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Cutland, Clare Louise

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Epidemiology and Prevention of Sepsis in Young Infants and the Potential Impact of Maternal HIV Infection on Neonatal Sepsis

Clare Louise Cutland

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Doctor of Philosophy

Johannesburg, South Africa 2016

Declaration

I, Clare Louise Cutland, declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted previously for any degree or examination at this or any other university.

Signature: 

Date: 28th October 2016

Place: Johannesburg, South Africa

Dedication

I dedicate this work to my husband, Mike Reynolds, for your patience, unwavering encouragement and love. You have supported my decisions, despite knowing that they regularly involve sacrifice on your behalf. You have always helped me keep things in perspective and make me appreciate what is important in life.

To my children, Keira and Mark, who have both been born during my PhD path. You are the light of my life! Thank you for your understanding during my absences to present and write up this work.

To my parents, Julian and Sadie Cutland. Thank you for raising me with a love for medicine and a thirst for knowledge. Your unconditional support though many years of study, and life, has provided me with opportunities and strength to achieve my dreams. ‘What lies behind us and what lies before us are tiny matters compared with what lies within us’ - *Oliver Wendell Holmes*

To my sisters, Hillary Saville and Zoe Jewell, for always being there for me, in so many ways!

> “It’s in your hands to make our world a better one for all”
>
> \- Nelson Mandela

Publications contributing to this thesis and the role of the student

Paper I

Schrag SJ, **Cutland CL,** Zell ER, Kuwanda L, Buchmann EJ, Velaphi SC, Groome MJ, Madhi SA; the PoPS Trial Team. Risk Factors for Neonatal Sepsis and Perinatal Death among Infants Enrolled in the Prevention of Perinatal Sepsis Trial, Soweto, South Africa. Pediatr Infect Dis J. 2012 May 4.

Paper II

Cutland CL, Schrag SJ, Zell ER, Kuwanda L, Buchmann E, Velaphi SC, Groome MJ, Adrian PV, Madhi SA; PoPS trial team. Maternal HIV infection and vertical transmission of pathogenic bacteria. Pediatrics. 2012 Sep;130(3):e581-90. Epub 2012 Aug 6.

Paper III

Cutland CL, Schrag SJ, Thigpen MC, Velaphi SC, Wadula J, Adrian PV, Kuwanda L, Groome MJ, Buchmann E, Madhi SA. Increased risk for group B Streptococcus sepsis in young infants exposed to HIV, Soweto, South Africa, 2004-2008(1). Emerg Infect Dis. 2015 Apr;21(4):638-45. doi: 10.3201/eid2104.141562. PubMed PMID: 25812061; PubMed Central PMCID: PMC4378461.

Paper IV

Cutland CL, 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; the 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 Dec 5; 374 (9705):1909-16.

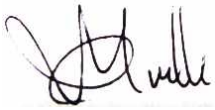
Paper III is from the Sepsis surveillance study.

Project title: 'Surveillance of culture-confirmed invasive infections among young infants and post-partum mothers at Chris Hani Baragwanath Hospital' (Sepsis surveillance)
Role of the student: Principal investigator. Contributed to the conception and design of the study. Developed the protocol and case report forms. Prepared and submitted ethics application. Implemented the surveillance system. Collected, cleaned, analysed and interpreted data. Prepared, revised and submitted manuscript for publication.

Papers I, II and IV are from the Prevention of Perinatal Sepsis (PoPS trial), a CDC- and BMGF-funded interventional, single-centre trial.
*Project title: '*Preventing serious neonatal and maternal peripartum infections in developing country settings with a high prevalence of HIV infection: Assessment of the disease burden and evaluation of an affordable intervention in Soweto, South Africa.' (PoPS trial)
Role of the student: Clinical lead investigator, under principal investigators Professor Shabir Madhi (Wits/ RMPRU) and Dr Stephanie Schrag (CDC, USA). Contributed to the conception and design of the trial and development of the protocol. Developed and revised case report forms. Prepared and submitted ethics application. Hired and trained staff, implemented and led the trial. Contributed to collection and cleaning of data and interpretation of results. Prepared, revised and submitted first-author manuscripts for publication, and contributed to preparation of second-author publication.
The co-authors have agreed to the use of the manuscripts in this thesis (See Appendix 8).



Clare L. Cutland (student)



Shabir A. Madhi (supervisor)



Stephanie J. Schrag
(Co-principal investigator with SA Madhi, PoPS trial)

Abstract

Introduction: Neonatal infections contribute to 25% of all neonatal deaths, which account for approximately 44% of all under-5 childhood deaths globally. Pathogens responsible for sepsis in neonates and young infants can be acquired vertically prior to or during labour, or from the environment (community or hospital).

This project evaluated the burden and aetiology of sepsis in neonates and young infants (≤90 days), and explored this association to in-utero exposure to human immunodeficiency virus. The study also included a specific focus on the epidemiology of invasive Group B Streptococcal disease in young infants.

Additionally, we assessed the efficacy of intrapartum chlorhexidine vaginal washes for: (i) preventing early-onset neonatal sepsis; and (ii) vertical transmission of potentially pathogenic bacteria to the newborns. Furthermore, we evaluated risk factors for poor outcomes due to neonatal sepsis.

Materials and methods: (i) A bacterial surveillance system was established at Chris Hani Baragwanath Academic Hospital (CHBAH) from 2004-2008 to identify young infants with bacterial sepsis hospitalised in the neonatal and paediatric wards. Medical and microbiological records were utilised to obtain clinical and laboratory data. Maternal HIV results were obtained from antenatal testing records or admission records.

(ii) A blinded, randomised, placebo-controlled trial of 0.5% chlorhexidine maternal vaginal intrapartum wipes and newborn skin wipes was conducted at CHBAH between 2004 and 2007. Consented, eligible participants were randomised during labour to receive either chlorhexidine vaginal wipes or water external genitalia wipes. Newborns received either chlorhexidine full-body wipes (intervention arm) or foot wipes (control

arm). Maternal and infant participants were followed up for admissions during the first month after delivery/ birth. A subset of 5144 maternal participants had an intrapartum lower vaginal swab collected, and skin swabs were collected from their newborns to assess colonisation with potentially pathogenic bacteria (Group B *streptococcus, Escherichia coli* and *Klebsiella pneumoniae*).

Results: Group B *streptococcus* (GBS) was the most commonly isolated bacterial pathogen, causing 35.2% of culture-confirmed sepsis in infants ≤90 days, 41.6% of early-onset disease (EOD, 0-6 days), 40.5% of late-onset neonatal disease (LOD, 7-27 days) and 18.7% of young-infant community-acquired disease (YI-CAD, 28-90 days). *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli)* and *Klebsiella pneumoniae (K. pneumoniae)* contribute 16.2%, 12.2% and 3.4% to sepsis in young infants.

Overall, incidence (per 1000 live births) of invasive GBS disease was 2.72 (95% confidence interval [95% CI]: 2.46 to 3.01), including an incidence of 1.50 and 1.22, respectively, in infants 0-6 days and 7-90 days of age. HIV-exposed infants were at greater risk of EOD (Relative risk [RR]: 1.69; 95% CI: 1.28-2.24) and LOD (RR= 3.18; 95% CI: 2.34-4.36) than HIV-unexposed infants. GBS serotypes Ia and III caused 84.0% of invasive GBS disease in young infants.

Intrapartum chlorhexidine interventional wipes was not efficacious in prevention of any of: (i) vertical transmission of pathogenic bacteria (54% vs. 55%; efficacy -0.05, 95% CI: -9.5 to 7.9) to the newborns; (ii) sepsis in first 3 days of life (3% vs. 4%; p=0.65,); (iii) sepsis in the later neonatal period (both <1%; p=0.4444); or (iv) maternal puerperal sepsis(both <1%; p=0.56).

Conclusion: GBS, *S. aureus, E. coli* and *K. pneumoniae* are the most commonly isolated bacterial pathogens in neonates and infants ≤90 days old. HIV-exposed infants are at greater risk of GBS sepsis. Intrapartum chlorhexidine intervention was not efficacious in reducing vertical transmission of pathogenic bacteria, neonatal or maternal sepsis. Alternative interventions to prevent sepsis in young infants, including maternal immunisation, need to be investigated in setting such as ours where there is a high prevalence of maternal HIV infection.

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The long road to finalisation of this thesis would not have come to an end without the incredible support I have received.

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Abbreviations

ARR	annual rate of reduction
BEmONC	Basic Emergency Obstetric and Neonatal Care
CACE	Complier Average Causal Effect
cART	Combination antiretroviral therapy
CDC	Centers for Disease Control and Prevention, Atlanta, USA
CFR	Case fatality proportion
CHBAH	Chris Hani Baragwanath Academic Hospital
CHC	Community Health Centres
CHX	Chlorhexidine
CLSI	Clinical Laboratory Standards institute
CPS	Capsular polysaccharides
CRFs	Case report Forms
CSF	Cerebrospinal fluid
CV	Conjugate vaccine
EOD	Early onset disease (0-6 days)
EPI	Expanded Program on Immunisation
GAIA	Global Alignment of Immunisation safety Assessment in pregnancy
GBS	Group B streptococcus (*Streptococcus agalactiae*)
HEU	HIV-exposed, uninfected
HICs	High income countries
HIV	Human immunodeficiency virus
HREC	Human Research Ethics Committee
HUU	HIV unexposed uninfected
IAP	Intrapartum antibiotic prophylaxis
ITT	Intent to treat
LMICs	Low- middle income countries
LOD	Late onset disease (3-28 days – PoPS)
MDG4	Millennium Development Goal number 4
MIC	Minimum inhibitory concentration
MOU	midwife obstetric units
MSAF	Meconium-stained Amniotic Fluid
NHLS	National Health Laboratory Services

N-LOD	Neonatal late onset disease (7-27 days)
NMR	Neonatal mortality rate
NPA	Nasopharyngeal aspirate
PCR	Polymerase chain reaction
PCV	Pneumococcal Conjugate Vaccine
PMR	Perinatal Mortality rate
PMTCT	Prevention of mother to child transmission (of HIV)
PoPS	Prevention of Perinatal Sepsis
PSBI	Possible Severe bacterial infection
PV	Per vaginal
RCT	Randomised controlled trial
RMPRU	Respiratory and Meningeal Pathogens Research Unit
ROM	Rupture of membranes
RR	Relative risk
SBA	Skilled birth attendant
SE-Asia	South East Asia
SS-Africa	sub-Saharan Africa
TBA	Traditional birth attendant
U5MR	Under-5 mortality rate
UTI	Urinary tract infection
VEOD	Very Early Onset disease (0-2 days)
WHO	World Health Organisation
YI-CAS	Young infant community-acquired sepsis (28-90 days)
95% CI	95% confidence interval

Preface

After my second year of medical school, I took a ‘gap year’, during which I completed a Bachelor of Science degree at the University of the Witwatersrand. I hoped to develop research skills to support my career as a medical doctor, not realising at the time the profound impact this decision would have on my future career path. I joined the Respiratory and Meningeal Pathogens Research Unit (RMPRU) in July 2000 to obtain post-graduate experience in research, initially on a 6-month contract, with every intention of returning to clinical medicine, to specialise and practice as a paediatrician. When I joined RMPRU, enrolment into a large trial assessing the impact of a 9-valent pneumococcal conjugate vaccine (PCV) was nearing completion, and I was involved in the follow up of participants until 5-years of age. Over the next few years, I was afforded the opportunity to set up and run other paediatric vaccine trials in Soweto and Eldorado Park, Gauteng, South Africa, including a phase III trial of Rotarix®. The results of the pivotal PCV and Rotarix® trials conducted by RMPRU have informed global (World Health Organisation) recommendations on introduction of these two life-saving vaccines into the Expanded Program on Immunisation (EPI). In 2009, PCV and Rotarix® were introduced into the South African EPI. The knowledge that work I have been directly involved in has impacted the lives of millions of children both in South Africa and globally is extremely rewarding as a medical practitioner, and has kept me in a full-time research position for the past 15 years!

Several interventions, including childhood vaccination, have led to a 53% reduction in under-5 child deaths between 1990 and 2015. The neonatal period (the first 28 days of life) is, however, the most treacherous time of life, during which the immense changes from intra-uterine life to life in the real world are encountered, and unfortunately,

millions of neonates annually do not weather the storm! Neonatal deaths contribute 44% of all under-5 child deaths globally.

Under the mentorship of Professor Shabir Madhi, I embarked on two projects in the field of sepsis in neonates and young infants, which have contributed to my PhD. These projects were both funded by the Centers for Disease Control and Prevention (CDC), USA and the Bill & Melinda Gates Foundation, and I have been fortunate to work intimately with experienced and influential public health care advisors and policy-makers. During and subsequent to the conduct of these projects, I have had the opportunity and privilege to interact with global leaders in neonatal sepsis and more recently in the growing and exciting field of immunisation of pregnant women, and to present my work in Europe, the United States, Asia and Africa.

The University of the Witwatersrand's recommended 'divided block' format has been used for this thesis. It includes a literature survey, a chapter describing the methods utilised, 5 discrete chapters describing the results of the work conducted, and a conclusion chapter. The methods section has not been repeated in each of the results chapters; rather a brief methods section has been included in some results chapters in which additional activities unique to that component of the research are described. Publications emanating from the work conducted have been used as the basis of 4 of the 5 results chapters (chapters 4-7). The unpublished chapter (chapter 3) is being prepared for publication. The references are not listed at the end of each chapter, but rather at the end of the thesis.

This thesis describes the aetiology of bacterial sepsis in neonates and young infants (≤90 days) in an urban low-middle income setting (Soweto, Gauteng, South Africa) with

a high neonatal mortality rate (21/ 1000 live births) and disease burden, and presents the results of an interventional randomised, blinded trial of chlorhexidine maternal intrapartum vaginal wipes and newborn surface wipes which was conducted at Chris Hani Baragwanath Academic hospital.

Subsequent to completing the projects described in this thesis, I have enjoyed continuing my work aimed at preventing infectious diseases in neonates and young infants, and am thrilled to be involved in the rapidly-progressing and exciting field of maternal immunisation.

A quote from our late inspirational leader, Nelson Mandela, is appropriate to the completion of this thesis: ‘It always seems impossible until it’s done’.

1. INTRODUCTION

The enormity of the burden of deaths in neonates and young infants was highlighted in *The Lancet's* Child survival series of 2003[1, 2] and Neonatal survival series in 2005[3-6], which reported that globally 3% (approximately 4 million) of all live-born babies died during the first 28 days of life in the year 2000[1, 3]. Ninety-nine percent of these neonatal deaths occurred in low- and middle-income countries (LMICs), where access to skilled care for the mother and newborn is limited[3]. The World Health Organization (WHO) regions of South-East Asia and Africa contributed 64% (2.57 million) of these deaths. Africa had the highest average neonatal mortality rate 44/1000 (range 9-70/1000 live births)[3].

The global neonatal mortality rate (per 1000 live births) declined by 37% from 33 to 21 between 1990 and 2012. However, this has lagged behind the more rapid year-on-year reduction of other causes of under-5 deaths in this time period. Consequently, the proportion of under-5 childhood deaths occurring during the neonatal period has increased from 37.6% in 2000 to 43.9% (2.761 million) in 2013[7-9].

The Millennium Development Goals (MDGs) (http://www.un.org/millenniumgoals/bkgd.shtml) are structured, time-bound targets adopted by world leaders in 2000, as a commitment on behalf of their nations to reduce extreme poverty and improve health and welfare of their population by 2015. The fourth of the eight Millennium Development Goals (MDG4) aimed to 'Reduce by

two thirds, between 1990 and 2015, the under-five mortality rate'. The MGD4 target was not achieved globally in 2015: there had been a 53% reduction in mortality in children under 5 years of age, rather than the targeted 66.7% reduction[10]. A 50% reduction in the Under-5 mortality rate (U5MR) between 1990 and 2015 was achieved by all regions of the world, but only 2 regions (East Asia & Pacific and Latin America & Caribbean) met the MDG4 target[11]. On a country level, only 32% (62/195) of countries managed to achieve their MDG4 target, including twelve low-income countries, ten of which fall in sub-Saharan Africa (Mozambique, Malawi, Ethiopia, Madagascar, Eritrea, Liberia, Niger, Rwanda, Uganda and Tanzania)[11]. South Africa did not achieve MDG4, however, with sustained efforts in scaling up of the prioritised interventions, the MDG4 goal could be achievable in South Africa soon after 2015[12].

In 2013, the rate of under-5 deaths (per 1000 live births) globally was 44.0, with the neonatal period at 18.4 and post-neonatal infant mortality of 13.2, which together contributed to almost 72% of all under-5 deaths[13]. South Africa had an under-5 mortality rate of 37.0 in 2013, including a neonatal mortality rate of 14.6 and post-neonatal infant mortality rate of 14.7[13]. The neonatal mortality rate in Soweto during 2013/ 2014 was, however, 21 per 1000 live births (Maternal influenza trials, unpublished data), which is 44% higher than reported nationally.

The annual rate of reduction (ARR) in under-5 mortality in South Africa was four times higher in 2000-2015 period, than in the 1990's, mainly due to decrease in Human immunodeficiency virus (HIV)-related childhood deaths[11], largely due to the implementation and strengthening of mother-to-child HIV transmission prevention programs (PMTCT)[14], and early antiretroviral therapy of HIV-infected children[11, 15].

Sixteen cost-effective interventions spanning preconception, antenatal, intrapartum and postnatal periods were identified during a systematic review as being effective in reducing mortality in the perinatal (including stillbirths and deaths in first 7 days of life) and neonatal periods[4]. Implementation or expansion of these interventions in LMICs is challenging, especially intrapartum interventions, which have the greatest impact in reducing neonatal mortality but have a high financial cost[4]. Recognition of the importance of newborn health as an important indicator of population health[16], has encouraged country leaders to prioritise maternal and newborn health programs[17]. Government and community commitment to strengthening of health systems aimed at maternal and newborn health highlighted in 2005[5, 6], has led to a reduction of the absolute number of annual neonatal deaths to ~2.6-2.9 million deaths in 2013[7, 13, 18].

The South African National Department of Health adopted and implemented 15 cost effective interventions (including antenatal corticosteroids during preterm labour, improved management of labour and delivery, early detection and treatment of HIV-infected women, oral rehydration solution) in 2013 which were identified and prioritised in a study to reduce maternal, neonatal and child deaths[12].
Allocation of resources and efforts to reducing mortality in neonates and young infants requires an in-depth knowledge of the causes of death.

1.1. Causes of mortality in neonates and post-neonatal infants

Unfortunately, the global regions with highest neonatal and infant mortality rates also have marked paucity of data, making accurate reporting difficult[3]. Civil registration

systems are administrative systems utilised by governments to record major vital events, most notably births and death. Many countries have legislated the registration of vital events, however, limited capacity to roll out these administrative systems in many poorer countries hampers adequate, timely registration of births and deaths. Data from civil registration systems are used to generate vital statistics for countries[19], and well established and functioning Civil Registration and Vital Statistics (CRVS) systems can contribute to improved health outcomes in the population, especially when data generated are utilised to plan resource availability and allocation [20]. Unfortunately, CRVS systems in many LMICs are inadequate, with up to 35% of births and 60% of deaths occurring globally being unregistered[21]. Statistical modelling has, therefore, been utilised to estimate the distribution of causes of child mortality[8] and neonatal cause-of-death distributions for countries without high-quality CRVS systems[22].

Globally, preterm birth (35.0%- 35.7%), intrapartum complication (23.4-24.0%) and sepsis (15.3-15.6%) are the main causes of neonatal death, and collectively account for approximately 75% of neonatal deaths[8, 22]. Oza et al[22] have recently published cause-of-neonatal-death estimates for 194 countries and have stratified neonatal deaths into early (0-6 days) and late (7-27 days) neonatal period deaths. The distribution of the causes of death differ remarkably between the early and late neonatal periods, with preterm birth complications contributing 40.8% and 21%; intrapartum complications contributing 27.0% and 12.9% and sepsis contributing 14.2% and 47.6% to early and late neonatal deaths respectively[22]. Despite sepsis ranking third in global causes of neonatal death, just over half of newborn deaths that occur at a community level in India were assessed to be sepsis-related[23]. A

review of community-based studies conducted in LMICs and published since 1990 reported infection-specific mortality rates ranging from 2.7 (95% CI: 1.6 to 4.2) to 38.6 (95% CI: 16.8 to 74.7), with 8-80% (median 36.5%, interquartile range 26%-49%) of all neonatal deaths being attributable to infections[24]. In a population-based verbal autopsy study conducted in India, Malawi, Bangladesh and Nepal, 63% to 82% of neonatal deaths occurred in the first week of life. The neonatal mortality rates were 9- 59 per 1000 live births, with infection causing 27.4% to 32.9% of deaths[25].

Approximately 36% of all neonatal deaths take place in the first 24 hours of life[7], accounting for approximately 1 million deaths annually. The addition of stillbirths to these neonatal deaths on first day of life doubles this number annually, to almost 2.2 million perinatal (stillbirths and first 7 days of life) deaths globally[7]. The main causes of post-neonatal under 5 mortality are lower respiratory tract infection, malaria and diarrhoeal disease (~1.75 million deaths annually)[18].

In an effort to assist community-based health care workers to identify and manage sick infants at entry-level health care facilities, the WHO developed the 'Integrated Management of Childhood Illness' (IMCI) algorithms during the mid-1990s. Management of neonatal illness during the first week of life was not included in the original IMCI guidelines, and a large multi-centre study (including a South African site) was conducted during the early 2000's to identify clinical predictors of severe illness requiring hospitalisation in infants under 60 days of age. Severe infection (including sepsis, meningitis and pneumonia) was identified as the cause of hospitalisation in 43% of neonates <7 days of age and 61% of infants aged 0-59 days old in Durban, South Africa[26, 27].

1.2. Sepsis in neonates and post-neonatal infants

Sepsis in young infants (<3 months of age) is delineated into specific age groups, according to the normal physiological changes that occur during childhood[28], and includes early-onset disease (EOD, 0-6 days of life) and late onset disease (LOD, 7-90 days). LOD can also be stratified into late onset *neonatal* disease (7-27 days) and post-neonatal disease (28-90 days).

The foetus and young infants' vulnerability to infections is dependent on the relationship between the triad of host, environment (pre- and post-delivery) and organism. In order to allow for successful outcomes of pregnancy, the mother's immune system adapts to tolerate the presence of the foetus, whilst still continuing to function effectively in the event of an infection[29]. This adapted immune system, however, makes the mother more susceptible to infection, which can in turn increase the risk of infection in foetus and newborn.

Maternal antibodies, which provide passive protection against infection to the infant during the first months of life, cross the placenta by an active receptor-mediated process which increases from 30 weeks of gestational age[30, 31]. Hypergammaglobulinemia[32], placental malaria[33], maternal HIV-infection[34] and preterm birth[35] reduce transplacental transfer of antibodies between mothers and infants, increasing risk of infections in infants.

The immune system of the foetus and newborn is specifically developed and adapts to enable the foetus and newborn to cope with the challenges he/ she encounters,

including self-protection against maternal alloantigens in utero, and progressing to a controlled or muted inflammatory response to the onslaught of exposures to previously un-encountered microorganisms during and after delivery.

Physical barriers including skin, mucous membranes and secreted mucous layer provide the first anatomical and physiological barrier to invasion by pathogens[36]. Susceptible ports of entry through this physical barrier include the umbilical cord attachment, eyes, nose, mouth, gastro-intestinal and uro-genital tracts.

The innate immune system has a cellular component comprising neutrophils, dendritic cells, monocytes and macrophages, and a humoral component made up of biologically active molecules (e.g. complement proteins) which are involved in regulation of inflammatory response[36, 37]. The foetal immune system needs to remain tolerant to the maternal alloantigens[38], which is probably one of the reasons why the innate immune system has muted functionality at birth[38-40], and which in turn increases the newborn's susceptibility to bacterial and viral infections[38, 40].

The cellular component of the adaptive immune system of a newborn comprises T- and B-lymphocytes, which develop from the bone marrow. T-lymphocytes develop further in the thymus[38]. Although mechanisms are not well understood, development of foetal and neonatal T cells deviates towards T-helper type 2 or regulatory responses (T_{reg}), which do not adequately protect against intracellular pathogens[38, 41]. This response if further heightened in HIV-exposed than HIV-unexposed infants. Additionally, B-cell numbers are increased and natural killer cell numbers are decreased in HIV-exposed infants compared to HIV-unexposed infants, [29] which

could contribute to increased susceptibility to infections in HIV-exposed infants even in the absence of them being HIV-infected .

The humoral component of the adaptive immune system in the neonate comprises predominantly of maternally-derived IgG antibodies[36, 40], which cross the placenta in increasing number as gestational age approaches term. Additionally, secretory IgA and IgG antibodies from breastmilk may confer protection against infections in the newborn[42, 43]. This passively-acquired immunity, which wanes over the first few months of life, is able to protect the newborn against pathogens to which the mother had been exposed.

HIV-exposed infants are at higher risk of being born prematurely or at low birth weight[44, 45]. Chronic inflammation due to HIV infection and other opportunistic pathogens could lead to an increase in the number of activated cytokine-producing T-cells in HIV-infected compared to HIV-uninfected individuals. In pregnant HIV-infected women, there is an increase in pro-inflammatory cytokines and growth factors in the placenta, which can also increase the risk of mother-to-child-transmission of HIV and other infections[29].

Maternal recto-vaginal colonisation with potentially pathogenic organisms, prolonged and/or premature rupture of membranes, preterm delivery, chorioamnionitis and maternal urinary tract infections are the most commonly recognised risk factors for early-onset disease (EOD, 0-6 days of life)[40, 46]. Pathogenic organisms including Group B streptococcus (GBS) and *Escherichia coli (E. coli)* are usually acquired by vertical transmission from mother to infant, either by migration of colonising bacterial isolates into the amniotic fluid through ruptured or macroscopically intact membranes

which could have micro-tears[47], acquisition during birth process[48] or rarely by haematogenous spread from a mother with GBS bacteraemia[49].

Late-onset disease (LOD, 7-90 days of life) could be acquired from the mother, but is more commonly community-acquired or nosocomially-acquired. Risk factors for community-acquired LOD include exposure to environmental pathogens from family or community[50] and interruptions of natural barriers (skin and mucous membranes)[40]. Exposure of neonate to invasive procedures, indwelling catheters and prolonged use of antibiotics predispose neonates to nosocomial LOD[40].

1.2.1. **Neonatal sepsis incidence, aetiology and antibiotic resistance patterns**

Identification of a causative pathogen from a normally sterile body fluid is considered the gold standard for diagnosis of sepsis[28, 51, 52]. The sensitivity to detect pathogens in blood culture samples from infants with sepsis is low (0.6% to 7.9%)[53, 54]. This may be in part related to the low blood sample volumes available from infants and children (up to 6ml blood should be used for blood culture testing)[53, 55], use of incorrect culture bottles[56], low bacterial load/ low level bacteraemia[56, 57] and additionally impacted by antibiotic administration to mother prior to delivery of infant[58], or to the infant prior to drawing blood for culturing[56]. Despite the difficulties encountered in trying to establish the bacterial cause of sepsis in neonates and young infants, data are available from many regions of the globe. Some publications do not, however, differentiate between sepsis acquired from mother (vertically transmitted, usually EOD), community or environment (EOD or LOD) and health-care

facility (nosocomial, usually LOD). Pathogen distribution and antibiotic sensitivity patterns can vary markedly between these groups.

Neonatal sepsis in high income countries (HIC)

Early onset neonatal sepsis rates in high income country (HIC) settings ranges from 0.59 to 1.6 per 1000 live births[40, 59-63], with GBS being the most commonly isolated pathogen[64-66] , which together with *Escherichia coli* account for almost 75% of cases[40, 64]. Preterm neonates are at increased risk of developing sepsis, with black preterm neonates having highest incidence in USA with rate of 5.14 per 1 000 live births and a case fatality ratio of 24.4%[40]. *E. coli* is the most common cause of EOD in preterm and very low birth weight (VLBW, birth weight <1500 grams) neonates, and the leading cause of sepsis-related mortality in VLBW infants[40].

Late onset neonatal sepsis rates in HIC settings range from 3.0 to 3.7 per 1000 live births, with *E.coli* and *Klebsiella* species being the main bacterial pathogens[64]. Preterm and low birth weight neonates are at highest risk for late onset sepsis, which is predominantly nosocomial and related to prolonged hospitalisation and indwelling foreign bodies including catheters[40].

Neonatal sepsis in low and middle income countries (LMIC)

In a population-based study conducted in Bangladesh, the culture-confirmed neonatal sepsis rate was 3.0 per 1000 live births, with *Staphylococcus aureus (S. aureus)* reported as the most common neonatal pathogen. However, this was considered an under-estimation of true burden of neonatal sepsis since more than half of the reported neonatal deaths occurred in the first 24 hours after delivery, and

62% of these neonates were not assessed by a health care worker prior to death[67]. The incidence of sepsis in neonates and post-neonatal young infants assessed in hospitals may not be representative of community-based incidence, especially in LMICs. Culture-confirmed- and clinically-suspected neonatal sepsis rates of 16 per 1000- and 49-170 per 1000 live births, respectively, have been reported from community-based studies in LMICs[24, 63].

The WHO Young Infants Study group conducted a study in four LMIC countries (The Gambia, Philippines, Ethiopia and Papua New Guinea) in the early 1990s, to describe the causes of sepsis in infants under 3 months of age and clinical features which predicted severe disease[68]. The bacterial pathogens most commonly isolated from blood were *Staphylococcus aureus (S. aureus;* 23%*), Streptococcus pneumoniae (S. pneumoniae*; 30%*), Streptococcus pyogenes (S. pyogenes*; 20%*)* and *E. coli* (18%) in infants aged 1 to 3 months. *S. pneumoniae* was the most common cause of meningitis in young infants, the majority (12/17, 71%) of which occurred in the second and third months of life. GBS was conspicuous in its absence, with only two neonatal bacteraemia and one neonatal meningitis cases being identified in 2398 enrolled sick infants[69]. Notably, however, this study only enrolled neonates following their discharge from health facilities after birth, and consequently likely under-recognised the burden and aetiology of EOD[68].

More recently, several literature reviews and meta-analyses have been conducted to describe the incidence and aetiology of sepsis in neonates and post-neonatal infants in LMIC, however, heterogeneity of studies and definitions used limited comparability of studies included in reviews[70]. Bacteraemia was identified in 3-16% of sick infants

presenting to first-referral hospitals, and from 20-60% of infants presenting to tertiary hospitals[70]. Between 62% and 75% of positive blood cultures in infants were from neonates, highlighting the large burden of disease in this age-group[70, 71].

The reported incidence (per 1 000 live births) of neonatal sepsis in LMICs varied from 3.5 to 23, including 7.1-38 in Asia, 6.5-23 in Africa and 3.5-8.9 in South America and Caribbean[64, 70-72]. The majority of identified pathogens in these studies were Gram-negative bacteria (53% - 61%), including *E. coli* (8% - 19%) and *Klebsiella species* (14% - 25%). Gram-positive bacteria contributed to 39% - 47% of the identified pathogens, of which *S. aureus* (13.3% - 26.4%) was the most common[70, 71].

Two meta-analyses of published[70, 71] and unpublished studies[71] from LMICs, indicated that *S. aureus (*13.3- 26%), *E. coli (*8-17.8%) and *Klebsiella* species (13.5-21%) were the most commonly-isolated bacteria identified in neonates with sepsis. In contrast, identification of GBS was uncommon, being identified in only 2-8% of bacterial pathogens in neonates with sepsis.

In the post-neonatal period, the meta-analysis by Zaidi et al[71] identified *S. pneumoniae* (27%), *S. aureus* (11-13%) and *S. pyogenes* (11-13%) as the most common pathogens, whereas the meta-analysis by Downie et al[70] identified *S. aureus* (22%), *E.coli* (10%) and *Klebsiella* species (10%) as the most dominant post-neonatal bacterial isolates[70]. GBS was identified in <1% to 11.5% of post-neonatal infants.

The age of the infants included in the post-neonatal period and years of data

collection differed between these two meta-analyses. Zaidi et al[71] limited review to infants aged 0-90 days from articles published between 1980 and 2007, however, Downie et al[70] included infants up to 1-year of age identified in articles published between 1993 and 2009. Both meta-analyses concluded that data on culture-confirmed sepsis in young infants from LMICs was extremely limited and quality of studies included in meta-analyses varied.

There is a paucity of data on antibiotic resistance patterns of bacterial pathogens causing neonatal and post-neonatal infant sepsis in LMIC. Bacteria isolated from neonates were more likely to be resistant to penicillin-gentamicin combinations (43%) and third-generation cephalosporins (44%) than bacteria isolated from post-neonatal infants (37% and 36% respectively)[70]. Included among these bacteria, however, were studies which included nosocomial sepsis cases.

Varied patterns of susceptibility were identified in the meta-analysis, with 72-74% of *E. coli* isolates being resistant to penicillin or ampicillin and 20-64% being resistant to third generation cephalosporins. Twenty to 80% of *S. aureus* isolates were resistant to penicillin or ampicillin, and approximately 37% were resistant to third generation cephalosporins. Furthermore, 70- 100% of *Klebsiella* species isolates were resistant to penicillin or ampicillin, and 43-66% were resistant to third generation cephalosporins[70, 73].

Cause of sepsis in neonates and young infants

Streptococcus agalactiae* or Group B *streptococcus (GBS) is a gram-positive coccus which was first identified in 1885 as the main cause of bovine mastitis by

Nocard and Mollereau[74]. In 1934, GBS was reported as a cause of severe, sometimes fatal puerperal uterine infection, and as a non-pathogenic vaginal coloniser in pregnant women by Rebecca Lancefield and Ronald Hare[75]. GBS has been reported as the principal cause of early-onset disease since the 1970s[76].

There are ten serotypes identified by their specific sialic acid-rich capsular polysaccharides (CPS: Ia, Ib, II, III, IV, V, VI, VII, VIII, IX and X); the most pathogenic serotypes in young infants are III (48.9%) and Ia (22.9%)[77]. The pathogenicity of GBS is based on the presence of several virulence factors. Capsular polysaccharides (CPS) expressed by GBS assists in evading ingestion by host phagocytes[40, 78]. Adherence to the epithelial cells and trans-epithelial migration is a pre-requisite for colonisation and invasion, and is mediated by pilus-like structures in the GBS surface[78, 79]. Additionally, C5a peptidase produced by GBS interferes with the host defence systems by inhibiting human C5a- neutrophil chemo attractant produced during complement activation[40, 78].

Prior to implementation of GBS prevention measures, the incidence (per 1000 live births) of GBS EOD in USA was 1.3-1.7[48]. In the United Kingdom, overall GBS incidence was 0.72 (95% confidence interval 0.66-0.78) in 2000- 2001, with the incidence of EOD being 0.47 (95% confidence interval 0.42-0.52)[80].

In a meta-analysis of 74 studies published between January 2000 and September 2011, the incidence (per 1000 live births) of GBS disease in young infants globally was 0.53 (95% confidence interval [95% CI]: 0.44 to 0.62)[77]. Africa reported the highest incidence (1.21; 95% CI: 0.50 to 1.91) and south-east Asia the lowest (0.02;

95% CI: 0 to 0.07)[77]. Other studies conducted in Africa which were not included in this meta-analysis also reported high incidence of GBS-related disease[81-84], including a retrospective study of GBS in children in Soweto, South Africa between 1997 and 1999, which reported a GBS-EOD incidence of 2.06 and GBS-LOD incidence of 1.0 live births[81].

The reasons for this marked difference in GBS-EOD rates are probably multifactorial. Recto-vaginal colonisation with GBS is a major risk factor for developing GBS-EOD[85]. The prevalence of recto-vaginal colonisation with GBS varies globally, and ranges from 11.1% (95% CI: 6.8-15.3) in South-East Asia to 22.4% (95% CI: 18.1-26.7) in Africa (Personal communication: Gaurav Kwatra, unpublished meta-analysis). Factors contributing to this variation may include (i) geographic variability in prevalence of GBS colonisation, (ii) variability in density of colonisation with GBS, which could impact on infection of the foetus and newborn, (iii) geographic variability in colonising GBS serotypes, which have differing invasive potential (iv) differences in naturally-acquired protective serotype-specific capsular antibody levels and (v) time and method of swab collection and processing.

Other risk factors for GBS-EOD, including prematurity[25], prolonged rupture of membranes may also vary with geographic region. Practices which may impact the rate of colonisation, vertical transmission and detection of GBS-EOD may vary between global communities. Availability of over-the-counter antibiotics in many regions[86-89] is a global concern, as misuse of antibiotics leads to emergence of antibiotic resistance. Various reasons including customers' demands, complacency, insufficient knowledge and financial benefit motivate pharmacists to dispense antibiotics without prescriptions[87, 89]. Despite policies regarding sale of non-

prescription medicines including antibiotics being in place, adequate enforcement of regulations may be lacking. In 2010, enforcement of existing laws restricting the over-the-counter sale of antibiotics in Mexico and Brazil was strengthened. A decreased seasonal variation in penicillin use was noted in Mexico[90], and a 30% reduction in amoxicillin sales was noted in Brazil[91] after enforcement of policies, which may indicate more appropriate use of antibiotics. Traditional medicines, which are widely used in communities in Africa and Asia, may include medicinal plant extracts which have antimicrobial properties[92-94], which in turn may impact GBS colonisation and transmission rates.

Identification of GBS-EOD is challenging, especially in regions with limited health care facilities. The majority (70-74.8%) of GBS-EOD cases are identified within the first 12 hours of life, with up to 100% presenting in first 48 hours of life[95]. In areas with high rates of home deliveries, GBS-EOD cases may be missed, as has been illustrated by a study conducted in Bangladesh where more than half of the neonatal deaths occurred in the first 24 hours of life, and 62% of infants who died were not investigated for bacteraemia[67]. This potential detection bias in neonatal sepsis epidemiological studies was recently corroborated by the neonatal sepsis aetiology study in South Asia (ANISA), where GBS was seldom identified. Despite the intensity of community-based surveillance in ANISA, no blood samples were obtained from 80% of the neonatal deaths, 70% of whom died within 6 hours of birth (personal communication Samir Saha).

GBS remains the most common cause of bacterial meningitis in young infants in the USA (0.003/1000 live births; 95% CI: 0.0021 to 0.0031) [96] and the United Kingdom

(0.16/1000 live births, 95% CI: 0.13 to 0.18) [97], and one of the most common causes of sepsis in neonates and young infants globally[71, 77, 95, 98]. Between 60% and 70% of invasive GBS cases across all age groups, occur in infants <3 months of age[66]. Neurological sequelae are more common in 6-month old survivors of GBS invasive disease than in controls (aOR 13.18, 95% CI: 1.44 to 120.95). A greater proportion of infants who had GBS-meningitis (23.5%) than sepsis (9.8%) have neurological abnormalities[99].

The true incidence of GBS-EOD may, however, be under-represented by studies which only include positive sterile site cultures (definite GBS-EOD). Inclusion of newborns with clinical features of sepsis and a GBS-positive swab from a non-sterile site (deep ear/ pharynx) swab (probable GBS-EOD) at least doubled the GBS-attributable disease burden in the UK (1.1/1000 to 3.6/ 1000 live births)[100] and Spain (0.39/ 1000 to 0.86/1000 live births)[101]. GBS sepsis can be a rapidly-progressive disease, resulting in death in 9.6% (95% CI: 7.5 to 11.8) of cases; twice as high in early onset cases (CFR 12.1%; 95% CI: 6.2 to 18.3) than late-onset cases (6.8%; 95% CI: 10.8 to 14.9). The case fatality rate in LMIC was three times higher than in HIC (12.6% vs. 4.6%)[77].

Staphylococcus aureus (S. aureus) is a gram positive coccus which was first described in 1880 by Scottish surgeon, Alexander Ogston (1844-1929), who hypothesised that the round organisms he observed in pus from surgical wounds were the cause of post-surgical abscesses and putrefaction[102]. *S. aureus* is a common colonising bacterium with almost half of all newborns being colonised (skin, mucous membranes) within the first few days of life[103]. *S. aureus* produces biofilms

which assist the pathogen evade the immune system and prevents antibiotic penetration[40]. Cell wall-anchored proteins and membrane- damaging toxins aid *S. aureus* in adhering to and invading cells. A robust T-cell response, especially Th1 and Th17 which are attenuated in newborns, is required to protect against *S. aureus* infection[104].

S. aureus has been isolated from more than half (52%) of the purulent umbilical exudates in Pakistani newborns with omphalitis[105]. It is one of the main causes of bacteraemia in neonates and post-neonatal infants, especially LOD in very low birth weight (VLBW) infants. Mortality and morbidity related to *S. aureus* infections in young infants is significant. Mortality of up to 25% has been reported in VLBW infants, and neurodevelopmental impairment observed in 18-37% of surviving infants[103].

Escherichia coli (E. coli) was first isolated and characterised from an infant's stool sample by Theodor Escherich in 1885[106]. It is a gram negative rod-shaped bacterium which colonises the gastrointestinal system of humans and other warm-blooded animals[106], often being the first bacterium to colonise infantile gut soon after birth[107], either by vertical transmission from mother or by environmental acquisition from other infants, care-givers' hands or other fomites[108].

Enterotoxigenic *E. coli* is one of the 4 main causes of moderate to severe diarrhoea in children in LMIC, which leads to approximately 800 000 deaths annually[109]. *E. coli* is also the most common cause of urinary tract infections in neonates (40-72%) and older infants, who are at risk of concomitant *E. coli* meningitis and bacteraemia[110].

E. coli is the second most common cause of early-onset meningitis overall, and the most common cause of meningitis and early onset sepsis in very low birth weight infants[40, 49, 111].

More than half (n=43, 51%) of children under 18 years with *E. coli* bacteraemia were under 3 months of age, highlighting the burden of disease in this age group. Additionally, young infants were more likely to have severe bacteraemia than older children (32.6% vs. 7.3%, p=0.006)[112]. Infants under 90 days of age with *E.coli* bacteraemia had a 9.5% case-fatality rate[112], and *E. coli* meningitis is associated with a 14% mortality rate[113]. Of the surviving infants in a retrospective study conducted in France, 21% of infants had neurological sequelae at 8 months of age[113].Central venous thrombosis with subsequent neurological sequelae have also been associated with *E. coli* infections in neonates (meningitis or UTI)[114].

Klebsiella pneumoniae (K. pneumoniae) is a rod-shaped gram negative bacterium of the family Enterobacteriaceae which was first described by German microbiologist Carl Friedländer in 1882[115]. Although initially identified as a cause of community-acquired pneumonia (CAP), the incidence of *K. pneumoniae*-associated CAP has declined, however, it remains associated with HIV-infection in adults. *K. pneumoniae* bacterial liver abscesses and meningitis has been described in adults[116]. *Klebsiella* (species) has been recognised as an important (4% - 25%) cause of sepsis in young infants in HIC[64] and LMIC[70, 71]. Recently there has been an emergence of hypervirulent or antibiotic-resistant strains of *K. pneumoniae* globally[117, 118].

Several virulence factors have been well characterised in *K. pneumoniae*, including capsular factors, lipopolysaccharide, siderophores and fimbriae. These virulence

factors are largely defensive, allowing the bacteria to evade host immune responses[117].

Antibiotic resistance mechanisms include (i) the expression of extended spectrum beta-lactamases (ESBL) which leads to resistance to cephalosporins and monobactams and (ii) expression of carbapenemases, which leads to resistance to most ß-lactams, including carbapenems[117].

Neonatal intensive care units (NICUs) have reported an emergence in extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, most commonly *K. pneumoniae*. A recent review of ESBL-producing organisms causing outbreaks in NICUs[119] identified 75 studies, including 860 infections and 139 deaths (16% mortality rate). Outbreaks were most commonly triggered by admission of a colonised infant to NICU, however sub-optimal infection control measures, including poor hand washing or use of contaminated equipment, contributed to spread of bacteria to outbreak proportions[119].

There are three main carbapenemases produced by carbapenem-resistant *K. pneumoniae*: (i) *K. pneumoniae* carbapenemases (KPCs), (ii) carbapenemases of the oxacillinase-48 (OXA-48) and (iii) New Delhi metallo-ß-lactamase (NDM) carbapenemases. The geographic distribution of these antibiotic-resistant *K. pneumoniae* strains varies globally, however, all three strains have been reported in South Africa[118].

The multiple factors contributing to hypervirulence and antibiotic resistance of *K.*

pneumoniae have complicated the management of patients with *K. pneumoniae* infection, and useful antibiotics are now restricted to colistin, selected aminoglycosides, polymyxin B, fosfomycin and tigecycline[118].

1.2.2. **Challenges in identifying the burden of neonatal sepsis**

Lack of laboratory facilities and expertise in many LMIC settings impede the ability to confirm the presence and identification of invasive pathogens in septic neonates and post-neonatal infants[70, 71]. Sepsis has been described as the '*systemic inflammatory response (SIRS) to an active infectious process'* [51], which is manifest by physiological changes including but not limited to fever or hypothermia, tachypnoea, tachycardia and leucocytosis or leucopoenia in the presence of a proven or suspected infection[28, 51].

Lack of a standardised definition for sepsis in neonates and post-neonatal infants complicates the task of accurately reporting the disease burden, and reduces comparability of neonatal sepsis reports or study results[50, 120]. First-line health care workers' task of identifying severely ill infants who require referral for hospital admission or community-based treatment is also made more challenging by the lack of a globally-accepted definition[121].

Numerous definitions for neonatal sepsis have been proposed for use in varied settings including community-based health care settings[27] and hospital-based settings, which include combinations of clinical findings and/ or laboratory test results[122]. The sensitivity and specificity of laboratory tests (e.g. white cell count) and

clinical features (temperature- fever/ hypothermia) in predicting sepsis or its severity, varies between term and preterm neonates[122].

Several neonatal illness severity scoring systems are utilised, including the 'Score for Neonatal Acute Physiology (SNAP/ SNAP-PE [perinatal extension])[123], the Clinical Risk Index for Babies (CRIB/ CRIB-II)[124] and The Young infant Clinical Signs Study Group (YICSSG) for predicting severe illness in young infants[27].

Definitions for neonatal sepsis need to take into account the variations of laboratory and physiological features at different gestational ages, and different chronological age[125]. It has been proposed that a consensus definition of neonatal sepsis would assist in comparability of both observational studies and clinical trials[125], however, the dynamic nature of the disease requires a definition to allow for time lag between appearance and disappearance of numerous signs and symptoms[122]. Neonatal sepsis definition should include a scoring system to assess severity of sepsis and monitor improvement or deterioration in the neonate's condition[125]. Concerted efforts are being made to develop definitions which can be utilised (mainly in maternal immunisation field) in both well-resourced and poorly resourced settings[126, 127].

Possible serious (or severe) bacterial infection (pSBI) has been defined as 'a clinical syndrome used in the Integrated Management of Childhood Illness package referring to a sick young infant (<60 days old) who requires urgent referral to hospital'[128]. Signs used for the diagnosis of pSBI include inability to feed well, tachypnoea (>60 breaths per minute in neonates <7 days old), convulsions, severe chest indrawing, fever (≥38°C), hypothermia (<35.5°C) and movement only when stimulated[128].

Seale et al[129] conducted a systematic review and meta-analysis of pSBI in neonates born at ≥32 weeks gestation in LMICs, and estimated that there were 6.9 million cases of PSBI in 2012 (incidence risk 7.6%; 95% CI: 6.1-9.2%). Approximately 0.68 million neonatal deaths were attributable to PSBI (case fatality risk 9.8%).

In a more recent analysis of prospectively-collected data from 7 rural community-based Global Network sites in low and middle income countries (Argentina, Guatemala, Kenya, Zambia, India and Pakistan) of births occurring between 2010 and 2013[130], 12.9% (95% CI 12.8-13,0%) of all live-born infants fulfilled criteria for pSBI during the first 6 weeks of life. There was a wide variation in incidence (3% to 36%) across sites, suggesting possible over-reporting of respiratory symptoms, especially in preterm infants. The case fatality rate was 14% overall in pSBI cases, but varied widely between sites (5% to 42%), suggesting under-reporting of signs and symptoms of pSBI at some sites. Higher rates of pSBI reported in the Hibberd paper[130], compared to Seale's paper[129] were probably due to inclusion of all live-born infants, irrespective of gestational age, with preterm infants being more likely than term infants to have respiratory difficulty.

The WHO has published recommendations for identification and management of children with possible severe bacterial infections[131].Difficulties in confirming / diagnosing sepsis due to low yield of- or poor access to culture techniques, and lack of a standardised definition place health care providers under pressure to initiate antibiotics without confirmation of a causative pathogen, and diagnosis. This has led to overuse of empiric broad-spectrum antibiotics, which in turn increases antimicrobial resistant organisms.

1.2.3. **Antimicrobial Stewardship**

Discovery and use of antibiotics 8 decades ago revolutionised medical care. Pneumonia-related deaths decreased significantly, and complicated interventions including organ transplantation and cancer treatments became more acceptable due to decreased risk of infection[132]. Inappropriate use of antibiotics, including use of incorrect antibiotic, use of antibiotics when not required, and excessive duration of antibiotic use, leads to increased resistance patterns[40].

The increase in antimicrobial resistance of bacterial pathogens is a cause of great concern globally. In high-income countries, pathogens resistant to first-line antibiotics are treated with more expensive second- or third-line antibiotics; however, resistance to these antibiotics and the shortage of additional effective antibiotics entering the market will soon lead to spread to completely-resistant pathogens. In LMICs, resistance to first-line antibiotics may lead to an increase in preventable mortality, as cost of second- and third-line antibiotics is prohibitive and therefore not available to populations with highest burden of infectious diseases[132, 133]. The mechanism by which antibiotic resistance could emerge include when the pathogen develops one or more of the following adaptions: altered target site, antibiotic inactivation, reduced membrane permeability of efflux pumps[133].

Administration of antibiotics in pregnant women close to or during labour has been proven to decrease the incidence of GBS-associated early onset sepsis[134], however, it has also been associated with an increased risk of bacteraemia and urinary tract infections due to *E. coli* isolates with reduced antibiotic susceptibility profiles[110].The

WHO recommends ampicillin (or penicillin) and gentamicin for 10 days as first line treatment for neonatal sepsis. Cloxacillin is added if a *Staphylococcal* infection is suspected[131, 135], and a third generation cephalosporin is used to treat neonatal and infant sepsis in many countries[70]. Infants admitted to hospital, especially neonatal intensive care units, are high risk of infection with antibiotic resistant pathogens. Up to 95% of these infants will receive empiric antibiotics, despite low (<5%) culture-confirmed sepsis rates[133].

Some countries have implemented local antimicrobial stewardship guidelines. Public Health England has implemented a 'Start smart, then focus' strategy which guides health care providers on rapid implementation of antibiotic treatment only when a patient presents with clear evidence of infection, and review and continuation criteria for antimicrobial treatment[136]. Variations in antibiotic resistance patterns in different regions, and concerns about emerging multi-drug resistance needs to be considered carefully when recommending antibiotic treatment[137].

Simplified 7-day long regimens of combinations of oral and injectable antibiotics[138], or twice-daily oral antibiotics[139] have been found to be as effective in preventing treatment failure (including death, deterioration of symptoms or no improvement) as injectable procaine benzyl-penicillin-gentamicin in the treatment of possible serious bacterial infection (PSBI)[138] or tachypnoea[139] respectively in neonates and young infants in LMICs.

1.3. Strategies for prevention of sepsis in young infants

Sepsis in neonates and post-neonatal infants is multi-factorial, and there are several interventions, from basic to advanced care, which can reduce the risk of an infant from developing sepsis, and ultimately reduce burden of mortality in young infants.

1.3.1. **Clean birthing practices and skilled birth attendance**

Approximately 60 million newborns are delivered at home each year[140]. Unhygienic birthing practices often observed in home-based deliveries, places the newborn at substantial risk for infection due to exposure to opportunistic environmental pathogens. The major obstacles hindering increased rates of facility-based birth include cost charged by delivery centre, transportation challenges and large distances to birthing facility[141].

Traditional birth attendants (TBAs) are community members who provide care for 23%-40% of pregnant and labouring women in sub-Saharan Africa (SS-Africa) and south-east Asia (SE-Asia), despite having no formal medical training. Approximately half of the TBAs in SS-Africa and SE- Asia have received some basic training, including the importance of clean delivery and modern medical birthing techniques[142].

Skilled Birth Attendants (SBAs) are medically-trained health care providers (e.g. nurse, midwife, doctor) who are facility- or community-based, and can provide at least basic emergency obstetric care. Access to skilled birth attendance and care is

inversely proportional to the neonatal and maternal mortality rates; in areas of highest mortality, only 46% of women have access to SBA[143]. In South Africa, 84% of births are facility-based and attended by a skilled birth attendant; however, the disparity between rich and poor women is striking, with only 68% of women in the poorest quintile, compared to 98% of women in richest quintile having a SBA present at delivery in 2002[144].

1.3.2. **Umbilical cord care**

Traditional umbilical cord care methods are practised by many communities globally and are intended to lubricate brittle cords, accelerate detachment, or for non-specific medicinal or cultural reasons [145-149]. Cord care methods include covering the cord with cloth and applying substances including powders (made from a variety of ingredients including insects and their nests, fruit, mud, ash and herbs), liquid petroleum, breast milk, oil, butter and animal dung [145-149].

Omphalitis (umbilical infections) predisposes newborns to life-threatening sepsis. It occurs in up to 21.7% of community-based newborns in Pakistan, with *S. aureus* (52%), *Streptococcus pyogenes* (18%) and GBS (10%) being the most commonly isolated pathogens from purulent umbilical secretions[105].

Chlorhexidine is a widely available antiseptic solution, and is on the WHO essential drug list[150]. The effectiveness of chlorhexidine is related to the combination of its bacteriostatic and bactericidal effects[151]. Chlorhexidine is inexpensive, safe, stable,

acceptable to and compatible with health systems and simple to use[152]. Umbilical cord cleansing with a 4% solution of chlorhexidine has been proven to significantly reduce all-cause neonatal mortality (RR 0.81, 95% CI: 0.71 to 0.92) and omphalitis (RR 0.48, 95% CI: 0.4 to 0.57) in home-cared newborns when compared to dry cord care[153]. Culture-proven sepsis in neonates hospitalised in intensive care[154] in developing countries was reduced, however, minimal benefit has been observed when comparing chlorhexidine to dry cord care in hospital settings in HICs[155]. Chlorhexidine application has been associated with reduction [156] and lengthening[157] the cord separation time from the newborn. Hygienic cord care is one of six effective interventions recommended by the WHO for improving neonatal and child survival [158, 159]. The WHO recommended dry cord care in 1998[160], but amended this recommendation in 2013 to daily chlorhexidine cord cleansing for home-born neonates in settings with a high neonatal mortality rates[161].

Community members in many LMICs recognise the vulnerability of the newborn to infection of the umbilicus (omphalitis) or related to unhygienic umbilical cord care, and the potential harmfulness to the newborn of applying various substances to the cord stump [145-147]. Widespread behavioural change to reduce the use of traditional cord care methods and increase the use of chlorhexidine cord cleansing will, however, require extensive training of medical personnel, health care workers and community members especially if cord separation time is delayed compared to other cord care methods [145-149].

1.3.3. **Peripartum Maternal vaginal and infant skin disinfection**

Disinfection of the birth canal during labour using chlorhexidine intravaginal washes

and cleansing of the newborn skin immediately after birth were proposed as an inexpensive and simple method of reducing sepsis in neonates[162-165]. These interventions could be implemented relatively easily into clean birth practice kits, which could be used in facility as well has home-based deliveries[166]. Pathogens causing early onset sepsis are usually acquired by vertical transmission from mother to foetus/ newborn, just prior to or during the birth process[48]. Cleansing of the vagina during labour could therefore potentially reduce the exposure that the foetus/ newborn has to pathogenic organisms, therefore reducing vertically-acquired sepsis. Chlorhexidine is a suitable product for this purpose, as it rapidly reduces urethral and vaginal organism counts within an hour of application, and the effect is sustained for many hours[162].

Numerous clinical trials conducted in hospital-delivery settings have examined the impact of intrapartum vaginal chlorhexidine disinfection with or without newborn wipes on maternal endpoints including perinatal sepsis and morbidity, vertical transmission of colonisation with pathogenic and neonatal outcomes including rates of admission, infection and death. Trials completed and published prior to 2008 have been summarised in Table 1. Results from trials published after 2008 will be discussed in a later chapter.

A trial conducted in Sweden[167] included 4483 women randomised at 10 hospitals and showed a significant decrease in the primary end-point of neonatal admission in newborns of women who received the chlorhexidine washes, however, these results have been criticised as the criteria for neonatal admission were not standardised across the participating hospitals, which could have introduced bias[168]. Although

some trials demonstrated an impact on vertical transmission of GBS[169, 170], other did not corroborate those results[171, 172]. The impact of chlorhexidine intrapartum vaginal wipes on infection in the mothers has been inconclusive (Table 1).

Only two of these trials were performed in LMICs, both in Africa (Malawi and Egypt)[173, 174]. In comparison to the other trials summarised in table 1 which were randomised controlled, blinded trials, the trials conducted in Malawi and Egypt were non-randomised, and included period of intervention administration and periods of no intervention (control). Both of these trials used manual chlorhexidine vaginal wipes during labour, and a surface wipe of the newborn, which also differed from the trials conducted in Europe and the USA, which had utilised chlorhexidine vaginal irrigation/ washes[170, 172, 175-178], gel[169] or cream lubricant[171] .

The two African studies[173, 174] showed a significant reduction in neonatal and maternal morbidity associated with sepsis, as well as neonatal mortality. The concentration of chlorhexidine used, dosing interval, or application method may have improved the protective effect of the intervention. These trials, however, had several limitations including being non-randomised design, with periods of intervention and no intervention, resulting in the differences in outcome observed between chlorhexidine and control groups not be cleanly attributable to the intervention. Additionally, neonatal sepsis and peri-partum infection were defined based on subjectively determined clinical criteria, and were diagnosed by individuals who could not be effectively blinded as to what treatment the subjects received. Due to these limitations, the results of these two trials were not considered sufficient demonstration of effectiveness and widespread recommendation or implementation of the intervention was hindered. Several reviews on chlorhexidine maternal vaginal

and newborn skin cleansing[179-182] concluded that a randomised controlled trial, preferably performed in a LMIC, would be required prior to the intervention being accepted globally.

Part of the body of work for this thesis was the conduct of a randomised controlled trial of chlorhexidine maternal vaginal and infant skin wipes, the results of which will be discussed in later chapters.

Table 1: Summary of chlorhexidine vaginal intrapartum disinfection trials completed prior to 2008

Study	Country	Outcome aims	Study design	Participants	Intervention during labour	Outcome
Bakr AF, Karkour T. J Womens Health (Larchmt). 2005[174]	Egypt, hospital	To determine if cleansing birth canal with antiseptic at delivery reduces infections in mother and baby	Non-randomised Intervention/ non-intervention phases	Women in labour. N=4415	0.25% CHX-soaked wipes at each vaginal examination and newborn wipe	**Neonatal admissions**: no difference (13.3% vs 13.8%, p=0.63) **Neonatal Infection-related admissions**: Reduced in CHX vs. control (0.65% vs. 1.9%, p=0.0002) **Total neonatal Deaths**: Reduced in CHX vs. control (2.8% vs. 4.2%, p=0.01) **Infection-related neonatal deaths**: Reduced in CHX vs. control (0.22% vs. 0.84%, p=0.004) **Maternal admissions**: Reduced in CHX vs. control (3.1% vs. 5.1%, p=0.0008)
Rouse DJ, et al. Am J Obstet Gynecol. 2003[175]	USA, hospital	To determine whether intrapartum CHX vaginal irrigations prevented peripartum infection in nulliparous women ≥32 weeks	RCT	Labouring women, 6-hourly irrigations. N= 1041 (525 CHX, 516 placebo)	0.2% (200ml) CHX, vaginal irrigations vs. normal saline irrigations	**Peripartum infection**: 19.3% in CHX vs. 17.3% in control (RR 1.1; 95% CI: 0.9-1.4)
Facchinetti, et al. J Matern Fetal Neonatal Med. 2002[172]	Italy, hospital	To investigate intrapartum CHX vaginal flushings vs. intravenous ampicillin in preventing GBS transmission in neonates	RCT	GBS-colonised women at term. singleton, vaginal deliveries N=244.	0.2% (140ml) CHX solution vaginal flushings, 6-hourly. 2g IVI ampicillin 6-hourly.	**GBS Neonatal colonisation**: No change noted. (CHX 15.6%, Ampicillin 12%). ***E.coli* colonisation** reduced in CHX (1.8%) vs. ampicillin group (7.4%, p<0.05)
Stray-Pedersen B, et al. Int J Antimicrob Agents. 1999[170]	Norway, hospital	Determine whether CHX douche during labour reduced vertical transmission of vaginal micro-organisms and infectious morbidity in neonates and mothers	RCT	Women in labour, normal vaginal deliveries N=1130	0.2% (120ml) CHX vs. sterile saline Vaginal douching, 6-hourly	**VT colonisation**: Reduced in CHX vs. control (18% vs. 35%, p<0.0001) **VT GBS-colonisation**: Reduced in CHX vs. control (11% vs. 32%, p<0.05) **Neonatal infections**: Reduced in CHX vs. control (4.9% vs. 7.9%, p<0.05) **Maternal post-partum UTI:** Reduced in CHX vs. control (3.4% vs. 6.9%, p<0.05) **Maternal fever**: Reduced in CHX vs. control (3.3% vs. 6.6%, p<0.05)

Study	Country	Outcome aims	Study design	Participants	Intervention during labour	Outcome
Taha TE, et al. BMJ 1997[173]	Malawi, hospital	To determine if CHX vaginal cleansing reduces infections in mothers and newborns	Non-randomised clinical trial, 2 months no intervention, 3 months intervention, 1 month no intervention	Women in labour and their infants: Intervention: n=3635 women & 3743 newborns; Non-intervention: N=3330 women & 3417 newborns	0.25% CHX solution wipe at each vaginal examination (4 hourly) and newborn wipe	**Neonatal admissions:** Reduced in CHX vs control (16.9% vs. 19.3%, p<0.01), **Neonatal sepsis** admissions Reduced in CHX vs control (7.8% vs 17.9%, p<0.0002), **Neonatal mortality** Reduced in CHX vs control (28.6 vs 36.9/ 1000 LB, p<0.06), **Infection-related neonatal mortality** Reduced in CHX vs control (2.4 vs. 7.3/ 1000 LB, p<0.02), **Postpartum maternal infections**: Reduced in CHX vs control (1.7 vs. 5.1/1000, p=0.02)
Rouse DJ, et al. Am J Obstet Gynecol. 1997[176]	USA, hospital	To determine whether CHX vaginal irrigation prevents maternal peripartum infection (chorioamnionitis and endometritis)	RCT	Women in labour N=1024 (508 in CHX, 516 in placebo)	0.2% (200ml) CHX irrigations vs sterile water	**Maternal infection**: No difference observed (10% vs. 13%, RR 0.8, 95% CI: 0.5 to 1.1) in CHX vs. control
Sweeten KM, et al. Am J Obstet Gynecol. 1997[177] AND Eriksen NL, et al. Infect Dis Obstet Gynecol. 1997[178]	USA, hospital	To determine whether a single CHX intrapartum vaginal wash can reduce intra-amniotic infections in women, and neonatal infections and antibiotic use	RCT	Women in term labour. N=947 (481 CHX, 466 control)	0.4% (20ml) CHX vs. 20ml sterile water	**Intra-amniotic maternal infection:** No difference between CHX and control 5.2% vs. 4.5%, (95% CI: 0.82-1.14, p=0.65); **Endometritis:** No difference between CHX and control: 1.9% vs. 1.9% (95% CI: 0.62-1.56, p=1.0) **Neonatal antibiotic use:** No difference between CHX and control (3.2% vs. 1.9%, 95% CI: 0.72-3.72, p=0.32)
Adriaanse AH, et al. Eur J Obstet Gynecol Reprod Biol. 1995[169]	The Netherlands, hospital	To evaluate the effect of CHX gel during labour to reduce vertical transmission of GBS, maternal infectious morbidity and neonatal sepsis	RCT	Women in labour N=1020	0.3% (10ml) CHX gel vs. placebo gel (10ml), vs. no intervention. Repeated after 10 hours	**VT of GBS**: 52.4% vs. 71.4% vs. 66.7% (p=0.069) in CHX vs. placebo vs. control groups. **VT of GBS**: CHX vs. combined: 52.4% vs. 69.3% (p=0.026) **VT other bacteria**- not significant **Neonatal sepsis, antibiotic therapy, duration of hospitalisation**: no difference **Maternal morbidity** (GBS+ moms): no difference

Study	Country	Outcome aims	Study design	Participants	Intervention during labour	Outcome
Hennequin Y, et al. Acta Obstet Gynecol Scand. 1995[171]	Belgium, hospital	To assess effect on vertical transmission of GBS by CHX cream during per vaginal examinations	RCT	GBS-colonised women in labour N=59	1% CHX cream to coat gloves for routine vaginal examination vs. uncoated gloves.	**VT of GBS colonisation: No difference between CHX and control.** (39% vs. 42%, not significant)
Burman LG, et al. Lancet 1992[167]	Sweden, hospital	To determine effect on neonatal disease due to vertical transmission of GBS	RCT	Women in term labour, singleton pregnancies N=4483	0.2% (60ml) CHX vaginal flushing 6-hourly Vs. saline placebo	**Overall Neonatal admissions**: Reduced in CHX vs. control (2% vs. 2.9%, p=0.04) **Neonatal Infection:** Reduced in CHX vs. control (<0.1% vs. 0.3%, p=0.03) **Neonatal infection among infants of GBS+ moms**: Reduced in CHX vs. control (1% vs. 3.2%, p=0.04)

CHX- Chlorhexidine

RCT: Randomised controlled trial

1.3.4. **Risk based management for neonatal sepsis**

Certain factors including prolonged rupture of membranes (>18 hours), preterm labour and signs and symptoms of chorioamnionitis (maternal fever, uterine tenderness) have been proven to place newborn infants at significant risk of developing neonatal sepsis[40, 46]. GBS bacteriuria (indicating heavy colonisation) and history of a previous GBS-infected baby also increase the risk of GBS-EOD in newborns of affected women[183]. In the UK, 67% of neonates with GBS-EOD were exposed to at least one of these risk factors, compared to 17% of neonates without GBS-EOD[80].

Risk-based management of pregnant women with any of these factors is recommended and practised in some high-income countries, including the United Kingdom[184], many European countries[185] and New Zealand[186], and when possible, in LMICs[187]. Intravenous benzyl penicillin (or amoxicillin) is recommended 4-hourly during labour. Cephazolin or vancomycin can be used in penicillin-allergic women[184, 186]. Implementation of a nation-wide single risk-based management policy of pregnant women in New Zealand led to a 54% reduction (0.5/1000 to 0.23/1000 live births) in GBS-EOD[188].

1.3.5. **Intrapartum antibiotic prophylaxis**

GBS, which asymptomatically colonises the genital and gastrointestinal tract of 10-30% of adults[189-191], poses a significant risk of sepsis to foetuses and newborns, and is a pre-requisite for GBS-EOD[66]. Recto-vaginal colonisation with GBS in South African women changes over time during the last half of pregnancy, range between 28.7% and 33% at any one time point, but cumulatively 49.7% of women were colonised at one or more time points between 20 and 39 weeks gestation[192]. Almost 26% of women, who were not colonised at 20-25 weeks, acquired GBS colonisation before the end of their pregnancy, and the mean duration of colonisation was 6.4 weeks[192].

During pregnancy, GBS can ascend into the uterine cavity, and invade the amniotic fluid, leading to colonisation or infection of the foetus[47, 193]. Vertical transmission of colonising GBS isolates to the infant is dependent on intensity of colonisation of mother[194]. Guidelines for the prevention of perinatal GBS disease were first recommended by the Centers for Disease Control and Prevention (CDC)[195], the American Academy of Pediatrics[196] and American College of Obstetricians and Gynecologists in 1996. These guidelines were revised in 2002[197] and again in 2010[183], and have been proven to be cost effective[198, 199] in reducing GBS-EOD in the USA[134, 200]. Intrapartum Antibiotic Prophylaxis (IAP) must be administered to the mother at least 2 hours prior to delivery to reduce vertical transmission of GBS to the foetus/ newborn[201].

The revised US CDC guidelines[183] now include identification of pregnant women who

are GBS-colonised in late pregnancy (35-37 weeks gestation) by means of rectovaginal swab collection and processing, and the administration of intravenous antibiotics during labour; commonly known as intrapartum antibiotic prophylaxis (IAP). This strategy has led to a 76.5% in reduction of GBS-EOD cases, however, has had no significant impact on GBS-LOD incidence[202].

In a retrospective cohort study conducted in USA, GBS-EOD was significantly lower (adjusted relative risk 0.46; 95% CI: 0.36 to 0.60) in newborns whose mothers received IAP (0.33/ 1000 live births) compared to mothers who were managed by the risk-based approach (0.59/ 1000 live births)[203]. Australia implemented universal screening for GBS colonisation during pregnancy and IAP, which resulted in 30-35% of women receiving intrapartum antibiotics, compared to only 15-20% being eligible to received IAP if a risk-based approach was used[204]. The cost and logistics required to implement IAP are, however, extensive and different countries have developed their own recommendations regarding the implementation of IAP based on local GBS colonisation data, the incidence of GBS-EOD and cost effectiveness studies[204]. Additionally, concerns about increasing antibiotic resistance patterns in bacteria commonly associated with sepsis in newborns including *E. coli* and coagulase-negative staphylococcus, and immunological implications including the potential disruption of the development of the infant's intestinal microbiome that IAP may have on mothers and newborns has prevented global implementation of IAP[204].

Logistical challenges to IAP in LMIC include that both IAP and risk-based management strategies require births to take place at health care facilities, at which intravenous antibiotics are available, making implementation of these strategies impractical in many LMICs. Furthermore, even in settings where universal screening

and IAP and risk-based strategies for prevention of GBS-disease have implemented, GBS remains a significant cause of sepsis in young infants[77, 96].

1.3.6. **Vaccination of pregnant women**

The experience in reducing neonatal tetanus-related deaths from 787,000 in 1988 to 49,000 in 2013 is partly due to targeted tetanus-toxoid vaccination of pregnant women in low-income settings. This highlights the potential of vaccinating pregnant women for the control of infectious diseases in their neonates[205]. Additionally, recent studies have proven that immunisation of pregnant women with influenza vaccine[206] and acellular pertussis vaccine[207] are effective in reducing disease in their newborns and young infants.

Seasonal Influenza vaccination is considered a priority vaccination for pregnant women, with the WHO recommending vaccination with trivalent inactivated influenza vaccine at any stage of pregnancy[208]. This recommendation has been implemented by many countries including South Africa[209].

Administration of pertussis-containing vaccines during pregnancy is probably the most cost-effective strategy for preventing disease in young infants[210], and some countries, including the USA[211], UK[212] and Australia[213] recommend pertussis vaccination of pregnant women. Pertussis vaccination of pregnant women has not been implemented in South Africa.

Vaccine candidates for use in pregnant women to protect their newborn infants are under development for several diseases including GBS [66, 214] and respiratory syncytial virus[215]. The unique situation of vaccinating a woman to protect her offspring generates challenging situations including timing of vaccination to maximise benefit to foetus/ newborn[66], which endpoints should be considered (e.g. maternal or infant)[66] and how they should be reported[127]. Clinical trials assessing efficacy and immunology endpoints for vaccination of pregnant women need to be conducted in sites where disease burden surveillance is adequate and burden is high, but access to other prophylactic strategies is limited (usually LMIC)[214]. In order to ensure high quality data which could be utilised for global licensure, study sites need to have the capacity and experience to adhere to stringent ethical and Good Clinical Practice (GCP) guidelines and be able to identify adverse events in maternal participant and infants[214, 216].

Maternal immunisation for reduction of disease in mothers and infants is promising, but not without its challenges. Insurers are often reluctant to provide insurance for trials involving pregnant women[66]. Regulatory authorities, which are mandated to ensure safety and consider efficacy of vaccine for both the maternal vaccine recipient and the developing foetus/ newborn, are often perceived to hinder development of vaccines for pregnant women[217]. Engagement with regulators and policy makers in countries in which vaccines are most needed is required early in the development pathway to ensure timeous registration and implementation of vaccines once licensure and WHO prequalification are complete. Development of vaccines which are affordable to countries with the highest burden of disease (LMICs) is essential to ensure success of maternal immunisation programs[216].

Justification and objectives

Sepsis is one of the three main causes of mortality (together with intrapartum complications and prematurity) in neonates and post-neonatal infants. There is, however, a paucity of data on the aetiology of neonatal sepsis, especially in LMICs where disease burden is highest. Background data on the bacterial aetiology, incidence rates and antimicrobial resistance patterns of pathogens causing sepsis in young infants is important to enable health care providers plan and implement preventative strategies, and choose the most appropriate antibiotics for management, as determined by locally-acquired data.

Identification of risk factors for sepsis-related neonatal and post-neonatal morbidity and mortality allows health care providers to implement appropriate preventative and treatment measures. Maternal HIV-infection is known to lead to reduced transplacental antibody transfer, however, the impact that HIV-exposure has on the burden of bacterial sepsis in neonates and post-neonatal young infants has not been well described.

Cost-effective interventions are required for the prevention of sepsis in young infants, especially in LMICs where access to clean birthing facilities and skilled birth attendants is often limited. However, inadequately designed or conducted trials to assess efficacy in reducing neonatal and maternal sepsis and mortality has hampered global acceptance and implementation of some promising interventions such as chlorhexidine maternal vaginal- and newborn skin cleansing.

South Africa, despite high rates of facility-based deliveries and free access to

reasonable health care for pregnant women and children, has a high neonatal mortality rate. High HIV-prevalence in pregnant women combined with inadequate access (until recently) to antiretroviral therapy also contributed to the country's inability to reduce child mortality rates adequately to achieve the country's MDG4 target. The overall aim of this research was to describe the impact of maternal HIV-infection on the aetiology and risk factors of neonatal sepsis, and to evaluate an inexpensive intrapartum interventional strategy for reduction of neonatal sepsis.

The specific objectives of this project were:

1. To describe the aetiology and clinical spectrum of culture-confirmed invasive bacterial disease in neonates and post-neonatal young infants and the effect of maternal HIV-infection on disease incidence.
2. To describe maternal and neonatal factors associated with very early onset neonatal sepsis, late-onset neonatal sepsis and perinatal death in HIV-exposed and HIV-unexposed neonates (paper I).
3. To assess the impact of maternal HIV infection on (i) the prevalence of maternal vaginal colonisation with potentially pathogenic bacteria in neonates, (ii) vertical transmission of bacterial pathogens to newborns and (iii) sepsis rates during the very early and late neonatal periods (Paper II).
4. To describe the clinical and microbiological epidemiology, incidence and serotype distribution of invasive GBS disease in young infants in a setting with high prevalence of maternal HIV (paper III)

5. To determine the efficacy of intrapartum vaginal wipes plus neonatal body wipes with 0.5% chlorhexidine in reducing neonatal sepsis (culture-confirmed and clinically defined sepsis) and vertical transmission of GBS (Paper IV).

The body of work contributing to this thesis is divided into two distinct parts. The study design most appropriate to the specific research question was selected for each section of this work and is detailed in this thesis. The Human Research Ethics Committee (Medical) of the University of The Witwatersrand approved this PhD protocol (HREC number M150501, Appendix 1).

2. MATERIALS AND METHODS

2.1. Study Population

South Africa has a high rate of morbidity and mortality in neonates and post-neonatal infants. We have been unable to achieve MDG4, which 10 other Sub-Saharan countries with lower GDPs have managed. Despite 84% of all births taking place in health care facilities in South Africa, the perinatal mortality rate is high. There is great disparity between accessibility and quality of health care available to poor and rich South Africans. More than 85% of the country's population accesses public health care facilities including community health clinics, district-, regional- and provincial tertiary hospitals, however, only 40% of the total health care expenditure is allocated to these facilities[144]. South Africa's maternity services have been reviewed and compared to acceptable norms (by population number) for the number and level of facilities available, staff allocation, and minimum number of deliveries annually. Despite an acceptable number of staff being allocated to maternity services, there are numerous facilities which are understaffed and other facilities do not have enough deliveries to make them cost effective to operate[218].

Approximately 60% of births take place at primary care facilities including community health centers (CHC) and district hospitals[218, 219]. A survey of maternity care facilities in South Africa highlighted inadequate levels of care available, even at district hospital level. Complete Basic Emergency Obstetric and Neonatal Care (BEmONC) could not be conducted completely (fulfilling all 7 signal functions) at any of the 53 surveyed CHCs. Assisted vaginal deliveries could only be conducted by 2% of CHCs, and parenteral antibiotics were not available at 68% of CHCs. Almost a

quarter of district hospitals surveyed were unable to perform caesarean section deliveries due to a lack of doctors or non-functioning theatres[219].

Soweto was established in 1905 as a black African township, and to this day, remains an urban area of predominantly black-African residents. Tapped water is accessible to 98.2% of residents and electricity to 87.4%[220].The majority of the residents fall into low and low-middle socio-economic brackets, and very few Soweto residents have private medical insurance. Only 10.2% of South African children under-5 year of age, and 19.0% of children in Gauteng, are covered by private medical insurance.

Chris Hani-Baragwanath Academic Hospital (CHBAH) is a 3200-bed secondary-tertiary government-run hospital located in Soweto, Johannesburg, South Africa. CHBAH serves the 1.2-1.5 million people living in Soweto[220].

All maternal and child health at government institutions including CHBAH and primary health care facilities, is offered free-of-charge. The birth cohort of Soweto is approximately 30 000 per annum, three quarters (~22 500) of these births take place at CHBAH, and the remaining ~7500 births take place at one of 5 midwife obstetric units (MOUs), which are located at primary health care clinics in Soweto. Planned home-deliveries in Soweto are rare, and most births in Soweto (95.0%, Personal communication, EJ Buchmann) and in South Africa (84% to 87.3%)[144, 221] occur in a health care facility.

Approximately 35% of all deliveries that take place at CHBAH are caesarean sections, and all deliveries at MOUs are normal vaginal deliveries (i.e. 26.3% of all Soweto deliveries are caesarean deliveries). Healthy newborns are routinely

discharged home 12 hours post vaginal-, or 72 hours post caesarian delivery at CHBAH. Until mid-2014, CHBAH was the only government medical facility offering neonatal and paediatric in-patient care in Soweto.

Antenatal screening for GBS recto-vaginal colonisation during pregnancy with intrapartum antibiotic prophylaxis (IAP) for GBS-colonised women are not standard-of-care in South Africa. A maternal risk factor-based strategy is advocated at birthing facilities including CHBAH, for the management of prolonged rupture of membranes ($\geq$18 h), chorioamnionitis, a subset of preterm deliveries (26-33 weeks gestation), and pre-labour rupture of membranes. Prior to 2007, CHBAH Department of Obstetrics IAP guidelines recommended ampicillin 1g IV 6 hourly and metronidazole 400mg orally 3 times daily for suspected chorioamnionitis and prolonged rupture of membranes. In January 2007, targeted risk-based IAP for possible GBS infection (including women with preterm labour, previous GBS-affected infant or positive culture) was implemented, including an initial dose of intravenous ampicillin (2g), followed by 4-hourly intravenous ampicillin (1g) until delivery.

South Africa has a high HIV prevalence, the majority of which is spread heterosexually[222]. Risk factors for HIV-infection in antenatal attendees include older male partners, especially in women <20 years, economic dependence on partners, high-risk alcohol consumption and inconsistent condom use[223]. Prevention of Mother-to-child transmission of HIV (PMTCT) pilot sites were established in South Africa in 2001 by the National Department of Health. Neither of the two pilot sites in Gauteng were based in Soweto[224].

In July 2002, the Constitutional Court of South Africa ruled that HIV-positive pregnant woman had a constitutional right to access health care services to prevent mother to child transmission of HIV[225]. In 2001, PMTCT programs were established with non-governmental funding at CHBAH and 13 antenatal clinics in Soweto by the Perinatal HIV Research Unit (www.phru.co.za). Subsequent to the Constitutional court ruling in 2002, the Department of Health took over the responsibility of offering all pregnant women routine voluntary counselling and testing for HIV in antenatal clinics. More than 96.0% of pregnant women accept voluntary counselling and testing for HIV during antenatal visits (C. Mnyani, personal communication 28th July 2014). The HIV prevalence in pregnant women during the period in which the studies contributing to this thesis were performed (2004- 2008) remained stable at approximately 30%[226, 227]. The standard of care for prevention of mother-to-child transmission of HIV was amended in 2007 from single dose nevirapine administered to HIV-infected women at onset of labour and to their newborn infants, to triple antiretroviral therapy (stavudine, lamivudine and nevirapine) from 34 weeks gestation to women with a CD4+ count of less than 350cells/mm^3 [14].

Newborns presenting with signs and symptoms of severe illness at birth or prior to discharge from the postnatal wards are admitted to the neonatal unit at CHBAH, whereas infants discharged home after delivery and who subsequently present with suspected bacterial infections are hospitalized in the general pediatric wards at CHBAH. Investigation and management of neonates and young infants presenting with suspected invasive bacterial disease is conducted by attending physicians as per standard-of-care. This included complete blood count and blood culture in all infants. Cerebrospinal fluid tap for biochemistry, microscopy, culture and sensitivity is limited to infants with positive blood

cultures presenting at birth and all infants with suspected sepsis if admitted from the community. Sterile site cultures are processed at the National Health Laboratory Service (NHLS), with blood culture evaluated using the BacT/Alert microbial system (Organon Teknika, Durham, NC). Empiric treatment of neonates with suspected sepsis during the study period was intravenous penicillin and gentamicin, or ampicillin and gentamicin in infants between 1 and 12 months of age, whilst suspected meningitis cases were treated empirically with ampicillin and cefotaxime.

2.2. Study design and Methods: Sepsis surveillance study

2.2.1. **Study design and enrolment**

A bacterial surveillance system was established at CHBAH in the neonatal and paediatric wards in collaboration with the staff in the Departments of Paediatrics and Microbiology to determine incidence, aetiology and clinical spectrum of culture-confirmed sepsis in young infants and the impact of maternal HIV-infection. Pathogenic bacteria isolated from sterile site samples (blood, cerebrospinal fluid) collected by attending physicians during admission of young infants (≤90 days old), were identified by surveillance and review of clinical and microbiology records at CHBAH between January 2004 and December 2008.

Pathogenic bacteria were defined as organisms which are capable of causing disease, and are uncommonly considered to being contaminants. Bacterial contaminants included, but were not limited to, the following: *Bacillus* species, *Micrococcus*, *Propionibacterium* species, *Corynebacterium* species and Coagulase

negative *Staphylococcus* unless identified from 2 or more cultures obtained at separate times. Additional isolates which were potential contaminants were evaluated for clinical significance by the investigational team based on associated clinical and laboratory signs.

Young infants with GBS were identified through daily screening of admissions to neonatal and paediatric wards and microbiological reports. Additionally, all GBS isolates from sterile sites of any patient at CHBAH were kept aside by microbiology staff for collection by RMPRU staff. Young infants with pathogenic bacteria other than GBS were identified predominantly retrospectively through the surveillance system of microbiological records.

The parents or legal guardians of prospectively-enrolled infants were informed about the surveillance study, and signed written, informed consent forms. If the infant had died or been discharged prior to identification in the wards and obtaining parental consent, data were collected retrospectively. An audit of the NHLS database of all invasive pathogens isolated from paediatric patients over the study period was conducted to ensure that all patients were identified.Study investigators obtained information about each patient identified as having an invasive bacterial infection from their medical records and microbiological reports.

2.2.2. **Routine Blood Culture Sample collection and processing**

Blood samples for culture obtained by attending physicians were placed into BacT/Alert microbial system (Organon Teknika, Durham, NC, USA) paediatric blood

culture bottles and evaluated by the microbiology department of the National Health Laboratory Services (NHLS) at CHBAH. Cerebrospinal fluid (CSF) samples were obtained from infants admitted from the community with suspected sepsis, and from infants identified to have GBS-bacteraemia from blood sample collected at birth. CSF sample analysis included tests in clinical chemistry, haematology and microbiology as per routine practice by the NHLS. GBS isolates from blood or CSF were plated onto on blood agar plates by NHLS staff and were kept aside for collection by RMPRU staff. Pure cultures of these GBS isolates were stored at -70°C in RMPRU laboratory and serotyped in batches by latex agglutination as decribed[228]. Serotyping of GBS is not performed routinely by NHLS.

Antimicrobial susceptibility testing of isolated bacteria is performed routinely by the NHLS Department of Microbiology. A Gram stain is performed on samples from BacT/Alert blood culture bottles which display positive results, and on pathogens isolated from CSF samples. The results of the Gram stain guide the decision of which antimicrobial profile to select for susceptibility testing.

The Kirby-Bauer disk diffusion susceptibility test method is used in NHLS. Pathogenic organisms identified from blood or CSF cultures are grown on Mueller-Hinton agar, in the presence of antimicrobial-infused filter paper disks. A zone of absence of growth around the disk indicates inhibition of the organism by the specific antimicrobial. An automated Microscan Microbiology System (Beckman Coulter, previously Siemens) is also utilised for both the identification of the organism and the susceptibility testing using relevant panels in cases where the Kirby-Bauer method has not been utilised. Etest® strips are used to supplement the Kirby-Bauer and

Microscan results for certain pathogens and antibiotics (e.g. Vancomycin Etest® used for Vancomycin-resistant Enterococci and methicillin resistant *Staphylococcus aureus*.)

Minimum inhibitory Concentration (MIC) of antimicrobials is reported according to the Clinical Laboratory Standards Institute (CLSI) standards (www.clsi.org).

Invasive disease was categorized as bacteraemia if identified in blood only or as meningitis if (i) identified from CSF or (ii) there was CSF cytological evidence of purulent meningitis (>5 leucocytes/mm^3, adjusted in traumatic lumbar punctures to allow 1 leucocyte per 500 erythrocytes) in an infant with bacteremia.

2.2.3. **Data collection and analysis**

Data, including demographic and admission information, were abstracted from medical records of infants admitted to the neonatal or paediatric wards of CHBAH and entered onto paper case report forms (CRFs) by study doctors. Delivery information and maternal HIV-infection status was collected when possible from neonatal admission records, maternal delivery notes, or from the mother herself. HIV exposure was determined by abstracting antenatal HIV test results of mothers and supplemented by HIV ELISA results from maternal or infant blood tests conducted by attending physicians. The HIV infection status of HIV-exposed infants was determined using qualitative HIV PCR tests at the discretion of the attending physician. Gestational age assessment was based on the available obstetric details (last menstrual period date, ultrasound, serial symphysis- fundal height) or neonatal assessment by the attending physicians.

Data were entered into a customised Microsoft access database. Data on live births in Soweto were obtained from CHBAH and community clinics, and antenatal survey HIV-prevalence data were utilised to estimate denominators for HIV-infected and HIV-uninfected women.

Data were analysed using STATA/ IC 13.0 (Statacorp, College Station, TX, USA), and details of analyses are included in the methods sections of following chapters.

2.2.4. **Ethics**

The surveillance study was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC) on 2nd November 2003 (HREC references M03-10-07 and M100367, appendix 2) and the institutional review board of the Centers for Disease Control and Prevention, Atlanta, USA (protocol # 4128).

Written informed consent was obtained from a parent (usually mother) of infants enrolled prospectively into the surveillance study. If a patient was only identified by study staff after demise or discharge, the HREC waivered consent requirement, and data and GBS isolate (if relevant) were collected retrospectively.

2.2.5. **Funding**

The surveillance study was funded by the Centers for Disease Control and Prevention (Cooperative Agreement number: U50/CCU02196 and U01 CI000318)) and the Bill and Melinda Gates Foundation (Grant number 39415).

2.3. Study design and Methods: Prevention of Perinatal Sepsis (PoPS)

2.3.1. **Trial design**

The Prevention of Perinatal Sepsis (PoPS) trial was a blinded, randomised controlled trial conducted at CHBAH between 1st April 2004 and 25th October 2007 to evaluate the efficacy of a chlorhexidine intrapartum intervention in reducing neonatal and maternal sepsis. A nested cohort of participants was enrolled to evaluate vertical transmission of pathogenic bacteria, including GBS, *E. coli* and *K. pneumoniae*. Promising results from previous trials assessing this intervention[173, 174] did not culminate in the implementation of the intervention as the investigators had not utilised a randomised controlled trial design, which downgraded the quality of the evidence.

Study staff had no role in patient management. Study midwives administering the intervention were unblinded. The rest of the study team, including those collecting endpoint data, were blinded to intervention group. Laboratory staff was additionally blinded to mother-neonate pairings.

2.3.2. **Inclusion and exclusion criteria**

All pregnant women attending antenatal clinic at the participating antenatal clinic or presenting to the CHBAH labour admissions ward were eligible to register for the study if they met all of the following inclusion and none of the exclusion criteria:

Inclusion criteria for enrolment

- Planned to deliver at CHBAH or one of its satellite clinics
- Planned to remain in Soweto for at least two months after delivery
- Was able to understand and give written informed consent
- Was at least 15 years old

Exclusion criteria for enrolment

- Was expected to deliver by caesarean section
- Had antenatal ultrasound examination revealing major fetal congenital anomalies
- Had known or suspected condition in which vaginal exams are contraindicated, e.g. placenta praevia
- Had a history of allergic reaction to any topical antiseptic solution

On arrival in labour ward complex, enrolled participants were assessed for eligibility for continuation on the trial. In addition to review of inclusion and exclusion criteria for enrolment, the following criteria were assessed prior to randomisation:

Inclusion criteria for randomisation

- Continued consent for trial procedures for both maternal and infant participants

Exclusion criteria for randomisation

- Presented to labour ward with infant born before arrival
- Noted to have significant vaginal bleeding pre- or during labour
- Known intrauterine fetal death
- Full cervical dilatation or have baby's head on perineum
- Infant noted to be in face presentation
- Noted to have genital ulcers present

2.3.3. **Enrolment and randomisation**

Pregnant women were approached in the antenatal clinic at CHBAH and informed about the PoPS trial. Written informed consent for participation for themselves and their newborns was obtained from interested, eligible pregnant women after a one-on-one information and consenting session with a staff member. The antenatal card of each consented participant was marked with a trial-specific sticker, which included a participant identification number.

When consented participants presented to the labour ward complex of CHBAH, they were identified by research staff based in labour ward, and reminded of the trial and procedures that they had consented for. Eligibility criteria for continuation in the trial were assessed on arrival in labour, prior to randomisation.

Women who were in active phase of labour and fulfilled eligibility criteria during labour were randomised in a 1:1 ratio to receive either intrapartum vaginal wipes with 0.5% chlorhexidine-soaked cotton swabs (interventional arm), or and external

genitalia wipe with sterile water (placebo arm). Infants of women randomised to the interventional arm received a full body wipe with 0.5% chlorhexidine-soaked cotton swabs. Infants of women in placebo arm received a foot wipe with 0.5% chlorhexidine.

Swabbing of the vagina may cause micro-abrasions of the mucous membranes, which, if swabbing was performed without disinfectant solution, may have increased risk of infection in the mother and her infant. This concern pre-empted the single-blinded design of the trial. The limitations of the single-blinded design were mitigated by a strict randomisation process and the procurement of commercial colourless, mildly odoured chlorhexidine gluconate solution for use the trial, to ensure blinding of study team members not involved in performing interventional wipes.

Participants were randomised in large blocks (50 per block) to ensure equal representation of participants in each treatment arm. SAS® was used to randomly assign treatment groups to randomisation numbers. Randomisation forms, which included randomisation number and treatment group allocation, were pre-printed by the unblinded study statistician, and sealed in consecutively-numbered opaque randomisation envelopes. Randomisation envelopes were opened in sequential randomisation number order by the attending study midwife once a consented participant was confirmed to fulfil inclusion and exclusion criteria in labour. Non-sequential allocation of randomisation numbers was investigated during trial conduct to ensure that no bias was introduced intentionally or inadvertently by unblinded study midwives.

2.3.4. **Study procedures and sample collection**

Midwives, who were hired specifically for this trial, were based in labour and delivery complex of CHBAH throughout the year for the duration of the study. These research midwives conducted trial procedures and collected samples and data.

Colonisation swab

A colonisation sub-study was included in the main PoPS trial to determine maternal vaginal colonisation rates with bacteria known to be pathogenic in infants, including Group B *streptococcus, Escherichia coli* and *Klebsiella pneumoniae*, and transmission of colonisation to newborn. Knowledge of GBS-colonisation status of pregnant women in Soweto was limited, as the collection and testing of antenatal swabs and implementation of IAP is not routine, and not feasible in a setting like ours.

Women included in the colonisation cohort had a vaginal introitus swab collected prior to any interventional wipes being performed. Infants of women included in the colonisation cohort had a surface (skin) swab collected from the umbilical area, peri-auricular area and nares soon after birth, prior to the newborn receiving an interventional wipe or a bath. Swabs for colonisation were placed into Amies without charcoal transport medium and transported at room temperature to the laboratory of RMPRU for processing within 48 hours of collection.

Trial intervention

Per vaginal (PV) examinations to assess progress of labour are performed approximately 4 hourly in labouring women by CHBAH-employed attending physicians or midwives. Maternal interventional and control wipes were performed

by research midwives immediately after the routine PV examinations to avoid increasing the numbers of times the participant had to be repositioned for procedures. Maternal wipes were performed approximately 4 hours apart, but not more frequently than 3-hourly. The research midwives monitored participants during and after study wipes for possible reactions to wipes. A decision to terminate wipes early could have been made by the study midwife or attending physician, mainly in the event of a change in eligibility for trial continuation or moderate or severe reaction to chlorhexidine.

Chlorhexidine Interventional wipes:

Commercially-available 5% chlorhexidine gluconate was diluted weekly to 0·5% with autoclaved drinking-quality tap-water and stored in one litre opaque bottles at room temperature. Autoclaved tap-water was used for control-arm wipes. Chlorhexidine was tested for activity by RMPRU laboratory using spectrophotometric and biological activity tests, and both solutions were tested for sterility before and after a week's use.

Study midwives wrapped cotton wool soaked in 0.5% chlorhexidine solution around their index and middle fingers of their gloved examining hand, and gently wiped the cervix and vaginal walls in a clockwise motion. The presenting part of the foetus and mother's external genitalia were also wiped with chlorhexidine-soaked cotton wool pads. Following birth and collection of colonisation cohort skin swabs (if applicable), infants of participants randomised to chlorhexidine interventional wipes were wiped from head to toe (avoiding face and ears) with 0.5% chlorhexidine-soaked cotton wool swabs.

Placebo/ control wipes: Study midwives wiped the external genitalia of women randomised to control arm with sterile water- soaked cotton swabs. Chlorhexidine was not used for control wipes in these maternal participants, as disinfection of external genitalia may have had some benefit to mother and infant, and may have raised questions on the true impact of internal vaginal wipes. Infants of participants randomised to control arm had their feet wiped with chlorhexidine-soaked cotton swabs. Although an infant wipe was not essential in the placebo arm, it assisted in maintaining blinding of study investigators, who were reviewing case report forms of wipe dates and times. Before neonatal wipes all babies received a water bath per standard-of-care.

Participant follow up

Neonatal participants were followed up for hospitalisation for sepsis until 28 days of life, and maternal participants until 14 days post-delivery. Active monitoring of admissions to the neonatal unit and paediatric department at CHBAH was conducted daily by research trial staff members. Medical officers employed for the trial, and blinded to interventional arm, reviewed medical records of all admitted neonates, and abstracted information related to sepsis end-point.

Sterile site cultures from admitted trial participants were collected at the discretion of attending physicians and processed per standard practice at the hospital microbiology laboratory. Routine methods were used for culture and identification of invasive pathogens from sterile sites, including the BacT/Alert microbial system (Organon Teknika, Durham, NC) for blood culture. Active laboratory-based surveillance was also conducted to confirm that all sterile site cultures from study

neonates were captured. Information related to the neonatal sepsis endpoints was abstracted from medical records by trained study physicians. Hospital mortuary logs were also reviewed regularly to ensure deaths among trial participants were captured.

Due to the complexity of diagnosis in different settings, with variable access to diagnostic capacity or tests, no standardised definition of clinically-diagnosed neonatal sepsis was universally used, and the PoPS team developed its own definitions with the assistance of a team of neonatologists, which were used to determine rates of clinical- and culture-confirmed neonatal sepsis in study participants [229, 230]. Neonates of women who continued participation on PoPS at the time of labour were assessed for culture-confirmed- and clinically-confirmed neonatal sepsis endpoints.

2.3.5. **Endpoint definitions**

Maternal vaginal colonisation was defined as isolation of GBS, *E. coli* or *K. pneumoniae* from a vaginal introitus swabs collected during labour prior to the first interventional wipe. Vertical transmission of bacteria was defined as neonatal surface colonisation at birth with the same bacterium isolated from the mother's vaginal swab.

Very Early-onset disease (VEOD) was defined as sepsis occurring on day 0-2 of life and community-acquired late-onset neonatal disease (LOD) as that with onset from day 3 to 28 of life. Clinical sepsis definition required at least one laboratory and one

clinical sign (See Table 2). Medical records of neonates with culture confirmed sepsis episodes which did not fulfill the clinical sepsis criteria were reviewed by three neonatologists to determine if the case represented a sepsis episode. The medical records of all infant trial participants who were stillborn or died within 2 hours of birth were also reviewed to determine whether the cause of death could be attributed to neonatal sepsis. Data reviewed from maternal and infant medical records included documentation of signs of foetal distress (meconium stained liquor, decreased variability or severe, prolonged decelerations of foetal heart rate recorded by cardiotocography), complications in labour (prolonged labour/ poor progress in labour, foetal malpresentation, maternal death) and signs of neonatal asphyxia (poor Apgar scores, arterial blood gas results).

Very Early-onset culture confirmed sepsis: isolation of a micro-organism that is not a common contaminant from a normally sterile body site within the first 3 days of life

Very Early-onset clinical sepsis: A neonate hospitalised within 3 days of life and who in the absence of another recognizable congenital infection had at least one laboratory criteria and either: respiratory distress (one criterion required) or at least two clinical criteria (see Table 2).

Late-onset sepsis: Either culture-confirmed sepsis or clinical sepsis in an infant with symptom onset between three and 28 days of life.

Late-onset culture confirmed sepsis: isolation of a micro-organism that is not a common contaminant from a normally sterile body site between three and 28 days of

life.

Late-onset clinical sepsis: A neonate hospitalised between three and 28 days of life with at least one laboratory criteria and either: respiratory distress (two criteria required), OR one feature of respiratory distress and one other clinical criterion OR at least two other clinical criteria.

Perinatal deaths: Intrapartum stillbirths and deaths within the first 6 days of life

Table 2: Neonatal sepsis criteria [229, 230]

Clinical criteria	Definition
Respiratory distress	Respiratory rate >60 breaths/min; cyanosis, chest wall indrawing, grunting on expiration, respiratory distress noted in medical records
Hypotension	(defined as mean arterial pressure < 2 standard deviations (S.D.) from mean for weight/age
pyrexia or hypothermia	axillary temperature >38·0°C, not attributable to external warming, or axillary temperature <36·0°C
Abdominal/ feeding problems	abdominal distension OR feeding intolerance (>20% residual over 24 hours), or poor feeding after feeding well, or > 2 episodes of emesis
Bleeding diathesis	defined as petechiae, ecchymosis, mucous membrane bleeding, pulmonary hemorrhage, or excessive oozing from venipuncture sites
Lethargy or irritability	noted by medical staff in absence of other central nervous system symptoms
Central nervous system	seizures, or bulging fontanelle, or single witnessed episode of apnea
Laboratory criteria	
White blood cell count (WCC)	WCC <5 x10^9/l **OR** >25 x10^9/l in the absence of receiving corticosteroids;
Absolute neutrophil count (ANC)	ANC <1·75 x10^9/l or >15 x10^9/l
Platelet count	<150 x10^9/l
C-reactive protein	> 10·0 mg/l (early onset sepsis) OR >40mg/l (late-onset sepsis)
Elevated CSF white blood cell (WBC) count	>30 x10^6/l WBC in absence of significant red blood cells

The decision to restrict the very early-onset period for PoPS to the first 3 days of life was guided by the assumption that the potential impact of chlorhexidine interventional intrapartum wipes would be more pronounced in the first 3 days of life, as most vertically-acquired neonatal sepsis presents in this time period.

Postpartum sepsis was defined as maternal hospitalization within 14 days of delivery for endometritis (at least two of: fever, uterine tenderness, foul-smelling/ purulent lochia or vaginal discharge), sterile site culture-confirmed infection, or perineal wound infection among women who delivered vaginally.

2.3.6. **Laboratory methods**

Colonisation cohort swabs were transported to the RMPRU laboratory in Amies without charcoal transport medium were processed to identify GBS, *E. coli* and *K. pneumoniae* as follows. The swab was used to inoculate (i) a 5% horse blood agar plate, (ii) a MacConkey agar plate, (ii) a selective 5% horse blood agar plate containing colistin (10μg/ml) and nalidixic acid (15μg/ml), and (iv) the swab tip was placed in Todd-Hewitt broth supplemented with gentamicin (8 μg/ml) and nalidixic acid (15 μg/ml). All agar plates were incubated for 48 hours, and inspected for growth after 24 and 48 hours.

Samples were processed for GBS isolation as follows:

i. Blood agar plates and selective blood agar plates were incubated at 35-37°C in 5% CO_2 incubator.

ii. Todd- Hewitt broth was incubated for 24 hours at 35-37°C in a 5% CO_2 incubator. A blood agar plate was inoculated with the broth and inspected after 24 hours and 48 hours.

iii. Gram-positive β-haemolytic colonies present on blood agar and selective blood agar plates were isolated and identified as Group B streptococci by CAMP (Christie, Atkins and Munch-Petersen) positive test and inability of hydrolyse esculin.

iv. GBS isolates were serotyped using Group B *streptococcus* latex agglutination method with antisera against GBS serotypes Ia, Ib, II, III, IV, V, VI, VII, VIII, IX and X capsular polysaccharide (Statens Serum Institute, SSI, Sweden). Non-typeable GBS isolates were processed with PCR[228].

Swabs were processed for Gram-negative organisms as follows:

v. Blood agar plate and the MacConkey agar (incubated in ambient air) were inspected for gram negative bacteria. Isolated colonies will be identified by standard biochemical tests, and with the API20E identification system.

Colonies of pure cultures of GBS, *E. coli* and *Klebsiella spp.* isolated from swabs were inoculated into STGG and stored at -70°C. Specimen processing and results case report forms were completed for each specimen received in the laboratory, and entered into study-specific database.

2.3.7. **Data collection and safety monitoring**

The antenatal card, labour and delivery records of maternal participants, and delivery notes were reviewed and key prenatal and intrapartum variables were recorded on paper case-report forms. Underlying and pregnancy-induced maternal conditions including hypertensive disorders and gestational diabetes, and intrapartum events were routinely assessed and documented by attending physicians. Gestational age was determined either by extrapolation of an early antenatal ultrasound or by a Ballard score assessment performed by attending physicians within the first 72 hours of life.

CRFs were reviewed for completeness, accuracy and consistency by the study investigators and study co-ordinator. External observers monitored all trial procedures, all informed consent forms and a sub-set of complete records. Additionally, trial- independent to the trial reviewed medical records of all maternal deaths and neonatal deaths as well as serious adverse events assessed by study

investigators to be possibly related to trial intervention.

Data were double-entered into a custom-designed Microsoft Access database, and data validation and consistency checks between the two databases were performed on an on-going basis. Data queries were resolved by members of the study team, including investigators, study co-ordinator and nurses.

A statistical analysis plan was developed for the study, and was approved by the Scientific Steering committee (SSC) prior to locking and unblinding of the database. The SSC was established prior to study initiation to assist the investigators, and included an international obstetrician- gynaecologist (Marleen Temmermann- chair), international clinical epidemiologist (Paolo Miotti), two local obstetrician-gynaecologist (G. Justus Hofmeyr and Robert Pattinson) and a local paediatrician (Haroon Saloojee).

An independent Data and Safety Monitoring Board (DSMB) was established prior to initiation of the trial, and comprised of an international paediatric infectious disease specialist/ vaccinologist (Kathryn Edwards, Vanderbilt university, Nashville, Tennessee, USA), South African paediatrician/ neonatologist (Peter Cooper, University of the Witwatersrand, Johannesburg), South African Obstetrician-gynaecologist and researcher (James McIntyre, Perinatal HIV Research Unit, Soweto, Johannesburg) and an international epidemiologist (Maria Deloria Knoll, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA).

The DSMB continuously monitored serious adverse events throughout the trial. An

interim analysis was conducted after 2000 mother-infant pairs were randomised to allow for assessment of some baseline assumptions that guided the study design, including rates of clinical and culture-confirmed neonatal sepsis in control arm. A second interim data analysis was performed after 6000 mother-infant pairs were enrolled on the study to compare treatment groups for the primary outcome measure of confirmed or clinical neonatal sepsis in the first 72 hours of life. If a statistically significant benefit had manifested at that stage, the DSMB was mandated to consider stopping the trial had there been overwhelming efficacy. The DSMB did not unblind investigators or stop the study at either of these interim data analyses.

2.3.8. **Sample size calculation and statistical analysis**

A pre-study audit of neonatal admissions was performed at CHBAH, and estimated that the early-onset neonatal sepsis rate was approximately 30 per 1000 live births. In order to detect an efficacy of the intervention of 40% and using a power of 90% and an alpha of 0·05, a sample size of 3581 mother-newborn pairs per trial arm was required. In order to allow for approximately 10% drop-out from the trial, a sample size of 4000 mother-newborn pairs was targeted. For the colonisation sub-study, a baseline prevalence of 25% for maternal GBS colonisation at the time of delivery was assumed. Vertical transmission of GBS had been reported to vary between 30% and 70% in previous studies[169, 170, 231], and we assumed that vertical transmission would occur in 50% of deliveries among GBS-colonised women. We powered the colonization endpoint to detect at least a 30% reduction in vertical transmission of GBS, based upon which we planned on enrolling 1470 mother-newborn pairs per study arm. This was adjusted to 1800 per arm (3600 overall) to allow for compliance failures.

The statistical analyses conducted for the different end-points in the PoPS trial are detailed in the relevant chapter below, and in Rubin & Zell[232].

The PoPS data were analysed using SAS version 9.1 and 9.2 (Carey, NC, USA). A p-value of <0.05 was considered significant throughout, twins were counted as independent births, and stillborn neonates were included in ITT analyses.

2.3.9. **Ethics**

This trial was approved by the University of the Witwatersrand Human Research Ethics committee (HREC) on 13th March 2003 (HREC reference number 030207; Appendix 3) and the Institutional review board of the Centers for Disease Control and Prevention, Atlanta, USA (protocol # 3842). The PoPS trial was registered with Clinicaltrials.gov (NCT00136370).

All pregnant women enrolled on PoPS study were consented at the antenatal clinic of CHBAH. Inclusion and exclusion criteria and willingness to continue with study participation were reviewed by study staff at the time the woman arrived in labour ward for delivery.

2.3.10. **Funding**

The PoPS trial was funded by the Centers for Disease Control and Prevention (Cooperative Agreement number: U50/CCU02196 and U01 CI000318) and the Bill and Melinda Gates Foundation (Grant number 39415).

3. AETIOLOGY OF EARLY-ONSET AND COMMUNITY- ACQUIRED SEPSIS IN NEONATES AND YOUNG INFANTS.

The non-specific symptoms and signs of sepsis which young infants (<3 months of age) present with, makes it difficult to evaluate the incidence of clinically-confirmed sepsis. In addition, culture-confirmed neonatal sepsis may underestimate the incidence of neonatal sepsis, due to low sensitivity of blood culture, which is affected inter alia by inadequate or limited volume of blood obtained from young infants[53, 55], low bacterial load[56, 57] and antibiotic administration to infant or mother[58] prior to blood sampling.

The distribution of bacteria isolated from septic young infants differs between HICs and LMICs. In HICs, Group B Streptococcus (GBS) and *Escherichia coli (E. coli)* cause almost 75% of early-onset invasive disease (EOD)[40, 64], whilst *S. aureus* (6% - 18%), *E. coli* (5% - 13%) and *K. pneumoniae* (4% - 9%) are the most common bacterial pathogens of late onset disease (LOD)[64]. In contrast, in LMICs, *S. aureus* (13% - 26%), *E. coli* (8% - 18%) and *K. pneumoniae* (13.5% - 21%) have been reported as the most common bacterial pathogens causing neonatal sepsis, with GBS being rarely (2% - 8%) identified, especially in South-East Asia[70-72].

In this chapter, the epidemiology of early-onset (newborns 0-6 days) and community-acquired sepsis in neonates and young infants (≤90 days of age) will be presented. Included is an evaluation of the impact of maternal HIV-exposure on the burden of sepsis, and antibiotic susceptibility profile of the most common bacteria identified. The overall methods are detailed in Chapter 2. The denominator for incidence

calculations was obtained from administrative databases of live births from CHBAH and Soweto community clinics. Additionally, the odds ratio of culture-confirmed sepsis in different age categories being impacted by HIV-exposure was calculated, based on the assumption that the population prevalence of HIV-exposure of the neonates was 29.9%, as documented in Soweto antenatal attendees during the course of the study.

3.1. Results

3.1.1. Overall

Overall, 699 infants ≤90 days age with a significant pathogenic bacterium isolated from a sterile site within the first 72 hours of hospitalisation, were hospitalised at CHBAH between January 2006 and December 2008. The overall incidence (per 1000 live births) was 7.30; Table 3. Full clinical details were unavailable for 34% of the sepsis episodes (238/699). Fifty five percent (350/634) of cases occurred in males. The case fatality ratio of infants with a known outcome was 15% (69/461) (Table 4).

Overall, 26.4% (146/699) of the sepsis cases were associated with meningitis. Of the meningitis cases, 42.5% (62/146) had organism isolated from CSF but not blood, 24.7% (36/146) from CSF and blood, whilst remaining 48 cases (32.9%) were diagnosed by a positive blood culture only in the presence of CSF cytological profile suggestive of bacterial meningitis. The overall case fatality rate for meningitis cases was 18.8% (Table 4).

Maternal HIV-infection status was available for 57.9% (405/699) of cases, of whom 39.5% (160/405, 95% CI: 34.7% to 44.5%) were HIV-exposed; Table 3. Infants with

and without HIV-exposure results available did not differ substantially in age at diagnosis or gender in overall cohort or when stratified by age category (EOD, LOD and YI-CAS, data not shown). The prevalence of HIV-exposure for sepsis due to individual bacteria are detailed in Table 3, and included 47.1% for GBS, 41.2% for *S. pneumoniae,* 35.6% for *E. coli* and 53.3% for *K. pneumoniae*. HIV-exposed infants were at higher risk of developing sepsis than HIV-unexposed infants (OR 1.53, 95% CI: 1.13 to 2.07, p=0.004). The CFR for individual bacteria are shown in Table 4.

Due to the large number of missing HIV results, the incidence of invasive disease was calculated assuming that (i) infants with missing results had same HIV-exposure prevalence as population (29.9%), (ii) infants with missing results had same HIV-exposure prevalence as cases with HIV-exposure results (39.5%), (iii) all infants with missing HIV results were HIV-exposed and (iv) all infants with missing HIV results were HIV-unexposed. The odds of an HIV-exposed infant developing sepsis was 1.52 times greater than that of an HIV-unexposed infant (OR 1.52, 95%CI: 1.31-1.78) (Table 5).

Gram positive bacteria were present in 68.5% (n=479/699, 95%CI: 64.9% - 72.0%) of sepsis episodes, whereas 31.5% (n=220/699, 95%CI: 28.0% - 35.1%) were due to Gram-negative bacteria. The most common bacterial pathogen was GBS (246 cases, 35.2% of all isolates; incidence [per 1000 live births] 2.57), followed by *Staphylococcus aureus* (113 cases, 16.2% overall; incidence 1.18). The most commonly-isolated Gram-negative bacteria was *Escherichia coli* (85 cases, 12.2% overall; incidence 0.89) (Table 3,

Figure **1**). These three bacteria contributed to 60.9% (196/322), 78.9% (150/190) and 52.4% (98/187) of EOD, late- onset disease (LOD, 7-27 days age) and young infant community-acquired sepsis (YI-CAS, 28-90 days old), respectively; Figure 2 A-C.

Table 3: Incidence of vertically/ community-acquire pathogenic bacteria isolated from young infants aged 0-90 days

Bacteria isolates	**2006 N**	**2007 N**	**2008 N**	**Overall N**	**Overall Incidence**		**Total with HIV-exposure results**	**HIV exposed**	**HIV unexposed**	**% of HIV-exposed**
Soweto live births	31 338	32 350	32 033	95 721	per 1 000 live births (95% CI)					(95% CI)
Gram positive										
Staphylococcus aureus	23	41	49	113	1.18 (0.97-1.41)		60	15	45	25.0%
Streptococcus agalactiae (GBS)	76	79	91	246	2.57 (2.25-2.91)		221	104	117	47.1%
Streptococcus pneumoniae	6	11	12	29	0.30 (0.20-0.44)		17	7	10	41.2%
Streptococcus viridans	15	12	43	70	0.73 (0.57-0.92)		21	4	17	19.0%
Other Gram positives	2	8	11	21	0.22 (0.14-0.34)		3	0	3	0.0%
Gram negative										
Escherichia coli	24	29	32	85	0.89 (0.71-1.10)		45	16	29	35.6%
Klebsiella pneumoniae	10	5	9	24	0.25 (0.16-0.37)		15	8	7	53.3%
Haemophilus influenza type b	2	3	4	9	0.09 (0.04-0.18)		3	1	2	33.3%
*Acinetobacte*r spp.	18	9	30	57	0.60 (0.45-0.77)		8	1	7	12.5%
Other Gram negatives	9	12	24	45	0.47 (0.34-0.63)		12	4	8	33.3%
Total	185	209	305	699	7.30 (6.77-7.86)		405	160	245	39.5% (34.7- 44.5%)

Other Gram positives: *Streptococcus anginosus* (2), *Streptococcus bovis* (3), *Group A-, C-, D- F and G streptococcus (*1 each), *Streptococcus salivarius (*5)*, Streptococcus pyogenes (*3), *Streptococcus milleri* (2*), Streptococcus mitis* (1)
Other Gram negatives: *Enterobacter cloacae (*19)*, Klebsiella oxytoca* (5), *Listeria monocytogenes* (1), *Proteus mirabilis (*2)*, Pseudomonas aeruginosis* (7), *Enterobacter agglomerans (*1)*, Salmonella typhi* (1), *Serratia marcescens (*1)*, Shigella flexnerii (*1)

Table 4: Demographics of young infants (≤90 days of age) with vertically- or community-acquired bacterial sepsis

		Gender			Clinical presentation			Outcome Overall			
Bacteria isolated	**Total N**	**Female**	**Male**	**M:F**	**Bacteraemia**	**Meningitis**	**% of meningitis per organism**	**Discharged**	**Died**	**CFR**	**CFR meningitis**
Gram positive											
Staphylococcus aureus	113	55	48	0.9	105	8	7.1%	68	4	5.6%	0%
Streptococcus agalactiae (GBS)	246	108	137	1.3	148	98	39.8%	207	37	15.2%	19.6% (19/97)
Streptococcus pneumoniae	29	10	13	1.3	19	10	34.5%	16	2	11.1%	22.2% (2/9)
Streptococcus viridans	70	28	29	1.0	70	0	0.0%	24	1	4.0%	0%
Other Gram positives	21	12	7	0.6	19	2	9.5%	3	0	0.0%	
Gram negative											
Escherichia coli	85	24	50	2.1	67	18	21.2%	43	15	25.9%	31.2% (5/16)
Klebsiella pneumoniae	24	10	11	1.1	18	6	25.0%	13	3	18.8%	0%
Haemophilus influenza type b	9	5	1	0.2	8	1	11.1%	2	2	50.0%	0%
Acinetobacter spp.	57	18	31	1.7	55	2	3.5%	5	2	28.6%	0%
Other Gram negatives	45	14	23	1.6	44	1	2.2%	11	3	21.4%	0%
Total	699	284	350	1.2	553	146	26.4%	392	69	15.0%	18.8% (26/138)

CFR= Case fatality rate

Other Gram positives: *Streptococcus anginosus* (2), *Streptococcus bovis* (3), *Group A-, C-, D- F and G streptococcus (*1 each), *Streptococcus salivarius (*5)*, Streptococcus pyogenes (*3), *Streptococcus milleri* (2*), Streptococcus mitis* (1)
Other Gram negatives: *Enterobacter cloacae (*19)*, Klebsiella oxytoca* (5), *Listeria monocytogenes* (1), *Proteus mirabilis (*2)*, Pseudomonas aeruginosis* (7),

*Enterobacter agglomerans (*1)*, Salmonella typhi* (1), *Serratia marcescens (*1)*, Shigella flexnerii (*1)

Table 5 Incidence of invasive bacterial sepsis in 0-90 day old infants stratified by HIV-exposure

Bacteria isolated		HIV-exposed		HIV-unexposed		
		n	Incidence (95% CI)	n	Incidence (95% CI)	OR (95%CI)
Overall (Proration 29.9% of infants with missing HIV results presumed to be HIV-exposed)	Overall	248	5.79	451	4.51	1.28
			(5.09-6.55)		(4.10- 4.94)	(1.10- 1.51)
	EOD	109	2.54	213	2.13	1.19
			(2.09- 3.07)		(1.85- 2.44)	(0.94-1.51)
	LOD	65	1.52	125	1.25	1.23
			(1.17- 1.93)		(1.04- 1.49)	(0.89- 1.65)
	YI-CAS	74	1.73	113	1.13	1.59
			(1.36- 2.17)		(0.93-1.36)	(1.12- 2.07)
Overall (Proration 39.5% of infants with missing HIV results presumed to be HIV-exposed)	Overall	276	6.44	423	4.23	1.52
			(5.70- 7.24)		(3.84- 4.65)	(1.31-1.78)
	EOD	126	2.94	196	1.96	1.50
			(2.45- 3.50)		(1.70-2.25)	(1.19- 1.89)
	LOD	72	1.68	118	1.18	1.42
			(1.31-2.11)		(0.98- 1.41)	(1.05- 1.93)
	YI-CAS	78	1.82	109	1.10	1.67
			(1.44- 2.27)		(0.89- 1.31)	(1.23- 2.26)
HIV unknown assumed exposed	Overall	454	10.59	245	2.45	4.36
			(9.64- 11.61)		(2.15- 2.78)	(3.71- 5.11)
HIV unknown assumed unexposed	Overall	160	3.73	539	5.39	0.69
			(3.18- 4.36)		(4.94- 5.86)	(0.58- 0.83)

Proration based on prevalence of HIV exposure in community (29.9%) or in cases with HIV-exposure results (39.5%)
EOD- Early onset disease (0-6 days), LOD- Late onset (neonatal) disease (7-27 days), YI-CAS- Young infant community acquired sepsis (28-90 days)

Figure 1: Bacterial causes of vertically- or community-acquired culture- confirmed sepsis in infants (0-90 days)

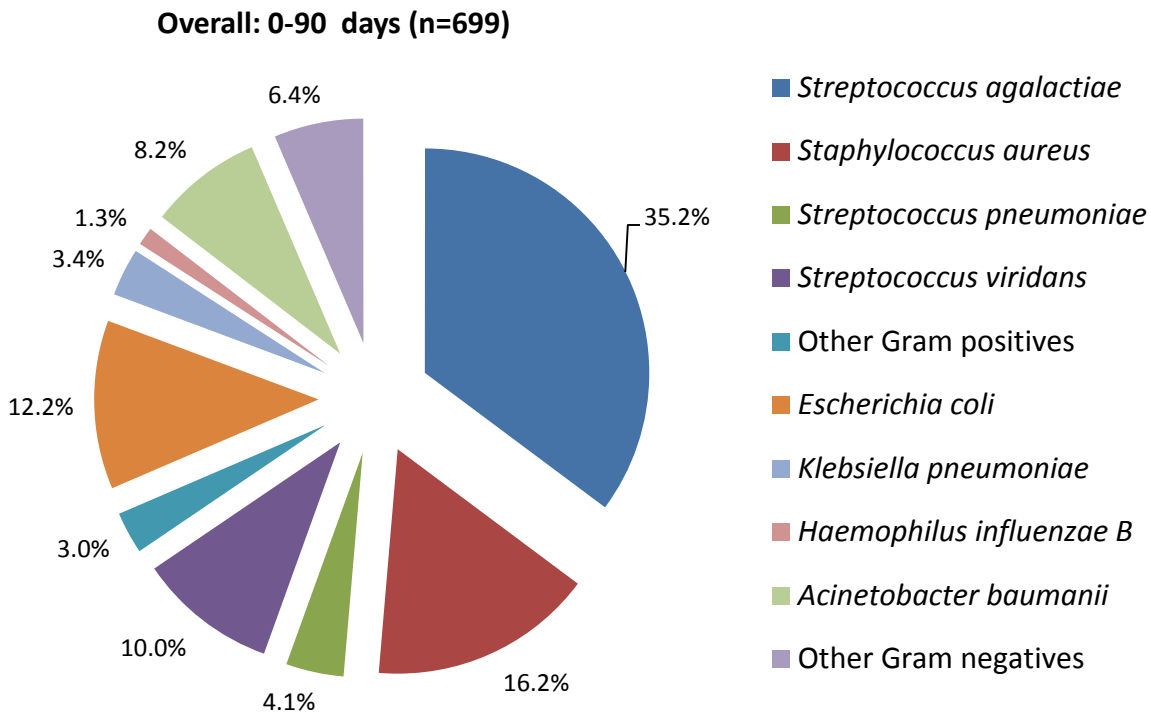
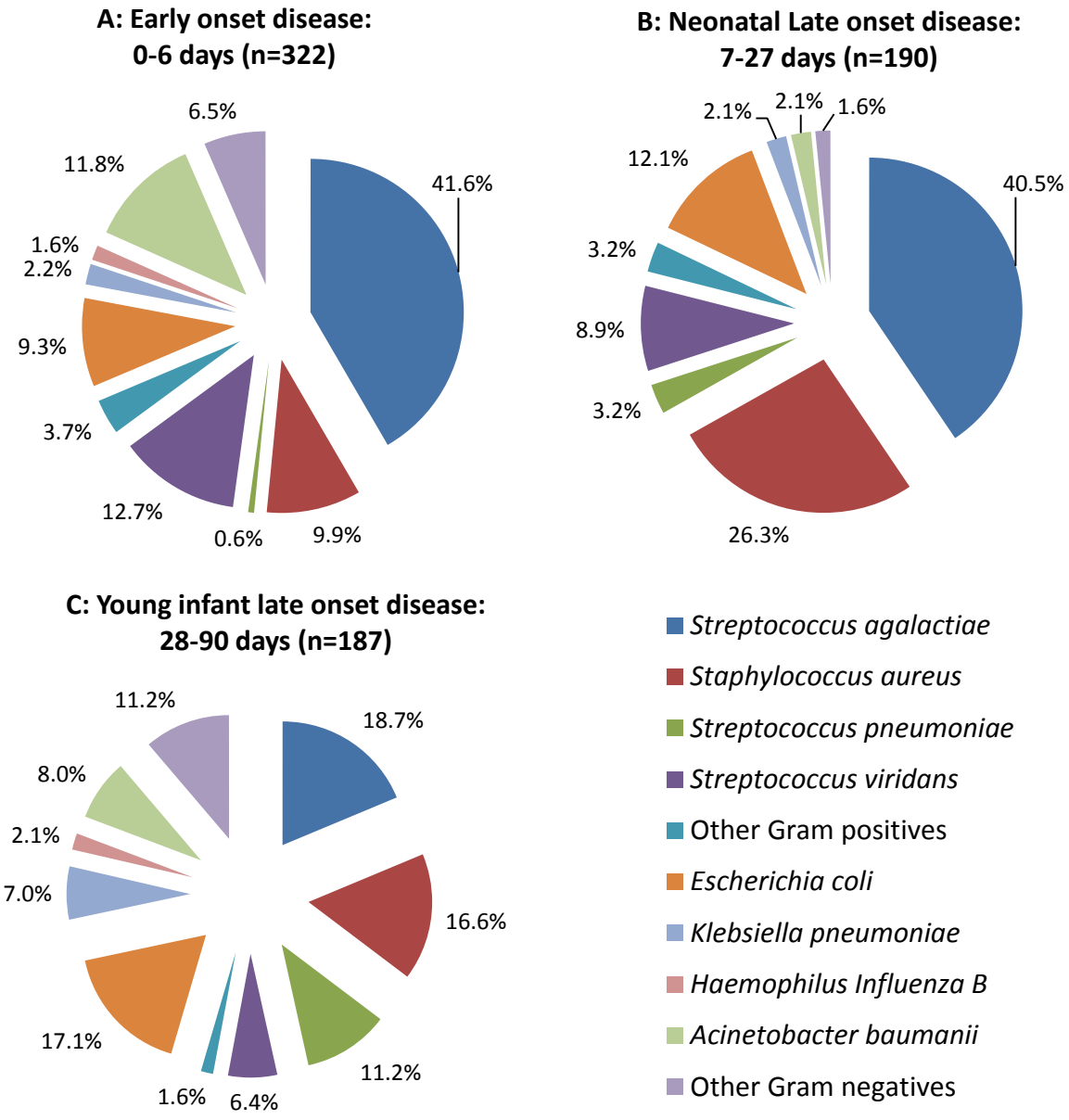


Figure 2: Bacterial causes of vertically- or community-acquired culture- confirmed sepsis in young infants (0-90 days) stratified by age



3.1.2. **Early onset disease**

Newborns admitted to the neonatal unit at CHBAH immediately after birth, and who had a pathogenic bacteria identified from a sterile site culture in first 72 hours of life contributed 83.6% (270/ 322) of the early-onset disease (EOD, 0-6 days) cases. The remaining EOD cases were admitted to the general paediatric ward at CHBAH between days 0 and 6 of life, and had a bacterium isolated from sterile site within 72 hours of admission. The incidence (per 1 000 live births) of culture-confirmed EOD was 3.36 (Table 6). Almost thirty-nine percent (59/152, 95% CI: 31.0 to 47.0%) of the EOD cases with known HIV results were HIV-exposed. In the sensitivity analysis, HIV-exposed young neonates had a higher odds of presenting with bacterial sepsis than HIV-unexposed infants (OR= 1.50, 95% CI: 1.19 – 1.89) (Table 5). The CFR was 17% (29/142); (Table 6, Table 7).

Gram positive bacteria were present in 68.6% (n=221, 95% CI: 63.3% - 73.7%) of EODs episodes, whilst 31.4% (n=101, 95% CI: 26.3% - 36.7%) were due to Gram-negative bacteria. The most common cause of EOD was GBS (41.6%, Figure 2, incidence 1.40), of whom 35.7% were HIV-exposed and 15.8% (21/112) died. Sixty three percent (88/140) of the GBS-EOD cases presented on day 0 of life and contributed 66% (88/133) of all bacterial isolates identified in sepsis cases presenting on day 0 Figure 3). Furthermore, 23.1% of the GBS EOD presented with meningitis.
The next most prevalent EOD bacterial pathogen were *Acinetobacter baumanii* (n=38, incidence: 0.40), *S. aureus* (n=32, incidence: 0.33) and *E. coli* (n=30, incidence: 0.31; CFR 14.3%) (Table 6). The majority of EOD cases (88.8%; 286/322) had bacteraemia,

whilst the rest (n=36) presented with meningitis; 16 (44.4%) of which were culture-confirmed on CSF and 20 (55.6%; all GBS cases) were cultured only from blood culture and the CSF cyto-chemistry profile was suggestive of bacterial meningitis; Table 7.

Figure 3: Distribution of bacterial pathogens causing early-onset neonatal bacterial sepsis by day of life (n= 322)

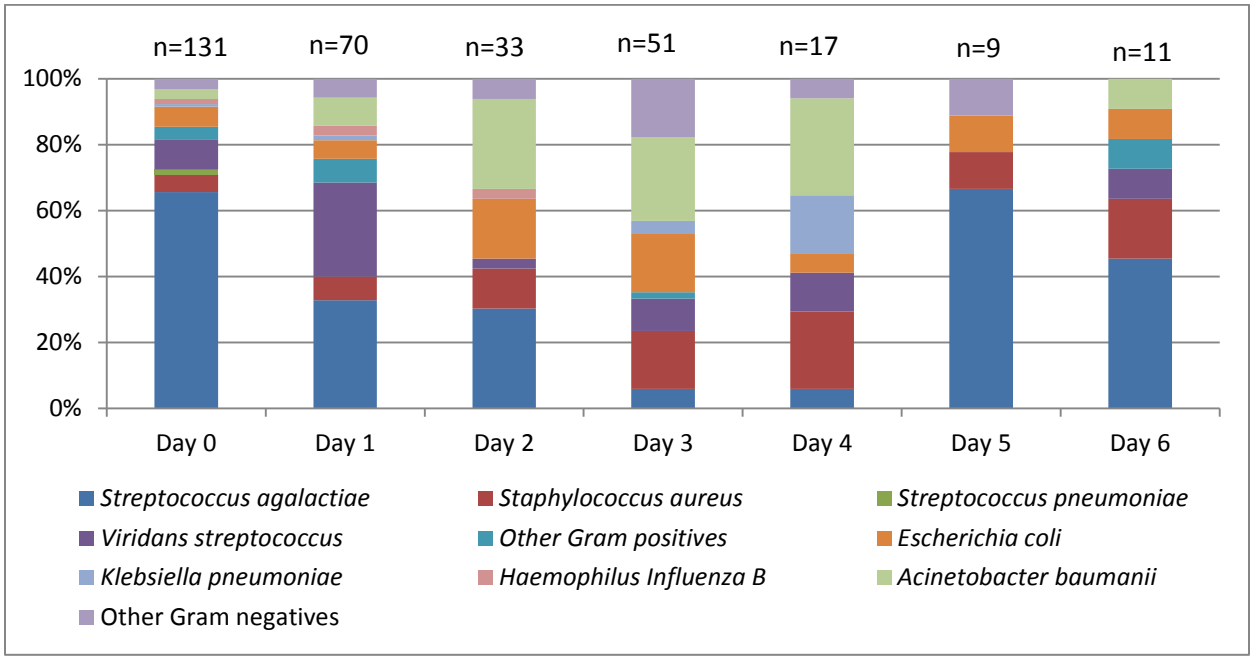


Table 6: Incidence of vertically/ community-acquire pathogenic bacteria isolated from neonates aged 0-6 days

Bacteria isolates	**2006 N**	**2007 N**	**2008 N**	**Overall N**	**Incidence (95% CI)**		**Total with HIV-exposure results**	**HIV exposed**	**HIV unexposed**	**% of HIV-exposed**
Soweto live births	31338	32350	32033	95721	per 1000 live births					
Gram positive										
Staphylococcus aureus	8	14	10	32	0.33 (0.23-0.47)		9	3	6	33.3%
Streptococcus agalactiae (GBS)	39	41	54	134	1.40 (1.17-1.66)		123	50	73	40.7%
Streptococcus pneumoniae	0	2	0	2	0.02 (0.002-0.08)		0	0	0	
Streptococcus viridans	9	7	25	41	0.43 (0.31-0.58)		2	0	2	0.0%
Other Gram positives	2	4	6	12	0.13 (0.06-0.22)		1	0	1	0.0%
Gram negative										
Escherichia coli	14	10	6	30	0.31 (0.21-0.45)		9	4	5	44.4%
Klebsiella pneumoniae	2	1	4	7	0.07 (0.02-0.15)		2	0	2	0.0%
Haemophilus influenza type B	1	1	3	5	0.05 (0.01-0.12)		1	0	1	0.0%
Acinetobacter spp.	9	6	23	38	0.40 (0.28-0.54)		1	0	1	0.0%
Other Gram negatives	3	5	13	21	0.22 (0.14-0.34)		4	2	2	50.0%
Total	87	91	144	322	3.36 (3.00-3.75)		152	59	93	38.8%

Other Gram positives: *Streptococcus anginosus*, *Streptococcus bovis*, *Group A-, C-, D- F and G streptococcus*, *Streptococcus salivarius, Streptococcus pyogenes*, *Streptococcus milleri, Streptococcus mitis*
Other Gram negatives: *Enterobacter cloacae, Klebsiella oxytoca*, *Listeria monocytogenes*, *Proteus mirabilis, Pseudomonas aeruginosis*, *Enterobacter agglomerans, Salmonella typhi*, *Serratia marcescens, Shigella flexnerii*

Table 7: Demographic and clinical presentation of early-onset (<7 days age) vertical or community-acquired bacterial sepsis.

		Gender			Clinical presentation			Outcome		
Bacteria isolated	**Total N**	**Female**	**Male**	**M:F**	**Bacteraemia**	**Meningitis**	**% of meningitis per organism**	**Discharged**	**Died**	**CFR**
Gram positive										
Staphylococcus aureus	32	13	15	1.2	32	0	0.0%	10	0	0.0%
Streptococcus agalactiae (GBS)	134	60	73	1.2	103	31	23.1%	112	21	15.8%
Streptococcus pneumoniae	2	1	1	1.0	2	0	0.0%	1	0	0.0%
Streptococcus viridans	41	16	15	0.9	41	0	0.0%	2	0	0.0%
Other Gram positives	12	9	2	0.2	12	0	0.0%	1	0	0.0%
Gram negative										
Escherichia coli	30	11	11	1.0	28	2	6.7%	12	2	14.3%
Klebsiella pneumoniae	7	4	3	0.8	7	0	0.0%	1	1	50.0%
Haemophilus influenza type b	5	3	0	0.0	4	1	20.0%	1	2	66.7%
Acinetobacter spp.	38	11	20	1.8	37	1	2.6%	0	1	100.0%
Other Gram negatives	21	9	8	0.9	20	1	4.8%	2	2	50.0%
Total	322	137	148	1.1	286	36	11.2%	142	29	17.0%

Other Gram positives: *Streptococcus anginosus*, *Streptococcus bovis*, *Group A-, C-, D- F and G streptococcus*, *Streptococcus salivarius, Streptococcus pyogenes*, *Streptococcus milleri, Streptococcus mitis*
Other Gram negatives: *Enterobacter cloacae, Klebsiella oxytoca*, *Listeria monocytogenes*, *Proteus mirabilis, Pseudomonas aeruginosis*, *Enterobacter agglomerans, Salmonella typhi*, *Serratia marcescens, Shigella flexnerii*

3.1.3. **Neonatal- late onset disease**

All community-acquired Neonatal late-onset disease (LOD, 7-27 days) cases were hospitalized to the paediatric wards of CHBAH. The incidence (per 1 000 live births) of culture-confirmed N-LOD was 1.98; Table 8. The prevalence of HIV-exposure was 36.9% (48/130; 95% CI: 28.6% to 45.8%) among those in whom the maternal HIV infection status was known (130/190; 68.4%). The overall CFR for LOD was 14.2% (23/162), including 14.5% for GBS-LOD cases and 26.3% for *E. coli* associated sepsis (Tables 8 & 9).

Gram positive bacteria were present in 82.1% (n=156, 95%CI: 75.9% - 87.3%) of sepsis episodes, whilst 17.9% (n=34, 95% CI: 12.7%- 24.1%) were due to Gram-negative bacteria. The most commonly identified bacteria associated with LOD were GBS (40.5%; incidence: 0.80), followed by *S. aureus* (23.6%; incidence: 0.52) and *E. coli* (12.1%; incidence: 0.24); Table 8. The overall prevalence of maternal HIV-infection exposure was 36.9%, including being 56.9% in GBS LOD. HIV-exposed infants had an increased odds of developing sepsis compared to HIV-unexposed infants (OR 1.42, 95%CI: 1.05- 1.93) (Table 5).

Table 8: Incidence of vertically/ community-acquire pathogenic bacteria isolated from neonates aged 7-27 days

Bacteria isolates	**2006 N**	**2007 N**	**2008 N**	**Overall N**	**Incidence (95% CI)**		**Total with HIV-exposure results**	**HIV exposed**	**HIV unexposed**	**% of HIV-exposed**
Soweto live births	**31338**	**32350**	**32033**	**95721**	**per 1000 live births**					
Gram positive										
Staphylococcus aureus	10	19	21	50	0.52 (0.39-0.69)		29	3	26	10.3%
Streptococcus agalactiae (GBS)	24	28	25	77	0.80 (0.63-1.01)		65	37	28	56.9%
Streptococcus pneumoniae	2	2	2	6	0.06 (0.02-0.14)		5	2	3	40.0%
Streptococcus viridans	0	2	15	17	0.18 (0.10-0.28)		10	1	9	10.0%
Other Gram positives	0	2	4	6	0.06 (0.02-0.14)		1	0	1	0.0%
Gram negative										
Escherichia coli	4	9	10	23	0.24 (0.15-0.36)		16	4	12	25.0%
Klebsiella pneumoniae	2	1	1	4	0.04 (0.01-0.11)		3	1	2	33.3%
Haemophilus influenza type B	0	0	0	0	0.00		0	0	0	
Acinetobacter spp.	1	1	2	4	0.04 (0.01-0.11)		0	0	0	
Other Gram negatives	0	0	3	3	0.03 (0.006-0.09)		1	0	1	0.0%
Total	43	64	83	190	1.98 (1.71-2.29)		130	48	82	36.9%

Other Gram positives: *Streptococcus anginosus*, *Streptococcus bovis*, *Group A-, C-, D- F and G streptococcus*, *Streptococcus salivarius, Streptococcus pyogenes*, *Streptococcus milleri, Streptococcus mitis*
Other Gram negatives: *Enterobacter cloacae, Klebsiella oxytoca*, *Listeria monocytogenes*, *Proteus mirabilis, Pseudomonas aeruginosis*, *Enterobacter agglomerans, Salmonella typhi*, *Serratia marcescens, Shigella flexnerii*

Table 9: Demographics of young infants with vertically- or community-acquired late onset neonatal bacterial sepsis: 7-27 days

		Gender			Clinical presentation			Outcome		
Bacteria isolated	**Total N**	**Female**	**Male**	**M:F**	**Bacteraemia**	**Meningitis**	**% of meningitis per organism**	**Discharged**	**Died**	**CFR**
Gram positive										
Staphylococcus aureus	50	28	19	0.7	46	4	8.0%	38	3	7.3%
Streptococcus agalactiae (GBS)	77	37	40	1.1	26	51	66.2%	65	11	14.5%
Streptococcus pneumoniae	6	2	3	1.5	6	0	0.0%	4	1	20.0%
Streptococcus viridans	17	6	10	1.7	17	0	0.0%	14	0	0.0%
Other Gram positives	6	2	3	1.5	5	1	16.7%	1	0	0.0%
Gram negative										
Escherichia coli	23	4	16	4.0	17	6	26.1%	14	5	26.3%
Klebsiella pneumoniae	4	0	3		3	1	25.0%	2	2	50.0%
Haemophilus influenza type b	0	0	0		0	0		0	0	
Acinetobacter spp.	4	1	3	3.0	4	0	0.0%	0	0	
Other Gram negatives	3	0	2		3	0	0.0%	1	1	50.0%
Total	190	80	99	1.2	127	63	31.6%	139	23	14.2%

Other Gram positives: *Streptococcus anginosus*, *Streptococcus bovis*, *Group A-, C-, D- F and G streptococcus*, *Streptococcus salivarius, Streptococcus pyogenes*, *Streptococcus milleri, Streptococcus mitis*
Other Gram negatives: *Enterobacter cloacae, Klebsiella oxytoca*, *Listeria monocytogenes*, *Proteus mirabilis, Pseudomonas aeruginosis*, *Enterobacter agglomerans, Salmonella typhi*, *Serratia marcescens, Shigella flexnerii*

3.1.4. **Young infant community acquired sepsis**

The incidence (per 1 000 live births) of Young infant community-acquired sepsis (YI-CAS, 28-90 days) was 1.95, of which 21.4% presented as meningitis. Gram positive bacteria were present in 54.5% (n=102, 95% CI: 47.1% - 61.8%) of sepsis episodes compared to 45.5% (n=85, 95% CI: 38.2% - 52.9%) being due to Gram-negative bacteria (Tables 10 & 11).

Of the meningitis cases, 90% (36/40) had bacteria cultured from the cerebrospinal fluid; and 10% (4/40) had a bacteraemia with CSF cytology suggestive of meningitis (Table 11). The prevalence of maternal HIV-exposure among YI-CAS cases was 43.1% (53/123, 95% CI: 34.2% to 52.3%) among those in whom the maternal HIV infection status was known. HIV-exposed infants had a greater odds of developing sepsis than HIV-unexposed infants on sensitivity analysis (OR 1.67, 95%CI: 1.23- 2.26, Table 5). The overall CFR for YI-CAS was 13.3%, including 14.3% for GBS and 32.0% for *E.coli* sepsis; Tables 10 and 11.

The three most common bacterial pathogens were GBS (18.7%; incidence 0.37), *E. coli* (17.1%; incidence 0.33) and *S. aureus* (16.6%; incidence 0.32), which contributed to 52.4% of the overall cases; Tables 10 & 11, Figure 2c.

Table 10: Incidence of vertically/ community-acquire pathogenic bacteria isolated from neonates aged 28-90 days

Bacteria isolates	**2006 N**	**2007 N**	**2008 N**	**Overall N**	**Incidence**		**Total with HIV-exposure results**	**HIV exposed**	**HIV unexposed**	**% of HIV-exposed**
Soweto live births	31338	32350	32033	95721	per 1000 live births					
Gram positive										
Staphylococcus aureus	5	8	18	31	0.32 (0.22-0.46)		22	9	13	40.9%
Streptococcus agalactiae (GBS)	13	10	12	35	0.37 (0.26-0.51)		33	17	16	51.5%
Streptococcus pneumoniae	4	7	10	21	0.22 (0.14-0.34)		12	5	7	41.7%
Streptococcus viridans	6	3	3	12	0.13 (0.06-0.22)		9	3	6	33.3%
Other Gram positives	0	2	1	3	0.03 (0.006-0.09)		1	0	1	0.0%
Gram negative										
Escherichia coli	6	10	16	32	0.33 (0.23-0.47)		20	8	12	40.0%
Klebsiella pneumoniae	6	3	4	13	0.14 (0.07-0.23)		10	7	3	70.0%
Haemophilus influenza type B	1	2	1	4	0.04 (0.01-0.11)		2	1	1	50.0%
Acinetobacter spp.	8	2	5	15	0.16 (0.09-0.26)		7	1	6	14.3%
Other Gram negatives	6	7	8	21	0.22 (0.14-0.34)		7	2	5	28.6%
Total	55	54	78	187	1.95 (1.68-2.25)		123	53	70	43.1%

Table 11: Demographics of young infants with vertically- or community-acquired late onset young infant bacterial sepsis: 28-90 days

		Gender			Clinical presentation			Outcome		
Bacteria isolated	**Total N**	**Female**	**Male**	**M:F**	**Bacteraemia**	**Meningitis**	**% of meningitis per organism**	**Discharged**	**Died**	**CFR**
Gram positive										
Staphylococcus aureus	31	14	14	1.0	28	3	9.7%	20	1	4.8%
Streptococcus agalactiae (GBS)	35	11	24	2.2	19	16	45.7%	30	5	14.3%
Streptococcus pneumoniae	21	7	9	1.3	14	7	33.3%	11	1	8.3%
Streptococcus viridans	12	6	4	0.7	12	0	0.0%	8	1	11.1%
Other Gram positives	3	1	2	2.0	2	1	33.3%	1	0	0.0%
Gram negative										
Escherichia coli	32	9	23	2.6	22	10	31.3%	17	8	32.0%
Klebsiella pneumoniae	13	6	5	0.8	11	2	15.4%	10	0	0.0%
Haemophilus influenza type b	4	2	1	0.5	4	0	0.0%	1	0	0.0%
Acinetobacter spp.	15	6	8	1.3	14	1	6.7%	5	1	16.7%
Other Gram negatives	21	5	13	2.6	21	0	0.0%	8	0	0.0%
Total	187	67	103	1.5	147	40	21.4%	111	17	13.3%

3.1.5. **Antibiotic susceptibility of top six leading causes of bacterial sepsis.**

The antibiotic susceptibility profile for the most common bacterial pathogens is shown in Table 11. Among the Gram positive bacteria, all GBS isolates were sensitive to penicillin, whilst 9.1% were resistant to erythromycin. *S. aureus* isolates were generally resistant (92.5%) to penicillin/ ampicillin, with 15.0% (16/107) being also resistant to methicillin. Also, 28.9%, 31.6% and 13.9% of *S. aureus* isolates were resistant to gentamicin, erythromycin and Fluorquinolones (Table 12).

Among the Gram-negative bacteria, approximately a quarter of *E. coli* isolates were resistant to penicillin/ ampicillin, gentamicin and fluoroquinolones, and 7.3% were also resistant to third generation cephalosporins. Resistance to penicillin/ ampicillin amongst other Gram negative pathogens (*K. pneumoniae* and *Aceintobacter spp.)* was 60-66%; 55% to fluoroquinolones and one-third were also resistant to third generation cephalosporins; Table 12.

Table 12: Antibiotic resistance profile for most common bacterial pathogens in young infants (0-90 days old) with vertically- or community-acquired sepsis

Bacteria isolated	**Penicillin/ ampicillin**			**Gentamicin**			**3rd generation cephalosporin**			**Erythromycin**			**Fluoroquinolones**		
	n/N	**% resistance**	**95% CI**	**n/N**	**% resistance**	**95% CI**	**n/N**	**% resistance**	**95% CI**	**n/N**	**% resistance**	**95% CI**	**N**	**% resistance**	**95% CI**
Gram positive															
Staphylococcus aureus	99/ 107	92.5%	(85.8-96.7)	11/ 38	28.9%	(15.4-45.9)	0/4	0.0%	(0-60.2)	25/ 79	31.6%	(21.6-43.1)	5/ 36	13.9%	(4.7-29.5)
Streptococcus agalactiae	0/ 134	0.0%	(0-2.7)	0/0	Not applicable		0/ 35	0.0%	(0-10.0)	6/ 66	9.1%	(3.4-18.7)	0/ 0	Not applicable	
Streptococcus pneumoniae	0/ 25	0.0%	(0-1.4)	0/0	Not applicable		0/0	Not applicable		0/ 10	0.0%	(0-30.8)	0/ 0	Not applicable	
Gram negative															
Escherichia coli	1/4	25.0%	(0.6-80.6)	14/ 55	25.5%	(14.7-39.0)	6/ 82	7.3%	(2.7-15.2)	2/ 3	66.7%	(9.4-99.2)	4/ 17	23.5%	(6.8-49.9)
Klebsiella pneumoniae	2/3	66.7%	(9.4-99.2)	8/ 17	47.1%	(23.0-72.2)	8/ 24	33.3%	(15.6-55.3)	0/ 0			5/ 9	55.6%	(21.2-86.3)
Acinetobacter spp.	6/10	60.0%	(26.2-87.8)	38/ 48	79.2%	(65.0-89.5)	16/49	32.7%	(19.9-47.5)	4/ 4	100.0%	(39.8-100)	17/ 31	54.8%	(36.0-72.7)

3.2. Discussion

GBS (incidence 2.57 per 1 000 live births) was the most commonly-identified bacterial pathogen in neonates and young infants overall in our setting, followed by *S. aureus* (incidence: 1.18) and *E. coli* (incidence: 0.89). These three pathogens contribute to 63.5% (444/699) of all sepsis cases in infants <90 days of age. Our observation differs from previous reports from both HICs, where GBS and *E.coli* are the most commonly isolated pathogens[40, 64-66], and LMICs where *S. aureus* and *E. coli* dominate, but very few GBS cases are identified[63, 67, 69-71]. HIV-exposed infants had a higher than expected risk of developing sepsis than HIV-unexposed infants (OR 1.52, 95% CI: 1.31 to 1.78) in sensitivity analysis based on the prevalence of HIV-exposure of cases with known HIV-results (39.5%).

The incidence (3.36 per 1000 live births) of culture-confirmed EOD in Soweto was 2-5 fold greater compared to that reported from HICs (0.59-1.6)[40, 59, 61, 62]. Also, the incidence (per 1000 live births) was midway between the wide range reported from LMICs, which are reported from a low of 0.93[233] to as high as 9.8[234]. The incidence of EOD in our study, however, likely represents an underestimate since up to 10% of women delivering at CHBAH receive intrapartum antibiotics[229], which may reduce the sensitivity of blood cultures for diagnosing bacterial sepsis. Additionally, almost 65% of GBS cases present on the day of birth and 21% between 24- 48 hours of age[235]. If newborn infants are not adequately investigated in epidemiological studies, the role of GBS could be significantly under-estimated.

GBS, *S. aureus* and *E. coli* contributed 76.2% of the LOD cases, and meningeal

involvement was present in 31.6% of cases, including 66.2% due to GBS. Despite the higher rate of meningitis in this age group than in EOD, the case fatality ratio was lower for LOD (14.2%) than EOD (17.0%). A higher case-fatality rate in EOD cases (25%- 55.5%) compared to cases presenting between days 7 and 60 of life (10% - 26.1%) has also been noted in several other studies conducted in Africa [54, 236]. GBS, *E. coli* and *S. aureus* were also the most common bacteria isolated between 29 and 90 days of life, and together contributed 52.4% of the cases, and the case fatality ratio was 13.3%.

Pathogens causing LOD and YI-CAS differ within and between regions. Waters et al[237] have reported that *S. aureus* (14.6%), *S. pneumoniae* (13.9%), *E. coli* (11%) and GBS (6.9%) are the predominant pathogens in Africa, but *Klebsiella* species (33.5%), *S. aureus* (10.0%), *E. coli* (9.0%) and *Pseudomonas* species (9.0%) are most common in South East Asia, whilst GBS contributed to only 3.0% of isolates in South East Asian studies[237]. Other meta-analyses[70, 71], also report *S. aureus* (11-22%) as a dominant pathogen, with *E.coli* (10%) and *Klebsiella* species (10%) also being among the leading pathogens, however, the sequence of pathogens varies between regions, as does the contribution of other common bacteria including *S. pneumoniae and S. pyogenes*. GBS was uncommonly isolated (<1%- 11%[70, 71]) in post-neonatal septic infants.

For all young infants (0-90 days old) with HIV exposure results available, 39.5% were HIV-exposed. Due to the large proportion of missing data, a sensitivity analysis was performed. HIV-exposed infants had a 1.52 fold greater odds of developing culture-confirmed sepsis than HIV-unexposed infants (95% CI: 1.31- 1.78).

When stratified by age categories, this observation was noted in EOD, LOD and YI-CAS categories (Table 5).

When the impact of HIV-exposure on specific pathogens was considered, GBS sepsis was prominent, as the number of cases with available HIV results was large (n=221, 89.8% of all GBS cases), and the proportion HIV-exposure of overall cases was high (47.1%). The incidence of GBS sepsis in HIV-exposed infants was 3.63/ 1000 live births, compared to 1.74/1000 live births in HIV-unexposed infants. A study conducted in Belgium also reported high incidence of GBS sepsis in HIV-exposed infants(15.5/1000 live births)[238]. The risk for GBS-EOD, GBS-LOD and GBS-YI-CAS were 1.55-fold (95% CI: 1.06 – 2.26); 2.99-fold (95% CI: 1.78- 5.08) and 2.41-fold (95% CI: 1.14 – 5.09) greater in HIV-exposed infants than HIV-unexposed infants respectively. This analysis was not conducted for other pathogens, due to high number with missing HIV-exposure results.

The HIV-infection status of HIV-exposed infants was not assessed in this study, as the standard of care during the study period did not include neonatal HIV-PCR testing. HIV-PCR results were not available for the majority of HIV-exposed study participants. During the study period, however, the early vertical transmission rate of HIV to infants in Gauteng province decreased from 15.8% in 2004 to 8.1% in 2008 due to successful implementation of PMTCT[239], therefore the actual number of HIV-infected infants would have been low. The recent implementation (in 2015/ 2016) of HIV-PCR testing at birth of HIV-exposed newborns in South African public health care facilities will mitigate this limitation in future, similar studies.

This study was conducted in a secondary-tertiary care facility in a relatively well-resourced urban area of South Africa. Residents of Soweto, South Africa have good access to health care facilities for labour and delivery and for care of sick neonates and post-neonatal infants, however, the neonatal mortality rate (14.6 per 1000 live births) is higher than expected for an upper- middle-income country (10 per 1000 live births)[240]. A recent study in Soweto reported that the neonatal mortality rate for Soweto could be as high as 20 per 1000 births[206].

Many neonates and young infants who present with episodes of sepsis have other potentially life-threatening conditions, including complications of prematurity. The mortality rates reported in this chapter are crude mortality rates, and might have been compounded by underlying conditions unrelated to the sepsis.

This study restricted enrolment to sepsis cases which most likely to be acquired either vertically or from the community. Nosocomial sepsis cases and probable contaminants were excluded. Physiological features and exposures to potential pathogens varies remarkably during the neonatal period, and stratification of culture-confirmed cases into recognized categories (e.g. EOD, 0-6 days; N-LOD, 7-27 days; YI-LOD, 28- 90 days) is vital to understanding pathogenesis of disease and implementing appropriate preventative interventions or treatments. Despite the majority of births in Soweto being facility-based, this study may have under-estimated the burden of culture-confirmed sepsis in neonates and young infants, as up to 40% of child-deaths in Soweto occur at home[15], many of whom are not assessed at a health care facility prior to death.

Despite the most commonly-isolated bacterial pathogen, GBS, being susceptible to

first-line antibiotics, antimicrobial resistance is high for other bacteria, notably high resistance of *S. aureus* to penicillin/ ampicillin (92.5%). This was higher than the resistance noted in a recent meta-analysis (86%)[70]. All the *S. aureus* isolates tested at CHBAH were, however, sensitive to third generation cephalosporins. Empiric antibiotic treatment does not adequately cover this pathogen.

K. pneumoniae (33.3%) and *Acinetobacter* spp. (32.7%) displayed high proportions with resistance to third generation cephalosporins, which was, however, lower than reported in a meta-analysis (57% for *Klebsiella* spp. and 53% for *Acinetobacter* spp.) [70]. The number of isolates included in this surveillance study was small, and ongoing monitoring of resistance patterns in common bacterial pathogens is essential to inform local antimicrobial use policies.

The main limitation of this study was the retrospective identification of cases and data collection, which led to missing important data for many of the young infants, which in turn limits robustness of data presented. Nevertheless, the results of this project contribute to the limited data available describing aetiology of community acquired sepsis in young infants from LMIC. In order to reduce infection-related neonatal deaths globally, prevention and management of neonatal infections in community settings must be prioritized[241]. This diverse spectrum of pathogens, especially reported in young infants 28-90 days of age, warrants consideration of a multifactorial approach to prevention and treatment of sepsis in young infants. Resource and expertise constraints make antenatal screening and IAP/risk based management an unachievable goal in many LMIC countries. Other strategies to reduce sepsis in young infants, including cord care and maternal immunisation

should be explored.

Vaccines for administration to pregnant women to prevent GBS sepsis in their newborns are in clinical trial phase of development[66, 214, 242]. *S. aureus*, which was the second-most commonly isolated bacteria from young infants in our setting, colonises almost 50% of newborns within days of birth[103] and is implicated in at least half of the omphalitis cases reported in a study from Pakistan[105]. Cord care with 4% chlorhexidine solution significantly reduces omphalitis[243, 244] and neonatal mortality[244], and should be recommended especially in LMICs where *S. aureus* sepsis is common.

4. RISK FACTORS FOR NEONATAL SEPSIS

Characterization of the risk factors associated with neonatal sepsis may shed light on potential prevention strategies. Major risk factors associated with early-onset neonatal group B streptococcal (GBS) disease in high-income countries have been well described and include prolonged membrane rupture, intrapartum fever, preterm delivery, and maternal colonisation with the pathogen[245-247]. Factors associated with other pathogens known to cause neonatal sepsis are less well described. Prematurity (<33 weeks), prolonged rupture of membranes and intrapartum fever have also been identified as risk factors for invasive *Escherichia coli* early-onset sepsis in the United States[111]. Although data from LMICs are limited, there is evidence that prolonged rupture of membranes, preterm delivery, intrapartum fever, birth asphyxia and labour complications are important contributors to early onset sepsis[248, 249,250].

In this chapter, the maternal and neonatal factors associated with very early-onset neonatal sepsis (VEOD, 0-2 days), late-onset community acquired sepsis (3-28 days), and perinatal death in a cohort of more than 8000 mother-baby dyads delivered at a large public hospital in Soweto, South Africa are described by performance of a secondary analysis of the Prevention of Perinatal trial (published paper is in Appendix 4).

4.1. Statistical analysis

Maternal variables considered to be potential risk factors included: age (<20 years, ≥

20 years), prenatal care, start date of prenatal care (<21 weeks' gestation, ≥ 21 weeks' gestation), first birth, previous neonatal death, HIV status, diagnosed urinary tract infection during pregnancy, anemia, pre-gestational or gestational diabetes mellitus, underlying cardiac conditions, and syphilis infection. Intrapartum variables included: referral for delivery at CHBAH from a satellite birthing clinic, total number of vaginal exams, duration of membrane rupture, duration of labour (approximated by the time between randomization on admission for active labour, and delivery), intra-amniotic infection (defined as any two of the following: fever, uterine tenderness, foul smelling vaginal discharge, maternal tachycardia, fetal tachycardia), multiple birth, mode of delivery (vaginal, emergency caesarean due to a failed vaginal birth in a woman with a prior caesarean, emergency caesarean for other reasons), fever, meconium stained amniotic fluid (MSAF) and receipt of intrapartum antibiotics (documented administration of antibiotics after admission for active labour and excluding women with emergency caesarean deliveries who received antibiotics less than 1 hour before delivery). Infant variables analyzed included: birth weight, gestational age (preterm: <37 weeks, term: ≥ 37 weeks) and infant gender.

Univariate and multivariable analyses were performed using logistic regression in SAS Version 9.2 (Carey, NC, USA). Multivariable models were evaluated starting with all factors that were significant at $p<0.15$ in univariate analysis and dropping non-significant factors using stepwise backwards selection. Collinearity of independent variables was evaluated. All two-way interactions in final multivariable models were evaluated. Throughout, two-sided P-values <0.05 were considered statistically significant and 95% confidence intervals were calculated. Relative risks were approximated by odds ratios because sepsis and perinatal death endpoints

were rare in this population. [251]

4.2. Results

Median maternal age was 26 years (interquartile range 22-31), the median gestational age at delivery was 39 weeks (range 23 to 44 weeks) and 3.8% (309/8129) infants were born prematurely (<37 weeks gestation). Eight percent (615/8129) of newborns had low birth weight (<2500 grams), including 0.4% (35/8129) who had a very low birth weight (<1500 grams). HIV results were available for 99% (7902/8011) women, of whom 26.4% (2090/7902) were HIV-infected. Only 11 women (0.1%) had not attended antenatal clinic. Women with planned caesarian section deliveries were excluded from continuing on the trial at arrival in labour, however, 23% (1867/8011) of women delivered by emergency caesarean section. On medical history and examination, 10.7% (854/8011) women had a urinary tract infection and 21.0% (1678/8011) were hypertensive during late pregnancy.

Of the 8129 infants born to trial participants, 26 were stillborn and 4.8% (388/8129) required resuscitation just after birth. In addition to the 26 stillbirths, 56 infants died during the neonatal period (by 28 days of life), 80.4% (45/56) of whom died during the first week of life. Fifteen of these newborns died within hours of delivery, mainly due to severe birth asphyxia or extreme prematurity. The median age at death was 0 days (interquartile range: 0-28). The leading causes of death in live-born neonates were birth asphyxia (n=30, 52.6%), and respiratory distress of the newborn (n=7; 12.5%).

Intrapartum antibiotics (IAP) were administered to 10.5% (837/ 8011) women. Of these women, 60% (498/837) received intravenous ampicillin (1g 6 hourly), the majority (>66%) in combination with metronidazole. Two women received penicillin. The median duration between administration of the first dose of IAP and delivery was 14.8h (IQR: 7.8-26.7h). The most commonly documented reasons for IAP administration were prolonged rupture of membranes (410/837 or 49%); purulent or offensive vaginal discharge (90/837 or 11%); and preterm labour (48/837 or 6%). Only 62.9% (449/714) of women with prolonged rupture of membranes (>18 hours) received IAP.

Hospital policy stipulates administration of IAP to women who deliver between 26 and 33 weeks gestation, however, only 49.4% (42/85) of women eligible for IAP for prematurity were adequately treated. Women who present with at least one sign of chorioamnionitis should receive IAP as per hospital policy, however, only 10.0% (129/1295) of these women received IAP for the following indications: Meconium-stained amniotic fluid ((MSAF: 92/1200 or 7.7%); offensive or purulent vaginal discharge (32/79 or 40.5%); maternal tachycardia (8/22 or 36.3%); fetal tachycardia (8/32 or 25%); maternal fever (12/17 or 70.6%) or uterine tenderness (1/1 or 100%).

Of the 8103 live-born newborns, 289 fulfilled the definition (see Table 2 in methods chapter) for early-onset sepsis (35.7/ 1000 live births) and 34 fulfilled criteria for late onset disease (4.2/1000 live births). Ten percent (29/289) of the early-onset cases were culture confirmed with GBS being the most common pathogen (16/29) (Table 13).

Table 13: Characteristics of neonates with sepsis, Soweto[252]

Characteristic[a] (% unless otherwise stated)	Clinical very early-onset sepsis (n=260)	Culture-confirmed very early-onset sepsis[b] (n=29)	Clinical late-onset sepsis (n=14)	Culture-confirmed late-onset sepsis[b] (n=20)
Birthweight (%)				
<1500 g	4	0	0	0
1500-2499 g	15	21	7	10
≥2500g	80	79	93	85
Preterm delivery (% <37 weeks)	15	10	0	5
Apgar at 5 minutes (median, IQR)	8 (6-9)	9 (7-10)	10 (9-10)	10 (10-10)
Resuscitation at birth (%)	39.6%	37.9%	7.1%	0%
Median age at onset (days, IQR)	0 (0-0)	0 (0-1)	17 (13-22)	17 (14-24)
Median length of hospital stay (days, IQR)	7 (5-10)	10 (8-13)	6 (3-7)	16 (6-22)
Infant HIV infection status[c]	5	10	7	10
Clinical signs of sepsis				
Respiratory distress (%)	98	79	86	55
Hypotension (%)	9	14	36	10
Pyrexia or hypothermia (%)	9	14	29	30
Abdominal feeding problems (%)	4	17	36	50
Bleeding diathesis (%)	0	0	0	0
Lethargy or irritability (%)	17	28	43	45
Central nervous system signs (%)	21	17	21	20
Laboratory signs of sepsis				
Abnormal white blood cell count (%)	20	3	50	25
Abnormal neutrophil count (%)	19	7	57	20
Low platelet count (%)	28	17	7	5
Elevated C reactive protein (%)	54	52	43	60
Elevated CSF white blood cell count (%)	1	7	0	30
Outcome (%)				
Survived	94	90	93	100
Died by day 28 of life	6	10	7[d]	0[c]

[a]All variables had fewer than 1% missing values except for HIV infection status which had 9% (28/323) missings
[b]Among the culture confirmed cases, 2 early onset and 7 late onset cases were identified by CSF
[c] Infant HIV infection status was determined by PCR at 6 weeks of age
[d]2 infants in each of these groups died shortly after the 28 day period (days 29-32 of life), before hospital discharge

Almost 59% (20/34) of late onset cases were culture-confirmed, with *Escherichia coli* being identified as the leading cause (n=9), followed by GBS (n=5). The median age at onset for late onset cases was 14 days (interquartile range: 11-20) and only 1 late onset case was born prematurely.

Fifteen percent (1205/8129) of neonates were exposed to meconium-stained amniotic fluid (MSAF) and 3.3% (40/ 1205) of these were diagnosed with meconium aspiration syndrome. Meconium aspiration syndrome was present in 10% (30/289) of early onset cases, and 11.3% (8/71) of perinatal deaths, but none of the neonates with late onset disease.

In univariate analysis, low birthweight, preterm delivery and maternal factors, including primiparity, emergency caesarian delivery, prolonged labour and pre-gestational diabetes mellitus, were associated with increased risk of early-onset sepsis (Table 14). Fewer factors including emergency caesarian delivery and primiparity were associated with late onset sepsis in univariate analysis, but only MSAF (aOR 2.4; 95% CI: 1.1-5.0) remained significantly associated with LOD in multivariable analysis (Table 15). When analysis was restricted to culture-confirmed cases of early and late onset sepsis (29 early onset and 20 late onset cases), MSAF (aOR 2.7, 95% CI: 1.4-5.0), low birthweight (aOR 2.5, 95% CI: 1.2-5.4), and first birth (aOR 2.1, 95% CI: 1.2-3.8) were associated with increased sepsis risk on multivariable analysis (Table 16).

Table 14: Factors associated with very early-onset sepsis, PoPS trial cohort[252]

Characteristic	Cases, % exposed (n=289[a])	Non-cases, % exposed (n=7840[a])	OR§ (95% CI)	aOR[b] (95% CI)
Maternal				
Age <20 years	18.0	13.6	1.4 (1.0-1.9)	
Pre-gestational diabetes mellitus	0.7	0.2	3.4 (0.8-14.9)	
First birth	59.5	42.2	2.0 (1.6-2.6)	1.8 (1.4-2.3)
Multiple gestation	5.2	2.8	1.9 (1.1-3.3)	
Rupture of membranes ≥ 18 h	13.5	8.8	1.6 (1.1-2.3)	
Duration of labour (h, quartiles)[c]			P=0.003	
<2.9h	21.1	24.7	REF	
2.9-5.4h	18.3	24.9	0.9 (0.6-1.3)	
5.5-9.4h	27.7	25.5	1.3 (0.8-2.3)	
>=9.5h	32.9	24.8	1.6 (1.1-2.2)	
Number of vaginal exams (quartiles)			P=0.0005	P=0.02
<2	6.3	8.7	REF	REF
2-3	31.5	39.8	1.1 (0.7-1.9)	1.1 (0.6-1.8)
4	18.2	19.0	1.3 (0.8-2.3)	1.2 (0.7-2.1)
>4	44.1	32.5	1.9 (1.1-3.1)	1.6 (1.0-2.7)
Mode of delivery				
Emergency caesarean due to prior cesarean	0.7	2.0	0.4 (0.1-1.6)	
Emergency caesarean for other reasons	29.1	21.1	1.5 (1.2-2.0)	
Vaginal delivery	70.2	77.0	REF	
Intrapartum antibiotics[d]	14.5	10.4	1.5 (1.0-2.0)	
Meconium stained amniotic fluid	30.5	14.3	2.6 (2.0-3.4)	2.8 (2.2-3.7)
Infant				
Birthweight				
<1500 g	3.8	0.3	14.3 (6.9-29.5)	6.5 (2.4-17.3)
1500-2499 g	15.7	7.3	2.5 (1.8-3.4)	1.8 (1.1-2.9)
≥2500g	80.5	92.3	REF	
Preterm delivery (<37 weeks')	14.2	3.4	4.7 (3.3-6.6)	2.6 (1.4-4.8)

[a]All variables had fewer than 15 missing values except for birthweight which had 49 missings (47 among non-cases)

[b] Univariate: all factors with p< 0.15 are shown; all were entered into multivariable model, only those with p<0.05 are shown. Due to missing values, the multivariable model included 287 cases and 7793 non-cases. Apgar score was excluded from model because of overlap with endpoint. Only 1 case of intrapartum fever was recorded so the variable could not be analyzed

[c] Duration of labour is approximated by time between randomization into the PoPS trial (which for most mothers coincided with onset of true labour) and delivery

[d] IAP defined as any duration, excluding cesarean prophylaxis (<1 hour of antibiotics before delivery)

Perinatal death was associated with pregnancy-induced hypertension, pre-gestational diabetes mellitus and anaemia on univariate analysis. Preterm delivery (aOR 5.9, 95% CI: 3.1-11.2), primiparity (aOR 1.7, 95% CI: 1.0-2.7), and MSAF (aOR 2.1, 95% CI: 1.2-3.7) had the strongest risk of death on multivariable analysis. (

Table 17).

Primiparous women were more likely than multiparous women to have characteristics, including prolonged labour and higher number of per vaginal examinations; which were associated with poor neonatal outcomes. Characteristics were compared between primiparous- and multiparous women, and illustrated in Figure 4. In general the magnitude of the differences were small, except for young maternal age (<20 years), labour duration longer than the median length, and the related number of vaginal exams (Figure 4).

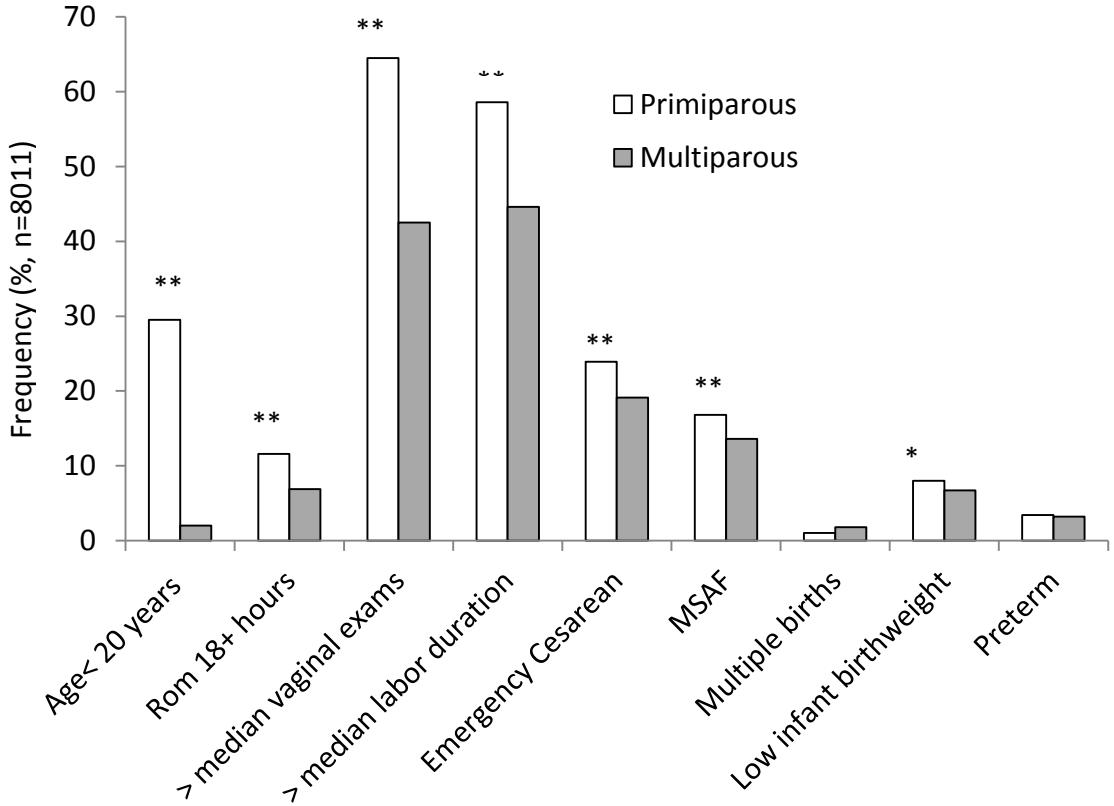


Figure 4: Characteristics of primiparous and multiparous women in the Prevention of Perinatal Sepsis Trial, delivering at Chris Hani Baragwanath Academic Hospital, Soweto, South Africa.

Groups differing by p<=0.001 are denoted by **; group differing by P<0.05 are denoted by *. ROM=rupture of membranes; MSAF=meconium stained amniotic fluid

Table 15: Factors associated with community-acquired late onset sepsis

Characteristic	**Cases, % exposed (n=34)**	**Non-cases, % exposed (n=8087)**	**OR[a] (95% CI)**	**aOR[a] (95% CI)**
Maternal				
First birth	58.8	42.7	1.9 (1.0-3.8)	
Mode of delivery		P=0.054		
Vaginal delivery	58.9	76.8	REF	
Emergency caesarean due to prior cesarean	2.9	1.9	2.0 (0.3-15.1)	
Emergency caesarean for other reasons	38.2	21.3	2.3 (1.2-4.7)	
Male sex	67.7	52.2	1.9 (0.9-3.9)	
Meconium stained amniotic fluid	29.4	14.8		2.4 (1.1-5.0)

[a] Univariate: all factors with p< 0.15 are shown; all were entered into multivariable model, only those with p<0.05 are shown. Apgar score was excluded from model because of overlap with endpoint. Only 1 case of intrapartum fever was recorded so the variable could not be analyzed

Table 16: Factors associated with culture confirmed very early or late onset sepsis

Characteristic	Cases, % exposed (n=49[a])	Non-cases, % exposed (n=8080[a])	OR[b] (95% CI))	aOR[b] (95% CI)
Maternal				
Age <20 years	22.5	13.7	1.8 (0.9-3.6)	
Mode of delivery			P=0.04	
Vaginal delivery	61.2	76.8	REF	
Emergency caesarean due to prior cesarean	2.0	1.9	2.2 (1.2-3.9)	
Emergency caesarean for other reasons*	36.7	21.2	1.3 (0.2-9.9)	
Duration of labour (h, quartiles)[c]				
<2.9h	12.2	24.7	REF	
2.9-5.4h	18.4	24.7	1.5 (0.5-4.2)	
5.5-9.4h	28.6	25.6	2.3 (0.9-5.9)	
>=9.5h	40.8	25.0	3.3 (1.3-8.2)	
Number of vaginal exams (quartiles)				
<2	10.2	8.6	REF	
2-3	34.7	39.5	0.7 (0.3-2.0)	
4	8.2	19.0	0.4 (0.1-1.4)	
>4	46.9	32.9	1.2 (0.5-3.2)	
First birth	63.3	42.7	2.3(1.3 -4.1)	2.1 (1.2-3.8)
Meconium	30.6	14.7	2.6 (1.4-4.7)	2.7 (1.4-5.0)
Infant				
Birthweight				
<2500 g	16.7	8.0	2.3 (1.1-4.9)	2.5 (1.2-5.4)
Preterm delivery (<37 weeks')	8.2	3.8	2.3 (0.8-6.3)	
Male	63.3	52.2	1.6 (0.9-2.8)	

[a] All variables had fewer than 15 missing values, except for birthweight which had 49 missings including 1 case

[b] Univariate: all factors with p< 0.15 are shown; all were entered into multivariable models, only those with p<0.05 in the final model are shown. The final multivariable model is based on 8080 observations (48 cases). Apgar score was excluded from model because of overlap with endpoint. Only 1 case of intrapartum fever was recorded so the variable could not be analyzed.

c Duration of labour is approximated by time between randomization into the PoPS trial (which for most mothers coincided with onset of true labour) and delivery.

Table 17. Factors associated with perinatal death

Characteristic	Cases, % exposed (n=71[a])	Non-cases, % exposed (n= 8047[a])	OR[b] (95% CI))	aOR[b] (95% CI)
Maternal				
Pregnancy-induced hypertension	29.6	21.0	1.6 (0.9-2.6)	
Pre-gestational diabetes mellitus§	1.4	0.2	6.7 (0.9-51.4)	
Anaemia	14.1	8.7	1.7 (0.9-3.4)	
First birth	56.3	42.7	1.7 (1.1-2.8)	1.7 (1.0-2.7)
Meconium stained amniotic fluid	25.4	14.8	2.0 (1.1-3.4)	2.1 (1.2-3.7)
Infant				
Preterm delivery (<37 weeks')	16.9	3.6	5.4 (2.9-10.1)	5.9 (3.1-11.2)

[a]All variables had fewer than 9 missing values, except for birthweight which had 49 missings, including 35 among cases. Because of this, birthweight was only evaluated in the univariate models.

[b] Univariate: all factors with p< 0.15 are shown; all were entered into multivariable models, only those with p<0.05 in the final model are shown. Apgar score was excluded from model because of overlap with endpoint. Only 1 case of intrapartum fever was recorded so the variable could not be analyzed. Cell sizes for diabetes mellitus were too small to use in the multivariable model

4.3. Discussion

Neonatal sepsis contributes significantly to neonatal deaths globally; however, we present one of the first evaluations of risk factors associated with all-cause neonatal sepsis and perinatal death in a large cohort of facility-based births in sub-Saharan Africa.

As this was a secondary analysis of a clinical trial cohort, it was neither a population-based sample nor representative of all births at the facility. The rate of stillbirths on the PoPS trial (3.2/ 1000) was lower than the reported rate for CHBAH (26.9/1000, unpublished data 2005-2008, Department of Obstetrics and Gynaecology, CHBAH, Perinatal stats. Prof E. Buchmann), as any woman with an intrauterine death confirmed prior to PoPS randomisation in labour ward complex was excluded from continuation in the trial. The neonatal mortality rate (NMR, 6.9/1000 live births) recorded for the PoPS trial is lower than the national NMR (14.6/1000 live births). It has been reported that up to 40% of children in Soweto die at home, despite good access to health care facilities[15]. One limitation of the PoPS trial was the lack of an active follow-up contact at the end of the neonatal period to record maternal and infant adverse events and outcomes, especially ones which occurred outside CHBAH. The lower-than-expected NMR in the PoPS trial was probably due to inadequate identification and recording of neonatal deaths, rather than improved care or benefit of the intervention offered.

Despite the perinatal mortality rate (PMR, stillbirths plus neonatal deaths in first week of life; 8.7/1000 births) in PoPS trial participants being an under-estimation of the actual PMR at CHBAH, factors associated with perinatal death were assessed.

Poor neonatal outcomes (death and sepsis) are often associated with prolonged duration of labour and prolonged rupture of membranes[40, 46], which are in turn associated with higher number of vaginal examinations and higher rate of caesarean section deliveries. These features are more commonly observed in primiparous women, who in our setting, are also often young (<20 years).

A rigorous sepsis definition, including both clinical and laboratory signs, was used. Preterm birth and low birthweight are recognized risk factors for neonatal sepsis, and our findings corroborate what has been reported from high-income countries [111, 200, 247, 253]. Two thirds of *E. coli* –confirmed neonatal sepsis in the USA is diagnosed in preterm infants[111]. Although effective prevention strategies for preterm delivery remain elusive, improved access to prenatal care, and improved maternal nutrition and health in LMICs might be of some benefit in reducing low birthweight.

Primiparity and MSAF were each associated with approximately double the risk of sepsis and death in our neonatal trial participants. Meconium is rarely passed by preterm foetuses, and MSAF is an indicator of in-utero foetal distress[254]. MSAF is associated with intra-amniotic infection and early- and late onset sepsis in LMIC[248], and HIC settings[255]. It is unclear whether the presence of meconium in the amniotic fluid stimulates growth of pathogens, or whether foetal infection is the stress-trigger which leads to passing of meconium in utero[254, 256]. Our rigorous clinical sepsis definition captured some non-infectious syndromes including meconium aspiration syndrome.

Primiparity has occasionally been identified as a risk factor for neonatal sepsis,

usually only in univariate analysis or studies with limited intrapartum variables for evaluation[257]. Women delivering their first baby usually have slower progress of cervical dilatation during labour than multiparous women[258], leading to a longer duration of labour. Additionally, duration between rupture of membranes and delivery is longer in primiparous than multiparous women. We collected information on duration of labour, duration between membrane rupture and delivery and total number of vaginal exams received; all known risk factors for sepsis; and noted that small differences in these characteristics exist between primiparous and multiparous women. Primiparous women are additionally more likely to have a combination of these characteristics, which in turn led to first birth remaining robustly associated with sepsis and perinatal death in multivariable analysis.

On multivariable analysis, factors associated with neonatal sepsis were also associated with perinatal death. CHBAH is the referral center for satellite clinics in Soweto, and the threshold for referral of women with pregnancy- or labour-complications is low. We noted that there was an over-representation of several “high risk” maternal conditions, for example pregnancy induced hypertension in our trial participants, yet none of these conditions were associated with increased risk of perinatal death. The outcome diagnosis for 11% of deaths in foetal/ infant trial participants was documented as meconium aspiration syndrome, which may partially explain our observation with MSAF. Despite a pathogen not always being isolated, intrauterine and neonatal infections appear to contribute to a significant proportion of perinatal deaths.

Intrapartum antibiotic exposure has been established to be effective at preventing

GBS-VEOD, however, is ineffective at reducing GBS-LOD and early-onset *E. coli* sepsis[111, 259]. The lack of a protective effect of IAP against neonatal sepsis and death during the perinatal period in our setting observed on multivariable analysis is consistent with other studies which assessed all-cause neonatal sepsis and similarly found no impact of IAP[253].

Challenges with identification of features of chorioamnionitis in a busy, regularly under-staffed labour ward has been highlighted by our observations that only half of women in preterm labour and 10% of women with at least one sign of chorioamnionitis received adequate risk-based IAP prior to delivery. Even in HIC settings, reduction of GBS-VEOD by risk based strategies was lower with culture-based GBS screening strategies[203].

Several limitations were present in our analysis. Despite using a clinical sepsis definition which was more rigorous than that used in previous studies, the non-specific signs and symptoms present in neonatal sepsis reduced the specificity of the definition and other conditions including birth asphyxia may have been misclassified as sepsis. Additionally, despite our large sample size (>8000 mother-baby dyads), the sample size for some endpoints, including late onset disease and culture-confirmed sepsis, was small, therefore limiting our ability to evaluate some variables for associations. Most importantly, however, our trial participants differed from the overall CHBAH birth cohort. Trial enrolment practices restricted the number of preterm births on the trial compared to CHBAH births. Additionally, the population delivering at CHBAH had more high-risk deliveries than in the community clinics in Soweto, leading to these deliveries being over-represented in our trial cohort. Despite

this, adequate preterm, low birthweight and very low birthweight infants were included in the trial cohort to allow us identify these factors as significant risks for the endpoints under consideration. Additionally CHBAH births capture 75% of all births in Soweto, so despite them not being representative of the full population, they capture a large portion. Moreover, the over-representation of high risk maternal conditions was considered a benefit to evaluate whether or not these conditions were associated with increased risk of sepsis or perinatal death.

Most previous sepsis risk-factor analyses have been conducted in HICs and have focused on pathogen-specific endpoints. Few previous studies in LMICs have had a robust comparison group for formal risk factor assessment. Despite the South African Soweto population being wealthier and having better access to facility-based deliveries and maternity and neonatal care than many other settings in sub-Saharan Africa, these features also facilitated a rigorous evaluation of prenatal, intrapartum and demographic risk factors in an urban African setting with high maternal HIV prevalence.

The difficulties demonstrated of implementing a risk-based IAP strategy in a busy labour ward in a LMIC and the failure of IAP to prevent all-cause neonatal sepsis prevention highlights the challenges of neonatal sepsis prevention in LMICs. Primiparity and MSAF are easily-identifiable risk factors for neonatal sepsis and death and further exploration of the mechanisms behind the association noted is warranted. Additionally, promising antenatal strategies aimed at preventing neonatal sepsis, including maternal immunisation need to be investigated.

5. MATERNAL HIV INFECTION AND VERTICAL TRANSMISSION OF PATHOGENIC BACTERIA

Vertical transmission of potentially pathogenic bacteria from a colonised mother to her infant is a pre-requisite for vertically- acquired bacterial sepsis in neonates, therefore a clear understanding of the burden of maternal colonisation and timing and risk factors for vertical transmission is essential to assist in reducing neonatal sepsis.

There is a paucity of data on the impact of maternal HIV exposure on clinical- or pathogen-specific burden of neonatal sepsis[249]. Aberrations of the immune system in HIV-infected women may lead to reduced transplacental transfer of antibodies to the foetus in-utero[260, 261] thereby potentially increasing neonatal susceptibility to sepsis. HIV infection has not been associated with differences in the prevalence of GBS vaginal colonisation compared to HIV-uninfected women[262].

HIV-infection in childhood led to a reversal of decades of improvement in child survival[263]. PMTCT programs have been successfully implemented in many countries, including South Africa, and under-5 childhood mortality rates have reduced, however, HIV-exposed uninfected infants and children have significantly higher morbidity and mortality than HIV-unexposed infants and children. This effect is related to poorer maternal health including a compromised immunological status, increased maternal mortality and early weaning of HIV-exposed infants[264-268].

In this chapter, the impact of maternal HIV infection on: (i) the prevalence of maternal vaginal colonisation with pathogens associated with neonatal sepsis; (ii) vertical transmission of bacterial pathogens to the newborn; and (iii) sepsis rates during the

very early (0-2 days) and late neonatal (3-27 days) periods is described (published paper attached: Appendix 5).

5.1. Statistical considerations

Propensity score matching was used to reduce bias with respect to important covariates for each defined endpoint and was used to evaluate maternal HIV infection as a risk factor for neonatal sepsis[269,270]. We chose propensity score matching because covariates considered key to endpoints under consideration differed between HIV-infected and HIV-uninfected women. Standard modeling approaches provide estimates even in the absence of an appropriate comparison population.

For maternal colonisation, the following covariates were considered:

- Maternal age categorized as either >25 years of ≤ 25 years
- Rupture of membranes (ROM) prior to swab collection and
- Antibiotic use in week before delivery

For vertical transmission, the following covariates were considered:

- prolonged ROM (>18 hours),
- antibiotic use in the week preceding labour or intrapartum antibiotic administration
- number of per vaginal examinations performed during labour

For early- and late onset neonatal sepsis, the following covariates were considered:

- mode of delivery,
- gestational age at delivery,
- prolonged rupture of membranes,
- maternal fever ≥38.0ºC,
- intrapartum antibiotic use,
- known maternal GBS colonisation and
- urinary tract infections (UTIs).

Multivariable logistic regression was used to estimate the propensity score for each mother or infant (as appropriate). The propensity score is the conditional probability of the mother being HIV-infected given the variables in the model. Each HIV-infected maternal participant was matched to an HIV-uninfected maternal participant with the closest propensity score. Balance for each covariate included in the relevant propensity score model was evaluated for HIV-infected and HIV-uninfected maternal participants and the model that achieved the best balance was used.

The model for maternal colonisation was limited to mothers with known HIV-infection status included in the colonisation cohort for whom a swab result was known. The vertical transmission model was limited to mothers colonised with GBS, *E. coli* or *K. pneumoniae*, who had a known HIV-infection status, delivered vaginally, and whose newborn had a microbiologic swab with a result.

For the VEOD and LOD sepsis endpoints, two additional comparison groups were considered: HIV-unexposed, uninfected (HUU) versus HIV-exposed, uninfected

(HEU) neonates; and HEU versus HIV-infected neonates. Propensity score models were constructed as above, with the appropriate outcome variable for the comparison, balancing on the variables listed above for VEOD and LOD.

As CD4+ cell count results were only available for 34.7% (725/2090) of HIV-infected women, maternal CD4+ cell count was not included as a covariate.

An unadjusted analysis on the total cohort was conducted to understand the influence of covariates associated with HIV exposure or infection status on the endpoints evaluated.
Proportions were compared using Chi-square tests and relative risks and 95% confidence intervals were used to assess HIV as a risk factor. The denominator for incidence was per 1000 births. SAS version 9·1 was used for analyses.

5.2. Results

The HIV infection results were available for 98.5% (7894/8011) of maternal participants, 26.5% (2090/ 7894) of whom were HIV-infected. CD4+ results were only available for 34.7% (725/2090) HIV-infected mothers, the majority of whom (64.4%; 467/725) were enrolled between June 2006 and October 2007. Of these women, just over half (50.8%; 368/725) had a CD4+ count of >350 cells/ mm^3, 27.4% (199/725) had CD4+ count between 200 and 350 cells/mm^3 and 21.8% (158/725) had CD4+ counts <200 cells/mm^3. According to national guidelines, antiretroviral treatment to prevent mother-to-child transmission of HIV was single-dose of nevirapine (sd-NVP)

to mother at onset of labour and to newborn infants until 2007, when triple antiretroviral therapy including stavudine, lamivudine and nevirapine was offered from 34 weeks gestation. Of the 2090 HIV-infected women enrolled in PoPS trial, 92.3% (1929/2090) received PMTCT medication. Most (97.6%, 1882/1929) HIV-infected women received sd-NVP and 2.4% (46/1929) received triple antiretroviral therapy which was initiated a mean of 151 (range 1 to 1095) days prior to delivery. There were 2130 HIV-exposed infants born to 2090 HIV-infected mothers.

HIV-infected – and HIV-uninfected maternal participants had several significantly different baseline demographic and clinical characteristics noted. HIV-infected women were older ($p<0.0001$), more likely to have urinary tract infections (13% vs. 10%, $p<0.0001$), anaemia (hemoglobin <10 mg/dl, 13% vs. 7%, $p<0.0001$), tuberculosis (1.5% vs. 0.2%, $p<0.0001$), received antibiotics before labour-onset (30% vs. 21%, $p<0.0001$) and received antibiotics during labour (12% vs. 10% for women with vaginal delivery, $p<0.0001$) than HIV uninfected women (Table 18). Although maternal fever was uncommon (0.2% overall), HIV-infected women were significantly more likely to have fever recorded than HIV-uninfected women (0.7% vs. 0.05%, $p<0.0001$). HIV-infected women also had higher frequencies of non-elective caesarian sections (26% vs. 23%, $p=0.006$); and preterm deliveries (5.9% vs. 3.1%, $p<0.0001$) than HIV-uninfected women. The median birth weight of HIV-exposed newborns was significantly lower than HIV-unexposed newborns (3050 grams vs. 3160 grams; $p<0.0001$) (Table 18).

Table 18 Maternal and newborn demographic and clinical characteristics stratified by maternal HIV status[230]

Characteristic	Overall	HIV uninfected mothers n(%)	HIV infected mothers n(%)	p-value
Mothers	**N=8011**	**n=5812**	**n=2090**	
Median age in years (range)	26 (12-51)	26 (12-49)	27 (14-51)	<0.0001
Median parity (range)	1 (0-9)	1 (0-9)	1 (0-7)	<0.0001
Median gravidity (range)	2 (1-10)	2 (1-10)	2 (1-8)	<0.0001
Median gestational age (range)	39 (23-44)	39 (24-44)	39 (23-44)	
Medical history (%)				
Urinary tract infection	854 (11)	573 (10)	275 (13)	<0.0001
Haemoglobin <10 mg/dl	692 (9)	420 (7)	264 (13)	<0.0001
Gestational diabetes	20 (0.2)	17 (0.3)	3 (0.1)	0.25
Maternal tuberculosis	42 (0.5)	10 (0.2)	32 (1.5)	<0.0001
Received antibiotics during pregnancy (%)	1856 (23)	1218 (21)	617 (30)	<0.0001
Intrapartum antibiotics (IAs) in women with vaginal delivery. (% of IAs)	629/6137	436/4494 (10)	189/1554 (12)	0.006
Antibiotics in 7 days prior to delivery	475 (6)	318 (5)	152 (7)	0.48
Intrapartum fever (%)	17 (0.2)	3 (0.05)	14 (0.7)	<0.0001
Prolonged rupture of membranes of ≥ 18 hours at delivery (%)	710 (9)	525 (9)	178 (9)	0.48
Unbooked/No prenatal care (%)	11 (0.1)	6 (0.1)	3 (0.1)	0.64
Meconium stained liquor (%)	1200 (15)	925 (16)	256 (12)	<0.0001
Delivery (%) [1]				
Spontaneous vaginal delivery	6136 (77)	4494 (77)	1554 (74)	0.007
Emergency caesarian section	1874 (23)	1318 (23)	536 (26)	0.006
Vaginal vacuum/Forceps	165 (2)	116 (2)	49 (2)	0.31
Number of per vaginal exams during labour				
<3	3020	2213 (38)	760 (36)	0.17
≥3	4991	3599 (62)	1330 (64)	
Newborn characteristics	**n= 8129**	**n= 5886**	**n= 2130**	
Female gender	3873 (47)	2796 (48)	1028 (48)	0.57
Twin birth (%)	118 (1)	74 (1)	40 (2)	0.038
Pre-term[2]	313 (4)	181 (3)	124 (6)	<0.0001
Median birth weight (range) (grams)	3130 (480-5630)	3160 (670-5630)	3050 (480-4690)	<0.0001
Median Apgar 5 mins (range)	10 (0-10)	10 (0-10)	10 (0-10)	0.27
Outcome				
Stillborn	26 (<0)	19 (<0)	7 (<0)	0.97
Died shortly after delivery	15 (<0)	12 (<0)	3 (<0)	0.56
Surviving newborn admitted to neonatal ward	788 (10)	578 (10)	210 (10)	0.80

[1] Includes mode of delivery for all singletons and first-born twins only
[2] Note: Term infants: 7811 overall; 5701 HIV unexposed infants, 2005 HIV exposed infants

As per routine standard of care, infant HIV PCR testing was undertaken at a median of 42 days of age (1- 347 days). HIV PCR results were only collected by study staff for 64.2% (1367/2130) of HIV-exposed infants, of whom 8.2% (112/1367) were HIV-infected. Chlorhexidine interventional wipes had no impact on vertical transmission rate of HIV (65/713 [9.1%] vs. 47/654 [7.2%] PCR positive in chlorhexidine vs. control arms, p=0.19). HIV-infected infants had a lower birth weight (median 2995 vs. 3100 grams; p=0.001), lower median gestational age (38 vs. 39 weeks; p=0.005), and higher frequency of exposure to meconium stained liquor (21% vs. 11%; p=0.002) during labour than HIV-exposed uninfected infants.

5.2.1. **Vaginal colonisation and vertical transmission of genital-tract bacteria**

A total of 5146 women were enrolled onto the colonisation cohort, 99.1% (5099/5146) of whom had known HIV-infections status. There were 3752 HIV-uninfected and 1347 HIV-infected women on the colonisation cohort. All but one of the HIV-infected women on the colonisation cohort were included in the matched subset analysis for assessment of vaginal colonisation (1346 HIV-uninfected and 1346 HIV-infected women). In the total cohort, HIV-uninfected women were more likely to be colonised with GBS than HIV-infected women (22.0% vs. 17.1%, p=0.0002), however HIV-uninfected women were less likely to be colonised with *E. coli* (43% vs. 47%, p=0.0385) than HIV-infected women. There was no difference in colonisation with *K. pneumoniae* (8% vs. 7%, p=0.2189) between HIV-uninfected and HIV-infected women in total cohort (Table 19).

In the matched analysis, HIV-uninfected women were more likely to be colonised with GBS (23% vs. 17%, p=0.0002) and *K. pneumoniae* (10% vs. 7%, p=0.008) than HIV-infected women. The difference seen in *E. coli* colonisation in total cohort was not observed in the matched analysis (45% vs 47%, p=0.37) (Table 19).

Vertical transmission of GBS and *K. pneumoniae* was not affected by HIV-exposure status of the infant. The rate of vertical transmission of *E. coli* was, however, higher in HIV-exposed than HIV-unexposed neonates in the total cohort (60% vs. 53%; p=0.0066) and matched-subset (60% vs. 52%, p=0.015) populations (table 20).

Table 19 Prevalence of bacterial vaginal colonisation in HIV-infected and –uninfected women during labour.[230]

	Total colonisation cohort			**Matched subset**		
	HIV negative n=3752 n (%)	**HIV positive n=1347 n (%)**	**p-value**	**HIV negative n=1346 n (%)**	**HIV positive n=1346 n (%)**	**p-value**
Group B streptococcus (GBS)						
GBS colonised mothers (total)	824 (22)	231 (17)	0.0002	307 (23)	230 (17)	**0.0002**
Escherichia Coli (E.coli)						
***E. coli* colonised mothers (total)**	1624 (43)	677 (47)	0.0385	603 (45)	626 (47)	**0.37**
Klebsiella pneumoniae (K. pneumoniae)						
***K pneumoniae* colonised mothers (total)**	**301 (8)**	**94 (7)**	**0.2189**	**132 (10)**	**94 (7)**	**0.008**

Table 20: Vertical transmission of pathogenic bacteria from mother to newborn stratified by maternal HIV-infection status[230].

	Total colonisation cohort			**Matched subset**		
	HIV negative n (%)	**HIV positive n (%)**	**p-value**	**HIV negative n (%)**	**HIV positive n (%)**	**p-value**
Group B streptococcus (GBS)						
Total number infants born vaginally to GBS colonised mother.	648	181		177	177	
GBS colonised vaginally born newborn[1]	372/641 (58)	93/179 (52)	0.1467	94/174 (54)	90/175 (51)	**0.63**
Escherichia coli (E. coli)						
Total number infants born vaginally to *E.coli* colonised mother.	1277	460		449	449	
***E. coli* colonised, vaginally born newborn**	665 (53)	269 (60)	0.0066	232 (52)	263 (60)	**0.015**
Klebsiella pneumoniae (K. pneumoniae)						
Total number infants born vaginally to *K. pneumoniae* colonised mother.	241	73		69	69	
***K. pneumoniae* colonised, vaginally born newborn[2]**	**74 (31)**	**20 (27)**	**0.5887**	**20 (29)**	**19 (27)**	**0.85**

[1]Nine infants born vaginally to GBS colonised mothers did not have swab collected/ processed for GBS: 7 HIV unexposed and 2 HIV exposed infants. [2] One infant born to an HIV-uninfected mother colonised by *K pneumoniae* did not have swab processed.

5.2.2. **Maternal HIV-infection status and sepsis within three days of age (VEOD)**

Of the 8129 infants born to enrolled mothers, 26 were stillborn and 3.6% (290/8103) of the live-born infants were hospitalised for sepsis within the first three days of life. The majority of these infants (89.0%, 258/290) had clinical sepsis, 29 had culture-confirmed sepsis, and 3 early neonatal deaths (<3 days of age) which did not fulfill the clinical sepsis definition were included after review by neonatal panelists.

HIV-exposure did not affect the incidence of clinical or overall early-onset disease in the total cohort or matched sub-set analysis. The incidence of culture-confirmed early-onset disease was, however, 3.3 fold greater among HIV exposed- compared to HIV-unexposed infants in the matched analysis (p=0.05), and 1.67 fold increased in the total-cohort analysis (p=0.167; Table 21). The incidence of VEOD (per 1000 births) in HIV-exposed infants born to mothers with known CD4+ results was inversely associated with the immunological status of the mother (CD4+ cells/mm^3<200: 75.9; 200 to 350: 40.2; >350:19.0; p=0.0065, trend is linear).

The incidence (per 1000 births) of clinically-confirmed early-onset sepsis in HIV-infected newborns (102) was significantly higher than in HIV-exposed, PCR-negative newborns (20.4, p=0.033) in the matched subset analysis and in the total cohort analysis (116 vs. 17.6; p<0.0001; table 22). HIV-exposed, uninfected newborns had a significantly lower incidence of early onset disease (20.6) than HIV-unexposed newborns (33.7; p=0.046) in the matched subset analysis and in the total cohort analysis (21.5 vs. 38.0; p=0.004, table 23). Culture-confirmed early-onset disease was unaffected by HIV-exposure in HIV-uninfected newborns in the matched-subset

and total-cohort analysis (Table 23).

5.2.3. **Maternal HIV-infection status and neonatal sepsis between days 4 and 28 (LOD)**

The incidence of LOD was unaffected by HIV-exposure in the matched subset analysis and total cohort analysis (table 21). HIV-infected neonates had a higher incidence of overall LOD (26.8) than HIV-exposed, PCR-negative neonates (5.6; p=0.042) neonates in the total-cohort, with a similar trend observed in the matched analysis (30.6 vs. 10.2; p=0.62, table 22). HIV-exposure did not affect the incidence of LOD in HIV-uninfected infants in the matched or total-cohort analysis (table 23).

The incidence of LOD was inversely associated with maternal immunological status (CD4+ <200 cells/mm^3: 19.0; 200 to 350 cells/mm^3: 10.0; and >350 cells/mm^3: 8.1; p=0.55).

Table 21 Impact of in-utero HIV exposure on incidence of very early- (within 3 days) and late-onset (between 4 and 28 days of age) neonatal sepsis

	TOTAL				MATCHED SUBSET		
VERY EARLY ONSET DISEASE (VEOD)	**Total n=8129 n (rate; 95% CI)**	**HIV unexposed n=5886 (rate; 95% CI)**	**HIV exposed n=2130 (rate; 95% CI)**	**P-value (HIV unexposed vs. HIV exposed)**	**HIV unexposed n=2054 n (rate)**	**HIV exposed n=2054 n (rate)**	P-value (HIV unexposed vs. HIV exposed)
Culture-confirmed sepsis	29 (3.6; 2.4, 5.1)	18 (3.1; 1.8, 4.8)	11 (5.2; 2.6, 9.2)	0.165	3 (1.5; 0.3, 4.3)	10 (4.9; 2.3, 8.9)	**0.05**
Group B streptococcus (GBS)	16 (2.0; 1.1, 3.2)	12 (2.0; 1.0, 3.6)	4 (1.9; 0.5, 4.8)		2 (1.0; 0.1, 3.5)	4 (1.9; 0.5, 5.0)	
Escherichia coli	2 (0.2; 0.03, 0.9)	0 (0, 0.63)	2 (0.9; 0.1, 3.4)		0	1 (0.5)	
Staphylococcus aureus	2 (0.2; 0.03, 0.9)	1 (0.2, (0.004, 0.9)	1 (0.5; 0.01, 2.6)		0	1 (0.5; 0.0, 2.7)	
Klebsiella pneumoniae	1 (0.1; 0.0003, 0.7)	0 (0, 0.63)	1 (0.5; 0.01, 2.6)		0	1 (0.5; 0.0, 2.7)	
Other	8 (1.0; 0.4, 1.9)	5[a] (0.8; 0.3, 2.0)	3[b] (1.4; 0.3, 4.1)		3 (1.5; 0.3, 4.3)	3 (1.5; 0.3, 4.3)	
Clinical sepsis only	258(31.7; 28.0, 35.8)	202 (34.3; 29.8, 39.3)	55 (25.8; 19.5,33.5)	0.056	57 (27.8; 21.1, 35.8)	51 (24.8; 18.5,32.5)	**0.56**
Deaths within 3 days of age[c]	3 (0.4; 0.1, 1.1)	3 (0.5; 0.1, 1.5)	0 (0, 1.7)	0.570	1 (0.5; 0.0, 2.7)	0 (0.0; 0.0, 1.8)	**1.000 (Exact)**
Overall	290 (35.7; 31.7,39.9)	223 (37.9; 33.2, 43.1)	66 (31.0; 24.0,39.3)	0.117	61 (29.7; 22.8, 38.0)	61 (29.7; 22.8,38.0)	**1.000**

LATE ONSET DISEASE (LOD)							
Culture-confirmed (CC) cases	20 (2.5; 1.5, 3.8)	12 (2.0; 1.1, 3.6)	7 (3.3; 1.3, 6.8)	0.310	3 (1.5; 0.3, 4.3)	6 (2.9; 1.1, 6.3)	**0.51(EXACT)**
Group B streptococcus	5[d] (0.6; 0.2, 1.4)	3 (0.5; 0.1, 1.5)	1 (0.5; 0.01, 2.6)				
Escherichia coli	8 (1.0; 0.4, 1.9)	6 (1.0; 0.4, 2.2)	2[e] (0.9; 0.1, 3.4				
Staphylococcus aureus (S. aureus)	2 (0.2, 0.03, 0.9)	2 (0.3; 0.04, 1.2)	0 (0, 1.7)				
Klebsiella spp.	2 (0.2; 0.03, 0.9)	0 (0, 0.63)	2[f] (0.9; 0.1, 3.4)				
Other	3 (0.4; 0.1, 1.1)	1[g] (0.2; 0.004, 0.9)	2[h] (0.9; 0.1, 3.4)				
Clinical sepsis only	14 (1.7; 0.9, 2.9)	8 (1.4; 0.6, 2.7)	6 (2.8; 1.0, 6.1)	0.167	4 (1.9; 0.5, 5.0)	7 (3.4; 1.4, 7.0)	**0.37**
Overall	**34 (4.82; 2.9, 5.8)**	**20 (3.4; 2.1, 5.2)**	**13 (6.1; 3.3, 10.4)**	**0.095**	**7 (3.4; 1.4, 7.0)**	**13 (6.3; 3.4, 10.8)**	**0.18**

[a] *Enterococcus faecalis* (x3*), Acinetobacter baumanii* x2;
[b] Streptococcus viridans (x2), *Acinetobacter lwoffii*.
[c] Early deaths (<3 days) which did not fulfill EOD-CC or EOD-Clinical sepsis definitions, but included as EOD after panel review
[d] One case of LOD GBS with unknown HIV result
[e] One case co-infected with *S. aureus*
[f] One co-infected with *S. aureus*, one with *Enterococcus faecium*
[g] Streptococcus species
[h] Enterococcus faecalis x 2 cases

Table 22 Incidence of very early and late onset sepsis in HIV exposed HIV-infected (HIV+) and HIV exposed uninfected (HEU) neonates.

	Sepsis categorization	**TOTAL infants with HIV PCR results**			**MATCHED SUBSET**		
		HIV+ n=112 n (rate; 95% CI)	**HEU n=1253 n (rate; 95% CI)**	**P-value**	**HIV+ n= 98 n (rate; 95% CI)**	**HEU n=98 n (rate; 95% CI)**	P-value
Very EARLY ONSET SEPSIS	Culture-confirmed	2 (17.9; 2.2, 63.0)	5 (4.0; 1.3, 9.3)	0.107	0 (0; 0, 36.9)	1 (10.2, 0.3, 55.5)	**1.00**
	Clinical sepsis only	13 (116; 63.3,190.3)	22 (17.6; 11.0, 26.5)	<0.0001	10(102.0, 50.0,179.7)	2 (20.4, 2.5, 71.8)	**0.033**
	Overall	15 (134; 76.9, 211.3)	27 (21.5; 14.2, 31.2)	<0.0001	10 (102.0; 50.0,179.7)	3 (30.6, 6.4, 86.9)	**0.08**
LATE ONSET SEPSIS	Culture-confirmed	2 (17.9; 2.2, 63.0)	5 (4.0; 1.3, 9.3)	0.107	2 (20.4; 2.5, 71.8)	0 (0; 0, 36.9)	**0.50**
	Clinical sepsis only	1 (8.9; 0.2, 48.7)	2 (1.6; 0.2, 5.8)	0.227	1 (10.2; 0.3, 55.5)	1 (10.2; 0.3, 55.5)	**1.00**
	Overall	**3 (26.8; 5.6, 76.3)**	**7 (5.6; 2.2, 11.5)**	**0.042**	**3 (30.6; 6.4, 86.9)**	**1 (10.2; 0.3, 55.5)**	**0.62**

Table 23 Impact of maternal HIV infection on very early and late onset neonatal sepsis in HIV-exposed, uninfected (HEU) and HIV-unexposed, uninfected (HUU) neonates.

	Sepsis categorization	**TOTAL HIV uninfected infants**			**MATCHED SUBSET**		
		HUU n=5867 n (rate; 95% CI)	HEU n=1253 n (rate; 95% CI)	P-value	HUU n=1216 n (rate; 95% CI)	HEU n= 1216 n (rate; 95% CI)	P-value
VERY EARLY ONSET SEPSIS	Culture-confirmed	21 (3.6; 2.2, 5.5)	5 (4.0; 1.3, 9.3)	0.797	4 (3.3; 0.9, 8.4)	4 (3.3; 0.9, 8.4)	1.000
	Clinical sepsis only	202 (34.4; 30.0, 39.4)	22 (17.6; 11.0, 26.5)	0.002	37 (30.4; 21.5, 41.7)	21 (17.3; 10.7, 26.3)	0.034
	Overall	223 (38.0; 33.3, 43.2)	27 (21.5; 14.2, 31.2)	0.004	41 (33.7; 24.3, 45.5)	25 (20.6; 13.3, 30.2)	0.0459
LATE ONSET SEPSIS	Culture-confirmed	12 (2.0; 1.1, 3.6)	5 (4.0; 1.3, 9.3)	0.203	3 (2.5; 0.5, 7.2)	5 (4.1; 1.3, 9.6)	0.726
	Clinical sepsis only	8 (1.4; 0.6, 2.7)	2 (1.6; 0.2, 5.8)	0.692	2 (1.6; 0.2, 5.9)	2 (1.6; 0.2, 5.9)	1.000
	Overall	20 (3.4; 2.1, 5.3)	7 (5.6; 2.2, 11.5)	0.306	5 (4.1; 1.3, 9.6)	7 (5.8; 2.3, 11.8)	0.563

Very early onset sepsis: 0-2 days
Late onset sepsis: 3-<28 days

5.3. Discussion

To our knowledge, this was the first study to have reported on the impact of maternal HIV infection on vertical transmission of potentially pathogenic bacteria to newborns, and on the relative incidence of clinical- and culture-confirmed early-onset and late-onset disease between HIV-exposed and HIV-unexposed neonates. During the conduct of this trial, the maternal HIV prevalence was 29%, and antiretroviral therapy to prevent mother- to child transmission of HIV offered as part of routine care was changed in latter part of the trial from single dose nevirapine to triple therapy. HEU neonates have been thought to be at higher risk of developing neonatal sepsis due to impaired maternal transfer of antibody[260], data from this study do not corroborate this speculation. On the contrary, VEOD rates were marginally lower in HEU than HUU in the total cohort and matched subset analysis; and no difference was observed for LOD rates between these groups.

Overall 20.7% (1055/5099) of women with known HIV results enrolled on to the colonisation cohort were colonised vaginally with GBS at the time of labour. This GBS colonisation rate observed in this trial are in line with what has been reported from other countries during the third trimester of pregnancy (10-30%)[191, 271, 272].

A study from Malawi reported a non-significantly lower rate of GBS colonisation in HIV-infected- versus HIV-uninfected pregnant women (19.4% vs. 21.7%, p=0.32)[262]. HIV-infected women with CD4+ counts >500cells/mm^3 did, however, have a significantly higher GBS colonisation rate than HIV-infected women with CD4+ counts <200cells/mm^3. (AOR 2.55; 95% CI: 1.10-5.90). Co-trimoxazole use was

dismissed as a confounding variable for differences in colonisation observed between HIV-infected women with high and low CD4+ counts[262]. Our observation of higher GBS colonisation in HIV-uninfected women than HIV-infected women in both matched and total cohorts may be indicative of differences in characteristics not controlled for in the propensity score analysis which may have reduced GBS colonisation in HIV-infected women, for example antibiotic use earlier in pregnancy. On the whole, rates of maternal colonisation and vertical transmission for leading sepsis pathogens did not differ considerably between HIV-infected and HIV-uninfected pregnant women.

The number of culture-confirmed VEOD cases in this cohort was small, which limited our power to detect a difference in this endpoint. A marginal trend showing increased risk of culture-confirmed early-onset sepsis in HIV-exposed compared to HIV-unexposed neonates was observed. Surveillance of a larger cohort of culture-confirmed sepsis cases was conducted in Soweto during the time period when the PoPS trial was being conducted and the results have been described in chapter 3 (overall sepsis) and chapter 6 (GBS) of this thesis. Although CD4+ testing of participants on the PoPS study was limited, we did note that HIV exposed neonates born to severely immunocompromised mothers (CD4+ <200 cells/mm^3) were at higher risk of developing VEOD and LOD than those born to mothers with CD4+ >350 cells/mm^3. Additionally, in the total cohort, there was an increased risk of VEOD and LOD in HIV-infected neonates compared to HIV-exposed, uninfected neonates, with a similar trend noted in the matched analysis, emphasizing the requirement to further reduce mother-to child transmission of HIV.

The clinical sepsis endpoint has lower specificity than a culture-confirmed sepsis endpoint.

Several other studies have reported an increase in morbidity and mortality in HIV-exposed infants, including during early infancy[268, 273], which contrasts with our results. Almost 30% of deaths in African infants occur during the first 2 months of life [266]. HIV-infected infants have a very high mortality rate of 309.1 to 420.8 per 1000, compared to HIV-exposed, uninfected (72.5 to 98.7/1000) and HIV-unexposed (48.0 to 91.0/ 1000)[266, 265]. Disease progression in African HIV-infected infants is rapid, with 85% of HIV-infected infants progressing to AIDS by the age of 6 months[274].

Our observation that some risk factors commonly associated with VEOD, including UTIs and prolonged ROM, were more prevalent in HIV-infected women, highlights the importance of adjusting for maternal factors when assessing the association between maternal HIV-infection and neonatal sepsis.

Our use of propensity score methods, which allowed us to select an appropriate referent group with similar risk factors for each outcome evaluated, while still remaining blinded, was a strength of the study. When the conclusions obtained from analysis of the entire cohort differed from the matched subset, we believe that the matched subset analysis offers the most valid comparison between the groups with regard to whether maternal-infection status, newborn HIV-exposure- or HIV-infection status is responsible for the differences between groups in the applicable comparisons. The differences between the overall cohort and matched subset analyses highlights the dangers of drawing conclusions about the impact of HIV

exposure on neonatal morbidity (or mortality) without appropriately adjusting for other factors.

There were several limitations to our study. Due to standard of care practices in antenatal clinics at the time that our study was conducted, CD4+ counts were only available for 34.7% of HIV-infected women. Additionally, HIV-PCR testing of HIV-exposed infants was performed at well-baby clinics in Soweto at 6 weeks of age. The study protocol included follow up to 4 weeks of age only, and contact with some maternal participants was not successful after the neonatal period. HIV-PCR results were only available for 64% of HIV-exposed infants. What is reassuring, however, is that HIV-exposed infants with and without HIV-PCR results were similar for many demographic and delivery variables. Vertical HIV transmission, which was not impacted by chlorhexidine interventional wipes[229], was 8.2% in trial participants, which is similar to what was reported in other settings (10.3%)[274] which utilized the same PMTCT regimen (single dose Nevirapine to mother and newborn).

Our trial enrollment procedures and inclusion and exclusion criteria skewed enrollment away from inclusion of preterm infants on the PoPS trial (4% in PoPS vs. 15% in CHBAH) (Personal communication: SC Velaphi). Prematurity is a recognized risk factor for neonatal sepsis, and our trial may have under-estimated the true rates in our setting. Nevertheless, a review by Thaver et al[24], reported the incidence of clinical sepsis in infants less than 60 days of age in developing countries to be 49 to 170 per 1 000 and 5.5/1000 for culture-confirmed sepsis. Although the incidence of neonatal sepsis observed in our study was close to the lower bound of this incidence for clinical sepsis (33.4 per 1000), we report a similar incidence of culture-confirmed sepsis (5.9 per 1000). The lower incidence of clinical sepsis rates in our study may

have been impacted by more stringent criteria used in our study for diagnosing "clinical sepsis".

In conclusion, reducing neonatal morbidity and mortality has been identified as a key focus area for public health under MDG4 which aimed to reduce under-5 mortality by two-thirds compared to 1990 levels[275]. Whilst there was a high incidence of neonatal sepsis in our study population, including among the highest reported incidence rates of invasive GBS sepsis from any LMIC, HIV-exposure does not appear to increase the risk of either very early- or late-onset neonatal sepsis, except among HIV-infected newborns and possibly among newborns of severely immunocompromised mothers. Consequently, we do not predict the HIV prevention roll-out in sub-Saharan Africa will result in important reductions in neonatal sepsis in this region, and sepsis specific interventions such as GBS vaccines may hold most promise in reducing neonatal infection-associated mortality.

6. GROUP B STREPTOCOCCUS SEPSIS IN YOUNG SOUTH AFRICAN INFANTS

In 2012, almost 10% of possible severe bacterial infection in neonates globally were fatal, leading to an estimated 680 000 neonatal deaths[129]. There is a paucity of data on pathogen-specific causes of neonatal sepsis from low-middle income countries (particularly during the first day of life) and whether maternal HIV-exposure increases susceptibility to severe neonatal bacterial infections.

The global incidence of Group B *Streptococcus* (GBS) was 0.53/ 1000 live births for 2000 to 2011, making GBS a leading cause of sepsis. A meta-analysis of data mainly from high-income countries demonstrated marked inter- and intra-regional variation in the incidence of EOD (within 7 days of birth) ranging from 1.21 (95% CI: 0.5-1.91) in Africa to 0.02 (95% CI: 0-0.07) in South-East Asia[77]. Maternal GBS colonisation is a major risk factor for EOD, with maternal GBS colonisation prevalence varying from 13.4% in South-East Asia to 20.9% in Africa[276]. Vertical transmission of GBS colonisation has been reported as approximately 50% from many countries including thee USA[48], Turkey[277] and Pakistan[278] but lower transmission rates (38.7%) has been reported from Tanzania[279] and higher vertical transmission rate (61%) has been reported from Sweden[280]. The marked variability of GBS- EOD is inconsistent with the smaller difference noted in maternal colonisation prevalence and vertical transmission. Maternal HIV-infection has not been associated with higher prevalence of GBS colonisation[229, 262] except among women with CD4+ lymphocyte >500 cells/mm^3 [262].

The development of a trivalent GBS polysaccharide-protein conjugate vaccine (GBS-

CV) targeted at pregnant women, aims to enhance transplacental transfer of capsular antibody to the foetus, which could protect against EOD and LOD[66, 281]. Knowledge of the incidence of invasive GBS- EOD and –LOD is warranted from LMICs to determine which serotypes should be included in a vaccine, and to prioritize vaccination against GBS disease.

In this chapter, the clinical and microbiological epidemiology, incidence and serotype distribution of invasive GBS disease among young infants in a setting of high maternal HIV-infection prevalence are described. Additionally, the potential impact of a trivalent GBS-CV in reducing the national burden of invasive GBS disease was estimated. (Published paper attached: appendix 6)

6.1. Statistical considerations

The incidence was calculated as cases per 1000 live births. Population denominators for HIV-infected and HIV-uninfected pregnant women were determined by utilizing administrative live birth data from CHBAH and community clinics for Soweto and HIV-prevalence survey data for the study period. There are well-established patient-referral protocols in Soweto, which would have limited the chance that neonates, especially newborns, resident outside the hospital catchment area were admitted to CHBAH. A sensitivity analysis of incidence was undertaken to account for those GBS cases in which maternal HIV-status was unknown. For the overall (2004-2008) and annual incidence calculations, we attributed the same prevalence of HIV-exposure to cases in whom HIV-exposure status was unknown as was present in those for whom it was

documented, with the alternate sensitivity analysis assuming either all cases with unknown maternal HIV-status were HIV-exposed or conversely HIV-unexposed.

Live birth population data from 2012 for South Africa (1,168,403)[282], national antenatal HIV prevalence data[283], and GBS-disease surveillance results were utilized to estimate the annual national number of invasive GBS cases and deaths in HIV-unexposed and HIV-exposed infants. Based on the conservative assumption that infants born at- or prior to 33 weeks gestation would not be protected against invasive GBS disease by vaccination of pregnant women with a GBS-CV[284] and adjusting for the proportion of serotypes included in the current investigational trivalent GBS-CV (serotypes Ia, Ib and III) and a proposed pentavalent vaccine (addition of serotypes II and V), we estimated the number of annual vaccine-preventable invasive GBS cases and deaths in South Africa. As vaccine efficacy has not yet been established for GBS-CV, hypothetical vaccine efficacy assumptions of 75% for the overall population, 85% for HIV-unexposed and 65% for HIV-exposed infants (based on the lower immunogenicity of the trivalent GBS-CV in HIV-infected pregnant women[284]) were utilized for these estimates.

Proportions were compared using the χ^2 test and Fisher's exact test as appropriate and the Wilcoxon rank sum test (non-parametric) was applied for continuous variables. Univariate analysis was performed to determine factors associated with mortality due to invasive GBS disease. Throughout, two-sided p-values ≤0.05 were considered statistically significant and 95% confidence intervals (CIs) were calculated. Analyses were conducted using Stata/IC 13.0 (StataCorp, 4905 Lakeway Drive College Station, Texas 77845 USA).

6.2. Results

In a 5-year period (2004-2008), a total of 389 invasive GBS cases were identified among young infants under 90 days old. Fifty-five percent (214/ 389) were GBS-EOD cases. Complete medical records were unavailable for 17 (10 EOD, 7 LOD) cases. These cases were included in incidence calculations but were excluded from further analysis. The overall incidence (per 1 000 live births) of invasive GBS disease was 2.72 (95% CI: 2.46-3.01), with GBS-EOD incidence of 1.50 (95% CI: 1.30-1.71) and GBS-LOD incidence of 1.22 (95% CI: 1.05-1.42). The incidence of both GBS-EOD and GBS-LOD were similar across years (table 24).

Overall, 26.6% of cases were born prematurely (<37 weeks gestation), including 29.8% of GBS-EOD and 22.3% of GBS-LOD cases. The majority (69.4%) of preterm births occurred at or prior to 33 weeks gestation; including 63.0% of GBS-EOD and 80.6% of GBS-LOD cases (Table 25). Among GBS-EOD cases, 64.5% (138/214) had a positive culture from samples obtained on the day of birth (median age: 0 days; IQR 0 to 1; Figure 5). The second week of life contributed the most number of cases after the early-onset period, with 44% of GBS-LOD cases presenting during this time period (GBS-LOD median age 16 days; IQR: 11, 29; Figure 5). Infants with GBS-LOD were 5.57 (95% CI: 3.50-8.90) fold more likely to present with meningitis (61.7%) compared to GBS-EOD cases (22.4%; p<0.0001).

Table 24 Group B Streptococcus disease in young infants stratified by year and HIV-exposure status, Soweto, South Africa. 2004-2008[235]

	Overall	Early onset disease				Late onset disease				Overall
		Overall	**HIV-unexposed**	**HIV-exposed**		**Overall**	**HIV-unexposed**	**HIV-exposed**		**HIV exposed vs. HIV unexposed**
		n, incidence* (95% CI)	**n, incidence* (95% CI)**	**n, incidence* (95% CI)**	**RR (95% CI)**	**n, incidence* (95% CI)**	**n, incidence* (95% CI)**	**n, incidence* (95% CI)**	**RR (95% CI)**	**Risk ratio (95% CI)**
2004	2.54	38, 1.51	22, 1.25	16, 2.11	1.70	26, 1.03	12, 0.68	14, 1.85	2.72	2.06
	(1.95;3.24)	(1.07;2.07)	(0.78; 1.88)	(1.21;3.43)	(0.83; 3.38)	(0.67;1.51)	(0.35;1.19)	(1.01;3.10)	(1.17; 6.44)	(1.22; 3.47)
2005	3.09	45, 1.70	28, 1.51	17, 2.14	1.42	37, 1.39	12, 0.65	24, 3.01	4.67	2.39
	(2.46; 3.83)	(1.24;2.27)	(1.00; 2.18)	(1.24; 3.41)	(0.73; 2.68)	(0.98;1.92)	(0.33;1.13)	(1.93; 4.48)	(2.24; 10.24)	(1.51; 3.79)
2006	2.56	40, 1.35	21, 1.01	19, 2.14	2.11	36, 1.21	12, 0.58	24, 2.70	4.67	3.04
	(2.02; 3.21)	(0.96;1.84)	(063; 1.55)	(1.29;3.33)	(1.07; 4.12)	(0.85;1.68)	(0.30;1.01)	(1.73;4.01)	(2.24; 10.24)	(1.89; 4.94)
2007	2.57	40, 1.30	26, 1.21	14, 1.52	1.26	39, 1.27	22, 1.02	18, 1.95	1.91	1.56
	(2.03; 3.20)	(0.93;1.77)	(0.79; 1.77)	(0.83;2.54)	(0.61; 2.50)	(0.90;1.73)	(0.64;1.55)	(1.16;3.08)	(0.97; 3.73)	(0.96; 2.48)
2008	2.87	51, 1.66	27, 1.26	24, 2.60	2.07	37, 1.21	16, 0.74	21, 2.28	3.06	2.44
	(2.23;3.53)	(1.24;2.18)	(0.83; 1.83)	(1.67;3.87)	(1.15; 3.73)	(0.85;1.66)	(0.43;1.21)	(1.41;3.48)	(1.52; 6.28)	(1.57; 3.80)
Overall (N)	2.72	214, 1.50	124, 1.24	90, 2.10	1.69	175, 1.22	74, 0.74	101, 2.36	3.18	2.25
	(2.46;3.01)	(1.30;1.71)	(1.03;1.48)	(1.69;2.58)	(1.28; 2.24)	(1.05;1.42)	(0.58;0.93)	(1.92;2.86)	(2.34; 4.36)	(1.84; 2.76)

Incidence* per thousand live births

Figure 5: Age distribution of young infants (0-90 days of age) with invasive group B *Streptococcus* (GBS) sepsis, Soweto, South Africa, 2004-2008

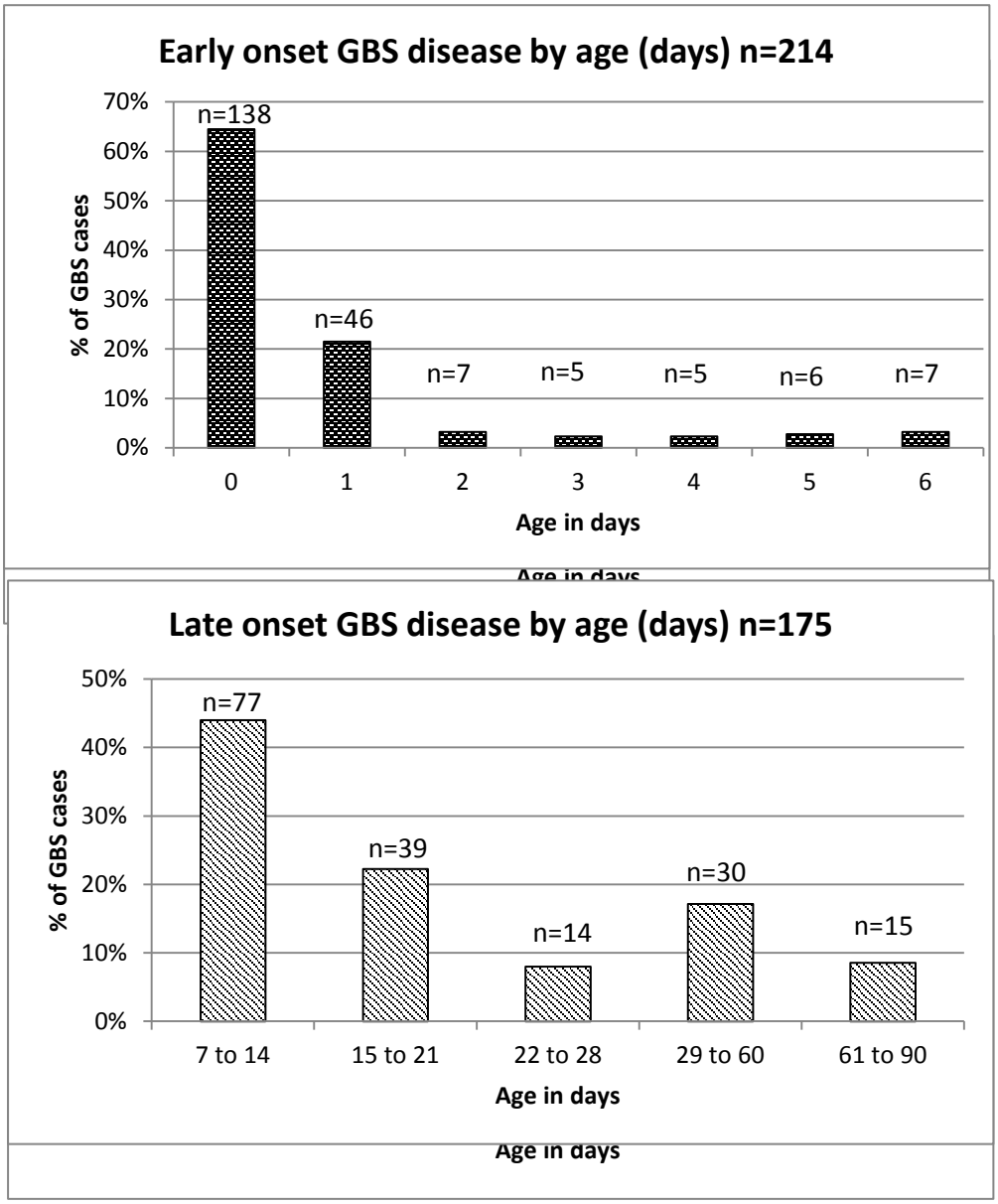


Table 25 Demographics and outcomes of young infants with invasive Group B Streptococcus disease[235]

	Overall n=372 cases	Early Onset Disease (EOD; age <7days) n= 204			Late Onset Disease (LOD; age 7-90 days) n= 168		
		Overall	HIV-unexposed	HIV-exposed	Overall	HIV-unexposed	HIV-exposed
	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)
Male	198/ 371 (53.4)	109/ 204 (53.4)	50/103 (48.5)	42/ 73 (57.5)	89/167 (53.3)	35/62 (56.5)	45/85 (52.9)
HIV exposed	161/327 (49.2)	75/179 (41.9)			86/148 (58.1)		
Birth weight (grams)							
Median (range)	2795 (605-4300)	2755 (605-4240)	2897.5 (710-4240)	2660 (605-4220)	2800 (760-4300)	2850 (820-4300)	2785 (1270-4245)
<1500g	37/322 (11.5)	24/ 186 (12.9)	10/ 94 (10.6)	10/ 71 (14.1)	13/136 (9.6)	7/55 (12.7)	5/67 (7.5)
1500- <2500g	80/322 (24.8)	48/ 186 (25.8)	22/ 94 (23.4)	20/ 71 (28.2)	32/136 (23.5)	10/55 (18.2)	20/67 (29.9)
≥2500g	205/322 (63.7)	114/ 186 (61.3)	62/94 (66.0)	41/ 71 (57.7)	91/136 (66.9)	38/55 (69.1)	42/67 (62.7)
Gestational age (weeks)							
≤33	59/320 (18.4)	34/181 (18.8)	19/96 (19.8)	11/68 (16.2)	25/139 (18.0)	10/57 (17.5)	11/68 (16.2)
>33- <37	26/320 (8.1)	20/181 (11.0)	9/96 (9.4)	8/68 (11.8)	6/139 (4.3)	2/57 (3.5)	4/68 (5.9)
≥37	235/320 (73.4)	127/181 (70.2)	68/96 (70.8)	49/68 (72.1)	108/139 (77.7)	45/57 (78.9)	53/68 (77.9)
Caesarean delivery	64/331 (19.3)	42/190 (22.1)	24/96 (25.0)	13/73 (17.8)	22/141 (15.6)	7/57 (12.3)	12/70 (17.1)
Meconium stained amniotic fluid	33/105 (31.4)	33/88 (37.5)	21/49 (42.9)	10/33 (30.3)			
Mortality							
All cases	63/372 (16.9)	30/ 204 (14.7)	11/ 102 (10.8)	12/ 74 (16.2)	33/168 (19.6)	10/62 (16.1)	18/ 85 (21.2)
Bacteremia cases	26/220 (11.8)	18/156 (11.5)	7/84 (8.3)	6/50 (12.0)	8/64 (12.5)	0/24* (0.0)	6/34* (17.6)
Meningitis cases	37/152 (24.3)	12/48 (25.0)	4/14 (28.6)	4/24 (16.7)	25/104 (24.0)	10/38 (26.3)	12/51 (23.5)
Median days of hospitalization (range)	n=307; 15 (0,216)	n=172; 13 (0, 216)	N=90; 12 (1, 216)	N=62; 13 (0, 67)	N=135; 20; (2, 66)	N=52, 16.5 (2,55)	N=67; 20 (4, 66)
Median duration of hospitalization deaths (days, range)	n=62; 1 (0, 93)	n=30; 1.5 (0,93)	N=11; 2 (0, 29)	N=12; 2 (0, 93)	N=32; 1 (0, 72)	N=10; 1 (0,44)	N=17; 2 (0, 72)

* P-value comparing HIV-exposed and HIV unexposed significant (p= 0.02)

6.2.1. **Invasive GBS disease and the impact of HIV-exposure**

The maternal HIV-infection status was available for 84.1% (327/389) infants with invasive GBS disease, 49.2% (161/327) of whom were HIV-exposed (Table 25). Maternal HIV infection status results were unavailable for 12.3% of GBS-EOD cases and 11.9% of LOD cases. PMTCT regimens administered to mother and baby were not well documented in infants' medical records (41.6%) and were therefore not analyzed in this study. HIV PCR results were available for 46/161 (28.6%) of HIV-exposed infants with GBS invasive disease, including six with GBS-EOD (all HIV PCR non-reactive) and 40 GBS-LOD cases, 8 (20%) of whom were HIV infected.

Infants with GBS-LOD were more likely to be HIV-exposed (58.1%) than infants with GBS-EOD (41.9%; p=0.004; Table 25). HIV-exposed and HIV-unexposed cases did not differ significantly overall or when stratified by EOD and LOD with regard to mode of delivery, preterm birth, low birth weight, exposure to meconium stained liquor (Table 25), prolonged rupture of membranes, or IAP during labour.

HIV-exposed infants had a 2.25 fold (95% CI: 1.84-2.76) higher incidence of invasive GBS disease (incidence: 4.46, 95% CI: 3.85-5.13) than HIV-unexposed infants (incidence: 1.98, 95% CI: 1.71-2.28, Table 26). This was evident for GBS-EOD (2.10 vs. 1.24, respectively; RR 1.69; 95% CI: 1.28-2.24) and more so for GBS-LOD (2.36 vs. 0.74, respectively; RR 3.18; 95% CI: 2.34-4.36); as well as specifically for bacteremia and meningitis (Table 26). These differences in incidence of invasive GBS disease remained significant in all sensitivity analyses for missing maternal HIV-infection status (Table 26), except for GBS-EOD when all infants in whom HIV-exposure status was unknown were assumed to be HIV-unexposed (Table 26).

Table 26 Incidence (per 1000 live births) of Invasive Group B Streptococcus sepsis (0-90 days old) stratified by in-utero HIV-exposure status[235]

	Overall		Bacteraemia		Meningitis	
HIV exposure status	**n, incidence (95% CI)** †	**RR* (95% CI)**	**n, incidence (95% CI)** †	**RR* (95% CI)**	**n, incidence (95% CI)** †	**RR* (95% CI)**
Early onset disease						
Proration of HIV-exposure‡						
Unexposed	124, 1.24 (1.03;1.48)	1.69 (1.28; 2.24)	103, 1.03 (0.84;1.25)	1.43 (1.03; 1.97)	21, 0.21 (0.13;0.32)	3.00 (1.63; 5.58)
Exposed	90, 2.10 (1.69;2.58)		63, 1.47 (1.13;1.88)		27, 0.63 (0.41;0.92)	
Unknown, assume exposed						
Unexposed	104, 1.04 (0.85; 1.26)	2.47 (1.87; 3.26)	ND		ND	
Exposed	110, 2.57 (2.11,3.09)		ND		ND	
Unknown, assume unexposed						
Unexposed	139, 1.39 (1.17, 1.64)	1.26 (0.94; 1.68)	ND		ND	
Exposed	75, 1.75 (1.38;2.19)		ND		ND	
Late onset disease						
Proration of HIV-exposure‡						
Unexposed	74, 0.74 (0.58;0.93)	3.18 (2.34; 4.36)	27, 0.27 (0.18;0.39)	3.37 (2.01; 5.73)	47, 0.47 (0.35;0.62)	3.08 (2.07; 4.60)
Exposed	101, 2.36 (1.92;2.86)		39, 0.91 (0.65;1.24)		62, 1.45 (1.11;1.85)	
Unknown, assume exposed						
Unexposed	62, 0.62 (0.48; 0.79)	4.25 (3.09; 5.89)	ND		ND	
Exposed	113, 2.64 (2.17,3.17)		ND		ND	
Unknown, assume unexposed						
Unexposed	89, 0.89 (0.71, 1.09)	2.25 (1.66; 3.07)	ND		ND	
Exposed	86, 2.01 (1.61,2.48)		ND		ND	
Early onset plus late-onset disease, exposed vs. unexposed	2.25 (1.84; 2.76)		1.83 (1.40, 2.39)		3.05 (2.20, 4.25)	

*RR, relative risk; ND, not done.
† Incidence = no. cases/1,000 live births.
‡ Based on prevalence of HIV exposure among those tested

6.2.2. **Factors associated with mortality among invasive GBS cases**

The overall case fatality proportion (CFP) was 16.9%; 14.7% (30/204) of GBS-EOD cases died compared to 19.6% (33/168) of GBS-LOD cases (table 25). Infants with meningitis had a 2.4-fold greater risk of death (24.3%) than infants with bacteremia (11.8% OR 2.24; 95% CI: 1.33-4.35, p=0.0015; Table 25). The median duration of hospitalization for infants who died was one day, compared to 15 days among survivors (Table 25). Meningitis (p=0.002) and very low birth weight (<1500g, p=0.003) were associated with higher overall CFP on univariate analysis. Infants born ≤33 weeks gestation had a higher CFP in GBS-EOD cases (p=0.008), but not in GBS-LOD cases (p=0.68; Table 27).

Table 27 Infant factors associated with mortality due to invasive Group B Streptococcus disease[235]

	Overall				EOD				LOD			
Characteristic	**Survived n/N (%)**	**Died n/N (%)**	**Univariable OR (95% CI)**	**P-value**	**Survived n/N(%)**	**Died n/N(%)**	**Univariable OR (95% CI)**	**P-value**	**Survived n/N(%)**	**Died n/N(%)**	**Univariable OR (95% CI)**	**P-value**
Birth weight (grams)	N=266	N=52			N=155	N=28			N= 111	N=24		
<1500	23 (8.7)	13 (25.0)	3.26 (1.49;7.13)	0.003	12 (7.8)	12 (42.9)	8.33 (3.07; 22.64)	<0.001	11 (9.9)	1 (4.2)	0.37 (0.04; 3.04)	0.35
1500-2499	70 (26.3)	9 (17.3)	0.74 (0.33; 1.64)	0.46	43 (27.7)	4 (14.3)	0.78 (0.24; 2.54)	0.67	27 (24.3)	5 (20.8)	0.75 (0.25; 2.22)	0.61
≥2500	173 (65.0)	30 (57.7)	REF		100 (64.5)	12 (42.9)	REF		73 (65.8)	18 (75.0)	REF	
Gestational age (weeks)	N=265	N=51			N=154	N=24			N= 111	N=27		
≤33	24 (9.1)	2 (3.9)	1.79 (0.89; 3.61)	0.10	19 (12.3)	1 (4.2)	3.55 (1.40; 9.1)	0.008	5 (4.5)	1 (3.7)	0.78 (0.24; 2.52)	0.68
>33-<37	44 (16.6)	14 (27.5)	0.47 (0.11; 2.07)	0.47	24 (15.6)	10 (41.7)	0.45 (0.06; 0.64)	0.45	20 (18.0)	4 (14.8)	0.78 (0.09; 7.04)	0.83
>=37	197 (74.3)	35 (68.6)	REF		111 (72.1)	13 (54.2)	REF		86 (77.5)	22 (81.5)	REF	
HIV exposed	129/272 (47.3)	30/51 (58.8)	1.58 (0.86, 2.90)	0.14								
Male gender	160/304 (52.6)	37/63 (58.7)	1.28 (0.73, 2.22)	0.38								
EOD (%)	174/309 (56.3)	30/63 (47.6)	REF									
LOD (%)	135/309 (43.7)	33/63 (52.4)	1.42 (0.82, 2.44)	0.21								
Bacteraemia only	194 (62.8)	26 (41.3)	REF									
Meningitis	115 (37.2)	37 (58.7)	2.40 (1.38, 4.17)	0.002								

6.2.3. **Antimicrobial susceptibility and Serotyping of GBS**

Antimicrobial sensitivity profiles were available for 98.9% (385/ 389) of isolates; all of which were sensitive to penicillin, but 5.5% (15/273) of isolates demonstrated macrolide resistance.

Unfortunately, only 54.8% (213/389) of GBS isolates were available for serotyping, including 125 (58.6%) GBS-EOD and 88 (41.3%) GBS-LOD isolates. The proportion of isolates available for serotyping increased year-on-year (2004: 15.6%; 2005: 45.1%; 2006: 65.8%; 2007: 63.3%; 2008: 75%).

GBS serotypes Ia, Ib and III, which are included in the trivalent GBS-CV under development, caused 78% of GBS-EOD and almost 100% of GBS-LOD cases between 2004 and 2008. The addition of GBS serotypes II and V to a trivalent vaccine would increase coverage of GBS-EOD to 93%.

Serotype III was the most commonly-identified serotype (64.5%, 138/213), followed by serotype Ia (19.2%, 41/213) and together, these two serotypes accounted for 84.0% of all serotypes, including 74.4% of GBS-EOD and 97.7%% of GBS-LOD cases ($p<0.001$, Figure 6). The serotype distribution remained similar throughout the five years; and did not differ by HIV-exposure status.

Serotype III was responsible for a greater proportion of GBS-meningitis (77.8%; 63/81) than GBS-bacteremia cases (56.8%; 75/132; $p=0.002$). Serotype V was more commonly identified in GBS-bacteremia (7.6%; 10/132) than GBS-meningitis cases (3/81; 3.7%; $p=0.25$, Figure 7). There was no difference in serotype distribution between survivors and those who died ($p=0.51$).

Figure 6: Group B Streptococcus serotype distribution amongst young infants with early-onset disease (EOD) and late onset disease (LOD) in Soweto, South Africa, 2004-2008.

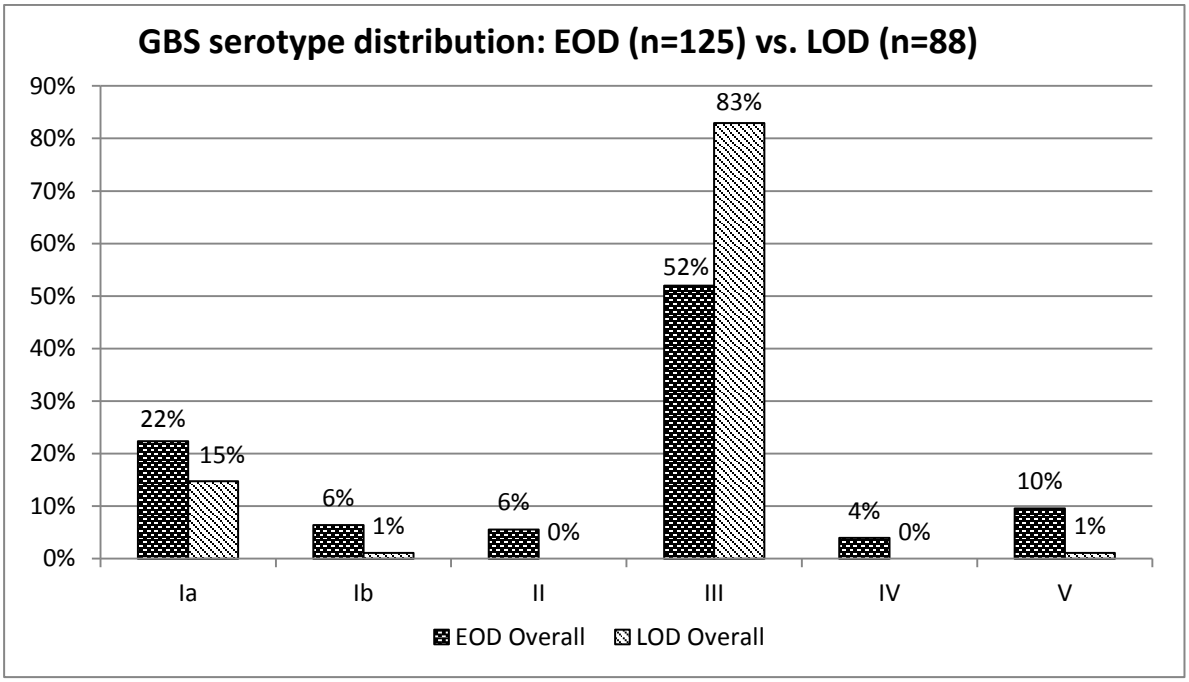
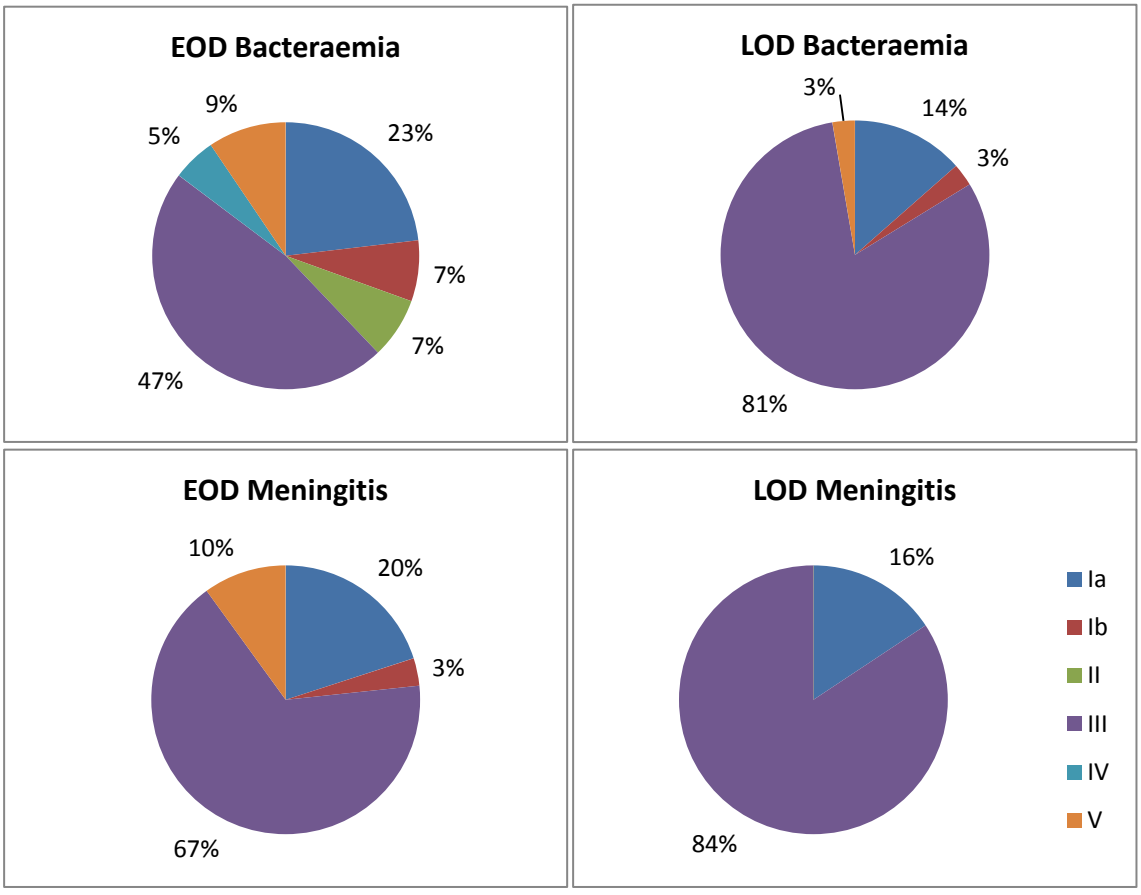


Figure 7*:* Distribution of group B *Streptococcus* serotypes causing early- and late- onset bacteraemia and meningitis, Soweto, South Africa, 2004-2008



6.2.4. **Estimation of national burden and potential vaccine-preventable fraction of invasive GBS disease**

We estimate there were approximately 3 178 invasive GBS cases and 549 GBS-related deaths in South Africa in 2012 (Table 28). This included approximately 1 639 cases and 283 deaths in HIV-unexposed infants. Assuming a vaccine efficacy of 85% in these infants, approximately 1230 cases and 163 deaths could have been prevented by a trivalent (Ia, Ib & III) GBS-conjugate vaccine and 1354 cases and 179 deaths could have been prevented by a pentavalent (Ia, Ib, II, III & V).

An estimated 1544 GBS-related cases and 266 GBS-related deaths occurred in South African HIV-exposed infants in 2012. The vaccine efficacy proposed for HIV-exposed infants was 65%. Using this efficacy, 866 cases (including 117 deaths) and 976 cases (including 129 deaths) could have been prevented using the trivalent and pentavalent GBS-CV respectively (table 28).

Table 28 Estimation of national burden of invasive Group B Streptococcus disease and potential annual vaccine-preventable fraction

	Overall		HIV unexposed		HIV exposed	
National estimates	**n**	**Incidence (95% CI)**	**n**	**Incidence (95% CI)**	**n**	**Incidence (95% CI)**
Total births	1 168 403 *		823724 †		344679†	
Number of invasive GBS cases	3178 ‡	2.72 (2.62; 2.81)	1639 §	1.99 (1.89; 2.09)	1544 ¶	4.48 (4.26; 4.71)
Number of deaths from invasive GBS #	549	0.47 (0.43; 0.51)	283	0.34 (0.30; 0.39)	266	0.77 (0.68; 0.87)
Number of deaths in infants >33 weeks gestation **	420	0.36 (0.33; 0.40)	217	0.26 (0.23; 0.30)	204	0.59 (0.51; 0.68)
	Assume VE of 75%		**Assume VE of 85%**		**Assume VE of 65%**	
Number of cases preventable by trivalent †† GBS-CV	2105	1.80 (1.73; 1.88)	1230	1.49 (1.14; 1.58)	886	2.57 (2.40; 2.75)
Number of deaths preventable by trivalent GBS-CV	278	0.24 (0.21; 0.27)	163	0.20 (0.17; 2.31)	117	0.34 (0.28; 0.41)
Number of cases preventable by pentavalent ‡‡ GBS-CV	2317	1.99 (1.90; 2.07)	1354	1.64 (1.56; 1.73)	976	2.83 (2.66; 3.01)
Number of deaths preventable by pentavalent GBS-CV	306	0.26 (0.23; 0.29)	179	0.22 (0.19; 0.25)	129	0.37 (0.31; 0.44)

* 2012 live births.
†HIV exposed and unexposed calculated based on national HIV prevalence in pregnant women (29.5%).
‡Overall GBS incidence 2.72/1000 live births.
§GBS incidence in HIV-unexposed infants: 1.99/1000.
¶ GBS incidence in HIV-exposed infants: 4.48/1000.
# Total deaths in infants ≤90 days old, assuming that 15.2% are ≤33 weeks gestation with a CRF of 26.5% and 84.8% are >33 weeks CFR of 15.6%.
** Deaths in infants <90 days old, born at >33 weeks gestation (84.8% of infants), CFR = 15.6%
†† Trivalent GBS-CV contains serotypes Ia, Ib& III and accounts for 88.3% of cases.
‡‡ Pentavalent GBS-CV contains serotypes Ia, Ib, II, III &V and accounts for 97.2% of cases.

6.3. Discussion

The incidence (per 1000 live births) of invasive GBS disease (2.72) reported in this surveillance study is higher than the global (0.53) and African (1.21) incidence estimates reported in a meta-analysis of studies conducted between 2000 to 2011[77]. The incidence of invasive GBS disease in HIV-unexposed infants was 1.98 (95% CI:1.71- 2.28), which is similar or greater than the incidence reported in many HICs prior to the widespread use of IAP[48]. The overall incidence observed was similar to that observed in the same population from 1997-1999 (3.0)[81], and in South African women of South-Asian descent (2.65) during the 1980s[83].

Despite similarity in the prevalence of GBS maternal vaginal colonisation at delivery[276], the high incidence of GBS invasive disease in South Africa contrasts with the lower incidence reported in South East Asia and the Western Pacific. Differences in access to facility-based birth, presence of trained birth-care attendants, and access to health facilities with adequate capability to diagnose and treat invasive GBS disease are possible reasons for the differences noted. This is illustrated by a study conducted in Bangladesh in which GBS was isolated from only 1 of 30 neonates with culture-confirmed neonatal bacteremia. More than half of the 259 reported neonatal deaths occurred within 24 hours of birth (many of which were community-births) and 62% were not investigated for bacteremia[67]. In our study, 65% of the GBS-EOD cases were diagnosed within 24 hours of birth, and the majority of these cases would likely have been missed if deliveries had occurred outside health care facilities or in facilities with limited capacity for investigating for invasive disease in newborns. Additionally, in our study which was conducted in a secondary- tertiary care facility, death due to invasive GBS disease was rapid (median 1 day from presentation).

These data suggest that sepsis is often established while the foetus is in-utero, therefore highlighting that antenatal or intrapartum interventions are required to prevent GBS-EOD. Additionally, up to 12% of stillbirths have been attributed to GBS[285] and could be prevented by antenatal or intrapartum interventions.

Up to 30% of infants in Soweto, South Africa are HIV-exposed, and this is contributing to the high incidence of GBS- invasive disease. We noted an incidence of GBS-invasive disease in HIV-exposed infants is 4.46/ 1000 live births. In addition to the higher incidence of GBS-LOD observed in HIV-exposed infants compared to HIV-unexposed infants (2.36 vs. 0.74; RR=3.18), we also observed a 1.69-fold (2.10 vs. 1.24, 95% CI: 1.28-2.24) greater risk of GBS-EOD in HIV-exposed compared to HIV–unexposed infants. This occurred despite our previous observation of lower prevalence of GBS vaginal colonisation in HIV-infected (17%) compared to HIV-uninfected women (23%; p=0.002) at delivery and with similar rates of vertical colonisation of their newborns (52%-58%)[230].
A study conducted in Belgium[238] and a study conducted between 2012 and 2014 at three large academic hospitals in Johannesburg after roll-out of combination antiretroviral therapy (cART) for pregnant women[99] have corroborated our observation.

The reasons for this increased risk of GBS-related sepsis in HIV-exposed infants is unclear, however it is possibly related to the disturbance of the delicate immunogenic equilibrium normally present between mother and foetus. A chronic inflammatory process, which is established in HIV-infected individuals to control the HIV-infection, stimulates increased placental pro-inflammatory cytokines and growth factors which

are associated with an increase in perinatal mother to child transmission of HIV and other co-infections. The use of cART during pregnancy recommended by the WHO has had varied results, with studies reporting both positive and negative impact on the outcome of HIV-exposed, uninfected infants. An immune reconstitution syndrome precipitated by initiation of cART, especially in severely immunocompromised pregnant women, as well as other metabolic changes can further disturb the immunogenic equilibrium between mother and foetus, increasing the infant's risk of infection and other adverse outcomes[29].

Many HIV-infected women in South Africa are only diagnosed during routine antenatal screening HIV screening. Prenatal diagnosis of HIV and initiation of combination ART may lead to improved foetal and infant outcomes in HIV-exposed infants, including a reduction in the GBS-LOD incidence.

We did not identify other differences in the prevalence of risk-factors for invasive GBS disease between HIV-exposed and HIV-unexposed cases[286], however, we did not have population level data on prevalence rates of these maternal risk factors for HIV-infected and –uninfected women. We observed a greater difference in risk of GBS-LOD than GBS-EOD in HIV-exposed compared to HIV-unexposed newborns, suggesting that factors other than well-established peri-partum EOD-associated risk-factors probably contribute to the increased susceptibility of GBS-invasive disease in HIV-exposed infants. HIV-PCR testing of HIV-exposed infants was usually only conducted at 4 to 6 weeks of age, much later in life than the age of most of our GBS-invasive disease cases. We had HIV-PCR results for only 28.1% of HIV-exposed infants on the study, and were therefore unable to assess whether HIV-infection in young infants contributed

to an enhanced susceptibility to invasive GBS disease. However, none of the GBS-EOD cases and 20% of the GBS-LOD cases with known HIV-PCR results were diagnosed as being HIV-infected. The vertical transmission rate of HIV in the population during the course of the study was 9.6%[287].

We did not have adequate data on the clinical, immunological and HIV-virological characteristics for mothers of the HIV-exposed GBS cases on our study, and were unable to analyst whether aberrations of these characteristics may have contributed to the heightened susceptibility of invasive disease noted in HIV-exposed infants.

Numerous studies have reported an inverse association between maternal GBS serotype-specific antibody levels and the risk of EOD or composite of EOD/LOD in their infants[288]. Lower maternal derived-antibody levels to a number of childhood vaccine epitopes among HIV-exposed, uninfected infants at birth up to at least 6 weeks of age has been reported[260, 289]. As such, it is plausible that lower naturally-acquired capsular antibody levels in HIV-infected women may be contributing to the increased susceptibility to invasive GBS disease in HIV-exposed infants. This warrants further investigation.

The serotype distribution of GBS isolates from GBS-EOD and GBS-LOD in this study was similar to that observed previously in the same setting[81], and did not differ by the infant's HIV-exposure status. Serotypes Ia, Ib and III, which are included in a trivalent GBS-CV currently in clinical development, caused 78% of GBS-EOD and 100% of GBS-LOD in this study. The overall potential disease reduction of a vaccine against invasive GBS disease may, however, be lower than this potential coverage due to it being

unlikely to confer protection through antibody acquisition in neonates born at <34 weeks of age[290]. Protection against GBS-EOD in premature newborns would require prevention of acquisition of GBS colonisation in the mother. This is pertinent to settings such as ours, where 29.8% of all GBS-EOD and 31.4% of all GBS-related deaths occurred in premature infants despite the rate of premature birth in the community only being 18%.

Continuous surveillance of GBS disease and serotype distribution is vital to identify changes which may impact on efficacy of GBS-CV under development. This has been well illustrated by ongoing surveillance in Johannesburg, which has reported a shift in serotype distribution in GBS-EOD cases with serotype Ia now being the most commonly observed invasive serotype (48.5% in 2012-2014 vs. 21.6% in 2004-2008), followed by III (19.7% in 2012-2014 vs. 52.8% in 2004-2008) and serotype V (18.2% in 2012-2014 vs. 9.6% in 2004-2008)[99, 235].

Conjugate vaccines (CV) for administration to pregnant women with the specific aim of reducing GBS –disease in young infants are under development or in clinical trials presently[214, 284]. Vaccine efficacy has not yet been established, however, utilising assumptions that the current investigational trivalent (serotypes Ia, Ib & III) GBS-CV would have no efficacy in reducing disease in infants born ≤33 weeks gestation, and would have 85% efficacy in HIV-unexposed infants and 65% efficacy in HIV-exposed infants, we estimated that 3178 GBS cases and 549 GBS-related deaths could be prevented annually in South Africa. With the shift in serotypes causing GBS-EOD more recently[99], development of a pentavalent rather than trivalent GBS-CV should be prioritised. An effective GBS vaccine could also be used to probe the role of GBS morbidity and mortality in countries with limited laboratory epidemiological capacity [291] as well as role of GBS in causing stillbirths[292].

7. PREVENTION OF SEPSIS WITH INTERVENTION

The burden of morbidity and mortality in neonates and young infants is concentrated in LMICs, were access to facility-based births and interventions to prevent sepsis is limited. The aetiology of sepsis in LMICs is also more diverse than that reported from HICs. Simple and inexpensive intrapartum interventions to reduce neonatal and maternal post-partum sepsis have been proposed for use in LMICs. Chlorhexidine intravaginal washes during labour and infant skin wipes have been associated with significant reductions in neonatal and maternal sepsis and neonatal mortality in non-randomised trials conducted in Malawi[173] and Egypt[174]. Chlorhexidine solutions of up to 1% solution have been used safely as vaginal cleanser[293], and up to 4% solution has been used for umbilical cord cleansing[243]. The non-randomised design of the two studies previously conducted in Africa hampered the implementation of this promising intervention. We conducted a large randomised, blinded trial to evaluate the efficacy of an intrapartum and neonatal chlorhexidine intervention in reducing early-onset neonatal sepsis and vertical transmission of GBS. The primary results manuscript is included (appendix 7).

7.1. Statistical considerations

The primary early-onset neonatal sepsis endpoint was evaluated based on intent-to-treat (ITT). Efficacy of chlorhexidine wipes was calculated using the formula:

$$\frac{(R_{Ctl} - R_{Trt})}{R_{Ctl}} \times 100$$

Where R_{Trt} was the incidence rate in infants in chlorhexidine intervention group, and

R_{Ctl} was the incidence rate in infants in control group.

For the colonisation sub-study analysis, a 'Complier Average Causal Effect' (CACE)[294-296] analysis was performed to determine the biological effect of the chlorhexidine intervention in the population of participants defined as 'true compliers'. The complexities inherent in the PoPS trial, which included both maternal and neonatal participants, with varied time durations between randomisation and delivery, necessitated the definition of compliance to allow for meaningful analysis. Women who received at least one interventional wipe, administered according to protocol and randomisation group between one and 6 hours prior to vaginal delivery were considered 'true compliers'.

We considered the mode of delivery to be important in the vertical transmission analysis, as infants born by via emergency caesarean section were considered to be unaffected by the interventional wipe performed on the mother, as infant did not pass through the vagina. The maternal participants were randomised to treatment group prior to confirmation of mode of delivery (normal vaginal delivery vs. emergency caesarean delivery) by attending physician based on maternal- and/ or foetal well-being, therefore, mode of delivery was not considered to be affected or biased by randomisation group, and could be used as a legitimate covariate for stratification[232]. For these reasons, only infants born vaginally and their mothers were included in the analysis for vertical transmission of pathogenic bacteria.

CACE analysis was preferred to intent-to-treat analysis, as an ITT analysis would have reflected the combination of the biological effect of the intervention and non-biological effects related to imperfect compliance to the protocol, rather than just

assessing biological effect of intervention. Details of the CACE analysis performed for the PoPS trial have been published in a statistical journal[232].

7.2. Results

7.2.1. **Participants**

A total of 21 723 women signed informed consent at an antenatal visit or admission prior to delivery during the 3.5-year trial recruitment period. Of these consented women, 10 927 women were assessed for continued trial eligibility on arrival in labour ward and 8 011 were randomised (Figure 8). A total of 2916 consented women were excluded on arrival in labour ward, as they did not fulfil the inclusion and exclusion criteria for trial continuation and receipt of interventional wipes. Sixty five consented women refused to participate further during labour, mainly due to discomfort of contractions. The majority of the remaining consented women delivered at another health care facility. Some were missed on arrival at CHBAH, as we only had two study staff members on duty in labour ward complex at any time.

A total of 8129 infants, including 118 sets of twins were born to randomised participants. Swabs for assessment of colonisation with potentially pathogenic bacteria were collected from 5146 women prior to delivery (Figure 9).

Baseline characteristics of maternal participants were similar between treatment arms (Table 29). The median age at randomization was 26 years (range: 12-51 years), and the median gestational age at delivery was 39 weeks (range: 23-44 weeks). HIV results from routine antenatal testing or pre-natal testing was available for 98.6% (7902/8011) participants, 26.5% (2090/7902) of whom were HIV-infected. Almost 10% (778/8011) women received intravenous antibiotics during labour, at least 2 hours

prior to delivery. Seventy seven percent (6136/8011) of randomised women delivered vaginally.

Approximately 18% of all babies born at CHBAH during the study period were premature (<37 weeks gestation) (Unpublished data: CHBAH Obstetric and Neonatal departments), however only 4% (309/8129) babies born to PoPS participants were premature. The lower-than-expected rate of prematurity on the PoPS trial was in part related to the study procedure of consenting women at antenatal clinic, mainly during the third trimester of pregnancy. Additionally, many women presenting in preterm labour did not fulfil inclusion and exclusion criteria at labour, often due to presentation in late phases of labour (e.g. full cervical dilatation).
When further stratified by maternal HIV-infection status, baseline characteristics remained similar between treatment arms.

Maternal participants received between one and eight interventional wipes (median: 1) prior to delivery, and 80% of women (6423 of 8011) received at least one wipe at least one but less than 6 hours prior to delivery. Interventional wipes were terminated early in 22 women overall, 18 of whom delivered vaginally (0·3%, 18/6135 vaginal deliveries). Reasons for early termination of wipes included face presentation (9/22), strong contractions (10/22) and discomfort (3/22, all of which were in chlorhexidine group).

Neonatal interventional wipes were performed on 99% (8070/8129) of neonates born to maternal trial participants, at a median age of 50 minutes. Neonatal wipes were not performed on 41 infants who were either stillborn or severely ill at birth. Interventional

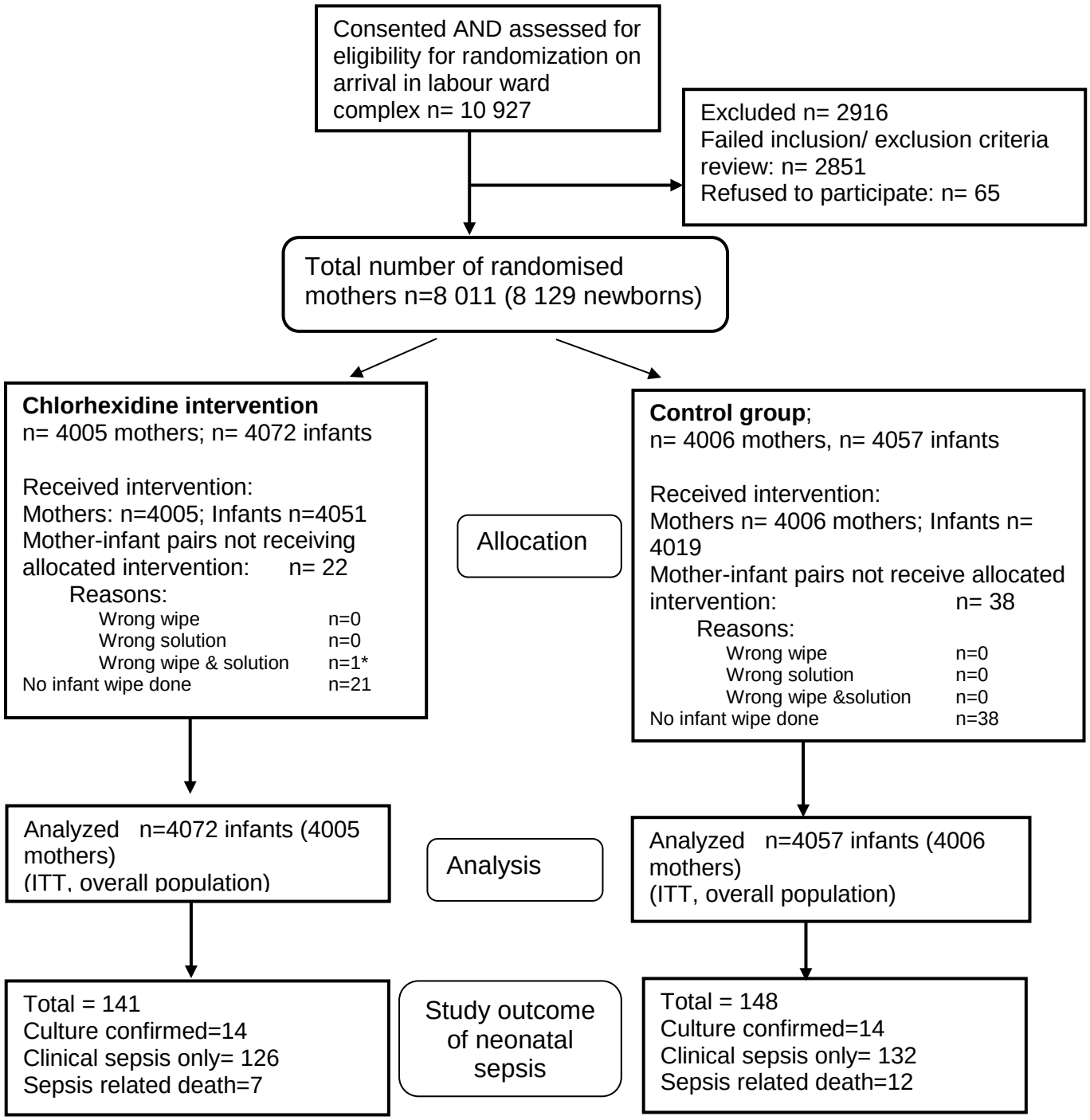
wipes were not performed on an additional 18 newborns during the trial for various reasons including staff shortages. Neonates were assessed prior to discharge home for adverse reactions to interventional wipes and no reactions or adverse events related to wipes were noted.

A total of 958 neonates were admitted 976 times; 16 neonates were admitted twice, and one was admitted 3 times. There was no difference in rate of hospitalisation between chlorhexidine (485/4072 or 119/1000 births) and control neonatal participants (491/4057 or 121/1000 births; p=0.79).

There were fewer perinatal and neonatal deaths in the chlorhexidine arm (34/4072 or 8.3/1000 births) than in the control arm (52/4057 or 12.8/ 1000 births; p=0.05), including 12 and 14 stillbirths in chlorhexidine and control arms respectively. Fifteen (2 in chlorhexidine and 13 in control arms) neonates died within hours of birth in the resuscitation room from severe birth asphyxia or extreme prematurity (25 weeks gestation).

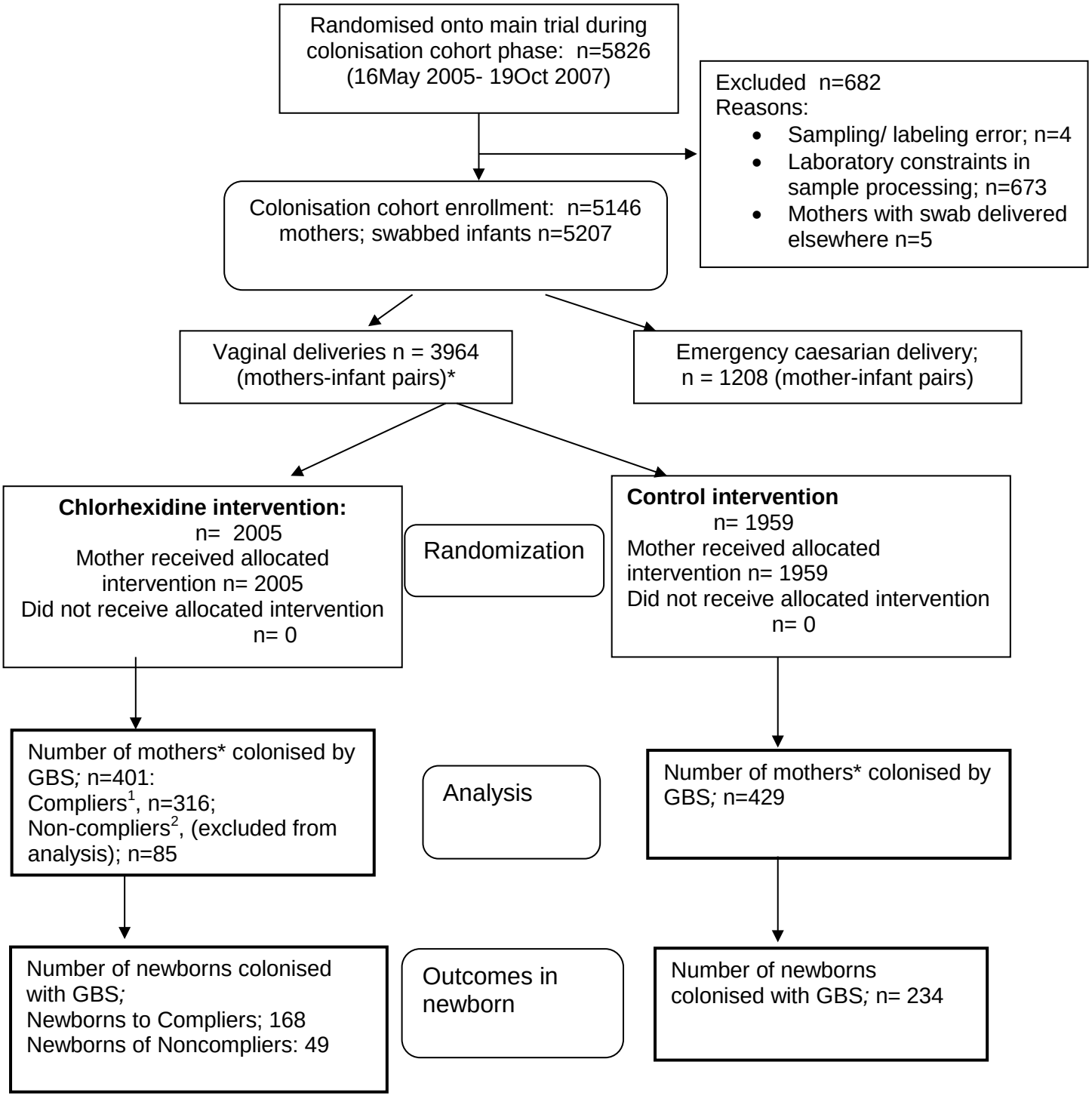
There were an additional 45 neonatal participants who died; 20 in the chlorhexidine arm and 25 in the control arm (p=0·45) at a mean age of 11.2 days (median = 7.0 days, SD 17.4). HIV-PCR results were available for 65.4% (1367/2090) of the HIV-exposed neonates, 8.2% (112/ 1367) were HIV-infected.

Figure 8: Flowchart of mothers and newborns enrolled into the study evaluating efficacy of chlorhexidine against early-onset neonatal sepsis.



*Participant was randomised, discharged and re-admitted at a later date. On readmission, a new randomisation number was allocated. The wipe not done according to protocol was performed according to first randomisation group, which was different to final group.

Figure 9: Flowchart of cohort enrolled into the colonisation sub-study



* Mother counted twice if twins both born vaginally.
[1]Complier= Mother received at least one wipe ≥ 1 hour and ≤ 6 hours prior to delivery and received only the correct intervention.
[2]Non-complier= Mother did not receive chlorhexidine wipe fulfilling criteria of compliers

Table 29: **Selected demographic and clinical characteristics of mothers and newborns.**

Characteristic	Chlorhexidine group %(n)	Control group %(n)
Mothers	n=4005	**N=4006**
Median age in years (range)	26 (14-47)	**26 (12-51)**
Median gravidity (range)	2 (1-9)	**2 (1-10)**
Medical history % (n)		
HIV-positive	26·2 (1050)	**26·2 (1036)**
Urinary tract infection	11·2 (449)	**10·1 (405)**
Gestational diabetes	0·2 (9)	**0·3 (11)**
Maternal tuberculosis	0·5 (19)	**0·6 (23)**
Received intrapartum antibiotics (IAs), excluding caesarean section prophylaxis	9·7 (388)	**9·8 (390)**
IAs in women with vaginal delivery (% of IAs)	75·0 (291)	**75·9 (296)**
Received antibiotics 7 days prior to delivery	25·4 (241)	**25·7 (234)**
Intrapartum fever	0·2 (8)	**0·2 (8)**
Prolonged rupture of membranes of U≥U 18 hours at delivery	9·6 (385)	**8·1 (325)**
Unbooked/No prenatal care	0·3 (10)	**0·03 (1)**
Twin birth	1·7 (67)	**1·3 (51)**
Meconium stained liquor	14·9 (598)	**15·1 (602)**
Delivery		
Spontaneous vaginal delivery	76·9 (3079)	**76·4 (3057)**
Emergency caesarian section	23·1 (924)	**23·6 (943)**
Vaginal vacuum/Forceps assisted	2·0 (80)	**2·1 (84)**
Babies	n=4072	**n=4057**
Female gender	46·8 (1906)	**48·5 (1967)**
Pre-term	3·9 (158)	**3·7 (151)**
Median birth weight (range)	3140g (480-5630)	**3125 (670-5090)**
Median Apgar at 5 minute (range)	10 (0-10)	**10 (0-10)**
HIV-exposed infants	25·8 (1050)	**25·7 (1040)**
HIV PCR positive (% of tested)	9·1 (65 of 716)	**7·2 (47 of 654)**
Outcome		
Stillborn	0·3 (12)	**0·4 (14)**
Died shortly after delivery	0·1 (2)	**0·3 (13)**
Surviving newborns admitted to neonatal war	**9·8 (397)**	**9·6 (390)**

* Median (range)

Intrapartum antibiotics: given at least 2 hours prior to delivery (excludes prophylaxis for caesarean section)

+ Includes mode of delivery of all singletons and first twin only. Four second twins (2 CHX; 2 control) were born by caesarian section when first twin born by vaginally. All other second twins born by same route as first twin.

7.2.2. **Very Early-onset disease**

Overall 289 very early-onset sepsis cases occurred, 28 of which were culture-confirmed cases. Group B streptococcus (16/28; 57.1%, incidence 1.98/1000 live births) was the most commonly isolated bacterial pathogen (10 in chlorhexidine arm and 6 in control arm; Table 30). The rate of culture-confirmed sepsis did not differ between chlorhexidine and control arms (14 cases in each arm). Similarly, overall very early onset sepsis rates did not differ between chlorhexidine arm (126 cases; 34.6/1000 births) and control arm (132 cases; 36.5/1000 births).

There was no difference (efficacy 5% (-19%, 24%)) in rate between the chlorhexidine arm (35/1000 births or 141/4072) and control arm (36/1000 births or 148/4057; p=0·65; Table 31). The very early-onset sepsis rates did not differ between chlorhexidine and control arms when stratified by HIV-exposure status (Table 31).

Eight-seven percent (252/289) of newborns with very early-onset sepsis were admitted within 4 hours of birth.

Table 30: Bacteria isolated from early-onset culture-confirmed cases

	Chlorhexidine	Control
Neonates	**n= 4072**	**n= 4057**
Group B *streptococcus*	10	6
Escherichia coli	0	1
Klebsiella pneumoniae	1	0
Staphylococcus aureus	0	1
Streptococcus viridans	1	1
Acinetobacter baumanii* and *lwoffii	1	2
Enterococcus faecalis* and *faecium	1	2
Enterobacter	0	1

7.2.3. **Late-onset sepsis**

In total, 39 late-onset neonatal sepsis cases were identified (4.8/1000 births), 22 (56·4%) of them in chlorhexidine arm (efficacy -29%, Table 31).

Table 31: Intent to treat analysis of very early onset sepsis in neonates and maternal post-partum sepsis by intervention group and HIV exposure status.

	Overall n (rate)		**Maternal HIV Negative Status n (rate)**		**Maternal HIV Positive Status n (rate)**	
	Chlorhexidine	Control	Chlorhexidine	Control	Chlorhexidine	**Control**
Neonates	**n= 4072**	**n= 4057**	**n= 2939**	**n= 2947**	**n= 1072**	n= 1058
Very Early-onset sepsis						
Culture-confirmed cases* meeting clinical sepsis definition	11	7	6 §	5	4 §	**2**
Culture-confirmed cases NOT meeting clinical sepsis definition ¶	3	7	2	4	1	**3**
Cases with clinical sepsis only	126	132	98	105	28	**27**
Early deaths (<3 days) not meeting culture-confirmed of clinical definitions, but included after panel review	1	2	1	2	0	**0**
Overall	141 (3·5%)	148 (3·6%)	107(3·6%)	116 (3·9%)	33 (3·1%)	**32 (3·0%)**
Efficacy (95% CI) overall Early onset sepsis	**5% (-19%, 24%); p= 0·65**		8% (-20%, 29%); p= 0·55		**-2% (-64%, 37%); p= 0·94**	
Late-onset neonatal sepsis						
Culture-confirmed sepsis meeting clinical sepsis definition	4	8	1	3	3	**4**
Culture-confirmed cases NOT meeting clinical sepsis definition	9	3	6	2	3	**1**
Cases with clinical sepsis only	9	6	7	1	2	**5**
Overall	22 (0·5%)	17 (0·4%)	14	6	8	**10**
Efficacy (95% CI) overall late-onset sepsis	-29% (-142%, 31%); p=0·43		-134% (-508%. 10%); p=0·07		**21% (-99%, 69%); p=0·62**	
Maternal post-partum sepsis ǂ						
	N=4005	**N=4006**	**n=2896**	**n=2916**	**n = 1050**	n = 1040
Culture-confirmed cases that fulfilled clinical criteria	0	0	0	0	0	**0**
Culture-confirmed only	1 ##	1 #	1	1	0	**0**
Endometritis only	14	11	8*	6	5	**5**
Overall	15 (0.3%)	12 (0.3%)	9 (0.3%)	7 (0.2%)	5 (0.5%)	**5 (0.5%)**
Efficacy (95% CI) overall postpartum sepsis	**-25% (-167%, 41%)**		**-29% (-247%, 52%)**		**1% (-240%, 71%)**	

Data are number or number (%) unless otherwise indicated. All neonates including second twins and stillbirths who were delivered at hospital are included.

***Very Early onset culture-confirmed sepsis** Isolation of a micro-organism that is not a common contaminant from a normally sterile body site within the first 3 days of life.

¶ Very Early onset culture-confirmed sepsis in the absence of study-specified clinical criteria of very early-onset neonatal sepsis.

‡ **Maternal postpartum sepsis** was defined as maternal hospitalization within 14 days of delivery for endometritis (at least two of: uterine tenderness, fever, foul-smelling/ purulent lochia or vaginal discharge), sterile site culture-confirmed infection, or perineal wound infection among vaginal parturients.

Citrobacter freundii # and *E. coli* ##

§ HIV exposure results missing for one newborn with culture-confirmed, clinical early onset sepsis in chlorhexidine arm and for one maternal postpartum sepsis case fulfilling the clinical criteria

7.2.4. **Maternal Postpartum sepsis**

Twenty-seven cases of postpartum sepsis were identified among the 8011 randomised women, 15 in the chlorhexidine arm (3·7/1000 women) and 12 in the control arm (3·0/1000 women, Table 31). None of the maternal deaths (1 in chlorhexidine and 3 in control group) or postpartum admissions (1 013 in chlorhexidine and 1 034 in control group; p=0·60) were attributed to the intervention. The majority (1876/2047; 91·6%) of maternal hospitalizations were for routine post-caesarean care.

7.2.5. **Vertical transmission**

As part of the PoPS study, 5146 women and their 5207 infants were swabbed to determine rates of maternal vaginal colonisation and vertical transmission of potentially pathogenic bacteria in our population. HIV-infection status was known for 99.1% (5099/ 5146) of the maternal participants on the colonisation cohort. Seventy seven percent (3946/5146) of women enrolled into the colonisation cohort delivered vaginally and 20.9% (825/3946) were colonised with GBS. GBS was cultured from 54.2% (450/830) of newborns born to GBS-colonised mothers. Amongst mother-infant dyads in colonisation cohort randomised to the chlorhexidine arm, 78.8% (316/401) met the “true complier” definition. No significant reduction in vertical transmission of GBS colonisation was noted in the chlorhexidine arm compared to the control arm (Efficacy -0·05% (-9·2%,7·9%), Table 32); results were similar when mothers receiving >2 hours intrapartum antibiotics (n=47), primarily for meconium-stained liquor, were excluded. Vertical transmission results for *E. coli* and *K. pneumoniae* were similar in chlorhexidine and control arms (Table 32).

Table 32: Intent-to-treat analysis of vertical transmission of selected maternal vaginal colonizing bacteria from mother to newborns

Neonatal Colonisation Status	**Chlorhexidine group**				**Control group**		Overall Efficacy (95% CI)
	Complier		Non-complier				
Group B streptococcus							
Positive	168	(53%)	49	(58%)	234	(55%)	-0·05% (-9·2 to 7·9%)
Negative	135	(43%)	28	(33%)	175	(41%)	
Missing result	13	(4%)	8	(9%)	20	(5%)	
Total	**316**		**85**		**429**		
Escherichia coli							
Positive	361	(51%)	92	(50%)	442	(51%)	0.3% (-6.0 to 5.3)
Negative	298	(42%)	74	(40%)	362	(42%)	
Missing result	45	(6%)	45	(24%)	60	(7%)	
Total	**704**		**184**		**864**		
Klebsiella pneumoniae							
Positive	37	(29%)	6	(23%)	49	(30%)	10.6% (-5.9 to 24.1)
Negative	88	(69%)	18	(69%)	103	(63%)	
Missing result	3	(2%)	2	(8%)	11	(7%)	
Total	**128**		**26**		**163**		

Denominators in each column are the number of women colonised with the respective pathogen.

7.3. Discussion

Published reviews of chlorhexidine maternal vaginal- and newborn skin cleansing[180, 182] concluded that, although two non-randomised trials from Africa[173, 174] showed promising results in reducing neonatal sepsis, a randomised controlled trial, preferably conducted in a LMIC was required before implementation of the intervention was accepted globally.

The randomised controlled trial of maternal-newborn chlorhexidine or control wipes that we conducted in a LMIC failed to show benefit of chlorhexidine maternal and neonatal wipes in preventing early-onset sepsis, despite using a chlorhexidine solution double the concentration of that used in the Malawi[173] and Egypt[174] trials. This was corroborated by lack of impact on vertical transmission of leading sepsis pathogens and on serious maternal postpartum sepsis[229].

Another randomised controlled trial of chlorhexidine vaginal and neonatal wipes to assess efficacy of intervention on neonatal sepsis and perinatal mortality was conducted in Karachi, Pakistan between 2005 and 2008, utilising 0.6% chlorhexidine[297]; a slightly higher concentration than what we used for the PoPS trial (0.5%)[229]. The results of the Pakistan trial[297], which showed no significant reduction in neonatal sepsis, perinatal mortality or maternal mortality, supported our PoPS trial findings[229]. A significant reduction in neonatal skin infections (3.3% vs. 8.2%, $p<0.0001$) was the only benefit observed[297].

Therefore, two large randomised, controlled trials, conducted in LMIC settings[229, 297]

with high neonatal mortality rates (South African NMR= 14.6/1000, Pakistan NMR = 54/1000 live births[105]), in which over 13000 women and their infants were enrolled have both proven that chlorhexidine interventional vaginal and neonatal skin wipes do not reduce the incidence of neonatal sepsis or mortality, despite previously reported benefits[173, 174].

Several reasons for the differences in results observed between the other African trials[173, 174] and our trial have been considered, and include the individual randomised, controlled design of our trial compared to the non-randomised design of the other trials and the more stringent neonatal sepsis definition that we used. Additionally, the previous African trials utilized hospital staff to deliver the interventional wipes, and the participants in the chlorhexidine group may have inadvertently benefited from more intensive monitoring and general care than those in the non-intervention group. The other African trials did not stratify neonatal sepsis episode by age categories within the neonatal period. An intrapartum intervention like chlorhexidine wipes is unlikely to have any effect beyond the first few days of life.

Neither of the other African trials[173, 174] assessed vertical transmission of bacteria to corroborate their findings. Our finding that chlorhexidine does not interrupt vertical transmission suggests it is unlikely to prevent vertically-acquired neonatal infections in any setting or population.

Pathogenic bacteria can ascend into the amniotic fluid and placenta, leading to colonisation of neonatal skin, chorioamnionitis and sepsis in foetus. Ascending infections are more commonly observed in the presence of certain risk factors

including prolonged rupture of membranes, however, pathogenic bacteria can also cross intact membranes and infect a foetus in-utero[40, 46, 47]. These factors allow for infectious processes to become established while the foetus is still in-utero. The large proportion of culture-confirmed and clinically evident sepsis episodes which present during the first 24 to 48 hours of life[71, 95, 235] is evidence of this intra-uterine infectious process. Additionally, intra-uterine infections contribute to the large number of stillbirths that occur each year[285, 298].

Although there were significantly fewer neonatal deaths in the chlorhexidine arm than in the control arm in our trial, the effect on mortality was greatest in deaths that occurred in the first few hours after birth. Most of these early neonatal deaths were caused by birth asphyxia, as determined after blinded record review by a panel of neonatologists. This effect on mortality was unlikely to be related to the chlorhexidine intervention, in the light of the lack of efficacy on sepsis and vertical transmission endpoints.

Intrapartum antibiotic use in our population (9.7% of mothers, primarily for meconium-stained liquor) could have blunted the efficacy of chlorhexidine by reducing rates of sepsis and vertical transmission in both treatment and control arms. The overall incidence of sepsis (36/1000 live births), the GBS-culture-confirmed early-onset sepsis incidence (1.98/1000 live births) and the vertical transmission seen in our trial were similar to those observed in HICs prior to widespread use of IAP.

Although we failed to show a reduction of vertical transmission of pathogenic

bacteria when using chlorhexidine vaginal wipes, reduction of vertical transmission of GBS has been reported using similar interventions, though administered as gels or douches[169, 299]. It is unlikely that the wipe method used in our trial was less effective in reducing vertical colonisation than the other methods used. Our trial included a cohort of GBS-colonised women which was substantially larger than cohorts from other trials, which individually had inadequate power to evaluate vertical transmission.

Although this trial was randomised, a limitation of the study was the lack of blinding of midwives administering the intervention; we did not implement a true placebo due to concerns about detrimental effects of vaginal wipes without antisepsis. Numerous safeguards were established and carefully implemented to counteract this limitation, including the use of colourless chlorhexidine solution, and trial midwives not being involved in patient-care or collection of endpoint data.

Despite several trials having raised hopes that chlorhexidine vaginal/ neonatal cleansing would be beneficial in saving newborn lives, the results of our PoPS trial[229], which have been corroborated by a trial conducted in Pakistan[297] suggest that implementation of this intervention is unlikely to reduce neonatal mortality from vertically-acquired sepsis. Other interventions are needed to reduce neonatal mortality.

8. THESIS CONCLUSION

There were several major findings described in this thesis. The results of the surveillance study undertaken as part of this thesis, have added to the growing body of evidence describing the aetiology of sepsis in young infants in a middle-income setting. The high incidence of culture-confirmed bacterial sepsis in neonates and post-neonatal young infants presenting to CHBAH, and how maternal HIV-infection in the era prior to anti-retroviral roll-out may have increased this burden were described. Ongoing surveillance of bacterial pathogens causing invasive disease in young infants, and antibiotic susceptibility patterns is essential to assist health care providers develop appropriate treatment guidelines. This thesis has included two definitions of early onset neonatal sepsis: (i) < 3 days of age and (ii) <7 days of age. The source of the pathogen causing neonatal sepsis is usually maternal in neonates under 3 days of age, and environmental in older neonates, which supports the use of the <3-day cut-off, however, the most commonly utilised definition of early onset disease is <7 days of age. It has been recently recommended for studies to report separately for cases occurring <3 days and between 3-7 days of age[300].

Maternal recto-vaginal colonisation rates with pathogenic organisms including GBS, *E. coli* and *K. pneumoniae* had not been described previously in this population. In this thesis we reported on the prevalence of colonisation by these bacteria in the women at time of delivery, as well as the rate of vertical transmission of colonising bacteria to their newborns. The prevalence of maternal GBS colonisation, vertical transmission and invasive disease incidence in our population mirrored that observed in other settings with high incidence of invasive GBS disease prior to

intervention of antenatal screening and IAP. There is a paucity of data on GBS colonisation rates in other LMICs, especially in SE Asia, which also reports low incidence of invasive GBS disease. In order to establish whether the low reported incidence of invasive GBS disease in SE Asia is related to low GBS colonisation rates, or missed case identification, we are currently co-ordinating a Bill and Melinda Gates Foundation funded multi-centre study which aims to describe the prevalence of maternal recto-vaginal colonisation, vertical transmission of GBS colonisation and serotype-specific capsular antibody levels in mother-infant dyads with term deliveries in Africa and South-East Asia.

The incidence of GBS sepsis reported in South Africa[235] is amongst the highest currently being reported globally, and has provided vital data for the planning of new interventions which aim to reduce GBS-EOD and LOD. Serotype data available from this study and subsequent GBS disease surveillance[99] have informed the composition of polysaccharide based vaccines for administration to pregnant women for prevention of invasive disease in their newborns, that would be appropriate in our setting and are currently under clinical development. HIV-exposure significantly increased the infant's risk of invasive GBS disease, and whether early diagnosis and initiation of antiretroviral treatment of HIV infected pregnant women, would assist in reducing risk of GBS associated morbidity and mortality in their infants require further investigation.

An inexpensive chlorhexidine intervention which had previously been reported to be effective in reducing neonatal sepsis and mortality[173, 174], was assessed in this project. The results of the PoPS trial[229], which were corroborated by a similar trial

subsequently conducted in Pakistan[297], described the lack of efficacy of chlorhexidine maternal vaginal and infant skin swabs in reducing neonatal sepsis, mortality and vertical transmission of pathogenic bacteria. These definitive results support evidence that intra-uterine infection is a significant contributor to neonatal sepsis, and possibly infection-related stillbirths.

The outcomes of the work conducted for this thesis have supported a shift towards more focused research on other interventions which show promise for reducing the large burden of sepsis in neonates and young infants, specifically the acceleration in research on and development of vaccines for administration to pregnant women. Immunisation of pregnant women has been described as the 'missing link' in the previous efforts to identify and implement antenatal interventions to protect mothers and newborn infants[301].

Subsequent to completing the data collection for this thesis, I have been a lead clinical investigator in several maternal immunisation trials, including seasonal influenza vaccine[206] and GBS-vaccine[242, 302] trials. The vaccine efficacy rates for a trivalent influenza vaccine administered to pregnant women in reducing confirmed influenza were 50.4% (95% CI: 14.5 – 71.2) and 48.8% (95% CI: 11.6 – 70.4) in HIV-uninfected women and their infants, and 57.7% (95% CI: 0.2 - 82.1) in HIV-infected women respectively[206].

Phase Ib/ II randomised trials (V98_08, Clinicaltrials.gov identifier: NCT01193920, V98_05; Clinicaltrials.gov identifier NCT01412801) of a trivalent GBS conjugate vaccine (GBS-CV) were conducted in Africa, including Soweto, South Africa between

2010 and 2012, and have reported that this GBS-CV is well tolerated and immunogenic in HIV-uninfected non-pregnant and pregnant women[302] and HIV-infected pregnant women[242]. The next step towards licensure of a GBS-CV is to conduct a phase III efficacy trial, however, challenges in conducting this trial including the large sample size required (~70 000 mother-infant pairs), identification and selection of appropriate clinical trial sites in areas of high disease burden[214] and other programmatic issues remain to be addressed. In the interim, however, we are conducting a large observational study at CHBAH to establish an immune correlate of protection against invasive GBS-EOD and GBS-LOD (Clinicaltrials.gov number: NCT02215226).

The pathway to develop and introduce a GBS-CV is gaining momentum, with support from organizations including the Bill & Melinda Gates Foundation[216].

Contributing to this effort is a manuscript which I published on the lessons learnt during conduct of maternal immunisation trials in Soweto[303], as an extension to the work conducted for this thesis.

Additionally, the Brighton Collaboration and World Health Organisation established the Global Alignment of Immunisation safety Assessment in pregnancy (GAIA) network in 2014, which is developing definitions for neonatal and maternal adverse events following maternal immunisation. I have been involved in the GAIA activities since July 2014, and led the working group which developed the 'Neonatal death' definition and was a member of four other working groups in 2015 (preterm birth, neonatal infections, pathways to preterm birth and maternal death; submitted for publication, Vaccine). I am also leading the 'Low birth weight' GAIA working group in 2016, and am a member of the 'microcephaly' working group.

In conclusion, the results of the work undertaken for this thesis have supported the acceleration of research on maternal immunisation as a suitable method to prevent infections in neonates and young infants, and possibly infection-related stillbirths.

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Appendices

1. Ethics clearance certificate: PhD protocol
2. Ethics clearance certificate: Sepsis surveillance study
3. Ethics clearance certificate: PoPS trial
4. Paper I
5. Paper II
6. Paper III
7. Paper IV
8. Co-author approvals
9. Copyright permission for published papers

Appendix 1: Ethics clearance certificate: PhD protocol

R14/49 Dr Clare Cutland

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150501

NAME:
(Principal Investigator) Dr Clare Cutland

DEPARTMENT: DST/NRF Vaccine Preventable Diseases/ Respiratory and Meningeal Pathogens Research Unit
Chris Hani Baragwanath Academic Hospital

PROJECT TITLE: Epidemiology and Prevention Sepsis in Young Infants and the Potential Impact of Martenal HIV Infection on Neonatal Sepsis

DATE CONSIDERED: Adhoc

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Prof Shabir Madhi

APPROVED BY: Professor P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 20/05/2015

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature

Date 01 JUNE 2015

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 2:

Ethics clearance certificate: Sepsis surveillance study

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Cultland et al

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M03-10-07

PROJECT Surveillance of Culture-Confirmed Invasive Infections Among Young Infants and Post-Partum Mothers at CH Baragwanath Hospital

INVESTIGATORS Drs CL/et al Cultland et al

DEPARTMENT RMPRU, CH Baragwanath Hospital

DATE CONSIDERED 03-10-31

DECISION OF THE COMMITTEE Approved unconditionally

Unless otherwise specified the ethical clearance is valid for 5 years but may be renewed upon application
This ethical clearance will expire on 1 January 2008.

DATE 03-11-02 CHAIRMAN.....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof H Crewe-Brown
Dept of School of Pathology, CH Baragwnath Hospital
Works2\Iain0015\HumEth97.wdb\M 03-10-07
==========
DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/~~we~~ fully understand the conditions under which I am/~~we are~~ authorized to carry out the abovementioned research and I/~~we~~ guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/~~we~~ undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress form. I/~~we~~ agree to inform the Committee once the study is completed.

DATE 21 November 2003 SIGNATURE .....

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Clare Cutland

CLEARANCE CERTIFICATE	**M10367**
PROJECT	Surveillance of Culture-Confirmed Invasive Infections among Young Infants and Postpartum Mothers at CH Baragwanath Hospital (Previously M031007)
INVESTIGATORS	Dr Clare Cutland.
DEPARTMENT	Respiratory & Meningeal Pathogens Research U.
DATE CONSIDERED	26/03/2010
DECISION OF THE COMMITTEE*	Renewal Approved

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 26/03/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

13 APR 2010

Appendix 3:

Ethics clearance certificate: PoPS trial

University of the Witwatersrand, Johannesburg

Human Research Ethics Committee; (Medical)
FWA Registered No IRB00001233

SECRETARIAT: Suite 189, Private Bag x2600, Houghton 2041, South Africa • Tel: +27-11-717-2800 • Fax: +27-11-717-2833

13 March 2003

COURIERED

Dr S Madhi,
Principal Investigator
Paediatric Infectious Diseases Research Unit
Chris Hani Baragwanath Hospital
SAIMR Room 11
Diepkloof, Soweto
2013

Fax: 011 489 8692

Dear Dr Madhi,

PROTOCOL: CHLORHEXIDINE - PREVENTING SERIOUS NEONATAL AND MATERNAL PERIPARTUM INFECTIONS IN DEVELOPING COUNTRY SETTINGS WITH A HIGH PREVALENCE OF HIV INFECTION: ASSESSMENT OF THE DISEASE BURDEN AND EVALUATION OF AN AFFORDABLE INTERVENTION IN SOWETO, SOUTH AFRICA (CHLORHEXIDINE).

ETHICS REFERENCE NO: 030207

RE : FINAL ETHICS APPROVAL:

The abovementioned clinical trial has been approved by the Ethics Committee for Research on Human Subjects (Medical): Clinical Trials; on the 28 February 2003.

1. This is to certify that the above-mentioned trial was reviewed and approved by the University of the Witwatersrand, Human Research Ethics Committee (HREC) and the Protocol Review Committee (PRC).

2. THIS APPROVAL IS SUBJECT TO THE FOLLOWING PROVISOS:
* A copy of the MCC approval letter must be submitted to the Ethics Regulatory Office Secretariat before the study commences / the changes are implemented.

* The study is conducted according to the protocol submitted to the University of the Witwatersrand, Human Research Ethics Committee. Any amendments to the protocol must first be submitted to the Human Research Ethics Committee for approval.

* During the study, the University of the Witwatersrand, Human Research Ethics Committee is informed immediately of :
- Any Unexpected Serious Adverse Events or Unexpected Adverse Drug Reactions, which, in the Investigator and/or the Sponsor's opinion are suspected to be related to the study drug. (International and Local Reports).
- Any data received during the trial which, may cast doubt on the validity of the continuation of the study .

* The University of the Witwatersrand, Human Research Ethics Committee is notified of any decision to discontinue the study and the reason stated.

* The Investigators authorised by this approval participate in this study. Additional Investigators shall be submitted to the University of the Witwatersrand, Human Research Ethics Committee for approval prior to their participation in the study.

* In the event of an authorised Investigator ceasing to participate in the study, the University of the Witwatersrand, Human Research Ethics Committee must be informed and the reason for such cessation given.

PRINCIPLES OF INFORMED CONSENT:
3. The University of the Witwatersrand, Human Research Ethics Committee requires that in all studies, the Principles of Informed Consent are adhered to. This applies to volunteers as well as patients.

PROGRESS REPORTS:
4. The University of the Witwatersrand, Human Research Ethics Committee requests that the MCC Progress Reports be submitted twice a year (March and September) and a report of the final results. At the conclusion of the study.

THE SUPPORTING APPROVAL DOCUMENTS ARE ATTACHED:

a. Ethics Approval Form signed by the Chairperson of HREC - Kindly return the copy of the Approval Form signed by the Principal Investigator per fax 011 711-2833 for our records.

b. Protocol Review Committee Approval Signature page signed by the Chairperson of the PRC.

c. List of the members present at the HREC meeting held 28 FEBRUARY 2003.

* WE AWAIT YOUR RESPONSES AS REQUESTED:
* MCC Approval.
* Copy of Approval Form signed by the Principal Investigator.

The above has been noted for the Ethics Committee information and records.

KINDLY FORWARD TO THE RELEVANT INVESTIGATORS / CRA / SPONSOR / STUDY CO-ORDINATORS - WHERE APPLICABLE

Regards,

Naidoo.

MISS MERLEESA NAIDOO
For and on behalf of the Human Research Ethics Committee: (Medical)

Wits Clinical Research

27 Eton Road, Healthcare Park, Parktown, 2193, South Africa
Tel. +27-11-717-2800, Fax: +27-11-482-1088
Postnet Suite 189, Private Bag x2600, Houghton, 2041

COURIERED

Dr S Madhi,

Paediatric Infectious Diseases Research Unit
Chris Hani Baragwanath Hospital
SAIMR Room 11
Diepkloof, Soweto
2013

Fax: 011 489 8692

Dear Dr Madhi,

PROTOCOL NO: Chlorhexidine

PROTOCOL TITLE: Preventing serious neonatal and maternal peripartum infections in developing country settings with a high prevalence of HIV infection: Assessment of the disease burden and evaluation of an affordable intervention in Soweto, South Africa (Chlorhexidine).

PRC REFERENCE NUMBER: 030207

****

Please be advised that your trial application was:

APPROVED

The Expert Reviewers were: Prof PA Cooper
Prof G Norton

Also reviewed by: Dr M Joffe: Chairperson Protocol Review Committee
Dr J Moorman : Gauteng Department of Health
Dr S Kahn: Gauteng Department of Health
Dr Wojciechowska / Dr Manning - Superintendent - Johannesburg Hospital
Dr Naidoo - Superintendent - Chris Hani Baragwanath Hospital

Yours sincerely

DR MAUREEN JOFFE
Chairperson: Protocol Review Committee

13 March 2003

cc.
CDC Center for Disease Control and Dr C Cutland **Tel.** 011 489 8786 **Cell.** 082 **Fax.** 011 489 8692

INDEPENDENT ETHICS COMMITTEE APPROVAL FORM 2003

Ethics Reference No.	**030207**	**Date of Meeting**	28 February 2003
Principal Investigators:	Dr S Madhi	**Investigators:**	Dr CL Cutland Dr S Velaphi

Protocol Title:	**Preventing serious neonatal and maternal peripartum infections in developing country settings with a high prevalence of HIV infection: Assessment of the disease burden and evaluation of an affordable intervention in Soweto, South Africa (Chlorhexidine).**

DOCUMENTS REVIEWED		Tick As Appropriate		Yes	No
Protocol Name	**Chlorhexidine**	**Date:**	07 February 2003		
Protocol Amendment No	Chlorhexidine Version 1	**Date:**	07 February 2003	☑	☐
Investigator's Brochure					
Subject Information/Consent Form	Consent Form for HIV Testing of Hospitalized Infants			☑	☐
	Consent Form for HIV Testing of HIV Exposed Infants			☑	☐
	Informed Consent Form - CHLORHEX Version 2 dated 11 March 2003			☑	☐
Advertisements				☐	☑
Insurance/Compensation				☐	☑
Relevant Trial Hospital/(s)	Lillian Ngoyi Community Clinic			☑	☐
	Chris Hani Baragwanath			☑	☐
Syndicate Name	Respiratory and Meningeal Pathogens Research Unit Syndicate			☑	☐
opsis of Study/Trial Summary	Neonatal and Maternal Peripartum Infections.			☑	☐
Other	Neonatal Admission Form			☑	☐
	Colonisation Cohort Swab Requisition Form			☑	☐
	Maternal Admission Form			☑	☐
	L&D Chart Review Form			☑	☐
	L&D Infant Data Form			☑	☐
	L&D Maternal Data Form			☑	☐
	Antenatal Registration Form			☑	☐
	Inclusion / Exclusion Criteria Screening			☑	☐
	Study Flow Chart			☑	☐
	Protocol Summary dated 7 February 2003			☑	☐
	Adverse Event Reporting Form			☑	☐

DETAILS OF COMMITTEE	
Name	University of the Witwatersrand Human Research Ethics Committee: (Medical)
Address	Division of the Deputy Registrar (Research), Department of Research, Senate House University of the Witwatersrand , 1 Jan Smuts Avenue, BRAAMFONTEIN, Johannesburg, 2000

DETAILS OF MEETING	Yes	No
Is the Investigator a member of the committee ?	☐	☑
s" did he/she vote ?	☐	☑
Is the Committee organised and operated according to applicable laws and regulations together with ?	☑	☐
Local GCP requirements ?	☑	☐
ICH GCP requirements ?	☑	☐
FDA GCP requirements ? FWA Registered No. IRB00001223	☑	☐
Progress reports required on a 6 monthly basis ?	☑	☐

DECISION ON APPROVAL : is approval given to conduct the trial ?	Tick As Appropriate
Yes - with no conditions	☑
Yes - with conditions	☐
Specify conditions :	
No	☐
Specify reasons	

SIGNATURES

INDEPENDENT ETHICS COMMITTEE APPROVAL FORM 2003

I confirm that the details on this form are correct:		Date
Name: **Prof PE Cleaton-Jones** **Chairperson of Committee**	**Signature:**	**13 March 2003**

DECLERATION OF INVESTIGATOR/(S)

To be completed and ONE COPY returned to the Secretariat for the HREC at Wits Health Consortium, Healtcare Park, 27 Eton Road, Parktown, 2193 or Fax To: 011 717-2833..

I/We fully understand the conditions under which I am/we are authorised to carry out and complete the above-mentioned research and I/we agree to ensure full compliance with these conditions. Should any amendment, alteration or departure be contemplated from the research procedure methodology or manner of execution I/we will communicate with the Chairman of the Human Research Ethics Commit (Medical) for approval prior to acting on any of the above mentioned proposed amendments, alterations or departures. I am/we are fully aware that any anauthosised amendment, alteration or departure as above will amount to misconduct and may lead to the institution of disciplinary procedures.

Any approval given by the HREC is conditional upon consent being obtained by the Investigator/s from the Superintendent (or equivalent official) of the Hospital, Clinic or Institution in which the research is, in part or full, to take place.

The Chairman may of course at his discretion place the matter before the full Committee.

DATE: 26 MARCH 03 SIGNATURE: _____ NAME: SHABIR A. MAHDI

PROTOCOL NUMBER **Chlorhexidine** ETHICS REF.: **030207**

Attendance Register for the Ethics Meeting held on 28 February 2003 from 13:00 - 15:00

Venue: PPS Boardroom, Faculty of Health Sciences, Medical School,

AFFILIATED TO THE UNIVERSITY IF THE WITWATERSRAND

Surname	Initials	Title	Discipline/s	Academic Qualifications	Gender	Present
Bhagwanjee	S	Prof	Anaesthesia	MBBCh, FCA, DA (SA), FFA (SA)	M	Present
Cleaton-Jones (Chairperson)	PE	Prof	Medical Practitioner	BDS, MBBCh, PhD, DTM&H, DPH, DA (SA), DSc (Dent), Hon PhD, MASSAfr	M	Present
Cooper	PA	Prof	Paediatrics	MBBCh, PhD, DCH (SA), DCH, FCPaed (SA)	M	Absent
Donde	B	Prof	Radiation Oncology	MBBCh, MMed Rad (T)	M	Present
Drower	SJ	Prof	Social Worker	BSocSci (Hons), PhD, RSW	F	Present
Eagle	GT	Prof	Physiology	BA (Hons) MA (Clin Psych), PhD, FRCP	F	Absent
Feldman (Deputy Chairperson)	C	Prof	Pulmonology	MBBCh, PhD, FCP (SA) (Respiratory) FRCP	M	Absent
Langley	G	Mrs	Nursing	MSc (Nursing)	F	Present
Lownie	MA	Dr	Maxillo-Facial & Oral Surgery	BDS, BA (Hons), DipMFOS, FCMFOS (SA)	F	Present
McLean	GR	Dr	Philosopher and Ethicist	BA (Hons) MA B Phil DPhil	M	Present
Mokhachane (Deputy Chairperson)	M	Dr	Paediatrics	FCP (Paeds) SA, MMed (Wits), Neonatology (SA)	F	Absent
Oettle	GJ	Prof	Surgery	BSc (Hons), MBBCh, FRCS	M	Present
Paizes	A.	Prof	Lawyer	BCom, LLB, PhD	M	Absent
Penn	C	Prof	Speech Pathology	BA (S&HT), PhD	F	Absent
Ross	E	Prof	Social Worker	BA, MA, PhD	F	Present
Schuklenk	U	Prof	Biomedical Ethics	PhD	M	Absent
Skeen	AS	Prof	Lawyer	BA (Hons), BL (Hons), LLB, MPhil	M	Present
Szabo	CP	Prof	Psychiatry	MBBCh, MMed, PhD	M	Absent
Van Gelderen	CJ	Prof	Obstetrics & Gynaecology	MBBCh, FRCOG, FCPsych	M	Absent
Velaphi	S	Dr	Paediatrics	MBBCh, FCPaeds, Mmed	M	Absent
Vorster (Deputy Chairperson)	M	Prof	Psychiatry	BA (Crim), MBBCh, MMed, FCPsych(SA), PhD	F	Present
Wadee	A	Prof	Immunology	BSc, MSc, PhD	M	Present
Woodiwiss	A	Prof	Cardiovascular Pathophysiology	BSc, BSc (Physio) MSc, PhD	F	Present

NOT AFFILIATED TO THE UNIVERSITY IF THE WITWATERSRAND

Surname	Initials	Title	Discipline/s	Academic Qualifications	Gender	Present
[illegible]urgh	C	Prof	Educationist	B.Sc (Hons), M.Com DEd NED	M	Absent
[illegible]ggenpoel	M	Prof	Psychiatry Nurse	RN, PhD	F	Present

Note 1: This committee has been in continuous operation since October 1966
Note 2: The large committee size is to ensure a good attendance at meetings
Note 3: A Quorom consists of 5 members, of which 1 non-affiliate and 1 non-medical member to be present
Note 4: The following members alternate: Prof A S Skeen and Prof A Paizes
Prof C Penn and Dr E Ross
Prof C P Szabo and Prof M Vorster
Prof P A Cooper and Dr S Velaphi

This is to certify that the Human Research Ethics Committee: (Medical) of the University of the Witwatersrand operates according to the following guidelines of good clinical practice: 1. ICH Harmonised Tripartite Guideline for Good Clinical Practice (1996): 2. SA National Department of Health Guidelines for Good practice in the conduct of clinical trials in human participants in South Africa (2000): The Committee's United States Federal Wide Assurance details are: 1. Country code SF: 2. University of the Witwatersrand: IORG0000862: 3. Human Reserach Ethics Committee: (Medical): IRB00001223.

Appendix 4: Paper I

Risk Factors for Neonatal Sepsis and Perinatal Death Among Infants Enrolled in the Prevention of Perinatal Sepsis Trial, Soweto, South Africa

Stephanie J. Schrag, DPhil, Clare L. Cutland, MD,† Elizabeth R. Zell, MStat,* Locadiah Kuwanda, MSc,† Eckhart J. Buchmann, MD,‡ Sithembiso C. Velaphi, MD,§ Michelle J. Groome, MD,† Shabir A. Madhi, MD, PhD,† and the PoPS Trial Team*

Background: Factors associated with neonatal sepsis, an important cause of child mortality, are poorly described in Africa. We characterized factors associated with early-onset (days 0–2 of life) and late-onset (days 3–28) sepsis and perinatal death among infants enrolled in the Prevention of Perinatal Sepsis Trial (NCT00136370 at ClinicalTrials.gov), Soweto, South Africa.

Methods: Secondary analysis of 8011 enrolled mothers and their neonates. Prenatal and labor records were abstracted and neonatal wards were monitored for hospitalized Prevention of Perinatal Sepsis–enrolled neonates. Endpoint definitions required clinical and laboratory signs. All univariate factors associated with endpoints at $P < 0.15$ were evaluated using multivariable logistic regression.

Results: About 10.5% (837/8011) of women received intrapartum antibiotic prophylaxis; 3.8% of enrolled versus 15% of hospital births were preterm. Among 8129 infants, 289 had early-onset sepsis, 34 had late-onset sepsis, 49 had culture-confirmed neonatal sepsis and 71 died in the perinatal period. Factors associated with early-onset sepsis included preterm delivery [adjusted relative risk (aRR) = 2.6; 95% confidence interval (CI): 1.4–4.8]; low birth weight (<1500 g: aRR = 6.5, 95% CI: 2.4–17.3); meconium-stained amniotic fluid (MSAF) (aRR = 2.8, 95% CI: 2.2–3.7) and first birth (aRR = 1.8; 95% CI: 1.4–2.3). Preterm, low birth weight, MSAF and first birth were similarly associated with perinatal death and culture-confirmed sepsis. MSAF (aRR = 2.4, 95% CI: 1.1–5.0) was associated with late-onset sepsis.

Conclusions: Preterm and low birth weight were important sepsis risk factors. MSAF and first birth were also associated with sepsis and death, warranting further exploration. Intrapartum antibiotic prophylaxis did not protect against all-cause sepsis or death, underscoring the need for alternate prevention strategies.

Key Words: group B streptococcus, newborns, sepsis, pneumonia

(*Pediatr Infect Dis J* 2012;31: 821–826)

Accepted for publication April 11, 2012.

From the *Centers for Disease Control and Prevention, Atlanta, GA; †Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases & Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Soweto, South Africa; and ‡Department of Obstetrics and Gynaecology and §Department of Paediatrics, Division of Neonatology, Chris Hani Baragwanath Hospital, University of the Witwatersrand, Soweto, South Africa.

The study was funded by the US Agency for International Development, National Vaccine Program Office, Centers for Disease Control and Prevention's Antimicrobial Resistance Working Group via CDC Cooperative Agreement numbers U50/CCU021960 and 5U01CI000318, and the Bill and Melinda Gates Foundation Grant number 39415. The sponsors of the study had no role in study design, data gathering, analysis, interpretation, dissemination or in the decision to submit this report for publication. The authors have no other funding or conflicts of interest to disclose.

Address for correspondence: Stephanie J. Schrag, DPhil, MS C25, Centers for Disease Control and Prevention, Atlanta, GA 30333. E-mail: sschrag@cdc.gov.

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DOI: 10.1097/INF.0b013e31825c4b5a

Neonatal mortality accounts for 41% of deaths among children <5 years of age, globally. Among neonatal deaths, 900,000 annually (approximately one-fourth) are due to neonatal sepsis and pneumonia, primarily in the first week of life among infants in developing countries.[1] The African region includes some of the highest neonatal mortality rates, yet prevention strategies remain unsatisfactory.[2] Because etiology data are limited and there may be a more diverse spectrum of neonatal sepsis-causing pathogens in Africa,[2,3] prevention strategies targeting all-cause neonatal sepsis are particularly attractive.

In Africa, and more broadly for developing countries, few data are available on risk factors associated with neonatal sepsis. There is evidence that preterm delivery, prolonged rupture of membranes and intrapartum fever likely play important roles in early-onset sepsis, as they do in resource-rich countries.[2,4] Labor complications and birth asphyxia have also been suggested as early-onset sepsis risk factors in a Nigerian study.[5]

Although HIV-infected women have a higher incidence of factors that may predispose to neonatal sepsis,[6–9] a recent analysis[10] confirmed that maternal HIV infection is not a direct risk factor for neonatal sepsis.

We assessed maternal and neonatal factors associated with early-onset neonatal sepsis, late-onset community acquired sepsis and perinatal death in a cohort of over 8000 mother-baby pairs delivered at a large public hospital in Soweto, South Africa.

PATIENTS AND METHODS

This is a secondary analysis of the Prevention of Perinatal Sepsis trial (NCT00136370 at ClinicalTrials.gov). The trial's primary goal was to assess the efficacy of maternal and newborn chlorhexidine washes in preventing neonatal sepsis. Neither the intervention[11] nor maternal HIV infection status (C.L. Cutland, MD, et al, submitted for publication) affected neonatal sepsis, thus the full trial cohort was used here.

Study Population and Setting

Eight thousand eleven mothers delivering at Chris Hani-Baragwanath Academic Hospital (CHBAH), a secondary-tertiary level hospital, in Soweto, South Africa, and their 8129 neonates were enrolled between April 1, 2004, and October 25, 2007. The hospital delivers three-quarters of the 30,000 annual births in Soweto, South Africa, providing free maternal and child care to an urban, low-middle and low-income population. Most of the remaining births are conducted by midwives in public antenatal clinics that transfer women with difficult labor to CHBAH. CHBAH is the only public facility that admits neonates requiring hospital care in the Soweto region. All newborn infants who are respiratory depressed at birth are resuscitated according to the South African Paediatric Association Guidelines that are adapted from the American Academy of Paediatrics Neonatal Resuscitation Program.[12] Infants who need respiratory support are admitted to the neonatal

intensive care unit for mechanical ventilation, except for extremely low birth weight infants and those with severe congenital abnormalities and/or severe intrapartum asphyxia. Maternal screening for group B streptococcus (GBS) colonization and intrapartum antibiotic prophylaxis (IAP) is not implemented, but IAP is advocated for the management of chorioamnionitis, prolonged rupture of membranes (≥18 hours), a subset of preterm deliveries (26–33 weeks gestation) and prelabor rupture of membranes. Trial exclusion criteria included planned cesarean section, antepartum hemorrhage, known severe congenital malformation, intrauterine death confirmed before randomization, known allergy to chlorhexidine, face presentation, significant genital warts or ulcers, full cervical dilatation at randomization and age <15 years. While refusals for trial participation were rare (65/10,927 women refused participation), trial enrolment procedures skewed away from enrolling preterm deliveries. The incidence of preterm neonates in the study population (3.8%) was lower than in the hospital population (15%; S.C. Velaphi, MD, personal communication).

Study Procedures

Participants were enrolled into the trial on presentation for labor.[11] Study staff did not provide clinical care. Prenatal and labor records were abstracted for key prenatal and intrapartum variables. Underlying and pregnancy-induced conditions, such as gestational diabetes, as well as intrapartum signs and newborn gestational age were based on documented physician diagnosis as opposed to application of standardized study definitions. At CHBAH, physicians generally make clear clinical notes with problem lists on hypertensive disorders, diabetes mellitus in pregnancy and other medical disorders, and intrapartum events such as pyrexia, and passage of meconium-stained amniotic fluid (MSAF). Gestational age was determined either from records of an early antenatal ultrasound or a Ballard score done soon after birth or within the first 72 hours of life.

Study doctors conducted active surveillance of the neonatal and pediatric wards to identify neonates hospitalized from birth to 28 days of age. Sterile site cultures from study participants were collected at the discretion of attending physicians and processed per standard practice at the hospital microbiology laboratory. Routine methods were used for culture and identification of invasive pathogens from sterile sites. Active laboratory-based surveillance was also conducted to confirm that all sterile site cultures from study neonates were captured. Information related to the neonatal sepsis endpoints was abstracted from medical records by trained study physicians. Logs at the hospital morgue were also reviewed routinely to ensure deaths among study participants were captured.

Endpoint Definitions

Early-onset sepsis (EOS) was defined as sepsis occurring on days 0–2 of life and community-acquired late-onset sepsis (LOS) as that from days 3–28 of life. At least one clinical and one laboratory sign were required for the fulfillment of clinical sepsis, as previously published (see Appendix, Supplemental Digital Content 1, http://links.lww.com/INF/B211).[11] Admission records of newborns with positive blood cultures who did not fulfill the clinical sepsis criteria were reviewed by 3 neonatologists to determine whether they captured a sepsis episode. The records of all trial infants who were stillborn or died within 2 hours of birth were also reviewed to determine whether the cause of death could be attributed to neonatal sepsis; no stillbirths and 2 live born, early deaths were deemed as clinical early-onset sepsis. Intrapartum stillbirths and deaths within the first 6 days of life were considered perinatal deaths.

Statistical Analysis

Maternal, intrapartum and neonatal variables analyzed as potential risk factors are shown in Supplemental Digital Content 5 (http://links.lww.com/INF/B212). Univariate and multivariable analyses were performed using logistic regression in SAS Version 9.2 (Carey, NC). Multivariable models were evaluated starting with all factors that were significant at $P < 0.15$ in univariate analysis and dropping nonsignificant factors using stepwise backward selection. Collinearity of independent variables was evaluated. All 2-way interactions in final multivariable models were evaluated. Throughout, 2-sided $P < 0.05$ was considered statistically significant and 95% confidence intervals (CIs) were calculated. Relative risks were approximated by odds ratios because sepsis and perinatal death endpoints were rare in this population.[13]

RESULTS

Basic characteristics of the cohort (8011 mothers, 8129 infants) have been described previously.[11] Median maternal age was 26 years [interquartile range (IQR): 22–31]; only 11 (0.1%) enrolled women had no prenatal care; 26.1% (2690/8011) had documented HIV infection (1.4% or 109 had unknown HIV status) and 21.0% (1678/8011) had hypertension. Among infants, 3.8% (309/8129) were born at <37 weeks' gestation and 8.0% (615/8129) had a birth weight <2500 g (including 0.4% or 35 with a birth weight <1500 g), and 4.8% (388/8129) required resuscitation upon birth.

About 10.5% (837) of women received IAP. Among these, 60% (498/837) received intravenous ampicillin (1 g 6 hourly), over two-thirds in combination with metronidazole. Only 2 women received penicillin. The median duration between administration of the first dose of IAP and delivery was 14.8 hours (IQR: 7.8–26.7 hours). Documented reasons for IAP included prolonged rupture of membranes (410/837 or 49%), foul-smelling vaginal discharge (90/837 or 11%) and preterm labor (48/837 or 6%). Among women with a preterm indication (per hospital policy, delivery from 26–33 weeks' gestation), 49.4% (42/85) received IAP. Among women with rupture of membranes ≥18 hours, 62.9% (449/714) received IAP. Among women with at least one sign of chorioamnionitis as per hospital policy, 10.0% (129/1295) received IAP (MSAF: 92/1200 or 7.7%; foul-smelling discharge: 32/79 or 40.5%; maternal tachycardia: 8/22 or 36.3%; fetal tachycardia: 8/32 or 25%; maternal fever: 12/17 or 70.6%; uterine tenderness: 1/1 or 100%).

There were 289 cases of early-onset sepsis (35.6/1000 births). Ten percent (29/289) were culture confirmed; GBS was the most common pathogen (16/29; detailed list of pathogens in Table 1). There were 34 late-onset sepsis cases (4/1000 births); 20 were culture-confirmed, with *Escherichia coli* the leading cause (n = 9) followed by GBS (n = 5) (Table 1). The median age at onset for late-onset cases was 14 days (IQR: 11–20). Only 1 late-onset case was preterm. Eighty-two infants died by day 28 of life (10/1000 births). The median age at death was 0 days (IQR: 0–28). The leading causes of death were intrapartum stillbirth (n = 26; 32%), birth asphyxia (n = 30; 37%) and respiratory distress of the newborn (n = 7; 9%). Among infants who died after the first week of life (n = 11; 13%), the primary causes were respiratory distress, sepsis and probable necrotizing enterocolitis. Among the trial cohort, 14.8% (1205/8129) were exposed to MSAF and 3.3% (40/ 1205) of exposed were diagnosed with meconium aspiration syndrome. Ten percent of early-onset cases (30/289), 0% of late-onset cases, 0% of culture-confirmed neonatal sepsis cases and 11.3% (8/71) of perinatal deaths had meconium aspiration syndrome.

In univariate analysis, low birth weight, preterm delivery and several maternal factors were associated with increased risk of early-onset sepsis (Table 2). In multivariable analysis, low birth weight had the strongest association with early-onset sepsis and showed a

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TABLE 1. Characteristics of Infants With Neonatal Sepsis, Soweto, South Africa

Characteristic*	Clinical Early-onset Sepsis (n = 260)	Culture-confirmed Early-onset Sepsis† (n = 29)	Clinical Late-onset Sepsis (n = 14)	Culture-confirmed Late-onset Sepsis (n = 20)
Birth weight (%)				
<1500 g	11 (4)	0	0	0
1500–2499 g	39 (15)	6 (21)	9 (7)	2 (10)
≥2500 g	208 (80)	23 (79)	13 (93)	17 (85)
Preterm delivery (% <37 weeks)	38 (15)	3 (10)	0	1 (5)
Resuscitation at birth (%)	103 (40)	11 (38)%	1 (7.1)	0
Median age in days at onset (IQR)	0 (0–0)	0 (0–1)	17 (13–22)	17 (14–24)
Median days of hospital stay (IQR)	7 (5–10)	10 (8–13)	6 (3–7)	16 (6–22)
Infant HIV infection status‡	12 (5)	3 (10)	1 (7)	2 (10)
Clinical signs of sepsis				
Respiratory distress (%)	255 (98)	23 (79)	12 (86)	11 (55)
Hypotension (%)	23 (9)	4 (14)	5 (36)	2 (10)
Pyrexia or hypothermia (%)	24 (9)	4 (14)	4 (29)	6 (30)
Abdominal feeding problems (%)	10 (4)	5 (17)	5 (36)	10 (50)
Bleeding diathesis (%)	0	0	0	0
Lethargy or irritability (%)	45 (17)	8 (28)	6 (43)	9 (45)
Central nervous system signs (%)	54 (21)	5 (17)	3 (21)	4 (20)
Laboratory signs of sepsis				
Abnormal white blood cell count (%)	52 (20)	1 (3)	7 (50)	5 (25)
Abnormal neutrophil count (%)	50 (19)	2 (7)	8 (57)	4 (20)
Low platelet count (%)	73 (28)	5 (17)	1 (7)	1 (5)
Elevated C-reactive protein (%)	141 (54)	15 (52)	6 (43)	12 (60)
Elevated CSF white blood cell count (%)	2 (1)	2 (7)	0	6 (30)
Outcome (%)				
Died by day 28 of life	14 (6)	3 (10)	1 (7)§	0§

*All variables had <1% missing values except for HIV infection status which had 9% (28/323) missings.

†Among the culture-confirmed cases, 2 early-onset and 7 late-onset cases were identified by CSF. Early-onset pathogens included group B streptococcus (n = 16), *Enterococcus faecalis* (n = 3), *Escherichia coli* (n = 2), *Staphylococcus aureus* (n = 2), *Acinetobacter baumannii* (n = 2), viridans Streptococci (n = 2), *Klebsiella pneumoniae* (n = 1) and *Acinetobacter lwoffii* (n = 1). Late-onset pathogens included *E. coli* (n = 8), group B streptococcus (n = 5), *Enterococcus faecium* (n = 2), *S. aureus* (n = 2), Klebsiella species (n = 2) and Streptococcus species (n = 1). Three culture-confirmed infections were coinfections (*E. coli*/*S. aureus*; Klebsiella/*S. aureus*, Klebsiella/*E. faecium*).

‡Infant HIV infection status was determined by polymerase chain reaction at 6 weeks of age.

§Two infants in each of these groups died shortly after the 28-day period (days 29–32 of life), before hospital discharge.

dose response with infants <1500 g at highest risk [adjusted relative risk (aRR) = 6.5, 95% CI: 2.4–17.3; Table 2]. In addition, MSAF (aRR = 2.8; 95% CI: 2.2–3.7), first birth (aRR = 1.8; 95% CI: 1.1–2.9) and ≥4 vaginal examinations (aRR = 1.6; 95% CI: 1.0–2.7) remained significantly associated with early-onset sepsis (Table 2). Fewer factors were associated with late-onset sepsis, including first birth, emergency cesarean (not due to prior cesarean), male sex and MSAF (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/B213). In multivariable analysis, the only significant association was with MSAF (aRR = 2.4; 95% CI: 1.1–5.0). When both early- and late-onset sepsis were limited to culture-confirmed cases (29 early-onset and 20 late-onset cases), MSAF (aRR = 2.7, 95% CI: 1.4–5.0), low birth weight (aRR = 2.5, 95% CI: 1.2–5.4) and first birth (aRR = 2.1, 95% CI: 1.2–3.8) were again associated with increased sepsis risk (see Table, Supplemental Digital Content 3, http://links.lww.com/INF/B214).

Univariate factors associated with perinatal death included pregnancy-induced hypertension, pregestational diabetes mellitus and anemia, as well as first birth, MSAF and preterm delivery (see Table, Supplemental Digital Content 4, http://links.lww.com/INF/B215). On multivariable analysis, preterm delivery was associated with the strongest risk of death (aRR = 5.9, 95% CI: 3.1–11.2). MSAF (aRR = 2.1, 95% CI: 1.2–3.7) and first birth (aRR = 1.7, 95% CI: 1.0–2.7) also remained significantly associated.

To explore further the association between primiparous status and the sepsis and perinatal death endpoints, we compared the characteristics of women delivering a first birth to those of women with prior deliveries. Primiparous women were more likely to have a number of characteristics associated with poor neonatal outcome, including age <20 years, rupture of membranes ≥18 hours, above the median number of vaginal examinations, above the median labor duration, emergency cesarean delivery, MSAF and low infant birth weight. While the differences between primiparious and multiparous women were statistically significant ($P < 0.05$) for these characteristics, in general the magnitude of the differences was small, except for young maternal age (29.5% versus 20%, $P < 0.001$), greater than the median labor duration (58.6% versus 44.6%, $P < 0.001$), and greater than the median number of vaginal examinations (64.5% versus 42.5%, $P < 0.001$).

IAP was not significantly associated with any of the endpoints on univariate or multivariable analysis, even when forced into final multivariable models. When the endpoint was limited to early-onset GBS disease (n = 16), the point estimates for IAP were consistent with protection in univariate (RR = 0.57, 95% CI: 0.08–4.3) and multivariable models (aRR = 0.38, 95% CI: 0.04–3.6), but did not attain statistical significance.

DISCUSSION

Despite the important contribution of neonatal sepsis to global neonatal deaths, we present one of the first evaluations of risk factors associated with all-cause neonatal sepsis and perinatal death in a large cohort of facility-based births in sub-Saharan Africa. This was a secondary analysis of a clinical trial cohort that was neither population-based nor representative of all births at the facility; nevertheless, use of a sepsis definition involving both clinical and laboratory signs and capture of an array of maternal, intrapartum and newborn characteristics allowed for a more rigorous

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TABLE 2. Factors Associated With Early-onset Sepsis, PoPS Trial Cohort, Soweto, South Africa

Characteristic	Cases, % Exposed (n = 289*)	Noncases, % Exposed (n = 7840*)	OR† (95% CI)	aOR† (95% CI)
Maternal				
Age <20 yr	18.0	13.6	1.4 (1.0–1.9)	
Pregestational diabetes mellitus	0.7	0.2	3.4 (0.8–14.9)	
First birth	59.5	42.2	2.0 (1.6–2.6)	1.8 (1.4–2.3)
Multiple gestation	5.2	2.8	1.9 (1.1–3.3)	
Rupture of membranes ≥18 h	13.5	8.8	1.6 (1.1–2.3)	
Duration of labor (h, quartiles)‡			*P* = 0.003	
<2.9	21.1	24.7	REF	
2.9–5.4	18.3	24.9	0.9 (0.6–1.3)	
5.5–9.4	27.7	25.5	1.3 (0.8–2.3)	
≥9.5	32.9	24.8	1.6 (1.1–2.2)	
Number of vaginal examinations (quartiles)			*P* = 0.0005	*P* = 0.02
<2	6.3	8.7	REF	REF
2–3	31.5	39.8	1.1 (0.7–1.9)	1.1 (0.6–1.8)
4	18.2	19.0	1.3 (0.8–2.3)	1.2 (0.7–2.1)
>4	44.1	32.5	1.9 (1.1–3.1)	1.6 (1.0–2.7)
Mode of delivery				
Emergency cesarean due to prior cesarean	0.7	2.0	0.4 (0.1–1.6)	
Emergency cesarean for other reasons	29.1	21.1	1.5 (1.2–2.0)	
Vaginal delivery	70.2	77.0	REF	
Intrapartum antibiotics§	14.5	10.4	1.5 (1.0–2.0)	
Meconium-stained amniotic fluid	30.5	14.3	2.6 (2.0–3.4)	2.8 (2.2–3.7)
Infant				
Birth weight				
<1500 g	3.8	0.3	14.3 (6.9–29.5)	6.5 (2.4–17.3)
1500–2499 g	15.7	7.3	2.5 (1.8–3.4)	1.8 (1.1–2.9)
≥2500 g	80.5	92.3	REF	
Preterm delivery (<37 weeks)	14.2	3.4	4.7 (3.3–6.6)	2.6 (1.4–4.8)

*All variables had <15 missing values except for birth weight which had 49 missings (47 among noncases).

†Univariate: all factors with $P < 0.15$ are shown; all were entered into multivariable model, only those with $P < 0.05$ are shown. Due to missing values, the multivariable model included 287 cases and 7793 noncases. Apgar score was excluded from model because of overlap with endpoint. Only 1 case of intrapartum fever was recorded so the variable could not be analyzed.

‡Duration of labor is approximated by time between randomization into the PoPS trial (which for most mothers coincided with the onset of true labor) and delivery.

§IAP defined as any duration, excluding cesarean prophylaxis (cesarean with <1 hour of antibiotics before delivery).

aOR indicates adjusted odds ratio; OR, odds ratio; PoPS, Prevention of Perinatal Sepsis.

evaluation than is possible in most sub-Saharan Africa settings. Our findings that preterm birth and low birth weight were strongly associated with increased risk of sepsis and perinatal death, and that MSAF and primiparity were each associated with approximately twice the risk of sepsis and death, provide a strong foundation for further investigation into the role of these factors in African newborns.

Preterm delivery and low birth weight are well-established neonatal sepsis risk factors in industrialized countries, and our finding that lowest birth weights were associated with the most risk is similar to what has been reported in the United States.[14–17] Biological mechanisms likely include immature skin barrier and immune system. For some pathogens, such as *E. coli*, low gestational age is a driving risk factor with two-thirds of *E. coli* in the United States among preterm infants.[14] Unfortunately, effective prevention strategies for preterm delivery remain elusive, although in resource poor settings improved maternal nutrition and access to prenatal care might be of some benefit in reducing low birth weight.

In contrast, industrialized country evaluations have not commonly identified significant associations between MSAF and sepsis. However, a strong association between MSAF and culture-confirmed early and late-onset sepsis was reported from a tertiary care hospital in Tanzania,[4] and a population-based evaluation of infants receiving a septic workup in a California health maintenance organization also reported an association between MSAF and culture-confirmed sepsis.[18] MSAF is more common among women delivering at term[19] and was a common exposure among the infants in our study population (approximately 15% of deliveries). There are several possible explanations for the association between MSAF and neonatal sepsis. First, the clinical sepsis definitions we applied captured noninfectious syndromes including meconium aspiration syndrome. However, the fact that we observed significant, similar associations between MSAF and culture-confirmed infections suggests that misclassification does not fully explain our observations. In addition, meconium may enhance the growth of sepsis pathogens[20] and result in neurological damage that predisposes to late-onset sepsis. Finally, MSAF may represent sepsis-associated fetal distress *in utero*.

Primiparity is rarely identified as a sepsis risk factor, often in univariate analysis or studies with limited intrapartum variables for evaluation.[21] Because women delivering a first birth typically have longer duration between membrane rupture and delivery, an established sepsis risk factor, associations with first birth have been attributed to this. However, in our cohort, we collected information on duration of membrane rupture, duration of labor and total number of vaginal examinations received. First birth remained robustly associated with sepsis and perinatal death in multivariable analysis and was not simply a surrogate for duration-related variables. Instead, when we compared primiparous women with those with previous births, we found that first births were more likely to have a suite of characteristics established in past studies as sepsis risk factors. Because the magnitude of these differences was generally small, we believe the combination of these small differences may be best captured by primiparous status.

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Interestingly, on multivariable analysis, the same factors associated with neonatal sepsis were similarly associated with perinatal death. Because CHBAH serves as a referral center for satellite clinics, several "high risk" maternal conditions such as pregnancy-induced hypertension were overrepresented in our cohort, yet none were associated with increased risk of perinatal death. The association with MSAF may be partially explained by the observation that 11% of deaths had a discharge diagnosis of meconium aspiration syndrome. The overlap between factors associated with sepsis and perinatal death may also suggest that neonatal infections contributed importantly to a portion of deaths, even though a pathogen was not always isolated.

Intrapartum antibiotic exposure was not associated with reduced risk of neonatal sepsis or perinatal death in this setting. The lack of a protective effect of IAP on multivariable analysis is consistent with other studies of all-cause neonatal sepsis, which have similarly found no impact of IAP.[16] IAP currently is only established to be effective in preventing early-onset GBS disease; when we limited the endpoint to culture-confirmed GBS early-onset sepsis, the point estimates for IAP protection were consistent with this although the confidence intervals were wide, and the sample size was small. Moreover, IAP has been shown to have no effect on late-onset invasive GBS sepsis or on early-onset *E. coli* sepsis.[14,22] Our findings also demonstrate the challenges of implementing a risk-based IAP policy even in a referral center labor ward such as CHBAH. Only half of women delivering preterm and 10% of women with signs of chorioamnionitis received IAP. In addition, intrapartum fever, an important maternal risk factor for puerperal and neonatal sepsis, was only documented in the labor record of <1% of women, highlighting the difficulties of reliably measuring and capturing signs in a busy labor ward. Even in industrialized country settings, IAP implementation under risk-based strategies has proven lower than under culture-based GBS screening strategies.[23]

There are several limitations to our analysis. Although we used a more rigorous clinical sepsis case definition than past studies, the definition still was not specific for infection and likely captured birth asphyxia and other forms of respiratory distress. In addition, although our cohort of over 8000 mother-baby pairs was large, the sample size for some endpoints, particularly late-onset disease and culture-confirmed sepsis, remained small, limiting our ability to evaluate some variables for associations. Most importantly, however, our trial cohort differed from the overall CHBAH birth cohort, most notably in the smaller percentage of preterm births; it also differed from all Soweto births due to the overrepresentation in the CHBAH population of high risk deliveries. However, there were enough preterm, low birth weight and very low birth weight infants in the trial cohort to identify these factors as significant risks for the endpoints under consideration. In addition, CHBAH births capture three-fourths of all births in Soweto, so while they may not represent the full population, they capture a large portion. Moreover, the overrepresentation of high risk maternal conditions was a benefit in terms of evaluating whether these conditions were associated with risk of sepsis or perinatal death.

Most previous sepsis risk factor analyses have been limited to resource-rich settings and have focused on pathogen-specific endpoints. In sub-Saharan Africa, few studies have had a robust comparison group for formal risk factor assessment. While the Soweto, South Africa population is wealthier and has more access to care than many settings in sub-Saharan Africa, these same features facilitated a rigorous evaluation of demographic, prenatal and intrapartum risk factors. The failure of IAP to prevent all-cause neonatal sepsis and the challenges of implementing a risk-based IAP strategy for targeted pathogen prevention shed light on the challenges of neonatal sepsis prevention in Africa through existing strategies. Further exploration of the mechanisms behind the association with first birth and MSAF are warranted as these are easily identifiable risk factor. In addition, exploration of maternal immunization as a strategy to prevent early newborn infections holds promise and Phase II trials of a GBS vaccine are currently underway in southern Africa.

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ANNOUNCEMENT: ESTABLISHMENT OF THE SOCIETY FOR MIDDLE EAR DISEASE

The Society for Middle Ear Disease (SMED) is an international non-profit community advocacy society dedicated to promoting public and professional awareness of the importance of middle-ear disease (otitis media) as a major health problem. SMED enables individuals with middle-ear disease, as well as parents and other family members of infants and children who have otitis media, to meet the challenges of the disease and its associated hearing loss and possible complications through information, advocacy, and support.

SMED provides general information on otitis media and official diagnosis and management guidelines from countries around the world on the website www.societyformiddleeardisease.com. SMED actively raises funds for a foundation in which the interest provides money for expenses incurred by SMED and for future, peer-reviewed competitive grants to investigators for independent otitis media research. SMED also promotes funding for otitis media research by lobbying government agencies, and solicits funds from foundations, the health-care industry, and private philanthropy. Currently, there are eight advocates (Ruth Gabig Auld, EdD – Chair) and eight advisors (otolaryngologists, pediatricians, and scientists) from the U.S. and 35 advisors from 27 other countries. SMED Headquarters: Children's Hospital of Pittsburgh Foundation, One Children's Hospital Drive, 4401 Penn Avenue, Faculty Pavilion, 7th Floor (Pediatric Otolaryngology), Pittsburgh, PA, 15224. E-mail: (Debi.Buza@chp.edu). Comments and questions: Tasnee Chonmaitree, MD (tchonamai@UTMB.EDU); Jerome O Klein, MD (jerome.klein@bmc.org).

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Appendix 5: Paper II

Maternal HIV Infection and Vertical Transmission of Pathogenic Bacteria

AUTHORS: Clare L. Cutland, MBBCh,[a] Stephanie J. Schrag, DPhil,[b] Elizabeth R. Zell, MStats,[b] Locadiah Kuwanda, MMedStats,[a] Eckhardt Buchmann, MBBCh, FCOG(SA), PhD,[c] Sithembiso C. Velaphi, MBChB, FCPaeds(SA),[d] Michelle J. Groome, MBBCh, MSc(Epi&Biostats),[a] Peter V. Adrian, PhD,[a] Shabir A. Madhi, MBBCh, FCPaeds(SA), PhD,[a,e] and the PoPS trial team

[a]Department of Science and Technology/National Research Foundation, Vaccine Preventable Diseases & Medical Research Council, Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Soweto, South Africa; [b]Centers for Disease Control and Prevention, Atlanta, Georgia; Departments of [c]Obstetrics and Gynaecology, and [d]Paediatrics, Division of Neonatology, Chris Hani Baragwanath Hospital, University of the Witwatersrand, Soweto, South Africa; and [e]National Institute for Communicable Diseases, National Health Laboratory Services, Sandringham, South Africa

KEY WORDS
group B *Streptococcus* (GBS), newborns, sepsis, HIV, pneumonia

ABBREVIATIONS
EOS—early-onset sepsis
GBS—group B *Streptococcus*
HEU—HIV-exposed uninfected
HUU—HIV-unexposed uninfected
LOS—late-onset sepsis
PCR—polymerase chain reaction
PoPS—Prevention of Perinatal Sepsis
ROM—rupture of membranes
UTI—urinary tract infection

Drs Cutland, Schrag, Zell, Buchmann, Velaphi, and Madhi participated in the conception of the trial, study design, protocol development and amendment, study planning and implementation; Drs Cutland and Groome followed up participants and collected data; Dr Adrian managed sample processing; Dr Zell and Kuwanda analyzed the data; all authors participated in interpretation of the results; Drs Cutland, Zell, Schrag, and Madhi drafted the manuscript and all authors contributed to critical review and revision of the manuscript; all authors have seen and approved the final version of the manuscript; and Dr Madhi, as corresponding author, had full access to data and final responsibility for the decision to submit manuscript for publication.

(Continued on last page)

WHAT'S KNOWN ON THIS SUBJECT: Neonatal sepsis is an important cause of under-5 childhood mortality. Infants born to HIV-infected mothers are at increased risk of morbidity and mortality, even if not having acquired HIV. This association needs further study during the neonatal period.

WHAT THIS STUDY ADDS: Maternal HIV infection was associated with increased vaginal colonization by *Escherichia ecoli* but not group B *Streptococcus*. Neonates born to HIV-infected mothers were only at increased risk of sepsis if they had acquired HIV-infection, but not if HIV-uninfected.

abstract

BACKGROUND: HIV-exposed newborns may be at higher risk of sepsis because of immune system aberrations, impaired maternal antibody transfer and altered exposure to pathogenic bacteria.

METHODS: We performed a secondary analysis of a study (clinical trials.gov, number NCT00136370) conducted between April 2004 and October 2007 in South Africa. We used propensity score matching to evaluate the association between maternal HIV infection and (1) vaginal colonization with bacterial pathogens; (2) vertical transmission of pathogens to the newborn; and (3) sepsis within 3 days of birth (EOS) or between 4–28 days of life (LOS).

RESULTS: Colonization with group B *Streptococcus* (17% vs 23%, $P = .0002$), *Escherichia coli* (47% vs 45%, $P = .374$), and *Klebsiella pneumoniae* (7% vs 10%, $P = .008$) differed modestly between HIV-infected and uninfected women, as did vertical transmission rates. Maternal HIV infection was not associated with increased risk of neonatal EOS or LOS, although culture-confirmed EOS was >3 times higher among HIV-exposed infants ($P = .05$). When compared with HIV-unexposed, neonates, HIV-exposed, uninfected neonates (HEU) had a lower risk of EOS (20.6 vs 33.7 per 1000 births; $P = .046$) and similar rate of LOS (5.8 vs 4.1; $P = .563$). HIV-infected newborns had a higher risk than HEU of EOS (134 vs 21.5; $P < .0001$) and LOS (26.8 vs 5.6; $P = .042$).

CONCLUSIONS: Maternal HIV infection was not associated with increased risk of maternal bacterial colonization, vertical transmission, EOS, or LOS. HIV-infected neonates, however, were at increased risk of EOS and LOS. *Pediatrics* 2012;130:1–10

Neonatal sepsis is a leading (26%–49%) cause of neonatal mortality in developing countries and contributes disproportionately to mortality among children <5 years of age.[1,2] A recent systematic review reported a paucity of data on the incidence of neonatal sepsis in sub-Saharan Africa.[3] Estimates of culture-confirmed neonatal sepsis ranged between 5.46 to 21 per 1000 live births.[4–6] Published studies rarely distinguished between sepsis occurring during the early neonatal period (first 3 or 7 days of life), which likely reflects bacteria acquired directly from the mother's genital tract, and sepsis in older neonates which may be acquired by vertical transmission or through environmental exposures.

There is also a lack of data on the impact of maternal HIV exposure on clinical or pathogen-specific burden of neonatal sepsis.[3] A maternal immune system compromised by HIV infection may result in reduced transplacental transfer of antibodies to the fetus in utero,[7,8] possibly increasing neonatal sepsis susceptibility. Although HIV infection increases bacterial vaginosis[9] and the diversity of vaginal microbiota,[10] it has not been associated with any difference in overall prevalence of group B *Streptococcus* (GBS) vaginal colonization compared with HIV-uninfected women.[11]

High maternal HIV prevalence rates (5%–26%) in sub-Saharan Africa have led to a reversal of decades of improvement in child survival.[12] Morbidity and mortality in HIV-infected children is significantly higher than in HIV-unexposed children.[13,14] In addition, HIV-exposed uninfected (HEU) infants have a higher than expected risk of morbidity and mortality compared with HIV-unexposed uninfected (HUU) infants; an effect related to maternal immunologic status and early weaning among HIV-exposed infants.[14–17]

We performed a secondary analysis of a South African cohort of >8000 mother-baby pairs to assess the impact of maternal HIV infection on (1) the prevalence of maternal vaginal colonization with pathogens associated with neonatal sepsis; (2) vertical transmission of bacterial pathogens to the newborn; and (3) sepsis rates during the early and late neonatal periods.

METHODS

This analysis reports on secondary objectives of a cohort of women and their newborns enrolled in the Prevention of Perinatal Sepsis (PoPS) trial.[18] PoPS was a randomized, placebo-controlled trial to determine the efficacy of intrapartum chlorhexidine maternal vaginal washes and newborn skin wipes for reducing early-onset neonatal sepsis and vertical acquisition of pathogenic bacteria by newborns. The study was conducted between April 2004 and October 2007 and found no effect of interventional wipes against primary end points of overall early-onset sepsis (EOS) and vertical transmission of colonizing pathogenic bacteria.[18]

Study Population

Pregnant women attending an antenatal clinic or presenting in labor at Chris Hani-Baragwanath Hospital, a secondary-tertiary level of care hospital, in Soweto, South Africa, were enrolled into the study. The prevalence of HIV in antenatal clinic attendees in the study population during the trial period was 29.9%.[19] The study staff had no input into clinical management of study participants, including management of HIV infection. The standard of care for prevention of mother-to-child transmission of HIV during the trial period included a single dose of nevirapine (sd-NVP) to both the HIV-infected mother at onset of labor and the newborn. Before triple antiretroviral treatment of HIV-infected individuals became routinely available in 2007, CD4+ and HIV viral load testing by attending physicians was limited. From 2007, pregnant women with WHO Stage IV disease or CD4+ count <200 cells/mm^3 were offered treatment that included stavudine, lamivudine, and nevirapine from at least 34 weeks gestation.[20] All mothers and newborns are provided with free public health care in South Africa. In total, 8011 mothers and their 8129 neonates were enrolled in the trial. HIV polymerase chain reaction (PCR) testing of HIV-exposed infants is offered routinely at 4 to 6 weeks of age.

Study Procedures

Details of study procedures have been described.[18] In brief, swabs of the lower vagina were obtained from maternal participants from May 2005 onward before administering the first study interventional wipe. A surface swab of the umbilicus, nares, and outer ear for culture was collected from neonates after delivery but before bath and study wipe. Swabs were cultured for *Streptococcus agalactiae* (GBS), *Escherichia coli,* and *Klebsiella pneumoniae* by using standard methods.[18]

Study doctors conducted active surveillance of neonatal and pediatric wards to identify hospitalized study neonates and abstracted information related to neonatal sepsis end points from medical records. Sterile site cultures were collected at the discretion of attending physicians before initiation of antibiotic treatment and processed per standard practice at the hospital microbiology laboratory. Blood was sampled for bacterial growth by using the BacT/Alert microbial system (Organon Teknika, Durham, NC). Routine methods were used for culture and identification of invasive pathogens from other sterile sites. Active laboratory-based surveillance was conducted to confirm that all sterile site cultures obtained from study neonates were captured. Maternal CD4+ T-lymphocyte count results

were documented when available as part of standard of care.

End Point Definitions

Maternal vaginal colonization was defined as isolation of GBS, *E coli,* or *K pneumoniae* from vaginal swabs collected during labor before the first interventional wipe. Vertical transmission of bacteria was defined as neonatal surface colonization at birth with the same bacterium isolated from the maternal vagina. Early-onset sepsis (EOS) was defined as sepsis occurring within the first 3 days of life and late-onset sepsis (LOS) as that from the fourth to 28th day of life (Table 1). Culture-confirmed episodes of sepsis not fulfilling clinical sepsis criteria and records of all stillborn infants and neonates dying within 2 hours of birth were reviewed by 3 neonatologists to determine if the case represented a sepsis episode. Neonatal and maternal HIV infection status was based on documented HIV testing results; those with unknown status were excluded from analyses involving HIV infection status.

Statistical Considerations

To specifically evaluate maternal HIV infection as a neonatal sepsis-related risk factor, we used propensity score matching to reduce bias with respect to important covariates for each defined end point.[21,22] Covariates considered for impact on end points included: (1) for maternal vaginal colonization: maternal age ($>$ or $\leq$25 years), rupture of membranes (ROM) before swab collection, and history of antibiotics in week before delivery; (2) for vertical transmission: prolonged ROM, antibiotic use in week preceding labor and intrapartum, number of per vaginal examinations; and (3) for early- and late-onset neonatal sepsis: mode of delivery, gestational age, prolonged ROM, maternal fever $\geq$38.0°C, intrapartum antibiotic use, known maternal GBS colonization and urinary tract infections (UTIs). We chose propensity score matching because key covariates differed between HIV-infected and HIV-uninfected women, and standard modeling approaches provide estimates even in the absence of an appropriate comparison population.

We used multivariable logistic regression to estimate the propensity score for each mother (or infant). The propensity score is the conditional probability of the mother being HIV-infected given the variables in the model. We matched each HIV-infected mother to an HIV-uninfected mother with the closest propensity score. For each propensity score matched analysis, balance for each covariate included in the model was evaluated for HIV-infected and HIV-uninfected mothers. The propensity score model that achieved the best balance was used. The model for maternal colonization was limited to mothers with known HIV result and

TABLE 1 Neonatal Sepsis End Point Definitions

Early-onset culture confirmed sepsis: isolation of a microorganism that is not a common contaminant (include, but are not limited by, the following list: Coagulase-negative *Staphylococcus*, *Bacillus*, *Micrococcus*, *Propionibacterium*, *Stomatococcus*, *Gamella*, *Prevotella*, *Corynebacterium*) from a normally sterile body site within the first 3 d of life

Early-onset clinical sepsis: A neonate hospitalized within 3 d of life and who, in the absence of another recognizable congenital infection, had at least one laboratory criterion and either respiratory distress (1 criterion required) or at least 2 clinical criteria (see below).

Late-onset sepsis: Either culture-confirmed sepsis or clinical sepsis in an infant with symptom onset between 3 and 28 d of life.

Late-onset culture confirmed sepsis: isolation of a microorganism that is not a common contaminant from a normally sterile body site between 3 and 28 d of life.

Late-onset clinical sepsis: A neonate hospitalized between 3 and 28 d of life with at least 1 laboratory criterion and either respiratory distress (2 criteria required), OR one feature of respiratory distress and one other clinical criterion OR at least 2 other clinical criteria.

Early-onset sepsis: Either early-onset culture-confirmed sepsis or clinical sepsis in the absence of another recognizable congenital infection, as defined below.

Clinical Criteria	Definition
Respiratory distress	Respiratory rate >60 breaths/min; cyanosis, chest wall indrawing, grunting on expiration, respiratory distress noted in medical records
Hypotension	Defined as mean arterial pressure < 2 SDs from mean for weight/age
Pyrexia or hypothermia	Axillary temperature >38.0°C, not attributable to external warming, or axillary temperature <36.0°C
Abdominal/ feeding problems	Abdominal distension OR feeding intolerance (>20% residual over 24 h), or poor feeding after feeding well, or > 2 episodes of emesis
Bleeding diathesis	Defined as petechiae, ecchymosis, mucous membrane bleeding, pulmonary hemorrhage, or excessive oozing from venipuncture sites
Lethargy or irritability	Noted by medical staff in absence of other central nervous system symptoms
Central nervous system	Seizures, or bulging fontanelle, or single witnessed episode of apnea
Laboratory criteria	
White blood cell count (WCC)	WCC $<5 \times 10^9$/L OR $>25 \times 10^9$/L in the absence of receiving corticosteroids;
Absolute neutrophil count (ANC)	ANC $<1{\cdot}75 \times 10^9$/L or $>15 \times 10^9$/L
Platelet count	$<150 \times 10^9$/L
C-reactive protein	> 10·0 mg/L (early-onset sepsis) OR >40 mg/L (late-onset sepsis)
Elevated cerebrospinal fluid white blood cell (WBC) count	$>30 \times 10^6$/L WBC in absence of significant red blood cells

Previously published as Supplementary webappendix.[18]

colonization swab with a result. The model for vertical transmission was limited to mothers with a known HIV result, vaginal delivery, a colonization swab with a positive result (ie, for GBS, *E coli,* or *K pneumoniae)* and a newborn with a microbiologic swab result.

For the EOS and LOS sepsis end points, we considered 2 additional comparison groups: HUU versus HEU neonates and HEU versus HIV-infected neonates. We built propensity score models as above, with the appropriate outcome variable for the comparison, balancing on the variables listed above for EOS and LOS. CD4+ cell count results were available for only 34.7% (725/2090) of HIV-infected women, thus maternal CD4+ cell count was not included as a covariate.

We also conducted unadjusted analyses on the total cohort to understand the influence of covariates associated with HIV exposure or infection status on the endpoints evaluated.

χ^2 tests were used to compare proportions. Relative risks and 95% confidence intervals were used to assess HIV as a risk factor. Denominator for incidence was per 1000 births. Analyses were conducted by using SAS version 9·1.

Ethics Consideration

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand, South Africa and the Institutional Review Board of the Centers for Disease Control and Prevention, USA. Written, informed consent was obtained from the mother before any study procedure was undertaken. The trial was registered at ClinicalTrials.gov (NCT00136370).

RESULTS

HIV infection status was available for 98.5% (7894/8011) of mothers, 26.5% (2090/ 7894) of whom were HIV infected. Among HIV-infected women with CD4+ results available (725/2090), 21.8% (158/725) had CD4+ counts <200 cells/mm^3, 27.4% (199/725) between 200 and 350 cells/mm^3, and 50.8% (368/725) >350 cells/mm^3. The majority of CD4+ results available (467/725, 64.4%) were from women enrolled between June 2006 and October 2007. Antiretroviral treatment to prevent mother-to-child transmission of HIV was provided to 92.3% (1929/2090) of HIV-infected mothers, including 97.6% (1882/1929) who received sd-NVP and 2.4% (46/1929) who received triple antiretroviral therapy, which was initiated a mean of 151 (range 1 to 1095) days before delivery. Overall, 2130 newborns were born to 2090 HIV-infected mothers.

Significant differences in baseline demographic and clinical characteristics of HIV-infected and -uninfected mothers included HIV-infected women being older ($P < .0001$) and more likely to have UTIs (13% vs 10%), hemoglobin <10 mg/dL (13% vs 7%), tuberculosis (1.5% vs 0.2%), receive antibiotics before labor onset (30% vs 21%) and during labor (12% vs 10% for women with vaginal delivery); $P < .01$ for all observations (Table 2). HIV-infected compared with -uninfected women had higher frequencies of fever during labor (0.7% vs 0.05%); nonelective caesarian sections (26% vs 23%); and premature deliveries (5.9% vs 3.1%). HIV-exposed newborns had lower birth weights than HUU neonates (Table 2).

Infant HIV PCR testing was undertaken at a median of 42 days of age (1–347 days) in 64.2% (1367/2130) of HIV-exposed infants, of whom 8.2% (112/1367) were HIV infected. Chlorhexidine wipes did not affect vertical HIV transmission rate (65/713 [9.1%] vs 47/654 [7.2%] PCR positive in interventional versus control arms, $P = .19$). Differences in baseline demographics among HIV-exposed neonates with PCR testing results included that HIV-infected compared with HEU neonates had a lower median gestational age (38 vs 39 weeks; $P = .005$), lower birth weight (median 2995 vs 3100 g; $P = .001$) and higher frequency of exposure to meconium-stained liquor (21% vs 11%; $P = .002$) during labor.

Vaginal Colonization and Vertical Transmission of Genital-Tract Bacteria

The total colonization cohort included 5099 (3752 HIV-uninfected, 1347 HIV-infected) women with known HIV status. The matched subset analysis for assessment of vaginal colonization included 1346 HIV-infected and 1346 HIV-uninfected participants. HIV-infected women were less likely to be colonized than HIV-uninfected women with GBS (17% vs 23%; $P = .0002$), or *K pneumoniae* (7% vs 10%; $P = .008$) in the matched subset analysis. In the total cohort, prevalence of GBS colonization was similar to that observed in the matched analysis, whereas higher rates of *E coli* colonization (47% v. 43%, $P = .039$) and no difference in *K pneumoniae* colonization were observed in HIV-infected compared with HIV-uninfected women (Table 3).

There was no difference in the rate of vertical acquisition of GBS or *K pneumoniae* in HIV-exposed compared with HUU newborns. The rate of vertical acquisition of *E coli* was, however, greater in HIV-exposed than HUU neonates in the matched-subset populations (60% vs 52%, respectively; $P = .015$) as well as in the total cohort (60% vs 53%; $P = .0066$; Table 4)

Maternal HIV-Infection Status and Sepsis Within 3 Days of Age (EOS)

Two hundred and ninety (3.6%) of the 8129 infants were hospitalized for sepsis within the first 3 days of life; 29 (0.36%) with culture-confirmed sepsis and 258 (3.2%) with clinical sepsis. The incidence of EOS did not differ significantly between HIV-exposed and HUU neonates in

TABLE 2 Maternal and Newborn Demographic and Clinical Characteristics Stratified by HIV Status

Characteristic	Overall	HIV-Uninfected Mothers, *n* (%)	HIV-Infected Mothers, n (%)	*P*
Mothers	*n* = 8011	*n* = 5812	*n* = 2090	
Median age in years (range)	26 (12–51)	26 (12–49)	27 (14–51)	<.0001
Median parity (range)	1 (0–9)	1 (0–9)	1 (0–7)	<.0001
Median gravidity (range)	2 (1–10)	2 (1–10)	2 (1–8)	<.0001
Median gestational age (range)	39 (23–44)	39 (24–44)	39 (23–44)	
Medical history (%)				
Urinary tract infection	854 (11)	573 (10)	275 (13)	<.0001
Hemoglobin <10 mg/dL	692 (9)	420 (7)	264 (13)	<.0001
Gestational diabetes	20 (0.2)	17 (0.3)	3 (0.1)	.25
Maternal tuberculosis	42 (0.5)	10 (0.2)	32 (1.5)	<.0001
Received antibiotics during pregnancy (%)	1856 (23)	1218 (21)	617 (30)	<.0001
Intrapartum antibiotics (IAs) in women with vaginal delivery (% of IAs)	629/6137	436/4494 (10)	189/1554 (12)	.006
Antibiotics in 7 d before delivery	475 (6)	318 (5)	152 (7)	.48
Intrapartum fever (%)	17 (0.2)	3 (0.05)	14 (0.7)	<.0001
Prolonged rupture of membranes of ≥18 h at delivery (%)	710 (9)	525 (9)	178 (9)	.48
Unbooked/no prenatal care (%)	11 (0.1)	6 (0.1)	3 (0.1)	.64
Meconium stained liquor (%)	1200 (15)	925 (16)	256 (12)	<.0001
Delivery (%)[a]				
Spontaneous vaginal delivery	6136 (77)	4494 (77)	1554 (74)	.007
Emergency caesarian delivery	1874 (23)	1318 (23)	536 (26)	.006
Vaginal vacuum/forceps assisted	165 (2)	116 (2)	49 (2)	.31
Number of per vaginal examinations during labor				
<3	3020	2213 (38)	760 (36)	.17
≥3	4991	3599 (62)	1330 (64)	
Newborns	*n* = 8129	*n* = 5886	*n* = 2130	
Female gender	3873 (47)	2796 (48)	1028 (48)	.57
Twin birth (%)	118 (1)	74 (1)	40 (2)	.038
Preterm[b]	313 (4)	181 (3)	124 (6)	<.0001
Median birth weight, g (range)	3130 (480-5630)	3160 (670-5630)	3050 (480-4690)	<.0001
Median Apgar 5 min (range)	10 (0-10)	10 (0-10)	10 (0-10)	.27
Outcome				
Stillborn	26 (<0)	19 (<0)	7 (<0)	.97
Died shortly after delivery	15 (<0)	12 (<0)	3 (<0)	.56
Surviving newborn admitted to neonatal ward	788 (10)	578 (10)	210 (10)	.80

[a] Includes mode of delivery for all singletons and first-born twins only.
[b] Note: Term infants: 7811 overall; 5701 HIV-unexposed infants, 2005 HIV-exposed infants.

the matched subset analysis or the total cohort. The incidence of culture-confirmed EOS was, however, 3.3-fold greater among HIV-exposed compared with HUU in the matched analysis ($P = .05$), and 1.67-fold increased in the total-cohort analysis ($P = .167$; Table 5). In the total cohort, among HIV-exposed neonates born to mothers with CD4+ results, the incidence of EOS (per 1000 births) was inversely associated with the immunologic status of the mother (CD4+ cells/mm^3 <200, 75.9; 200–350, 40.2; >350, 19.0; $P = .0065$, trend is linear).

Among HIV-exposed newborns tested by HIV PCR, there was a higher incidence of clinically diagnosed EOS in HIV-infected (102) compared with HEU newborns (30.6) in the matched subset analysis ($P = .033$), with a similar increase in the total-cohort analysis ($P < .0001$; Table 6). In the matched-subset analysis, HEU newborns had a lower incidence (20.6/1000 births) of EOS than HUU newborns

TABLE 3 Prevalence of Bacterial Vaginal Colonization in HIV-infected and -uninfected Women During Labor

	Total Colonization Cohort			Matched Subset		
	HIV-negative (*n* = 3752),*n* (%)	HIV-positive (*n* = 1347),*n* (%)	*P*	HIV-negative (*n* = 1346),*n* (%)	HIV-positive (*n* = 1346),*n* (%)	*P*
GBS						
GBS-colonized mothers (total)	824 (22)	231 (17)	.0002	307 (23)	230 (17)	.0002
E coli						
E coli-colonized mothers (total)	1624 (43)	677 (47)	.0385	603 (45)	626 (47)	.37
K pneumoniae						
K pneumoniae-colonized mothers (total)	301 (8)	94 (7)	.2189	132 (10)	94 (7)	.008

TABLE 4 Vertical Transmission of Pathogenic Bacteria From Mother to Newborn Stratified by Maternal HIV-Infection Status

	Total Colonization Cohort			Matched Subset		
	HIV-negative, *n* (%)	HIV-positive, *n* (%)	*P*	HIV-negative, *n* (%)	HIV-positive, *n* (%)	*P*
GBS						
Total number infants born vaginally to GBS-colonized mother.	648	181		177	177	
GBS-colonized vaginally born newborn[a]	372/641 (58)	93/179 (52)	.1467	94/174 (54)	90/175 (51)	.63
E coli						
Total number infants born vaginally to *E coli*-colonized mother.	1277	460		449	449	
E coli-colonized, vaginally born newborn	665 (53)	269 (60)	.0066	232 (52)	263 (60)	.015
K pneumoniae						
Total number infants born vaginally to *K pneumoniae*-colonized mother.	241	73		69	69	
K pneumoniae-colonized, vaginally born newborn[b]	74 (31)	20 (27)	.5887	20 (29)	19 (27)	.85

[a] Nine infants born vaginally to GBS-colonized mothers did not have swab collected/ processed for GBS: 7 HIV-unexposed and 2 HIV-exposed infants.
[b] One infant born to an HIV-uninfected mother colonized by *K pneumoniae* did not have swab processed.

(33.7; $P = .045$); with a similar difference observed in the total-cohort analysis ($P = .004$; Table 7). There was no difference in incidence of culture-confirmed EOS between HEU and HUU in the matched-subset or total-cohort analysis.

Maternal HIV-Infection Status and Neonatal Sepsis Between Days 4 and 28 (LOS)

The incidence of LOS was similar between HIV-unexposed and HIV-exposed neonates in the matched analysis and total cohort (Table 5). Among HIV-exposed neonates, the incidence of LOS was greater in HIV-infected (26.8) than HEU (5.6; $P = .042$) neonates in the total cohort, with a similar trend observed in the matched analysis (30.6 vs 10.2; $P = .62$; Table 6). The incidence of LOS did not differ between HEU and HUU in the matched or total-cohort analysis, Table 7.

Among neonates with maternal CD4+ results available, the incidence of LOS was inversely associated with maternal immunologic status (CD4+ <200 cells/mm^3, 19.0; 200–350 cells/mm^3, 10.0; and >350 cells/mm^3, 8.1; $P = .55$).

DISCUSSION

To our knowledge, this is the first study to have reported on the impact of maternal HIV infection on vertical transmission of bacteria to newborns, and on the relative incidence of EOS and LOS between HIV-exposed and HIV-unexposed neonates. Our data come from a setting of high maternal HIV infection (29%), high usage of nevirapine to prevent mother-to-child HIV transmission, and extremely low usage of triple antiretroviral therapy among mothers. Whereas it has been hypothesized that HEU neonates are at higher risk of developing sepsis due to impaired maternal transfer of antibody,[7] data from our study do not corroborate such speculation. On the contrary, EOS rates were marginally lower in HEU than HUU in the total cohort and matched subset analysis; and no difference was observed for LOS rates between these groups.

The absence of an increased risk of GBS colonization among HIV-infected women in our study is supported by another recently published study from Malawi, which found no evidence of increased GBS colonization associated with HIV, except in women with CD4+ counts >500mm^3.[11] Overall, our observations that maternal colonization and vertical transmission rates for leading sepsis pathogens did not differ considerably between HIV-infected and -uninfected mothers lend support to our observation that HIV-exposed and -unexposed newborns did not differ in neonatal sepsis risk. Although the number of culture-confirmed EOS cases in our cohort was small, limiting our power to detect a difference in this end point, we believe that the marginal trend in culture-confirmed EOS does not belie a true difference in risk between HIV-exposed and -unexposed newborns. However, we acknowledge that the clinical sepsis end point has lower specificity than an invasive sepsis end point, and thus, culture-confirmed trends may not match clinical sepsis trends.

Our study, however, did identify that EOS rates were higher in HIV-infected neonates. HIV exposed neonates born to severely immunocompromized mothers (CD4+ <200 cells/mm^3) were at higher risk of developing EOS and LOS than those born to mothers with CD4+ >350 cells/mm^3. This observation, however, requires further corroboration because CD4+ testing was very limited. The increased risk of EOS and LOS among HIV-infected compared with HEU in the total cohort and a similar trend in the matched analysis, emphasize the need to further reduce mother-to-child transmission of HIV.

Data from our study are in contrast to other studies that have reported an increase in morbidity and mortality in HIV-exposed infants, including during early infancy.[13,23] Mortality rate in African HEU infants is 72.5 to 98.7/1000, compared with 48.0 to 91.0/1000 in HUU infants and 309.1 to 420.8/1000 in HIV-infected infants,[14,16] with almost 30% of these deaths occurring in first 8 weeks

TABLE 5 Impact of In Utero HIV Exposure on Incidence of Early- (Within 3 d) and Late-Onset (Between 4 and 28 d of Age) Neonatal Sepsis

	Total				Matched Subset		
	Total (n = 8129), n (rate; 95%CI)	HIV Unexposed (n = 5886), n (rate; 95% CI)	HIV Exposed (n = 2130), n(rate; 95%CI)	P value(HIV Unexposed vs HIV Exposed)	HIV Unexposed (n = 2054), n (rate)	HIV Exposed (n = 2054), n (rate)	P value (HIV Unexposed vs HIV Exposed)
Early Onset Sepsis (EOS)							
Culture-confirmed sepsis	29 (3.6; 2.4, 5.1)	18 (3.1; 1.8, 4.8)	11 (5.2; 2.6, 9.2)	.165	3 (1.5; 0.3, 4.3)	10 (4.9; 2.3, 8.9)	.05
GBS	16 (2.0; 1.1, 3.2)	12 (2.0; 1.0, 3.6)	4 (1.9; 0.5, 4.8)		2 (1.0; 0.1, 3.5)	4 (1.9; 0.5, 5.0)	
E coli	2 (0.2; 0.03, 0.9)	0 (0, 0.63)	2 (0.9; 0.1, 3.4)		0	1 (0.5)	
Staphylococcus aureus	2 (0.2; 0.03, 0.9)	1 (0.2, (0.004, 0.9)	1 (0.5; 0.01, 2.6)		0	1 (0.5; 0.0, 2.7)	
K pneumoniae	1 (0.1; 0.0003, 0.7)	0 (0, 0.63)	1 (0.5; 0.01, 2.6)		0	1 (0.5; 0.0, 2.7)	
Other	8 (1.0; 0.4, 1.9)	5[a] (0.8; 0.3, 2.0)	3[b] (1.4; 0.3, 4.1)		3 (1.5; 0.3, 4.3)	3 (1.5; 0.3, 4.3)	
Clinical sepsis only	258 (31.7; 28.0, 35.8)	202 (34.3; 29.8, 39.3)	55 (25.8; 19.5,33.5)	.056	57 (27.8; 21.1, 35.8)	51 (24.8; 18.5,32.5)	.56
Deaths within 3 d of age[c]	3 (0.4; 0.1, 1.1)	3 (0.5; 0.1, 1.5)	0 (0, 1.7)	.570	1 (0.5; 0.0, 2.7)	0 (0.0; 0.0, 1.8)	1.000 (exact)
Overall	290 (35.7; 31.7,39.9)	223 (37.9; 33.2, 43.1)	66 (31.0; 24.0,39.3)	.117	61 (29.7; 22.8, 38.0)	61 (29.7; 22.8,38.0)	1.000
Late Onset Sepsis (LOS)							
Culture-confirmed cases	20 (2.5; 1.5, 3.8)	12 (2.0; 1.1, 3.6)	7 (3.3; 1.3, 6.8)	.310	3 (1.5; 0.3, 4.3)	6 (2.9; 1.1, 6.3)	.51 (exact)
GBS	5[d] (0.6; 0.2, 1.4)	3 (0.5; 0.1, 1.5)	1 (0.5; 0.01, 2.6)				
E coli	8 (1.0; 0.4, 1.9)	6 (1.0; 0.4, 2.2)	2[e] (0.9; 0.1, 3.4				
S aureus	2 (0.2, 0.03, 0.9)	2 (0.3; 0.04, 1.2)	0 (0, 1.7)				
Klebsiella sp.	2 (0.2; 0.03, 0.9)	0 (0, 0.63)	2[f] (0.9; 0.1, 3.4)				
Other	3 (0.4; 0.1, 1.1)	1[g] (0.2; 0.004, 0.9)	2[h] (0.9; 0.1, 3.4)				
Clinical sepsis only	14 (1.7; 0.9, 2.9)	8 (1.4; 0.6, 2.7)	6 (2.8; 1.0, 6.1)	.167	4 (1.9; 0.5, 5.0)	7 (3.4; 1.4, 7.0)	.37
Overall	34 (4.82; 2.9, 5.8)	20 (3.4; 2.1, 5.2)	13 (6.1; 3.3, 10.4)	.095	7 (3.4; 1.4, 7.0)	13 (6.3; 3.4, 10.8)	.18

[a] *Enterococcus faecalis* (×3), *Acinetobacter baumanii* ×2;

[b] Viridans *Streptococcus* (×2), *Acinetobacter lwoffii*.

[c] Early deaths (<3 d) which did not fulfill EOS-meconium liqor (ML) or EOS-Clinical sepsis definitions, but included as EOS after panel review.

[d] One case of LOS GBS with unknown HIV result.

[e] One case coinfected with *S aureus*.

[f] One coinfected with *S aureus*, one with *Enterococcus faecium*.

[g] *Streptococcus* spp.

[h] E faecalis ×2 cases.

TABLE 6 Incidence of Early- and Late-Onset Sepsis in HIV-exposed HIV-infected (HIV+) and HIV-exposed Uninfected (HEU) Neonates

Sepsis Categorization	Total Infants With HIV PCR Results			Matched Subset		
	HIV+ (n = 112),n (rate; 95% CI)	HEU(n = 1253),n (rate; 95% CI)	P	HIV+(n = 98),n (rate; 95% CI)	HEU(n = 98),n (rate; 95% CI)	P
Early-onset sepsis						
Culture-confirmed	2 (17.9; 2.2, 63.0)	5 (4.0; 1.3, 9.3)	.107	0 (0; 0, 36.9)	1 (10.2, 0.3, 55.5)	1.00
Clinical sepsis only	13 (116; 63.3190.3)	22 (17.6; 11.0, 26.5)	<.0001	10 (102.0, 50.0179.7)	2 (20.4, 2.5, 71.8)	.033
Overall	15 (134; 76.9, 211.3)	27 (21.5; 14.2, 31.2)	<.0001	10 (102.0; 50.0179.7)	3 (30.6, 6.4, 86.9)	.08
Late-onset sepsis						
Culture-confirmed	2 (17.9; 2.2, 63.0)	5 (4.0; 1.3, 9.3)	.107	2 (20.4; 2.5, 71.8)	0 (0; 0, 36.9)	.50
Clinical sepsis only	1 (8.9; 0.2, 48.7)	2 (1.6; 0.2, 5.8)	.227	1 (10.2; 0.3, 55.5)	1 (10.2; 0.3, 55.5)	1.00
Overall	3 (26.8; 5.6, 76.3)	7 (5.6; 2.2, 11.5)	.042	3 (30.6; 6.4, 86.9)	1 (10.2; 0.3, 55.5)	.62

of life.[14] This is particularly pertinent because disease progression in African HIV-infected infants is rapid, including 85% of infants progressing to severe AIDS by 6 months of age.[24] Our data are also not consistent with the data reported from a study in Belgium, in which the incidence of neonatal GBS sepsis, especially LOS, was greater in HIV-exposed (1.55%) than HIV-unexposed (0.08%) newborns.[25]

The importance of adjusting for maternal factors when assessing the association between maternal HIV infection and neonatal sepsis is evident from our observation that many risk factors commonly associated with EOS, including prolonged ROM and UTIs, were more prevalent in HIV-infected women. A major strength of our study was the utilization of propensity score methods to better evaluate associations between our comparison groups. Propensity score matching allowed us to select an appropriate referent group similar on known or suspected risk factors for each outcome evaluated while blinded to the outcome. When conclusions drawn from the entire cohort differ from the matched subset, we believe the matched subset gives the most valid comparison between the groups with regard to whether maternal-infection status, newborn HIV-exposure or HIV-infection status is responsible for the differences between groups in the applicable comparisons. The differences between the entire cohort and matched subset analyses highlights the dangers of drawing conclusions about the impact of HIV exposure on neonatal morbidity (or mortality) without appropriately adjusting for other factors.

Our study does, however, have some limitations including that CD4+ results were available for only 34.7% of HIV-infected women, and HIV testing was only undertaken in 64% of HIV-exposed infants. It is reassuring that infants with HIV testing were similar to infants without HIV testing for the variables available, although the paucity of maternal CD4+ count data prevented us from ascertaining whether this variable differed between the 2 groups. Incidentally, vertical transmission rate of HIV infection observed in our study (8.2%), where sd-NVP was recommended for the mother and newborn, was not affected by chlorhexidine interventional wipes and consistent with the transmission rate reported elsewhere with the same regimen (10.3%).[24] A further limitation of our study included that our enrollment procedures and inclusion and exclusion criteria skewed enrollment away from inclusion of preterm infants. The incidence of preterm neonates in our study population was lower than in the hospital population (4% vs 15%, respectively) (S. C. Velaphi, personal communication, January 10, 2011). Consequently, because prematurity is a recognized risk factor for neonatal sepsis, the rates of EOS and LOS

TABLE 7 Impact of Maternal HIV Infection on Early- and Late-Onset Neonatal Sepsis in HIV-exposed, Uninfected (HEU) and HIV-unexposed, Uninfected (HUU) Neonates

Sepsis Categorization	Total HIV Uninfected Infants			Matched Subset		
	HUU(n = 5867),n (rate; 95% CI)	HEU(n = 1253),n (rate; 95% CI)	P	HUU(n = 1216),n (rate; 95% CI)	HEU(n = 1216),n (rate; 95% CI)	P
Early-onset sepsis						
Culture-confirmed	21 (3.6; 2.2, 5.5)	5 (4.0; 1.3, 9.3)	.797	4 (3.3; 0.9, 8.4)	4 (3.3; 0.9, 8.4)	1.000
Clinical sepsis only	202 (34.4; 30.0, 39.4)	22 (17.6; 11.0, 26.5)	.002	37 (30.4; 21.5, 41.7)	21 (17.3; 10.7, 26.3)	.034
Overall	223 (38.0; 33.3, 43.2)	27 (21.5; 14.2, 31.2)	.004	41 (33.7; 24.3, 45.5)	25 (20.6; 13.3, 30.2)	.0459
Late-onset sepsis						
Culture-confirmed	12 (2.0; 1.1, 3.6)	5 (4.0; 1.3, 9.3)	.203	3 (2.5; 0.5, 7.2)	5 (4.1; 1.3, 9.6)	.726
Clinical sepsis only	8 (1.4; 0.6, 2.7)	2 (1.6; 0.2, 5.8)	.692	2 (1.6; 0.2, 5.9)	2 (1.6; 0.2, 5.9)	1.000
Overall	20 (3.4; 2.1, 5.3)	7 (5.6; 2.2, 11.5)	.306	5 (4.1; 1.3, 9.6)	7 (5.8; 2.3, 11.8)	.563

reported in our study may underestimate the true rates of neonatal sepsis in our setting. Nevertheless,a recent review reported that the incidence of clinical sepsis in infants <60 days in developing countries was 49 to 170 per 1000 and 5.5 per 1000 for culture-confirmed sepsis.[26] Although the incidence of neonatal sepsis observed in our study was toward the lower bound of this for clinical sepsis (33.4 per 1000), we report a similar incidence of culture-confirmed sepsis (5.9 per 1000). The lower incidence of clinical sepsis rates in our study may have been influenced by more stringent criteria used in our study for diagnosing "clinical sepsis."

CONCLUSIONS

Reducing neonatal morbidity and mortality has been identified as a key focus area for public health under Millennium development goal number 4, which aims at reducing under-5 mortality by two-thirds in comparison with 1990 levels.[1] Although there was a high incidence of neonatal sepsis in our study population, including among the highest reported incidence rates of GBS sepsis from any developing country, maternal HIV infection does not appear to increase the risk of either early- or late-onset neonatal sepsis, except among newborns with HIV infection and possibly among newborns of severely immunocompromized mothers. Consequently, we do not predict the HIV prevention roll-out in sub-Saharan Africa will result in important reductions in neonatal sepsis in this region, and sepsis-specific interventions such as GBS vaccines may hold most promise in reducing neonatal infection-associated mortality.

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Address correspondence to Shabir A Madhi, MBBCh, FCPaeds(SA), PhD, Chris Hani Rd, Chris Hani-Baragwanath Hospital, New Nurses Residence-11th Floor, Central-West wing, PO Box 90753, Bertsham, 2013 Soweto, South Africa. E-mail: shabirm@nicd.ac.za

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Appendix 6: Paper III

Increased Risk for Group B *Streptococcus* Sepsis in Young Infants Exposed to HIV, Soweto, South Africa, 2004–2008[1]

Clare L. Cutland, Stephanie J. Schrag, Michael C. Thigpen, Sithembiso C. Velaphi, Jeannette Wadula, Peter V. Adrian, Locadiah Kuwanda, Michelle J. Groome, Eckhart Buchmann, Shabir A. Madhi

Although group B *Streptococcus* (GBS) is a leading cause of severe invasive disease in young infants worldwide, epidemiologic data and knowledge about risk factors for the disease are lacking from low- to middle-income countries. To determine the epidemiology of invasive GBS disease among young infants in a setting with high maternal HIV infection, we conducted hospital-based surveillance during 2004–2008 in Soweto, South Africa. Overall GBS incidence was 2.72 cases/1,000 live births (1.50 and 1.22, respectively, among infants with early-onset disease [EOD] and late-onset [LOD] disease). Risk for EOD and LOD was higher for HIV-exposed than HIV-unexposed infants. GBS serotypes Ia and III accounted for 84.0% of cases, and 16.9% of infected infants died. We estimate that use of trivalent GBS vaccine (serotypes Ia, Ib, and III) could prevent 2,105 invasive GBS cases and 278 deaths annually among infants in South Africa; therefore, vaccination of all pregnant women in this country should be explored.

In 2013, a total of 41.6% (2.6 million) of deaths worldwide in children <5 years of age occurred in neonates; 76.7% occurred within 6 days after birth (*1*). Furthermore, in 2012, ≈6.9 million probable cases of severe bacterial infections and 680,000 associated deaths occurred among neonates (*2*). Nevertheless, there is a paucity of data from low- and middle-income countries on pathogen-specific causes of neonatal sepsis, particularly during the first day of life, and it is unknown whether in utero HIV exposure increases susceptibility to severe neonatal bacterial infections.

Author affiliations: University of the Witwatersrand, Johannesburg, South Africa (C.L. Cutland, S.C. Velaphi, J. Wadula, P.V. Adrian, L. Kuwanda, M.J. Groome, E. Buchmann, S.A. Madhi); Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, Johannesburg (C.L. Cutland, P.V. Adrian, L. Kuwanda, M.J. Groome, S.A. Madhi); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (S.J. Schrag, M.C. Thigpen); Chris Hani Baragwanath Academic Hospital, Johannesburg (S.C. Velaphi, J. Wadula, E. Buchmann); National Institute for Communicable Diseases, Sandringham, South Africa (S.A. Madhi)

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Group B *Streptococcus* (GBS) is a leading cause of severe invasive disease in young infants. A meta-analysis dominated by studies from high-income countries estimated global incidence of 0.53 cases/1,000 live births during 2000–2011 (*3*). Considerable intra- and interregional variation in the incidence of invasive early-onset disease (EOD; disease in 0- to 6-day-old infants) was observed (*3*–*5*), ranging from 1.21 cases/1,000 live births (95% CI 0.5–1.91) in Africa to 0.02 cases/1,000 live births (95% CI 0–0.07) in Southeast Asia (*3*). This variability is inconsistent with the lesser difference in prevalence of maternal GBS colonization, the major risk factor for EOD, in women from different regions (20.9% in Africa, 13.4% in Southeast Asia) (*6*). Maternal HIV infection has not been associated with a higher prevalence of GBS colonization (*7*,*8*), except among women with CD4+ lymphocyte counts of >500 cells/mm^3 (*8*).

Providing intrapartum antimicrobial drug prophylaxis (IAP) to women identified as rectovaginally colonized by GBS at 35–37 weeks' of pregnancy has been associated with a >80.0% reduction in EOD (*9*); however, this strategy is logistically challenging to implement and maintain in resource-constrained settings. Furthermore, IAP has not decreased the incidence of late-onset disease (LOD; disease in 7- to 90-day-old infants) (*10*).

Progress has been made in the development of a trivalent GBS polysaccharide–protein conjugate vaccine (GBS-CV), which is targeted for use in pregnant women; the goal is to enhance transplacental transfer of capsular antibody to the fetus (*1*,*8*–*10*), which could protect against EOD and LOD. Improved estimates of the incidence of invasive GBS disease are needed from low- and middle-income countries to contextualize the prioritization of GBS vaccination and determine whether temporal changes in invasive serotypes should be considered in the design of serotype-specific GBS vaccine.

We evaluated the clinical and microbiological epidemiology, incidence, and serotype distribution of invasive

[1]Preliminary results from this study were presented at the 8th World Congress of the World Society for Pediatric Infectious Diseases, November 19–22, 2013, Cape Town, South Africa.

GBS disease among young infants in a setting with a high prevalence of maternal HIV infection. A secondary aim was to estimate the potential effect of a trivalent GBS-CV in reducing the number of invasive GBS cases nationally.

Materials and Methods

Study Setting and Design

During 2004–2008, we undertook hospital-based surveillance of culture-confirmed invasive bacterial sepsis in infants 0–90 days of age at Chris Hani Baragwanath Academic Hospital (CHBAH), a public secondary–tertiary health care facility in Soweto, South Africa. CHBAH is the only public hospital in Soweto with neonatal care facilities; ≈90.0% of all hospitalizations from the community occur in this hospital. Soweto is a predominantly black-African community and has 1.4 million inhabitants, including 125,000 children <5 years of age and a birth cohort of ≈28,000/year (*11*); ≈21,000 are delivered at CHBAH and 7,000 are delivered at 1 of 6 community-based midwife obstetric units. Women with potentially complicated deliveries at midwife obstetric units and clinically ill newborns are referred to CHBAH by ambulance.

Health care for pregnant women and children is provided free of charge in South Africa (*12*). Most deliveries in Soweto (95.0%) (E. Buchmann, pers. comm., 2014 Jul 19) and in South Africa as a whole (87.3% in 2010) (*13*) occur in health facilities. Voluntary counseling and testing for HIV is offered at antenatal clinics; >96.0% of pregnant women accept testing (C. Mnyani, pers. comm,, 2014 Jul 28). Single-dose nevirapine, administered as standard of care to women in labor and their newborns to prevent mother-to-child HIV transmission, was supplemented in 2007 with triple antiretroviral therapy to immunocompromised women (<350 CD4+ cells/mm^3) from 34 weeks' gestation onward.

During the surveillance period, HIV prevalence in pregnant women remained stable at 29.9% (*14*), and ≈18.0% of all children were born prematurely (<37 weeks gestational age) or had a low birthweight (<2,500 g) (CHBAH, unpub. data). Gestational age was determined on the basis of the available obstetric or neonatal assessments by attending physicians. Healthy newborns are routinely discharged home 12 h after vaginal or 72 h after cesarian delivery.

At CHBAH, newborns with signs and symptoms of severe illness at birth or before discharge from postnatal wards are admitted to the neonatal unit; infants who are discharged home after delivery and subsequently return for suspected bacterial infections are hospitalized in the general pediatric wards. Investigation and treatment of neonates and young infants with suspected invasive bacterial disease were conducted according to standard of care by attending physicians. Investigations included complete blood cell counts and blood cultures for all infants. Lumbar punctures (to obtain cerebrospinal fluid [CSF] samples for biochemistry, microscopy, and antimicrobial drug sensitivity testing and culture) were limited to infants with GBS-positive cultures of blood samples obtained at birth and to all infants admitted from the community for suspected sepsis. Sterile-site cultures were processed at the National Health Laboratory Service (NHLS). Blood cultures were evaluated by using the BacT/Alert microbial system (Organon Teknika, Durham, NC, USA). GBS isolates were retrieved from NHLS, stored at –70°C, and serotyped by latex agglutination (*15*). During the surveillance period, empiric treatment for suspected sepsis consisted of intravenous penicillin and gentamicin for neonates and ampicillin and gentamicin for infants 1–12 months of age; case-patients with suspected meningitis received ampicillin and cefotaxime empirically.

Maternal screening for rectovaginal GBS colonization during pregnancy is not routinely performed in public health facilities in South Africa. Before 2007, CHBAH IAP guidelines recommended administration of intravenous ampicillin (1 g/6 h) and oral metronidazole (400 mg 3×/d) for suspected chorioamnionitis and prolonged rupture of membranes. In January 2007, targeted risk-based IAP was implemented for possible GBS infection in women with preterm labor, a previous infant infected with GBS, or a GBS-positive culture; treatment consisted of an initial 2-g dose of intravenous ampicillin, followed by intravenous ampicillin (1 g/4 h) until delivery. During the surveillance period, 10.5% of women received IAP during labor (*16*).

Infants 0–90 days of age who were admitted to CHBAH with GBS isolated from a normally sterile site were identified through screening of ward admissions and microbiological records within 24 h of identification of GBS. We also undertook an audit of the NHLS database to identify all invasive pathogens isolated from infants over the study period. Invasive GBS disease was categorized as bacteremia if identified in blood only and as meningitis if identified from CSF or if there was CSF cytologic evidence of purulent meningitis (>5 leukocytes/mm^3; adjusted in traumatic lumbar punctures to allow 1 leukocyte/500 erythrocytes) in an infant with GBS bacteremia.

Demographic, birth, maternal HIV infection status, and other clinical data were abstracted from infants' medical records by study doctors. HIV exposure was determined by abstracting antenatal HIV test results of mothers and supplemented by HIV ELISA results from maternal or infant blood tests conducted by attending physicians. The HIV infection status of HIV-exposed infants was determined by using a qualitative HIV PCR at the discretion of the attending physician.

Statistical Considerations

Incidence was calculated as cases per 1,000 live births. Administrative live birth data from CHBAH and community clinics for Soweto and HIV prevalence survey data for the surveillance period were used to determine population denominators for HIV-infected and -uninfected women. Established patient referral protocols limited the chance that neonates, especially newborns, living outside the hospital catchment area were admitted to CHBAH. A sensitivity analysis of incidence was undertaken to account for GBS case-patients for whom maternal HIV status was unknown. For overall and annual incidence calculations, we attributed the prevalence of HIV exposure among case-patients with known exposure status to case-patients with unknown exposure status. Alternate sensitivity analyses assumed all cases with unknown maternal HIV status were HIV-exposed or, conversely, HIV-unexposed.

On the basis of 2012 population data for number of live births in South Africa (1,168,403) (*17*) and national prevalence of HIV infection in pregnant women (29.5%) (*18*), we used our study data to estimate annual national number of invasive GBS cases and deaths and stratified estimates by HIV exposure. We estimated the number of annual vaccine-preventable invasive GBS cases and deaths in South Africa on the basis of the conservative assumption that administration of GBS-CV to pregnant women (*19*) would not protect against invasive GBS disease in infants born at ≤33 weeks' gestation, and we adjusted for the proportion of serotypes included in the current experimental trivalent GBS-CV (Ia, Ib, and III) and a future pentavalent vaccine (addition of serotypes II and V). These estimates were based on hypothetical vaccine efficacy assumptions of 75.0% for the overall population; 85.0% for HIV-unexposed infants; and 65.0% for HIV-exposed infants, as determined on the basis of the lower immunogenicity of trivalent GBS-CV in HIV-infected pregnant women (*19*).

Proportions were compared by using the χ^2 and Fisher exact tests, as appropriate; Wilcoxon rank sum test (nonparametric) was applied for continuous variables. Univariate analysis was performed to determine factors associated with GBS-related death. Two-sided p values ≤0.05 were considered statistically significant, and 95% CIs were calculated. Analyses were conducted by using STATA/IC 13.0 (StataCorp, College Station, TX, USA).

Ethics Consideration

The study was approved by the Human Research Ethics Committee, University of the Witwatersrand (M03–10–07 and M10–367) and the institutional review board of the Centers for Disease Control and Prevention. Mothers of infants prospectively identified with invasive GBS disease signed informed, written consent. Consent was waived by the ethics committees for retrospective review of records of cases identified after discharge or death of the infant.

Results

During the surveillance period, 389 invasive GBS cases were identified in infants 0–90 days of age; 214 (55.0%) cases were EOD. Complete medical records were unavailable for 17 cases (10 EOD, 7 LOD), which were included in incidence calculations but not in univariable analysis. Overall incidence of invasive GBS was 2.72 cases/1,000 live births (95% CI 2.46–3.01). EOD incidence was 1.50 cases/1,000 live births (95% CI 1.30–1.71), and LOD incidence was 1.22 cases/1,000 live births (95% CI 1.05–1.42); incidences for both were generally similar across years (data not shown). Overall, 26.6% of case-patients were born prematurely, including 29.8% of EOD and 22.3% of LOD case-patients. Most (69.4%) preterm births occurred at ≤33 weeks' gestation, including 63.0% and 80.6% of those for EOD and LOD case-patients, respectively (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/4/14-1562-Techapp1.pdf). Of the 214 EOD case-patients, 138 (64.5%) had positive culture results for blood samples obtained at birth (median age 0 days, interquartile range 0–1; Figure 1, panel A). Forty-four percent of LOD cases were detected during week 2 of life (median age 16 days, interquartile range 11–29; Figure 1, panel B). Infants with LOD were 5.57-fold (95% CI 3.50–8.90) more likely than infants with EOD to have meningitis (61.7% vs. 22.4%; p<0.0001).

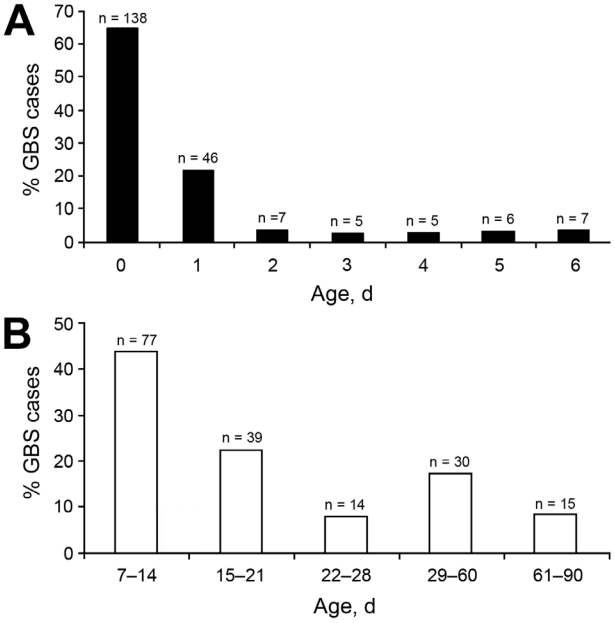


Figure 1. Age distribution of young infants (0–90 days of age) with invasive group B *Streptococcus* (GBS) sepsis, Soweto, South Africa, 2004–2008. A) Distribution for 214 infants with early-onset disease. B) Distribution for 175 infants with late-onset disease.

Invasive GBS Disease and HIV Exposure

Maternal HIV infection status was available for 327 (84.1%) GBS case-patients, of whom 161 (49.2%) were HIV-exposed (online Technical Appendix Table 1). HIV-exposure data were unavailable for 12.3% of EOD and 11.9% of LOD case-patients. Regimens to prevent mother-to-child HIV transmission were documented in only 41.6% of the infants' medical records and were therefore not analyzed in this study. HIV PCR results were available for 46 (28.6%) of 161 HIV-exposed infants: 6 had EOD (all nonreactive results) and 40 had LOD (8 [20.0%] had reactive results).

Infants with LOD were more likely than those with EOD to be HIV-exposed (58.1% vs. 41.9%; p = 0.004) (online Technical Appendix Table 1). HIV-exposed and -unexposed case-patients overall or when stratified by EOD and LOD did not differ substantially with regard to mode of delivery, preterm birth, and low birthweight (online Technical Appendix Table 1) or to exposure to meconium-stained liquor, prolonged rupture of membranes, or IAP during labor (data not shown).

The incidence of invasive GBS disease was 2.25-fold (95% CI 1.84–2.76) greater in HIV-exposed than HIV-unexposed infants (4.46 cases/1,000 live births [95% CI 3.85–5.13] vs. 1.98 cases/1,000 live births [95% CI 1.71–2.28]) (Table 1). The higher incidence of GBS disease in HIV-exposed compared with HIV-unexposed infants was evident for EOD case-patients (2.10 vs. 1.24 cases/1,000 live births, respectively; risk ratio 1.69, 95% CI 1.28–2.24) and more so for LOD case-patients (2.36 vs. 0.74 cases/1,000 live births, respectively; risk ratio 3.18, 95% CI 2.34–4.36). Bacteremia and meningitis incidence was also higher in HIV-exposed than HIV-unexposed infants (Table 1). These differences in incidence of invasive GBS disease remained significant in all sensitivity analyses in which missing maternal HIV infection status were extrapolated (Table 1), except for EOD when infants with unknown HIV-exposure status were assumed to be HIV-unexposed (Table 1).

Table 1. Incidence of invasive group B *Streptococcus* sepsis in 0- to 90-day-old infants, by in utero exposure to HIV, Soweto, South Africa, 2004–2008*

	Overall		Bacteremia		Meningitis	
HIV exposure status	No. cases, incidence (95% CI)†	RR (95 % CI)	No. cases, incidence (95% CI) †	RR (95%CI)	No. cases, incidence (95% CI) †	RR (95% CI)
Early-onset disease						
Proration of unknown exposure‡						
Unexposed	124, 1.24 (1.03–1.48)	1.69 (1.28–2.24)	103, 1.03 (0.84–1.25)	1.43 (1.03–1.97)	21, 0.21 (0.13–0.32)	3.00 (1.63–5.58)
Exposed	90, 2.10 (1.69–2.58)		63, 1.47 (1.13–1.88)		27, 0.63 (0.41–0.92)	
Unknown, assume exposed						
Unexposed	104, 1.04 (0.85–1.26)	2.47 (1.87–3.26)	ND		ND	
Exposed	110, 2.57 (2.11–3.09)		ND		ND	
Unknown, assume unexposed						
Unexposed	139, 1.39 (1.17–1.64)	1.26 (0.94–1.68)	ND		ND	
Exposed	75, 1.75 (1.38–2.19)		ND		ND	
Late-onset disease						
Proration of unknown exposure‡						
Unexposed	74, 0.74 (0.58–0.93)	3.18 (2.34–4.36)	27, 0.27 (0.18–0.39)	3.37 (2.01–5.73)	47, 0.47 (0.35–0.62)	3.08 (2.07–4.60)
Exposed	101, 2.36 (1.92–2.86)		39, 0.91 (0.65–1.24)		62, 1.45 (1.11–1.85)	
Unknown, assume exposed						
Unexposed	62, 0.62 (0.48–0.79)	4.25 (3.09–5.89)	ND		ND	
Exposed	113, 2.64 (2.17–3.17)		ND		ND	
Unknown, assume unexposed						
Unexposed	89, 0.89 (0.71–1.09)	2.25 (1.66–3.07)	ND		ND	
Exposed	86, 2.01 (1.61–2.48)		ND		ND	
Early-onset plus late-onset disease, exposed vs. unexposed	2.25 (1.84–2.76)		1.83 (1.40–2.39)		3.05 (2.20–4.25)	

*RR, relative risk; ND, not done.
†Incidence = no. cases/1,000 live births.
‡Based on prevalence of HIV exposure among those tested.

Factors Associated with Death among Infants with Invasive GBS

The overall case-fatality rate (CFR) was 16.9%; the CFR for meningitis (24.3%) was 2.4-fold greater than that for bacteremia (11.8%; odds ratio 2.24, 95% CI 1.33–4.35; p = 0.0015). The median duration of hospitalization was 1 day for infants who died and 15 days for those who survived (online Technical Appendix Table 1). In univariate analysis, meningitis and very low birthweight (<1,500 g) were associated with a higher overall CFR (p = 0.002 and p = 0.003, respectively). Prematurity (≤33 weeks' gestation) was associated with a higher CFR in EOD but not LOD case-patients (p = 0.008 and p = 0.68, respectively) (online Technical Appendix Table 2).

Antimicrobial Drug Susceptibility and Serotyping of GBS

Antimicrobial drug sensitivity profiles were available for 385 (98.9%) of 389 isolates; all were penicillin sensitive. Macrolide resistance was prevalent in 15 (5.5%) of 273 of isolates.

Of 389 isolates, 213 (54.8%) were available for serotyping, including 125 (58.6%) from EOD case-patients and 88 (41.3%) from LOD case-patients. The proportion of isolates available for serotyping increased each year: 2004, 15.6%; 2005, 45.1%; 2006, 65.8%; 2007, 63.3%; 2008, 75.0%. Overall, serotypes Ia and III accounted for 84.0% of all serotypes (19.2% [41/213] and 64.8% [138/213], respectively) and for 74.4% of EOD and 97.7% of LOD cases (p<0.001; Figure 2). Serotype distribution remained similar throughout the study and did not differ by HIV-exposure status (data not shown). Overall, a greater proportion of meningitis than bacteremia cases were caused by serotype III (77.8% [63/81] vs. 56.8% [75/132]; p = 0.002), and serotype V was more commonly identified in bacteremia than meningitis cases (7.6% [10/132] vs. 3.7% [3/81]; p = 0.25; online Technical Appendix Figure). Serotype distribution between survivors and nonsurvivors did not differ (p = 0.51; data not shown).

Nationwide Number and Potential Vaccine-Preventable Fraction of Invasive GBS Disease

We estimated ≈3,178 invasive GBS cases and 549 GBS-associated deaths among South Africa's 2012 birth cohort (Table 2). For HIV-unexposed and -exposed infants, these estimates represent ≈1,639 and 1,544 cases and 283 and 266 deaths, respectively. On the basis of the predefined estimated efficacy of trivalent GBS vaccine to protect infants born at >33 weeks gestation, we estimated that, each year, 1,230 cases of invasive GBS disease and 163 deaths could be prevented in HIV-unexposed infants and 886 cases and 117 deaths could be prevented in HIV-exposed infants (Table 2).

Discussion

We report a high overall incidence of invasive GBS disease in Soweto (2.72 cases/1,000 live births), which is greater than global (0.53 cases/1,000) and African (1.21 cases/1,000) incidence estimates reported in a meta-analysis of studies conducted during 2000–2011 (*3*). That overall estimate included an incidence of 1.98 cases/1,000 live births (95% CI 1.71– 2.28) among HIV-unexposed infants, which is similar to or greater than incidences reported in many resource-rich countries before the widespread use of IAP (*20*). Furthermore, the observed overall incidence was similar to that for the same population a decade earlier (3.0 cases/1,000 live births) (*21*) and to that for women of South Asian descent in South Africa during the 1980s (2.65 cases/1,000 live births) (*22*). Despite the 2007 implementation of a risk-based IAP strategy at CHBAH, the high incidence of GBS disease has persisted, indicating the limited effect of the strategy in a resource-restricted setting with high maternal HIV infection prevalence. The limited effect could indicate poor strategy adherence or that the strategy missed most women whose newborns were at risk for EOD.

The high incidence of invasive GBS disease in South Africa contrasts with the lower incidence reported in South

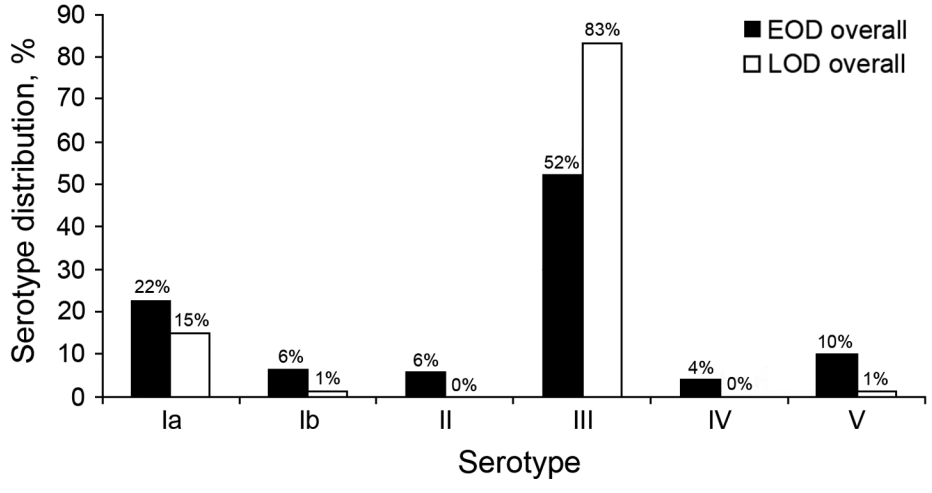


Figure 2. Group B *Streptococcus* serotype distribution among 125 patients with early-onset disease (EOD) and 88 patients with late-onset disease (LOD), Soweto, South Africa, 2004–2008.

Table 2. Estimated annual number of invasive GBS disease cases and associated deaths and potential annual vaccine-preventable fraction, South Africa*

	Overall		HIV unexposed†		HIV exposed†	
National estimates	No.	Incidence (95% CI)	No.	Incidence (95% CI)	No.	Incidence (95% CI)
Births	1,168,403‡		823,724		34,4679	
Invasive GBS cases	3,178§	2.72 (2.62–2.81)	1,639¶	1.99 (1.89–2.09)	1,544#	4.48 (4.26–4.71)
Invasive GBS-associated deaths						
No total**	549	0.47 (0.43–0.51)	283	0.34 (0.30–0.39)	266	0.77 (0.68–0.87)
No. in infants born at >33 weeks' gestation††	420	0.36 (0.33–0.40)	217	0.26 (0.23–0.30)	204	0.59 (0.51–0.68)
Vaccine-preventable cases and deaths						
Trivalent GBS-CV‡‡						
Cases	2,105§§	1.80 (1.73–1.88)	1,230¶¶	1.49 (1.14–1.58)	886##	2.57 (2.40–2.75)
Deaths	278§§	0.24 (0.21–0.27)	163¶¶	0.20 (0.17–2.31)	117##	0.34 (0.28–0.41)
Pentavalent GBS-CV***						
Cases	2,317§§	1.99 (1.90–2.07)	1,354¶¶	1.64 (1.56–1.73)	976##	2.83 (2.66–3.01)
Deaths	306§§	0.26 (0.23–0.29)	179¶¶	0.22 (0.19–0.25)	129##	0.37 (0.31–0.44)

*GBS, group B *Streptococcus;* GBS-CV, GBS polysaccharide–protein conjugate vaccine.
†HIV-exposed and -unexposed values were calculated on the basis of national HIV prevalence in pregnant women (29.5%). Incidence values represent cases/1,000 live births.
‡2012 live births.
§Overall GBS incidence is 2.72/1,000 live births.
¶GBS incidence for HIV-unexposed infants is 1.99/1,000 live births.
#GBS incidence in HIV-exposed infants is 4.48/1,000 live births.
**Total deaths in infants ≤90 days old, assuming 15.2% were born at ≤33 weeks of gestation and have a case-fatality rate (CFR) of 26.5% and assuming 84.8% were born at >33 weeks of gestation and have a CFR of 15.6%.
††Deaths in infants <90 days old who were born at >33 weeks of gestation (84.8% of infants); CFR 15.6%.
‡‡Trivalent GBS-CV contains serotypes Ia, Ib, and III, which account for 88.3% of cases.
§§Assuming vaccine efficacy of 75%.
¶¶Assuming vaccine efficacy of 85%.
##Assuming vaccine efficacy of 65%.
***Pentavalent GBS-CV contains serotypes Ia, Ib, II, III, and V, which account for 97.2% of cases.

Asia and the Western Pacific, despite similarity in prevalence of maternal vaginal GBS colonization at delivery (*6*). Possible reasons for this discrepancy include differences in delivery location, presence of trained birth-care attendants, and access to health facilities with adequate capability to diagnose and treat invasive GBS disease. Possible reasons for differences are exemplified by the findings in a study conducted in Bangladesh (*23*), which reported that only 1 of 30 culture-confirmed neonatal bacteremia cases was caused by GBS; however, >50.0% of the 259 reported neonatal deaths (many among infants born outside health care facilities) occurred within 24 h of birth, and 62.0% of those cases were not investigated for bacteremia. Our finding that 64.5% of EOD cases were diagnosed within 24 h of birth highlights the effect that births outside of health care facilities or where there is limited capacity for investigating invasive disease in newborns could have on measuring the incidence of EOD. Also, even though case-patients were treated in a secondary–tertiary hospital, death caused by invasive GBS disease was rapid (median 1 d from hospitalization).

In our study, the overall incidence of GBS disease in HIV-exposed infants was 4.46 cases/1,000 live births. The high prevalence of maternal HIV infection is contributing to the high incidence of GBS disease in South Africa, which corroborates data from a Belgium study that reported an incidence of 15.5 cases/1,000 live births (i.e., 5 cases/322 infants, predominantly LOD) in HIV-exposed infants, compared with 0.8 cases/1,000 live births (i.e., 16 cases/20,158 infants) in HIV-unexposed infants (*24*). Our study was not designed to evaluate whether the lower threshold used for investigating for sepsis in HIV-exposed than for HIV-unexposed infants may have contributed to ascertainment bias. However, such bias is unlikely because the threshold for investigating for sepsis among neonates is low in general at CHBAH; an investigation is done only when clinically indicated.

In addition to the higher incidence of LOD observed in HIV-exposed compared with HIV-unexposed infants, we observed that risk for EOD was 1.69-fold (95% CI 1.28–2.24) greater in HIV-exposed infants. This increased risk was present despite our previous observation that the prevalence of vaginal GBS colonization at delivery was lower in HIV-infected than HIV-uninfected women (17.0% vs. 23.0%; p = 0.002), even though rates of vertical colonization were similar for their newborns (52.0%–58.0%) (*25*). Although our study did not identify other differences in prevalence of risk factors for invasive GBS disease between HIV-exposed and -unexposed infants (*26*), we did not have population-level data on prevalence rates of these maternal risk factors for HIV-infected and -uninfected women. However, the observation of a greater difference in risk for LOD than EOD in HIV-exposed newborns compared with HIV-unexposed newborns suggests that risk factors other than peripartum

EOD–associated risk factors likely contribute to the heightened susceptibility of invasive disease in HIV-exposed infants. We were unable to determine whether HIV infection in the neonates contributed to an enhanced susceptibility to invasive GBS disease. None of the newborns with EOD for whom HIV testing was done were HIV-positive, whereas 20.0% (8/40) of tested LOD case-patients were HIV-positive. The population-based vertical transmission rate of HIV during the course of the study was 9.6% (*27*). A further limitation of our study was the lack of data on the clinical, immunologic, and HIV-virologic characteristics of the HIV-infected women and analysis of whether these characteristics could have contributed to a heightened susceptibility of invasive disease in their neonates.

Multiple studies have reported an inverse association between maternal GBS serotype–specific antibody levels and an infant's risk for EOD and LOD (*28*). Lower levels of maternally derived antibodies to several childhood vaccine epitopes have been reported in HIV-exposed but uninfected infants at birth to at least 6 weeks of age (*29*,*30*). Thus, it is plausible that lower naturally acquired capsular antibody in HIV-infected women may contribute to increased susceptibility to invasive GBS disease in HIV-exposed infants; this possibility warrants further investigation. The increased incidence of invasive GBS disease in HIV-exposed but uninfected infants could also be due to observed perturbations of their immune systems (*31*,*32*).

The serotype distribution of GBS isolates from EOD and LOD cases in this study was similar to that observed previously (*21*) and did not differ by HIV-exposure status. Serotypes Ia, Ib, and III, which are included in the current investigational trivalent GBS-CV, covered 78% of EOD and 100% of LOD invasive isolates in this study. However, the overall potential disease reduction of a vaccine against invasive GBS disease may be lower than this potential coverage because the vaccine is unlikely to confer protection through antibody acquisition in neonates born at <34 weeks' gestation (*33*). The protection of premature newborns against EOD would require prevention of GBS acquisition from the mother. This strategy is pertinent to settings like ours, in which 29.8% of all EOD-associated and 31.4% of all GBS-associated deaths occurred in premature infants despite the rate of premature birth in the community being only 18.0%.

Using a conservative approach of assuming no efficacy against invasive GBS disease in infants born at ≤33 weeks' gestation and vaccine efficacy of 85% and 65% in HIV-unexposed and HIV-exposed infants, respectively, we estimate that vaccination of pregnant women with the current investigational trivalent conjugate vaccine could potentially prevent 3,178 GBS cases and 549 GBS-associated deaths annually in South Africa. An effective GBS vaccine could also be used to probe the possible role of GBS in causing sickness and death in countries with limited epidemiologic laboratory capacity (*34*) and in causing stillbirths (*35*).

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Dr. Cutland is a senior research medical doctor in the Medical Research Council, Respiratory and Meningeal Pathogens Research Unit, and PhD student at the University of the Witwatersrand. Her research interests include epidemiology and prevention of vaccine-preventable diseases in children, particularly young infants.

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Address for correspondence: Clare L. Cutland, Respiratory and Meningeal Pathogens Research Unit, PO Box 90753, Bertsham, Johannesburg, 2013, South Africa; email: cutlandc@rmpru.co.za

Appendix 7: Paper IV

Chlorhexidine maternal-vaginal and neonate body wipes in sepsis and vertical transmission of pathogenic bacteria in South Africa: a randomised, controlled trial

*Clare L Cutland, Shabir A Madhi, Elizabeth R Zell, Locadiah Kuwanda, Martin Laque, Michelle Groome, Rachel Gorwitz, Michael C Thigpen, Roopal Patel, Sithembiso C Velaphi, Peter Adrian, Keith Klugman, Anne Schuchat, Stephanie J Schrag, and the PoPS Trial Team**

Summary

Background About 500 000 sepsis-related deaths per year arise in the first 3 days of life. On the basis of results from non-randomised studies, use of vaginal chlorhexidine wipes during labour has been proposed as an intervention for the prevention of early-onset neonatal sepsis in developing countries. We therefore assessed the efficacy of chlorhexidine in early-onset neonatal sepsis and vertical transmission of group B streptococcus.

Methods In a trial in Soweto, South Africa, 8011 women (aged 12–51 years) were randomly assigned in a 1:1 ratio to chlorhexidine vaginal wipes or external genitalia water wipes during active labour, and their 8129 newborn babies were assigned to full-body (intervention group) or foot (control group) washes with chlorhexidine at birth, respectively. In a subset of mothers (n=5144), we gathered maternal lower vaginal swabs and neonatal skin swabs after delivery to assess colonisation with potentially pathogenic bacteria. Primary outcomes were neonatal sepsis in the first 3 days of life and vertical transmission of group B streptococcus. Analysis was by intention to treat. The trial is registered with ClinicalTrials.gov, number NCT00136370.

Findings Rates of neonatal sepsis did not differ between the groups (chlorhexidine 141 [3%] of 4072 *vs* control 148 [4%] of 4057; p=0·6518). Rates of colonisation with group B streptococcus in newborn babies born to mothers in the chlorhexidine (217 [54%] of 401) and control groups (234 [55%] of 429] did not differ (efficacy –0·05%, 95% CI –9·5 to 7·9).

Interpretation Because chlorhexidine intravaginal and neonatal wipes did not prevent neonatal sepsis or the vertical acquisition of potentially pathogenic bacteria among neonates, we need other interventions to reduce childhood mortality.

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*Members listed at end of paper

Department of Science and Technology/National Research Foundation, Vaccine Preventable Diseases and Medical Research Council, Respiratory and Meningeal Pathogens Research Unit, University of Witwatersrand, Soweto, South Africa (C L Cutland MD, Prof S A Madhi MD, L Kuwanda MSc, M Laque MD, M Groome MD, P Adrian PhD, Prof K Klugman MD); **Centers for Disease Control and Prevention, Atlanta, GA, USA** (E R Zell MStat, R Gorwitz MD, M C Thigpen MD, R Patel MD, A Schuchat MD, S J Schrag DPhil); **Department of Paediatrics, Division of Neonatology, Chris Hani Baragwanath Hospital, University of Witwatersrand, Soweto, South Africa** (S C Velaphi MD); **and Department of International Health, Rollins School of Public Health and Division of Infectious Diseases, School of Medicine, Emory University, Atlanta, GA, USA** (Prof K Klugman)

Correspondence to: Dr Clare L Cutland, Department of Science and Technology/National Research Foundation, Vaccine Preventable Diseases and Medical Research Council, Respiratory and Meningeal Pathogens Research Unit, University of Witwatersrand, PO Box 90753, Bertsham, 2013, South Africa **cutlandc@hivsa.com**

Introduction

About 900 000 sepsis-associated neonatal deaths per year arise in developing countries, mainly in the first week of life.[1,2] Early-onset sepsis poses unique opportunities for prevention because of intrapartum, vertical transmission of bacteria to newborn babies. For example, widespread use of targeted prophylaxis with intrapartum antibiotics in the USA coincided with a 70% reduction in early-onset group B streptococcal disease.[3–5] Logistical and resource limitations, however, prevent use of intrapartum antibiotics in developing countries. Additionally, the increased diversity of sepsis-causing pathogens in developing countries[6] draws attention to the potential value of a syndrome-based rather than pathogen-specific approach to prevention. Maternal deaths are also increased in developing countries with about half the 500 000 deaths per year arising in sub-Saharan Africa.[7]

Use of intravaginal washes during labour with chlorhexidine, a commonly available wide-spectrum microbicide, has been proposed as a cheap, simple, and accessible intervention that could potentially reduce neonatal and maternal post-partum sepsis in developing countries.[8–10] Vaginal washes with intrapartum chlorhexidine are postulated to reduce neonatal sepsis by preventing newborn acquisition of bacteria that colonise the mother's vagina during labour and delivery. Chlorhexidine is safe and well tolerated as a vaginal cleanser in pregnant women in solutions of up to 1% concentration[11] and in solutions of up to 4% as a cleanser for the newborn umbilical cord.[12]

In two non-randomised studies in Africa, use of chlorhexidine wipes to clean the birth canal and newborn baby was associated with significant reductions (50–75%) in neonatal and maternal sepsis-associated morbidity and neonatal mortality.[13,14] Despite these findings, the absence of a definitive randomised, controlled trial has impeded widespread acceptance and implementation of chlorhexidine wipes.[9,15,16] The conclusions drawn from reviews about the use of chlorhexidine for cleansing the maternal vaginal canal and newborn skin[9,10,15,17] are that a randomised controlled trial, preferably done in a developing country, is needed before this intervention is accepted globally. In a

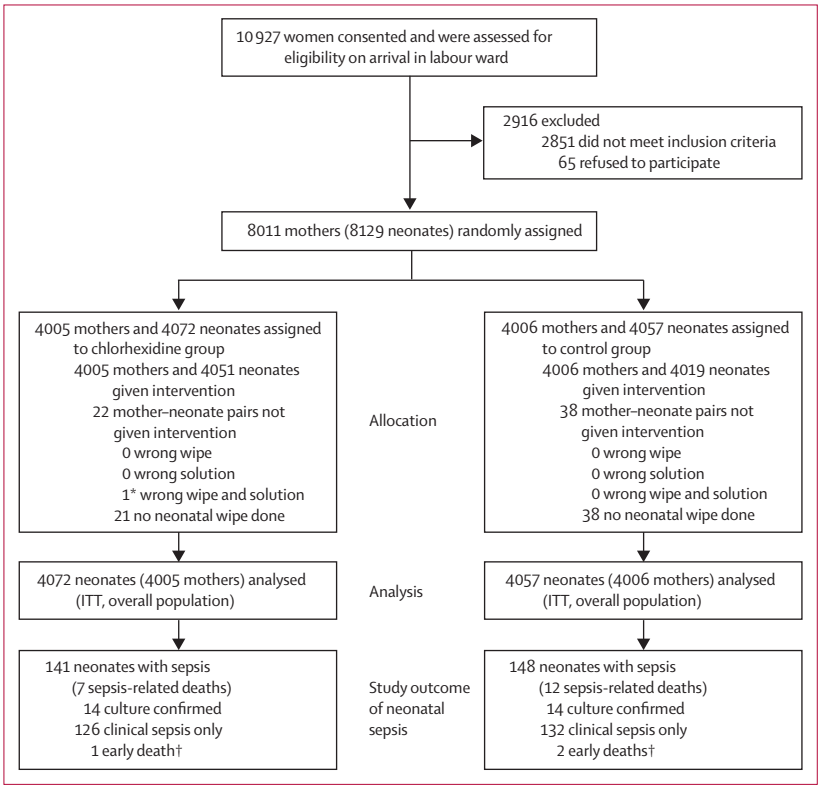


Figure 1: **Trial profile**
ITT=intention to treat. *Participant was randomly assigned, discharged, and readmitted at a later date, and was allocated a new randomisation number on readmission; the wipe that was not done according to protocol was done according to the first randomisation group, which was different to the final group. †Did not meet criteria.

Cochrane meta-analysis[18] of randomised or quasi-randomised trials, vertical transmission of group B streptococcus, but not early-onset infection with this bacterium[18] or maternal or neonatal infections caused by other pathogens, was reduced significantly by use of chlorhexidine.[17,19] We therefore assessed the efficacy of intrapartum and neonatal chlorhexidine in reducing early-onset neonatal sepsis and vertical transmission of group B streptococcus.

Methods

Study setting

The study was undertaken at Chris Hani-Baragwanath Hospital, Soweto, South Africa; three-quarters of 30 000 babies born every year in Soweto are delivered in this hospital. This referral centre provides free maternal and child care to an urban, middle-income and low-income population; it is the only local public hospital that admits neonates. Maternal screening for colonisation with group B streptococcus and prophylaxis with intrapartum antibiotics are not routine but are part of a maternal risk-factor-based strategy that is advocated for the management of chorioamnionitis. The facility provides an active HIV voluntary testing programme with an antenatal HIV prevalence of 35 360 (30%) of 118 188 pregnant women during the trial.

Participants

Pregnant women (aged 12–51 years) were screened for enrolment antenatally or in the labour ward, and were reassessed for eligibility during active labour before randomisation and planned migration from study area in the first month after delivery. Exclusion criteria were planned caesarean section, antepartum haemorrhage, known severe congenital malformation, intrauterine death confirmed before randomisation, known allergy to chlorhexidine, face presentation, many genital warts or ulcers, full cervical dilatation, and age younger than 15 years.

Mothers provided informed, written consent for trial participation for themselves and their newborns babies. The study was approved by the human research ethics committee of the University of Witwatersrand, Soweto, South Africa, and the institutional review board of the Centers for Disease Control and Prevention, Atlanta, GA, USA. The study was monitored by an independent data and safety monitoring board that undertook a masked review of the study conduct and safety of participants in the study, including one unmasked interim analysis for efficacy.

Randomisation and masking

Random assignment of patients into the trial was initiated on April 1, 2004, and completed on Oct 25, 2007. The sponsor generated the randomisation numbers using SAS (version 9.0) and assigned them in blocks of 50 participants. The forms were preprinted, sealed in opaque envelopes, and opened sequentially by study midwives when participants were randomly assigned. Commercially available, 5% colourless, and mildly odoured chlorhexidine gluconate was diluted every week to 0·5% with autoclaved tap water that was suitable for drinking and stored in 1 L opaque bottles at room temperature. Autoclaved tap water was used as the control. Bottles containing chlorhexidine solution and water were not labelled with the solution name but were labelled with a code and date of preparation. Study midwives were aware of the intervention they were administering. The rest of the study team, including those gathering endpoint data, were unaware of the treatment allocations. Laboratory staff were also unaware of mother and neonate pairings. Study staff had no role in the management of the patients.

Intervention

Chlorhexidine was tested for activity with spectrophotometric and biological tests. The control and chlorhexidine solutions were tested for sterility before and after use.

The midwives wrapped cotton pads soaked in water or chlorhexidine around their gloved fingers. In the chlorhexidine group, the midwives rotated their examining fingers circumferentially over the cervix and vaginal walls, and wiped the external genitalia; in the

control group, only the external genitalia were wiped to avoid any risk of vaginal wipes in the absence of antisepsis. Doctors were not allowed to enter the cubicle or nursery when a midwife was doing the interventional wipes.

Neonates in the chlorhexidine group were wiped from head to toe, avoiding the face and ears, as soon as possible after birth with cotton pads soaked in chlorhexidine solution. Neonates in the control group were given a chlorhexidine foot wipe (chlorhexidine was used as a neonatal control solution instead of water to assist in blinding and design of the case report form). All babies were bathed in water as per standard of care before being wiped.

Procedures

The midwives gathered information about baseline characteristics from labour records, and interviewed mothers about side-effects of the wipes. Trained doctors prospectively identified mothers admitted to hospital within 14 days of delivery, or neonates within 28 days of birth, and gathered information related to the sepsis endpoints from medical records. Sterile site cultures were gathered at the discretion of the attending physicians and processed at the hospital microbiology laboratory with routine methods including the BacT/Alert microbial system (Organon Teknika, Durham, NC, USA) for blood culture. Active laboratory-based surveillance was done to ensure that all sterile-site cultures from admitted neonates were captured. External clinical research associates monitored all trial procedures. Additionally, trial-independent clinicians reviewed all maternal and neonatal deaths, and serious adverse events that were assessed to be possibly related to trial intervention.

Colonisation substudy

On randomisation, a swab of the lower vagina was obtained from the mothers from May 16, 2005, to Oct 19, 2007. Surface swabs of the umbilicus, nares, and outer ear were gathered for culture from neonates after delivery but before the neonate was bathed and wiped.

Swabs were inoculated into Amies medium and processed within 48 h. For isolation of group B streptococcus, swabs were inoculated onto 5% horse blood agar with and without colistin (10 μg/mL) and nalidixic acid (15 μg/mL) and into Todd-Hewitt broth (2 mL) supplemented with gentamicin (8 μg/mL) and nalidixic acid (15 μg/mL), followed by inoculation onto 5% horse blood agar. Gram-positive β-haemolytic colonies were identified as group B streptococcus by CAMP (Christie, Atkins, and Munch-Petersen) positive test, inability to hydrolyse esculin, and group B antigen latex agglutination (Omega Diagnostics, Scotland, UK). *Escherichia coli* and *Klebsiella pneumoniae* were inoculated onto MacConkey agar, and colonies were identified by standard biochemical tests.

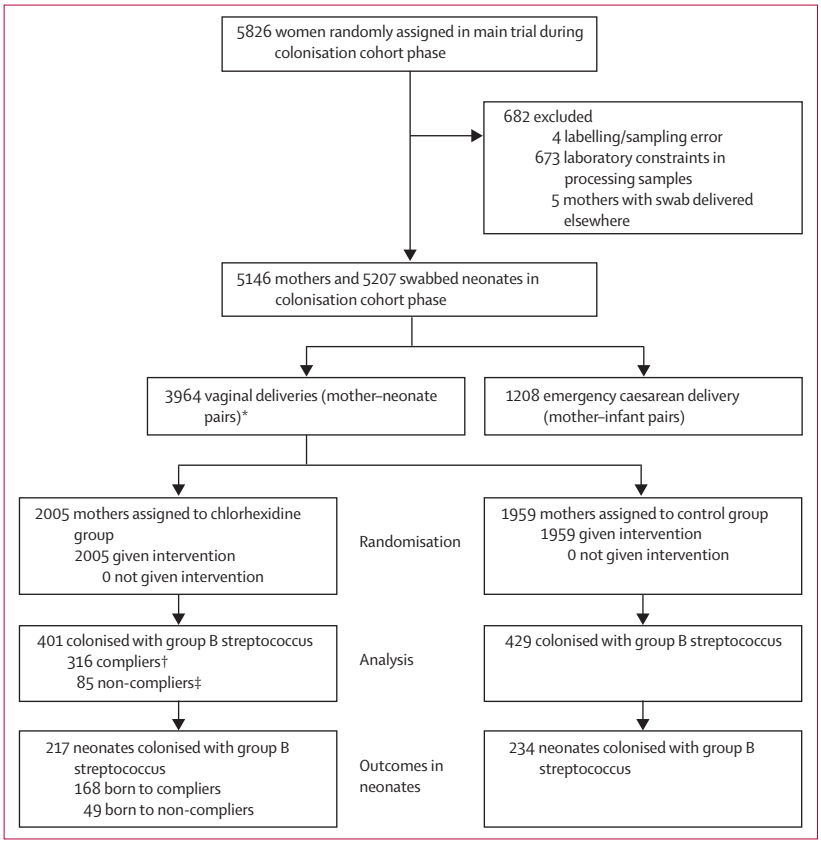


Figure 2: **Colonisation substudy profile**

*Mother was counted twice if both twins were delivered vaginally. †Mother given at least one wipe 1–6 h before delivery, and was given only the correct intervention. ‡Mother was not given chlorhexidine wipe 1–6 h before delivery, therefore did not meet the criteria for compliers.

Sepsis definitions

We defined early-onset sepsis as arising within the first 3 days of life and including culture-confirmed or clinical sepsis on the basis of clinical and laboratory signs. Cases of late-onset sepsis had symptom onset between 3 days and 28 days of age (webappendix p 1). Vertical transmission was defined as surface colonisation of the newborn baby at birth with the same bacterium isolated from the mother's vagina during vaginal delivery. Post-partum sepsis was defined as admission of the mother to hospital within 14 days of delivery for endometritis (at least two of uterine tenderness, fever, foul-smelling or purulent lochia, or vaginal discharge), culture-confirmed infection of sterile site, or perineal wound infection among vaginal parturients.

See Online for webappendix

Statistical analysis

A background rate of early-onset sepsis of 30 per 1000 livebirths was estimated during a prestudy hospital audit. On the basis of α 0·05 and 90% power, a sample size of 3581 mother–baby pairs was needed per group to detect a 40% efficacy of the intervention. Thus, we targeted a total of 8000 mother–baby pairs. For the colonisation substudy, 1470 mother–neonate pairs per group were needed to

	Chlorhexidine	Control
Mothers		
n	4005	4006
Age (years)	26 (22–31)	26 (22–31)
Gravidity	2 (1–3)	2 (1–3)
Medical history		
HIV-positive	1050 (26%)	1036 (26%)
Urinary tract infection	449 (11%)	405 (10%)
Gestational diabetes	9 (<1%)	11 (<1%)
Maternal tuberculosis	19 (<1%)	23 (<1%)
Given intrapartum antibiotics*	388 (10%)	390 (10%)
Intrapartum antibiotics in women with vaginal delivery†	291 (75%)	296 (76%)
Given antibiotics 7 days before delivery‡	241 (25%)	234 (26%)
Intrapartum fever	8 (<1%)	8 (<1%)
Rupture of membranes (delivery ≥18 h)	385 (10%)	325 (8%)
Unbooked or no prenatal care	10 (<1%)	1 (<1%)
Twin birth	67 (2%)	51 (1%)
Meconium-stained amniotic fluid	598 (15%)	602 (15%)
Delivery§		
Spontaneous vaginal delivery	3079 (77%)	3057 (76%)
Emergency caesarean delivery	924 (23%))	943 (24%)
Vaginal vacuum or forceps assisted	80 (2%)	84 (2%)
Newborn babies		
n	4072	4057
Girl	1906 47%)	1967 (48%)
Preterm	158 (4%)	151 (4%)
Birthweight (g)	3140 (2850–3465)	3125 (2850–3450)
Apgar at 5 min	10 (10–10)	10 (10–10)
HIV-exposed infants	1050 (26%)	1040 (26%)
HIV PCR-positive¶	65 (9%)	47 (7%)
Outcome		
Stillborn	12 (<1%)	14 (<1%)
Died soon after delivery	2 (<1%)	13 (<1%)
Surviving neonates admitted	397 (10%)	390 (10%)

Data are median (IQR) or number (%), unless otherwise indicated. *At least 2 h before delivery (excludes prophylaxis for caesarean section). †Denominator is the number of women given intrapartum antibiotics. ‡Denominator is the number of women who had been given any antibiotics during pregnancy: 949 in chlorhexidine group and 912 in control group. §Includes mode of delivery of all singletons and first twins only; four second twins (2 in chlorhexidine group, and 2 in control group) were delivered by caesarean section after the first twin was delivered vaginally; other second twins were delivered by the same route as the first twin; denominator was 4003 in the chlorhexidine group, and 4000 in the control group because eight mothers delivered at another centre and therefore these data were not available. ¶Data are number (%) of neonates tested—ie, 716 in chlorhexidine group, and 654 in control group.

Table 1: **Selected demographic and clinical characteristics of mothers and newborn babies in the chlorhexidine and control groups**

detect a 30% reduction in vertical transmission of group B streptococcus between the mother and child on the basis of a 25% prevalence of maternal colonisation with this bacterium. This number was adjusted to a total of 3600 to allow for compliance failures.

Data were reviewed for accuracy, consistency, and completeness before double data-entry into custom-designed databases. Consistency checks and data validation were done regularly.

An analytical plan was approved by the trial's steering committee before database locking and unmasking. Analyses were done with SAS (version 9.1). The primary endpoint of sepsis was assessed on an intention-to-treat basis. Efficacy of chlorhexidine wipes was calculated with the formula

$$\frac{\text{RCtl}-\text{RTrt}}{\text{RCtl}}\times 100\%$$

RTrt was the incidence rate in infants in the intervention group, and RCtl was the incidence rate in infants in the control group. The colonisation analysis focused on the subset of women with a vaginal delivery who had positive colonisation status and met inclusion criteria. Because the objective was to elucidate the mechanism by which chlorhexidine might affect sepsis, we did an analysis of complier-average causal effect[20–22] to estimate the intention-to-treat effect among true compliers (webappendix pp 2–3). We defined compliance as women being given only wipes of the assigned treatment and at least one wipe between 1 h and 6 h before delivery. A p value of less than 0·05 was considered significant. Throughout, twins were counted as independent births, and stillborn neonates were included in the intention-to-treat analyses.

The trial is registered with ClinicalTrials.gov, number NCT00136370.

Role of the funding source

The sponsors of the study had no role in study design, data gathering, analysis, interpretation, dissemination, or in the decision to submit this report for publication. The corresponding author had full access to the data and the final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile; figure 2 shows the substudy profile. Table 1 shows that the baseline characteristics of participants were similar in the chlorhexidine and control groups. The median gestational age at delivery was 39 weeks (range 23–44); about 10% of mothers were given intrapartum antibiotics (table 1). HIV results were available for 7902 (99%) of 8011 mothers, and 2090 (26%) of these were infected with HIV. 1867 (23%) of 8011 women had a caesarean delivery. Of 8129 infants born, 309 (4%) were born before 37 weeks' gestation. When further stratified by maternal HIV status, baseline characteristics remained similar in the chlorhexidine and control groups (data not shown).

Mothers were given a median of one (range 1–8) wipe during labour. 6423 (80%) of 8011 women had one or more wipes at least 1 h and not more than 6 h before delivery. Use of interventional wipes was stopped early in 18 (<1%) of 6135 women who had a vaginal delivery. Use of wipes was stopped early in nine mothers because of the presentation of the neonate's face, ten because of

strong contractions, and three (all in chlorhexidine group) as a result of discomfort.

8070 (99%) of 8129 neonates had wipes at a median of 50 min (IQR 30–70) after birth. 41 of 59 neonates did not have a wipe because they were stillborn or severely ill. No reactions to wipes were noted at discharge. No neonatal adverse or serious adverse events related to the intervention were reported. 958 neonates were admitted 976 times, 16 were admitted twice, and one was admitted three times. Overall, fewer neonates died in the chlorhexidine group

	Overall		Maternal HIV-negative status		Maternal HIV-positive status	
	Chlorhexidine	Control	Chlorhexidine	Control	Chlorhexidine	Control
Neonates						
n	4072	4057	2939	2947	1072	1058
Bacteria cultured						
Group B streptococcus	10	6	..	..	..	..
Escherichia coli	0	1	..	..	..	..
Klebsiella pneumoniae	1	0	..	..	..	..
Staphylococcus aureus	0	1	..	..	..	..
Streptococcus viridans	1	1	..	..	..	..
Acinetobacter baumannii and *lwoffii*	1	2	..	..	..	..
Enterococcus faecalis and *faecium*	1	2	..	..	..	..
Enterobacter	0	1	..	..	..	..
Early-onset sepsis						
Culture-confirmed cases* meeting definition for clinical sepsis	11	7	6†	5	4†	2
Culture-confirmed cases not meeting definition for clinical sepsis definition‡	3	7	2	4	1	3
Cases with clinical sepsis only	126	132	98	105	28	27
Early deaths (<3 days) not meeting culture-confirmed* or clinical definitions, but included as early-onset sepsis after panel review	1	2	1	2	0	0
Total	141 (3%)	148 (4%)	107 (4%)	116 (4%)	33 (3%)	32 (3%)
Overall efficacy (95% CI)	5% (–19 to 24); p=0·6518	..	8% (–20 to 29); p=0·5527	..	–2% (–64 to 37); p=0·9425	..
Late-onset sepsis						
Culture-confirmed sepsis meeting criteria for clinical sepsis	4	8	1	3	3	4
Culture-confirmed cases not meeting definition for clinical sepsis	9	3	6	2	3	1
Cases with clinical sepsis only	9	6	7	1	2	5
Total	22 (<1%)	17 (<1%)	14 (<1%)	6 (<1%)	8 (<1%)	10(<1%)
Overall efficacy (95% CI)	–29% (–142 to 31); p=0·4289	..	–134% (–508 to 10); p=0·0722	..	21% (–99 to 69); p=0·6161	..
Mothers with post-partum sepsis						
n	4005	4006	2896	2916	1050	1040
Bacteria cultured						
Citrobacter freundii	0	1	..	..	..	..
Escherichia coli	1	0	..	..	..	..
Culture-confirmed cases meeting clinical criteria	0	0	0	0	0	0
Culture-confirmed only	1	1	1	1	0	0
Endometritis only	14	11	8†	6	5	5
Total	15 (<1%)	12 (<1%)	9 (<1%)	7 (<1%)	5 (<1%)	5(<1%)
Overall efficacy (95% CI)	–25% (–167 to 41); p=0·5626	..	–29% (–247 to 52); p=0·6069	..	1% (–240 to 71); p=0·9879	

Data are number or number (%), unless otherwise indicated. All neonates (including second twins and stillborn neonates) who were delivered in a hospital are included.
*Early-onset culture-confirmed sepsis—ie, isolation of a microorganism that is not a common contaminant from a site on the neonate's body that is usually sterile within the first 3 days of life. †Data for HIV were missing for one newborn baby with culture-confirmed, clinical early-onset sepsis in the chlorhexidine group and for one mother with post-partum sepsis meeting the clinical criteria. ‡Early onset culture-confirmed sepsis in the absence of study-specified clinical criteria of early-onset neonatal sepsis.

Table 2: **Intent-to-treat analysis of early-onset sepsis in neonates and maternal post-partum sepsis by intervention group and HIV exposure status**

	Chlorhexidine		Control	Overall efficacy (95% CI)
	Complier	Non-complier		
Group B streptococcus				
Positive	168 (53%)	49 (58%)	234 (55%)	..
Negative	135 (43%)	28 (33%)	175 (41%)	..
Missing result	13 (4%)	8 (9%)	20 (5%)	..
Total	316	85	429	0·05% (–9·2 to 7·9)
Escherichia coli				
Positive	361 (51%)	92 (50%)	442 (51%)	..
Negative	298 (42%)	74 (40%)	362 (42%)	..
Missing result	45 (6%)	18 (24%)	60 (7%)	..
Total	704	184	864	0·3% (–6·0 to 5·3)
Klebsiella pneumoniae				
Positive	37 (29%)	6 (23%)	49 (30%)	..
Negative	88 (69%)	18 (69%)	103 (63%)	..
Missing result	3 (2%)	2 (8%)	11 (7%)	..
Total	128	26	163	10·6% (–5·9 to 24·1)

Data are number (%) or number, unless otherwise indicated; denominators in each column are the number of women colonised with the respective pathogen.

Table 3: **Intent-to-treat analysis of vertical transmission of selected maternal-vaginal colonising bacteria from mother to newborn babies in the chlorhexidine and control groups, by neonatal colonisation status**

(34 of 4072 [8·3 per 1000 births]) than in the control group (52 of 4057 [12·8 per 1000 births]; p=0·0490) but there were no differences in the rates of admission to hospital (485 of 4072 [119 per 1000 births]) *vs* 491 of 4057 [121 per 1000 births]; p=0·7901). 26 (3·2 per 1000 births) deliveries resulted in stillbirths (12 in chlorhexidine group and 14 in control group; p=0·6852) and 15 (2 in chlorhexidine group and 13 in control group; p=0·0043) neonates died in the resuscitation room within 9 h after birth. 13 neonates (11 in control group) died of birth asphyxia, and twins in the control group died of extreme prematurity (25 weeks' gestation). Of 45 remaining neonatal deaths that occurred, 20 were in the chlorhexidine group and 25 in the control group (p=0·4473). Mean age at outcome was 11·2 days (SD 17·4) and median was 7 days (IQR 4–11). 112 (8%) of 1370 HIV-exposed, tested neonates were infected with this virus.

Overall, 289 cases of early-onset sepsis occurred, with no difference in rates in the chlorhexidine (34·6 per 1000 births) and control groups (36·5 per 1000 births; table 2). Similarly, the rates of culture-confirmed sepsis (14 episodes in each group) and clinically diagnosed early-onset sepsis did not differ (table 2). Group B streptococcus was the most commonly identified pathogen from sterile sites in early-onset sepsis (16 [57%] of 28 cultured bacteria; incidence 2·0 per 1000 livebirths). Rates of early-onset sepsis did not differ in the chlorhexidine and placebo groups when further stratified by the status of maternal HIV infection (table 2). 252 (87%) of 289 newborn babies with early-onset sepsis were admitted within 4 h after birth. 39 cases (4·8 per 1000 births) of late-onset sepsis occurred—22 (56%) in the chlorhexidine group (table 2).

27 of 8011 women had post-partum sepsis (15 in chlorhexidine group [3·7 per 1000]; 12 in control group [3·0 per 1000]; table 2). Maternal deaths (1 in chlorhexidine group and 3 in control group) or post-partum admissions (1013 in chlorhexidine group and 1034 in control group; p=0·5951) were not attributed to the intervention. Most mothers (1876 [92%] of 2047) were admitted to hospital for routine care after caesarean delivery.

3964 (77%) of 5146 women in the colonisation substudy had vaginal deliveries; 825 (21%) of these were colonised with group B streptococcus. This bacterium was cultured from 450 (54%) of 830 newborn babies born to mothers colonised with group B streptococcus. 316 (79%) of 401 mother–infant pairs in the chlorhexidine group met the definition of a true complier. The rates of colonisation with group B streptococcus in neonates were similar in the chlorhexidine and control groups, without substantial reduction in vertical transmission (table 3); results were similar when data were excluded for mothers who were given intrapartum antibiotics for more than 2 h (n=47), mainly for meconium-stained amniotic fluid. Results for *E coli* and *K pneumoniae* were similar in the two groups (table 3).

Discussion

Use of maternal and neonatal chlorhexidine wipes did not prevent the occurrence of early-onset sepsis. This absence of benefit was corroborated by the lack of effect on vertical transmission of the main sepsis-causing pathogens, and on serious maternal post-partum sepsis.

Differences between our study and other African studies[13,14] include our use of a stringent definition of neonatal sepsis, controlled randomisation of individuals, and use of 0·5% chlorhexidine solution instead of 0·25%. Additionally, even though substantial reductions were reported in neonatal and maternal sepsis in the chlorhexidine groups in these studies in Malawi and Egypt,[13,14] vertical transmission of bacteria was not assessed to corroborate the results. Furthermore, because staff were not employed to deliver the intervention in these African trials,[13,14] mothers who were assigned to the intervention group might have inadvertently benefited from more intensive monitoring and general care than might have those in the non-intervention group. In these studies,[13,14] episodes of sepsis were not stratified by neonatal age, even though a chlorhexidine intervention is unlikely to have an effect after the first few days of life. Only 57·8% of cases of neonatal sepsis in the Malawi study occurred in neonates younger than 48 h.[13] Similarities between studies include timing and number of wipes, and eligibility criteria, although we included mothers who were in preterm labour.

Our results suggest that chlorhexidine is unlikely to prevent vertically acquired neonatal infections in any setting or population. Because vertically acquired

pathogens can cross intact membranes, causing infections of the amniotic fluid and stillbirths,[23] vaccination of mothers against dominant bacterial pathogens warrants further study. The results of a review[6] of pathogens associated with neonatal sepsis in developing countries suggest that many neonatal infections might be acquired environmentally. Few data are available from developing countries about sepsis during the first 3 days of life; since this period is when most vertically acquired sepsis occurs, such data are urgently needed to clarify the contribution of vertical transmission to neonatal mortality.

Although much fewer neonates died in the chlorhexidine group than in the control group in our trial, the effect on mortality was greatest within hours of birth, at which time the main cause of death was birth asphyxia, as assessed after a panel of neonatologists, who were unaware of treatment assignment, reviewed the records. The lack of effect of chlorhexidine on neonatal sepsis or vertical transmission of bacteria suggests that the effect on mortality was not likely to be the result of the chlorhexidine intervention. The use of intrapartum antibiotics in our population of mothers (mainly for meconium-stained amniotic fluid) could have reduced the efficacy of chlorhexidine through the reduction in the rates of sepsis and vertical transmission in treatment and control groups. However, the incidence of overall sepsis remained high, and the incidences of culture-confirmed infection with group B streptococcus and vertical transmission were similar to those reported in industrialised countries before the widespread use of prophylaxis with intrapartum antibiotics.

Although we did not show a reduction in vertical transmission of pathogenic bacteria with use of chlorhexidine vaginal wipes, reduction of vertical transmission of group B streptococcus has been reported with similar interventions.[18,19,24] Although unlikely, the chlorhexidine wipe method that we used might have been less effective at reducing vertical transmission than the use of douching or gel application in other studies, However, our cohort of mothers colonised with group B streptococcus was substantially larger than the cohorts in other studies,[18,19,24] which on their own were inadequately powered for the assessment of vertical transmission.

A limitation of our study was that midwives were aware of the intervention that they were administering. Also, we did not use a true placebo because of the concerns about the detrimental effects of using vaginal wipes without antisepsis. Several safeguards, including the use of colourless chlorhexidine solution, and nursing staff not being involved in patient care or gathering endpoint data, were established and maintained to counteract this limitation.

Although several trials have raised hopes that chlorhexidine vaginal and neonatal cleansing would be beneficial in saving the lives of newborn babies, the results from our trial suggest that use of chlorhexidine wipes is unlikely to reduce neonatal mortality from vertically acquired sepsis. Other neonatal interventions are needed to achieve the Millennium Developmental Goal of reduction in childhood mortality.

Contributors

CLC, SAM, ERZ, RG, MCT, RP, SCV, KK, AS, and SJS participated in the conception of the trial, study design, protocol development and amendment, and study planning and implementation. CLC, ML, MG, MCT, and RP followed up participants and gathered data; SAM participated in gathering data; PA managed laboratory set-up and sample processing; and ERZ, LK, and SJS analysed the data. All authors participated in the interpretation of the results. CLC, SAM, ERZ, LK, ML, MG, PA, and SJS drafted the report and all authors contributed to critical review and revision of the report. LK, ERZ, and MCT participated in database development, and SJS participated in database design. SCV participated in review of neonatal cases. CLC, SAM, ML, MG, MCT, and RP participated in monitoring and cleaning data and assisted in data analysis. All authors have seen and approved the final version of the report.

PoPS Trial Team

Data safety monitoring board: Kathryn Edwards (chair), Peter Cooper, Maria Deloria Knoll, James McIntyre.
Scientific steering committee: Marleen Temmerman (chair), G Justus Hofmeyr, Paolo Miotti, Robert Pattinson, Haroon Saloojee.
Advisers and collaborators: Cynthia Whitney, Anne von Gottberg, Eckhart Buchmann, Donald Rubin.
Serious adverse events reviewers: Boris Jivkov, Aneetha Moodley. *Respiratory and Meningeal Pathogens Research Unit team:* Waarisa Fareed, Suzett Fourie, Veebha Gosai, Razia Hassan, Gavin Jaques, Waasila Jassat, Stephanie Jones, Marianne Kohler, Pravesh Lakha, Kerry Marran.

Conflicts of interest

We declare we have no conflicts of interest.

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Appendix 8: Co-author approvals

Article 1

Risk Factors for Neonatal Sepsis and Perinatal Death among Infants Enrolled in the Prevention of Perinatal Sepsis Trial, Soweto, South Africa.

Pediatric Infectious Diseases Journal. 2012 Aug; 31 (8):821-6.

doi: 10.1097/INF.0b013e31825c4b5a.

Authors	Name	Signature	Date
1st author	Stephanie J. Schrag		1st Feb 2016
2nd author	Clare L. Cutland		28 JAN 2016
3rd author	Elizabeth R. Zell		31st Jan 2016
4th author	Locadiah Kuwanda		1st Feb 2016
5th author	Eckhart J. Buchmann		31st Jan 2016
6th author	Sithembiso C. Velaphi		31st Jan 2016
7th author	Michelle J. Groome		2nd Feb 2016
8th author	Shabir A. Madhi		1st Feb 2016

Comments:

First author: S. J. Schrag:

Clare Cutland, although she was not lead author on this paper, played a substantive role in all aspects from protocol and primary data collection through to analysis plan, interpretation and critical review of the manuscript. Thus as lead author I view her as entitled to use this paper for degree purposes.

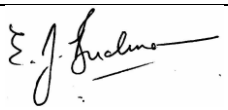
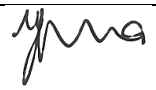
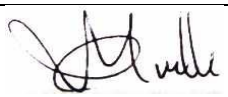
Primary supervisor: S. A. Madhi:

Article I

Risk Factors for Neonatal Sepsis and Perinatal Death among Infants Enrolled in the Prevention of Perinatal Sepsis Trial, Soweto, South Africa.

Pediatric Infectious Diseases Journal. 2012 Aug; 31 (8):821-6.

doi: 10.1097/INF.0b013e31825c4b5a.

Authors	Name	Signature	Date
1st author	Stephanie J. Schrag		1st Feb 2016
2nd author	**Clare L. Cutland**		
3rd author	Elizabeth R. Zell		31st Jan 2016
4th author	Locadiah Kuwanda		1st Feb 2016
5th author	Eckhart J. Buchmann		31st Jan 2016
6th author	Sithembiso C. Velaphi		31st Jan 2016
7th author	Michelle J. Groome		2nd Feb 2016
8th author	Shabir A. Madhi		1st Feb 2016

Comments:

First author: S. J. Schrag:

_____

_____

Primary supervisor: S. A. Madhi:

Clare Cutland was the lead clinician on the PoPS trial, and contributed significantly to protocol development, trial set-up, case report design, staff hiring, training and management, daily trial conduct, data collection, cleaning and analysis. Although she was not the first author on this paper, her contribution to the trial entitles her to include it in her PhD thesis work.



Article II

Maternal HIV infection and vertical transmission of pathogenic bacteria.
Pediatrics. 2012 Sep;130(3):e581-90. Epub 2012 Aug 6.

Authors	Name	Signature	Date
1st author	Clare L. Cutland		28 OCT 2016
2nd author	Stephanie J. Schrag		1st Feb 2016
3rd author	Elizabeth R. Zell		31st Jan 2016
4th author	Locadiah Kuwanda		1st Feb 2016
5th author	Eckhart J. Buchmann		31st Jan 2016
6th author	Sithembiso C. Velaphi		31st Jan 2016
7th author	Michelle J. Groome		2nd Feb 2016
8th author	Peter V. Adrian		22nd Feb 2016
9th author	Shabir A. Madhi		1st Feb 2016

Article III

Increased risk for group B Streptococcus sepsis in young infants exposed to HIV, Soweto, South Africa, 2004-2008(1).

Emerging Infectious Diseases 2015 April; 21(4):638-45. doi: 10.3201/eid2104.141562.
PubMed PMID: 25812061; PubMed Central PMCID: PMC4378461.

Authors	Name	Signature	Date
1st author	Clare L. Cutland		28 APR 2016
2nd author	Stephanie J. Schrag		1st Feb 2016
3rd author	Michael C. Thigpen		8th Feb 2016
4th author	Sithembiso C. Velaphi		31st Jan 2016
5th author	Jeannette Wadula		9th Feb 2016
6th author	Peter V. Adrian		22nd Feb 2016
7th author	Locadiah Kuwanda		1st Feb 2016
8th author	Michelle J. Groome		2nd Feb 2016
9th author	Eckhart J. Buchmann		31st Jan 2016
10th author	Shabir A. Madhi		1st Feb 2016

Article

Chlorhexidine maternal-vaginal and neonate body wipes in sepsis and vertical transmission of pathogenic bacteria in South Africa: a randomised, controlled trial. Lancet. 2009 Dec 5; 374 (9706):1909-16.

Authors	Name	Signature	Date
1st author	Clare L. Cutland		
2nd author	Shabir A. Madhi		1st Feb 2016
3rd author	Elizabeth R. Zell		31st Jan 2016
4th author	Locadiah Kuwanda		1st Feb 2016
5th author	Martin Laque		14th Mar 2016
6th author	Michelle J. Groome		2nd Feb 2016
7th author	Rachel Gorwitz		9th Feb 2016
8th author	Michael C. Thigpen		8th Feb 2016
9th author	Roopal Patel		9th Feb 2016
10th author	Sithembiso C. Velaphi		31st Jan 2016
11th author	Peter V. Adrian		22nd Feb 2016
12th author	Keith Klugman		9th Feb 2016
13th author	Anne Schuchat		9th Feb 2016
14th author	Stephanie J. Schrag		1st Feb 2016

Article IV

Chlorhexidine maternal-vaginal and neonate body wipes in sepsis and vertical transmission of pathogenic bacteria in South Africa: a randomised, controlled trial. Lancet. 2009 Dec 5; 374 (9706):1969-16.

Authors	Name	Signature	Date
1st author	Clare L. Cutland		28 APR 2016
2nd author	Shabir A. Madhi		1st Feb 2016
3rd author	Elizabeth R. Zell		31st Jan 2016
4th author	Locadiah Kuwanda		1st Feb 2016
5th author	Marilin Laque		4th Mar 2016
6th author	Michelle J. Groome		2nd Feb 2016
7th author	Rachel Gerwitz		9th Feb 2016
8th author	Michael C. Thigpen		8th Feb 2016
9th author	Roopal Patel		9th Feb 2016
10th author	Sithembiso C. Velaphi		31st Jan 2016
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Title: Risk Factors for Neonatal Sepsis and Perinatal Death Among Infants Enrolled in the Prevention of Perinatal Sepsis Trial, Soweto, South Africa.

Author: Schrag, Stephanie; Cutland, Clare; Zell, Elizabeth; Kuwanda, Locadiah; Buchmann, Eckhart; Velaphi, Sithembiso; Groome, Michelle; Madhi, Shabir; MD, PhD

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