but also a higher inoculum due to denser GBS colonisation. Dense maternal GBS colonisation can be indicated by maternal GBS bacteriuria, which has been associated with increased risk of neonatal GBS disease. Other risk factor increasing exposure to GBS, and hence incidence of GBS disease are prolonged rupture of membranes ($\geq$18h),[157,160] prolonged labour, obstetric procedures and examinations,[78] as well as maternal chorioamnionitis (indicated by intra-partum fever $\geq 38.0^{0}$C).[157,160]

Neonates are more susceptible to GBS infection if they have lower levels of anti-capsular type-specific antibody, either because their mothers have low circulating levels, (which can be indicated by having had a previous neonate with GBS disease),[160] or because they were born preterm. Maternal placental IgG transfer is limited before 34 weeks' gestation, and so less protection is conferred to the neonate. However the relationship is complicated by the fact that GBS infection may also cause preterm delivery through inflammation, as a result of ascending infection.[51,161] There are other factors which may decrease immunological protection; HIV infection reduces immunity to invasive bacterial disease, and in a study of neonatal infection in HIV unexposed, HIV exposed-uninfected and HIV exposed-infected neonates in South Africa, it was reported that HIV exposed-infected neonates were at greater risk of early and late onset sepsis compared to HIV exposed uninfected neonates (134 cases/1000 live births compared to 21.5 case/1000 live births; $p < 0.0001$), as well as HIV uninfected neonates.[162]

Treatment of GBS in infants

Current WHO guidelines for first line antibiotic treatment of possible severe bacterial infection, usually diagnosed on the basis of the presence of clinical signs,[5] are ampicillin (or penicillin) with gentamicin.[25] Group B Streptococci are sensitive to penicillin treatment, which may be synergistic with gentamicin.[163] However, antibiotic susceptibility testing is important for both treatment and intrapartum antimicrobial prophylaxis. Although prevalence of penicillin non-susceptible GBS (PNSGBS) is currently very low, it is essential to monitor, as penicillin remains the first line antimicrobial for both treatment and intra-partum antimicrobial prophylaxis (IAP). Susceptibility testing for PNSGBS by disc diffusion testing can have low specificity,[164] and reports of PNSGBS from the United States and Japan suggest reduced susceptibility to penicillin is due to substitutions in penicillin binding proteins (PBP2x[165-168] and PBP2B).[169] In addition, resistance to macrolides, such as erythromycin, is

increasing. This is particularly important as cross-resistance to lincosamides such as clindamycin can be induced, [170] and clindamycin is used as a second line antimicrobial for IAP in people with penicillin allergy. Resistance to macrolides is usually conferred by either erm genes (B, A/TR or C), resistance phenotype macrolide–lincosamide–streptogramin (MLSB); or mef genes which usually just confer resistance to macrolides (M phenotype) as either mef A or mef E.[171] Resistance to tetracycline has been described in the context of the emergence of GBS,[76] with the acquisition of TetM on transposon Tn916.[76]

GBS types

The risk of GBS disease may depend on the GBS serotype which the neonate is exposed to. In the United States and Europe serotype III, MLST type-17 (within clonal complex 17) has been identified as a hyper virulent clone, associated with late onset GBS disease, and meningitis.[34,87] [167,172] [173] There are limited data from Africa and Asia on invasive GBS serotypes causing disease, and no published data on MLST types. The recent review of young infant GBS disease identified only two studies with serotype data in Africa, [48,143] which reported that serotypes Ia, and III caused most disease, with some serotype V and serotype II.[153] There were approximately equal proportions of early onset GBS disease associated with serotype Ia as serotype III, whilst serotype III caused a higher proportion of late onset neonatal GBS disease.[174]

Stillbirth

GBS has been reported as a cause of stillbirth in Europe and the USA, but the incidence of stillbirth associated with GBS is unknown even in high-resource settings. Similarly to early-onset sepsis, most stillbirths resulting from invasive GBS disease are thought to be due to ascending infection, with GBS passing through (intact) amniotic membranes.[149,175-177]

Data on GBS as a cause of stillbirth in sub-Saharan Africa are very limited. There is one published case report, of a mother from West Africa, who delivered in the United States. The case report describes the investigations required to reach the final diagnosis of GBS disease (full autopsy and histology), and without which the diagnosis would have been missed.[176]

A case-control study from Zimbabwe examined stillbirth in the context of maternal chorioamnionitis (inflammation of the fetal membranes due to infection), identifying maternal chorioamnionitis more frequently in stillbirths (n=66) than in controls (n=66) (OR 8.5, 95% C.I. 4.0-18).[178] In addition, cultures were taken from the maternal cervix, placental disk, umbilical cord, the throat and ear in well new-borns and from these sites and the internal organs in stillbirths. GBS was cultured from any site in three well new-borns and eight stillbirths.[178]

Preterm birth and low birth weight

The association between preterm delivery and maternal GBS colonisation has recently been systematically reviewed. Results differed according to study design, and were limited in number. A pooled estimate of the odds ratio for preterm delivery with maternal GBS colonisation was 1.06 (95% CI 0.95-1.19) in 11 cohort studies; 1.75 (95% CI 1.43-2.14) in five cross-sectional studies and 1.59 (95% CI 1.03-2.44) in four case control-studies.[179] The differences in the findings may relate to the study design, and the time GBS screening was undertaken. In cohort studies GBS screening was antenatal, in cross-sectional and case-control studies it was at delivery. Screening for GBS earlier in pregnancy is less predictive of maternal GBS colonisation at delivery than sampling later in pregnancy, at 35-37 wks,[180] so this could introduce misclassification and bias the result towards the null hypothesis of no association. However, the studies were small, especially for case-control studies (48 GBS positive and 211 GBS negative in the meta-analysis), and only three studies adjusted for confounding factors. In addition, knowledge of GBS status may have introduced information

or recruitment bias, particularly in case-control studies. It has also been suggested that preterm delivery is only seen in the context of dense GBS colonisation, indicated by asymptomatic GBS bacteriuria,[181] and subsequently reported in a large study in the United States where only mothers heavily GBS colonised had higher odds of delivering a preterm infant (OR 1.5, 95% CI 1.1- 1.9).[182]

1.4.6 Prevention of GBS neonatal disease

The mainstay of prevention of GBS perinatal disease has been intra-partum antimicrobial prophylaxis (IAP) with intravenous penicillin-G or ampicillin administered to the mother at least four hours prior to delivery, although alternatives (usually clindamycin) are used in the case of penicillin allergy.[183] There are two approaches to determining to whom IAP should be offered; either antenatal screening of mothers for GBS colonisation (35-37 weeks' gestation) to detect mothers who are GBS colonised, or using risk factors. The presence of any one risk factor for neonatal GBS disease (maternal fever intra-partum ($\geq$38.0^{o}C), preterm delivery (<37 weeks), or prolonged rupture of membranes ($\geq$18 hours)) is used to identify those mostly likely to benefit from IAP. In addition, women with GBS bacteriuria during their current pregnancy,[184] or who previously gave birth to an infant with early-onset GBS disease, are offered IAP.[185] Intra-partum antibiotic prophylaxis has reduced early onset GBS disease by over 80% in the USA.[105] Since 2002, this has been based on screening for maternal GBS colonisation at 35-37 weeks' gestation as USA data suggested that screening was more effective in reducing early onset GBS disease.[185] European countries, however, continue to use either screening or risk-based methods.[186]

Maternal vaccines are an increasingly attractive strategy to prevent specific neonatal infections, including GBS, as discussed previously (page 21). A GBS vaccine may have the additional benefit, compared to IAP, that it may prevent ascending GBS infection, and potentially help reduce preterm birth and stillbirth, as well as protect neonates from late

onset sepsis. In addition, in sub-Saharan Africa vaccination may be more feasible than a GBS screening programme, or even a risk-factor based IAP approach, given the high proportion of home deliveries.[24] There are considerable challenges, however, in developing an immunogenic vaccine which is considered safe to trial and use in pregnancy, as well as determining the time at which it should be given. The most advanced GBS vaccine candidate, a serotype specific vaccine including serotypes Ia, Ib and III, is about to enter phase three trials.[187] In order to determine the need and likely effectiveness of such a vaccine, more data on the burden and disease causing GBS serotypes in sub-Saharan Africa are urgently required.

1.5 Summary of Study Rationale and objectives

The burden of mortality and morbidity in children in sub-Saharan Africa is highest in the perinatal-period. Group B Streptococcus has emerged as an important perinatal and neonatal pathogen in resource-rich countries, and is now doing so in sub-Saharan Africa. However, data on GBS in sub-Saharan Africa are lacking, both in terms of maternal and new-born GBS colonisation, risk factors, transmission and the contribution of GBS to the high burden of perinatal deaths, including stillbirths. Data on serotypes and MLST types are also lacking, both from maternal GBS colonisation studies and from studies of invasive perinatal and neonatal GBS disease. It is unclear whether a serotype-specific vaccine would protect against GBS disease in this region. The aim of this study is to provide the first comprehensive description of the clinical and molecular epidemiology of GBS: maternal colonisation and perinatal outcomes in Kenya.

This study will answer the following specific questions concerning GBS in Coastal Kenya:

Maternal GBS Colonisation

1. What is the prevalence of maternal GBS colonisation at delivery?
2. What are the risk factors for maternal GBS colonisation at delivery?
3. What are the serotypes and MLST types of GBS isolates colonising mothers at delivery?

Maternal GBS Colonisation and Perinatal outcomes

4. Is maternal GBS colonisation associated with gestation at delivery?
5. Is maternal GBS colonisation associated with birth weight at delivery?
6. Is maternal GBS colonisation associated with possible severe bacterial infection?
7. Is maternal GBS colonisation associated with stillbirth?

Invasive GBS disease

8. Is invasive GBS associated with stillbirth?
9. What is the incidence of invasive GBS disease in neonates?
10. What are the clinical characteristics of invasive GBS disease in neonates?
11. What are the serotypes and MLST types causing perinatal and neonatal disease?

Vertical transmission of GBS

12. What is the prevalence of neonatal GBS surface colonisation after delivery?
13. What is the molecular evidence for vertical transmission of GBS resulting in neonatal surface colonisation?
14. What is the molecular evidence for vertical transmission of GBS resulting in perinatal disease?

Chapter 2: Methods

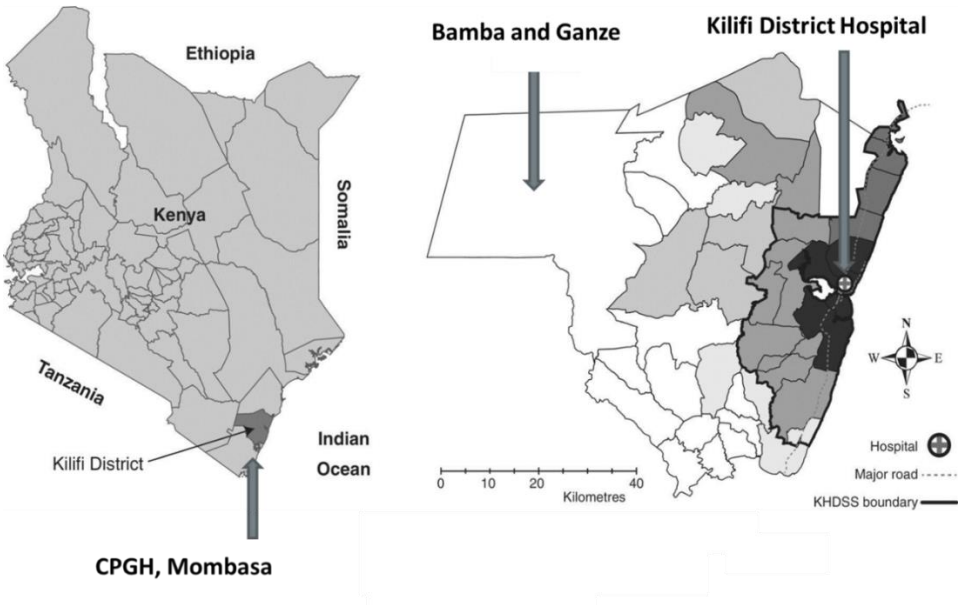
2.1 Study design

A set of studies were designed to meet the overall aim and the specific questions: a cohort study of maternal GBS colonisation at delivery and adverse perinatal outcomes, a sub-study of transmission of colonisation of GBS from mothers to new-borns, surveillance of neonatal invasive GBS disease, and a nested case control study to examine invasive GBS disease in stillbirths.

2.1.1 Setting

The study sites were government funded health facilities, run by the Ministry of Medical Services in Kenya. They included an urban provincial hospital, Coast Provincial General Hospital, Mombasa; a semi-rural district hospital, Kilifi District Hospital; and rural health facilities, Bamba sub-district hospital and Ganze health facility (see Figure 7). Bamba and Ganze health facilities are close in geographic location and serve similar populations, and were therefore combined as the "rural" site in analyses.

Figure 7 Study sites in Coast Province, Kenya[2]*



*Reprinted (minor amendment to show sites) from International Journal of Epidemiology, 41 (3), Scott, J. A., Bauni, E., Moisi, J. C., Ojal, J., Gatakaa, H., Nyundo, C., Molyneux, C. S., Kombe, F., Tsofa, B., Marsh, K., Peshu, N., Williams, T. N., Profile: The Kilifi Health and Demographic Surveillance System (KHDSS), p650-7., Copyright (2012), with permission from Oxford University Press.

Coast Provincial General Hospital (CPGH)

This hospital is situated in Mombasa on the Kenyan Coast, the second largest city in Kenya (population 1.2 million), with 44% of the population living under the urban poverty line.[188] CPGH is the second largest hospital in Kenya, a teaching hospital and referral centre for Coast Province. It includes three maternity wards, which provide comprehensive emergency obstetric care. This includes basic procedures for emergency obstetric care (including antibiotics, anticonvulsants/sedatives, manual removal of the placenta, removal of retained products, and assisted vaginal delivery), in addition to blood transfusion and caesarean section. There are two full-time obstetrician-gynaecologists at the hospital, and medical officers providing continuous on-call cover, as well as nursing staff. There are around 10000 deliveries per year.

Kilifi District Hospital (KDH)

This hospital is based in Kilifi County, a rural county with a population of 540000 in 2009, covering an area of 12246 km^2 where the main economy is subsistence farming.[2] It is estimated that 72% of the population live below the poverty line.[188] A part of Kilifi County is included in detailed health and demographic surveillance (KHDSS) from which accurate population data are available.[2] KDH is a referral centre for peripheral health facilities and provides comprehensive obstetric care. There is one full time obstetrician-gynaecologist at the hospital, with medical officers providing continuous on-call cover, and nursing staff. There are approximately 3000 deliveries per year.

Bamba Sub-District hospital

The sub-district hospital is based in Bamba, a division of Kilifi County, with a population of 36000. It is a rural area, with an economy based on subsistence farming and 83% of people live below the poverty line.[188] The sub-district hospital has 12 in-patient beds and it provides basic obstetric care. It is staffed by a clinical officer and nursing staff, with a weekly clinic by

a medical officer. There are approximately 400 mothers delivering at this sub-district hospital over one year.

Ganze health facility

This health facility is based in Ganze, a division of Kilifi District, with a population of 33000. It is a rural area, with an economy based on subsistence farming and 81% of the population live below the poverty line.[188] There are no inpatient beds, but it provides basic obstetric care. It is staffed by a clinical officer and nursing staff. There are approximately 250 mothers delivering at this health facility each year.

2.1.2 Participants

Women were eligible for inclusion if they were admitted for delivery at study sites at designated times of recruitment, and if written informed consent was given (included in the appendices, page 223). There were no additional exclusion criteria. Recruitment was originally planned to cover one calendar year at the urban (Coast Provincial General Hospital) and rural sites (Bamba sub-district hospital and Ganze health facility) and two years at the semi-rural site (Kilifi District Hospital). However, the period of recruitment was extended where possible to meet recruitment targets, as there was national strike action closing all government health facilities twice during the course of the study (September 2012 and December 2012-February 2013).

Women were recruited from Kilifi District Hospital between 1st August 2011 and 31st July 2013 for the cohort study, and recruitment was undertaken 24 hours a day, 7 days a week. For the sub-study of neonatal colonisation after delivery, recruitment was from within this cohort, from 1st May 2012 to 31st July 2013, between 5am and 4pm, to allow sampling within 6 hours of delivery by study staff. For the nested case-control study of invasive GBS in stillbirths, participants were recruited from the 1st May 2012 to the 1st October 2013. The

end date of this study was extended from 31st July to the 1st October 2013 as the calculated sample size had not been reached.

Women were recruited over a 48 hour period each week from Coast Provincial General Hospital between the 1st April 2012 and the 31st July 2013. This recruitment strategy was used to include women arriving in and out of normal working hours, and recruit over a one year period in case of seasonality. It was also for logistical reasons, including frequent (twice in a 24h period) transport of samples to the laboratories at KWTRP for processing.

Women were recruited for five days each week (Sunday to Thursday with 24hr recruitment) at Bamba sub-district health facility and Ganze health facility from 1st July 2012 to the 31st July 2013. This strategy was to include women delivering in and out of working hours, and meet the recruitment target over a year. It was also for logistical reasons, with a courier taking samples daily (Monday to Friday) to KEMRI-Wellcome Trust Research Laboratories.

2.1.3 Variables

What are the risk factors for maternal GBS colonisation at delivery?

The outcome of interest was maternal GBS colonisation. Potential confounders to be included *a priori* were maternal age and parity. Exposures of interest to be included were demographic factors (age) and indicators of socio-economic status (education, marital status, household SES); genetic factors (ethnicity), factors relating to pregnancy (parity, previous stillbirth), co-morbidities (anaemia, HIV infection, syphilis infection, under or over nutrition) and factors relating to GBS exposure (contact with cattle, gutting fish) or treatment (antibiotics within 4 weeks of delivery). In addition factors at delivery affecting maternal GBS colonisation status were considered (rupture of membranes $\geq$18h, vaginal examination prior to sample taking) as well as emergency-referral and obstetric complications. No effect modifiers were hypothesised *a priori*.

Is maternal GBS colonisation associated with gestation at delivery?

The exposure was maternal GBS colonisation at admission for delivery. The outcome of interest was gestation, categorised into quartiles. Effect modification between the exposure, and prolonged rupture of membranes (≥18h), urinary tract infection (measured by leukocytes and nitrites at delivery, +/- clinical symptoms) and maternal fever ≥38.0^{0}C were investigated, as factors increasing exposure to GBS either through dense colonisation or reduced barriers to infection. Confounders included *a priori* were maternal age, parity and the sex of the baby. Other confounders included in models included a minimum set determined by causal diagrams (directed acyclic graphs): indicators of demographic and socio-economic status (education, household SES), co-morbidities (HIV infection, nutritional status) and factors at delivery (obstetric complications, multiple delivery).

Is maternal GBS colonisation associated with birth weight at delivery?

The exposure was maternal GBS colonisation at admission for delivery. The outcome of interest was birth weight, categorised into quartiles. Effect modification between the exposure and prolonged rupture of membranes (≥18h), urinary tract infection (measured by leukocytes and nitrites at delivery, +/- clinical symptoms) and maternal fever ≥38.0^{0}C were investigated, as factors increasing exposure to GBS either through dense colonisation or reduced barriers to infection. Confounders included a priori were maternal age, parity and the sex of the baby. Other confounders included in models included a minimum set determined by causal diagrams (directed acyclic graphs): indicators of demographic and socio-economic status (education, household SES), co-morbidities (HIV infection, nutritional status) and factors at delivery (obstetric complications, multiple delivery).

Is maternal GBS colonisation associated with possible severe bacterial infection?

The exposure was maternal GBS colonisation at admission for delivery. The outcome of interest was possible severe bacterial infection. Effect modification between the exposure and prolonged rupture of membranes (≥18h), urinary tract infection (measured by leukocytes and nitrites at delivery, +/- clinical symptoms) and maternal fever ≥38.0^{0}C were investigated, as factors increasing exposure to GBS either through dense colonisation or reduced barriers to infection. Confounders included *a priori* were maternal age, parity and the sex of the baby. Other confounders included in models included a minimum set determined by causal diagrams (directed acyclic graphs): indicators of demographic and socio-economic status (education, household SES), co-morbidities (HIV infection, nutritional status) and factors at delivery (obstetric complications, multiple delivery).

Is maternal GBS colonisation associated with stillbirth?

The exposure was maternal GBS colonisation at admission for delivery. The outcome of interest was stillbirth. Effect modification between the exposure and prolonged rupture of membranes (≥18h), urinary tract infection (measured by leukocytes and nitrites at delivery, +/- clinical symptoms) and maternal fever ≥38.0^{0}C were investigated, as factors increasing exposure to GBS either through dense colonisation or reduced barriers to infection. Confounders included *a priori* were maternal age, parity and the sex of the baby. Other confounders included in models included a minimum set determined by causal diagrams (directed acyclic graphs): indicators of demographic and socio-economic status (education, household SES), co-morbidities (HIV infection, nutritional status) and factors at delivery (obstetric complications, multiple delivery).

What are the clinical characteristics of invasive GBS disease in neonates?

Cases were defined as neonates (admitted to Kilifi District Hospital) who had GBS isolated from either blood or cerebrospinal fluid. Clinical descriptive data included demographic details (age at admission, sex), disease type (sepsis, meningitis or mixed), and co-morbidities such as admission weight and HIV infection. Since 2007, HIV tests are offered to all paediatric admissions in line with national guidelines[189] and referred to the hospital comprehensive care clinic if required. Prior to 2007, systematic HIV screening was not national policy. However for children with severe invasive disease ethical approval was sought and obtained for a trained counsellor to visit households and offer voluntary counselling and testing to children previously admitted, using the KHDSS database of household locations. For those who were untraceable or who had died a stored blood sample was tested for HIV by PCR. Clinical outcome was included in terms of discharge or death.

2.1.4 Data collection and management

Clinical data were collected prospectively and systematically using standardised maternal admission records (MARs) across the three study sites (see appendices, page 241). The MARs were completed by fieldworkers (demographic and lifestyle data), nurses and clinicians (clinical data) and included standard demographics (age, location, education); lifestyle (cattle farming, gutting fish); antenatal history (parity, antibiotic use in pregnancy); medical history; investigations (HIV status, syphilis serology, haemoglobin and urine dipstick results); maternal anthropometry (height, weight, mid upper-arm circumference (using SECA 861 Electronic Flat Scales and standardised mid-upper arm circumference tapes); delivery data (mode of delivery, prolonged rupture of membranes ($\geq$18h), maternal temperature, maternal drug administration (including antibiotic use both at delivery and during pregnancy), complications; and perinatal outcomes (stillbirth, early neonatal death, hospital admission, birth-weight and gestation at delivery).

Neonatal admission data were also standardised, using existing surveillance platforms in paediatric care in KDH, and introducing standardised neonatal admission records (NAR) to CPGH. Neither Bamba sub-district Hospital nor Ganze health facility admit neonates, but transfer for admission to KDH for treatment was recorded. Accurate data were facilitated through training and use of digital birth scales (SECA baby scale 376) accurate to <5.0 grams. Clinical data from admission records were entered into databases designed for the study (MySQL/PHP in KDH; Epi Info in CPGH and Bamba and Ganze) by fieldworkers or (for Bamba and Ganze) a clinical officer. All data were checked from clinical records for accuracy in KDH and CPGH by study clinical officers after data entry, but not re-entered.

Data from KDH, CPGH and Bamba and Ganze were backed up on the secure server at KEMRI-Wellcome Trust Research Laboratories. Microbiological data were entered into existing databases (Filemaker) at the KEMRI-Wellcome Trust Research Laboratories. At the end of the study, data were extracted from all of these databases and linked using unique numerical study identifiers.

2.1.5 Procedures

Recto vaginal swab: These were undertaken at routine vaginal examination at admission for delivery, so that there was minimal discomfort associated with the procedure. Where possible the swab was taken prior to rupture of membranes.[190] A small cotton swab was used to wipe the lower third of the vaginal mucosa and then the inside surface mucosa of the anus,[191] according to standard operating procedures. The swab was placed into Amies transport medium with charcoal to maximise sensitivity.[107] Samples were stored in refrigerators and transported in cool containers to KEMRI-Wellcome Trust Research Programme (KWTRP) Laboratories to maximise sensitivity.[107]

Neonatal surface swabs: An external ear swab, swab of nares and umbilicus was undertaken with one swab on neonates within 6 hours of delivery. A small cotton swab was wiped around, inside the nares, the external ear canal and umbilicus (in that order). The swab was then placed into Amies transport medium with charcoal. Samples were stored in refrigerators and transported in cool containers to KWTRP laboratories.

Cord blood culture: Cord blood cultures for GBS infection were taken from the umbilical cord after cleaning with 70% ethanol. Blood samples were inoculated into a blood culture bottle (BACTEC PedsPlus, Becton Dickinson, UK). Samples were transported to KEMRI-Wellcome Trust Laboratories.

Lung aspirate: Using a sterile technique, needle aspiration of the stillborn lung (90^0 to the skin, inserted whilst drawing back into the right lung) was undertaken within 4 hours of delivery. Samples were transported to KEMRI-Wellcome Trust Laboratories with 30 minutes of sampling. If a delay in processing was unavoidable (between 00:00h and 06:00h) samples were refrigerated.

Neonatal blood culture: Peripheral blood cultures for infection were taken from neonates admitted to KDH paediatric ward. Blood was sampled with an aseptic technique and inoculated into blood culture bottles (BACTEC PedsPlus, Becton Dickinson, UK) as part of routine care.

Neonatal cerebrospinal fluid culture: Cerebrospinal fluid cultures for infection were taken from neonates admitted to KDH paediatric ward using aseptic technique as part of routine clinical care.

2.1.6 Microbiological methods

Conventional Microbiology

All samples were transported to KWTRP Laboratories for processing. These laboratories are externally assessed for quality, meeting UK NEQAS standards. The summary laboratory analysis plan, supported by detailed standard operating procedures, is included in the appendices, page 244.

Recto-vaginal swab and neonatal surface swabs: Swabs were registered on arrival to KEMRI-Wellcome Trust Research Laboratories (KWTRP). The swab was removed aseptically and placed in LIM broth, incubated at 35^0C-37^0C, 5-10% CO_2 for 18-24 hours. After incubation, it was sub-cultured with a sterile loop and inoculated onto blood agar. Further incubation at 35^0C-37^0C 5-10% CO_2 for 18-24 hours was the undertaken. After this time the plate was examined for possible GBS based on morphology and the presence of ß-haemolysis. If there was no growth, the blood agar plate was incubated for another 24h. Any possible GBS were identified based on the CAMP test and definitive grouping using Streptococcal grouping latex kit (PRO-LAB diagnostics, USA). GBS isolates were frozen in 1mL vials and stored at -80^0C.

Cord and neonatal blood culture: Blood cultures were processed using an automated blood-culture system (BACTEC 9050, Becton Dickinson, UK). Positive blood samples were cultured on standard mediums (Blood Agar and Chocolate Agar) using standard microbiological techniques. Antibiotic susceptibility testing was undertaken for isolated organisms, which were also frozen and stored.

Lung aspirate: Lung aspirate samples were registered on arrival to KEMRI-Wellcome Trust Research Laboratories and their processing prioritised. They were examined by gram stain, and inoculated onto media plates (Blood Agar, Chocolate Agar and MacConkey Agar, Colistin Nalidixic Agar, Anaerobic Agar) and incubated at 35^0C-37^0C. Aerobic plates were read at 24

hrs and if there was no growth they were incubated again, and examined at 48, 72 and 96 hours. Anaerobic plates were examined at 72h. In addition, 0.5mL of aspirate was inoculated into a BACTEC bottle and incubated in the automated blood culture system (BACTEC, BD, UK). If the blood culture bottle flagged positive subcultures were made on Blood and Chocolate Agar. Organisms were identified according to standard methods and antibiotic susceptibility testing undertaken for isolated organisms which were also frozen and stored.

Neonatal cerebrospinal fluid culture: These were registered on arrival to KEMRI-Wellcome Trust Research Laboratories and their processing prioritised. They were examined by gram stain, and inoculated onto media plates (Blood Agar, Chocolate Agar and MacConkey Agar, Colistin Nalidixic Agar, Anaerobic Agar) and incubated at 35^0C-37^0C. Aerobic plates were read at 24 hrs and if no growth were incubated again, and examined at 48, 72 and 96 hours. Anaerobic plates were examined at 72h.

Molecular microbiology

Preparation, DNA Extraction and sequencing

Frozen GBS isolates were shipped to the Microbiology Department, John Radcliffe Hospital, Oxford, UK, in two batches, following approval from the Ethical Review Committee, Nairobi (see Public Engagement and Ethics section, page 69). These isolates were stored at -80^0C prior to sub-culture and DNA extraction.

DNA extraction was undertaken with a commercial kit (QuickGene, Fujifilm, Tokyo, Japan) from a single colony cultured on a Columbia blood agar plate after incubation for 24-48 hours. Pools of 96 samples were submitted to the Wellcome Trust Centre for Human Genetics (Oxford University, UK) for high throughput sequencing using HiSeq2500, generating 150 base paired-end reads. Sequence reads were analysed and assembled using a pipeline developed specifically for bacterial genomes. Each isolate was mapped using to

the *Streptococcus agalactiae* reference genome; 2603V/R,[192] and DNA quality assessed looking at mapcalls to the reference genome (>60%). In order to confirm reproducibility of sequencing, 32 samples were sequenced twice. Those GBS isolates with low mapcalls were re-isolated, re-grouped and re-sequenced (if re-grouping confirmed the isolate as GBS).

Sequetyping and MLST typing

Serotype allocation methods were developed by Dr Anna Sheppard (Bioinformatics, University of Oxford) based on capsular gene sequences of the ten known GBS serotypes. For de novo assembly of each isolate, a blast database was generated and queried with the variable region of the capsular locus region for each serotype (corresponding to cpsG-cpsK for serotypes Ia, Ib and II-VII, and cpsR-cpsK for serotype VIII), as given for each capsular type in Table 4. MLST allocation was undertaken by Dr Adam Giess (Bioinformatics, University of Oxford) based on sequences of the seven housekeeper genes,[87] as conventionally used to allocate MLST type.

Method Validation

The first validation analyses concerned the time to processing for GBS swabs, which were transported to KWTRP Laboratories. The three sites were at different distances and time to processing was likely to be longer for the rural sites than the urban site, which was likely to be longer than the semi-rural site (the same site as the KWTRP laboratories). A descriptive analysis of the time to processing was undertaken according to whether GBS was isolated or not, by site. The Wilcoxon rank sum test was used to determine if there were differences in the two groups (GBS culture positive or negative) according to time to processing, as data were not normally distributed. In addition, a logistic regression analysis was undertaken to determine if the odds of GBS maternal colonisation were associated with the time taken to processing, allowing for clustering between sites, and for each site individually.

Serotype allocation was validated against 61 serotyped GBS isolates typed using conventional latex agglutination assays, and 60 previously serotyped GBS isolates using PCR based methods with capsular gene typing. Serotypes VIII and IX were rare in the reference isolates and two additional reference isolates of these serotypes were therefore obtained from the Statens Serum Institute. Agreement between serotype allocations based on capsular gene sequence from WGS was tested against that obtained from latex agglutination and PCR methods, with the kappa statistic. After validation, the GBS isolates from this study were then assigned a capsular type based on the WGS data.

Table 4 Capsular typing of *Streptococcus agalactiae*

Serotype	**Accession**	**Region**
Ia[193]	AB028896.2	6982-11695
Ib[194]	AB050723.1	2264-6880
II[35]	EF990365.1	1915-8221
III[195]	AF163833.1	6592-11193
IV[196]	AF355776.1	6417-11656
V[196]	AF349539.1	6400-12547
VI[196]	AF337958.1	6437-10913
VII[196]	AY376403.1	3403-8666
VIII[196]	AY375363.1	2971-7340
IX[85,197]*		

* Only a partial region of the capsular locus sequence has been described so the relevant region from the *de novo* assembly of the serotype IX type strain was extracted.

2.1.7 Study size

The sizes of the series of studies undertaken were determined by the following calculations:

Cohort study: Prevalence of maternal GBS colonisation and risk factors for maternal GBS colonisation

Assuming recruitment of 8000 mothers, (5500 from KDH, 2000 from CPGH and 500 from rural sites), over at least one calendar year at all sites (to account for any seasonal variation) and assuming a 20% prevalence of maternal GBS at delivery, the precision of the overall maternal prevalence estimate would be +/- 1% (α=0.05). With the same assumptions, the power of the study to detect risk factors associated with maternal GBS colonisation for odds ratios of 1.5, 2 and 4 (α=0.05), using data on maternal risk factors from retrospective pilot data from Kilifi District Hospital (2002-2006) was assessed to be >90% for all exposures apart from maternal syphilis infection, see Table 5.

Table 5 Power to detect maternal risk factors for GBS colonisation

Risk factor (exposure)	Mothers (%) with risk factor at delivery*	OR 1.5	OR 2	OR 4
Maternal age over 35 years	8.3	>90	>90	>90
Not married	10.2	>90	>90	>90
No education	25.3	>90	>90	>90
Grand multiparity (≥5 previous births)	6.5	>90	>90	>90
HIV infection	5.1	>90	>90	>90
Syphilis infection (VDRL positive)	1.8	52	>90	>90
Anaemia (Hb <8g/dl at delivery)	10.6	>90	>90	>90

*From pilot data in Kilifi District Hospital for maternal admissions 2002-2006

Cohort study: Risk factors for adverse perinatal outcomes

The power of the study to detect odds ratios of 1.5, 2 and 4 for the association of maternal GBS colonisation with adverse perinatal outcomes, based on maternal GBS colonisation prevalence of 20% (α=0.05), would be >90% for all outcomes apart from culture positive neonatal GBS disease, see Table 6.

Sub-study within cohort study: maternal colonisation to neonatal surface colonisation

Recruiting 1000 mothers over one calendar year in KDH, assuming 20% of the mothers were colonised, the study would identify an estimated 50% vertical transmission risk with a precision of +/- 10% (α=0.05).

Surveillance of neonatal GBS disease

The sample size was determined by the number of neonates admitted to KDH since systematic investigation of child admissions for invasive bacterial disease (July 1998- July 2013).[47] In terms of estimating incidence, within those born in KHDSS, and assuming 8000 deliveries in KHDSS per year, and 0.5 invasive GBS neonatal cases per 1000 deliveries, over 15 years, 60 cases would be expected. The estimated incidence would be able to be given to a precision of +/- 0.12 cases per 1000 deliveries (α=0.05).

Nested case-control study to determine if there was an association between GBS isolation from a sterile site and stillbirth

In order to identify an odds ratio of 5 for the association of the exposure (GBS isolated from sterile site) and the outcome (stillbirth), and assuming that GBS was isolated in 1% of controls, using a ratio of 2 live births to 1 stillbirth (as stillbirths are a rare event), the study would have 80% power (α=0.05) by recruiting 168 cases (stillbirths) over one year and 336 controls (live new-borns).

Table 6 Power to detect an association between maternal GBS colonisation and perinatal outcomes

Perinatal outcomes	Deliveries (%) with adverse outcome*	OR 1.5	OR 2	OR 4
Neonatal admission (<7 days)	4.7	>90	>90	>90
Neonatal culture positive GBS disease	0.5	<50	<50	<50
Stillbirth	7.0	>90	>90	>90
Birth weight <2500g	17.4	>90	>90	>90
Perinatal death	9.0	>90	>90	>90

*From pilot data in Kilifi District Hospital for maternal admissions 2002-2006

2.1.8 Public Engagement and Ethics

Engagement

The study was based in government health facilities in Coast Province, Kenya. In order to undertake this work, the permission of the hospital administration was requested in CPGH and KDH, and the permission of the District Ministry of Health officer for Kilifi District to undertake the work in Bamba sub-District Health Facility and Ganze Health Facility. Community engagement was undertaken through discussions with community representatives, through an established network developed in Kilifi District. Additional meetings were set up for information giving to remote community representatives in Bamba and Ganze sub-locations of Kilifi County, in English and Kiswahili. Engagement with health facilities and all health care staff involved was undertaken at the start, at six-monthly intervals through the study, at the end of recruitment, and at the end of the study, reporting findings.

Consent

Written informed consent was requested for all eligible mothers to be included in the study. In circumstances where the mother was illiterate, consent was requested orally, and witnessed and signed by an observer. In addition, written informed consent was requested in KDH for cord blood samples prior to delivery for all mothers and in a subset of these, cord blood was used for the case-control study of stillbirths and controls.

Additional written informed consent was requested, after a stillbirth had occurred, for a lung aspirate to be taken in Kilifi District Hospital. The timing of requesting the consent for stillbirth investigations was determined following discussions with social scientists and the community liaison group, who advised that if consent were requested “in the event of a

stillbirth," if a stillbirth subsequently occurred this could be understood by parents as the prediction that it might happen, and actually cause it to happen.

Obtaining informed consent at delivery presented challenges, particularly if the mother presented at a late stage of delivery or with obstetric complications. Previous work on obtaining informed consent at delivery suggested a staged process.[198 199] In situations where it was inappropriate to take full informed consent (for example a mother presenting late, or presenting with complications, or any condition giving the admitting midwife cause for concern), a two phased consent process was used, where verbal assent for a recto-vaginal swab was requested (appendices, page 222) and later written consent requested (appendices, page 223). If consent was refused after assent had been given, samples would be discarded. In the case of maternal deaths, however (after this was raised by Oxford Tropical Research Ethics Committee), if assent had been given, the samples were retained on the basis that consent was unlikely to have been subsequently withheld, and these mothers and their new-borns were then able to be included in the study, representing an important group to include. Assent was requested by the admitting nurse, as all government nurses were trained in study procedures. Consent was requested by fieldworkers, who were highly trained in the consent procedure, and were from the locality in which the study was undertaken. They were able to request consent in English, Kiswahili or Kigiriama (consent forms in English included in appendices, page 223).

Ethics

Ethical approval for this study was obtained from the National Ethical Review Committee, Nairobi, Kenya (SSC/ERC 2030), page 239) and the Oxford Tropical Research Ethics Committee (OXTREC 53-11), page 240. Written informed consent was obtained from all participants. Permission for the export of GBS isolates to the University of Oxford was given by the National Ethical Review Committee, Nairobi, Kenya.

2.1.9 Analytical Methods

Statistical analyses for clinical epidemiology were undertaken using Stata (Version 13) and analyses of whole genome sequence data were undertaken using existing pipelines, python programming scripts and the statistical software R.

Descriptive analyses

Clinical, microbiological and whole genome sequence summarised data were extracted from databases and merged. Binomial and categorical data were summarised, and checked for extreme outliers. Continuous variables were examined graphically for normality. Categorisation was undertaken according to existing standard groupings, or quartiles. Where values were perceived to be impossible, they were replaced with missing values. Duplicate entries were removed. Simple descriptive tabulations were undertaken to include characteristics of mothers and neonatal outcomes and proportions of missing data were examined.

Principal Component Analysis for socio-economic status

Principal Component analysis (PCA) was used to create a set of linear uncorrelated variables to measure socio-economic status. PCA creates orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables.[200] It has been used in relation to socio-economic indicators, where many similar indicators exist, as it highlights the differences in the data, reducing the number of variables, but without losing power. Variables used included type of house, water source, type of fuel used, and number of people sharing the dwelling, after recoding these variables to dummy variables. Data were missing for some variables, and so the PCA was undertaken in two ways; firstly on complete cases, and secondly recoding a missing indicator as absent. The results of the techniques were compared, with no difference in Eigenvalues. The full dataset was

therefore used in multivariable analyses, with indicators that were missing described as absent. The number of principal components was chosen based on the eigenvalues from the covariance matrix to maximise information on variability but maintain a parsimonious model.

Adjustment for clustering

The data for this study came from three sites, urban, semi-rural and rural, with the potential for clustering, as women attending the same facilities may be more similar to each other than to women from different sites; thus the data are not truly independent. Different approaches were considered to address this in the analysis.[201] One method considered was to include study site as a variable, but this does not reflect the fact that the data are not truly independent, therefore the probability of associations being identified by chance is underestimated. Other methods considered were generalised estimating equations, and random effects models. These methods are often used for highly correlated data, for example repeat measurements on the same individual. However, they rely on having a relatively high number of clusters (usually over 30), otherwise bias is introduced. Due to this, and the fact that although there will be some clustering in this dataset, but it is unlikely to be as important as if the same individuals were repeatedly sampled, the standard errors for risk factors according to site were adjusted (cluster variable),[202] which allowed for the lack of independence of data to be reflected in the standard errors, but did not affect the parameter estimates.

The same problem was encountered with multiple pregnancies, when considering risk factors for adverse perinatal outcomes. In this instance, because the proportion of multiple deliveries was low (<5%) and the lack of independence introduced therefore likely to be limited, a variable for multiple delivery was included in the multivariable analyses, but no further adjustments were made.

Missing data

The proportion of missing data across variables was first examined, and methods to mitigate missing data, which could bias results,[203] were considered. Multiple imputation with chained equations was chosen to mitigate the problems of missing data, as it represented a method to maximise power and minimise bias. However, its validity depends on the data being missing at random (versus missing not at random) and the imputation is only as good as the model on which it is based.[204] This depends on preserving relationships between exposures and outcomes,[204] (important in non-linear continuous variables) and including auxiliary variables which may not be included in the final regression model, but inform the value of the missing variable.[205,206] The association of missing data with other variables was evaluated, including maternal education, and emergency referral, which were important to include in the model as auxiliary variables, whether or not they were included in the final multivariable model. The imputation model included all the outcome variables (for separate regression analyses) maintaining the relationships of the data; regressing continuous variables as natural splines to account for non-linearity, using ordered regression where appropriate for categorical data (educational status, parity) and including auxiliary variables that were associated with missingness (such as emergency referral) as well as all risk factors to be included in multivariable modelling in all the analyses included in this thesis. The model was imputed stratified by maternal GBS status, to preserve effect modification relationships for this variable. Fifty datasets were imputed using chained equations, as many imputations increase the power of the analysis.[207] Imputed data were compared to real data after imputation process. Participants with outcome data missing were not included in analyses as this can increase Monte Carlo error in correctly imputed variables, and increase error in incorrectly imputed variables.[206]

The full imputing model included a regression of continuous variables (using natural splines): maternal temperature, maternal age, antenatal haemoglobin, birth weight, mid-upper arm circumference, gestation; ordinal logistic regression of ordered categorical variables: number of previous pregnancies, educational level and HIV status; logistic regression of: prolonged rupture of membranes, stillbirth, previous stillbirth, urinary tract infection, cattle tending, emergency referral, vaginal examination before recto-vaginal swab, ethnicity (non Mijikenda or Mijikenda), sex of baby and perinatal GBS disease. Complete variables included multiple delivery, obstetric complication, study site, possible severe bacterial infection, socio-economic status, and maternal GBS colonisation at delivery (Stata code, page 250).

Univariable analyses

Initial analyses were undertaken using traditional methods (Mantel-Haenszel),[208] and univariable logistic regression. For nominal, binomial, and ordinal data the baseline group was the "normal" category, if this was able to be defined, or, if not, the largest group. For ordinal data, departure from linearity was assessed using the likelihood ratio test. These analyses were undertaken prior to adjusting standard errors for clustering, because these standard errors are the square root of the variances of the variance-covariance matrix, and are not likelihood based methods, so this test cannot be used with these adjustments.

For continuous variables (maternal age, mid-upper arm circumference, haemoglobin), the relationship between the variable and the outcome was examined using natural splines. This was undertaken in order to understand the relationship, maximise power, and to keep these terms in imputed models to reflect non-linear relationships. For ease of interpretation of results, continuous variables were also categorised in regression analyses, after checking they concurred with the results using continuous variables and natural splines.

Effect modification

Risk factors hypothesised *a priori* to act as effect modifiers were examined, and these were included in the regression analyses if there was some evidence the effect was not due to chance ($p < 0.1$ in univariable analyses), after checking the absolute numbers that were being examined in interactions, and where the relationship was biologically plausible with large effect (OR >1.5 or <0.5) sizes.

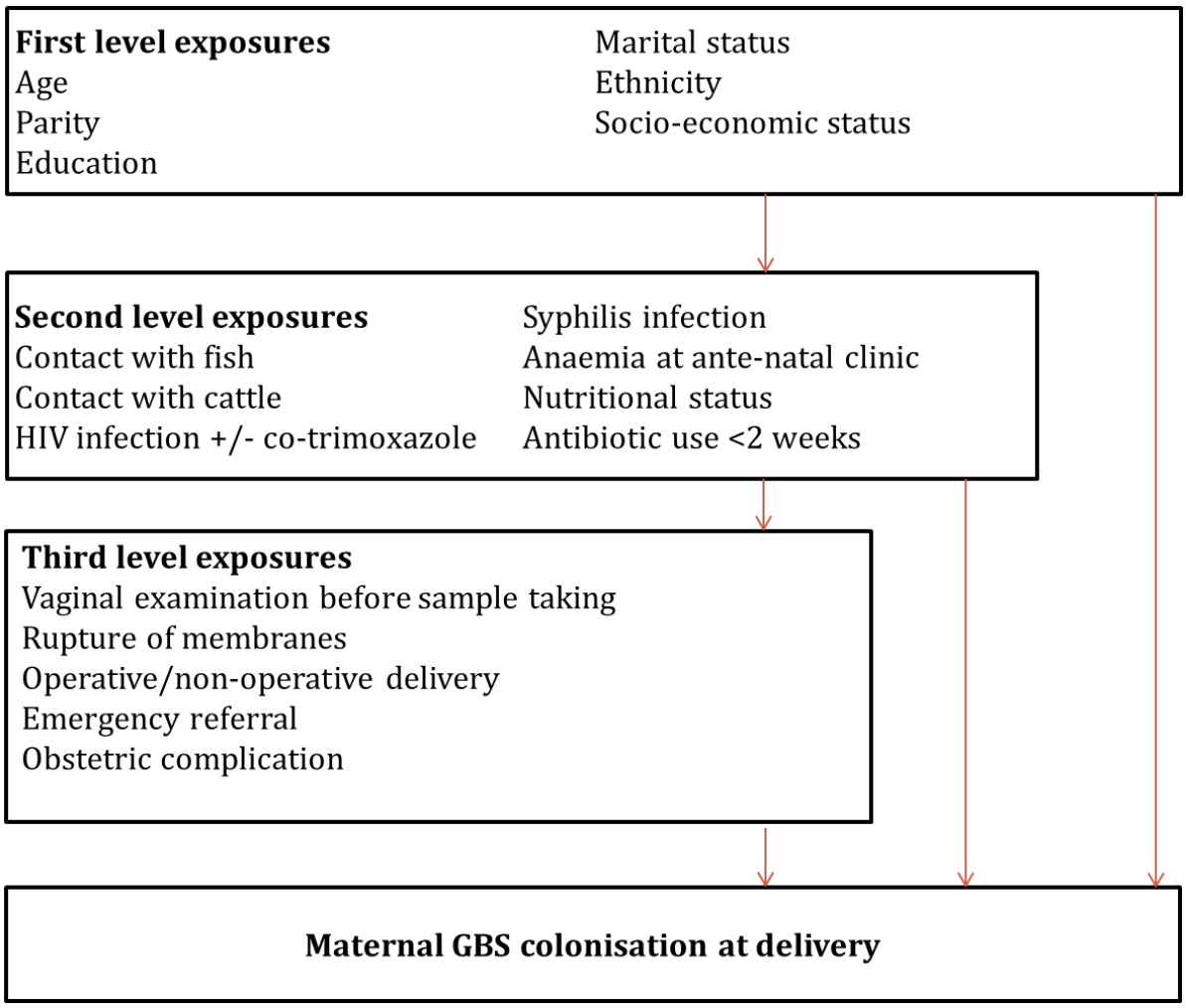
Modelling Strategies for multivariable analyses

Risk factors for maternal GBS Colonisation

In order to identify risk factors for maternal GBS colonisation a conceptual framework was developed as a basis for explanatory models of the influence of the exposures on the outcome (Figure 8). Multivariable modelling was undertaken in two ways, firstly on complete cases and secondly on all cases using imputed data.

Multivariable regression on complete cases and imputed data was undertaken including all factors associated with the outcome in univariable analyses and using backwards stepwise elimination of risk factors. Exposures with no evidence against the null hypothesis of no association between the exposure and outcome ($p > 0.1$) and no effect on other covariates (~10% change in strength of associations) were removed individually out of the multivariable model, except for *a priori* confounders (maternal age and parity). Pre-specified effect modifiers (interactions) were tested as described above. The combined Wald test was used because the standard errors reported in the table of parameter estimates are the square root of the variances (diagonal elements) of the variance-covariance matrix, not likelihood based methods.

Figure 8 Conceptual framework for the exposures associated with maternal GBS colonisation

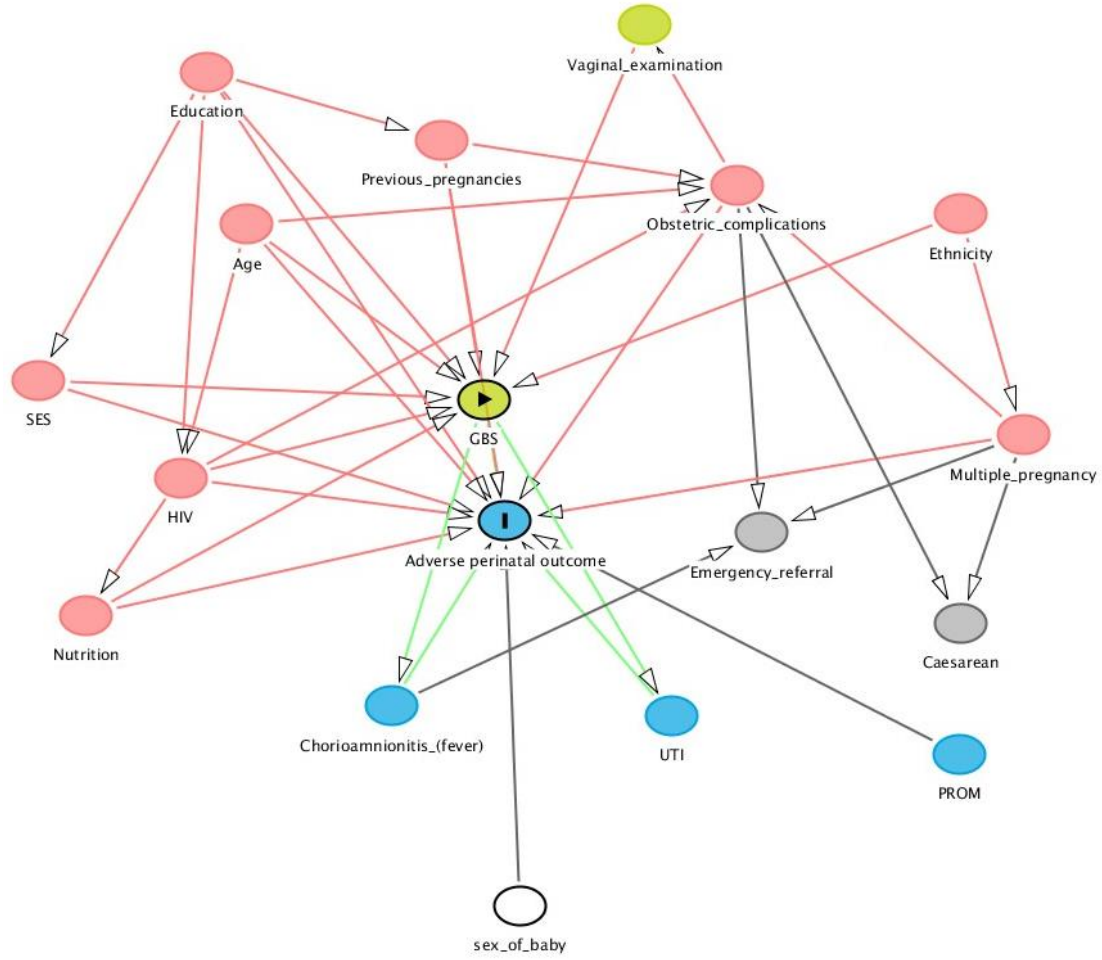


Maternal GBS as a cause of adverse perinatal outcomes

In order to determine the degree to which maternal GBS colonisation was a cause of adverse perinatal outcomes (stillbirth, low birth weight, preterm birth, pSBI) a causal model was developed to try and describe the relationships between exposures and the outcome. This can be undertaken using graphical methods, counterfactual models, sufficient-component cause models, and structural equations.[209] A graphical method was used here,[210] based on directed acyclic graphs of the exposure, outcome and possible confounders (see Figure 9) to create a Bayesian network.[211] This was used as it is a clear depiction of the relationships behind the analysis and enables sufficient adjustment variables to be identified such that adjustment would reduce confounding, and minimize problems from collinear variables when estimating the causal effect of the exposure on the outcome. Maternal fever (sign of chorioamnionitis) and urinary tract infection were on the causal pathway and were therefore only included in the model (with the minimum adjustment set of variables) if there was evidence of effect modification, not as confounders. PROM (prolonged rupture of membranes >18h) was also included as an effect modifier (it can increase exposure to GBS in utero). The "moral graph" of all possible networks is given in the appendices, page 249.

For perinatal mortality, stillbirth and possible severe bacterial infection, the outcome was a binary variable. However for gestation and birth weight the outcome variable was continuous. These outcomes and associated exposures could therefore have been modelled using linear regression, but because the linear regression model weights clusters (based on site) equally, and the rural site was small, considerable power was lost. Ordinal logistic regression was initially therefore used, to keep the ordering of the data, but there was strong evidence against the assumption of proportional odds. Multinomial analysis for categories of gestation and birth weight were therefore undertaken.

Figure 9 Causal Model: Directed Acyclic Graphs forming the basis for a Bayesian Network to show the relationships between the exposures and adverse perinatal outcomes.



Incidence of neonatal and perinatal GBS disease

The incidence of neonatal disease over 15 years (1998-2013) was calculated based on the number of live births in the mid-study year, using data from demographic surveillance; the Kilifi Health and Demographic Surveillance Survey (KHDSS).[2] This analysis only included neonates admitted to KDH with GBS disease who were residing in the KHDSS census area.

The incidence of perinatal disease during the cohort study (2011-2013) was determined from the number of births in KDH maternity and the number of stillbirths with GBS isolated from cord blood or lung aspirate, plus the number of neonates in 0-6 days who were born in maternity and had GBS isolated from clinical investigations (blood culture or cerebrospinal fluid culture) following paediatric admission.

Genetic comparison

Whole genome sequence data were checked for quality (percentage mapping to the reference genome) before MLST allocation and capsular typing based on genetic sequence. Established pipelines and python scripts were used to generate UPGMA (Unweighted Pair Group Method with Arithmetic Mean) files,[212] (based on genetic pairwise distances), assuming a constant rate of evolution. These FASTA format files were then used to illustrate relationships visually in R statistical software.[213] Genetic pairwise differences between GBS isolates from mothers and neonates were analysed to calculate single nucleotide polymorphisms (SNPs) between GBS isolates from mothers and new-borns.

Chapter 3: Maternal GBS colonisation

3.1 Results

This chapter describes the results of the cohort study of mothers, recruited across three study sites in coastal Kenya. It addresses the following three questions:

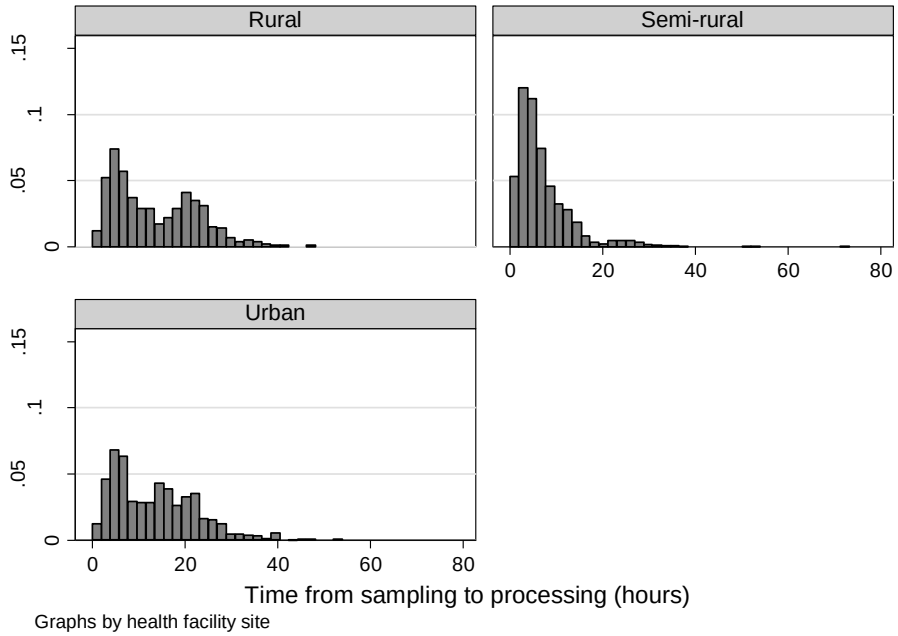
1. What is the prevalence of maternal GBS colonisation at delivery?
2. What are the risk factors for maternal GBS colonisation at delivery?
3. What are the serotypes and MLST types of GBS isolates colonising mothers at delivery?

3.1.1 Method validation

Sample transport

Almost all (7832/7967 (98.3%)) recto-vaginal swabs were processed in the KWTRP laboratories within 48 hours of sampling. Only 7/7967 were not, and there were 128/7967 samples with time data missing. Sample times were increased from rural and urban centres, compared to the semi-rural centre (adjacent to KWTRP laboratories) due to transport time (Wilcoxon rank sum tests for processing time compared to semi-rural site, $p<0.0001$ for rural and urban sites, Figure 10). However, there was no difference in the processing time for negative swabs compared to positive swabs (Wilcoxon rank sum test, $p=0.98$). In logistic regression analyses for the association between a positive swab and time to process there was no evidence against the hypothesis of no association in all sites (OR 1.00 (0.98-1.01) $p=0.4$), or looking at individual sites; rural (OR 0.99 (0.95-1.02) $p=0.5$), urban (OR 0.99 (0.98-1.01) $p=0.3$) or semi-rural (OR 0.99 (0.98-1.01) $p=0.3$).

Figure 10 Time from recto-vaginal swab sampling to laboratory processing by study site in hours



Sequetyping method

A sequetyping method was designed based on published gene sequences of known capsular types of GBS and tested against a validation set of 121 GBS isolates which had been previously serotyped by classical methods,[87] and PCR-based methods.[144] Serotype VIII and IX were rare in these validation sets and 2 reference isolates were obtained from the Statens Serum Institute, Denmark, to include in the comparison dataset (Table 7).

The kappa statistic for all previously serotyped isolates with a known capsule type was 0.93 (very high). Using capsular gene sequence data to allocate serotypes, two serotypes that did not have a capsular type assigned by latex agglutination methods had serotypes Ib and VI assigned. The kappa statistic for agreement including these isolates was 0.92 (also very high). The serotypes described in the analyses that follow were therefore based on this method.

Table 7 Comparison of serotype allocation by WGS compared to latex agglutination or PCR*

		Serotype allocated by WGS										
		Ia	**Ib**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**Total**
Serotype by latex agglutination or PCR	**Ia**	**28**	0	0	0	0	0	0	0	0	0	**28**
	Ib	0	**6**	1	0	0	0	0	0	0	0	**7**
	II	0	0	**22**	0	0	0	0	0	0	0	**22**
	III	3	0	0	**30**	0	0	0	0	0	1	**34**
	IV	0	0	0	0	**1**	0	1	0	0	0	**2**
	V	0	0	0	0	0	**15**	0	0	0	0	**15**
	VI	0	0	0	0	0	1	**6**	0	0	0	**7**
	VII	0	0	0	0	0	0	0	**5**	0	0	**5**
	VIII	0	0	0	0	0	0	0	0	**1****	0	**1**
	IX	0	1	0	0	0	0	0	0	0	**1****	**2**

*Includes only isolates that were assigned a serotype by conventional methods

**Reference isolates from the Statens Serum Institute, Denmark

3.1.2 Participants

There were 10131 women who attended the health facilities during the period of recruitment, of whom 618 were ineligible (no delivery). There were 1546 women who were eligible but not recruited: 815 did not give permission for inclusion and 731 were missed opportunities. In total there were 7967 women recruited, representing 84% of eligible women (Figure 11).

The characteristics of women for age, marital status, ethnicity, and parity were comparable between those included and those excluded (see Table 8). However, there were some differences; a higher percentage of mothers with tertiary education refused consent (90/691, 13.0%) compared to mothers with primary education (413/5258, 7.9%).

Figure 11 Recruitment of mothers to the cohort study to examine risk factors for maternal GBS colonisation across urban, semi-rural and rural sites (2011-2013)

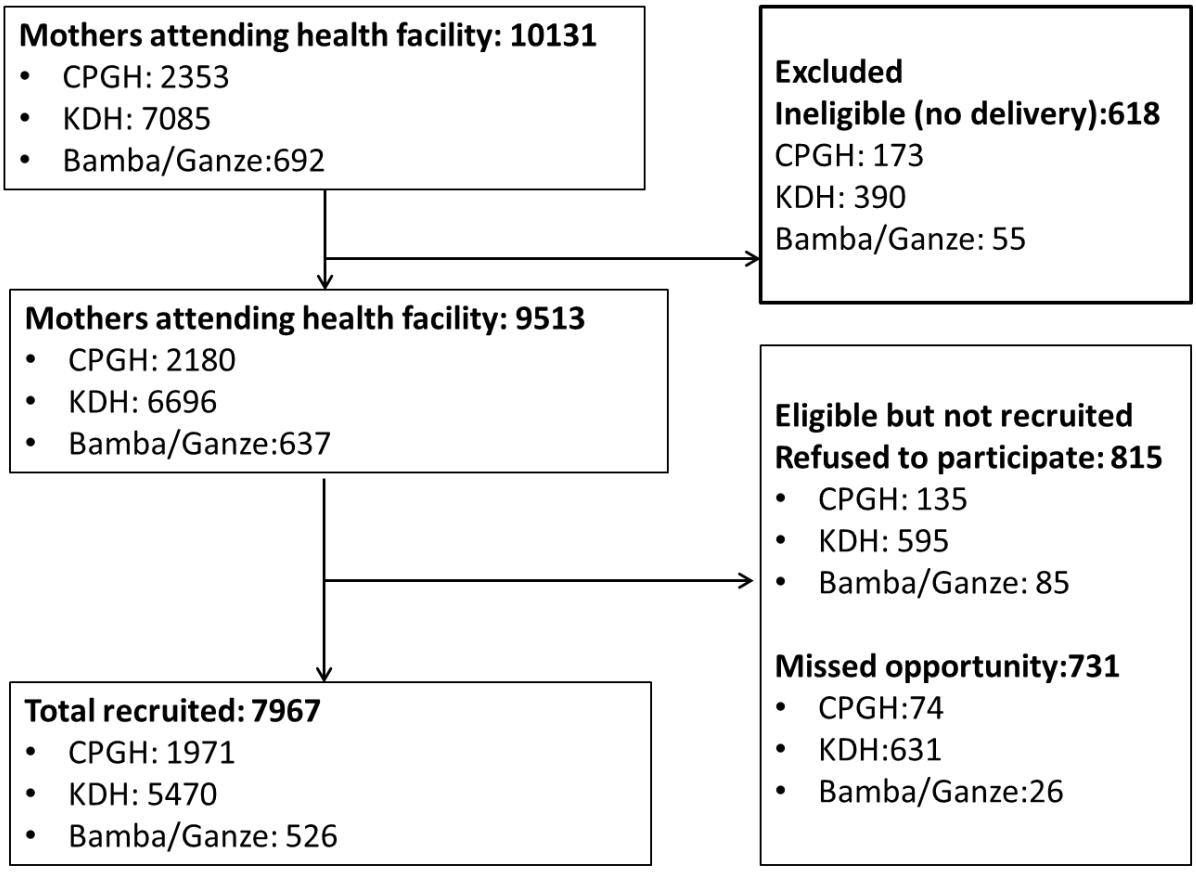


Table 8 Characteristics of women attending for delivery who were excluded due to lack of consent or a missed opportunity

	Category	Total	Included		No consent		Missed opportunity	
			N	**(%)**	N	**(%)**	N	**(%)**
		N=9513	**N=7967**		**N=815**		**N=731**	
Age in quartiles	1	1932	1676	(86.7)	145	(7.5)	111	(5.7)
	2	1931	1664	(86.2)	159	(8.2)	108	(5.6)
	3	1964	1658	(84.4)	187	(9.5)	119	(6.1)
	4	2016	1672	(82.9)	217	(10.8)	127	(6.3)
	Missing	1670	1297	(77.7)	107	(6.4)	266	(15.9)
Education	None	1382	1147	(83.0)	119	(8.6)	116	(8.4)
	Primary	5258	4433	(84.3)	413	(7.9)	412	(7.8)
	Secondary	1864	1561	(83.7)	165	(8.9)	138	(7.4)
	Tertiary	691	562	(81.3)	90	(13.0)	39	(5.6)
	Missing	318	264	(83.0)	28	(8.8)	26	(8.2)
Married	Yes	8641	7245	(83.8)	728	(8.4)	668	(7.7)
	No	713	589	(82.6)	73	(10.2)	51	(7.2)
	Missing	159	133	(83.6)	14	(8.8)	12	(7.5)
Mijikenda ethnicity	No	2603	2226	(85.5)	221	(8.5)	156	(6.0)
	Yes	6757	5617	(83.1)	570	(8.4)	570	(8.4)
	Missing	153	124	(81.0)	24	(15.7)	5	(3.3)
Parity (> 28 weeks)	0	3493	2987	(85.5)	256	(7.3)	250	(7.2)
	1-4	3271	2550	(78.0)	398	(12.2)	323	(9.9)
	≥5	1624	1340	(82.5)	146	(9.0)	138	(8.5)
	Missing	125	90	(72.0)	15	(12.0)	20	(16.0)
Emergency referral	No	8420	7104	(84.4)	710	(8.4)	606	(7.2)
	Yes	894	705	(78.9)	94	(10.5)	95	(10.6)
	Missing	199	158	(79.4)	11	(5.5)	30	(15.1)

3.2 What is the prevalence of maternal GBS colonisation at delivery?

The characteristics of the 7967 women included in the study are given in Table 9 below. Of those recruited 526/7967 (6.6%) of women were from the rural health care centres (Bamba sub-district hospital and Ganze health facility); 5470/7967 (68.7%) were from the semi-rural site (Kilifi District Hospital) and 1971/7967 (24.7%) were from the urban site (Coast Provincial General Hospital). Of the 7967 women recruited, there were 934/7967 (11.7%, 95%CI 11.0-12.5%) colonised with GBS at delivery. There was variation across the sites, with the lowest prevalence of GBS colonisation in the rural site 47/526 (8.9%, 6.6-11.7%), the semi-rural site 608/5470 (11.1%, 10.2-12.0%) and highest in the urban site 279/1971 (14.2%, 12.6-15.8%).

3.3 What are the risk factors for maternal GBS colonisation at delivery?

The risk factors for maternal GBS colonisation at delivery were examined in univariable analyses and multivariable analyses of complete cases and using imputed data. Relationships of continuous variables with maternal GBS colonisation were examined graphically (Figure 12). For maternal age, haemoglobin measurement, and principal components analysis of household socioeconomic variables, the relationships were non-linear (Figure 12). They were included in imputation models using natural spline variables, and regression models were undertaken with both continuous variables (as natural splines) and categorised variables; results did not differ, and the results using categorical variables are presented for ease of interpretation.

The characteristics of the mothers, proportion of women in each category by GBS colonisation status, proportion of missing data across variables, and results of univariable analyses are given in Table 9. Maternal age and parity were included in all analyses as *a priori* confounders. Variables included in the initial model ($p<0.1$ for combined Wald test across categories) were first level variables: educational status, ethnicity (Mijikenda or other), household socio-economic status; second level variables: haemoglobin level, HIV status, nutritional status, mother looking after cattle; and third level variables: vaginal examination (VE) before swab, emergency referral, and obstetric complication at delivery.

The results of the multivariable complete case analyses are presented in Table 10 and imputed data analyses in Table 11 at each of these three levels. The results of the multivariable analyses on complete cases and imputed data are very similar; with more power being maintained in the imputed analysis and therefore stronger evidence against the null hypotheses, as p values were smaller, and socio-economic status and nutrition remained strongly associated with the outcome in the third level of the complete case analysis.

Level 1 variables

Level 1 variables included maternal age and parity (also *a priori* confounders) as well as ethnicity and household socio-economic status. Parity was associated with GBS colonisation in univariable and multivariable analyses; women who were multiparous (parity $\geq$5) were less likely to be colonised with GBS at delivery; (OR 0.81 (0.70-0.94), p <0.001 imputed model). Although quartiles of maternal age were not associated with maternal GBS colonisation in the univariable analysis, when maternal age was examined as a continuous variable there was evidence of a non-linear association (p=0.016 for linear component and p=0.003 for natural spline function), and in multivariable analyses it was associated with maternal GBS colonisation as both a categorical variable (as presented) and a continuous variable.

Ethnicity was associated with GBS colonisation; the Mijikenda, the indigenous population, had lower odds of being GBS colonised at delivery (OR 0.74 (0.62-0.88)). This association was explored in a sub-analysis of data from Kilifi District Hospital (the largest site) looking at the subdivisions of the Mijikenda (Giriama, Kauma, Kambe, Duruma, Ribe, Rabai, Jibana, and Chonyi). There was no evidence against the null hypothesis for no association between maternal GBS colonisation and these ethnic groups (Chi2 test, p=0.2) and no evidence against the null hypothesis for maternal GBS colonisation being associated with ethnicity across the non-Mijikenda groups (Arab, Asian, Bajun, Boran, Digo, Embu, Gabbra, Gosha, Gurreh, Hawaiyah, Kalenjin, Kamba, Kikuyu, Kisii, Kuria, Luhya, Luo, Masai, Mbere, Meru, Mnubi, Saraja, Pokomo, Sakue, Shiraz, Somali, Swahili, Taita, Taveta, Teso, Turkana, Chi2 test, p=0.4).

The odds of maternal GBS colonisation were highest in the highest SES group (first level of the conceptual model OR 1.22 (1.07-1.40) in complete cases, OR 1.20 (1.04-1.40) in the imputed analysis), with the effect persisting across the three levels of the conceptual model.

There was evidence of confounding between socio-economic status and ethnicity, as effect sizes were both reduced after adjustment. There was no evidence of collinearity, based on standard errors, but the two variables were strongly associated; the odds ratio of being of Mijikenda ethnicity in the lowest quartile for socio-economic status was 48.1 (22.4-103.3), compared to the highest quartile of socio-economic status as the reference group.

Level 2 variables

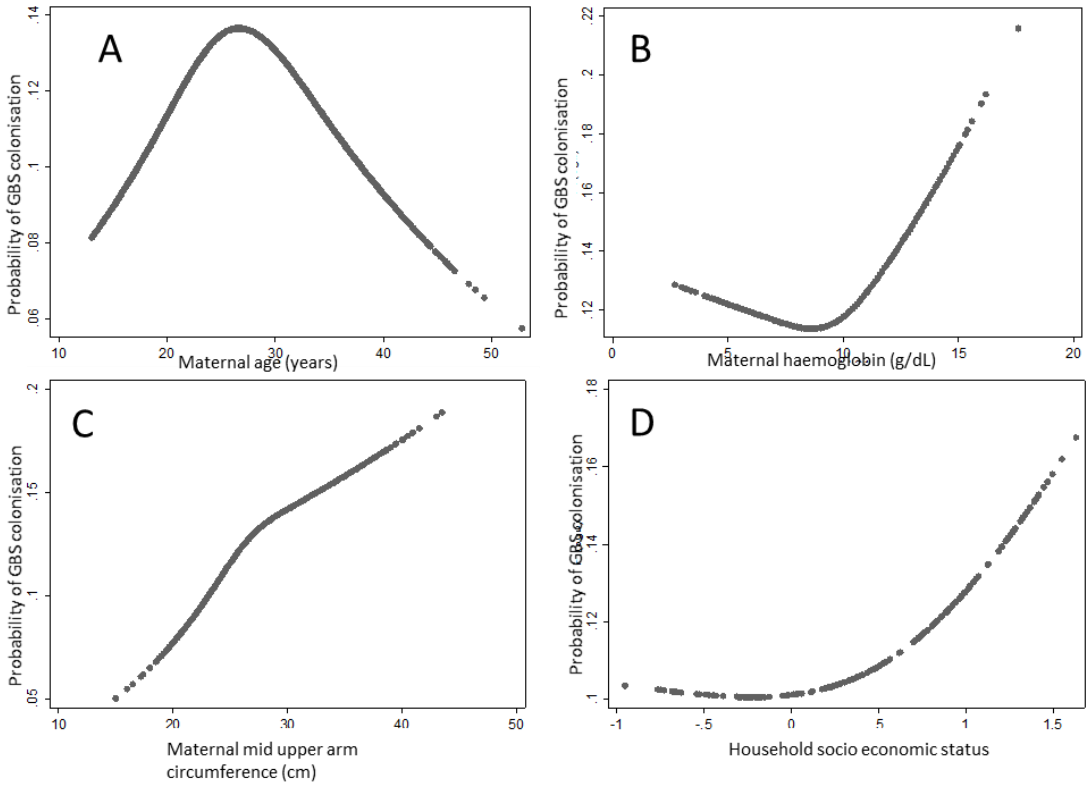
Level 2 variables included in multivariable models were cattle tending, nutritional status and HIV status. Cattle tending was associated with increased maternal GBS colonisation at delivery; odds were increased by 29% (17-42%, imputed model). Nutritional status, determined by mid-upper-arm circumference (MUAC), showed a dose-response relationship with maternal GBS colonisation, with women who have the smallest MUACs being the least likely to be GBS colonised (OR 0.73 (0.60-0.89)), and women who have the largest MUACs being the most likely to be GBS colonised (OR 1.05, (0.78-1.40), the p value for overall association was <0.001, imputed model).

HIV status was categorised into participants who were HIV negative, those women who were HIV positive and on prophylactic co-trimoxazole, and HIV positive women not on co-trimoxazole. There was good evidence that women who were HIV positive and on prophylactic co-trimoxazole were less likely to be colonised with GBS at delivery (OR 0.23 (0.16-0.33)) compared to HIV negative women. There was weaker evidence against the null hypothesis of no association for HIV positive women not on co-trimoxazole and the multivariable complete case analysis differed to the imputed model. In the complete case analysis, the 95%CI did not include 1 (ORs 0.67 (0.47-0.98)) and therefore did not include a null result, whereas the imputed analysis included 1 in the 95% confidence intervals (OR 0.70, 95%CI 0.44-1.10) suggesting there may not be an association between being HIV positive and not on co-trimoxazole and GBS colonisation.

Level 3 variables

Level three variables had little effect on the other variables included in the model, however, a vaginal examination at delivery was associated with lower odds of maternal GBS colonisation, (OR 0.83 (0.69-0.98) imputed model). A mother with any obstetric complication had reduced odds of maternal GBS colonisation compared to a mother without an obstetric complication at delivery (OR 0.85 (0.79-0.92) imputed model).

Figure 12 Relationship between continuous variables and maternal GBS colonisation at delivery*



*A: maternal age (years) against probability of maternal GBS colonisation, B: maternal haemoglobin (g/dL) against probability of maternal GBS colonisation, C: maternal mid-upper arm circumference (cm) against probability of maternal GBS colonisation and D: household socio-economic status (principal components analysis) against probability of maternal GBS colonisation.

Table 9 Exposures associated with maternal GBS colonisation: univariable analyses

Variable	Category	GBS colonisation					
		N (7697)	N (934) (%)		OR	95%CI	p
Age in quartiles	1	1674	166	(9.9)	0.75	(0.50-1.11)	0.2
	2	1663	223	(13.4)	1.05	(0.92-1.20)	
	3	1656	213	(12.9)	1		
	4	1672	186	(11.1)	0.85	(0.69-1.04)	
	Missing	1302	146	(11.2)			
Parity	0	2986	365	(12.2)	0.98	(0.90-1.06)	<0.001
	1-4	3550	442	(12.5)	1		
	≥5	1341	119	(8.9)	0.68	(0.55-0.85)	
	Missing	90	8	(8.9)			
Education	None	1147	99	(8.6)	0.75	(0.59-0.95)	0.046
	Primary	4433	498	(11.2)	1		
	Secondary	1561	211	(13.5)	1.23	(1.02-1.50)	
	Tertiary	562	88	(15.7)	1.47	(1.06-2.02)	
	Missing	264	38	(14.4)			
Married	Yes	7245	867	(12.0)	1		0.2
	No	589	56	(9.5)	0.77	(0.53-1.13)	
	Missing	133	11	(8.3)			
Mijikenda	No[##]	2226	345	(15.5)	1		<0.001
	Yes	5617	578	(10.3)	0.63	(0.52-0.76)	
	Missing	124	11	(8.9)			
Household SES	Very low	1086	96	(8.8)	0.80	(0.62-1.03)	0.008
	Low	2720	294	(10.8)	1		
	Medium	2123	229	(10.8)	1.00	(0.84-1.19)	
	High	2038	315	(15.5)	1.51	(1.35-1.69)	
Haemoglobin quartiles	1	1624	189	(11.6)	1.02	(0.90-1.06)	0.058
	2	1485	157	(10.6)	0.91	(0.58-1.77)	
	3	1731	199	(11.5)	1		
	4	1666	226	(13.6)	1.21	(1.03-1.41)	
	Missing	1461	163	(11.2)			
Iron supplements	No	2100	254	(12.1)	1		0.4
	Yes	5863	680	(11.6)	0.95	(0.86-1.06)	
	Missing	4	0	(0.0)			
HIV status	-ve	7285	879	(12.1)	1		<0.001
	+ve	239	20	(8.4)	0.67	(0.46-0.97)	
	+ve +c[#]	161	5	(3.1)	0.24	(0.18-0.30)	
	Missing	282	30	(10.6)			
MUAC in quartiles	1	1428	125	(8.8)	0.68	(0.54-0.85)	0.001
	2	2219	264	(12.4)	1		
	3	1662	183	(11.0)	0.87	(0.73-1.04)	
	4	2170	309	(14.2)	1.18	(1.01-1.37)	
	Missing	578	53	(9.2)			

Antibiotics <4 weeks before delivery	No	7028	828	(11.8)	1		1
	Yes	507	62	(12.2)	1.00	(0.73-1.35)	
	Missing	432	44	(10.2)			
Syphilis serology positive	No	6706	780	(11.6)	1		
	Yes	35	3	(8.6)	0.71	(0.09-5.59)	0.7
	Missing	1226	151	(12.3)			
Mother guts fish	No	5626	657	(11.9)	1		0.9
	Yes	2300	274	(12.1)	1.02	(0.74-1.41)	
	Missing	41	3	(7.5)			
Mother looks after cattle	No	7471	873	(11.9)	1		<0.001
	Yes	449	56	(12.4)	1.08	(1.05-1.11)	
	Missing	47	5	(10.6)			
VE before swab	No	4952	609	(12.3)	1		<0.001
	Yes	780	73	(9.4)	0.74	(0.63-0.87)	
	Missing	2235	252	(11.3)			
Rupture of membranes	No	6399	748	(11.7)	1		
	Yes	1522	184	(12.1)	1.04	(0.95-1.14)	0.4
	Missing	46	2	(4.4)			
Emergency referral	No	7104	854	(12.0)	1		0.069
	Yes	705	68	(9.7)	0.78	(0.60-1.02)	
	Missing	158	12	(7.6)			
Obstetric complication	No	6913	823	(11.9)	1		
	Yes	1054	111	(10.5)	0.87	(0.83-0.91)	<0.001
Operative delivery	No	6195	738	(11.9)	1		0.2
	Yes	1610	182	(11.3)	0.94	(0.86-1.03)	
	Missing	162	14	(8.6)			

* Robust standard errors to account for intracluster correlation within sites

**combined Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

[##] Arab, Asian, Bajun, Boran, Digo, Embu, Gabbra, Gosha, Gurreh, Hawaiyah, Kalenjin, Kamba, Kikuyu, Kisii, Kuria, Luhya, Luo, Masai, Mbere, Meru, Mnubi, Saraja, Pokomo, Sakue, Shiraz, Somali, Swahili, Taita, Taveta, Teso, Turkana

Table 10 Exposures associated with maternal GBS colonisation: multivariable complete case analyses

		First level (N=6496)			Second level (N=5657)			Third level (N=3979)		
Variable	**Category**	**OR**	**95%CI***	**p value**	**OR**	**95%CI***	**p value**	**OR**	**95%CI***	**p value**
Age in quartiles	1	0.74	(0.52-1.04)	<0.001	0.75	(0.49-1.13)	<0.001	0.77	(0.40-1.80)	<0.001
	2	1.00	(0.91-1.11)		1.06	(0.87-1.27)		1.15	(0.79-1.69)	
	3	1			1			1		
	4	0.93	(0.75-1.15)		0.93	(0.77-1.12)		0.92	(0.72-1.17)	
Parity	0	1.03	(0.93-1.10)	<0.001	01.03	(1.00-1.05)	<0.001	1.06	(1.00-1.14)	<0.001
	1-4	1			1			1		
	≥5	0.79	(0.70-0.90)		0.81	(0.71-0.93)		0.85	(0.63-1.15)	
Mijikenda	No	1		<0.001	1		<0.001	1		<0.001
	Yes	0.71	(0.65-0.78)		0.71	(0.67-0.76)		0.65	(0.57-0.74)	
Household SES	Very low	0.89	(0.62-1.26)	0.004	0.85	(0.71-1.02)	0.001	0.88	(0.72-1.09)	0.2
	Low	1			1			1		
	Medium	0.90	(0.81-1.00)		0.95	(0.84-1.07)		1.00	(0.81-1.23)	
	High	1.22	(1.07-1.40)		1.22	(1.15-1.29)		1.24	(1.05-1.47)	
Mother looks after cattle	No				1		<0.001	1		<0.001
	Yes				1.30	(1.19-1.42)		1.45	(1.40-1.50)	
MUAC in quartiles	1				0.66	(0.53-0.83)	<0.001	0.76	(0.48-1.21)	0.3
	2				1			1		
	3				0.82	(0.67-1.00)		0.80	(0.62-1.03)	
	4				1.04	(0.79 -1.38)		1.02	(0.72-1.46)	
HIV status	-ve				1		<0.001	1		<0.001
	+ve				0.67	(0.47-0.98)		1.16	(0.92-1.46)	
	+ve +c[#]				0.19	(0.13-0.27)		0.20	(0.14-0.26)	
VE before swab	No							1		0.018
	Yes							0.57	(0.36-0.91)	
Obstetric complication	No							1		<0.001
	Yes							0.78	(0.70-0.88)	

*based on robust standard errors to account for intracluster correlation within recruitment sites

**combined Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

Table 11 Exposures associated with maternal GBS colonisation: multivariable imputed analyses (N=7967)

		First level (N=7967)			Second level (N=7967)			Third level (N=7967)		
Variable	**Category**	**OR**	**95%CI***	**p****	**OR**	**95%CI***	**p****	**OR**	**95%CI***	**p****
Age in quartiles	1	0.78	(0.57-1.8)	0.014	0.80	(0.55-1.15)	0.009	0.80	(0.56-1.15)	0.008
	2	1.02	(0.89-1.17)		1.04	(0.87-1.22)		1.03	(0.88-1.21)	
	3	1			1			1		
	4	0.94	(0.77-1.15)		0.96	(0.78-1.18)		0.95	(0.80-1.14)	
Parity	0	1.04	(0.99-1. 09)	<0.001	1.04	(0.99-1.09)	<0.001	1.05	(1.00-1.10)	<0.001
	1-4	1			1			1		
	≥5	0.81	(0.70-0.94)		0.80	(0.69-0.92)		0.81	(0.69-0.94)	
Mijikenda	No	1		0.001	1		0.002	1		0.002
	Yes	0.74	(0.62-0.88)		0.73	(0.60-0.90)		0.73	(0.60-0.94)	
Household SES	Very low	0.87	(0.66-1.15)	<0.001	0.88	(0.66-1.16)	<0.001	0.88	(0.67-1.15)	<0.001
	Low	1			1			1		
	Medium	0.88	(0.82-0.95)		0.87	(0.82-0.92)		0.88	(0.82-0.93)	
	High	1.21	(1.05-1.39)		1.18	(1.06-1.30)		1.19	(1.08-1.30)	
Mother looks after cattle	No				1		<0.001	1		<0.001
	Yes				1.29	(1.17-1.42)		1.29	(1.16-1.43)	
MUAC in quartiles	1				0.73	(0.60-0.89)	<0.001	0.73	(0.59-0.89)	<0.001
	2				1			1		
	3				0.84	(0.66-1.07)		0.85	(0.67-1.08)	
	4				1.05	(0.78-1.40)		1.06	(0.79-1.42)	
HIV status	-ve				1		<0.001	1		<0.001
	+ve				0.70	(0.44-1.10)		0.69	(0.43-1.11)	
	+ve +c[#]				0.23	(0.16-0.33)		0.23	(0.16-0.33)	
VE before swab	No							1		0.033
	Yes							0.83	(0.69-0.98)	
Obstetric complication	No							1		<0.001
	Yes							0.85	(0.79-0.92)	

*based on robust standard errors to account for intracluster correlation within recruitment sites

**combined Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

3.4 What are the serotypes and MLST types of GBS isolates colonising mothers at delivery?

There were 910/934 (97%) GBS isolates from recto-vaginal swabs successfully sequenced and included in this analysis. There were seven out of the ten known serotypes present; serotype III (38.8%) was the most common, then 1a (20.9%), V (17.0%), Ib (12.3%), II (8.7%), IV (2.0%) and VII (0.3%), illustrated in Figure 13.There were no GBS isolates of serotype VI, VIII or IX.

There were no differences in proportions of GBS serotypes by site (chi2 p=0.3), and all serotypes were present in all the three sites, apart from the rural site where no serotype VII was identified (Figure 14). Figure 15 illustrates the genetic relationships of the capsular types; there are strains where recombination has occurred at the capsular locus, bringing a capsular type into a branch predominantly associated with another capsular type. Figure 16 illustrates the fact that there are no clear clones of GBS in particular sites.

The MLST types are described in terms of clonal complex in Table 12. Clonal complex-17 was the most common 271/910 (29.8%), which is mainly serotype III (the other clonal complexes do not correspond closely to capsular type). Clonal complex 23 was the next most common (230/910, 25.3%) of the five major clonal complexes, then clonal complex 10 (147/910, 16.2%), then clonal complex 19 (112/910, 12.3%), and clonal complex 1 (95/910, 10.4%).

The results of a post-hoc analysis of the phylogeny of GBS strains associated with handling cattle compared to strains from mothers who did not handle cattle, are included in Figure 17. The strains associated with women handling cattle were dispersed across the GBS phylogeny, not clearly associated with one particular clonal complex.

Figure 13 Pie diagram of the percentages of GBS serotypes (N=910) colonising mothers in coastal Kenya (all sites)

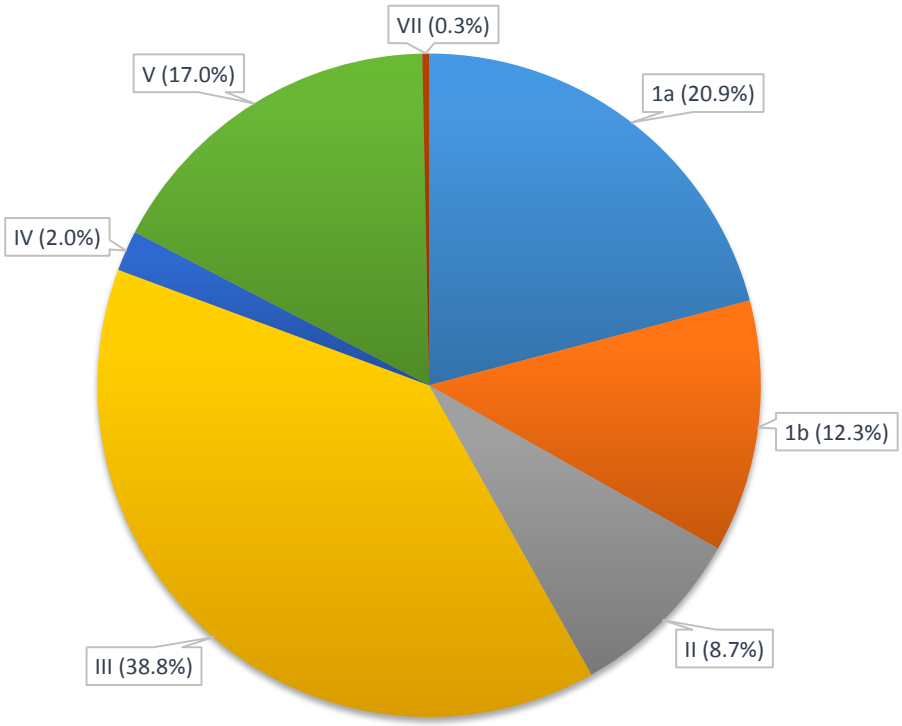
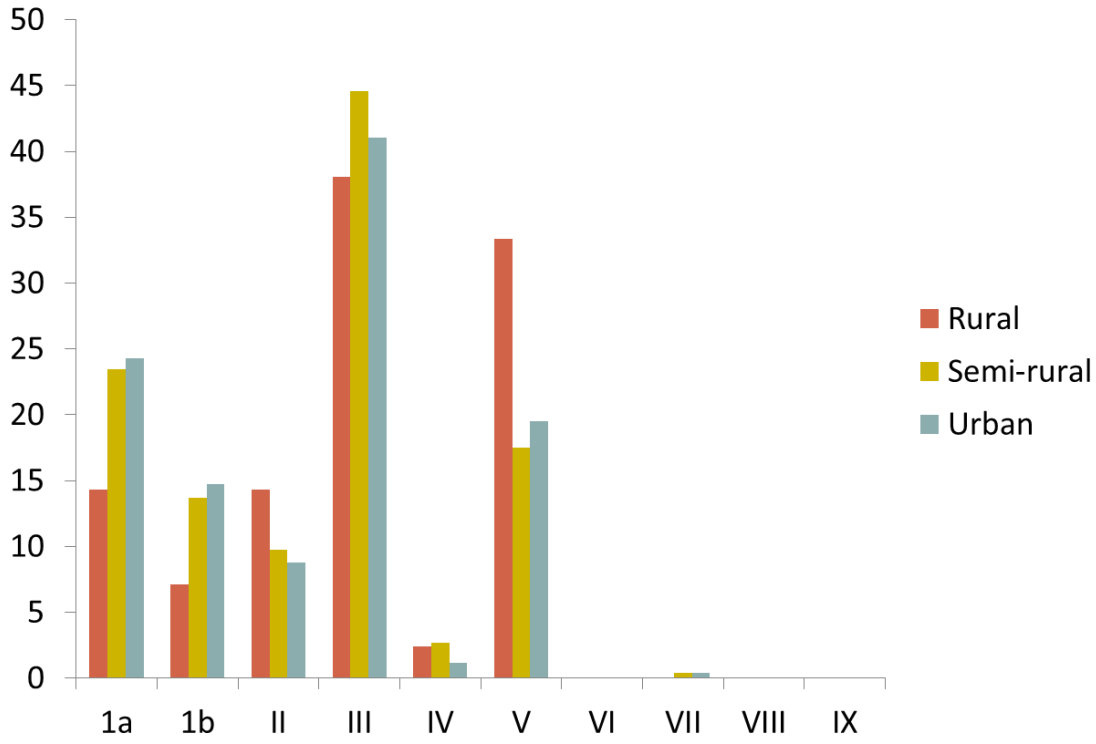


Figure 14 Bar chart of the GBS serotypes colonising mothers (N=910) at delivery by study site



**Rural N= 46, Semi-rural N=588, Urban N=276

Figure 15 UPGMA tree of serotype distribution of maternal GBS colonising isolates across all three sites*

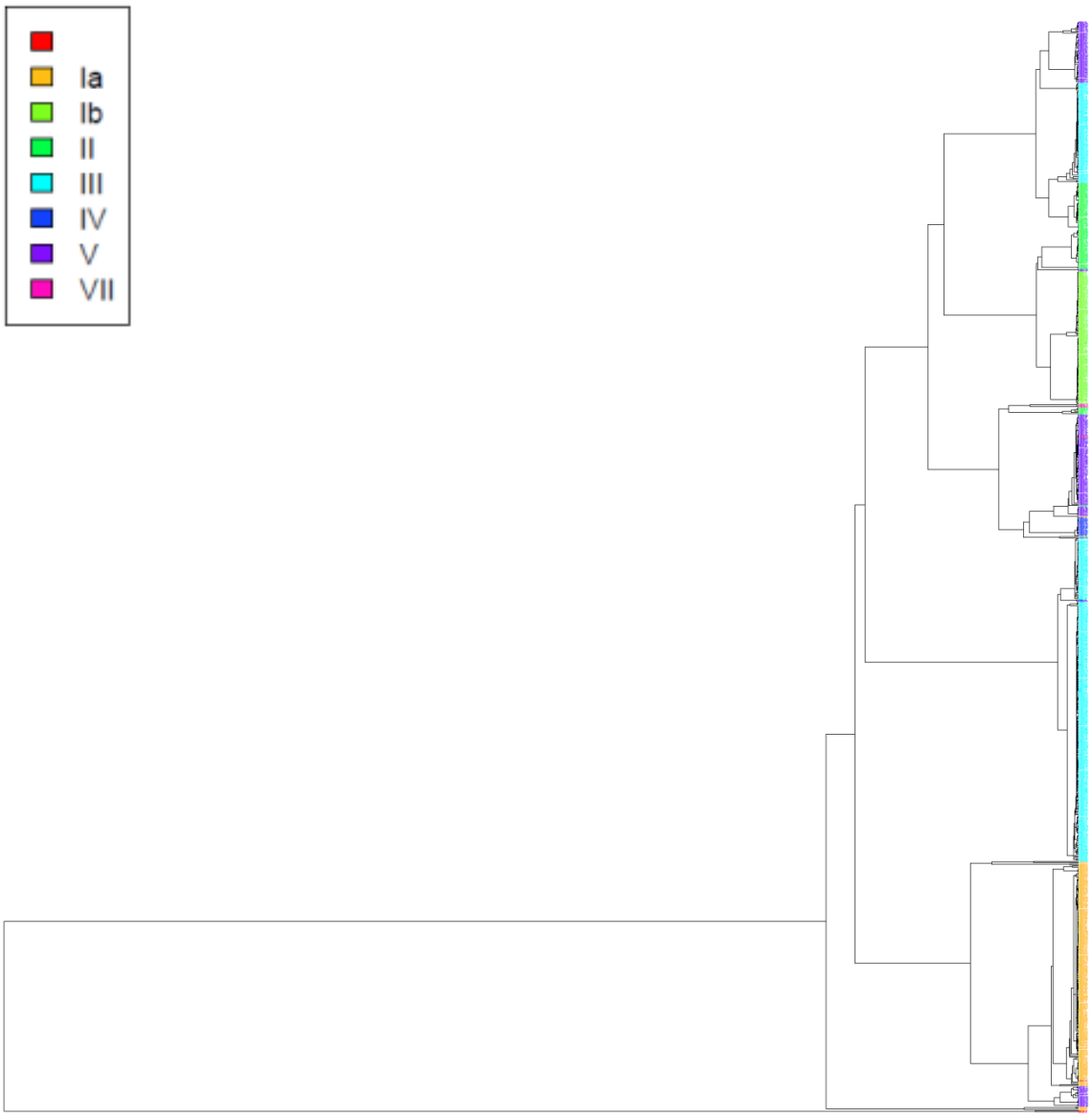
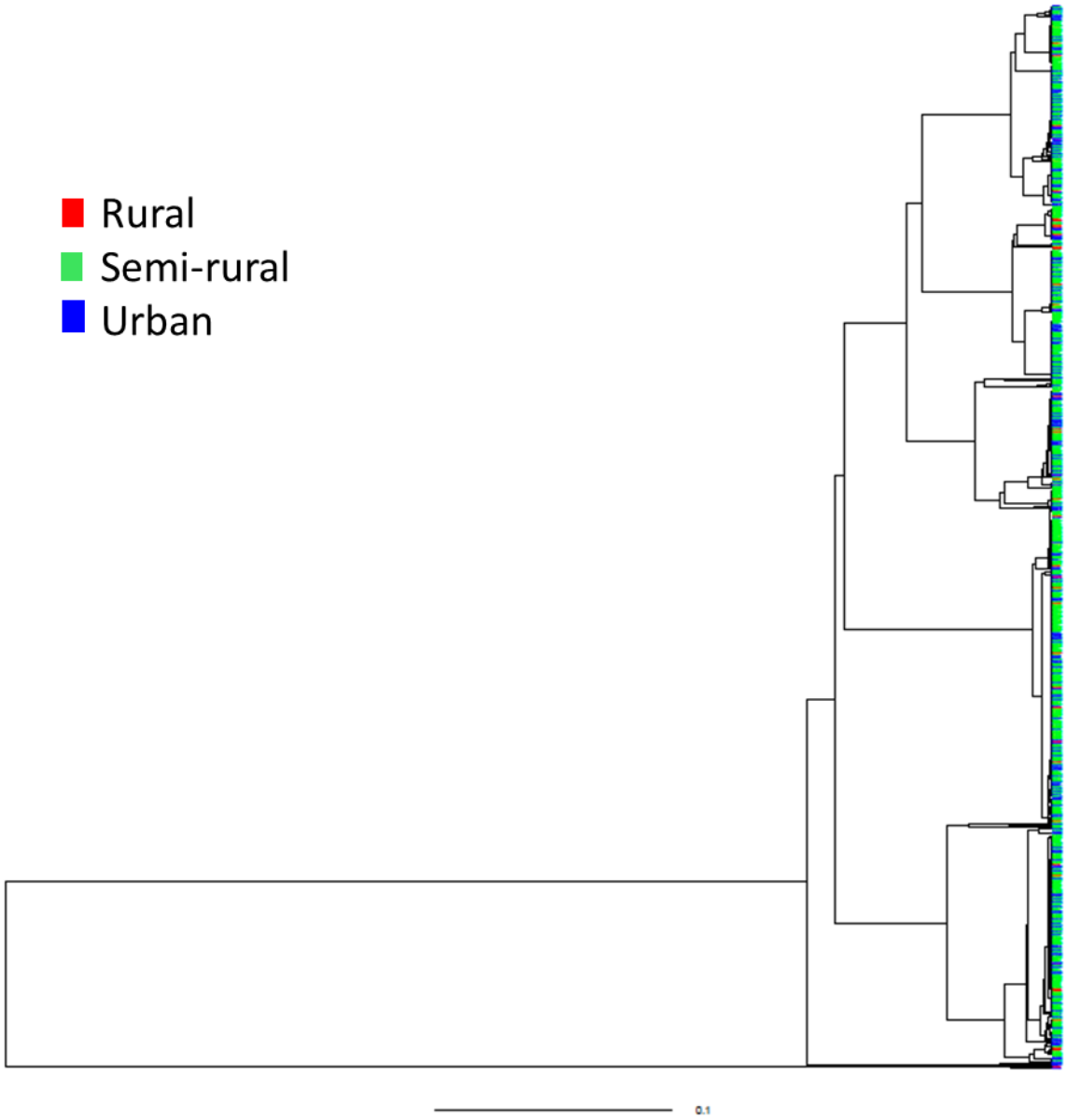


Figure 16 UPGMA tree of maternal GBS colonising isolates, coloured by site*



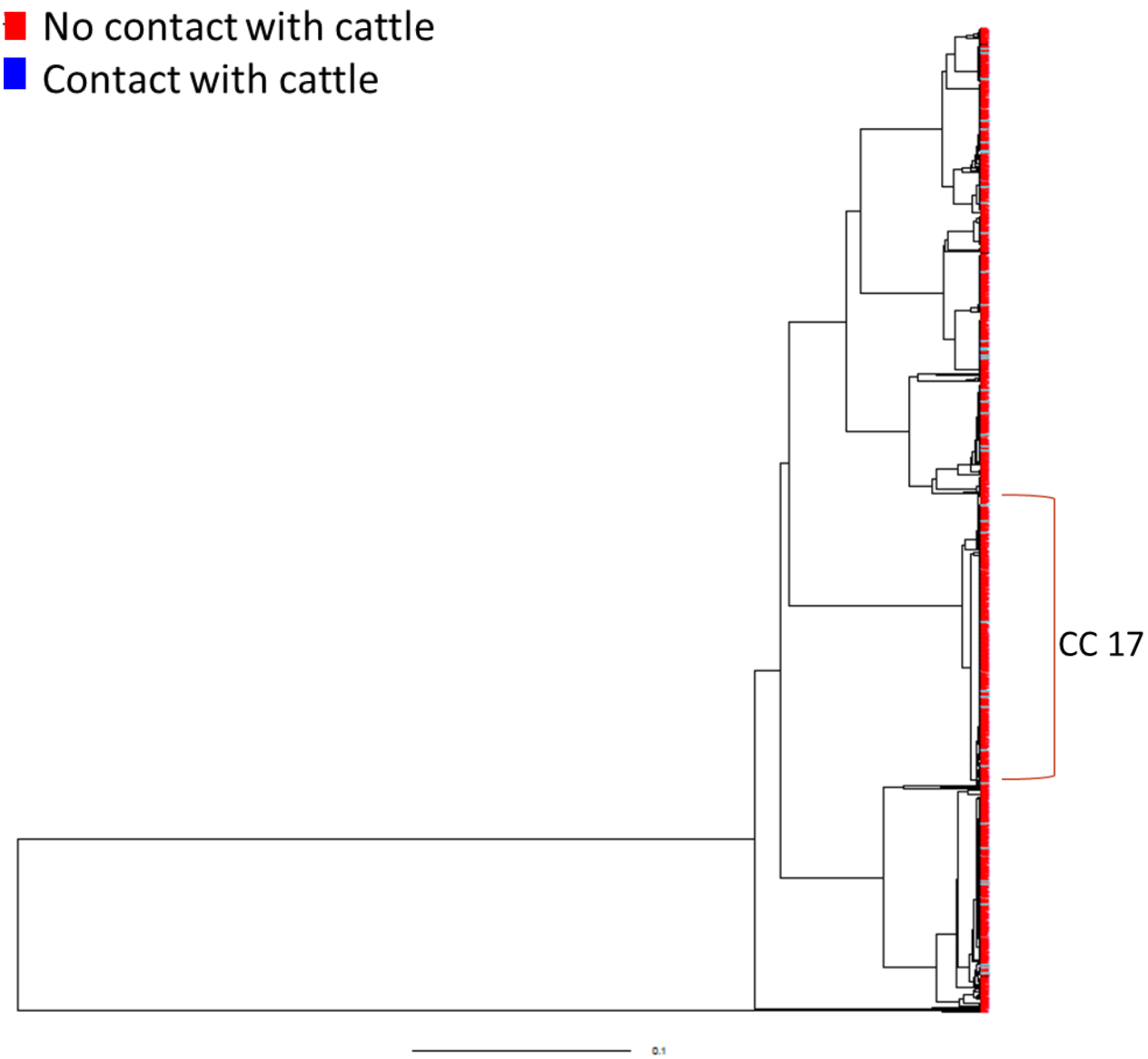
*The colours indicate the health facility the mother attended for delivery; rural (Bamba or Ganze), semi-rural (Kilifi District Hospital) and urban (Coast Provincial General Hospital). The UPGMA tree does not take into account recombination events and therefore may not fully reflect evolutionary time.

Table 12 Clonal complexes and MLST types of GBS colonising mothers at delivery across three study sites in coastal Kenya 2011-2013*

Clonal complex	MLST	N	(%)	N	(%)
	1	76	8.4		
	Close to 1	10	1.1		
	2	6	0.7		
	3	2	0.2		
	167	1	0.1		
1	196	16	1.8	111	12.2
	10	84	9.2		
	12	2	0.2		
	8	56	6.2		
	Close to 8	3	0.3		
10	Close to 10	2	0.2	147	16.2
	147	0	0.0		
	17	184	20.2		
	484	67	7.4		
	109	2	0.2		
	291	2	0.2		
	Close to 484	3	0.3		
17	Close to 17	13	1.4	271	29.8
	182	66	7.3		
	19	28	3.1		
	327	27	3.0		
	Close to 327	3	0.3		
	328	10	1.1		
19	28	38	4.2	172	18.9
	23	187	20.5		
	Close to 249	2	0.2		
	Close to 28	1	0.1		
	144	1	0.1		
	223	2	0.2		
	498	4	0.4		
	24	7	0.8		
23	55	1	0.1	205	22.5
	103	2	0.2		
Other	486	2	0.2	4	0.4

*There were no MLST types from clonal complex-26; where the MLST type is indicated "close to" all but one of the MLST genes match the MLST type.

Figure 17 UPGMA tree of maternal GBS colonising isolates across all sites*



*The isolates in red indicate the mother had no contact with cattle, in blue are GBS isolates from mothers who did have contact with cattle. This descriptive analysis suggests that the GBS isolates associated with cattle contact are spread across the clonal complexes, not clustered within clonal complex-17 (indicated). The UPGMA tree does not take into account recombination events and therefore may not fully reflect evolutionary time.

3.5 Discussion

3.5.1 Key Results

Maternal colonisation with GBS at delivery occurs across rural and urban regions in coastal Kenya, there were 934/7967 (11.7%, 95%CI 11.0-12.5%) mothers colonised with GBS at delivery, with the highest prevalence in the urban site 279/1971 (14.2%, 12.6-15.8%), compared to the semi-rural site 608/5470 (11.1%, 10.2-12.0%) and rural site 47/526 (8.9%, 6.6-11.7). Risk factors for maternal GBS colonisation fell into four groups; demographics and socio-economic status (age, parity, ethnicity, household SES), increased exposure (contact with cattle), co-morbidities (nutritional status, HIV infection) and factors at delivery (vaginal examination prior to recto-vaginal sampling, obstetric complication). There were a range of serotypes of GBS colonising mothers, although serotype Ia, Ib, III and V accounted for 89% of all serotypes isolated. There was genetic variation in GBS isolates within and between the three sites.

3.5.2 Interpretation

Prevalence

The prevalence of maternal GBS colonisation at delivery was at the lower end of the ranges reported from sub-Saharan Africa (9-47%) and in fact, worldwide.[94,96,110-114,151] However there were important differences in the sites investigated, with the highest GBS prevalence in the urban setting, and the lowest GBS prevalence in the rural setting. This reflected differences in demographics and exposures discussed below.

Level 1: Demographic factors and socio-economic status

Maternal GBS colonisation was lower in higher parity women, which concurred with the largest prospective study in the USA,[118] but not the large retrospective analysis, although this study may have been biased by misclassification of unscreened women as not GBS

colonised.[119] Maternal age was associated with maternal GBS colonisation, but the relationship was non-linear and this association would not necessarily have been seen depending on the categories used for maternal age, which may explain some of the different findings in the published literature, including those studies which reported no association.[94,119,120] A study from Korea found the reverse pattern reported here, with maternal GBS colonisation highest in the youngest and oldest age groups.[117] This could still be due to categorisation, but it may also be due to very different age structures in mothers included in the studies. There were only 93/2633 mothers under 25 years (3.6% of all participants) in the Korean study; in this study the majority of mothers were under 25 years of age. There were 390/2633 (14.8%) over 35 years of age in the Korean study, and 590/7967 (7.4%) in this study, which were included with over 30 year olds in the categorical analysis using quartiles of age.

Ethnicity was also identified as an important factor, as widely described in other studies,[118,119] although in this study, unlike most others, all the participants included were of African ethnicity. Within this, maternal GBS colonisation was found to be higher in women who were not Mijikenda (i.e. who were not the indigenous coastal population), but there was no difference in maternal GBS colonisation between the sub-divisions of the Mijikenda group and the non-Mijikenda group. In this population the Mijikenda are usually the most socio-economically disadvantaged group, and this association could be due to residual confounding, as adjusting for socio-economic status is difficult. Higher SES was associated with increased odds of maternal GBS colonisation, a finding supported by the large retrospective study from the USA, where increased educational attainment and highest income quintile were both associated with maternal GBS colonisation.[119] Genetic polymorphisms accounting for increased GBS colonisation in the non-Mijikenda seem less likely because of the heterogeneity of different ethnicities in this group. The supposition that

variations may be due to residual confounding is supported by the finding in other studies of similar ethnicities (New-York Hispanics and non-New York Hispanics) living in differing geographical regions having very different reported prevalence of maternal GBS colonisation.[118]

Level 2: Exposures and co-morbidities

Maternal GBS colonisation was highest in mothers with the highest mid-upper arm circumference (proxy for muscle and fat mass or body-mass index (BMI)). Three previous studies, including one from the Reunion islands, have also reported increased BMI being associated with maternal GBS colonisation.[119,120,123] The reasons for this are unclear; there could be a causal association, or residual confounding from socio-economic status.

For maternal HIV infection, there was a substantial decrease in maternal GBS colonisation in women who were HIV infected and used co-trimoxazole prophylaxis, an association which has not previously been shown. It was previously examined in a sub-analysis of a GBS maternal colonisation study in Malawi, which reported lower maternal GBS colonisation with lower CD4 counts. This study used lack of previous HIV testing or care before the participants' PMTCT test as a proxy for not taking co-trimoxazole,[124] and found no evidence for a difference in trends in maternal GBS colonisation with CD4 count between this group (n=277) and those who had previously been diagnosed with HIV (n=125). This could be due to misclassification (it was not reported how many previously diagnosed HIV infected individuals were taking co-trimoxazole) or chance, as the numbers in the sub-analysis were small, or residual confounding by nutritional or socio-economic status. The association of maternal GBS with co-trimoxazole prophylaxis found here may help explain differences in findings in other studies. For example, women with HIV infection in the United States are unlikely to be taking co-trimoxazole as they have better access to anti-retroviral therapy, or

which would also reduce the need for other antibiotic treatment for concurrent illnesses, and therefore an association between HIV and maternal GBS colonisation would be less likely identified, as reported.[120] In the study from South Africa where a reduction in maternal GBS colonisation was reported with HIV infection, it was also noted that this group was more likely to have had antibiotics before delivery,[162] and antibiotic treatment could be important in HIV women in the context of concurrent illnesses and more frequent antibiotic medication, even if not on regular co-trimoxazole prophylaxis. However, in this study there was no association between antibiotic use in the four weeks prior to admission for delivery and maternal GBS colonisation. This may be because many mothers were uncertain as to the medication (if any) that they had taken, which may have resulted in misclassification and biased the results towards a null finding.

The association of increased maternal GBS colonisation with cattle exposure was interesting because of the hypothesised transmission between humans and bovines reported,[214] and the hypothesis that hyper invasive neonatal GBS (MLST 17) arose from a bovine strain ancestor.[73] However the UPGMA tree showed that the strains isolated from mothers with contact with cattle were diverse, rather than clustered in clonal complex 17. The finding does, however, support the theory that interspecies transmission (either from bovines to humans or vice versa) is ongoing. This could be confounded by a dietary relationship with milk (milk seems more likely to be consumed (and unpasteurised) by those who have cattle) and further work would be required to explore this.

Level 3: Factors at delivery

There was a strong association between maternal vaginal examination before sample taking and maternal GBS colonisation, as reported elsewhere,[103] which could be related to anti-sepsis use or mechanical removal. The association between an obstetric complication and

lower odds of GBS isolation could relate to bias (women with lower socio-economic status were more likely to attend hospital for delivery only with a problem) or if the complication were haemorrhage, this could reduce the likelihood of detecting maternal GBS by mechanical washing away. No association was identified between prolonged rupture of membranes (≥18h) and maternal GBS colonisation status, but this factor may be more important in facilitating invasion of GBS and causing neonatal disease.

GBS Serotypes colonising mothers

The GBS serotypes colonising mothers reported here were as expected based on a recent review. In Europe, the Middle East, Africa, Australia and Asia serotype III predominated whilst in the Americas serotype Ia was predominant.[142] In Europe types III, Ia, II and V have been most commonly reported,[97,215] as in a recent study in South Africa,[143] but in contrast to data from the Thai-Burmese border where serotype II, and VI were commonly isolated.[144] Whilst the serotypes reported colonising mothers here were therefore consistent with other studies in Africa, Europe and to a slightly lesser extent, the United States, there may be greater geographical differences to other regions where there are fewer data, such as Southeast Asia.

3.5.3 Strengths and Limitations

The prevalence of maternal GBS colonisation was low, and although analyses were undertaken to validate the methods in terms of sensitivity of detection of GBS in terms of time to processing, recent advances have suggested that classical culture methods are less sensitive than nucleic-acid amplification tests in detection of maternal GBS colonisation,[216] and revised CDC guidelines in 2012 (since the start of the study) suggested that these nucleic-acid amplification tests, and selective media, should be considered for use in screening programmes.[106] The prevalence of maternal GBS colonisation may therefore have been

underestimated. However, as these were relatively recent guidelines, the studies to which we are comparing results, and which have reported higher GBS colonisation prevalence, were also using classical microbiological culture methods, rather than nucleic-acid amplification, so the comparison of prevalence with other settings would hold, as well as the risk factors for maternal GBS colonisation.

This study was health facility based, and whilst this was mitigated to some extent by recruitment at a range of health facilities, these findings cannot necessarily be extrapolated to women who delivered outside of a health facility. There was some evidence of selection bias in recruitment, either through self-selection (more educated women were more likely to withhold consent) or missed opportunities, which were more common in those mothers who presented as emergencies, despite the assent procedure which aimed to facilitate their inclusion. However, the proportion and number of women who were missed in sampling, or who withheld consent, was still low, and unlikely to affect the findings.

The clinical data were collected by clinical staff, and in this setting missing data were, to some extent, unavoidable. Bias that this could have introduced was reduced by using multiple imputations to as well as complete case analyses for multivariable analyses. It was notable that there was much greater consistency across the univariable and multivariable imputed models, compared to the complete case models where the number of participants varied considerably and estimates of effect size fluctuated. This suggests that the imputed model was appropriate and the results from the imputed data were more robust.

Although many exposures were adjusted for in multivariable analyses, there was likely to have been residual confounding. Principal component analysis was used to maximise the information on socio-economic status, but this remains a difficult exposure to capture, and the association of maternal GBS colonisation with ethnicity may be due to residual

confounding. There were also variables which were not included that could be important, either as exposures or as confounders, including the number of sexual partners, or smoking, although this is rare in this (female) population.

It is also possible that some results were identified by chance, even though this was a relatively large and adequately powered prospective study, with risk factors hypothesised prior to the study start. There were small numbers of HIV positive women, especially subdivided into those on co-trimoxazole (n=239) and those not (n=161); however the association between maternal GBS colonisation and co-trimoxazole use had a large effect size and was biologically very plausible, making it unlikely to be a chance finding.

In terms of the molecular analysis, the serotype allocation had very high agreement as measured by the kappa statistic, with both serological methods and GBS typed by PCR, so the serotypes were likely accurate. The UPGMA trees, although broadly representative of the phylogeny, do not taken into account recombination events, so the depth of some of the branches may be increased in the illustrations, not accurately reflecting evolutionary time.

3.5.4 Conclusions

This was the largest and most comprehensive study of the prevalence and risk factors for maternal GBS colonisation at delivery in sub-Saharan Africa to date. The participants differed considerably to other populations outside of sub-Saharan Africa, but this was a strength of the study in that maternal GBS colonisation could be examined in a population with extremes; the ranges in maternal demographics, socio-economic status, nutritional status, relatively common exposure to cattle, and exposure to co-morbidities such as HIV infection are not frequently seen in other settings, and allow examination of these risk factors.

Despite this, the findings were consistent with the larger studies of GBS colonisation, mostly from the United States, showing that increased socio-economic status and nutritional status

are important risk factors for maternal GBS colonisation. The relationship with ethnicity is complex, but it seems more likely that in this context it results from environmental factors rather than genetic differences.

These findings are important, as risk factors such as increased socio-economic and nutritional status may help explain increased maternal GBS colonisation in resource-rich settings. They also raise concern; as Africa becomes more urbanised and moves through the epidemiological transition, maternal GBS colonisation may increase, with the potential to increase associated neonatal GBS disease.

Chapter 4: Maternal GBS colonisation and perinatal disease

4.1 Results

4.1.1 Summary

This chapter describes the results of the cohort study but considers maternal GBS colonisation as the exposure of interest and gestation and low birth weight at delivery, possible severe bacterial infection, stillbirth and perinatal mortality as the outcomes of interest. It addresses the following questions:

1. Is maternal GBS colonisation associated with gestation at delivery?
2. Is maternal GBS colonisation associated with birth weight at delivery?
3. Is maternal GBS colonisation associated with possible severe bacterial infection?
4. Is maternal GBS colonisation associated with stillbirth?
5. Is maternal GBS colonisation associated with overall perinatal mortality?

4.1.2 Participants

There were 10131 women who attended the health facilities during the period of recruitment, of whom 618 were ineligible (no delivery); there were 1546 women who were eligible but not recruited. Of the 7967 women who were recruited, there were 8191 births, of which 7741 were singletons, 441 were twins and 9 were triplets. After exclusion of new-borns on whom outcomes were missing, there were 7833 deliveries (Figure 18).

The characteristics of the participants are given in Table 13. Overall there were 525/7833 (6.7%) perinatal deaths; 425/7833 (5.4%) were stillbirths. There were 390/7408 cases (5.3%) of possible severe bacterial infection, and 220/7409 (3.0%) neonates were very low birth weight (VLBW, <1500g), 1373/7409 (18.5%) neonates were low birth weight (LBW, 1500-2499g); 537/7408 (7.2%) neonates were very preterm (28-31 weeks) and 2005/7408 (27.1%) neonates were preterm (32-36 weeks). The percentage of all adverse perinatal outcomes, except low birth weight, was lower in mothers who were colonised with GBS.

Figure 18 Recruitment of mothers and new-borns to cohort study of perinatal outcomes

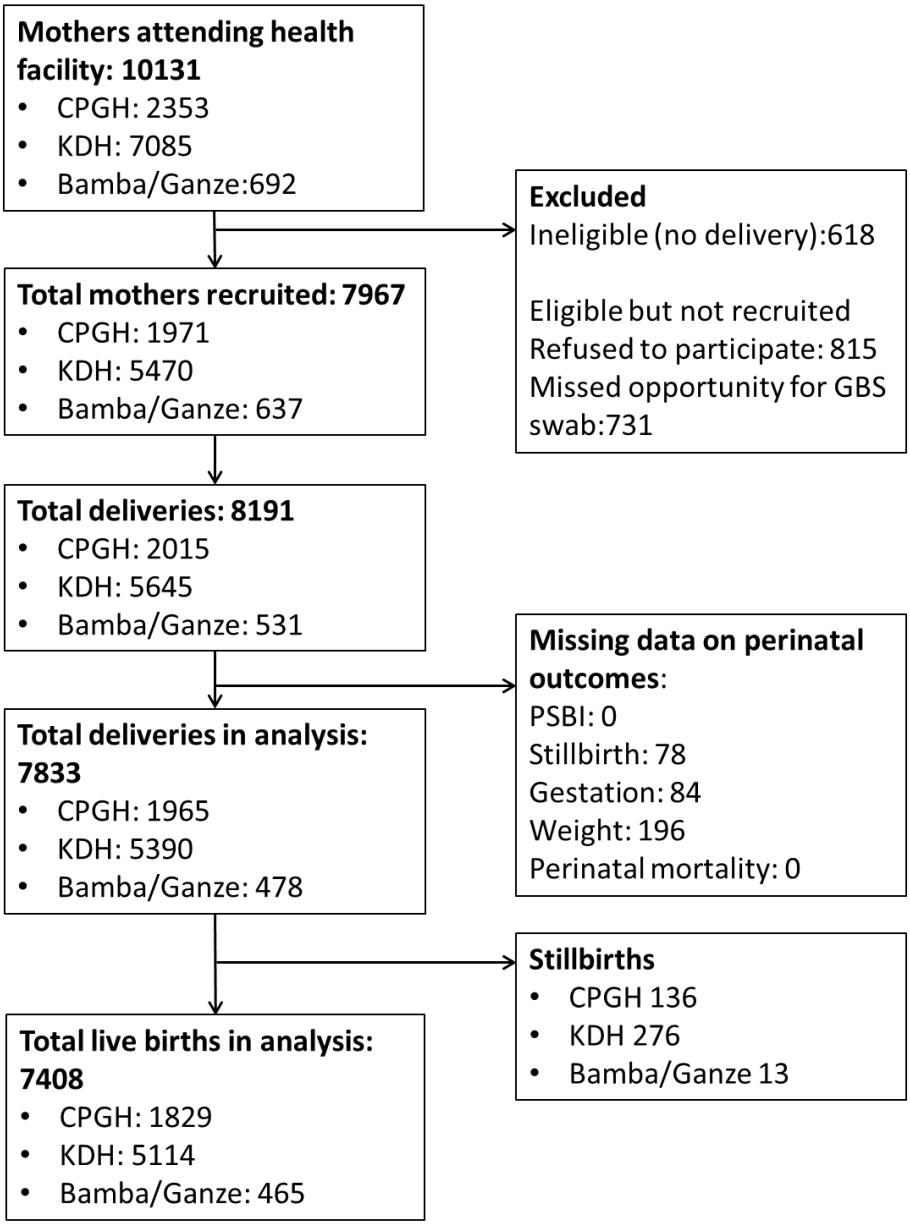


Table 13 Characteristics of participants and perinatal outcomes

Variable	Category	Births	Live births	Perinatal mortality		Stillbirth		PSBI		VLBW		LBW		Very Preterm		Preterm	
				N	%	N	%	N	%	N	%	N	%	N	%	N	%
	Total	**7833**	**7408**	**525**	6.7	**425**	5.4	**390**	5.3	**220**	3.0	**1373**	18.5	**537**	7.2	**2005**	27.1
GBS	Negative	**6903**	**6522**	**469**	6.8	**381**	5.5	**344**	5.3	**206**	3.2	**1205**	18.5	**495**	7.6	**1773**	27.2
	Positive	**930**	**886**	**56**	6.0	**44**	4.7	**46**	5.2	**14**	1.6	**168**	19.0	**42**	4.7	**232**	26.2
Age in quartiles	1	**1632**	**1564**	**89**	5.5	**68**	4.2	**92**	5.9	**49**	3.1	**323**	20.7	**144**	9.2	**440**	28.1
	2	**1640**	**1542**	**114**	7.0	**98**	6.0	**90**	5.8	**48**	3.1	**284**	18.4	**101**	6.5	**409**	26.5
	3	**1634**	**1547**	**109**	6.7	**87**	5.3	**98**	6.3	**41**	2.7	**245**	15.8	**101**	6.5	**380**	24.6
	4	**1651**	**1541**	**139**	8.4	**110**	6.7	**93**	6.0	**49**	3.2	**276**	17.9	**106**	6.9	**413**	26.8
	Missing	**1276**	**1214**	**74**	5.8	**62**	4.9	**17**	1.4	**33**	2.7	**245**	20.2	**85**	7.0	**363**	29.9
Parity	0	**2931**	**2777**	**183**	6.2	**154**	5.3	**148**	5.3	**77**	2.8	**614**	22.1	**194**	7.0	**703**	25.3
	1 to 4	**3498**	**3334**	**209**	6.0	**164**	4.7	**169**	5.1	**93**	2.8	**509**	15.3	**216**	6.5	**858**	25.7
	≥5	**1320**	**1219**	**126**	9.5	**101**	7.7	**63**	5.2	**48**	3.9	**232**	19.0	**123**	10.1	**414**	34.0
	Missing	**84**	**78**	**7**	8.3	**6**	7.1	**10**	12.8	**2**	2.6	**18**	23.1	**4**	5.1	**35**	44.9
Education	None	**1121**	**1049**	**85**	7.6	**72**	6.4	**40**	3.8	**38**	3.6	**192**	18.3	**116**	11.1	**334**	31.8
	1º	**4355**	**4108**	**305**	7.0	**247**	5.7	**233**	5.7	**137**	3.3	**802**	19.5	**325**	7.9	**1199**	29.2
	2º	**1543**	**1467**	**94**	6.1	**76**	4.9	**92**	6.3	**27**	1.8	**278**	19.0	**54**	3.7	**331**	22.6
	3º	**554**	**533**	**27**	4.9	**21**	3.8	**20**	3.8	**9**	1.7	**57**	10.7	**22**	4.1	**80**	15.0
	Missing	**260**	**251**	**14**	5.4	**9**	3.5	**5**	2.0	**9**	3.6	**44**	17.5	**20**	8.0	**61**	24.3
Socio-economic status	Very low	**1079**	**1009**	**89**	8.2	**70**	6.5	**49**	4.9	**38**	3.8	**195**	19.3	**96**	9.5	**323**	32.0
	Low	**2640**	**2503**	**172**	6.5	**137**	5.2	**91**	3.6	**90**	3.6	**505**	20.2	**199**	8.0	**741**	29.6
	Medium	**2100**	**1991**	**136**	6.5	**109**	5.2	**102**	5.1	**51**	2.6	**344**	17.3	**110**	5.5	**500**	25.1
	High	**2014**	**1905**	**128**	6.4	**109**	5.4	**148**	7.8	**41**	2.2	**329**	17.3	**132**	6.9	**441**	23.1

Variable	Category	Births	Live births	Perinatal mortality		Stillbirth		PSBI		VLBW		LBW		Very Preterm		Preterm	
				N	%	N	%	N	%	N	%	N	%	N	%	N	%
Nutritional status (quartiles of MUAC)*	1	**1398**	**1336**	**85**	6.1	**62**	4.4	**56**	4.2	**52**	3.9	**315**	23.6	**122**	9.1	**402**	30.1
	2	**2094**	**2005**	**109**	5.2	**89**	4.3	**95**	4.7	**56**	2.8	**382**	19.1	**158**	7.9	**556**	27.7
	3	**1652**	**1546**	**122**	7.4	**106**	6.4	**85**	5.5	**39**	2.5	**285**	18.4	**116**	7.5	**427**	27.6
	4	**2140**	**2014**	**161**	7.5	**126**	5.9	**132**	6.6	**58**	2.9	**284**	14.1	**97**	4.8	**494**	24.5
	Missing	**549**	**507**	**48**	8.7	**42**	7.7	**22**	4.3	**15**	3.0	**107**	21.1	**44**	8.7	**126**	24.9
HIV status	-ve	**7166**	**6796**	**463**	6.5	**370**	5.2	**348**	5.1	**194**	2.9	**1238**	18.2	**473**	7.0	**1806**	26.6
	+ve	**237**	**215**	**23**	9.7	**22**	9.3	**10**	4.7	**9**	4.2	**52**	24.2	**22**	10.2	**70**	32.6
	+ve+Cotrim	**157**	**148**	**12**	7.6	**9**	5.7	**13**	8.8	**4**	2.7	**28**	18.9	**11**	7.4	**36**	24.3
	Missing	**273**	**249**	**27**	9.9	**24**	8.8	**19**	7.6	**3**	1.2	**55**	22.1	**31**	12.4	**93**	37.3
Obstetric complications	No	**6793**	**6539**	**335**	4.9	**254**	3.7	**312**	4.8	**139**	2.1	**1051**	16.1	**407**	6.2	**1677**	25.6
	Yes	**1040**	**869**	**190**	18.3	**171**	16.4	**78**	9.0	**81**	9.3	**322**	37.1	**130**	15.0	**328**	37.7

*Mid-upper arm circumference (quartiles)

4.2 Is maternal GBS colonisation associated with preterm delivery?

Maternal GBS colonisation at delivery was examined as a factor associated with gestational age group at delivery in logistic regression analyses, allowing for clustering for site. Ordinal logistic regression analyses were first undertaken but the assumption of proportional odds was not met, so multivariate analyses were undertaken, making no assumptions about proportionality across gestational age categories. The univariable, multivariable complete case analyses (N=4993) and multivariable imputed analyses (N=7408) are given in detail in the three tables below, with the variables which were adjusted for in Table 15 and Table 16, based on causal modelling. The effects are presented as exponentiated coefficients from a multinomial model (Stata version 13); these multinomial odds ratios are also referred to as relative risk ratios, but as this may cause confusion with the term risk ratio, so they are called multinomial odds ratios ($_m$OR) here, to reflect their calculation by comparison to the reference category (here the "normal" gestation category).[217] Examination of continuous variables (maternal age, nutritional status, maternal haemoglobin and maternal temperature) was undertaken with natural splines prior to categorisation (presented here), graphical representations of these relationship are included in the appendices, page 247.

There was no evidence against the null hypothesis of no association between preterm ((<37 wks, ≥32 weeks), $_m$OR 0.85 (0.65-1.10) p=0.2) and very preterm ((<32 weeks), $_m$OR 0.63 (0.39-1.02) p=0.60) deliveries and maternal GBS colonisation in mothers with a temperature in the normal range (36.5^{o}C-37.5^{o}C) in univariable analyses. However, post-dates delivery was associated with higher odds of maternal GBS colonisation at delivery in univariable analysis ($_m$OR 1.20 (1.04-1.38), p=0.014), and in the imputed multivariable analysis ($_m$OR 1.17 (1.00-1.38), p=0.049).

There was evidence of effect modification with maternal axilliary temperature at delivery for very preterm deliveries. Although the absolute numbers were small, the effect size was large and consistent in univariable and imputed multivariable analyses. In the presence of fever (>37.5 ^{o}C) maternal GBS colonisation was associated with very preterm delivery in univariable analyses ($_{m}$OR 4.65 (2.39-9.03), p <0.001) and imputed multivariable analyses ($_{m}$OR 3.54 (1.23-10.2). p=0.019). In the complete case analysis, this association was not seen ($_{m}$OR 1.30 (0.45-3.81), p=0.6).

The exposures that were associated with increased maternal GBS colonisation (higher socioeconomic status, nutritional status, HIV uninfected mothers) were usually associated with term delivery (37-42 weeks). Exposures associated with decreased maternal GBS colonisation (high parity, obstetric complication at delivery) were associated with delivery either being preterm or post-dates (see Table 14, Table 15, Table 16).

Table 14 Association between maternal GBS colonisation and gestational age, in three sites in coastal Kenya – univariable analyses

		Post- dates (>42wks)			Preterm (≥32 wks <37 wks)			Very preterm (<32 wks)		
		$_{m}OR^{\#\#}$	CI*	p**	$_{m}OR^{\#\#}$	CI*	p**	$_{m}OR^{\#\#}$	CI*	p**
	Neg	1			1			1		
GBS	Pos	1.20	(1.04-1.38)	0.014	0.85	(0.65-1.10)	0.2	0.63	(0.39-1.02)	0.060
	<36.5	1.01	(0.93-1.09)	0.9	1.09	(0.99-1.19)	0.09	0.90	(0.88-0.92)	<0.001
	36.5-37.5	1								
GBS-ve	>37.5	1.48	(0.99-2.21)	0.06	1.79	(0.92-3.51)	0.09	1.25	(0.49-3.2)	0.6
	<36.5	0.93	(0.70-1.24)	0.6	1.23	(0.85-1.77)	0.3	1.10	(0.90-1.36)	0.4
GBS +ve	>37.5	1.42	(0.31-6.54)	0.7	0.67	(0.23-1.92)	0.5	4.65	(2.39-9.03)	<0.001
	1	1.10	(0.78-1.55)	0.6	1.31	(1.20-1.43)	<0.001	1.62	(0.83-3.12)	0.2
	2	1.09	(0.85-1.38)	0.9	1.11	(0.98-1.27)	0.1	1.06	(0.84-1.33)	0.6
Age in	3	1			1			1		
quartiles	4	1.06	(1.03-1.09)	0.9	1.12	(0.84-1.49)	0.4	0.97	(0.85-1.10)	0.6
	0	1.17	(1.06-1.28)	0.001	1.03	(0.87-1.21)	0.7	1.18	(0.93-1.50)	0.2
	1-4	1			1			1		
Parity	≥5	1.36	(1.22-1.51)	<0.001	1.56	(1.50-1.64)	<0.001	1.88	(1.74-2.03)	<0.001
	Female	1			1			1		
Sex	Male	0.93	(0.71-1.22)	0.6	0.98	(0.89-1.08)	0.7	0.91	(0.81-1.03)	0.1
	None	1.37	(1.19-1.58)	<0.001	1.24	(1.16-1.33)	<0.001	1.50	(0.92-2.46)	0.1
	1o	1			1			1		
	2o	0.69	(0.57-0.84)	<0.001	0.63	(0.54-0.75)	<0.001	0.34	(0.15-0.76)	0.008
Education	3o	0.89	(0.60-1.31)	0.6	0.43	(0.25-0.72)	0.002	0.42	(0.35-0.50)	<0.001

		Post- dates (>42wks)			Preterm (≥32 wks <37 wks)			Very preterm (<32 wks)		
		$_{m}$OR##	CI*	p**	$_{m}$OR##	CI*	p**	$_{m}$OR##	CI*	p**
Socio-economic status	V low	1.03	(0.83-1.27)	0.8	1.10	(1.04-1.17)	0.002	1.33	(0.99-1.78)	0.056
	Low	1			1			1		
	Med	0.79	(0.75-0.83)	<0.001	0.73	(0.70-0.76)	<0.001	0.65	(0.55-0.77)	<0.001
	High	0.75	(0.58-0.97)	0.027	0.66	(0.47-0.54)	0.018	0.74	(0.54-1.03)	0.079
Nutritional status (MUAC)	1	1.18	(0.90-1.54)	0.2	1.17	(1.12-1.22)	<0.001	1.32	(0.91-1.90)	0.1
	2	1			1			1		
	3	1.01	(0.90-1.13)	0.9	0.91	(0.80-1.04)	0.2	0.85	(0.79-0.92)	<0.001
	4	1.05	(0.96-1.16)	0.3	0.77	(0.69-0.86)	<0.001	0.47	(0.41-0.55)	<0.001
HIV status#	neg	1			1			1		
	pos	0.69	(0.64-0.75)	<0.001	1.24	(0.98-1.56)	0.074	1.14	(0.57-2.27)	0.7
	pos &c	1.44	(1.21-1.73)	<0.001	1.00	(0.68-1.47)	0.9	0.95	(0.77-1.17)	0.6
Obst. Comp.	No	1			1			1		
	Yes	0.98	(0.85-1.13)	0.8	1.42	(1.22-1.65)	<0.001	2.10	(1.18-3.74)	0.011
Multiple delivery	No	1			1			1		
	Yes	1.43	(0.96-2.13)	0.080	2.29	(1.68-3.13)	<0.001	2.96	(1.71-5.14)	<0.001

*based on robust standard errors to account for intracluster correlation within recruitment sites

** Wald test

#HIV positive and on co-trimoxazole prophylaxis

##exponentiated coefficients from an -mlogit- model, these multinomial odds ratios are also referred to as relative risk ratios (but these are not risk ratios)

Table 15 Association between maternal GBS colonisation and gestational age, in three sites in coastal Kenya – complete case analyses (N=4993)

		Post- dates (>42wks)			Preterm (≥32 wks <37 wks)			Very preterm (<32 wks)		
		$_{m}OR^{\#\#}$	CI*	p**	$_{m}OR^{\#\#}$	CI*	p**	$_{m}OR^{\#\#}$	CI*	p**
	Neg	1			1			1		
GBS	Pos	1.25	(0.98 -1.60)	0.073	1.06	(0.80-1.40)	0.7	0.77	(0.44-1.35)	0.4
	<36.5	1.06	(0.96-1.19)	0.3	1.19	(1.08-1.95)	0.001	0.97	(0.88-0.92)	<0.001
	36.5-37.5	1			1			1		
GBS-ve	>37.5	1.32	(0.80-2.20)	0.3	1.39	(0.99-1.95)	0.057	1.44	(0.59-3.52)	0.4
	<36.5	0.82	(0.52-1.30)	0.4	1.02	(0.63-1.64)	0.9	0.97	(0.81-1.16)	0.7
GBS +ve	>37.5	1.46	(0.38-5.55)	0.6	0.59	(0.18-1.91)	0.4	1.30	(0.45-3.81)	0.6
	1	1.00	(0.62-1.61)	0.9	1.27	(0.99-1.65)	0.060	1.53	(0.94-2.52)	0.090
	2	0.86	(0.46-1.60)	0.6	1.21	(1.00-1.47)	0.048	1.09	(0.89-1.33)	0.4
Age in	3	1			1			1		
quartiles	4	0.79	(0.65-0.97)	0.024	1.02	(0.69-1.49)	0.9	0.78	(0.56-1.10)	0.2
	0	1.12	(0.74-0.70)	0.6	0.95	(0.68-1.32)	0.7	1.06	(0.74-1.50)	0.8
	1-4	1			1			1		
Parity	≥5	1.28	(1.11-1.47)	0.001	1.29	(1.07-1.56)	0.009	1.85	(1.13-3.03)	0.014
	Female	1			1			1		
Sex	Male	0.91	(0.68-1.23)	0.6	1.01	(0.85-1.21)	0.9	0.83	(0.80-0.86)	<0.001
	None	1.38	(1.29-1.47)	<0.001	1.17	(1.06-1.28)	0.001	1.42	(0.84-2.38)	0.2
	1o	1			1			1		
	2o	0.81	(0.76-0.85)	<0.001	0.74	(0.62-0.89)	0.001	0.41	(0.24-0.70)	0.001
Education	3o	0.97	(0.59-1.61)	0.9	0.57	(0.38-0.85)	0.006	0.55	(0.34-0.90)	0.017

		Post- dates (>42wks)			Preterm (>32 wks <37 wks)			Very preterm (<32 wks)		
		mOR##	CI*	p**	mOR##	CI*	p**	mOR##	CI*	p**
Socio-economic status	V low	1.00	(0.69-1.45)	0.9	0.88	(0.83-0.92)	<0.001	1.19	(0.76-1.84)	0.4
	Low	1			1			1		
	Med	0.86	(0.70-1.04)	0.1	0.73	(0.65-0.82)	<0.001	0.90	(0.74-1.09)	0.3
	High	0.86	(0.80-0.92)	<0.001	0.93	(0.75-1.16)	0.5	1.53	(1.34-1.74)	<0.001
Nutritional status (MUAC)	1	1.06	(0.73-1.54)	0.7	1.06	(1.01-1.11)	0.016	1.19	(0.60-2.36)	0.6
	2	1			1			1		
	3	1.20	(1.01-1.42)	0.034	0.92	(0.81-1.04)	0.2	0.89	(0.74-1.07)	0.2
	4	1.32	(1.13-1.55)	0.001	0.91	(0.82-1.02)	0.1	0.61	(0.57-0.65)	<0.001
HIV status[#]	neg	1			1			1		
	pos	0.96	(0.82-1.14)	<0.001	1.19	(1.01-1.39)	0.033	1.11	(0.49-2.51)	0.8
	pos &c	1.56	(1.29-1.89)	<0.001	1.11	(0.78-1.58)	0.6	1.15	(1.06-1.24)	0.001
Obst. Comp.	No	1			1			1		
	Yes	1.08	(0.84-1.39)	0.7	1.46	(1.33-1.60)	<0.001	2.10	(1.26-3.51)	0.004
Multiple delivery	No	1			1			1		
	Yes	1.52	(1.30-1.77)	<0.001	2.81	(1.59-4.94)	<0.001	2.10	(1.39-3.18)	<0.001

*based on robust standard errors to account for intracluster correlation within recruitment sites

** Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

[##]exponentiated coefficients from an -mlogit- model, these multinomial odds ratios are also referred to as relative risk ratios (but these are not risk ratios)

Table 16 Association between maternal GBS colonisation and gestational age, in three sites in coastal Kenya– imputed analyses (N=7408)

		Post- dates (>42wks)			Preterm (≥32 wks <37 wks)			Very preterm (<32 wks)		
		$_{m}OR^{\#\#}$	CI	p	$_{m}OR^{\#\#}$	CI	p	$_{m}OR^{\#\#}$	CI	p
	Neg	1			1			1		
GBS	Pos	1.17	(1.00-1.38)	0.049	0.91	(0.69-1.21)	0.5	0.65	(0.40-1.06)	0.083
	<36.5	1.03	(0.93-1.14)	0.6	1.11	(1.03-1.19)	0.004	0.92	(0.84-1.01)	0.065
	36.5-37.5	1								
GBS-ve	>37.5	1.36	(0.84-2.20)	0.2	1.56	(0.86-2.84)	0.1	1.02	(0.44-2.36)	0.9
	<36.5	0.94	(0.73-1.21)	0.6	1.21	(0.77-1.89)	0.4	1.15	(0.88-1.51)	0.3
GBS +ve	>37.5	1.26	(0.31-5.10)	0.7	0.60	(0.21-1.73)	0.3	3.54	(1.23-10.2)	0.019
	1	1.03	(0.89-1.22)	0.6	1.24	(0.99-1.54)	0.056	1.59	(1.01-2.50)	0.045
	2	0.99	(0.66-1.54)	0.9	1.12	(0.92-1.36)	0.4	1.13	(0.89-1.43)	0.3
Age in quartiles	3	1			1			1		
	4	0.94	(0.70-1.24)	0.6	0.96	(0.73-1.26)	0.8	0.78	(0.67-0.90)	0.001
	0	1.21	(1.06-1.38)	0.004	0.98	(0.76-1.25)	0.8	0.99	(0.78-1.24)	0.9
	1-4	1			1			1		
Parity	≥5	1.18	(1.02-1.36)	0.026	1.43	(1.21-1.68)	<0.001	1.73	(1.16-2.58)	0.007
	Female	1			1			1		
Sex	Male	0.91	(0.71-1.17)	0.5	0.96	(0.87-1.06)	0.4	0.89	(0.79-1.00)	0.046
	None	1.41	(1.19-1.58)	<0.001	1.13	(1.02-1.25)	0.024	1.40	(0.85-2.29)	0.2
	1o	1			1			1		
	2o	0.71	(0.63-0.80)	<0.001	0.71	(0.63-0.80)	<0.001	0.38	(0.19-0.76)	0.006
Education	3o	0.92	(0.73-1.15)	0.5	0.51	(0.32-0.82)	0.005	0.53	(0.42-0.66)	<0.001

		Post- dates (>42wks)			Preterm (≥32 wks <37 wks)			Very preterm (<32 wks)		
		$_{m}$OR[##]	CI	p	$_{m}$OR[##]	CI	p	$_{m}$OR[##]	CI	p
Socio-economic status	V low	0.91	(0.71-1.17)	0.5	0.91	(0.83-1.00)	0.062	1.02	(0.69-1.51)	0.9
	Low	1			1			1		
	Med	0.84	(0.78-0.91)	<0.001	0.84	(0.81-0.88)	<0.001	0.91	(0.80-1.01)	0.1
	High	0.83	(0.72-0.95)	0.008	0.87	(0.69-1.08)	0.2	1.35	(1.03-0.78)	0.032
Nutritional status (MUAC)	1	1.11	(0.84-1.46)	0.5	1.10	(1.03-1.17)	0.003	1.24	(0.91-1.90)	0.3
	2	1			1			1		
	3	1.06	(0.94-1.21)	0.3	0.95	(0.82-1.10)	0.5	0.90	(0.80-1.01)	0.086
	4	1.21	(1.07-1.36)	0.002	0.89	(0.79-0.99)	0.034	0.60	(0.50-0.71)	<0.001
HIV status[#]	neg	1			1			1		
	pos	0.74	(0.61-0.88)	0.001	1.28	(0.96-1.70)	0.089	1.31	(0.67-2.55)	0.4
	pos &c	1.60	(1.25-2.04)	<0.001	1.11	(0.81-1.51)	0.5	1.09	(0.83-1.42)	0.5
Obst. Comp.	No	1			1			1		
	Yes	0.97	(0.87-1.08)	0.6	1.39	(1.27-1.54)	<0.001	2.14	(1.25-3.69)	0.006
Multiple delivery	No	1			1			1		
	Yes	1.42	(0.91-2.22)	0.1	2.08	(1.53-2.81)	<0.001	2.46	(1.60-3.79)	<0.001

*based on robust standard errors to account for intracluster correlation within recruitment sites

** Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

[##]exponentiated coefficients from an -mlogit- model, these multinomial odds ratios are also referred to as relative risk ratios (but these are not risk ratios)

4.3 Is maternal GBS colonisation associated with birth weight?

Maternal GBS colonisation at delivery was examined as a risk factor for birth weight in logistic regression analyses, allowing for clustering by site. Ordinal logistic regression was first undertaken, as previously, but the assumption of proportional odds was again not met, so multivariate analyses were undertaken so that no assumption about proportionality across birth weight categories was made. The effects are presented again as multinomial odds ratios ($_m$OR), as suggested elsewhere, to reflect their calculation by comparison to the reference category (here the "normal" weight category).[217] Examination of continuous variables (maternal age, nutritional status, maternal haemoglobin and maternal temperature) was undertaken with natural splines prior to categorisation; graphical representations of the continuous relationships of these variables with birth weight are included in the appendices, page 247.

The univariable analyses, multivariable complete case analyses (N=4993), and multivariable imputed case analyses (N=7408) are given in detail in the three tables below with the variables which were adjusted for based on the causal model (Figure 9). The unvariable analyses and imputed analyses were more consistent with each other than the complete case analysis, from which considerable power was lost due to incomplete data. The results discussed below therefore focus on the univariable and multivariable imputed data, which were more robust.

There was no evidence against the null hypothesis of no association between maternal GBS colonisation at delivery and birth weight category of high and low birthweight (categories >3500g and 2500-3499g) in mothers with a temperature in the normal range (36.5C-37.5C) in unvariable analyses. There was a suggestion that maternal GBS colonisation was associated with lower odds of very low birth weight in a mother with a temperature in the

normal range (OR 0.50, 95%CI 0.25-1.00). However, this was not seen in multivariable analyses, and was likely confounded as exposures that were associated with increased maternal GBS colonisation (higher socioeconomic status, nutritional status, HIV negative) were associated with lower odds of very low birth weight, and exposures associated with decreased maternal GBS colonisation (high parity, obstetric complication) were associated with increased odds of very low birth weight.

In contrast, for mothers with fever, the odds of very low birth weight were high across all analyses; in the univariable analysis ($_m$OR 49.3 (24.5-99.3), p <0.001) in the imputed dataset the $_m$OR 39.3 (7.45-208, p <0.001) and in the complete case analysis, the $_m$OR for the effect size for this association was >100. This was likely due to the small numbers of mothers with fever who had very low birth weight newborns, and almost all being colonised with GBS, so the odds ratios approach infinity. The numbers were larger in the univariable and imputed data analyses, as less power was lost, but the odds ratios even in these analyses were unlikely to reflect risk ratios. There was no evidence against the null hypothesis of no effect modification for other pre-specified effect modifiers (prolonged rupture of membranes ($\geq$18h) or urinary tract infection).

Whilst outside the scope of the *a priori* question posed here, there were some interesting associations with large effect sizes for exposures included in the model; high birth weight was strongly associated with male sex ($_m$OR 1.70 (1.44-2.01), p<0.001), but multiple deliveries had very low odds of high birth weight ($_m$OR 0.08 (0.02-0.34). p=0.001), and were also strongly associated with low birth weight ($_m$OR 9.29 (95%CI 6.66-13.0) p <0.001) or very low birth weight ($_m$OR 20.5 (95%CI 12.0-35.3), p<0.001).

Table 17 Associations between maternal GBS colonisation and birth weight in three sites in coastal Kenya – univariable analyses

		High birth weight (>3500g)			Low birth wt (1500 – 2499g)			Very low birth wt (<1500g)		
		$_{m}OR^{\#\#}$	CI*	p**	$_{m}OR^{\#\#}$	CI*	p**	$_{m}OR^{\#\#}$	CI*	p**
	Neg	1			1			1		
GBS	Pos	0.91	(0.44-1.89)	0.8	0.95	(0.76-1.18)	0.6	0.50	(0.25-1.00)	0.050
	<36.5	1.07	(0.23-9.69)	0.3	0.93	(0.85-1.02)	0.09	0.86	(0.86-0.92)	<0.001
	36.5-37.5	1								
GBS-ve	>37.5	0.70	(0.24-2.03)	0.5	1.20	(0.78-1.85)	0.4	0.48	(0.49-3.2)	0.6
	<36.5	1.06	(0.75-1.51)	0.7	1.15	(1.01-1.32)	0.037	0.48	(0.08-3.04)	0.4
GBS +ve	>37.5	1.50	(0.23-9.69)	0.7	1.19	(0.24-5.81)	0.8	49.3	(24.5-99.3)	<0.001
	1	0.53	(0.50-0.57)	<0.001	1.36	(1.18-1.55)	<0.001	1.36	(0.80-2.31)	0.3
	2	0.88	(0.72-1.06)	0.2	1.16	(0.99-1.37)	0.067	1.19	(0.95-1.48)	0.1
Age in quartiles	3	1			1			1		
	4	1.12	(1.06-1.18)	<0.001	1.17	(1.12-1.22)	<0.001	0.91	(0.63-1.31)	0.6
Pregnancy no. (> 28 wks)	First	0.66	(0.50-0.86)	0.002	1.52	(1.38-1.66)	<0.001	1.11	(0.98-1.26)	0.098
	2^{nd}-4^{th}	1			1			1		
	>5	0.89	(0.63-1.26)	<0.001	1.23	(1.11-1.34)	<0.001	1.29	(0.93-1.78)	0.1
	Female	1			1			1		
Sex	Male	1.63	(0.35-1.97)	<0.001	0.89	(0.78-1.01)	0.064	1.15	(0.77-1.71)	0.5
	None	0.91	(0.65-1.27)	0.6	0.90	(0.71-1.16)	0.4	0.73	(0.47-1.13)	0.2
	1o	1			1			1		
	2o	1.64	(1.44-1.87)	<0.001	1.05	(0.71-1.58)	0.8	0.54	(0.26-1.10)	0.091
Education	3o	1.89	(1.67-2.14)	<0.001	0.59	(0.45-0.76)	<0.001	0.37	(0.13-1.11)	0.076

		High birth weight (>3500g)			Low birth wt (1500 – 2499g)			Very low birth wt (<1500g)		
		$_{m}$OR[##]	CI*	p**	$_{m}$OR[##]	CI*	p**	$_{m}$OR[##]	CI*	p**
Socio-economic status	V low	1.15	(0.85-1.55)	0.4	0.94	(0.77-1.14)	0.5	1.01	(0.70-1.47)	0.9
	Low	1			1			1		
	Med	1.61	(1.31-1.97)	<0.001	0.86	(0.73-1.01)	0.074	0.74	(0.55-1.00)	0.046
	High	2.04	(1.81-2.31)	<0.001	0.89	(0.72-1.10)	0.3	0.44	(0.31-0.62)	<0.001
Nutritional status (MUAC)	1	0.89	(0.63-1.25)	0.5	1.27	(1.23-1.32)	<0.001	1.71	(1.20-2.44)	0.003
	2	1			1			1		
	3	1.51	(1.10-2.08)	0.010	0.90	(0.81-1.01)	0.074	0.57	(0.47-0.68)	<0.001
	4	2.40	(1.73-3.34)	<0.001	0.73	(0.63-0.86)	<0.001	0.85	(0.49-1.48)	0.6
HIV status[#]	neg	1			1			1		
	pos	0.75	(0.59-0.95)	0.015	1.28	(0.95-1.72)	0.1	0.56	(0.40-0.79)	0.001
	pos &c	1.02	(0.91-1.15)	0.7	1.02	(0.65-1.61)	0.9	0.41	(0.11-1.45)	0.2
Obst. Comp.	No	1			1			1		
	Yes	0.78	(0.48-1.29)	0.3	2.31	(2.10-2.55)	<0.001	4.35	(3.81-4.96)	<0.001
Multiple delivery	No	1			1			1		
	Yes	0.09	(0.02-0.38)	<0.001	9.02	(6.59-12.34)	<0.001	22.8	(14.00-37.17)	<0.001

*based on robust standard errors to account for intracluster correlation within recruitment sites

** Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

[##]exponentiated coefficients from an -mlogit- model, these multinomial odds ratios are also referred to as relative risk ratios (but these are not risk ratios)

Table 18 Associations between maternal GBS colonisation and birth weight in three sites in coastal Kenya - complete case analyses (N=4993)

		High birth weight (>3500g)			Low birth wt (1500 – 2499g)			Very low birth wt (<1500g)		
		$_{m}OR^{\#\#}$	CI*	p**	$_{m}OR^{\#\#}$	CI*	p**	$_{m}OR^{\#\#}$	CI*	p**
	Neg	1			1			1		
GBS	Pos	0.96	(0.46-1.99)	0.9	0.97	(0.67-1.41)	0.9	0.84	(0.25-2.75)	0.8
	<36.5	0.98	(0.87-1.10)	0.7	0.86	(0.83-0.89)	<0.001	0.98	(0.48-2.04)	1.0
	36.5-37.5	1								
GBS-ve	>37.5	0.76	(0.24-2.44)	0.6	1.16	(0.69-1.95)	0.6	<0.1	(<0.1)	<0.001
	<36.5	0.86	(0.64-1.16)	0.6	1.18	(0.78-1.77)	0.4	0.19	(0.10-0.36)	<0.001
GBS +ve	>37.5	0.93	(0.17-4.94)	0.9	0.95	(0.23-4.00)	0.9	>100	(>100)	<0.001
	1	0.82	(0.63-1.08)	<0.001	1.09	(0.92-1.29)	0.3	1.44	(0.99-2.10)	0.058
	2	1.02	(0.92-1.13)	0.7	1.09	((0.84-1.42)	0.5	1.60	(1.42-1.80)	<0.001
Age in	3	1			1			1		
quartiles	4	0.99	(0.83-1.18)	0.9	1.26	(1.16-1.36)	<0.001	0.79	(0.50-1.24)	0.3
	0	0.63	(0.40-0.99)	0.048	1.59	(1.44-1.76)	<0.001	0.83	(0.75-0.91)	<0.001
	1-4	1			1			1		
Parity	≥5	1.10	(0.80-1.52)	0.5	1.02	(0.85-1.21)	0.9	1.09	(1.01-1.18)	0.027
	Female	1			1			1		
Sex	Male	1.74	(1.31-2.31)	<0.001	0.88	(0.78-0.99)	0.031	1.04	(0.59-1.84))	0.9
	None	0.90	(0.76-1.07)	0.2	0.99	(0.82-1.19)	0.9	0.81	(0.66-1.00)	0.048
	1o	1			1			1		
	2o	1.46	(1.36-1.55)	<0.001	1.03	(0.53-2.00)	0.8	0.69	(0.25-1.93)	0.5
Education	3o	1.82	(1.16-2.88)	0.010	0.64	(0.54-0.76)	<0.001	0.19	(0.04-0.95)	0.044

		High birth weight (>3500g)			Low birth wt (1500 – 2499g)			Very low birth wt (<1500g)		
		$_{m}$OR[##]	CI*	p**	$_{m}$OR[##]	CI*	p**	$_{m}$OR[##]	CI*	p**
Socio-economic status	V low	1.04	(0.71-1.52)	0.8	0.76	(0.63-0.93)	0.008	0.92	(0.61-1.38)	0.7
	Low	1			1			1		
	Med	1.23	(1.14-1.33)	<0.001	0.86	(0.72-1.03)	0.1	1.11	(0.72-1.70)	0.6
	High	1.48	(1.34-1.63)	<0.001	1.01	(0.82-1.23)	0.9	0.85	(0.49-1.46)	0.6
Nutritional status (MUAC)	1	0.94	(0.51-1.75)	0.9	1.25	(1.18-1.33)	<0.001	1.46	(0.80-2.66)	0.2
	2	1			1			1		
	3	1.42	(1.00-2.77)	0.052	0.81	(0.74-0.89)	<0.001	0.37	(0.19-0.71)	0.003
	4	2.00	(1.44-2.77)	<0.001	0.73	(0.66-0.81)	<0.001	0.85	(0.35-2.10)	0.7
HIV status[#]	neg	1			1			1		
	pos	0.69	(0.59-0.95)	0.2	1.63	(0.93-2.87)	0.087	0.45	(0.23-0.87)	0.018
	pos &c	0.89	(0.91-1.15)	<0.001	1.04	(0.49-2.18)	0.9	0.50	(0.09-2.94)	0.4
Obst. Comp.	No	1			1			1		
	Yes	0.73	(0.44-1.22)	0.2	2.00	(1.43-2.79)	<0.001	3.31	(1.88-5.84)	<0.001
Multiple delivery	No	1			1			1		
	Yes	0.06	(0.01-0.30)	<0.001	10.20	(8.41-12.38)	<0.001	15.44	(7.71-30.94)	<0.001

*based on robust standard errors to account for intracluster correlation within sites

** Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

[##]exponentiated coefficients from an -mlogit- model, these multinomial odds ratios are also referred to as relative risk ratios (but these are not risk ratios)

Table 19 Association between maternal GBS colonisation and birth weight, in three sites in coastal Kenya – imputed analyses (N=7408).

		High birth weight (>3500g)			Low birth wt (1500 – 2499g)			Very low birth wt (<1500g)		
		$_{m}OR^{\#\#}$	CI*	p**	$_{m}OR^{\#\#}$	CI*	p**	$_{m}OR^{\#\#}$	CI*	p**
	Neg	1			1			1		
GBS	Pos	0.89	(0.48-1.67)	1.0	1.07	(0.77-1.48)	0.7	0.53	(0.26-1.10)	0.087
	<36.5	1.00	(0.90-1.11)	1.0	0.93	(0.85-1.02)	0.09	0.85	(0.60-1.21)	0.4
	36.5-37.5	1								
GBS-ve	>37.5	0.79	(0.30-2.07)	0.6	1.09	(0.61-1.94)	0.4	0.43	(0.07-2.78)	0.5
	<36.5	1.03	(0.69-1.53)	0.9	1.08	(0.74-1.59)	0.037	0.47	(0.08-3.04)	0.4
GBS +ve	>37.5	1.11	(0.18-6.84)	0.9	1.11	(0.33-3.77)	0.8	39.3	(7.45-207.7)	<0.001
	1	0.81	(0.61-1.08)	0.2	1.06	(0.88-1.29)	0.5	1.37	(0.91-2.06)	0.1
	2	1.02	(0.86-1.22)	0.8	1.04	(0.85-1.27)	0.7	1.32	(0.93-1.87)	0.1
Age in	3	1			1			1		
quartiles	4	1.03	(0.88-1.20)	0.7	1.16	(1.03-1.30)	0.017	0.74	(0.49-1.12)	0.2
	0	0.70	(0.51-0.97)	0.033	1.58	(1.37-1.82)	<0.001	0.92	(0.77-1.10)	0.4
	1-4	1			1			1		
Parity	≥5	1.14	(0.88-1.48)	0.3	0.99	(0.88-1.11)	<0.001	1.02	(0.86-1.20)	0.9
	Female	1			1			1		
Sex	Male	1.70	(1.44-2.01)	<0.001	0.84	(0.74-0.94)	0.004	1.02	(0.62-1.68)	0.9
	None	0.95	(0.84-1.07)	0.4	0.97	(0.79-1.18)	0.7	0.65	(0.49-0.87)	0.004
	1o	1			1			1		
	2o	1.41	(1.27-1.57)	<0.001	1.06	(0.67-1.67)	0.8	0.68	(0.28-1.64)	0.4
Education	3o	1.50	(1.19-1.89)	0.001	0.62	(0.55-0.70)	<0.001	0.53	(0.17-1.63)	0.3

		High birth weight (>3500g)			Low birth wt (1500 – 2499g)			Very low birth wt (<1500g)		
		$_{m}$OR[##]	CI*	p**	$_{m}$OR[##]	CI*	p**	$_{m}$OR[##]	CI*	p**
Socio-economic status	V low	1.07	(0.77-1.49)	0.7	0.90	(0.80-1.01)	0.085	0.93	(0.61-1.41)	0.7
	Low	1			1			1		
	Med	1.31	(1.17-1.47)	<0.001	0.90	(0.76-1.06)	0.2	0.79	(0.59-1.06)	0.7
	High	1.57	(1.47-1.68)	<0.001	1.00	(0.84-1.18)	1.0	0.57	(0.41-0.78)	<0.001
Nutritional status (MUAC)	1	0.92	(0.63-1.25)	0.5	1.26	(1.18-1.36)	<0.001	1.78	(1.22-2.58)	0.003
	2	1			1			1		
	3	1.37	(1.00-1.89)	0.010	0.88	(0.78-1.00)	0.047	0.59	(0.43-0.83)	<0.001
	4	1.95	(1.50-2.53)	<0.001	0.72	(0.66-0.796)	<0.001	1.03	(0.48-2.18)	0.9
HIV status[#]	neg	1			1			1		
	pos	0.65	(0.43-0.99)	0.045	1.52	(1.04-2.23)	0.032	0.84	(0.37-1.89)	0.7
	pos &c	0.86	(0.76-0.99)	0.033	1.13	(0.63-2.02)	0.7	0.56	(0.12-2.70)	0.5
Obst. Comp.	No	1			1			1		
	Yes	0.74	(0.46-1.19)	0.2	2.04	(1.92-2.17)	<0.001	3.75	(3.08-4.57)	<0.001
Multiple delivery	No	1			1			1		
	Yes	0.08	(0.02-0.34)	0.001	9.29	(6.66-13.00)	<0.001	20.54	(11.96-35.27)	<0.001

*based on robust standard errors to account for intracluster correlation within recruitment sites

**combined Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

[##]exponentiated coefficients from an -mlogit- model, these multinomial odds ratios are also referred to as relative risk ratios (but these are not risk ratios)

4.4 Is maternal GBS colonisation associated with possible severe bacterial infection?

Maternal GBS colonisation at delivery was examined as a risk factor for possible severe bacterial infection in logistic regression analyses. The denominator was live births. The univariable, multivariable complete case analysis and multivariable imputed analysis are given in detail in Table 20, with the confounders which were adjusted for, based on causal modelling (Figure 9). Examination of continuous variables (maternal age, nutritional status, maternal haemoglobin and maternal temperature) was undertaken with natural splines prior to categorisation (presented here), graphical representations of the relationship of these variables with possible severe bacterial infection are included in the appendices, page 247.

There was very little evidence against the null hypothesis of no association between maternal GBS colonisation at delivery and possible severe bacterial infection, although there was suggestion that prolonged rupture of membranes >18h could act as an effect modifier. In the univariable analysis with both PROM (>18h) and maternal GBS colonisation the odds ratio for pSBI was 2.53 (95%CI 1.04-6.14, p=0.040); in the complete case analysis there was only weak evidence for an association: with both PROM (>18h) and maternal GBS colonisation the odds ratio for association with pSBI was 3.29 (0.85-9.46, p=0.098), but in the imputed analysis the association was very weak, suggesting it may have been due to chance (OR 2.25 (0.79-6.45), p=0.130).

The discrepancies and wide confidence intervals were due to the small numbers within the categories for the interaction term. For those mothers not GBS colonised there were 270/6116 (4.4%) without pSBI and with PROM, and 13/331 (3.9%) with pSBI and with PROM. For those mothers colonised with GBS there were 30/817 (3.7%) without pSBI and with

PROM and 3/42 (7.1%) with pSBI and with PROM. There was no evidence against the null hypothesis of no effect modification for urinary tract infection or maternal fever.

Although outside the scope of the question posed *a priori*, male sex was consistently associated with pSBI, OR 1.22 (1.17-1.26), p <0.001 (imputed dataset). There were more neonates admitted with pSBI to women of higher socio economic status (OR 2.22 (1.24-1.18), imputed, p <0.001), and of higher nutritional status (OR 1.23 (1.02-1.49) p <0.001, imputed dataset). Although there was evidence of an association with HIV infection in the univariable analyses, this was not sustained in the imputed dataset. The numbers were small, and the confidence intervals cross one for individual categories, so it is difficult to draw firm conclusions. However, it was clear that an obstetric complication at delivery and multiple deliveries were associated with admissions with signs of pSBI, although with a smaller effect size than seen in preterm and low birth weight deliveries. The odds ratios for the association of pSBI with obstetric complication and multiple delivery were 1.64 (1.25-2.15), p<0.001) and 2.86 (2.44-3.35), p<0.001 respectively (imputed datasets).

Table 20 Association between maternal GBS colonisation and possible severe bacterial infection, in three sites in coastal Kenya.

		Univariable analyses			Multivariable complete case (n=5062)			Multivariable imputed (n=7408)		
Variable	**Category**	**OR**	**95%CI***	**p****	**OR**	**95%CI***	**p****	**OR**	**95%CI***	**p****
GBS (PROM neg)	Neg	1			1			1		
	Pos	0.91	(0.83-1.00)	0.046	0.81	(0.77-0.85)	<0.001	0.93	(0.82-1.06)	0.3
PROM (GBS neg)	Neg	1			1			1	1	
	Pos	0.83	(0.32-2.15)	0.7	1.09	(0.63-1.86)	0.8	0.91	(0.51-1.61)	0.7
PROM & GBS pos		2.53	(1.04-6.14)	0.040	2.84	(0.85-9.46)	0.098	2.25	(0.79-6.45)	0.130
Age in quartiles	1	0.93	(0.56-1.55)		0.90	(0.64-1.22)		1.08	(0.83-1.41)	
	2	0.94	(0.72-1.21)		0.89	(0.72-1.12)		1.00	(0.86-1.17)	
	3	1			1			1		
	4	0.91	(0.71-1.17)	0.099	0.81	(0.77-0.95)	0.005	0.86	(0.77-0.98)	0.070
Parity	0	1.03	(0.64-1.66)		1.06	(0.64-1.71)		0.99	(0.67-1.48)	
	1-4	1			1			1		
	≥5	0.99	(0.51-1.91)	0.9	1.11	(0.86-1.66)	<0.001	1.21	(0.89-1.63)	<0.001
Sex	Female	1			1			1		
	Male	1.19	(1.14-1.25)	<0.001	1.24	(1.13-1.36)	<0.001	1.22	(1.17-1.26)	<0.001
Education	None	0.65	(0.30-1.38)		0.79	(0.59-1.07)		0.73	(0.64-0.83)	
	1º	1.14	(0.91-1.44)		1			1		
	2º	1			0.83	(0.58-1.19)		0.96	(0.60-1.53)	
	3º	0.64	(0.25-1.65)	0.5	0.63	(0.29-1.38)	0.011	0.52	(0.23-1.18)	<0.001
Socio-economic status	Very low	1.34	(0.93-1.92)		1.43	(1.01-2.04)		1.29	(1.02-1.63)	
	Low	1			1			1		
	Medium	1.37	(0.65-2.90)		1.58	(0.87-2.86)		1.34	(0.63-2.85)	
	High	2.18	(1.12-4.23)	0.005	2.11	(1.51-2.95)	0.045	2.22	(1.24-3.99)	<0.001

		Univariable analyses			Multivariable complete case (n=5062)			Multivariable imputed (n=7408)		
		OR	**95%CI***	**p****	**OR**	**95%CI***	**p****	**OR**	**95%CI***	**p****
Nutritional status (MUAC)	1	0.87	(0.73-1.02)		0.82	(0.51-1.31)		0.9	(0.71-1.12)	
	2	1			1			1		
	3	1.18	(0.86-1.62)		1.13	(0.88-1.56)		1.13	(0.84-1.50)	
	4	1.37	(1.06-1.78)	0.008	1.24	(0.85-1.80)	<0.001	1.23	(1.02-1.49)	<0.001
HIV status	neg	1			1			1		
	pos	0.83	(0.39-1.78)		0.80	(0.51-1.25)		0.97	(0.56-1.64)	
	pos &c[#]	1.69	(0.61-4.65)	<0.001	1.48	(0.55-4.03)	<0.001	1.59	(0.56-4.54)	0.069
Obstetric complication	No	1			1			1		
	Yes	1.94	(1.42-2.66)	<0.001	1.41	(0.86-2.31)	0.017	1.64	(1.25-2.15)	<0.001
Multiple delivery	No	1			1			1		
	Yes	3.02	(2.51-3.64)	<0.001	3.98	(3.04-5.21)	<0.001	2.86	(2.44-3.35)	<0.001

*based on robust standard errors to account for intracluster correlation within recruitment sites

**combined Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

4.5 Is maternal GBS colonisation associated with stillbirth?

Maternal GBS colonisation at delivery was examined as a risk factor for stillbirth in logistic regression analyses, allowing for clustering by site. The univariable, multivariable complete case analysis and multivariable imputed analysis are given in detail in Table 21 below, with the confounders which were adjusted for, based on causal modelling. Examination of continuous variables (maternal age, nutritional status, maternal haemoglobin and maternal temperature) was undertaken with natural splines prior to categorisation (presented here), graphical representations of the relationship of these variables with possible severe bacterial infection are included in the appendices, page 247.

There was very little evidence against the null hypothesis of no association between maternal GBS colonisation at delivery and possible severe bacterial infection, although there was suggestion that urinary tract infection (UTI) could act as an effect modifier. In the univariable analysis the presence of both UTI and maternal GBS colonisation the OR was 1.33 (95%CI 1.22-1.45, p <0.001), in the complete case analysis there was some evidence for an association: odds ratio with UTI and GBS was 2.48 (2.34-2.63, p <0.001), but in the imputed analysis there was very little evidence, OR 1.43 (95%CI 0.90-2.25 p=0.126). The discrepancies and wide confidence intervals may again be due to the small numbers within the categories for the interaction term. For those mothers not colonised with GBS there were 226/5842 (3.9%) live births and with UTI and 12/341 (3.5%) stillborn and with UTI. For those mothers colonised with GBS there were 32/785 (4.1%) live born with UTI and 2/41 (4.9%) stillborn and with UTI. There was no evidence against the null hypothesis of no effect modification for PROM or maternal fever.

Other associations observed in the analysis (not included *a priori*) include a dose-response effect of maternal age, OR from 0.67 (95%CI 0.37-2.25) in the lowest quartile to OR 1.19

(0.85-1.66), p <0.001 in the highest quartile, and an inverse dose-response relationship for stillbirth in participants with no education (OR 1.10 (0.89-1.39) to those with higher education (OR 0.64, (0.38-1.12), p <0.001. For nutrition, the odds of stillbirth were higher in the third and fourth quartiles of mid-upper arm circumference (OR 1.44, 1.16-1.80 and 1.31 (1.06-1.60) compared to the second quartile (reference group). Obstetric complications were strongly associated with stillbirth; OR 4.98 (2.50-9.91), p<0.001.

Table 21 Association between maternal GBS colonisation and stillbirth in three sites in coastal Kenya.

		Univariable analyses			Multivariable complete case n=5191			Multivariable imputed (n=7833)		
Variable	**Category**	**OR**	**95%CI***	**p ****		**95%CI***	**p ****	**OR**	**95%CI***	**p****
	Negative	1			1			1		
GBS (no UTI)	Positive	0.88	(0.79-0.99)	0.036	0.89	(0.61-1.30)	0.5	0.89	(0.72-1.11)	0.3
	Negative	1			1			1	1	
UTI (GBS neg)	Positive	0.91	(0.63-1.30)	0.6	0.93	(0.86-1.02)	0.092	0.83	(0.62-1.11)	0.2
UTI & GBS pos	Positive	1.33	(1.22-1.45)	<0.001	2.48	(2.34-2.63)	<0.001	1.43	(0.90-2.25)	0.126
	1	0.77	(0.40-1.50)		0.54	(0.20-1.44)		0.67	(0.37-1.21)	
	2	1.13	(0.97-1.31)		0.97	(0.78-1.20)		1.06	(0.92-1.23)	
	3	1			1			1		
Age in quartiles	4	1.27	(1.08-1.48)	<0.001	1.28	(0.98-1.74)	<0.001	1.19	(0.85-1.66)	<0.001
	0	1.13	(0.86-1.48)		1.74	(1.61-1.88)		1.33	(1.07-1.66)	
	1-4	1			1			1		
Parity	≥5	1.68	(0.98-2.89)	<0.001	1.15	(0.81-1.65)	<0.001	1.32	(0.92-1.89)	<0.001
	Female	1			1			1		
Sex	Male	0.93	(0.89-0.97)	0.001	0.97	(0.82-1.13)	0.7	0.93	(0.89-0.98)	0.004
	None	1.14	(0.74-1.76)		1.08	(0.69-1.69)		1.10	(0.89-1.39)	
	1o	1			1			1		
	2o	0.86	(0.82-0.91)		0.65	(0.54-0.77)		0.77	(0.65-0.93)	
Education	3o	0.66	(0.31-1.39)	0.5	0.77	(0.51-1.17)	0.4	0.64	(0.38-1.12)	<0.001
	Very low	1.27	(0.80-2.01)		1.16	(0.99-1.25)		1.03	(0.84-1.26)	
	Low	1			1			1		
Socio-economic	Medium	1.00	(0.63-1.60)		0.82	(0.51-1.33)		0.95	(0.63-1.44)	
status	High	1.05	(0.60-1.81)	0.4	0.94	(0.57-1.62)	0.1	1.04	(0.68-1.60)	<0.001

		Univariable analyses			Multivariable complete case n=5191			Multivariable imputed (n=7833)		
		OR	**95%CI***	**p** **	**OR**	**95%CI***	**p** **	**OR**	**95%CI***	**p** **
Nutritional status (MUAC)	1	1.05	(0.94-1.65)		1.08	(0.74-1.57)		1.11	(0.88-1.39)	
	2	1			1			1		
	3	1.54	(1.27-1.87)		1.56	(1.15-2.13)		1.44	(1.16-1.80)	
	4	1.41	(1.12-1.77)	<0.001	1.24	(1.01-1.53)	<0.001	1.31	(1.06-1.60)	<0.001
HIV status	neg	1			1			1		
	pos	1.88	(1.42-2.50)		1.71	(1.30-2.23)		1.97	(1.31-3.00)	
	pos &c	1.12	(0.70-1.78)	<0.001	1.25	(0.58-2701)	<0.001	1.27	(0.73-2.13)	<0.001
Obstetric complication	No	1			1			1		
	Yes	5.07	(2.64-9.71)	<0.001	3.87	(2.11-7.06)	<0.001	4.98	(2.50-9.91)	<0.001
Multiple delivery	No	1			1			1		
	Yes	1.42	(1.23-1.64)	<0.001	1.22	(0.79-1.87)	0.4	0.94	(0.79-1.12)	0.5

*based on robust standard errors to account for intracluster correlation within recruitment sites

**combined Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

4.6 Is maternal GBS colonisation associated with perinatal mortality?

Maternal GBS colonisation at delivery was examined as a risk factor for perinatal mortality in logistic regression analyses, allowing for clustering by site. The univariable, multivariable complete case analysis and multivariable imputed analysis are given in detail in Table 22, with the confounders which were adjusted for, based on causal modelling. Examination of continuous variables (maternal age, nutritional status, maternal haemoglobin and maternal temperature) was undertaken with natural splines prior to categorisation (presented here), graphical representations of the relationship of these variables with possible severe bacterial infection are included in the appendices, page 247.

There was no evidence against the null hypothesis of no association between maternal GBS colonisation at delivery and perinatal mortality (see Table 22). There was no evidence against the null hypothesis of no effect modification for pre-specified effect modifiers (prolonged rupture of membranes ($\geq$18h), urinary tract infection or maternal fever).

Exposures that were associated with increased maternal GBS colonisation (higher nutritional status and HIV uninfected mothers) were associated with lower odds of perinatal mortality in this analysis (highest quartile of MUAC OR 1.39 (1.13-1.73), p<0.001; HIV infected and no co-trimoxazole OR1.60 (1.18-2.21) p <0.001). Exposures associated with decreased maternal GBS colonisation (high parity and obstetric complication) were associated with increased odds of perinatal mortality ((OR 1.31 (1.09-1.58), p<0.001) and (OR 4.17 (2.67-6.51), p <0.001).

Table 22 Analyses of association between maternal GBS colonisation and perinatal mortality, in three sites in coastal Kenya.

		Univariable analyses			Multivariable complete case (n=5668)			Multivariable imputed (n=7833)		
Variable	**Category**	**OR**	**95%CI***	**p ****	**OR**	**95%CI***	**p ****	**OR**	**95%CI***	**p ****
GBS	neg	1		0.3	1		0.9	1		0.7
	pos	0.88	(0.71-1.10)		0.99	(0.59-1.67)		0.94	(0.71-1.26)	
Age in quartiles	1	0.81	(0.47-1.40)	<0.001	0.66	(0.33-1.34)	<0.001	0.75	(0.48-1.16)	0.002
	2	1.05	(0.91-1.20)		0.95	(0.93-1.65)		1.02	(0.91-1.16)	
	3	1			1			1		
	4	1.29	(1.15-1.43)		1.24	(0.93-1.65)		1.17	(0.94-1.46)	
Parity	0	1.05	(0.71-1.55)	<0.001	1.50	(1.22-1.84)	<0.001	1.19	(0.85-1.68)	<0.001
	1-4	1			1			1		
	≥5	1.66	(1.10-2.52)		1.29	(1.11-1.48)		1.31	(1.09-1.58)	
Sex	Female	1		0.2	1		0.3	1		0.2
	Male	0.95	(0.89-1.02)		0.97	(0.92-1.03)		0.94	(0.97-1.02)	
Education	None	1.09	(0.69-1.71)	0.6	1.04	(0.74-1.47)	0.8	1.02	(0.81-1.68)	<0.001
	1º	1			1			1		
	2º	0.86	(0.78-0.95)		0.71	(0.62-0.82)		0.81	(0.69-0.95)	
	3º	0.68	(0.33-1.39)		0.86	(0.54-1.37)		0.70	(0.43-1.17)	
Socio-economic status	Very low	1.29	(0.82-2.03)	0.4	1.14	(0.89-1.46)	0.5	1.12	(0.69-1.85)	0.2
	Low	1			1			1.06		
	Medium	0.99	(0.62-1.59)		0.90	(0.55-1.48)		1	(0.70-1.61)	
	High	0.97	(0.59-1.61)		0.93	(0.57-1.53)		1.02	(0.98-1.06)	
Nutritional status (MUAC) in quartiles	1	1.18	(1.06-1.31)	<0.001	1.25	(0.85-1.85)	<0.001	1.23	(1.03-1.45)	<0.001
	2	1			1			1		
	3	1.45	(1.28-1.65)		1.48	(1.09-2.07)		1.36	(1.18-1.59)	
	4	1.48	(1.20-1.83)		1.42	(1.05-1.52)		1.39	(1.13-1.73)	

		Univariable analyses			Multivariable complete case (n=5668)			Multivariable imputed (n=7833)		
		OR	**95%CI***	**p** **	**OR**	**95%CI***	**p** **	**OR**	**95%CI***	**p** **
HIV status	neg	1			1			1		
	pos	1.45	(1.22-1.99)	<0.001	1.34	(1.03-1.74)	<0.001	1.60	(1.18-2.21)	<0.001
	pos &c[#]	1.03	(1.09-1.32)		1.36	(1.30-1.42)		1.33	(1.07-1.65)	
Obst. comp.	No	1		<0.001	1		<0.001	1		<0.001
	Yes	4.31	(2.83-6.57)		3.28	(2.41-4.45)		4.17	(2.67-6.51)	
Multiple delivery	No	1			1			1		
	Yes	1.69	(1.45-1.97)	<0.001	1.52	(1.23-1.87)	<0.001	1.17	(1.08-1.27)	<0.001

*based on robust standard errors to account for intracluster correlation within recruitment sites

**combined Wald test

[#]HIV positive and on co-trimoxazole prophylaxis

4.7 Sub-analysis: Is maternal GBS colonisation associated with invasive perinatal GBS disease?

The extent to which maternal GBS was associated with invasive perinatal disease was examined in a post-hoc analysis, cases of which were identified in other sub-studies (see Chapter 5, page 150). This analysis was not included *a priori* because the sample size was considered too small, based on the sample size calculations (see page 66).The invasive GBS cases (neonates and stillbirths) included here were only identified in Kilifi District Hospital, as this was the only site with routine high quality blood cultures for paediatric admissions, and cord blood cultures for stillbirths as part of the nested case-control study. Therefore only KDH deliveries (2011-2013) were included in the analysis.

There were 5390 deliveries in Kilifi District Hospital included (see Figure 18) and 8 perinatal cases of invasive GBS disease; 5 live borns and 3 stillbirths in this period (see Table 30).

Out of culture proven GBS disease (five cases) of early onset neonatal sepsis there were no early onset sepsis risk factors (urinary tract infection, prolonged rupture of membranes, maternal fever in mothers at delivery). There was one preterm neonate (gestation of 36 weeks') out of five. Of the three stillbirths with GBS isolated only one out of three mothers had any EOS risk factor; in this case the mother had both prolonged rupture of membranes ($\geq$18h) and a urinary tract infection (GBS culture positive); all stillbirths with GBS isolated were born at term or post-dates.

The univariable, multivariable complete case analysis and multivariable imputed logistic regression analyses examining the association of maternal GBS colonisation and invasive perinatal GBS disease are given in detail in Table 23; because of the small numbers some exposure categories (including multiple delivery) could not be included in analyses. The results show that maternal GBS colonisation was strongly associated with invasive GBS disease, with a large effect

size (OR 13.2 (95%CI 3.15-55.5) p <0.001) in the univariable analysis and in the imputed multivariable analysis (OR 12.6 (2.94-58.9) p <0.001). There were no other exposures consistently associated with invasive GBS disease. Although the suggestion of a link between maternal HIV status and invasive GBS disease was interesting, numbers were small, as one neonate with invasive GBS disease was born to a mother with HIV infection, out of the 8 cases of perinatal GBS disease.

Table 23 Association between maternal GBS colonisation and invasive perinatal GBS disease, in Kilifi District Hospital.

		Univariable analyses			Multivariable complete case (n=2667)			Multivariable imputed (n=5390)		
Variable	**Category**	**OR**	**95%CI***	**p ****	**OR**	**95%CI***	**p ****	**OR**	**95%CI***	**p ****
GBS	neg	1		<0.001	1		0.001	1		0.001
	pos	13.2	(3.15-55.5)		20.3	(3.23-128.0)		12.6	(2.94-58.9)	
Age in quartiles	1	3.82	(0.43-34.3)	0.4	2.11	(0.14-31.6)	0.9	3.51	(0.33-37.8)	0.6
	2	1.98	(0.18-21.9)		1.92	(0.15-24.0)		1.75	(0.15-20.1)	
	3	1			1			1		
	4	0.92	(0.06-14.70)		0.84	(0.04-18.8)		0.71	(0.03-14.9)	
Parity	0	3.03	(0.6-15.7)	0.2	1.72	(0.23-12.6)	0.7	1.97	(0.34-11.5)	0.6
	1-4	1			1			1		
	≥5	1.12	(0.10-12.39)		3.14	(0.16-60.0)		3.19	(0.20-50.0)	
Sex	Female	1			1			1		
	Male	0.65	(0.14-2.92)	0.6	1.02	(0.19-5.31)	1.0	0.7	(0.15-3.21)	0.6
Education	None	0.99	(0.11-8.84)	0.8	1.51	(0.74-1.47)	0.9			
	1º	1			1			1		
	2º	1.87	(0.34-10.22)		0.69	(0.07-7.29)				
	3º	1	empty			empty			omitted	
Socio-economic status	Very low	1	empty	0.8		empty	0.9		empty	0.3
	Low	1			1			1		
	Medium	0.61	(0.12-3.13)		1.31	(0.20-8.84)		0.52	(0.09-2.84)	
	High	0.66	(0.07-5.68)		1.54	(0.12-19.33)		0.19	(0.02-1.77)	

		Univariable analyses			Multivariable complete case (n=2667)			Multivariable imputed (n=5390)		
		OR	**95%CI***	**p** **	**OR**	**95%CI***	**p** **	**OR**	**95%CI***	**p** **
Nutritional status (MUAC in quartiles)	1	0.78	(0.07-8.62)	0.4	1.24	(0.10-14.92)	1.0	0.79	(0.07-8.87)	0.4
	2	1			1			1		
	3	0.67	(0.06-7.35)		0.84	(0.07-10.16)		0.76	(0.07-8.50)	
	4	2.66	(0.49-14.56)		1.57	(0.19-13.12)		2.92	(0.49-17.31)	
HIV status	neg	1		0.2	1		0.036			
	pos	3.84	(0.47-31.3)		13.6	(1.19-155.9)				
	pos &c[#]		empty			empty			omitted	
Obst. comp	No	1		0.4	1		0.092	1		0.4
	Yes	2.16	(0.43-10.71)		4.74	(0.78-28.86)		1.93	(0.37-10.04)	

4.8 Discussion

4.8.1 Key Results

Maternal GBS colonisation was a risk factor for perinatal GBS disease, and there were suggestions in these analyses that maternal GBS was a risk factor for other adverse perinatal outcomes, in the context of biologically plausible effect modifiers. For gestational age and birth weight, there was an association between maternal GBS colonisation and very preterm delivery and very low birth weight in the context of maternal fever; consistent with a biologically plausible association between chorioamnionitis and preterm delivery. For stillbirth, there was weak evidence that GBS colonisation in the context of urinary tract infection was associated with stillbirth, which is biologically plausible in terms of urinary tract infection being a marker of dense colonisation, more likely to ascend, and cause in-utero foetal death. For possible severe bacterial infection, there was evidence that GBS colonisation in the context of prolonged rupture of membranes was associated with pSBI, which was again plausible in the context of increased exposure to ascending GBS infection after rupture of membranes, leading to signs of infection at or shortly after birth.

4.8.2 Interpretation

Adverse perinatal outcomes were common in the participants included in this study, as was the exposure of interest, maternal GBS colonisation (~12% of women) at delivery. The study may not have detected direct associations between maternal GBS colonisation and adverse perinatal outcomes, as many of the adverse perinatal outcomes were not caused by maternal GBS, and many of the women who were colonised with GBS at delivery did not have adverse perinatal outcomes. In addition, the risk factors associated with maternal GBS colonisation include higher nutrition, higher socio-economic status and higher educational level; factors which were otherwise associated with good perinatal outcomes, and although these were adjusted for,

residual confounding was likely, as these exposures were strongly associated with perinatal outcomes.

In contrast, examination of a very specific outcome (invasive perinatal GBS disease) showed a very clear association between maternal GBS colonisation and invasive perinatal GBS disease, even with small numbers (OR 12.6 (2.94-58.9), p <0.001). This was consistent with a recent review and meta-analysis of global maternal GBS colonisation and neonatal infection (11 studies included in this meta-analysis, none were available from Africa); neonates of mothers with GBS colonisation had a pooled OR of 9.4 (95% CI 3.1-28.5) for laboratory culture confirmed GBS infection compared to neonates of non-GBS colonised mothers.[153]

The finding of weak evidence of large effect sizes for very biologically plausible effect modifiers, suggests that maternal GBS colonisation is associated with these adverse perinatal outcomes, but identified only in the context of another risk factor, as the association is more specific. However, the fact that risk factors were only identified in one of the eight perinatal cases of GBS disease in the duration of the cohort study suggests that although maternal fever, urinary tract infection and prolonged rupture of membranes may be effect modifiers, they are not required to be present for perinatal GBS disease.

The effect size for the association between very preterm deliveries (and very low birth weight) and maternal fever with GBS colonisation is large, but the absolute numbers are small. The association is however, biologically plausible, because chorioamnionitis is an established risk factor for preterm delivery;[218] the incidence of maternal chorioamnionitis was recently described in a large case control study as being inversely associated with gestational age.[219] In terms of maternal GBS colonisation and preterm birth, in a recent review studies have conflicting findings;[179] some find a direct association between maternal GBS colonisation and preterm birth (case-control studies), whilst others do not (mainly cohort studies, which sample

from mothers ante-partum). One large study from the United States found an association only with an additional factor – "heavy" colonisation.[220] It may be that sampling prior to delivery in the cohort studies introduces sufficient misclassification of maternal GBS colonisation status at delivery, that associations are not identified. Or it may be that the degree to which a direct association is identified depends on the number of other causes of preterm birth or low birth weight in a population. In some settings maternal GBS colonisation may be a leading cause of chorioamnionitis, but in our study setting there are likely many other causes, including other infections, such as malaria,[221] although overall incidence of malaria is decreasing as the region moves from stable to unstable transmission.[222]

In terms of the association of maternal GBS colonisation with stillbirth, there is again no direct association, but the suggestion of urinary tract infection acting as an effect modifier. Urinary tract infection is thought to be a marker of dense colonisation, and would therefore be consistent with ascending GBS infection and in-utero disease. However, the timing may be less acute, compared to preterm delivery, with less chance of maternal fever being identified (axillary temperatures were only recorded once, at admission for delivery). Because of the small numbers more work is needed to assess the contribution of urinary tract infection to stillbirth in this setting. No associations are detected between maternal GBS colonisation and overall perinatal mortality, which may be because of the differing mechanisms of disease resulting in perinatal death which make associations harder to identify, or because in fact, the associations in the context of effect modifiers are due to chance.

The association of maternal GBS colonisation and possible severe bacterial infection is only suggested in the context of PROM ($\geq$18h) in these analyses, which is a well-established risk factor for early onset disease.[157,223-225] This contrasts to the large effect size and strong association between maternal GBS colonisation and perinatal GBS disease in the sub-analysis. The difference is likely due to the spectrum of disease which pSBI encompasses; the diagnosis

is based on clinical signs,[5] and includes infections due to other pathogens, as well as viral infections and many non-infectious cases such as respiratory distress syndrome and birth asphyxia (see Figure 4).[17]

In terms of prevention, decreases in GBS disease have been well described in the United States through the use of intra-partum prophylaxis (IAP). Guidelines have evolved from a risk factor based approach to antenatal screening of pregnant women for GBS colonisation to determine whom should receive IAP. [226] [227] There was insufficient evidence to choose between the approaches in 1996, [228] but United States guidelines were revised in 2002[185] after an analysis of 629912 live births showed that the risk ratio for early onset GBS disease was 0.48 (95%CI 0.37 to 0.63) in the GBS screened group compared to the risk-factor based group.[229] In contrast, the approaches to determine IAP in Europe remain mixed.[186] Internationally, WHO recommends giving antibiotics to neonates with documented risk factors for infection (PROM $\geq$18h, maternal fever $\geq 38^0$C, offensive amniotic fluid),[25] but intra-partum prophylaxis (IAP) is not currently included in recommendations. Setting aside logistical issues, although the numbers here are small, it would seem from the neonatal GBS cases that more GBS disease may be prevented by screening, as risk factors were rarely identified.

4.8.3 Strengths and limitations

As in the discussion in the previous chapter, this study was health facility based, and whilst this was mitigated to some extent by recruitment at a range of health facilities, these findings cannot necessarily be extrapolated to women who delivered outside of a health facility. Again there were problems with missing data, and the potential for bias introduced by the exclusion of cases missing data on perinatal outcomes, even though these were a small proportion of deliveries (358/8191, 4.4%). The same multiple imputation model was used as previously described, to mitigate the effect of missing data, and the bias which would have been introduced and power lost. In this analysis the imputed analyses were particularly valuable in

assessing underpowered interaction terms. The estimates from the imputed analyses were more consistent with the univariable analyses, increasing their reliability and maintaining power in analyses such that interactions were not missed in multivariable analyses. Conversely, for very weak associations where numbers were very small, the imputed analyses indicated that the associations could have been due to chance. This again was useful, as it highlighted the fragility of these associations and the lack of power, indicating that further work is required to establish whether effect modifiers, included here as they were biologically plausible, and the effect sizes were relatively large, were in fact identified by chance.

There are other limitations to this study which may increase misclassification and increase the chance of a null finding. The classification of gestational age, for example, may include considerable misclassification as whilst gestational age was calculated from early ultrasound scans for a minority of the participants, the majority of gestational ages were determined from the mothers' last menstrual period for which dates may be uncertain. In addition, fundal height measurements late in pregnancy may be influenced by weight rather than gestation, making differences between intra-uterine growth restriction and preterm delivery difficult to disentangle. There was also likely residual confounding, and in these analyses this would be likely to reduce effect sizes, as the risk factors for increased odds of maternal GBS colonisation were often associated with decreased odds of adverse perinatal outcomes.

4.8.4 Conclusions

Maternal GBS colonisation was a risk factor for perinatal GBS disease, and there were suggestions in these analyses that maternal GBS may be a risk factor for other adverse perinatal outcomes in the context of biologically plausible effect modifiers.

The very limited data here suggest screening may be more effective than a risk factor approach if IAP were introduced, but the data were insufficient to draw any firm conclusions. A large

randomised controlled trial would be needed to compare these strategies. However, both of these strategies may be less effective and more difficult to implement than a Group B Streptococcal vaccine, which is discussed further in the discussion in Chapter 5, page 171.

Chapter 5: Invasive GBS disease

5.1 Results

This chapter describes the results of the stillbirth case-control study for invasive GBS disease (2012-13), a review of neonatal admissions with GBS disease to Kilifi District Hospital between 1998 and 2013 and the types of GBS causing invasive disease. It aims to answer the following questions

1. Is invasive GBS disease associated with stillbirth?

2. What is the incidence of invasive GBS disease in neonates?

3. What are the clinical characteristics of invasive GBS disease in neonates?

4. What are the serotypes and MLST types of GBS causing perinatal and neonatal disease?

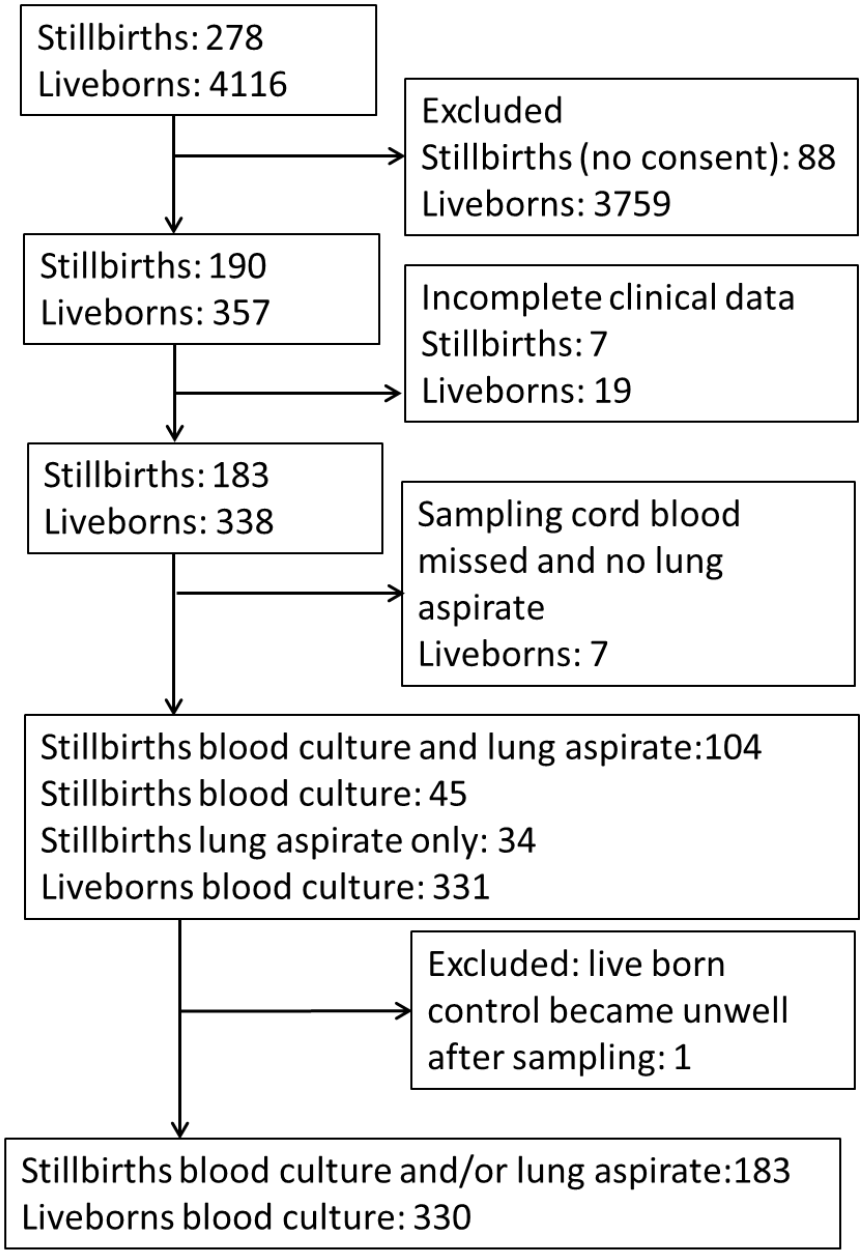
5.2 Is invasive GBS disease associated with stillbirth?

5.2.1 Participants in stillbirth case-control study

There were 278 stillbirths between 1st May 2012 and 1st October 2013 in Kilifi District Hospital. Of these, we recruited 190 stillbirths, and complete clinical data for inclusion were available for 183 (109 intra-partum stillbirths and 74 ante-partum stillbirths), Figure 19.

A cord blood culture and lung aspirate was undertaken on 104 stillbirths, a cord blood culture only on 45 stillbirths, and a lung aspirate only on 34 stillbirths. We recruited 357 controls (subsequent 2 live deliveries following a case) and complete data for inclusion were available for 338 live-borns. Cord blood cultures were taken from 331/338 of the well new-born controls at delivery. We excluded one further live born control baby who became ill shortly after delivery, and then died of *Klebsiella pneumoniae* sepsis. This pathogen was reported on both cord blood culture and peripheral blood culture at admission to the paediatric ward.

Figure 19 Recruitment to the stillbirth case-control study in Kilifi District Hospital 2012-13



5.2.2 Findings

There were four stillbirths (two ante-partum and two intra-partum stillbirths) with GBS isolated from cord blood or lung aspirate out of 183 stillbirths who were sampled, 2.2% (95%CI 0.6-5.5), see Table 24 below. One of these stillbirths (ante-partum stillbirth) had GBS isolated in both the lung and the cord blood. There was 1/330 (0.3%) live born neonate with GBS isolated from cord blood, who was not unwell. Comparing cord blood cultures in live births to stillbirths (intra-partum and ante-partum combined), the odds ratio for association of GBS from cord blood culture with stillbirth was 6.8 (95%CI 0.7-65), p=0.09 fisher's exact test. Details by birth type (ante or intra partum stillbirth) are in Table 25 and Table 26, showing a dose-response relationship in the association between odds of GBS isolation from cultures from live borns, intra-partum and ante-partum stillbirths, for GBS isolates from blood only (Table 25, p=0.055) and including cultures from blood or lung aspirate (Table 26 p=0.069). Assuming causation in the four cases of stillbirth where GBS was isolated as a pure culture, for hospital deliveries, there were 4/4394 births resulting in stillbirth due to invasive GBS disease in the period of sub-study (2012-2013); 0.91 cases per 1000 births (95%CI 0.25-2.3 per 1000) in hospital deliveries.

There were other bacterial organisms identified in cases and controls, as described in Table 24; sample taking was difficult in some cases, especially intra-partum stillbirths where blood was commonly clotted and tissue disintegrating. However, it is interesting to look at other common early neonatal pathogens: *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. *Klebsiella pneumoniae* was not isolated, apart from the live born neonate who subsequently developed EOS. *Staphylococcus aureus* was present as a paired isolate from lung and cord blood in one stillbirth (intra-partum) and there were no *Staphylococcus aureus* isolated from blood cultures of any live borns. *Escherichia coli* was identified in 3/137 lung aspirates and 11/153 cord blood cultures from stillbirths, and in 7/337 live births (comparing cord blood cultures in live births to stillbirths for *E Coli*, OR 3.7 (95%CI 1.4-9.8) Fisher's exact test p=0.008). Comparing

these four pathogens, in live births and stillbirths, the OR of association was 3.67 (1.5-8.9), p=0.002.

Table 24 Organisms isolated from cord blood in live borns and cord blood or lung aspirates of still borns (intrapartum and ante-partum stillbirths)

	Live born N=337		Stillbirth blood culture N=153			Stillbirth lung aspirate N=138		
		%	Intra-partum	ante-partum	%	Intra-partum	ante-partum	%
Acinetobacter sp.	1	0.3	0	0	0.0	0	0	0.0
Bacillus sp.	3	0.9	6	0	3.9	0	2	1.5
Coryneforms sp.	6	1.8	2	5	4.6	0	0	0.0
Enterobacterium cloacae	0	0	0	0	0	1	0	0.7
Escherichia coli	7	2.1	5	6	7.2	1	2	2.2
Enterococci sp.	0	0.0	5	0	3.3	0	0	0.0
Haemophilus sp.	1	0.3	0	0	0.0	1	0	0.7
Klebsiella oxytoca	1	0.3	0	0	0.0	0	0	0.0
Micrococcus sp.	0	0.0	0	1	0.7	0	0	0.0
Sphingomonas paucimobilis	1	0.3	0	0	0.0	0	0	0.0
Pseudomonas stutzeri	0	0.0	1	0	0.7	0	0	0.0
Staphylococcus aureus	0	0.0	1*	0	0.7	1*	0	0.7
Staphylococcus epidermidis	18	5.3	10	12	14.4	0	0	0.0
Streptococcus agalactiae	**1**	**0.3**	**1**	**2***	**2.0**	**1**	**1***	**1.4**
Streptococcus milleri	1	0.3	0	0	0.0	0	1	0.7
Streptococcus viridans	2	0.6	2	0	1.3	0	0	0.0
Other non-haemolytic Streptococcus	2	0.6	0	0	0.0	0	1	0.7
Mixed growth (>1 isolate)	6	1.8	3	9	7.8	4	4	5.8
Total	**50**	**14.8**	**36**	**35**	**46.4**	**9**	**11**	**14.6**

*paired isolates from one stillbirth

Table 25 Comparison of stillbirths (intrapartum and antepartum) and live born neonates with GBS isolated in cord blood cultures*

	GBS in blood	No GBS in blood	Odds ratio	(95%CI)
Well live borns (n=330)	1	329	1	
Stillborn (intrapartum n=94)	1	93	3.5	(0.2-57)
Stillborn (antepartum n=55)	2	53	12.4	(1.1-139)
Fisher's Exact Test	**P=0.055**			

*(excludes stillbirths with only a lung aspirate)

Table 26 Comparison of stillbirths and live born neonates with GBS isolated in cord blood culture (live borns) and cord blood culture or lung aspirate (stillbirths)

	GBS in blood	No GBS in blood	Odds ratio	(95%CI)
Well live borns (n=330)	1	329	1	
Stillborn (intrapartum, n=109)	2	107	6.1	(0.6-68)
Stillborn (antepartum, n=74)	2	72	9.1	(0.8-102)
Fisher's Exact Test	**P=0.069**			

5.3 What is the incidence of invasive GBS disease in neonates?

5.3.1 Participants in neonatal study

There were 83 neonates with invasive GBS disease identified on either culture of blood or cerebrospinal fluid, admitted between the 1st August 1998 and the 31st July 2013 to Kilifi District Hospital. Of these, 42/83 were residing in the KHDSS, with a known population denominator. There were 10 GBS isolates not available for whole genome sequencing from these neonates.

5.3.2 Findings

The minimum incidence of neonatal GBS disease in this population was 0.35 (0.25-0.47) cases per 1000 live births, based on community live births.

For hospital deliveries, there were 5/6598 live births with early onset GBS disease born within the cohort study of maternal GBS colonisation and adverse perinatal outcomes (2011-2013). Within this group, the incidence risk for early onset neonatal disease in hospital deliveries was 0.76 cases per 1000 live births (95%CI 0.25-1.77 per 1000).

5.4 What are the clinical characteristics of invasive GBS disease in neonates?

Between the 1st August 1998 and 31st July 2013 there were 83 neonates admitted to Kilifi District Hospital with invasive GBS disease. There were 36/83 (43%) neonates admitted in the perinatal period (days 0-6 of life), 17/36 died, a case fatality risk of 47% (95%CI 30-65%). There were 44/83 (53%) admitted in the post-perinatal period, and 5/44 died, a case fatality risk of 11% (4-25%), see Table 27 for clinical characteristics and Figure 20 for case fatality by age in days.

There were more male admissions (52/83, 63%) than female (28/83, 34%). There were 6/83 (7%) neonates known to have HIV infection, and case fatality risks were similar in both groups. Of those neonates with HIV infection, 2/25 (8%) were in neonates in the first 48h of life, and 3/44 (7%) were neonates over a week old. There were 25/83 (30%) neonates who were low weight (<2500g) at admission. Of those neonates with illness in the first 48h of life, 10/14 (71%) of the neonates who died were either very low birth weight (<1500g) or low birth weight (<2500g).

In terms of the disease type, sepsis was predominant in the perinatal period, see Figure 21. In the first 48h of life, all 25 cases were due to sepsis, in days 3-6 there were 8/11 cases of sepsis, 2/11 cases with sepsis and meningitis and 1/11 case of meningitis. After the perinatal period 22/44 neonates had sepsis, 14/44 had both sepsis and meningitis, and 8/44 had meningitis. Of the 22 neonates who died, 20 had sepsis (91%) and two had sepsis with meningitis (9%).

There was no strong evidence of a trend towards cases increasing or decreasing over the study period (based on moving averages, Figure 22).

Table 27 Characteristics of neonates admitted to Kilifi District Hospital with GBS disease (1998-2013)

		N=83	%	Deaths	CFR %
Age category	<48h	25	(30)	14	(56)
	2-6 days	11	(13)	3	(27)
	7-28 days	44	(53)	5	(11)
	Missing	3	(4)	1	(33)
Sex	Male	52	(63)	15	(29)
	Female	28	(34)	6	(21)
	Missing	3	(4)	1	(33)
HIV	No	66	(80)	17	(26)
	Yes	6	(7)	2	(33)
	Missing	11	(13)	4	(36)
Weight category	<1500g	9	(11)	6	(67)
	1500-2499g	16	(19)	9	(56)
	2500-3499g	40	(48)	3	(8)
	>3500g	12	(14)	1	(8)
	Missing	6	(7)	4	(67)
Disease type	Sepsis	58	(70)	21	(36)
	Meningitis	9	(11)	0	(0)
	Sepsis and meningitis	16	(19)	2	(13)

Figure 20 Invasive GBS disease and age at admission, by outcome (survival or death) in neonatal admissions to Kilifi District Hospital 1998-2013

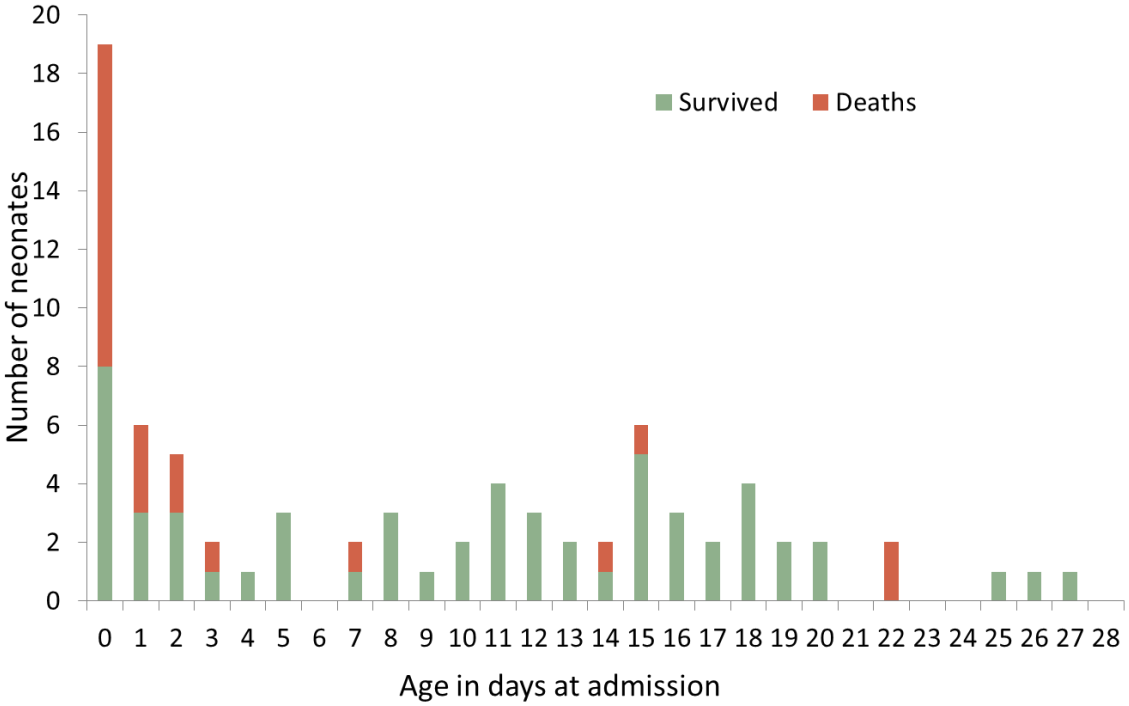


Figure 21 Invasive GBS disease and age at admission, by disease type (meningitis and/or sepsis) in neonatal admissions to Kilifi District Hospital 1998-2013

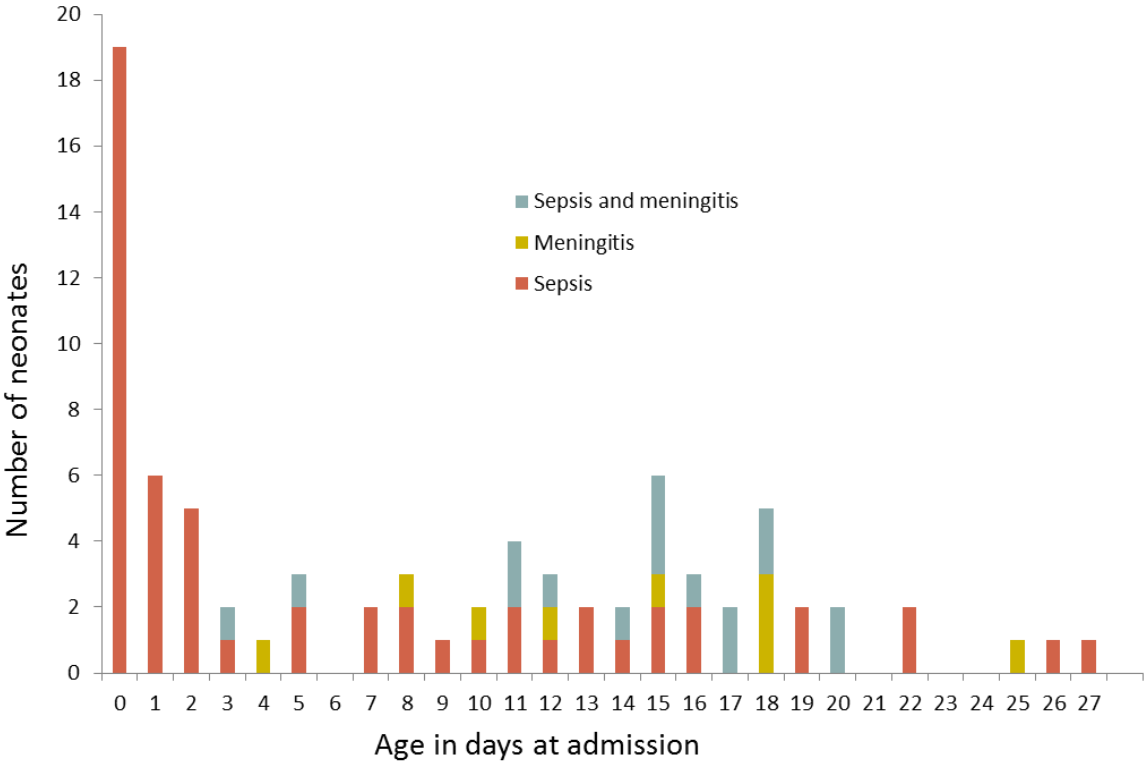
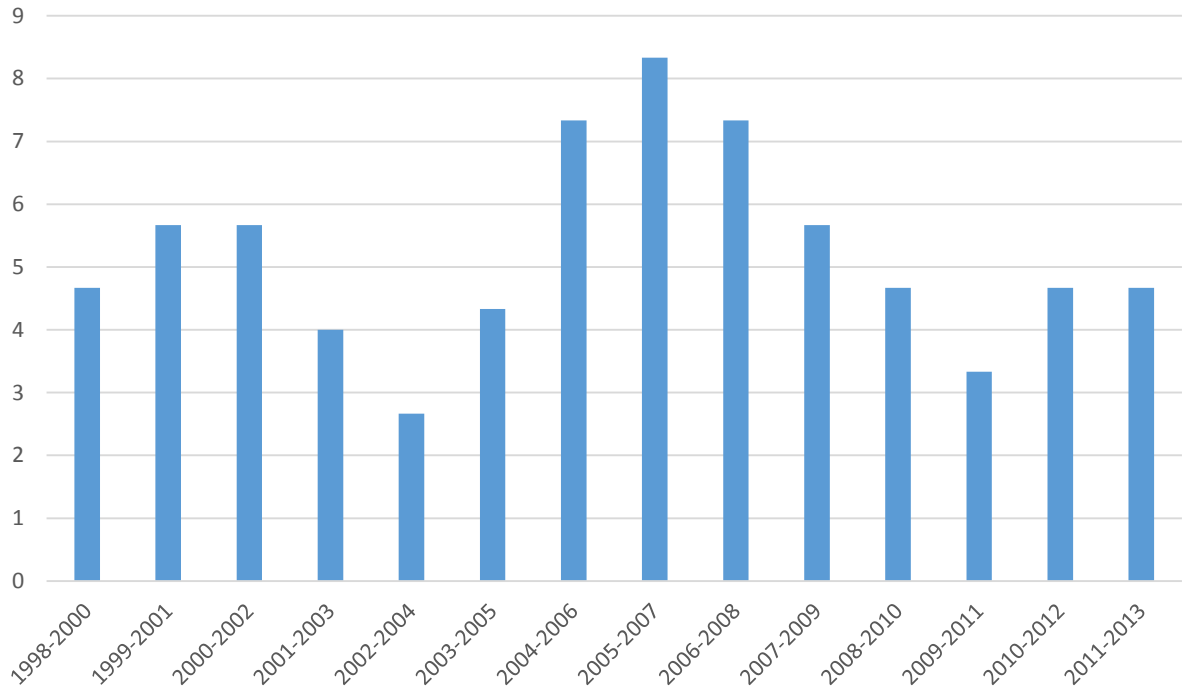


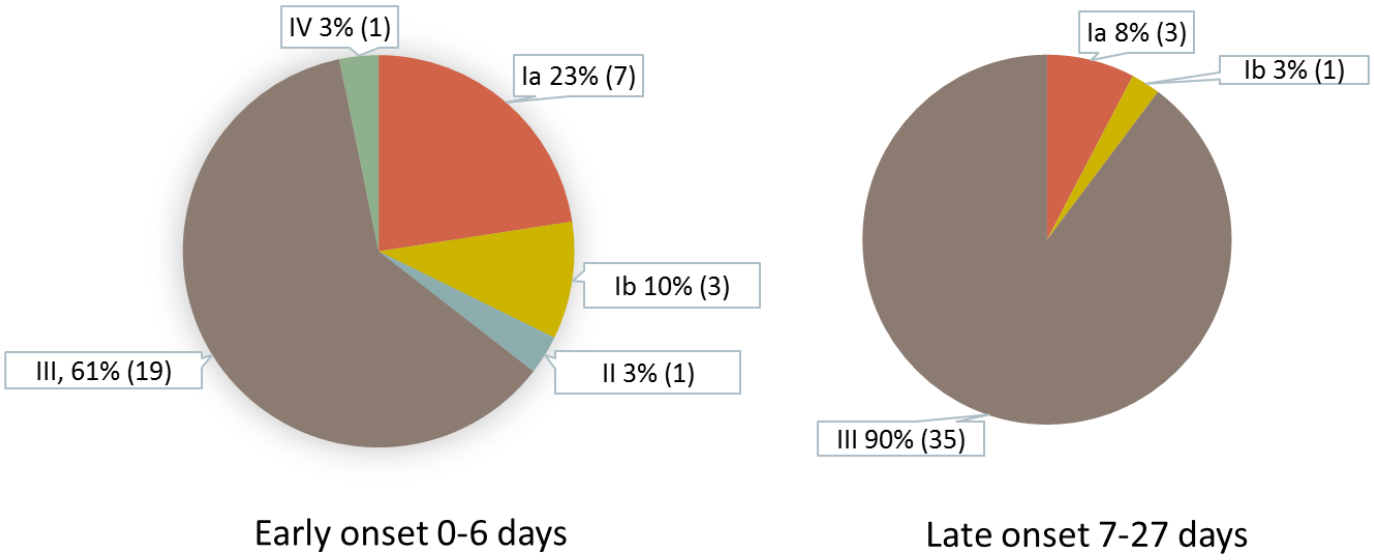
Figure 22 Neonatal admissions to Kilifi District Hospital 1998-2013 with GBS disease (three year moving averages)



5.5 What are the serotypes and MLST types causing neonatal disease?

There were 73 isolates available for whole genome sequencing and these were assigned a serotype. There were 3 neonates with age in days missing, so for those with a serotype and age in days, there were 70 serotypes; 31 in days 0-6 and 39 in days 7-27 of life. The serotypes causing disease in the first 0-6 days of life included serotypes Ia (7/31, 23%), Ib (3/31, 10%), II (1/31, 3%), III (19/31, 61%), IV (1/31, 3%); see Figure 23. Although serotype III predominated in early onset disease III (19/31, 61% (95%CI 42%-78%)) the proportion of late onset disease (days 7-27) caused by serotype III disease (35/39, 90% (95%CI 76%-97%)) was higher. Serotype III predominated in neonatal cases with meningitis; 19/20 cases were serotype III, 18/19 of which were clonal complex 17, as indicated in Table 28. The clonal complexes for invasive neonatal disease (1998-2013) were compared to maternal colonising isolates (2011-2013) from Kilifi District Hospital (Figure 24). Clonal complex-17 accounted for 54/70 77% (95%CI 66-86%) of neonatal invasive isolates, and 271/910 30% (95%CI 27-33%) of maternal colonising GBS isolates; the proportion of CC-1, CC-10, CC-19 were correspondingly lower in the neonatal invasive isolates, with similar proportions of CC-23 in both maternal colonising and neonatal invasive isolates. There were no GBS isolates from CC-26. Figure 25 illustrates the clustering of invasive GBS isolates in the CC-17 region, compared to GBS colonising isolates from mothers delivering in KDH.

Figure 23 GBS serotypes causing invasive early and late invasive neonatal disease (Kilifi District Hospital 1998-2013)*



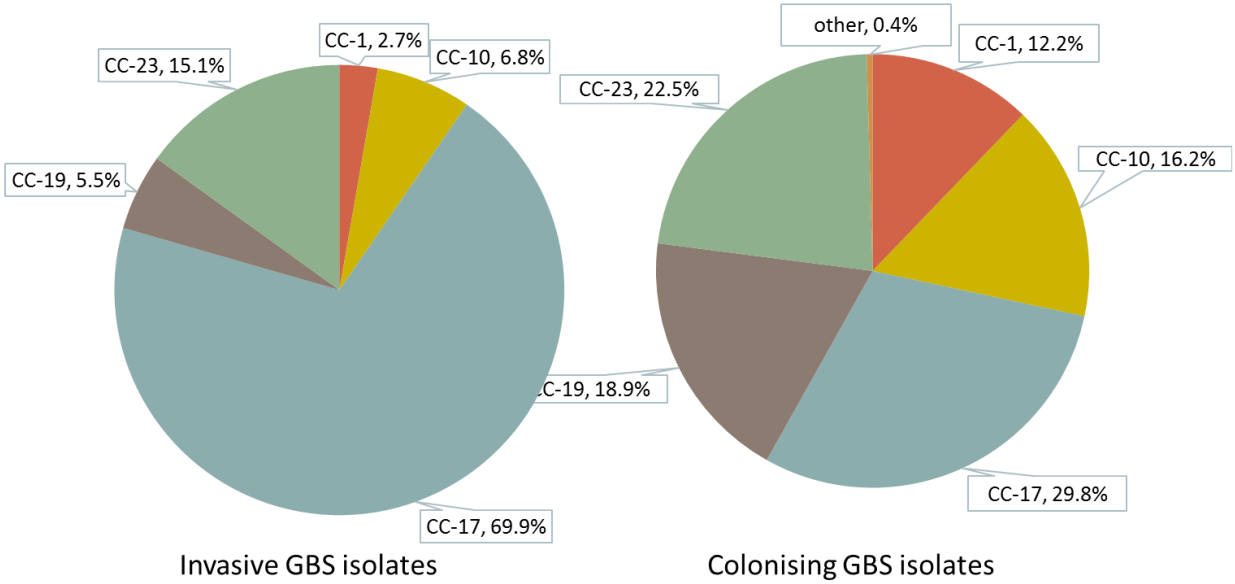
*Serotype and percentage (total number) of GBS isolates by serotype for early and late onset disease

Table 28 MLST types causing neonatal GBS disease (Kilifi District Hospital 1998-2013) grouped into clonal complexes

GBS isolates		Clinical syndrome					
Clonal Complex	MLST*	Sepsis	Meningitis	Sepsis and meningitis	N	Total	%
	2	1	0	0	1		
1	196	1	0	0	1	2	2.7
	10	2	0	0	2		
	8	2	0	0	2		
10	Close to 8	1	0	0	1	5	6.8
	147	1	0	0	1		
	17	27	1	13	41		
	484	4	2	2	8		
17	Close to 484	1	0	0	1	51	69.9
	182	2	1	0	3		
19	19	1	0	0	1	4	5.5
	23	10	0	0	10		
23	Close to 23	0	0	1	1	11	15.1

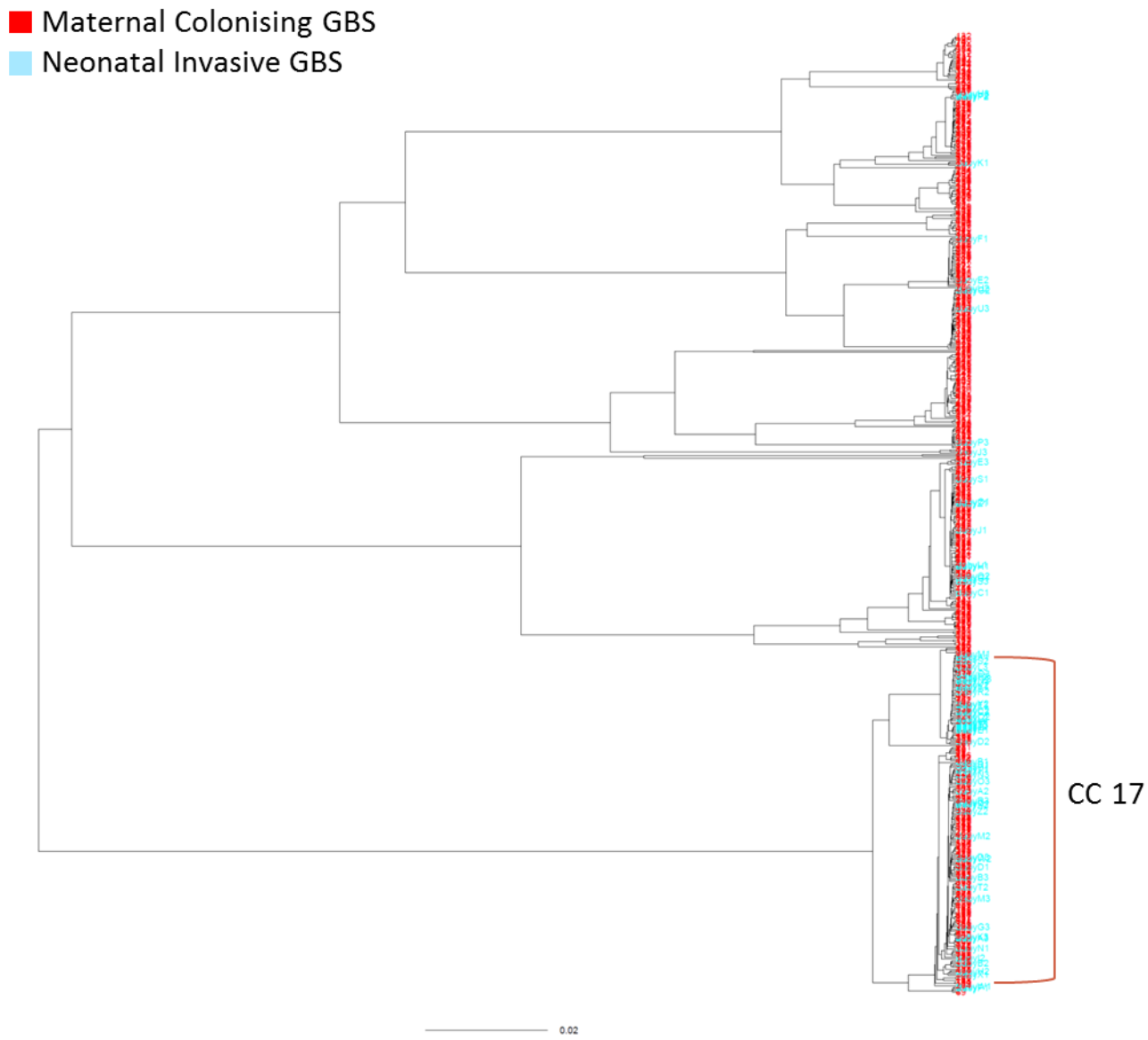
*Where the MLST type is indicated “close to” all but one of the MLST genes match the MLST type.

Figure 24 Clonal complexes of invasive neonatal GBS isolates (1998-2013) compared to maternal colonising isolates from Kilifi District Hospital (2011-13)*



*Clonal complex and percentage of GBS isolates for either invasive or colonising isolates

Figure 25 UPGMA tree of invasive neonatal GBS isolates (1998-2013) compared to maternal colonising isolates from Kilifi District Hospital (2011-13)*



*Maternal colonising GBS isolates are in red, and neonatal invasive isolates are coloured blue in this UPGMA tree. The UPGMA tree does not take into account recombination events and therefore may not fully reflect evolutionary time.

5.6 Discussion

5.6.1 Key results

Group B Streptococcus is an important cause of perinatal disease and death, likely causing a burden of stillbirth equal to that of neonatal GBS disease. The incidence of neonatal disease is lower than that estimated worldwide, but likely underestimated. The case fatality risk is strikingly high; over half of all neonates die from GBS disease in the first 48 hours of life.

There are differences in serotype distribution in early and late onset disease, with a marked dominance of serotype III, clonal complex 17, particularly in late onset neonatal disease, and as the almost universal cause of neonatal GBS meningitis.

A tri-valent vaccine (serotype Ia, Ib, III) would cover all serotypes causing late onset GBS neonatal disease in this study, and over 90% of serotypes causing early onset neonatal GBS disease in this study. There was only one GBS disease isolate (serotype IV) that would not be covered by a pentavalent vaccine (Ia, Ib, II, III, V), and has not previously been reported in sub-Saharan Africa.

5.6.2 Interpretation

This study strongly suggests that GBS is a cause of stillbirth in this setting, in around 2.2% (95%CI 0.6-5.5) of all stillbirths. The comparison with live born controls was useful as it suggested that these cases are unlikely to be due to contamination, with large effect sizes, particularly in the ante-partum stillbirths, and a dose-response relationship from live birth to intra-partum stillbirth to ante-partum stillbirth, the group hypothesised to be most likely stillborn due to infection. The incidence of neonatal GBS disease based on population denominator data was 0.35 cases per 1000 live births (95%CI 0.25-0.47). This estimate for neonatal disease is lower than estimates for neonatal GBS disease worldwide; for GBS EOD estimated worldwide (0.43/1000 live births (95% CI 0.37-0.49) and in Africa (0·53/1000 live births (95% CI 0·15–

0·92)).[153] For LOD, the pooled incidence risk was 0.24 (0.17-0.30) worldwide and 0.71/1000 (0.38-1.04) in Africa. [153]

However, the estimates from this study are minimum incidence estimates, as case identification relied on hospital attendance. Looking at hospital deliveries only, the incidence of GBS neonatal disease was higher, which supports this supposition, although confidence intervals were large because of the small number of cases; 0.76 cases per 1000 live births (95%CI 0.25-1.77 per 1000). Use of intravenous antibiotics during delivery is not uncommon, if a mother is unwell, but is not undertaken according to standard protocols for IAP. The use of intravenous antibiotics would also reduce the incidence of EOD reported here as they would likely sterilise neonatal blood cultures. Care seeking can also be difficult (culturally and practically) soon after a home delivery, and GBS EOD can be rapidly fatal, which may result in fewer cases of neonatal GBS disease being identified, particularly in EOD. Data on care seeking are limited, but a recent review suggested that in low and middle income countries only 59% (range 10-100%) of parents or guardians seek care for sick neonates, although the majority of the studies (17/22) included in this review were from Asia.[18] Care-seeking may influence the apparent higher proportion of late onset disease, compared to early onset disease. It is also possible that co-morbidities in this population are proportionately higher (low birth weight, HIV infection) and neonates remain susceptible to GBS disease to a greater degree than in resource-rich regions, resulting in more late onset GBS disease.

The overall case fatality risk (31%) here is higher than that reported from other African studies, even though these African studies[48,60,155] have a pooled case fatality risk (22%) over twice as high as other regions worldwide.[153] High mortality in the perinatal period is consistent with published data, as case fatality risks have been reported to be twice as high in the first week of life compared to later in infancy (7-89 days).[153]

The clinical presentations of disease described here were also consistent with the literature; sepsis predominates early in life, with more cases of meningitis later in the neonatal period.[230] There were increased cases in males, consistent with increased biological susceptibility to infection at this time.[17] Very low and low birth weights have been shown to be associated with increased risk of GBS disease. Although no formal analysis was undertaken, for GBS disease onset <48h there were 6/23 (26%) of VLBW neonates with GBS disease in this study, and 8/23 (35%) with low birth weight. As overall 3% of deliveries were very low birth weight, and 19% were low birth weight, this suggests that very low and low birth weight neonates are at higher risk of GBS disease in this setting, supporting findings from other regions.[50,231]

There were few cases of HIV infected neonates in this study (6/83, 7.0%), similar to the prevalence of HIV infection in mothers, 5.0% from data on mothers delivering in KDH 2011-2013), but vertical transmission would be expected in less than half of deliveries. Maternal HIV status was not known across the time period of this study (1998-2013) and it was therefore not possible to assess the numbers of neonates who were HIV exposed and uninfected. Of those HIV infected neonates, three were admitted with GBS disease after the perinatal period, the period thought to be of greater risk for HIV exposed uninfected infants.[232] Although these studies are small, and a more recent study in South Africa only identified HIV-exposed infected infants as being at greater risk.[23] More work is needed to determine whether HIV infection plays an important role in GBS disease by increasing susceptibility, both to GBS disease as a whole and in terms of serotypes causing disease; the relatively rare serotype IV GBS isolate was obtained from a neonate whose mother did not consent to HIV testing.

In the first 48h of life, and in stillbirths, the GBS serotypes were the most diverse, consistent with increased host vulnerability to less virulent GBS types at this time. The predominance of serotype III, CC-17 in later neonatal disease and its strong association with meningitis was

consistent with its identification as a hyper-virulent clone, as reported in other settings,[230,233-235] and the reason it has been targeted for a rapid diagnostic test.[234]

Group B Streptococcus has been shown to have a diverse set of virulence factors; it has the ability to invade host epithelial cells and avoid immunological clearance, which is supported by its polysaccharide capsule; however resisting phagocytosis in the blood stream may predispose its penetration of the blood brain barrier.[236] Additional virulence mechanisms may be diverse; one gene encoding surface-anchored protein, Spb1, required for maximal epithelial cell invasion has been identified in serotype III strains.[237] With increased use of WGS, more virulence genes may be identified.

Maternal immunity, and the trans-placental transfer of anti-GBS antibodies (serotype-specific) is an important protective mechanism against early onset neonatal GBS disease.[238] Glyco-conjugate vaccines are in development,[239] and the data here suggest that the trivalent vaccines in clinical trials would cover almost all disease. However, there is the possibility of serotype replacement, seen in pneumococcal disease, but less so with *Haemophilus influenzae*, after the introduction of serotype specific vaccines.[240,241] Protein based vaccines directed at conserved targets identified through WGS may offer more protection from GBS disease in the longer term.[236]

Changes in immune function at birth and in the immediate post-partum period are complex, but the response of the neonate to initial invasion likely determines whether the infection will be cleared or not.[242,243] Infection-induced production of inflammatory cytokines in utero are associated with preterm labour and delivery, and foetal immune systems are biased away from TH1 responses to TH2 responses,[244] which leave the neonate open to invasive bacterial infection before the immune system matures.[243] Toll-like receptors have been identified as important recognition molecules for microbial antigens, stimulating the innate immune

response, and clearing GBS inoculum which translocates across the gastro-intestinal mucosa.[245] Neonates have been shown to have a strong inflammatory cytokine response to GBS, but intracellular killing may be deficient, especially in preterms.[245] Improving understanding of the interplay between neonatal immunity and microbial invasion is important in understanding, and in the longer term, preventing, disease.

5.6.3 Strengths and limitations

Determining the cause of stillbirth in any setting is challenging, and in a semi-rural African setting this was certainly the case. A limited set of investigations were used to investigate the cause of stillbirth in order to meet the needs of the study question, yet also undertake a study that was acceptable in the population, and ethically appropriate. The limited nature of the investigations (not a full autopsy) limits the findings to support the conclusion that the GBS isolated from cord blood and lung aspirate was the cause of stillbirth. However, this is mitigated by the use of a case-control design, and although the numbers were small there was evidence that the isolation of GBS in antepartum stillbirth in particular, is unlikely to be due to chance contamination (Fishers' exact test p=0.055).

The incidences of neonatal GBS disease are, as discussed above, minimum estimates due to limitations in using hospital data and use of a population denominator. However, despite this, the systematic surveillance of all children admitted to hospital with routine blood cultures on admission provides a unique set of GBS isolates causing disease in this region which can be examined in both clinical and molecular terms providing important insights into the aetiology of GBS disease and the likely effectiveness of GBS vaccines in development.

5.6.4 Conclusions

GBS is an important cause of perinatal mortality in this region. Host susceptibility makes the foetus and new-born susceptible to a range of GBS types, after which most disease is caused by

the hyper-virulent strain serotype III, CC-17. Whilst this study provides important data, more data on the aetiology of neonatal sepsis in sub-Saharan Africa are needed and systems need to be strengthened to improve surveillance of severe neonatal disease, in particular before and shortly after, delivery.

Preventing GBS disease, through a risk factor based approach using intra-partum antibiotic prophylaxis, may reduce neonatal disease in the first 48 hours of life, but is unlikely to reduce stillbirth or late onset GBS disease. The most advanced vaccine in development, a trivalent conjugate vaccine (serotypes Ia, Ib and III) would prevent over 90% of all neonatal disease in this study, if its efficacy were 100%. Efficacy data are not yet available from Phase II trials, and a recent study of cost-effectiveness of prevention methods in South Africa suggested a combination of intra-partum prophylaxis based on risk factors as well as maternal GBS vaccination may be the most beneficial and cost-effective solution.[246]

In general terms, earlier case identification, improving supportive neonatal care and instigating effective treatment early is likely to reduce neonatal morbidity and mortality from infection. General strategies for prevention, including anti-sepsis measures such as cord cleansing with chlorhexidine;[27] [26] [247] would reduce mortality, although anti-sepsis measures at delivery (chlorhexidine maternal-vaginal wipes) were not effective in a large trial in South Africa.[30] Measures directed at preventing infection in low birth weight and preterm birth using emollient therapy,[248,249] need further trials to determine if widespread implementation is appropriate, and should be combined with strategies such as Kangaroo Mother care, shown to be effective in low birth weight neonates.[250]

Chapter 6: Vertical transmission of GBS

6.1 Results

This sub-study investigated GBS surface colonisation of neonates (in the ear, nose and umbilicus) within 6 hours of delivery and compared this to the GBS colonisation status of the mother. The genetic relationships between paired GBS isolates from mothers and their new-borns were then investigated, comparing whole genome sequences mapped to a reference genome, for both GBS isolates from neonatal surface colonisation, and GBS isolates from perinatal invasive disease.

The study aimed to answer the following questions:

1. What is the prevalence of neonatal GBS surface colonisation after delivery?
2. What is the molecular evidence for vertical transmission of GBS resulting in neonatal surface colonisation?
3. What is the molecular evidence for vertical transmission of GBS resulting in perinatal disease?

6.2 What is the prevalence of neonatal GBS surface colonisation after delivery?

6.2.1 Participants

There were 4464 mothers who attended KDH during this sub-study in Kilifi District Hospital between 1st May 2012 and 31st July 2013. Of these, 2385 of these delivered outside of neonatal sampling times, which were between 5am and 4pm, to allow neonatal surface sampling within 6 hours of delivery (see Figure 26). After exclusions for ineligibility (n=139), where consent was not obtained (n=469), and missed opportunities (n=641), there were 830 mother and baby pairs recruited.

6.2.2 Results

There were 104/830 mothers colonised with GBS at delivery (12.5%; 95%CI 10.4-15.0) and 44/830 neonates colonised with GBS (ear, umbilicus or nose) within 6 hours of delivery (5.3%, 95%CI 3.9-7.1%). There were 30 neonates colonised with GBS born to mothers colonised with GBS (presumed transmission 29%, 95%CI 22-38%); 14 neonates colonised with GBS were born to mothers not colonised with GBS (see Figure 27). The risk ratio for new-born surface colonisation if the mother was colonised with GBS at delivery was 15.0 (95%CI 8.2-27.3).

The study was not powered to assess risk factors for neonatal surface colonisation, and the participant characteristics with neonatal GBS colonisation are given as descriptive data. However, whilst the numbers were small (Table 29), the percentage of neonates with GBS surface colonisation after non-operative delivery (36/662, 5.4%) and operative delivery (7/161, 4.3%) were similar. For neonates with birth weight under 2500g, the prevalence of GBS surface colonisation was high (11/121, 9.1%). The number of neonates with birth weight under 1500g was small (n=10), and none were GBS surface colonised. For neonates 2500g or over, 33/686, (4.8%) were surface colonised with GBS after delivery. In terms of risk factors for early onset GBS disease, there were 35/830 mothers with prolonged rupture of membranes (2 neonates

colonised), 5/830 mothers with fever (>38^{o}C) (1 neonate colonised) and 25 mothers with a urinary tract infection (1 neonate colonised).

Figure 26 Recruitment of mother and neonatal pairs at delivery (Kilifi District Hospital 2012-13)

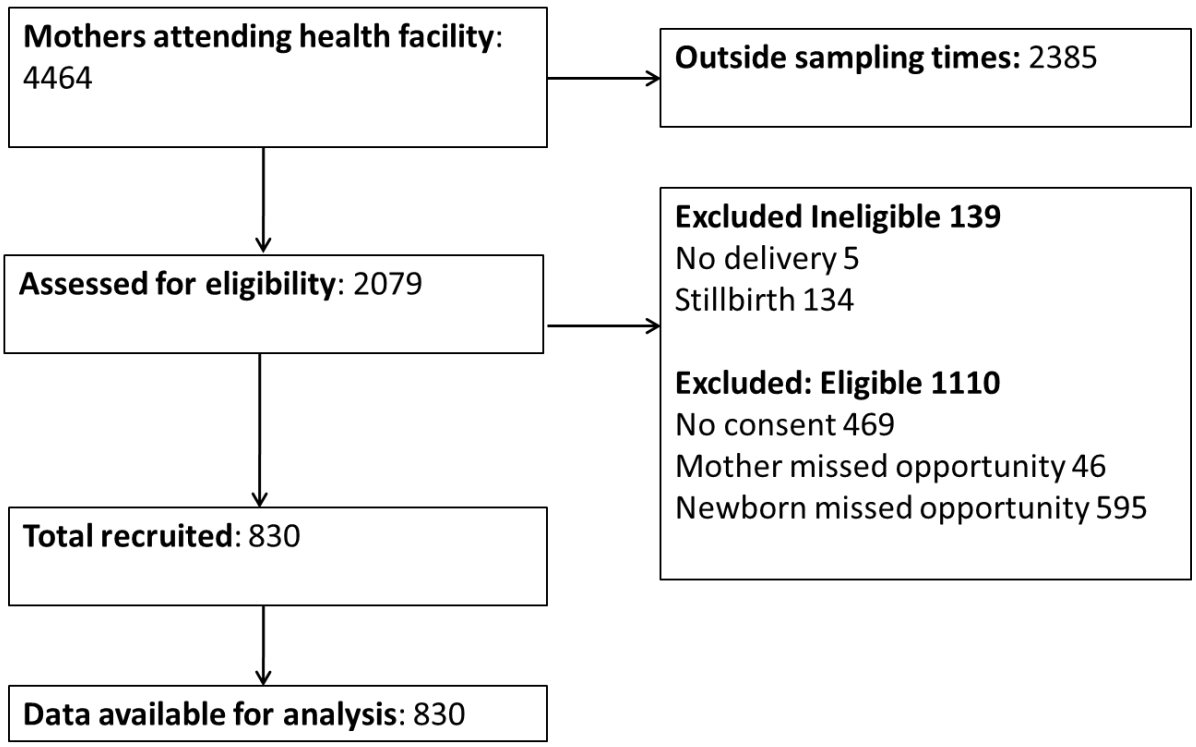
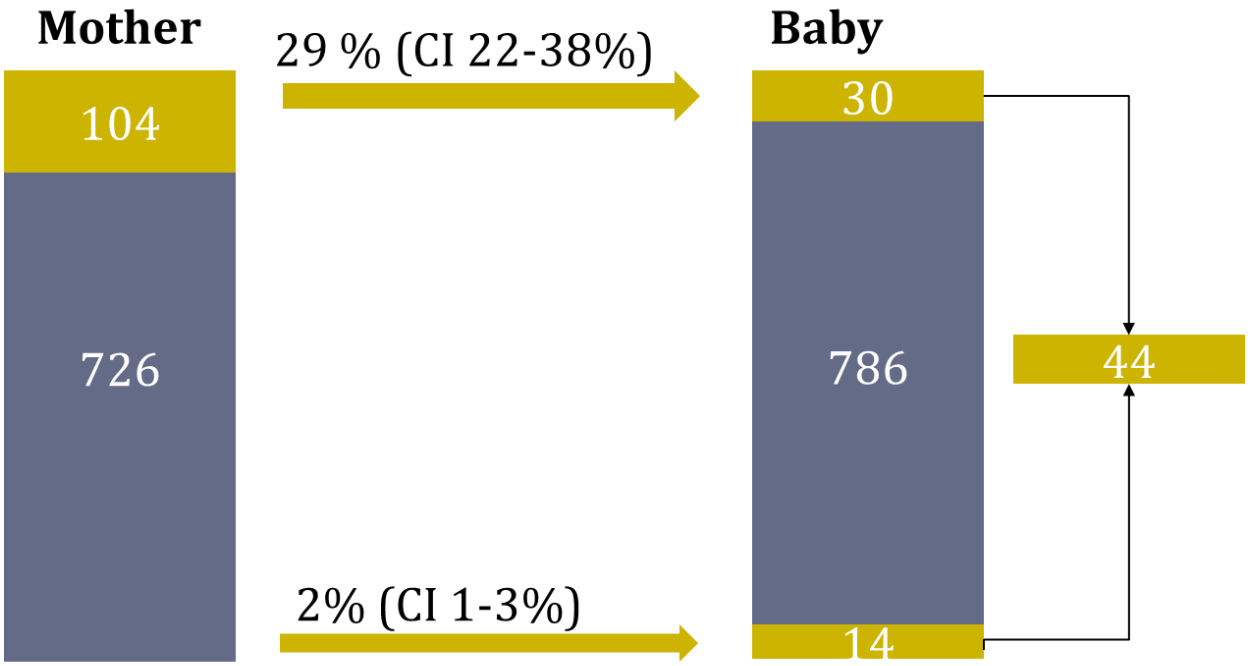


Table 29 Characteristics of mothers and newborns included in the study of GBS transmission (surface colonisation) in Kilifi District Hospital (2012-13)

Variable	Category	Total	Maternal GBS colonisation		Neonatal GBS colonisation	
		N	n	%	n	%
		830	**104**	**12.5**	**44**	**5.3**
Parity	1	301	47	15.6	20	6.6
	2-4	352	40	11.4	17	4.8
	>5	170	17	10.0	7	4.1
	Missing	7	0	0.0	0	0.0
Sex of baby	Male	431	48	11.1	19	4.4
	Female	385	56	14.5	25	6.5
	Missing	14	0	0.0	0	0.0
Mode of delivery	Non-operative	662	80	12.1	36	5.4
	Operative	161	23	14.3	7	4.3
	Missing	7	1	14.3	1	14.3
Gestation (weeks)	<34	27	5	18.5	0	0.0
	<37	200	20	10.0	11	5.5
	37-42	505	64	12.7	24	4.8
	>42	84	14	16.7	8	9.5
	Missing	14	1	7.1	1	7.1
Weight	<1500	10	1	10.0	0	0.0
	<2500	121	14	11.6	11	9.1
	2500-3500	592	76	12.8	30	5.1
	>3500	94	12	12.8	3	3.2
	Missing	13	1	7.7	0	0.0
PROM >18h	No	715	89	12.4	37	5.2
	Yes	35	3	8.6	2	5.7
	Missing	80	12	15.0	5	6.3
Maternal fever (>38.0^{o}C)	No	690	85	12.3	36	5.2
	Yes	5	2	40.0	1	20.0
	Missing	135	17	12.6	7	5.2
Maternal UTI	No	693	84	12.1	39	5.6
	Yes	25	6	24.0	1	4.0
	Missing	112	14	12.5	4	3.6

Figure 27 Maternal to neonatal transmission of GBS surface colonisation (Kilifi District Hospital 2012-13)



6.3 What is the molecular evidence for vertical transmission of GBS resulting in neonatal surface colonisation?

6.3.1 Participants

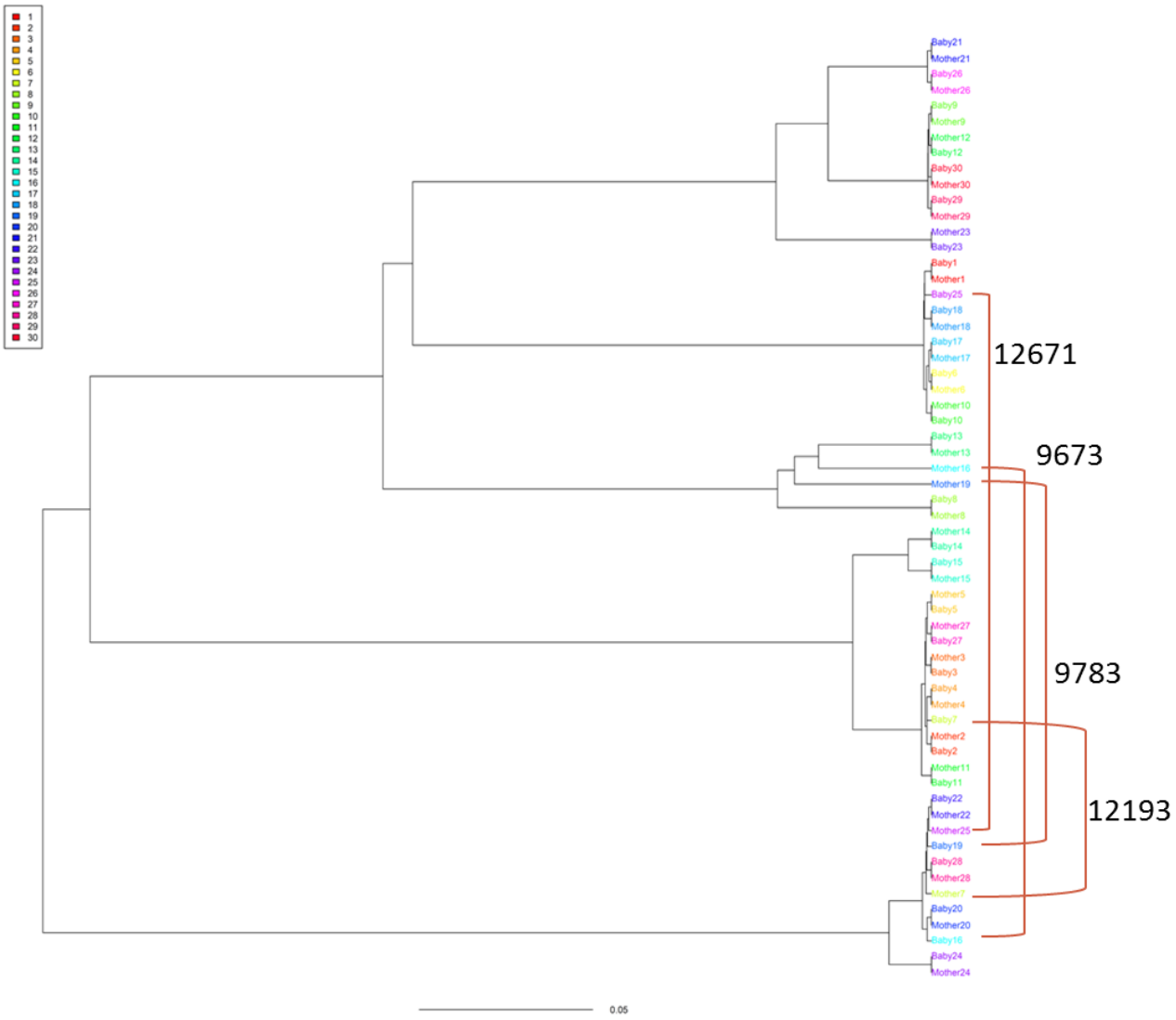
There were 830 mother and baby pairs recruited, as described and illustrated in Figure 27. Out of these 830 mother and new-born pairs, there were 30 pairs of GBS isolates from maternal GBS colonisation at delivery and new-born GBS surface colonisation.

6.3.2 Results

There were 30 pairs of GBS isolates from maternal GBS colonisation at delivery and new-born GBS surface colonisation, mapped to the reference genome 2603 VR.[192] There were 17/30 with 0 SNP differences, 6/30 had 1 SNP difference, 2/30 had 2 SNP differences and 1/30 had 4 SNP differences. There were 4/30 pairs with >5 SNP differences (9673, 9783, 12193, 12671) which may have been due to maternal GBS colonisation with mixed strains, or through nosocomial acquisition. The UPGMA tree illustrates these relationships (Figure 28), indicating the pairs of GBS isolates within which there is substantial variation and the number of SNP differences.

Of the seven neonates who were GBS colonised after delivery by caesarean section, 5/7 mothers were also colonised with GBS. One of these five was colonised by a GBS of a different serotype (9673 SNP differences), but 4/5 were colonised with the same GBS serotype and MLST type as colonising the neonate after delivery; 3/5 had 0 SNP differences, 1/5 had 1 SNP difference.

Figure 28 UPGMA tree showing the relationship between GBS isolates from maternal GBS recto-vaginal swabs and neonatal surface swabs*



*The maternal and neonatal pairs (1-30) are indicated by being the same colour, as per the key. There were <5 SNP differences between pairs, apart from those indicated, with the number of SNP differences given in the illustration. The UPGMA tree does not take into account recombination events and therefore may not fully reflect evolutionary time.

6.4 What is the molecular evidence for vertical transmission of GBS resulting in perinatal disease?

6.4.1 Participants

There were five neonates admitted to the paediatric ward with invasive GBS disease identified from routine clinical investigation (blood cultures and cerebrospinal fluid cultures), during the cohort study period (2011-2013). There were four stillbirths with GBS isolated from cord blood culture or lung aspirate in the stillbirth study. Three out of four of these stillborn babies' mothers were also tested for GBS maternal colonisation at delivery (one stillborn was delivered after maternal colonisation sampling had completed, see Table 30).

6.4.2 Results

Out of the five neonates with GBS disease admitted to paediatrics, three mothers were GBS colonised; the risk ratio for the association between maternal GBS colonisation and neonatal GBS disease was 11.6 (95%CI 1.9 – 69.1). For the stillbirths, two of the three mothers tested had GBS isolated before delivery, one of whom also had GBS isolated from urine culture. The risk ratio for the association between maternal GBS colonisation and stillbirth due to GBS disease was 7.6 (95%CI 1.1-52.6). There were therefore five pairs where GBS was isolated from the mother (colonising) and the neonate or stillborn baby (invasive) which could be compared based on pairwise nucleotide differences from sequence alignment (single nucleotide polymorphisms, SNPs).

The characteristics of the neonates and stillborn babies with invasive GBS disease, are described in Table 30. There were five neonates, and four stillbirths, two of whom were intra-partum stillbirths, and two were ante-partum stillbirths. All of these births were vaginal deliveries. There were 3/9 (33%) births with birth weight under 2500g, and 2/9 (22%) births with gestation under 37 weeks'. There was 1/9 (11.1%) mother who was HIV infected. There were two mothers

with urinary tract infection on dipstick, one of whom was confirmed to have GBS on urine culture. None of the mothers were reported to have fever, and only one mother (who also had the GBS urinary tract infection) had PROM (prolonged rupture of membranes ≥18h).

The types of GBS isolated from the neonates, who all had sepsis (peripheral blood cultures positive for GBS) were universally GBS serotype III. For the stillborn neonates, 2/4 were serotype V, 1/4 was serotype III, and 1/4 the isolate was not available for typing.

The UPGMA tree (Figure 29) shows the relationship of GBS isolates isolated from mothers and newborns and stillborns included in this sub-study, with pairs identified by number and colour. For the paired isolates, the two stillbirths (6 and 8) had 0 SNP differences between GBS isolates colonising the mother and GBS invading the foetus. For stillbirth 8, the GBS isolated from the lung, cord blood and the mothers' recto-vaginal swab and urine culture were all identical (0 SNP differences).

For neonates 1 and 2, GBS isolates were 0 SNPs different to the GBS isolates colonising the mother, and these neonates were admitted on day 0 and day 3 of life respectively. Neonate 3, admitted on day 0, had GBS isolated which was 1012 SNPs different to the maternal GBS colonising isolate. Mother 3 had a similar GBS isolate to mothers (and neonates) 1 and 2; there were four months between these admissions and no clear link in terms of household location. As the samples were sub-cultured at different times and processed on different 96 well sequencing plates the possibility of lab error was very low.

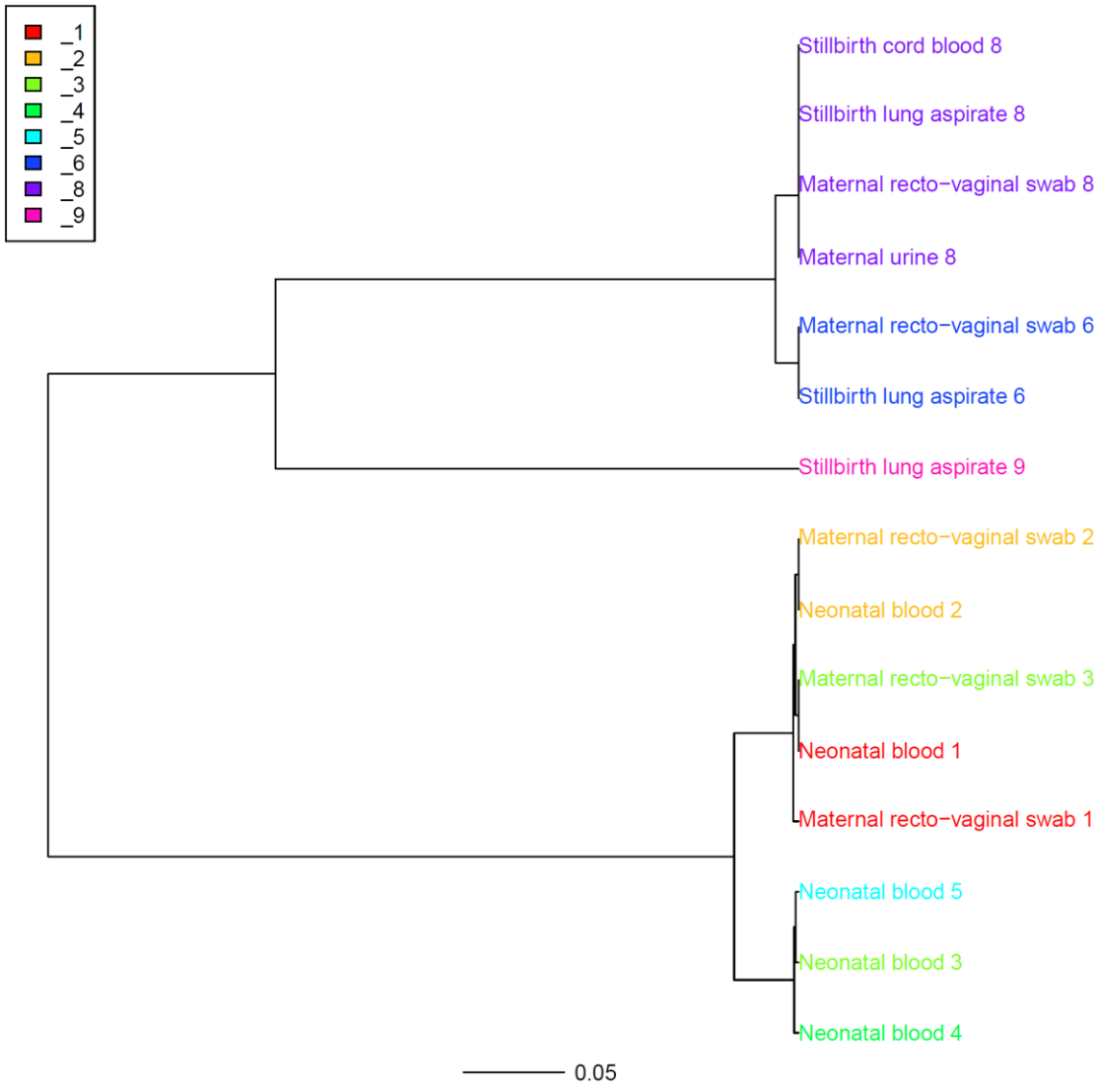
Table 30 Characteristics of live and stillborns with perinatal GBS disease in Kilifi District Hospital (2011-2013)

No.	Birth type*	Age in days	Maternal GBS status	Weight (grams)	Sex	Gestation (wks)	HIV status	PROM >18h	UTI	Maternal fever	Serotype
1	Live	0	Pos	3000	M	36	Neg	No	No	No	III
2	Live	3	Pos	3880	M	40	Pos	Missing	Missing	No	III
3	Live	0	Pos	2415	F	37	Neg	No	No	No	III
4	Live	2	Neg	3500	M	37	Neg	No	No	No	III
5	Live	2	Neg	3000	M	36	Neg	No	No	No	III
6	IPS	0	Pos	2455	F	39	Neg	No	No	No	V
7**	APS	0	Missing	2990	F	36	Neg	No	Dipstick +ve	No	N/A
8	APS	0	Pos	2850	F	39	Neg	Yes	GBS culture +ve	No	V
9	IPS	0	No	1705	F	35	Neg	Missing	No	No	III

*IPS intrapartum stillbirth; APS ante-partum stillbirth

**This stillbirth was not enrolled during the maternal colonisation study so only eight cases of perinatal GBS disease are included in the sub-analysis, described on page 140

Figure 29 UPGMA tree of invasive perinatal GBS isolates (sample types of cord blood, lung aspirate or peripheral blood culture), with maternal GBS pair (recto-vaginal swab) where available*



*The colour represent GBS isolates from either the mother or her baby. These "pairs" are numbered 1-9 in the key. One mother also had GBS identified from urine culture, which is also included in this UPGMA tree. The UPGMA tree does not take into account recombination events and therefore may not fully reflect evolutionary time.

6.5 Discussion

6.5.1 Key results

There were 44/830, 5% (95%CI 4-7%) neonates colonised with GBS (ear, umbilicus or nose) within 6 hours of delivery, and 30/104, 41% (29-53%) of new-borns were colonised with GBS whose mother was colonised with GBS. Whilst there was strong evidence of vertical transmission in 26/30 mother new-born pairs, in 4/30 pairs the GBS isolates were genetically dissimilar (>5 SNPs). In addition, around 2% (1-3%) of neonates whose mother was not colonised with GBS at delivery had surface GBS colonisation after delivery. There were nine neonates and stillbirths (2011-2013) with invasive GBS disease and of these there were five born to mothers who had GBS isolated from recto-vaginal swab at delivery. In four out of five of these pairs there was strong evidence of vertical transmission (0 SNP differences) and in one pair there were 1012 SNP differences.

6.5.2 Interpretation

The prevalence of neonatal GBS colonisation was around a half that reported in other studies,[30,94,151] consistent with the lower prevalence (11.4%, 95%CI 10.6-12.3) of maternal GBS colonisation at Kilifi District Hospital. However, transmission between mothers and neonates was also lower than that reported elsewhere; in Zimbabwe, 62%,[94] South Africa, 55%,[30] and Tanzania, 39%[109] of GBS colonised mothers had new-borns who were also surface-colonised with GBS.

There was good evidence of vertical transmission of GBS from from mother to new-born. However in those cases where neonates were GBS surface colonised with a genetically different GBS isolate, it could not be determined whether this was due to maternal GBS colonisation with more than one GBS strain, or GBS from the environment shortly after birth. Likewise, for neonates who were GBS surface colonised, whose mother was not

identified as being GBS surface colonised, this may be either due to lack of sensitivity in maternal recto-vaginal sampling and culture, or environmental contamination of the neonate.

It was interesting that neonates born either by operative (caesarean) or non-operative (vaginal) delivery were both surface colonised by GBS after delivery, and in 4/7 cases of operative delivery there was strong evidence that the GBS was acquired from the mother (<5 SNP differences). It is possible that ascending GBS may cause neonatal surface colonisation prior to delivery through the birth canal, especially as these would have been emergency caesarean sections, or that GBS surface colonisation was acquired from the mother or midwife handling the baby after delivery.

There was clear evidence of GBS transmission from mothers to new-borns causing perinatal disease, in terms of in-utero invasion (resulting in stillbirth) and cases of neonatal GBS disease in the first week of life. The isolation of four GBS isolates from four different culture sites (two from the mother and two from the stillbirth) all with 0 SNP differences was very strongly suggestive of a densely colonised mother with ascending invasive GBS disease causing stillbirth. There was one neonate with 1012 SNP differences in GBS isolated from peripheral blood culture, compared to the maternal colonising GBS isolate. This neonate had early onset sepsis (on day 0), which suggested environmental contamination was unlikely; there may have been diversity in the GBS strains colonising the mother, and ascending infection before delivery.

6.5.3 Strengths and limitations

The study was limited in that it did not include environmental sampling, or sampling of health care workers, which would be informative in those cases where GBS was not isolated from the mother, and could be important if there was evidence that neonatal GBS

surface colonisation was hospital acquired. In addition, the number of neonates colonised with GBS in this study was small, with insufficient power to analyse risk factors for neonatal GBS colonisation. There was the potential for selection bias, as 583/2088 neonates were not included due to missed opportunities. This may have been through early discharge, or the time frame for sampling being missed by the research clinician responding to other duties.

Despite these limitations, the study is important in its examination of maternal and neonatal GBS transmission in the context of both surface colonisation and perinatal disease, and use of whole genome sequence data to compare GBS isolates from mothers and newborns, demonstrating transmission.

6.5.4 Conclusions

Group B Streptococcus was transmitted vertically from mothers, resulting in perinatal GBS disease and GBS surface colonisation. GBS can cause ascending disease prior to passage through the birth canal. Perinatal GBS disease and GBS surface colonisation was not always associated with maternal GBS colonisation, which may be due to insensitivity in sampling and culture methods, or environmental acquisition, which further research is required to discern. In addition, more work is needed to understand where GBS causing disease in later onset neonatal disease comes from. This could be, for example, from mothers, other household members, or be hospital acquired.

Chapter 7: Summary and conclusions

On the Kenyan coast, maternal GBS colonisation at delivery was around 12% overall, with prevalence increasing from rural to urban settings. Although this is lower than found in North America and Europe, the methods were robust and study limitations seem unlikely to account for this. Maternal GBS colonisation was associated with higher socio-economic status and improved nutrition, as well as demographic factors (age and parity) and particular exposures (to cattle). The increase in maternal GBS colonisation with higher socio-economic status and nutritional status was an important finding, and is consistent with the largest and most recent study from the United States.[119] It seems plausible that changing socio-demographics may have contributed to the emergence of GBS disease, in North America and Europe over the last century, and that GBS prevalence is lower in a setting where the majority of the study population are below the poverty line, and have low nutritional status. Group B Streptococcus has, and is now emerging in the more developed parts of sSA, such as South Africa.[49] More importantly, maternal GBS colonisation may increase with further socio-demographic change, and the substantial urbanisation which sub-Saharan Africa is undergoing, and which is predicted to continue.[251, 10] The reasons behind the association of GBS colonisation with these socio-economic status and nutrition are unclear. It likely relates to a changing vaginal microbiome, but more work is needed to understand the vaginal microbiome and the community states during pregnancy, as well as the effect of diet, nutritional status and socio-economic factors.

Group B Streptococcus was an important cause of perinatal and neonatal disease, shown to be transmitted vertically from the mother to the foetus and new-born. Although overall incidence was low when using a population denominator from the community, the

incidence is likely underestimated through a combination of lack of access to care (especially for early-onset disease), which is supported by two key facts. First, that the proportion of late onset disease was higher than early onset disease (the opposite to developed countries with high care coverage) and secondly that the estimated incidence was twice as high for EOD in facility births. In addition, the use of antibiotics during delivery, which would sterilise neonatal blood cultures, could also reduce identified cases of EOD.

There was a spectrum of decreasing host vulnerability to GBS disease, and corresponding increase in the virulence of GBS strains causing disease across the perinatal and neonatal periods. This was seen in changing disease presentations and the serotypes and clonal complexes of GBS causing disease. In stillbirths and early onset neonatal disease, GBS disease was common in preterm (or low birth weight) neonates, and a range of GBS serotypes and clonal complexes caused disease, which was mostly sepsis. In late onset disease, and particularly meningitis, serotype III (almost all clonal complex 17) caused almost all cases of disease.

Maternal GBS colonisation was strongly associated with invasive perinatal GBS disease, but not directly linked with other adverse perinatal outcomes, outside the context of possible effect modifiers (maternal fever, prolonged rupture of membranes ($\geq$18h) and urinary tract infection). This relationship helps explain why intrapartum antibiotic prophylaxis has been able to be based on either a risk-based strategy (these effect modifiers) or screening for maternal GBS colonisation in the third trimester. However, as maternal GBS colonisation is a necessary cause for EOD, it would be expected, as has been found,[80] to be the most effective method of reducing EOD.

Although prevention of EOD could be through either intra-partum prophylaxis or vaccination; vaccination is an attractive strategy as it has the potential to reduce GBS

disease ante-partum (stillbirth), as well as late onset neonatal disease. Intra-partum prophylaxis would also require greater infrastructure and laboratory capacity for processing samples. In recent years, some of the perceived difficulties of introducing maternal vaccinations have been overcome, most recently with maternal influenza and pertussis vaccines. In sub-Saharan Africa there is a long standing maternal vaccination platform, with almost all women immunised against tetanus during pregnancy. In terms of coverage, for the GBS isolates causing disease in this study, a tri-valent GBS vaccine covering serotypes Ia, Ib and III, would have covered over 90% of GBS serotypes causing invasive disease. So although there is concern over potential serotype-replacement post vaccine-introduction (as seen with *S. pneumoniae* conjugate vaccine),[252] GBS serotypes are less divergent, and whilst this would require ongoing surveillance, may be less problematic.

There were limitations to this study. It was facility based, and although this was mitigated by including a range of health facilities across different communities, much less is known about home births and their outcomes. This is a potential avenue for future research, and although challenging, is increasingly feasible. A network of community health reporters would be required to identify pregnancies, and mobile phone technology utilised so that a study team could be informed of outcomes, which, with a careful community engagement process could allowing investigation of the cause of stillbirths and neonatal deaths, including use of techniques such as a minimally invasive autopsy.

The missing data within the clinical data was another limitation; although collected prospectively on standardised forms, clinical data were obtained by over 50 different health care professionals for the cohort study alone, and despite training and standard operating procedures, in a busy clinical context this introduced missing data. Whilst the bias missing data could introduce was reduced at analysis through multiple imputation,

this method had its own limitations in reducing post-estimation methods available, as likelihood based methods cannot be used.

Further research is required to deepen our understanding of Group B Streptococcus and the advance of whole genome sequencing offers the opportunity to investigate virulence genes, and examine the evolution of the GBS population, both from historic isolates and in future prospective studies. The reasons behind the virulence factors for CC-17 may be multiple, but one surface anchored protein, termed hypervirulent GBS adhesin (HvgA) has been identified and animal models suggest it needs to be expressed for CC-17 to be hypervirulent. This surface protein allows the GBS to adhere more effectively to endothelial cells, and cross the blood-brain barrier (which results in meningitis).[253]

With the introduction of maternal vaccination likely, it is essential to increase surveillance of both maternal colonising GBS isolates and those causing stillbirth and neonatal disease, so that the effectiveness of vaccination can be assessed, and an early response to changing capsular types made. In pragmatic terms, whole genome sequencing large sets of GBS isolates will allow rapid capsular tying (as used here) and antibiotic susceptibility prediction, which is the subject of future work, and is important for monitoring the emergence of resistance to the mainstay of current treatment, penicillin. Whole genome sequencing also allows studies of transmission in populations, as well as in the mother-baby pairs examined here. An interesting additional study would be a cross-sectional survey of GBS prevalence in a population, with whole genome sequencing used to examine transmission within and between households.

This was the most comprehensive study of GBS in sub-Saharan Africa to date. GBS is an important, potentially preventable, cause of perinatal and neonatal death. The results give insight into factors which may have contributed to the emergence of GBS in resource-rich

settings, with important implications in terms of increasing GBS disease in sub-Saharan Africa, as it undergoes urbanisation. Maternal GBS vaccinations in development are likely to cover GBS disease-causing serotypes. This is an opportunity to reduce perinatal mortality and morbidity in sub-Saharan Africa.

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References

1. Lancefield RC. A Serological Differentiation of Human and Other Groups of Hemolytic Streptococci. *The Journal of experimental medicine* 1933; 57(4): 571-95.

2. Scott JA, Bauni E, Moisi JC, et al. Profile: The Kilifi Health and Demographic Surveillance System (KHDSS). *Int J Epidemiol* 2012; 41(3): 650-7.

3. Lawn JE, Gravett MG, Nunes TM, Rubens CE, Stanton C, Group GR. Global report on preterm birth and stillbirth (1 of 7): definitions, description of the burden and opportunities to improve data. *BMC Pregnancy Childbirth* 2010; 10 Suppl 1: S1.

4. Murray CJ, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2013; 380(9859): 2197-223.

5. Young Infants Clinical Signs Study Group. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. *Lancet* 2008; 371(9607): 135-42.

6. Metzker ML. Sequencing technologies - the next generation. *Nature reviews Genetics* 2010; 11(1): 31-46.

7. Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* 2012; 379(9832): 2151-61.

8. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet* 2014.

9. Omran AR. The epidemiologic transition. A theory of the epidemiology of population change. *The Milbank Memorial Fund quarterly* 1971; 49(4): 509-38.

10. United Nations. World urbanization prospects: the 2009 revision. http://esa.un.org/unpd/wup/Documents/WUP2009_Highlights_Final.pdf (accessed 20.07.2014).

11. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? where? why? *Lancet* 2005; 365: 891-900.

12. Lawn JE, Blencowe H, Pattinson R, et al. Stillbirths: Where? When? Why? How to make the data count? *Lancet* 2011; 377(9775): 1448-63.

13. Lawn JE, Blencowe H, Oza S, et al. Every Newborn: Progress, priorities, and potential beyond survival. *Lancet* 2014; doi:10.1016/S0140-6736(08)61345-8.

14. Black RE CS, Johnson HL, Lawn JE, Rudan I, Bassani DG, Jha P, Campbell H, Walker CF, Cibulskis R, Eisele T, Liu L, Mathers C; for the Child Health Epidemiology Reference Group of WHO and UNICEF. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet* 2010.

15. Bang AT, Reddy HM, Bang RA, Deshmukh MD. Why do neonates die in rural Gadchiroli, India? (Part II): estimating population attributable risks and contribution of multiple morbidities for identifying a strategy to prevent deaths. *J Perinatol* 2005; 25 Suppl 1: S35-43.

16. Goldenberg RL, McClure EM, Saleem S, Reddy UM. Infection-related stillbirths. *Lancet* 2010; 375(9724): 1482-90.

17. Seale AC, Blencowe H, Manu AA, et al. Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, south Asia, and Latin America for 2012: a systematic review and meta-analysis. *Lancet Infect Dis* 2014.

18. Herbert HK, Lee AC, Chandran A, Rudan I, Baqui AH. Care seeking for neonatal illness in low- and middle-income countries: a systematic review. *PLoS Med* 2012; 9(3): e1001183.

19. Seale AC, Blencowe H, Zaidi A, et al. Neonatal severe bacterial infection impairment estimates in South Asia, sub-Saharan Africa, and Latin America for 2010. *Pediatr Res* 2013; 74 Suppl 1: 73-85.

20. Molyneux E. Severe neonatal bacterial infections: when numbers matter. *Lancet Infect Dis* 2014; 14(8): 665-7.

21. Seale AC, Mwaniki M, Newton CR, Berkley JA. Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa. *Lancet Infect Dis* 2009; 9(7): 428-38.

22. van Eijk AM, Ayisi JG, Ter Kuile FO, et al. HIV, malaria, and infant anemia as risk factors for postneonatal infant mortality among HIV-seropositive women in Kisumu, Kenya. *J Infect Dis* 2007; 196(1): 30-7.

23. Cutland CL, Schrag SJ, Zell ER, et al. Maternal HIV infection and vertical transmission of pathogenic bacteria. *Pediatrics* 2012; 130(3): e581-90.

24. UNICEF. Maternal Health. 2012. http://www.data.unicef.org/maternal-health/delivery-care (accessed 31.07.2014).

25. World Health Organisation. Pocket Book of Hospital Care for Children. Problems of the Neonate and Young Infant. Second Edition ed; 2013. p. 45-69.

26. Arifeen SE, Mullany LC, Shah R, et al. The effect of cord cleansing with chlorhexidine on neonatal mortality in rural Bangladesh: a community-based, cluster-randomised trial. *Lancet* 2012; 379(9820): 1022-8.

27. Tielsch JM, Darmstadt GL, Mullany LC, et al. Impact of newborn skin-cleansing with chlorhexidine on neonatal mortality in southern Nepal: a community-based, cluster-randomized trial. *Pediatrics* 2007; 119(2): e330-40.

28. Taha T, Biggar R, Broadhead RL, Mtimavalye LAR, Miotti PG, Justesen AB, Liomba GN, Chiphangwi JD. Effect of cleansing the birth canal with antiseptic solution on maternal and newborn morbidity and mortality in malawi: clinical trial. *British Medical Journal* 2007; 315: 216 - 20.

29. Winani S, Wood S, Coffey P, Chirwa T, Mosha F, Changalucha J. Use of a clean delivery kit and factors associated with cord infection and puerperal sepsis in Mwanza, Tanzania. *Journal of Midwifery and Women's Health* 2007; 98: 252 - 68.

30. Cutland C, Madhi SA, Zell ER, Kuwanda L, Laque M, Groome M, Gorwitz R, Thigpen MC, Patel R, Velaphi SC, Adrian P, Klugman K, Schuchat A, Schrag SJ; PoPS Trial Team. Chlorhexidine maternal-vaginal and neonate body wipes in sepsis and vertical transmission of pathogenic bacteria in South Africa: a randomised, controlled trial. *Lancet* 2009; 374(9705): 1909-16.

31. Sebitloane H, Moodley J, Esterhuizen, TM. Prophylactic antibiotics for the prevention of postpartum infectious morbidity in women infected with human immunodeficiency virus: a randomized controlled trial. *American Journal of Obstetrics and Gynaecology* 2008; 198(189): e1 - e6.

32. Roper MH, Vandelaer JH, Gasse FL. Maternal and neonatal tetanus. *Lancet* 2007; 370(9603): 1947-59.

33. Lindsey B, Kampmann B, Jones C. Maternal immunization as a strategy to decrease susceptibility to infection in newborn infants. *Curr Opin Infect Dis* 2013; 26(3): 248-53.

34. Baker CJ, Barrett FF. Group B streptococcal infections in infants. The importance of the various serotypes. *JAMA* 1974; 230(8): 1158-60.

35. Romero R, Espinoza J, Chaiworapongsa T, Kalache K. Infection and prematurity and the role of preventive strategies. *Seminars in neonatology : SN* 2002; 7(4): 259-74.

36. Gloyd S, Chai S, Mercer MA. Antenatal syphilis in sub-Saharan Africa: missed opportunities for mortality reduction. *Health Policy Plan* 2001; 16(1): 29-34.

37. Jongen VH, van Roosmalen J, Tiems J, Van Holten J, Wetsteyn JC. Tick-borne relapsing fever and pregnancy outcome in rural Tanzania. *Acta Obstet Gynecol Scand* 1997; 76(9): 834-8.

38. Katz V, Bowes WA, Jr. Perinatal group B streptococcal infections across intact amniotic membranes. *J Reprod Med* 1988; 33(5): 445-9.

39. Stoll BJ, Hansen NI, Sanchez PJ, et al. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. *Pediatrics* 2011; 127(5): 817-26.

40. Zaidi AK, Thaver D, Ali SA, Khan TA. Pathogens associated with sepsis in newborns and young infants in developing countries. *Pediatr Infect Dis J* 2009; 28(1 Suppl): S10-8.

41. Darmstadt GL, Saha SK, Choi Y, et al. Population-based incidence and etiology of community-acquired neonatal bacteremia in Mirzapur, Bangladesh: an observational study. *J Infect Dis* 2009; 200(6): 906-15.

42. The WHO Young Infants Study Group. Bacterial etiology of serious infections in young infants in developing countries: results of a multicentre study. Pediatr Infect Dis J. 1999; 18(10 Suppl):S17-22

43. Mulholland E, Ogunlesi OO, Adegbola RA, Weber M, Sam BE, Palmer A, Manary MJ, Secka OA, Aidoo M, Hazlett D, Whittle H, Greenwood BM. Etiology of serious infections in young Gambian infants. *Paediatric Infectious Diseases Journal* 1999; 18: S56 - 61.

44. Muhe L, Tilahun M, Lulseged S, Kebede S, Enaro D, Ringertz S, Kronvall G, Gove S, Mulholland EK. Etiology of pneumonia, sepsis and meningitis in infants younger than three months of age in Ethiopia. *Paediatric Infectious Diseases Journal* 1999; 18: S56- 61.

45. Quiambao BP, Simoes EA, Ladesma EA, et al. Serious community-acquired neonatal infections in rural Southeast Asia (Bohol Island, Philippines). *J Perinatol* 2007; 27(2): 112-9.

46. The WHO Young Infants Study Group. Bacterial etiology of serious infections in young infants in developing countries: results of a multicentre study. Pediatr Infect Dis J. 1999; 18(10 Suppl):S17-22

47. Berkley J, Lowe BS, Mwangi I, Williams T, Bauni E, Mwarumba S, Ngetsa C, Slack MPE, Njenga S, Hart CA, Maitland K, English M, Marsh K, Scott JAG. Bacteremia among Children Admitted to a Rural Hospital in Kenya. *The New England Journal of Medicine* 2005; 352: 39 - 47.

48. Gray K, Bennett SL, French N, Phiri AJ, Graham SM. Invasive Group B Streptococcal Infections in infants, Malawi. *Emerging Infectious Diseases* 2007; 13(2): 223 - 9.

49. Madhi S, Radebe K, Crewe-Brown H, Frasch CE, Arakere G, Mokhachane M, Kimura A high burden of invasive Streptococcus agalactiae in South African Infants. *Annals of Tropical Paediatrics* 2003; 23: 15 - 23.

50. Heath PT BG, Weisner AM, Efstratiou A, Lamagni TL, Tighe H, O'Connell LA, Cafferkey M, Verlander NQ, Nicoll A, McCartney AC; PHLS Group B Streptococcus Working Group. Group B streptococcal disease in UK and Irish infants younger than 90 days. *Lancet* 2004; 363: 292-4.

51. Schuchat A, Oxtoby M, Cochi S, et al. Population-based risk factors for neonatal group B streptococcal disease: results of a cohort study in metropolitan Atlanta. *J Infect Dis* 1990; 162(3): 672-7.

52. Mehl-Auget I, Vaillant V, Goulet V. Invasive streptococcal disease (group A, B, and *Streptococcus pneumoniae*) in France 1987-1994. *Adv Exp Med Biol* 1997; 418: 75-8.

53. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet* 2012; 379(9832): 2162-72.

54. Vergnano S, Menson E, Kennea N, et al. Neonatal infections in England: the NeonIN surveillance network. *Arch Dis Child Fetal Neonatal Ed* 2011; 96(1): F9-F14.

55. von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. *Lancet Infect Dis* 2002; 2(11): 677-85.

56. Aiken AM, Mturi N, Njuguna P, et al. Risk and causes of paediatric hospital-acquired bacteraemia in Kilifi District Hospital, Kenya: a prospective cohort study. *Lancet* 2011; 378(9808): 2021-7.

57. Downie L, Armiento R, Subhi R, Kelly J, Clifford V, Duke T. Community-acquired neonatal and infant sepsis in developing countries: efficacy of WHO's currently recommended antibiotics--systematic review and meta-analysis. *Arch Dis Child* 2012; 98: 146-54.

58. Airede A. Neonatal Septicaemia in an African city of high altitude. *Journal of tropical paediatrics* 1992; 38(4): 189-91.

59. Nathoo K, Mason PR, Chimbira TH. Neonatal Septicaemia in Harare Hospital: aetiology and risk factors. The puerperal sepsis study group. *Central Africa Journal of Medicine* 1990; 36: 150 - 6.

60. Milledge J, Calis JCJ, Graham SM, Phiri A, Wilson LK, Soko D, Mbvwinji M, Walsh AL, Rogerson SR, Molyneux ME, Molyneux EM. Aetiology of neonatal sepsis in Blantyre, Malawi: 1996–2001. *Annals of Tropical Paediatrics* 2005; 25: 101-10.

61. Laving A, Musoke RN, Wasunna AO, Revathi G. Neonatal Bacterial meningitis at the newborn unit of Kenyatta National Hospital. *East African Medical Journal* 2003; 2003(80): 456- 62.

62. English M, Ngama M, Musumba C, Wamola B, Bwika J, Mohammed S, Ahmed M, Mwarumba S, Ouma B, McHugh K, Newton C. Causes and outcome of young infant admissions to a Kenyan District Hospital. *Arch Dis Child* 2003;88(5):438-43

63. Udo J, Anah MU, Ochigbo SO, Etuk IS, Ekanem AD. Neonatal morbidity and mortality in Calabar, Nigeria: a hospital-based study. *Nigerial Journal of Clinical Practice* 2008; 11(3): 285-9.

64. Ghiorghis B. Neonatal sepsis in Addis Ababa, Ethiopia: a review of 151 bacteraemic neonates. Ethiopian Medical Journal 1997; 35(3): 169 - 76.

65. Adejuyigbe EA k-NA, Adisa B. Bacterial isolates in the sick young infant in Ile-Ife, Nigeria. *J Trop Pediatr* 2004; 50(6): 323-7.

66. Ojukwu JU, Abonyi LE, Ugwu J, Orji IK. Neonatal Septicaemia in high risk babies in South- Eastern Nigeria. *J Perinat Med* 2006; 34(2): 166 - 72.

67. Nocard M. Sur une mammite contagieuse des vaches laitieres. *Ann Inst Pasteur* 1887; 1: 109-27.

68. Fry RM. Fatal Infections by Haemolytic Streptococcus Group B. *Lancet* 1938; 1: 100-201.

69. Baker CJ, Barrett FF, Gordon RC, Yow MD. Suppurative meningitis due to streptococci of Lancefield group B: a study of 33 infants. *J Pediatr* 1973; 82(4): 724-9.

70. Barton LL, Feigin RD, Lins R. Group B beta hemolytic streptococcal meningitis in infants. *J Pediatr* 1973; 82(4): 719-23.

71. Communicable Disease Surveillance Centre. Neonatal meningitis: a review of routine national data 1975-83. *Br Med J (Clin Res Ed)* 1985; 290(6470): 778-9.

72. Ismail AQ, Yeates DG, Marciano A, Goldacre M, Anthony M. Cow's milk and the emergence of group B streptococcal disease in newborn babies. *Neonatology* 2011; 100(4): 404-8.

73. Bisharat N, Crook DW, Leigh J, et al. Hyperinvasive neonatal group B streptococcus has arisen from a bovine ancestor. *J Clin Microbiol* 2004; 42(5): 2161-7.

74. Lamy MC, Dramsi S, Billoet A, et al. Rapid detection of the "highly virulent" group B Streptococcus ST-17 clone. Microbes Infect 2006; 8(7): 1714-22..

75. Hery-Arnaud G, Bruant G, Lanotte P, et al. Mobile genetic elements provide evidence for a bovine origin of clonal complex 17 of Streptococcus agalactiae. *Appl Environ Microbiol* 2007; 73(14): 4668-72.

76. Da Cunha V, Davies MR, Douarre PE, et al. Streptococcus agalactiae clones infecting humans were selected and fixed through the extensive use of tetracycline. *Nature communications* 2014; 5: 4544.

77. Dillon HC, Jr., Gray E, Pass MA, Gray BM. Anorectal and vaginal carriage of group B streptococci during pregnancy. *J Infect Dis* 1982; 145(6): 794-9.

78. Schuchat A. Epidemiology of group B streptococcal disease in the United States: shifting paradigms. *Clinical microbiology reviews* 1998; 11(3): 497-513.

79. Phares CR, Lynfield R, Farley MM, et al. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. *JAMA* 2008; 299(17): 2056-65.

80. Schrag S, Zywicki S, Farley MM, Reingold AL, Harrison LH, Lefkowitz LB, Hadler JL, Danila R, Cieslak PR, Schuchat A. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med* 2000; 342(1): 15-20.

81. Madhi SA, Dangor Z, Heath PT, et al. Considerations for a phase-III trial to evaluate a group B Streptococcus polysaccharide-protein conjugate vaccine in pregnant women for the prevention of early- and late-onset invasive disease in young-infants. Vaccine 2013; 31 Suppl 4: D52-7.

82. Lancefield RC. A Serological Differentiation of Human and Other Groups of Hemolytic Streptococci. The Journal of experimental medicine 1933; 57(4): 571-95.

83. Lancefield RC. A Serological Differentiation of Specific Types of Bovine Hemolytic Streptococci (Group B). The Journal of experimental medicine 1934; 59(4): 441-58.

84. Yao K, Poulsen K, Maione D, et al. Capsular gene typing of Streptococcus agalactiae compared to serotyping by latex agglutination. *Journal of clinical microbiology* 2013; 51(2): 503-7.

85. Slotved HC, Kong F, Lambertsen L, Sauer S, Gilbert GL. Serotype IX, a Proposed New Streptococcus agalactiae Serotype. *J Clin Microbiol* 2007; 45(9): 2929-36.

86. Imperi M PM, Alfarone G, Baldassarri L, Orefici G, Creti R. A multiplex PCR assay for the direct identification of the capsular type (Ia to IX) of *Streptococcus agalactiae*. *J Microbiol Methods* 2010; 80(2): 212-4.

87. Jones N, Bohnsack JF, Takahashi S, et al. Multilocus sequence typing system for group B streptococcus. *J Clin Microbiol* 2003; 41(6): 2530-6.

88. Glaser P, Rusniok C, Buchrieser C, et al. Genome sequence of Streptococcus agalactiae, a pathogen causing invasive neonatal disease. *Mol Microbiol* 2002; 45(6): 1499-513.

89. Suara R, Adegbola RA, Baker CJ, Secka O, Mulholland EK, Greenwood BM. . Carriage of GBS in pregnant mothers and their neonates. *Journal of Infectious Diseases* 1994; 70: 1316 - 9.

90. Schmidt J HE, Halle H, Mohammed T, Gunther E. Colonization of pregnant women and their newborn infants with group B streptococci in the Gondar College of Medical Sciences. *Ethiop Med J* 1989; 27(3): 115-9.

91. Dawodo A, Damole IO, Onile BA. Epidemiology of Group B Streptococcal Carriage among pregnant women and their neonates. *Journal of Infectious Diseases* 1983; 1994(70): 1316 -9.

92. Onile BA. Group B Streptococcal Carriage in Nigeria. *Trans Royal Soc Tropical Medicine and Hygiene* 1980; 74: 367 - 70.

93. Uhiara J. Group B Streptococcus carriage among parturients and their neonates in Zavia, Nigeria. *African journal Med Med Sci* 1993; 22(3): 79 - 83.

94. Mavenyengwa R, Afset JE, Schei B, Berg S, Caspersen T, Bergseng H, Moyo SR. Group B Streptococcus colonization during pregnancy and maternal-fetal transmission in Zimbabwe. *Acta Obstet Gynecol Scand* 2010; 89(2): 250-5.

95. Joachim A MM, Massawe FA, Lyamuya EF. Maternal and neonatal colonisation of group B streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. *BMC Public Health* 2009; 9: 437.

96. Moyo S, Mudzori J, Tswana SA, Maeland JA. Prevalence, capsular type distribution, anthropometric and obstetric factors of group B Streptococcus (Streptococcus agalactiae) colonization in pregnancy. *Cent Afr J Med* 2000; 46(5): 115-20.

97. Barcaite E, Bartusevicius A, Tameliene R, Kliucinskas M, Maleckiene L, Nadisauskiene R. Prevalence of maternal group B streptococcal colonisation in European countries. *Acta Obstet Gynecol Scand* 2008; 87(3): 260-71.

98. Schuchat A, Wenger JD. Epidemiology of group B streptococcal disease. Risk factors, prevention strategies, and vaccine development. *Epidemiologic reviews* 1994; 16(2): 374-402.

99. Persson K, Bjerre B, Elfstrom L, Forsgren A. Longitudinal study of group B streptococcal carriage during late pregnancy. *Scand J Infect Dis* 1987; 19(3): 325-9.

100. Goodman JR, Berg RL, Gribble RK, Meier PR, Fee SC, Mitchell PD. Longitudinal study of group B streptococcus carriage in pregnancy. *Infect Dis Obstet Gynecol* 1997; 5(3): 237-43.

101. Baker CJ, Barrett FF, Yow MD. The influence of advancing gestation on group B streptococcal colonization in pregnant women. *Am J Obstet Gynecol* 1975; 122(7): 820-3.

102. Zamzami TY, Marzouki AM, Nasrat HA. Prevalence rate of group B streptococcal colonization among women in labor at King Abdul-Aziz University Hospital. *Archives of gynecology and obstetrics* 2011; 284(3): 677-9.

103. Nasri K, Chehrei A, Manavi MS. Evaluation of vaginal group B streptococcal culture results after digital vaginal examination and its pattern of antibiotic resistance in pregnant women. *Iranian journal of reproductive medicine* 2013; 11(12): 999-1004.

104. El A, . Tency I, Claeys G, Verstraelen H, Deschaght P, Decat E, Lopes dos Santos Santiago G, Cools P, Temmerman M, Vaneechoutte M. Comparison of culture with two different qPCR assays for detection of rectovaginal carriage of Streptococcus agalactiae (group B streptococci) in pregnant women. *Res Microbiol* 2011; 162(5): 499-505.

105. Verani JR, McGee L, Schrag SJ, Division of Bacterial Diseases NCfI, Respiratory Diseases CfDC, Prevention. Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. *MMWR Recomm Rep* 2010; 59(RR-10): 1-36.

106. Cagno CK, Pettit JM, Weiss BD. Prevention of perinatal group B streptococcal disease: updated CDC guideline. *American family physician* 2012; 86(1): 59-65.

107. Stoner K, Rabe LK, Hillier SL. Effect of Transport Time, Temperature, and Concentration on the Survival of Group B Streptococci in Amies Transport Medium. *J Clin Microbiol* 2004; 42(11): 5385-7.

108. Baker CJ, Goroff DK, Alpert S, et al. Vaginal colonization with group B streptococcus: a study in college women. *J Infect Dis* 1977; 135(3): 392-7.

109. Joachim A, Matee MI, Massawe FA, Lyamuya EF. Maternal and neonatal colonisation of group B streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. *BMC Public Health* 2009; 9: 437.

110. Suara RO, Adegbola RA, Baker CJ, Secka O, Mulholland EK, Greenwood BM. Carriage of group B Streptococci in pregnant Gambian mothers and their infants. *J Infect Dis* 1994; 170(5): 1316-9.

111. Uhiara JE. Group B streptococcal carriage among parturients and their neonates in Zaria, Nigeria. *Afr J Med Med Sci* 1993; 22(3): 79-83.

112. Dawodu AH, Damole IO, Onile BA. Epidemiology of group B streptococcal carriage among pregnant women and their neonates: an African experience. *Tropical and geographical medicine* 1983; 35(2): 145-50.

113. Onile BA. Group B streptococcal carriage in Nigeria. *Trans R Soc Trop Med Hyg* 1980; 74(3): 367-70.

114. Schmidt J, Halle E, Halle H, Mohammed T, Gunther E. Colonization of pregnant women and their newborn infants with group B streptococci in the Gondar College of Medical Sciences. *Ethiop Med J* 1989; 27(3): 115-9.

115. de Steenwinkel FD, Tak HV, Muller AE, Nouwen JL, Oostvogel PM, Mocumbi SM. Low carriage rate of group B streptococcus in pregnant women in Maputo, Mozambique. *Trop Med Int Health* 2008; 13(3): 427-9.

116. Anthony BF, Okada DM, Hobel CJ. Epidemiology of group B Streptococcus: longitudinal observations during pregnancy. *J Infect Dis* 1978; 137(5): 524-30.

117. Kim EJ, Oh KY, Kim MY, et al. Risk factors for group B streptococcus colonization among pregnant women in Korea. *Epidemiology and health* 2011; 33: e2011010.

118. Regan JA, Klebanoff MA, Nugent RP. The epidemiology of group B streptococcal colonization in pregnancy. Vaginal Infections and Prematurity Study Group. *Obstet Gynecol* 1991; 77(4): 604-10.

119. Stapleton RD, Kahn JM, Evans LE, Critchlow CW, Gardella CM. Risk factors for group B streptococcal genitourinary tract colonization in pregnant women. *Obstet Gynecol* 2005; 106(6): 1246-52.

120. Shah M, Aziz N, Leva N, Cohan D. Group B Streptococcus colonization by HIV status in pregnant women: prevalence and risk factors. *Journal of women's health* 2011; 20(11): 1737-41.

121. Manning SD, Neighbors K, Tallman PA, et al. Prevalence of group B streptococcus colonization and potential for transmission by casual contact in healthy young men and women. *Clin Infect Dis* 2004; 39(3): 380-8.

122. Ramos E, Gaudier FL, Hearing LR, Del Valle GO, Jenkins S, Briones D. Group B streptococcus colonization in pregnant diabetic women. *Obstet Gynecol* 1997; 89(2): 257-60.

123. Dahan-Saal J, Gerardin P, Robillard PY, et al. [Determinants of group B streptococcus maternal colonization and factors related to its vertical perinatal transmission: case-control study]. *Gynecologie, obstetrique & fertilite* 2011; 39(5): 281-8.

124. Gray KJ, Kafulafula G, Matemba M, Kamdolozi M, Membe G, French N. Group B Streptococcus and HIV infection in pregnant women, Malawi, 2008-2010. *Emerg Infect Dis* 2011; 17(10): 1932-5.

125. Joao EC, Gouvea MI, Menezes JA, et al. Group B Streptococcus in a cohort of HIV-infected pregnant women: prevalence of colonization, identification and antimicrobial susceptibility profile. *Scand J Infect Dis* 2011; 43(9): 742-6.

126. Manning SD, Springman AC, Million AD, et al. Association of Group B Streptococcus colonization and bovine exposure: a prospective cross-sectional cohort study. *PloS One* 2010; 5(1): e8795.

127. van der Mee-Marquet N DA, Salloum M, Violette J, Arnault L, Gaillard N, Bind JL, Lartigue MF, Quentin R; Bloodstream Infection Study Group of the Reseau des Hygienistes de la Region Centre. Molecular characterization of temporally and geographically matched *Streptococcus agalactiae* strains isolated from food products and bloodstream infections. *Foodborne Pathog Dis* 2009; 6(10): 1177-83.

128. Cohen L. Influence of pH on vaginal discharges. *The British journal of venereal diseases* 1969; 45(3): 241-7.

129. Döderlein A. Das Scheidensekret und seine Bedeutung fuerdas Puerperalfieber. *Die Arten des Scheidensekretes* 1892; (11): 699.

130. Boskey ER, Cone RA, Whaley KJ, Moench TR. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Human reproduction* 2001; 16(9): 1809-13.

131. Rogosa M, Sharpe ME. Species differentiation of human vaginal lactobacilli. *J Gen Microbiol* 1960; 23: 197-201.

132. Hyman RW, Fukushima M, Diamond L, Kumm J, Giudice LC, Davis RW. Microbes on the human vaginal epithelium. *Proc Natl Acad Sci U S A* 2005; 102(22): 7952-7.

133. Drake SM, Evans BA, Gerken A. Vaginal pH and microflora related to yeast infections and treatment. *The British journal of venereal diseases* 1980; 56(2): 107-10.

134. Hillier SL, Krohn MA, Rabe LK, Klebanoff SJ, Eschenbach DA. The normal vaginal flora, H2O2-producing lactobacilli, and bacterial vaginosis in pregnant women. *Clin Infect Dis* 1993; 16 Suppl 4: S273-81.

135. Jespers V, Menten J, Smet H, et al. Quantification of bacterial species of the vaginal microbiome in different groups of women, using nucleic acid amplification tests. *BMC Microbiol* 2012; 12: 83.

136. Zhou X, Brown CJ, Abdo Z, et al. Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. *The ISME journal* 2007; 1(2): 121-33.

137. Gajer P, Brotman RM, Bai G, et al. Temporal dynamics of the human vaginal microbiota. *Science translational medicine* 2012; 4(132): 132ra52.

138. Romero R, Hassan SS, Gajer P, et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome* 2014; 2(1): 4.

139. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 2011; 108 Suppl 1: 4680-7.

140. Ma B, Forney LJ, Ravel J. Vaginal microbiome: rethinking health and disease. *Annual review of microbiology* 2012; 66: 371-89.

141. Zarate G, Nader-Macias ME. Influence of probiotic vaginal lactobacilli on in vitro adhesion of urogenital pathogens to vaginal epithelial cells. *Letters in applied microbiology* 2006; 43(2): 174-80.

142. Ippolito DL, James WA, Tinnemore D, et al. Group B streptococcus serotype prevalence in reproductive-age women at a tertiary care military medical center relative to global serotype distribution. *BMC Infect Dis* 2010; 10: 336.

143. Madzivhandila M, Adrian PV, Cutland CL, Kuwanda L, Schrag SJ, Madhi SA. Serotype distribution and invasive potential of group B streptococcus isolates causing disease in infants and colonizing maternal-newborn dyads. *PloS One* 2011; 6(3): e17861.

144. Turner C, Turner P, Po L, et al. Group B streptococcal carriage, serotype distribution and antibiotic susceptibilities in pregnant women at the time of delivery in a refugee population on the Thai-Myanmar border. *BMC Infect Dis* 2012; 12: 34.

145. Honsa E, Fricke T, Stephens AJ, et al. Assignment of Streptococcus agalactiae isolates to clonal complexes using a small set of single nucleotide polymorphisms. *BMC Microbiol* 2008; 8: 140.

146. Najmi N, Jehan I, Sikandar R, Zuberi NF. Maternal genital tract colonisation by group-B streptococcus: a hospital based study. *JPMA The Journal of the Pakistan Medical Association* 2013; 63(9): 1103-7.

147. Linhares JJ, Cavalcante Neto PG, Vasconcelos JL, et al. [Prevalence of the colonization by Streptococcus agalactiae in pregnant women from a maternity in Ceara, Brazil, correlating with perinatal outcomes]. *Revista brasileira de ginecologia e obstetricia: revista da Federacao Brasileira das Sociedades de Ginecologia e Obstetricia* 2011; 33(12): 395-400.

148. Hakansson S, Axemo P, Bremme K, et al. Group B streptococcal carriage in Sweden: a national study on risk factors for mother and infant colonisation. *Acta Obstet Gynecol Scand* 2008; 87(1): 50-8.

149. Desa DJ, Trevenen CL. Intrauterine infections with group B beta-haemolytic streptococci. *British journal of obstetrics and gynaecology* 1984; 91(3): 237-9.

150. Bergqvist G, Holmberg G, Rydner T, Vaclavinkova V. Intrauterine death due to infection with group B Streptococci. *Acta Obstet Gynecol Scand* 1978; 57(2): 127-8.

151. Joachim A, Matee MI, Massawe FA, Lyamuya EF. Maternal and neonatal colonisation of group B streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. *BMC Public Health* 2009; 9: 437.

152. Eyre DW, Cule ML, Wilson DJ, et al. Diverse sources of C. difficile infection identified on whole-genome sequencing. *N Engl J Med* 2013; 369(13): 1195-205.

153. Edmond KM, Kortsalioudaki C, Scott S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. *Lancet* 2012; 379(9815): 547-56.

154. Ojukwu JU AL, Ugwu J, Orji IK. Neonatal septicemia in high risk babies in South-Eastern Nigeria. *J Perinat Med* 2006; 34(2): 166-72.

155. Sigaúque B RA, Mandomando I, Morais L, Quintó L, Sacarlal J, Macete E, Nhamposa T, Machevo S, Aide P, Bassat Q, Bardají A, Nhalungo D, Soriano-Gabarró M, Flannery B, Menendez C, Levine MM, Alonso PL. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. *Pediatr Infect Dis J* 2009; 28(2): 108-13.

156. Moses LM, Heath PT, Wilkinson AR, Jeffery HE, Isaacs D. Early onset group B streptococcal neonatal infection in Oxford 1985-96. *Arch Dis Child Fetal Neonatal Ed* 1998; 79(2): F148-9.

157. Oddie S, Embleton ND. Risk factors for early onset neonatal group B streptococcal sepsis: case-control study. *BMJ* 2002; 325(7359): 308.

158. Bedford H, de Louvois J, Halket S, Peckham C, Hurley R, Harvey D. Meningitis in infancy in England and Wales: follow up at age 5 years. *BMJ* 2001; 323(7312): 533-6.

159. Heath PT, Balfour G, Weisner AM, et al. Group B streptococcal disease in UK and Irish infants younger than 90 days. *Lancet* 2004; 363(9405): 292-4.

160. Beardsall K, Thompson MH, Mulla RJ. Neonatal group B streptococcal infection in South Bedfordshire, 1993-1998. *Arch Dis Child Fetal Neonatal Ed* 2000; 82(3): F205-7.

161. McGregor JA, French JI. Preterm Birth: The Role of Infection and Inflammation. *Medscape women's health* 1997; 2(8): 1.

162. Cutland CL SS, Zell ER, Kuwanda L, Buchmann E, Velaphi SC, Groome MJ, Adrian PV, Madhi SA; the PoPS trial team. Maternal HIV infection and vertical transmission of pathogenic bacteria. *Pediatrics* 2012; 130(3): e581-90.

163. Schauf V, Deveikis A, Riff L, Serota A. Antibiotic-killing kinetics of group B streptococci. *J Pediatr* 1976; 89(2): 194-8.

164. Kasahara K, Baltus AJ, Lee SH, Edelstein MA, Edelstein PH. Prevalence of non-penicillin-susceptible group B streptococcus in Philadelphia and specificity of penicillin resistance screening methods. *J Clin Microbiol* 2010; 48(4): 1468-9.

165. Dahesh S, Hensler ME, Van Sorge NM, et al. Point mutation in the group B streptococcal pbp2x gene conferring decreased susceptibility to beta-lactam antibiotics. *Antimicrob Agents Chemother* 2008; 52(8): 2915-8.

166. Kimura K, Suzuki S, Wachino J, et al. First molecular characterization of group B streptococci with reduced penicillin susceptibility. *Antimicrob Agents Chemother* 2008; 52(8): 2890-7.

167. Phares C, Lynfield R, Farley MM, Mohle-Boetani J, Harrison LH, Petit S, Craig AS, Schaffner W, Zansky SM, Gershman K, Stefonek KR, Albanese BA, Zell ER, Schuchat A, Schrag SJ; Active Bacterial Core surveillance/Emerging Infections Program Network. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. *JAMA* 2008; 299(17): 2056-65.

168. Simoes JA, Aroutcheva AA, Heimler I, Faro S. Antibiotic resistance patterns of group B streptococcal clinical isolates. *Infect Dis Obstet Gynecol* 2004; 12(1): 1-8.

169. Nagano N, Nagano Y, Toyama M, et al. Nosocomial spread of multidrug-resistant group B streptococci with reduced penicillin susceptibility belonging to clonal complex 1. *J Antimicrob Chemother* 2012; 67(4): 849-56.

170. Castor ML, Whitney CG, Como-Sabetti K, et al. Antibiotic resistance patterns in invasive group B streptococcal isolates. *Infect Dis Obstet Gynecol* 2008; 2008: 727505.

171. Marimon JM, Valiente A, Ercibengoa M, Garcia-Arenzana JM, Perez-Trallero E. Erythromycin resistance and genetic elements carrying macrolide efflux genes in Streptococcus agalactiae. *Antimicrob Agents Chemother* 2005; 49(12): 5069-74.

172. Weisner AM, Johnson AP, Lamagni TL, et al. Characterization of group B streptococci recovered from infants with invasive disease in England and Wales. *Clin Infect Dis* 2004; 38(9): 1203-8.

173. Zaleznik DF, Rench MA, Hillier S, et al. Invasive disease due to group B Streptococcus in pregnant women and neonates from diverse population groups. *Clin Infect Dis* 2000; 30(2): 276-81.

174. Phares CR LR, Farley MM, Mohle-Boetani J, Harrison LH, Petit S, Craig AS, Schaffner W, Zansky SM, Gershman K, Stefonek KR, Albanese BA, Zell ER, Schuchat A, Schrag SJ; Active Bacterial Core surveillance/Emerging Infections Program Network. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. *JAMA* 2008; 299(17): 2056-65.

175. Ahlenius I FJ, Thomassen P. Sixty-six cases of intrauterine fetal death. A prospective study with an extensive test protocol. *Acta Obstet Gynecol Scand* 1995; 74(2): 109-17.

176. Gibbs R, Roberts DJ. Case 27-2007: A 30-Year-Old Pregnant Woman with Intrauterine Fetal Death. *NEJM* 2007; 357: 918-25.

177. Embleton N, . Northern Region's Perinatal Mortality Survey. Fetal and Neonatal death from maternal acquired infection. *Paediatr Perinat Epiemiol* 2001; 15(1): 54-60.

178. Moyo SR, Hagerstrand I, Nystrom L, et al. Stillbirths and intrauterine infection, histologic chorioamnionitis and microbiological findings. *Int J Gynaecol Obstet* 1996; 54(2): 115-23.

179. Valkenburg-van den Berg AW, Sprij AJ, Dekker FW, Dorr PJ, Kanhai HH. Association between colonization with Group B Streptococcus and preterm delivery: a systematic review. *Acta Obstet Gynecol Scand* 2009; 88(9): 958-67.

180. Valkenburg-van den Berg AW, Houtman-Roelofsen RL, Oostvogel PM, Dekker FW, Dorr PJ, Sprij AJ. Timing of group B streptococcus screening in pregnancy: a systematic review. *Gynecol Obstet Invest* 2010; 69(3): 174-83.

181. Romero R, Mazor M, Oyarzun E, Sirtori M, Wu YK, Hobbins JC. Is there an association between colonization with group B Streptococcus and prematurity? *J Reprod Med* 1989; 34(10): 797-801.

182. Regan J, Klebanoff MA, Nugent RP, Eschenbach DA, Blackwelder WC, Lou Y, Gibbs RS, Rettig PJ, Martin DH, Edelman R. Colonization with group B streptococci in pregnancy and adverse outcome. VIP Study Group. *Am J Obstet Gynecol* 1996; 174(4): 1354-60.

183. Boyer KM, Gotoff SP. Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. *N Engl J Med* 1986; 314(26): 1665-9.

184. Thomsen AC, Morup L, Hansen KB. Antibiotic elimination of group-B streptococci in urine in prevention of preterm labour. *Lancet* 1987; 1(8533): 591-3.

185. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. *MMWR Recomm Rep* 2002; 51(RR-11): 1-22.

186. de la Rosa Fraile M, Cabero L, Andreu A, Rao GG. Prevention of group B streptococcal neonatal disease: a plea for a European consensus. *Clin Microbiol Infect* 2001; 7(1): 25-7.

187. Madhi SA, Dangor Z, Heath PT, et al. Considerations for a phase-III trial to evaluate a group B Streptococcus polysaccharide-protein conjugate vaccine in pregnant women for the prevention of early- and late-onset invasive disease in young-infants. *Vaccine* 2013; 31 Suppl 4: D52-7.

188. Central Bureau of Statistics Ministry of Planning and National Development. Geographic Dimensions of Well-Being in Kenya: Where are the Poor? From Districts to Locations (Volume One) 2003. http://go.worldbank.org/QQVD7EQTO0 (accessed 21/3/2014).

189. Ministry of Public Health and Sanitation Kenya. National Guidelines for HIV Testing and Counselling in Kenya. 2008. http://www.ilo.org/wcmsp5/groups/public/---

ed_protect/---protrav/---ilo_aids/documents/legaldocument/wcms_127533.pdf (accessed 21/08/2014)

190. Bergeron MG KD, Ménard C, Picard FJ, Gagnon M, Bernier M, Ouellette M, Roy PH, Marcoux S, Fraser WD. Rapid detection of group B streptococci in pregnant women at delivery. *N Engl J Med* 2000; 343(3): 175-9.

191. Schrag SJ, Verani JR. Intrapartum antibiotic prophylaxis for the prevention of perinatal group B streptococcal disease: Experience in the United States and implications for a potential group B streptococcal vaccine. *Vaccine* 2012.

192. Tettelin H, Masignani V, Cieslewicz MJ, et al. Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V Streptococcus agalactiae. *Proc Natl Acad Sci U S A* 2002; 99(19): 12391-6.

193. Yamamoto S, Miyake K, Koike Y, et al. Molecular characterization of type-specific capsular polysaccharide biosynthesis genes of Streptococcus agalactiae type Ia. *Journal of bacteriology* 1999; 181(17): 5176-84.

194. Watanabe M, Miyake K, Yanae K, et al. Molecular characterization of a novel beta1,3-galactosyltransferase for capsular polysaccharide synthesis by Streptococcus agalactiae type Ib. *Journal of biochemistry* 2002; 131(2): 183-91.

195. Chaffin DO, Beres SB, Yim HH, Rubens CE. The serotype of type Ia and III group B streptococci is determined by the polymerase gene within the polycistronic capsule operon. *Journal of bacteriology* 2000; 182(16): 4466-77.

196. Cieslewicz MJ, Chaffin D, Glusman G, et al. Structural and genetic diversity of group B streptococcus capsular polysaccharides. *Infect Immun* 2005; 73(5): 3096-103.

197. Imperi M, Pataracchia M, Alfarone G, Baldassarri L, Orefici G, Creti R. A multiplex PCR assay for the direct identification of the capsular type (Ia to IX) of Streptococcus agalactiae. *J Microbiol Methods* 2010; 80(2): 212-4.

198. Vawter D, Rogers-Chrysler G, Clay M, Pittelko L, Therkelsen D, Kim D, McCullough J. A phased consent policy for cord blood transfusion. *Transfusion* 2002; 42: 1268 - 74.

199. Hassall O, Ngina L, Kongo W, Othigo J, Mandaliya K, Maitland K, Bates I. The acceptability to women in Mombasa, Kenya, of the donation and transfusion of umbilical cord blood for severe anaemia in young children. *Vox Sanguinis* 2007; 94: 125 -31.

200. Filmer D, Pritchett LH. Estimating wealth effects without expenditure data--or tears: an application to educational enrollments in states of India. Demography 2001; 38(1): 115-32.

201. Austin PC. Estimating multilevel logistic regression models when the number of clusters is low: a comparison of different statistical software procedures. *The international journal of biostatistics* 2010; 6(1): Article 16.

202. Rodgers, WH. Regression standard errors in clustered samples. *Stata Technical Bulletin* 1993; 13: 19-23.

203. Rubin DB, Schenker N. Multiple imputation in health-care databases: an overview and some applications. *Stat Med* 1991; 10(4): 585-98.

204. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ* 2009; 338: b2393.

205. Wood AM, White IR, Thompson SG. Are missing outcome data adequately handled? A review of published randomized controlled trials in major medical journals. *Clinical trials* 2004; 1(4): 368-76.

206. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. *Stat Med* 2011; 30(4): 377-99.

207. Graham JW OA, Gilreath TD. How many imputations are really needed? Some practical clarifications of multiple imputation theory. *Prevention science : the official journal of the Society for Prevention Research* 2007; 8(3): 206-13.

208. Centers for Disease Control and Prevention. Adoption of hospital policies for prevention of perinatal group B streptococcal disease--United States, 1997. *JAMA* 1998; 280(11): 958-9.

209. Greenland S, Brumback B. An overview of relations among causal modelling methods. *Int J Epidemiol* 2002; 31(5): 1030-7.

210. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology* 1999; 10(1): 37-48.

211. Textor J, Hardt J, Knuppel S. DAGitty: a graphical tool for analyzing causal diagrams. *Epidemiology* 2011; 22(5): 745.

212. Sokal R, Michener C. A statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin* 1958; 38: 1409-38.

213. R Development Core Team. R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*, 2008. (accessed 2.07.2014).

214. Manning S, Springman AC, Million AD, Milton NR, McNamara SE, Somsel PA, Bartlett P, Davies HD. Association of Group B Streptococcus colonization and bovine exposure: a prospective cross-sectional cohort study. *PloS One* 2010; 5(1): e8795.

215. Florindo C, Damiao V, Silvestre I, et al. Epidemiological surveillance of colonising group B Streptococcus epidemiology in the Lisbon and Tagus Valley regions, Portugal (2005

to 2012): emergence of a new epidemic type IV/clonal complex 17 clone. *Euro Surveill* 2014; 19(23).

216. Couturier BA, Weight T, Elmer H, Schlaberg R. Antepartum Screening for Group B Streptococcus by Three FDA-Cleared Molecular Tests and Effect of Shortened Enrichment Culture on Molecular Detection Rates. *J Clin Microbiol* 2014.

217. Roberto G. Gutierrez StataCorp. Use of the term 'relative risk ratio'. 2005. http://www.stata.com/statalist/archive/2005-04/msg00678.html (accessed (30.07.2014).

218. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. *N Engl J Med* 1988; 319(15): 972-8.

219. Garcia-Munoz Rodrigo F, Galan Henriquez G, Figueras Aloy J, Garcia-Alix Perez A. Outcomes of very-low-birth-weight infants exposed to maternal clinical chorioamnionitis: a multicentre study. *Neonatology* 2014; 106(3): 229-34.

220. Regan J, Chao S, James LS. Premature rupture of membranes, preterm delivery, and group B streptococcal colonization of mothers. *Am J Obstet Gynecol* 1981; 141(2): 184-6.

221. Desai M, ter Kuile FO, Nosten F, et al. Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis* 2007; 7(2): 93-104.

222. O'Meara WP, Bejon P, Mwangi TW, et al. Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. *Lancet* 2008; 372(9649): 1555-62.

223. Schuchat A, Deaver-Robinson K, Plikaytis BD, Zangwill KM, Mohle-Boetani J, Wenger JD. Multistate case-control study of maternal risk factors for neonatal group B streptococcal disease. The Active Surveillance Study Group. *Pediatr Infect Dis J* 1994; 13(7): 623-9.

224. Boyer KM, Gadzala CA, Burd LI, Fisher DE, Paton JB, Gotoff SP. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. I. Epidemiologic rationale. *J Infect Dis* 1983; 148(5): 795-801.

225. Schuchat A, Zywicki SS, Dinsmoor MJ, et al. Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study. *Pediatrics* 2000; 105(1 Pt 1): 21-6.

226. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn: Guidelines for prevention of group B streptococcal (GBS) infection by chemoprophylaxis. *Pediatrics* 1992; 90(5): 775-8.

227. Hankins G, Chalas, E. Group B Streptococcal infections in pregnancy: ACOG's recommendations. *ACOG Newsletter* 1993; 37(2).

228. ACOG committee opinion. Prevention of early-onset group B streptococcal disease in newborns. Number 173--June 1996. Committee on Obstetric Practice. American College of Obstetrics and Gynecologists. *Int J Gynaecol Obstet* 1996; 54(2): 197-205.

229. Schrag SJ, Zell ER, Lynfield R, et al. A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. *N Engl J Med* 2002; 347(4): 233-9.

230. Poyart C, Reglier-Poupet H, Tazi A, et al. Invasive group B streptococcal infections in infants, France. *Emerg Infect Dis* 2008; 14(10): 1647-9.

231. Hyde TB, Hilger TM, Reingold A, et al. Trends in incidence and antimicrobial resistance of early-onset sepsis: population-based surveillance in San Francisco and Atlanta. *Pediatrics* 2002; 110(4): 690-5.

232. Epalza C, Goetghebuer T, Hainaut M, et al. High incidence of invasive group B streptococcal infections in HIV-exposed uninfected infants. *Pediatrics* 2010; 126(3): e631-8.

233. Musser JM, Mattingly SJ, Quentin R, Goudeau A, Selander RK. Identification of a high-virulence clone of type III Streptococcus agalactiae (group B Streptococcus) causing invasive neonatal disease. *Proc Natl Acad Sci U S A* 1989; 86(12): 4731-5.

234. Lamy MC, Dramsi S, Billoet A, et al. Rapid detection of the "highly virulent" group B Streptococcus ST-17 clone. *Microbes Infect* 2006; 8(7): 1714-22.

235. Gherardi G, Imperi M, Baldassarri L, et al. Molecular epidemiology and distribution of serotypes, surface proteins, and antibiotic resistance among group B streptococci in Italy. *J Clin Microbiol* 2007; 45(9): 2909-16.

236. Doran KS, Nizet V. Molecular pathogenesis of neonatal group B streptococcal infection: no longer in its infancy. *Mol Microbiol* 2004; 54(1): 23-31.

237. Adderson EE, Takahashi S, Wang Y, Armstrong J, Miller DV, Bohnsack JF. Subtractive hybridization identifies a novel predicted protein mediating epithelial cell invasion by virulent serotype III group B Streptococcus agalactiae. *Infect Immun* 2003; 71(12): 6857-63.

238. Baker CJ, Webb BJ, Kasper DL, Yow MD, Beachler CW. The natural history of group B streptococcal colonization in the pregnant woman and her offspring. II. Determination of serum antibody to capsular polysaccharide from type III, group B Streptococcus. *Am J Obstet Gynecol* 1980; 137(1): 39-42.

239. Schrag S J and Global Group B Streptococcal Vaccine Working Group. Group B streptococcal vaccine for resource-poor countries. *Lancet* 2011; 378(9785): 11-2.

240. Feikin DR, Kagucia EW, Loo JD, et al. Serotype-specific changes in invasive pneumococcal disease after pneumococcal conjugate vaccine introduction: a pooled analysis of multiple surveillance sites. *PLoS Med* 2013; 10(9): e1001517.

241. Mulholland K, Satzke C. Serotype replacement after pneumococcal vaccination. *Lancet* 2012; 379(9824): 1387; author reply 8-9.

242. Henneke P, Berner R. Interaction of neonatal phagocytes with group B streptococcus: recognition and response. *Infect Immun* 2006; 74(6): 3085-95.

243. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nature reviews Immunology* 2007; 7(5): 379-90.

244. Janeway CA, Jr., Medzhitov R. Innate immune recognition. *Annual review of immunology* 2002; 20: 197-216.

245. Henneke P, Takeuchi O, van Strijp JA, et al. Novel engagement of CD14 and multiple toll-like receptors by group B streptococci. *J Immunol* 2001; 167(12): 7069-76.

246. Kim SY, Russell LB, Park J, et al. Cost-effectiveness of a potential group B streptococcal vaccine program for pregnant women in South Africa. *Vaccine* 2014; 32(17): 1954-63.

247. Soofi S, Cousens S, Imdad A, Bhutto N, Ali N, Bhutta ZA. Topical application of chlorhexidine to neonatal umbilical cords for prevention of omphalitis and neonatal mortality in a rural district of Pakistan: a community-based, cluster-randomised trial. *Lancet* 2012; 379(9820): 1029-36.

248. Brandon DH, Coe K, Hudson-Barr D, Oliver T, Landerman LR. Effectiveness of No-Sting skin protectant and Aquaphor on water loss and skin integrity in premature infants. *J Perinatol* 2010; 30(6): 414-9.

249. Salam RA, Das JK, Darmstadt GL, Bhutta ZA. Emollient therapy for preterm newborn infants--evidence from the developing world. *BMC Public Health* 2013; 13 Suppl 3: S31.

250. Lawn JE, Mwansa-Kambafwile J, Barros FC, Horta BL, Cousens S. 'Kangaroo mother care' to prevent neonatal deaths due to pre-term birth complications. *Int J Epidemiol* 2010; 39(Suppl. 1): 144-54.

251. Lopez AD, Mathers CD. Measuring the global burden of disease and epidemiological transitions: 2002-2030. *Ann Trop Med Parasitol* 2006; 100(5-6): 481-99.

252. Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. *Lancet* 2011; 378(9807): 1962-73.

253. Tazi A, Disson O, Bellais S, et al. The surface protein HvgA mediates group B streptococcus hypervirulence and meningeal tropism in neonates. The Journal of experimental medicine 2010; 207(11): 2313-22.

Appendices

Appendix 1: Assent form

Assent form for research participation (KDH/Coast/Other)

Joint KEMRI / MoH Research into Clinical and Molecular Epidemiology of maternal Group B Streptococcus colonisation

For mothers requiring emergency treatment or rapid assessment.

A brief discussion with the mother by the admitting trial doctor or nurse must include the following phrases:

- KEMRI works with the MoH to make sure that each mother is treated to the best of our ability.
- KEMRI also conducts important research on this ward to find better ways of preventing and treating illnesses for all mothers and their children in the future.
- This research involves taking a recto-vaginal swab at routine vaginal examination
- It also involves taking a small sample of placenta.*
- This research has been approved in advance by national scientific and independent ethical committees in Nairobi and a scientific committee in Kilifi.
- If you do not wish to participate in this research, your treatment will not be affected. If you agree, we will explain more about the research after delivery, and you are free to change your mind about participating at any time.

Doctor or nurses signature	Print name	Date							
		D	*D*	*M*	*M*	*M*	**2**	**0**	*Y*	*Y*

Participant assents to research?	Yes / No

*In a subset only

Joint KEMRI / MoH Research into Clinical and Molecular Epidemiology of maternal Group B Streptococcus colonisation

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Centre for Disease Control and Prevention, USA	Stephanie Schrag
Coast Provincial General Hospital, Mombasa	Hemed Twahir, Victor Bandika, Musimbi Soita
Health Protection Agency, UK	Androulla Efstratiou

What is KEMRI and what is this study about?

This health care facility and KEMRI are working together to strengthen services at maternity. KEMRI is a national research organization whose work is to find better ways of preventing and treating illness for the benefit of everyone in the future.

One problem KEMRI is currently trying to learn more about is infections in babies. We know that particular bacteria, Group B Streptococci, can be present in mothers without any problems at all, but sometimes it may cause severe infections in babies. We want to find out how common and important these bacteria are in Kenya. This is important because vaccines are being developed to protect against these bacteria, and we want to know if the vaccine could help mothers in Kenya and their babies.

What will it involve for my baby and me if I agree?

- During your admission for delivery of your baby, we are asking to take a recto-vaginal swab at routine vaginal examination. This is not painful and involves wiping the lower inside surface of the vagina and anus with a cotton bud. If your baby becomes sick, the results will be made available to guide your baby's care.
- We are also asking for consent to take a small sample of placenta, so that we can look to see if there are any signs of infection.
- Everything else that is done during your stay in hospital will be part of normal tests and treatment requested by doctors in your health facility.

Are there any risks or disadvantages to me taking part?

KEMRI's priority for every woman is the care of her and her baby. When the recto-vaginal swab is taken, it may add a little time to the total time for vaginal examination, but the risks of the procedure are minimal. The sample from the placenta that we are requesting permission to take is taken after the baby is separated from the placenta.

Are there any benefits to me/my baby by taking part?

If your baby becomes sick during the first few days after birth, the swab results will be available to clinicians to help in the management of your baby.

What happens if I refuse to participate?

Participation in research is voluntary. If you do not want to take part, no recto-vaginal swab or placenta sample will be taken. You will have the same care whether you take part or not. If you do agree now, you can change your mind at any time and not take part in the research. This will not affect your care or your baby's care now or in the future.

What happens to the samples?

Swab samples collected will be tested in the laboratories in Kilifi to see if GBS bacteria are present. Placental samples will be tested to see if there is evidence of infection during pregnancy.

Most of the research tests that will be done on the samples will be done here in Kenya. However for some tests that cannot be done in Kenya, samples may be sent to laboratories overseas. *After the research, a small portion of the placental samples will be stored. We would like to store them for up to ten years. In this time, new research about maternal and infant health may be done on these samples. This will involve using new ways of looking for infection and how your body fights infection and what may have affected your baby's growth in the womb. All such research must first be approved by a national independent expert committee to ensure your safety, rights and privacy are respected.*

All the information and samples collected will be held confidentially. Personal identifiers will be removed so that samples can only be linked to mothers by people closely concerned with the research.

Who will have access to information about me/my child in this research?

All our research records are stored securely in locked cabinets and password protected computers. Only a few people who are closely concerned with the research will be able to view information from mothers.

Who has allowed this research to take place?

An independent national committee and a committee in Kilifi have looked carefully at this work and agreed that the research is important, that it will be conducted properly, and that mothers' safety and rights have been respected.

Will I have to pay for any of the tests?

You will not have to pay for any of the tests for research. Clinical care will be unaffected and charged as per the health facility that you are attending.

What if I have any questions?

You may ask any of our staff questions at any time. You will be given a copy of the information contained on this form to take away with you.

You can also contact those who are responsible for the care of you and your child and this research:

PI's name(s) and contacts

Dr. Anna Seale KEMRI- Wellcome Trust [Kilifi District Hospital,]

P.O.Box. 230, Kenya. Telephone: 041 7522063

If you want to ask someone independent anything about this research please contact

Community Liaison Manager, KEMRI – Wellcome Trust

P.O.Box 230, Kilifi. Telephone: 0723342780 or 041 7522063

Or

The Secretary - KEMRI/National Ethics Review Committee

P. O. BOX 54840-00200, Nairobi,

Tel number: 020 272 2541 Mobile: 0722205901 or 0733400003

Joint KEMRI / MoH Research into Clinical and Molecular Epidemiology of maternal Group B Streptococcus colonisation.

I, _____ (name), have had the research explained to me. I have understood all that has been read and had my questions answered satisfactorily.

Please insert the boxes below where relevant:

- ☐ **I agree to take part in this research**
- ☐ **I agree to samples being stored and used for future research**
- ☐ **I agree to samples being exported**

I understand that I can change my mind at any stage and it will not affect me or my baby in any way.

Subject's signature: _____ **Date** _____

Subject's name: _____**Time** _____
(Please print name)

I certify that I have followed the study SOP to obtain consent from the [participant]. She apparently understood the nature and the purpose of the study and consents to participation in the study. She has been given opportunity to ask questions which have been answered satisfactorily.

Designee/investigator's signature: _____ **Date** _____

Designee/investigator's name: _____ **Time** _____
(Please print name)

Only necessary if the participant cannot read:

I *attest that the information concerning this research was accurately explained to and apparently understood by the subject and that informed consent was freely given by the participant.

Witness' signature: _____ **Date** _____

Witness' name: _____ **Time** _____
(Please print name)

*__A witness is a person who is independent from the trial or a member of staff who was not involved in gaining the consent.__

Thumbprint of the subject as named above if they cannot write:

***THE PARTICIPANT SHOULD NOW BE GIVEN A SIGNED COPY TO KEEP*.**

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Coast Provincial General Hospital, Mombasa	Hemed Twahir, Victor Bandika, Musimbi Soita
Health Protection Agency, UK	Androulla Efstratiou

We are very sorry that you have lost your baby.

What is KEMRI and what is this study about?

This health care facility and KEMRI are working together to strengthen services at maternity. KEMRI is a national research organization whose work is to find better ways of preventing and treating illness for the benefit of everyone in the future.
We are trying to understand the reasons for the loss of babies in Kenya, which can include infections and problems that run in families, such as sickle cell disease.Babies can have problems for many reasons, and can happen to any mother and father. We are looking to identify causes, so that we can try and reduce stillbirths in the future.

What will it involve for me if I agree?

- We would like to test the cord blood taken after delivery, in addition to infection, for problems that run in families, including sickle cell disorder.
- We would like to take a sample of fluid (10mls or two teaspoons) from the lung of your baby, to look for infection. This is obtained with a normal needle and syringe (show equipment).
- If you wish, you can come back for the results of these tests when they are available. These results, and any implications of them, will be discussed with you at that time.

Are there any risks or disadvantages to me taking part?

If you choose to come back for the results, this will take time, but the costs of transport for you and your husband to return for results will be reimbursed.

Are there any benefits to me by taking part?

The results of the tests may help understand why your baby died.
If your baby was identified as having sickle cell disease, we would offer sickle cell testing to you and your husband and other children if they were unwell and had symptoms of sickle cell disease. This would enable them to seek appropriate treatment.
Depending on the results for infection, it may be advisable to have your next delivery in hospital, with medication to try and prevent this happening again. We would let you know if this was the case, and give you a card to identify yourself, if you return for another delivery.

What happens if I refuse to participate?

Participation in research is voluntary If you do not want to take part, the tests will not be undertaken and the sample of fluid will not be taken. You will have the same care whether you take part or not and you and your husband will be supported with counselling. If you do agree now, you can change your mind at any time and not take part in the research. This will not affect your care now or in the future.

What happens to the samples?

Samples taken (cord blood and lung fluid) will first be tested to look for infection, in Kilifi. Cord blood will be tested for sickle cell disease and other problems which run in the family. Most of the research tests that will be done on the samples will be done here in Kenya. However for some tests that cannot be done in Kenya, samples may be sent to laboratories overseas. *After the research, a small portion of the samples will be stored. We would like to store them for up to ten years. In this time, new research about maternal and infant health may be done on these samples. This will involve using new ways of looking for infection and how your body fights infection and what may have affected your baby's growth in the womb. All such research must first be approved by a national independent expert committee to ensure your safety, rights and privacy are respected.*
All the information and samples collected will be held confidentially. Personal identifiers will be removed so that samples can only be linked to mothers by people closely concerned with the research.

Who will have access to information about me in this research?

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Who has allowed this research to take place?

An independent national committee and a committee in Kilifi have looked carefully at this work and agreed that the research is important, that it will be conducted properly, and that mothers' safety and rights have been respected.

Will I have to pay for any of the tests?

You will not have to pay for any of the tests for research. Clinical care will be unaffected and charged as per the health facility that you are attending.

What if I have any questions?

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Please insert the boxes below where relevant:

- ☐ **I agree to take part in this research**
- ☐ **I agree to samples being stored and used for future research**
- ☐ **I agree to samples being exported**

I understand that I can change my mind at any stage and it will not affect me in any way.

Subject's signature: _____ **Date** _____

Subject's name: _____**Time** _____
(Please print name)

I certify that I have followed the study SOP to obtain consent from the [participant]. She apparently understood the nature and the purpose of the study and consents to participation in the study. She has been given opportunity to ask questions which have been answered satisfactorily.

Designee/investigator's signature: _____ **Date** _____

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What is KEMRI and what is this study about?

Kilifi District Hospital and KEMRI work together. KEMRI is a national research organization whose work is to find better ways of preventing and treating illness for the benefit of everyone in the future.
We are very happy for you that you have had a healthy baby at this health care facility. However, we are trying to understand the reasons that some mothers do not have healthy babies. To do this, we need to look at healthy babies and compare them to babies with problems. We are asking a sample of mothers here with healthy babies, if we can undertake some particular tests on the cord blood sample they agreed to have taken after delivery.

What will it involve for me and my baby if I agree?

- We would like to test the cord blood taken after delivery, in addition to infection, for problems that run in families, including sickle cell disorder.
- If you wish, you can come back for the results of these tests when they are available. These results, and any implications of them, will be discussed with you at that time.

Are there any risks or disadvantages to me taking part?

If you choose to come back for the results, this will take time, but the costs of transport for you and your husband to return for results will be reimbursed.

Are there any benefits to me/ my baby taking part?

If your baby was identified as having sickle cell disease, we would also offer sickle cell testing to you and your husband and other children if they were unwell and had symptoms of sickle cell disease. This would enable them to seek appropriate treatment.

What happens if I refuse to participate?

Participation in research is voluntary. If you do not want to take part, the tests will not be undertaken. You will have the same care whether you take part or not. If you do agree now, you can change your mind at any time and not take part in the research. This will not affect your care now or in the future.

What happens to the samples?

Samples taken (cord blood) will first be tested to look for infection, in Kilifi. It will also be tested for sickle cell disease and other problems which run in the family.
Most of the research tests that will be done on the samples will be done here in Kenya. However for some tests that cannot be done in Kenya, samples may be sent to laboratories overseas. After the research, a small portion of the samples will be stored. In the future, new research about problems for mothers or their babies' health may be done on these samples. Future research must first be approved by a national independent expert committee to ensure participants' safety and rights are respected.
All the information and samples collected will be held confidentially. Personal identifiers will be removed so that samples can only be linked to mothers by people closely concerned with the research.

Who will have access to information about me/my child in this research?

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Subject's signature: _____ **Date** _____

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Designee/investigator's name: _____ **Time** _____
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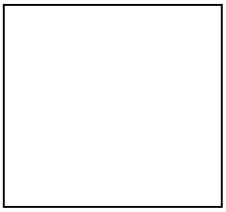
Thumbprint of the subject as named above if they cannot write:



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What is KEMRI and what is this study about?

Kilifi District Hospital and KEMRI are working together to strengthen services at maternity. KEMRI is a national research organization whose work is to find better ways of preventing and treating illness for the benefit of everyone in the future.
Although your baby is completely healthy, we are asking your help in research we are doing on infection in other babies. We know that a particular bacterium, group B Streptococcus, can be present without any problems at all, but sometimes it may cause severe infections in babies. We want to find out how common and important these bacteria are in Kenya. This is important because vaccines are being developed to protect against these bacteria, and we want to know if the vaccine could help mothers in Kenya and their babies. We are asking some mothers, permission to look at whether these bacteria are on the surface skin of their baby after birth.

What will it involve for my baby and me if I agree?

For your baby, it will involve wiping a cotton bud (swab) in the outside ear canal, nostril and on the skin around the umbilicus of your baby.

Are there any risks or disadvantages to me taking part?

There are no risks for this procedures.

Are there any benefits to me/my baby by taking part?

In situations where a healthy baby later becomes unwell during the first few days after being born, the swab results will be available to clinicians to help in the management of the baby.

What happens if I refuse to participate?

Participation in research is voluntary. If you do not want to take part, the swab will not be taken. You will have the same care whether you take part or not. If you do agree now, you can change your mind at any time and not take part in the research. This will not affect your care or the care of your baby now or in the future.

What happens to the samples?

The swab itself will be discarded. Any bacteria which grow from it will be stored and may undergo more detailed laboratory testing to see what particular types of bacteria they are. Most of the research tests that will be done on the samples will be done here in Kenya. However for some tests that cannot be done in Kenya, isolates may be sent to laboratories overseas.
All the information and samples collected will be held confidentially. Personal identifiers will be removed so that samples can only be linked to mothers and their babies by people closely concerned with the research.

Who will have access to information about me/my child in this research?

All our research records are stored securely in locked cabinets and password protected computers. Only a few people who are closely concerned with the research will be able to view information.

Who has allowed this research to take place?

An independent national committee and a committee in Kilifi have looked carefully at this work and agreed that the research is important, that it will be conducted properly, and that mothers' safety and rights have been respected.

Will I have to pay for any of the tests?

You will not have to pay for any of the tests for research. Clinical care will be unaffected and charged as per the health facility that you are attending.

What if I have any questions?

You may ask any of our staff questions at any time. You will be given a copy of the information contained on this form to take away with you.

You can also contact those who are responsible for the care of your child and this research:

PI's name(s) and contacts

Dr. Anna Seale KEMRI- Wellcome Trust [Kilifi District Hospital,]

P.O.Box. 230, Kenya. Telephone: 041 7522063

If you want to ask someone independent anything about this research please contact

Community Liaison Manager, KEMRI – Wellcome Trust

P.O.Box 230, Kilifi. Telephone: 0723342780 or 041 7522063

OrThe Secretary - KEMRI/National Ethics Review Committee

P. O. BOX 54840-00200, Nairobi,

Tel number: 020 272 2541 Mobile: 0722205901 or 0733400003

Joint KEMRI / MoH Research into Clinical and Molecular Epidemiology of maternal Group B Streptococcus colonisation.

I, _____ (name), have had the research explained to me. I have understood all that has been read and had my questions answered satisfactorily.

Please insert the boxes below where relevant:

- ☐ **I agree to take part in this research**
- ☐ **I agree to samples being stored and used for future research**
- ☐ **I agree to samples being exported**

I understand that I can change my mind at any stage and it will not affect me or my baby in any way.

Parent's 's signature: _____ **Date** _____

Parent's name: _____**Time** _____
(Please print name)

I certify that I have followed the study SOP to obtain consent from the [participant]. She apparently understood the nature and the purpose of the study and consents to participation of her baby in the study. She has been given opportunity to ask questions which have been answered satisfactorily.

Designee/investigator's signature: _____ **Date** _____

Designee/investigator's name: _____ **Time** _____
(Please print name)

Only necessary if the participant cannot read:

I *attest that the information concerning this research was accurately explained to and apparently understood by the subject and that informed consent was freely given by the participant.

Witness' signature: _____ **Date** _____

Witness' name: _____ **Time** _____
(Please print name)

*__A witness is a person who is independent from the trial or a member of staff who was not involved in gaining the consent.__

Thumbprint of the subject as named above if they cannot write:

***THE PARENT SHOULD NOW BE GIVEN A SIGNED COPY TO KEEP*.**

Appendix 3: Ethical Approval from Nairobi Ethics Review Committee

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **August 22, 2011**

TO: **DR. ANNA SEALE (PRINCIPAL INVESTIGATOR)**

THROUGH : **DR. NORBERT PESHU, DIRECTOR, CGMR-C, KILIFI**

forwarded

DIRECTOR CENTRE FOR GEOGRAPHIC MEDICINE RESEARCH, COAST

Dear Madam,

RE: **SSC PROTOCOL No. 2030 – REVISED (*RE-SUBMISSION*): THE CLINICAL AND MOLECULAR EPIDEMIOLOGY OF *STREPTOCOCCUS AGALACTIAE* (GROUP B STREPTOCOCCUS), MATERNAL COLONISATION IN THE KENYAN COAST (*VERSION 2.1*)**

Reference is made to your letter dated 16th August 2011.

We acknowledge receipt of:

a. The Revised Study Protocol – version 2.1 dated August 2011.
b. The Revised Informed Consent Document – English version 2.1.
c. The Revised Informed Consent Document – Kiswahili version 2.1.

This is to inform you that the Ethics Review Committee (ERC) reviewed the documents listed above and is satisfied that the issues raised at the initial review have been adequately addressed. Also noted is the inclusion in the summary table of the planned typing of GBS isolates from maternal colonization and to be compared with existing archived neonatal isolates from investigation of neonatal sepsis at Kilifi District Hospital.

The study is granted approval for implementation effective this **22nd day of August 2011**. Please note that authorization to conduct this study will automatically expire on **August 20, 2012**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **July 9, 2012**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC.

You are also required to submit any proposed changes to this protocol to the ERC to initiation and advise the ERC when the study is completed or discontinued.

Oxford Tropical Research Ethics Committee

University of Oxford
Joint Research Office, Block 60
Churchill Hospital, Oxford OX3 7LJT
Tel. +44 (0) 1865 (5)72228, fax +44 (0) 1865 (5)72224
E-mail: fiona.goulthorp@admin.ox.ac.uk

Dr A C Seale
Wellcome Trust Research Training Fellow
KEMRI-Wellcome CGMR-Coast
PO Box 230 Kilifi
Kenya

6th December 2011

Dear Dr Seale

Full Title of Study: The clinical and molecular epidemiology of *Streptococcus agalactiae* (Group B Streptococcus) maternal colonisation on the Kenyan Coast

OXTREC Reference: 53-11

Thank you for your letter 5th December 2011, in which you have responded to the committee's request for further clarification.

I am therefore happy as Chairman for OXTREC to give approval for this study.

OXTREC are grateful for annual and end of study reports for this study.

Yours sincerely,

Richard Mayon-White

Dr Richard Mayon-White

OXTREC Chair

Appendix 5: Maternal Admission Record

Admission Record – Coast Provincial General Hospital

1. Mother's admission details (midwife)

Mother's Name				Date of birth	
Hosp No.		Study ID		Home residence	
Home District		Division		Location	
Marital status	M☐S☐ D☐ W☐	Educational level	0 ☐ 1°☐ 2°☐ Hi☐	Mobile no.	
Emergency referral	Y N	Date of admission	___/___/____	Time admission	AM / PM
Time left ref. clinic	AM / PM	Next of Kin (NOK)			

2. Household /social history

How many people live in the building she sleeps in?		Ethnicity	
What is the average household monthly cash income? <1000 =1, 1000-3999=2, 4000-9999=3, 10,000-19,999 =4, >20,000=5, don't know=9			1 2 3 4 5 9
Type of house mother lives in?	Mud Wall=**1**, Stone Wall=**2,** Storey House=**3**, Iron Sheets=**4,** Other=**5,** Specify		1 2 3 4 5
What is the main water source?	Tap in house/ compound=**1**, Tap in community=**2,** Borehole in compound=**3**, Borehole in community=**4**, Water vendor=**5,** Natural source=**6**		1 2 3 4 5 6
Main source of fuel household uses for cooking?	Wood = **1**, charcoal = **2**, kerosene = **3**, gas/LPG = **4**, electricity = **5**		1 2 3 4 5
	If wood or charcoal, is it mainly used	In the house ☐	Outside the house ☐
Has the mother during pregnancy	Gutted fish? Y N Y N		Looked after cattle?
Does the father routinely	Gut fish? Y N Y N		Look after cattle?

3. Consent for treatment

I hereby give permission for anaesthesia and for any medical or surgical treatment which the Doctor may consider necessary to be performed upon me/my wife/my child.
Nakubali dawa ya kupoteza fahamu itumiwe na pia daktari aweza kufanya utibabu wowote ambao ataona ni lazima.

Date / /	Signature / Saini ..... Witness / Shahidi: Midwife ..... Student Midwife: .....

4. Antenatal History at admission

Is this the woman's first ever pregnancy (she is nulliparous)?			**Y N** If NO; fill table below.			**LMP**	/ /
Total number of pregnancies (incl.current)		No. of pregs >28 weeks	Born Alive		No. of pregs <28 weeks	Miscarriage	
			Stillborn			Termination	

No. **ANC attendances** this pregnancy?	0 1 2 3 4 5 >5 NK	Date first ANC visit	/ /
Which clinic?			NK
Number of doses **Malaria** prophylaxis:	0 1 2 3 4		NK
Number of doses of **TT** immunisation	0 1 2		NK
VDRL test result this pregnancy	R NR ND NK	Date of result	/ /
First **Hb** result this pregnancy	____ • __ g/dl ND NK	Date of result	/ /
Blood group	ND A B AB O +ve -ve		NK
Any transfusion this pregnancy?	Y N		NK
De-worming this pregnancy?	Y N		NK
Supplements this pregnancy?	None☐ Iron☐ Folic acid☐ Calcium ☐ Vitamins☐		NK
Antibiotics this pregnancy?	No☐ Yes☐		NK
Antibiotics in last 4 weeks:	None☐ for PROM☐ not for PROM☐		NK
Uterine Massage prior to this admission	None ☐ Most recent massage <6h☐ <24h☐ <1 week ☐ >1 week☐		NK

Mother's Name _____ Mother's Date of Birth___/___/_____

PMTCT test results this pregnancy (or status if already known to be R)?	**R** **NR** **ND** **NK**	Date of result	/ /
If mother is **Reactive**, is she on **Septrin?**	**Y** **N** **NK**	Is she on **ARVs?**	**Y** **N** NK
Other Medical problems?	Y N	If **Yes Specify:**	
Specific diagnosis at admission?	Y N	If **Yes Specify:**	

6. Examination on admission

Observations	HR	b/min	RR	b/min	BP	/	Temp	__•__ ^{0}C
Measurements	Weight	__•__ kg	Height	____•__ cm	MUAC	____•__ cm	SFH	_____cm
Pres./Lie	Cephalic OA ☐ Cephalic OP ☐ Breech ☐ Transverse/oblique ☐							
	Descent	/5	Cervix	_____ cm	Previous abdo scar?	Y N	FHR	/min
Membrane ruptured?	Y N **IF YES:**		Time ROM	: AM/PM	Liquor	Clear☐ Offensive☐ Mec☐ Not seen☐		
General Examination:								

7. Routine investigations results

Urine dipstick	ND	Protein 0 / + / ++ / +++	Glucose 0 / + / ++ / +++	Leukocytes 0 / + / ++ / +++	Nitrites Neg Pos	Blood Neg Pos

8. Research samples – assent/consent 7:30am Monday to 7:30am Wednesday inclusive

Assent given for research samples	Y N NA	Consent given	Y N NA

Vagino-Rectal Swab	Y N	Date and Time of Swab	/ / : AM / PM		
		Membranes Ruptured (At time swab taken)	Y N	If Y, time	AM / PM
		Has mother had a VE before the swab? (Includes VE at referring hospital)	Y N		NK

9. Delivery details (midwife/clinician)

1st Stage	Onset of labour?	No Labour☐ Spontaneous☐ Induced $_{oxy}$☐ Induced $_{PG}$☐ Induced $_{ARM}$☐		
	PROM (>=18hrs?)	Y N If Y: Duration _____ hours NK		
	Antibiotics before delivery?	☐ IV ☐ Oral ☐None NK		
2nd Stage	Fetal bradycardia? (<110 for 3 mins)	Y N	Fetal tachycardia? (>180 for 3 mins)	Y N
	Offensive amniotic fluid?	Y N	Thick meconium?	Y N
	Mode of delivery	Vaginal- $_{cephalic}$☐ Vaginal – $_{breech}$☐ Ventouse ☐ C/S – $_{elective}$ ☐ C/S – $_{emergency}$ ☐ Forceps ☐		
	Duration 2nd stage	H Mins NK		
3rd Stage	Perineal Tear	Y N	If YES: Grade of tear	1 2 3 4
	Episiotomy	Y N	Sutures	Y N NA
	Duration 3rd stage	H Mins NK	Placenta complete?	Y N NK
Delivery Complication	None☐ APH☐ PPH☐ Uterine rupture☐ Retained placenta☐ Obstructed labour☐ Dystocia☐ Cord prolapse☐ Retained twin☐ PET☐ Eclampsia☐ Other☐ Specify: _____			
	Blood loss	mls NK	Transfusion indicated?	Y N
	Blood available	Y N NA	Given Transfusion?	Y N NA
	Other delivery notes:			
Delivery attended by (tick all that apply):		Stud nurse☐ Nurse☐ COI ☐ CO☐ MOI ☐ MO☐ Obstet☐		

Mother's Name _____Mother's Date of Birth___/___/_____

10. Baby details

Multiple deliveries?	**Y N**		
Baby	**A**	**B**	**C**
Born alive	Y N	Y N	Y N
Date of birth	/ /	/ /	/ /
Time of birth	___:___ AM / PM	___:___ AM / PM	___:___ AM / PM
Born alive but died in maternity	Y N	Y N	Y N
	If Y: ____hrs ___mins from birth	If Y: ____hrs ___mins from birth	If Y: ____hrs ___mins from birth
FSB $_{fresh}$/MSB $_{skin\ broken}$	FSB MSB NA	FSB MSB	FSB MSB
Resuscitation	None☐ O2 ☐ BVM☐ CPR☐	None☐ O2 ☐ BVM☐ CPR☐	None☐ O2 ☐ BVM☐ CPR☐
Sex	M F	M F	M F
Gestation	/40	/40	/40
Weight	grams	grams	grams
OFC	_____•___ cm	_____•___ cm	_____•___ cm
MUAC	_____•___ cm	_____•___ cm	_____•___ cm
Admitted NNU <1 hr	Y N	Y N	Y N
Apgar Score	/1 /5 /10	/1 /5 /10	/1 /5 /10
Placental weight	_____g	_____g	_____g
TEO given	Y N	Y N	Y N
Vit K given	Y N	Y N	Y N
Nevirapine stat given	Y N NA	Y N	Y N

11. Outcome

Maternal Diagnosis(es) on discharge			
Maternal Outcome	Alive☐ Died ☐ Transfer (other hosp) ☐ Abscond ☐		
Date of Transfer to Postnatal ward (Date mother leaves the ward)	/ /	Time of transfer	AM / PM

Appendix 6: Laboratory analytical plan

The clinical and molecular epidemiology of *Streptococcus agalactiae* (Group B Streptococcus) maternal colonisation on the Kenyan coast.

(GBS) Kilifi Laboratory Analytical Plan

Introduction

Sub-Saharan Africa (sSA) has the highest regional rates of perinatal mortality worldwide. Group B Streptococcus (GBS) has been identified as a leading cause of early onset neonatal sepsis (EOS, in <7 days of life) in sSA. In other regions, maternal carriage is associated with early onset neonatal sepsis, but in addition, other adverse perinatal outcomes (stillbirths, early neonatal death, low birth weight and prematurity). Robust data on maternal GBS carriage in sSA and its burden on adverse perinatal outcomes are lacking, with important consequences for public health interventions.

Through investigation of maternal GBS carriage and perinatal outcomes at three different sites: rural, semi-rural and urban, this study will provide a comprehensive description of the burden of GBS in coastal Kenya. Risk factors for maternal colonisation and invasive neonatal disease will be assessed.

Transmission of GBS bacteria from mother to newborn will be assessed by assessing neonatal surface colonisation with neonatal surface swabs (nose, ear, and umbilicus) after birth.

Stillbirths will also be investigated, in individual cases, through additional detailed microbiological laboratory investigations (cord blood culture, sickle cell test and lung aspirates from stillbirths) to make an assessment of the contribution of GBS to stillbirths in Kenya and compared to controls (cord blood culture and sickle cell test only).

Subjects

June 2011 – Feb 2013: In KDH, Kilifi, all mothers who are admitted for delivery and consent for **recto-vaginal swab for GBS** are included (approx 3000 deliveries per year ~8/day)
Feb 2012 – 2013: In KDH, Kilifi neonates who are delivered from 7am to 5pm will be **surface swabbed (ear, nose, and umbilicus) for GBS** (~3/day).
Feb 2012 – 2013: In KDH, Kilifi stillbirths ("Group B" n=~150 in a year) who are delivered at any time will have **cord blood cultures, sickle cell test and lung aspirate samples** taken. For each stillbirth, 2 controls are recruited ("Group A" " n=~300 in a year) who have cord blood culture and sickle cell test taken (only – not a lung aspirate).
Feb 2012 – 2013: In CPGH, Kilifi mothers admitted from 7am Monday to 7am Wednesday morning inclusive will have **a recto-vaginal swab for GBS** taken at delivery. (approx 2800 deliveries per year ~25 on each day)
April 2012 – April 2013: In Bamba, Ganze, all mothers Sun – Thurs will have a **rectovaginal swab for GBS** taken at delivery. (~500).

Specimens and Lab tests:

	Which patients	When collected	When processed in lab	Test for	Comments
Microbiology					
Cord blood culture	**Stillbirth Cases, Live baby newborn controls**	**24/7**	**24/7**	**Bacteraemia**	**Midnight - 8am BACTEC bottles put into BACTEC by clinician. They must not be put into an ordinary incubator**
Lung aspirate	**Stillbirth cases**	**24/7**	**8am-6pm, 7 days/week**	**Bacterial culture, aliquot for storage as per SOP**	**Refrigerate if taken between midnight and 8am**
Neonatal surface swab (ear, nose, umbilicus)	**Newborns**	**7am-5pm in KDH**	**8am-midnight, 7 days/week**	**Bacterial culture for GBS**	**GBS culture only**
GBS recto-vaginal swab	**Mothers admitted for delivery: KDH, CPGH, rural sites**	**KDH - 24/7 CPGH – Mon to Weds Rural – Sun to Thurs**	**8am-midnight, 7 days/week**	**Bacterial culture for GBS**	**GBS culture only**
EDTA whole blood (purple top)	**Stillbirths and live birth controls**	**24/7**	**Twice weekly Tues and Fri**	**Sickle cell testing**	**Samples taken to CTL lab and then transferred direct to sickle cell lab as per current procedures.**

Specimen Transport Conditions

Specimen	Transport/storage conditions*	Until
Blood culture	≤24 h, room temperature	Placement in blood culture machine
Lung aspirate	≤24 h, 2-8°C (≤2 h, room temperature, preferably)	Inoculation onto culture media and other primary laboratory processing
Surface swab	≤24 h, 2-8°C	Inoculation onto culture media and other primary laboratory processing
Recto-vaginal swab	≤24 h, 2-8°C	Inoculation onto culture media and other primary laboratory processing
Whole blood	3 days 2-8°C	Processing in TW lab

**all specimens best if transported immediately at room temperature and processed immediately. Refrigeration is only if delays are unavoidable, because it can kill the pathogen we are looking for as well as the contaminants we want to prevent.*

Laboratory Methods

Cord Blood cultures: As per standard SOP LMIC 015.

- Time-to-positive must be recorded
- Volume must be recorded (weight method)

Surface swabs: These are processed for GBS only, as per SOP LMIC 03

- Refrigerate samples until processing 2-8°C
- Process <24h

Recto-vaginal swab: These are processed for GBS only, as per SOP LMIC 03

- Refrigerate samples until processing 2-8°C
- Process <24h

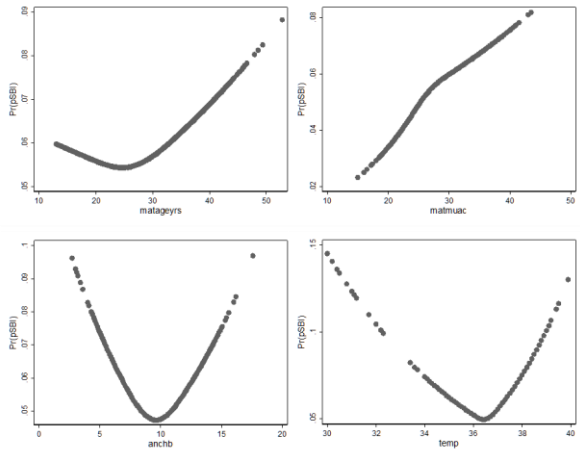
Lung aspirate fluid from stillbirth as per SOP LMIC 007:

- Microscopy and Bacterial culture by standard methods, (centrifuge if >1ml as per SOP, before gram stain and culture, but after making aliquot for PCR)
- Freeze aliquot at -70-80ºC
- NO TB culture

Whole blood: As per standard SOP for sickle cell Hb electrophoresis

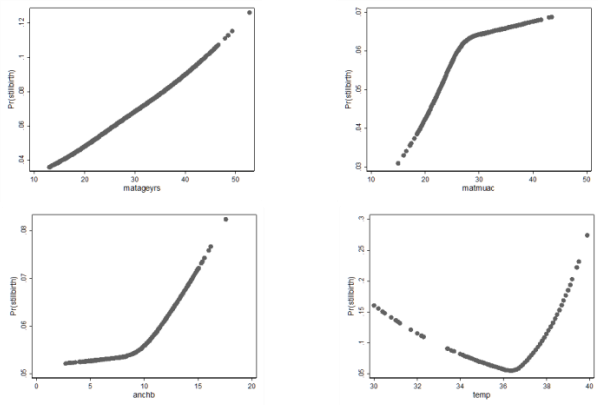
Appendix 7: Relationships of continuous variables with perinatal outcomes

Relationship of continuous variables and probability of neonatal admission with pSBI*



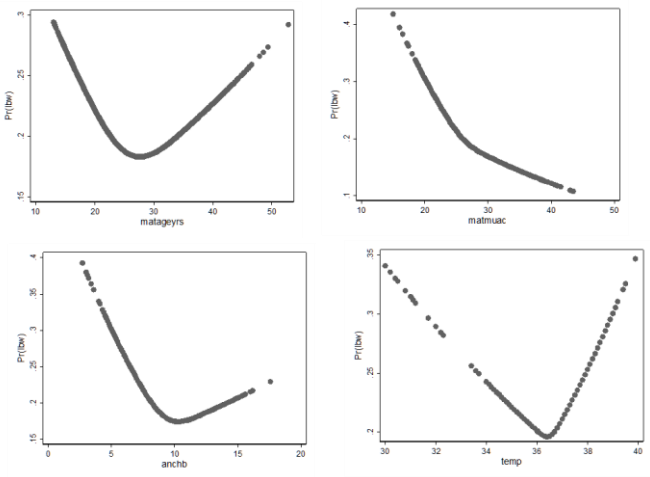
*Left top: maternal age (years) against probability of neonatal pSBI, right top: maternal mid-upper arm circumference (cm) against probability of neonatal pSBI, left bottom: maternal haemoglobin (g/dL) against probability of neonatal pSBI and right bottom: maternal temperature (^{0}C) against probability of neonatal pSBI.

Relationship of continuous variables and probability of preterm birth (<37 weeks) (live births only)*



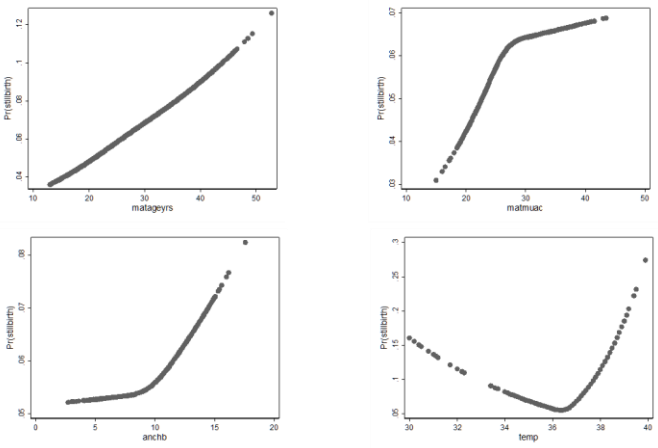
*Left top: maternal age (years) against probability of gestation <37 weeks, right top: maternal mid-upper arm circumference (cm) against probability of gestation <37 weeks; left bottom: maternal haemoglobin (g/dL) against probability of gestation <37 weeks and right bottom: maternal temperature (^{0}C) against probability of gestation <37 weeks.

Relationship of continuous variables and probability of low birth weight*



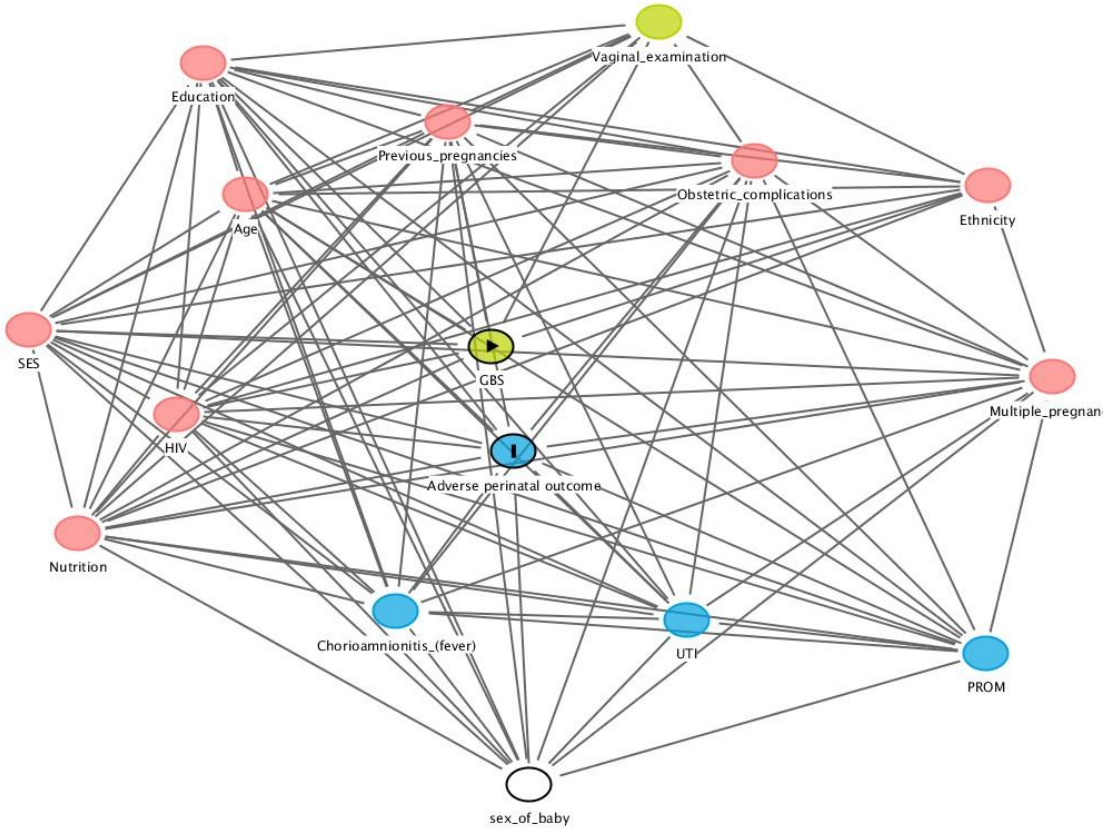
*Left top: maternal age (years) against probability of low birth weight, right top: maternal mid-upper arm circumference (cm) against probability of low birth weight, left bottom: maternal haemoglobin (g/dL) against probability of low birth weight and right bottom: maternal temperature (^{0}C) against probability of low birth weight.

Relationship of continuous variables and probability of stillbirth*



*Left top: maternal age (years) against probability of stillbirth, right top: maternal mid-upper arm circumference (cm) against probability of stillbirth, left bottom: maternal haemoglobin (g/dL) against probability of stillbirth and right bottom: maternal temperature (^{0}C) against probability of stillbirth.

Appendix 8: The Moral Graph



Appendix 9: Stata code for multiple imputations

```
sum
misstable summarize, gen(M_)
mi set wide
mi register imputed stillbirth2 longlab emerg_ref matyrs2* paritycat sexn educlevel
mijikenda mothercattle hivtmt muac2* anchb2* prevstillbn temp2* prom2 gestwks*
weight2* leukandnit vebeforeswab
mi register regular site sgb anyoutcome pc12* pSBI mumdups2 obstetcomp
mi register passive studyid stillbirth bornalivedied
mi impute chained (regress) temp2* matyrs2* anchb2* weight2* muac2* gestwks* (ologit)
paritycat educlevel hivtmt (logit) prom2 stillbirth2 prevstillbn leukandnit mothercattle
emerg_ref vebeforeswab mijikenda sexn = mumdups2 obstetcomp site pSBI pc12*, by(sgb)
add(50) rseed(532) chaindots augment force
save imputeall1d.dta, replace
***categorise with mi impute commands
use imputeall1d.dta, clear
set more off
*creating categories in imputed data
gen tempcats=temp21
mi xeq: recode temp21 -10/0=0 0.000000001/1=1 1.000000001/10=2
*tab1 _*_temp21
*mi xeq: recode anchb21 1/6.9999=0 7/10.9999=1 11/13.9999=2 14/34.99=3
mi xeq: recode anchb21 0/8.29999=0 8.3/9.599999=1 9.6/10.79999=2 10.8/20=3
*tab1 _*_anchb21
mi xeq: recode muac21 1/23.99999=0 24/25.99999=1 26/27.99999=2 28/50=3
*tab1 _*_muac21
mi xeq: recode matyrs21 1/21.523000000=0 21.523000001/25.37534000=1
25.375340001/29.999999=2 30/60=3
*tab1 _*_matyrs21
mi xeq: recode gestwks1 20/31.99999=3 32/36.99999=2 37/41.9999999=1  42/60=0
*tab1 _*_gestwks1
mi xeq: recode weight21 1/1499.99999=3 1500/2499.99999=2 2500/3499.99999=1
3500/6000=0
mi xeq: recode paritycat 0=0 1/3=1 4/6=2
recode pc121 -1/ -0.535278=0 -0.5352781/0.475694 =1 0.4756941/1.116948=2
1.1169481/2=
```