THE EPIDEMIOLOGY, RISK FACTORS AND DIAGNOSIS OF NEONATAL SEPSIS

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

Doctor of Philosophy

Johannesburg, South Africa 2017

DECLARATION

I, Sithembiso Christopher Velaphi declare that this thesis is my own, unaided 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 before for any degree or examination at this or any other University.

Signature of Candidate

14th day of September 2017 in Johannesburg

DEDICATION

I dedicate this work to my wife, Zimasa Velaphi, for her patience and support and for taking care of the children while I was focusing on conducting research and writing up this thesis.

To my children, Andiswa, Sango and Mulisi for their understanding during my absence to write up this work.

To my late mother, Tambeni Addinah Velaphi (1941-2014) and my late grandmother Nophamethe Velaphi (1910-1979) for teaching me patience and never to give up no matter how impossible the situation is.

PUBLICATIONS ARISING FROM THIS RESEARCH PROJECT

Abstracts presented at conferences

1. Maternal and neonatal vitamin D status and its association with early-onset neonatal sepsis in black South Africans. Presented at 36th Conference on Priorities in Perinatal Care in South Africa, 7-10 March 2017, Port Alfred, Eastern Cape, South Africa

2. Burden and Etiology of Sepsis in the First Three Days of Life in a Large Public Hospital: Preliminary Results from the Sepsis Aetiology in Neonates in South Africa Study — Soweto, South Africa, 2013–2014. Presented at the EIS conference 2016, Atlanta, USA

3. A Latent Class Modeling Approach to Characterize Neonatal Sepsis Etiology: Presented at the Women in Statistics and Data Science Conference, 21 October 2016, Charlotte, North Carolina, USA

Manuscripts planned for submission for publication

1. Maternal and neonatal vitamin D and its association with neonatal sepsis in Black South Africans (submitted to the journal 'Journal of Perinatology in July 2017).

2. Burden and aetiology of possible serious infection in the first 3-days of life, Soweto, South Africa: Results from the Sepsis Aetiology in Neonates in South Africa (SANISA) Study (in preparation for submission).

3. Incidence of early-onset and community acquired possible serious bacterial and culture-confirmed sepsis

4. Colonization rates and risk factors for colonization in blood and pharyngeal space in the first 28 days of life

ABSTRACT

Background: In sub-Saharan Africa, sepsis is the third most common cause of deaths during the neonatal period. Both maternal human immunodeficiency virus (HIV) infection and vitamin D deficiency (VDD) have been identified as risk factors for infection in children. The relationship of these risk factors, especially VDD among neonates in developing countries is not well documented. The "gold" standard to diagnose sepsis is blood culture; however, it has low sensitivity. Therefore there is a need for tests with improved sensitivity, to improve estimates of the incidence and aetiology of neonatal sepsis. This could enable prompt and targeted use of antibiotics to reduce both mortality and mitigate against emergence of antimicrobial resistance. The aims of this study were to determine the epidemiology of early-onset sepsis (EOS) and community acquired sepsis (CAS) using standard blood culture. Further, we evaluated the role of molecular diagnostic using polymerase chain reaction (PCR) based technology (Taqman array card), and evaluated role of maternal HIV infection and vitamin D status as risk factors for EOS and CAS in neonates born in Soweto, South Africa.

Methods and Procedures: Neonates born and/ or admitted with a diagnosis of possible serious bacterial infection (pSBI) with no previous hospital admission were prospectively enrolled into the study. They were grouped into EOS (onset of sepsis before 3 days of life) and CAS (onset of sepsis between 3-27 days of life). A subgroup of patients who met a predetermined definition of clinical or culture confirmed neonatal sepsis (protocol-defined sepsis), had blood and naso/oro-pharyngeal (NPOP) swabs tested using a PCR- based technique, Taqman array card (TAC) to identify possible causative pathogens. Healthy neonates were enrolled as controls, matched for age-group of cases with sepsis. In a separate cohort, mother-newborn dyads were enrolled soon after birth, and had blood taken to measure serum 25-hydroxyvitamin D [25(OH)D]. These newborns were grouped as being healthy or ill with sepsis. Sepsis in this cohort was defined as presence of clinical signs together with the presence of a positive blood culture and/or high C-reactive protein or interleukin-6. For both cohorts, cases and controls were stratified according to HIV exposure.

Results: There were 34,808 live births in Soweto over the study period, August 2013 to September 2014. A total of 3260 neonates were enrolled, 2624 (80%) and 636 (20%) with a diagnosis of early-onset pSBI (EO-pSBI) and community acquired pSBI (CA-pSBI) respectively. Blood culture positivity rate due to pathogens in neonates with EO-pSBI was 3.7% (96/2624). The incidence (per 1000 live births) of EO-pSBI was 106 (95%CI 102-109)

and 3.8 (95% CI 3.2-4.6) for culture-confirmed EOS. More than two thirds of putative pathogens isolated from neonates with culture-confirmed EOS (69.8%) were Gram positive bacteria. The common bacteria were Group B *streptococcus* (GBS; 35/105; 33%) , *Viridans streptococcus* (23/105; 22%), *Enterococcus species* (10/105; 10%) and *Escherichia coli (E. coli;* 10/105; 10%), with incidences (per 1000 live births) of 1.41 (95%CI 1.06-1.86), 0.92 (95%CI 0.65-1.30), 0.40 (95% CI 0.20-0.61) and 0.40 (95% CI 0.20-0.61) respectively. HIV exposed neonates had higher incidence of sepsis than HIV unexposed for EO-pSBI (OR:1.45; 95%CI 1.34-1.56). The overall case fatality rate was 9.0% (236/2624) for EOS. Blood culture positivity rate due to pathogens in neonates with CA-pSBI was 9% (55/636). The incidence of CA-pSBI and blood/CSF culture confirmed CAS were 33.4 (95%CI 31.6-35.4) and 3.53 (95%CI 2.96-4.22), respectively. More than three-quarters (76.7%) of putative pathogens isolated from CA-pSBI were Gram positive bacteria. Among, the culture-confirmed CAS, common organisms in blood were *Viridans streptococci* (17/60; 28%), GBS (14/60; 23%), *Staphylococcus aureus* (12/60; 20%), and *E.coli* (9/60; 15%); while in CSF the common organisms were GBS (9/25; 36%), *Staphylococcus aureus* (5/25; 20%), *Viridans streptococcus* (4/25; 16%) and *Enterococcus species* (4/25; 16%). The overall incidence for common organisms in blood and/ or CSF for CAS were 0.95 (95%CI 0.67-1.33), 0.90 (95%CI 0.63-1.27), 0.75 (95%CI 0.51-1.10) and 0.58 (95%CI 0.37-0.89) for *Staphylococcus aureus*, *Viridans streptococcus*, GBS and *Enterococcus* species respectively. HIV exposed neonates had higher incidence of blood/CSF culture confirmed CAS than HIV unexposed (OR:1.90;95%CI 1.32-2.74), including specifically for *Staphylococcus aureus* (OR:2.71; 95% CI 1.35-5.41), GBS (OR:4.82; 95%CI 2.13-10.9) and *E.coli* (OR:2.71; 95%CI 1.07-6.82). . The case fatality rate for CAS was 1.4% (9/636).

Among protocol-defined sepsis cases tested with TAC, bacteria or viruses were detected in blood in 37.1% of cases with EOS. Although similar organisms were identified in blood of cases and controls, proportion of cases with positive TAC was higher than in controls (37.1% vs 19.5%; OR: 2.35; 95%CI 1.72-3.21). The common organisms identified in blood of EOS cases using TAC were *Streptococcus pneumoniae* (14.2%), *Ureaplasma species* (9.2%), *Pseudomonas species* (8.5%) and GBS (7.0%). In pharyngeal swabs there were fewer cases that tested positive with TAC compared to controls (44.1% vs 53.1%; OR:0.69; 95%CI 0.59-0.90), and the common organisms identified in cases were *Ureaplasma species* (19.9%), *Klebsiella pneumoniae* (11.9%) and GBS (8.5%). After applying modelling factoring positive blood culture, one was able to attribute aetiology to a specific pathogen for 26.7% of cases using blood culture and TAC, and therefore 73.3% of cases did not have an identifiable

aetiology from the pathogens tested in culture or TAC. Among the positive TAC results in blood and pharyngeal swabs the organisms that were found to be attributable to EOS were *Ureaplasma species* (5.4%, 95% CI 3.6%-5.1%) , GBS (4.8%, 95%CI 4.1%-5.8%), and *Viridans streptococcus* (4.2%, 95%CI 3.5%-5.1%). There were no differences in number of cases and controls with positive TAC results between HIV exposed and unexposed neonates. In neonates with CAS protocol-defined sepsis cases tested with TAC, bacteria or viruses were detected in blood in 45.8% of cases. Proportion of cases with positive TAC in blood was higher than in controls (45.8% vs 27.4%; OR: 2.24; 95%CI 1.30-3.86). The common organisms identified included *Streptococcus pneumoniae* (15.7%), GBS (14.5%) and *E. coli* (8.3%). In pharyngeal swabs there were no differences in numbers with positive TAC results between cases and controls (75.0% vs 70.1%, OR:1.28; 95%CI 0.77-2.12), and the common organisms identified in cases were GBS (28.0%), *Klebsiella pneumoniae* (24.2%) and at equal rates (13.6%) were *E. coli*, *Ureaplasma species* and *Streptococcus pneumoniae*. Viruses were identified in 40% of cases in the pharyngeal swabs. There were no differences in number of cases and controls with positive TAC results between HIV exposed and unexposed neonates. Maternal and cord blood 25(OH)D levels were 54.7±30.1 and 39.0±21.3 nmol/L respectively, and prevalence of VDD (defined as a 25(OH)D level of <30 nmol/L) among the women and their newborns was 18.8% and 39.8% respectively. There were no significant differences in 25(OH)D levels or VDD between HIV infected and uninfected pregnant women. On multivariate analysis VDD in neonates was not associated with EOS.

Conclusions: There is high burden of neonatal sepsis in Soweto, including significant mortality. Based on blood culture, GBS is the most common pathogen causing EOS; *Viridans streptococcus* and *Staphylococcus aureus* the most common causes of culture-confirmed CAS. HIV exposure contributes significantly to a higher burden of bacterial sepsis in neonates. Although molecular detection using the TAC assay identified more bacteria organisms than from blood culture, including non-culturable organisms like *Ureaplasma species*, its use as a diagnostic tool for sepsis warrants further evaluation due to high positivity rates among healthy neonates for many of the targeted organisms in blood and NPOP swabs. Nevertheless, after correcting for positive controls, a combination of blood culture and TAC improved the detection of organisms in neonates with sepsis. In this study, maternal and newborn VDD was not associated with sepsis; however, this warrants further evaluation since this study had a limited number of neonates with culture confirmed disease.

ACKNOWLEDGEMENTS

With deep gratitude I wish to acknowledge:

- The Almighty God for His faithfulness and love which enabled me to complete this thesis
- The mothers and infants enrolled in this study
- The interns, medical officers and registrars who assisted in taking blood for this study
- My supervisors, Professors Shabir Ahmed Madhi and John Morley Pettifor for their valuable guidance and criticism, tireless effort, dedication and the constant encouragement I received from them during the study and write up of the thesis.
- Malefu Moleleki, Ann von Gottberg and Nicole Wolters from National Institute of Communicable Diseases for their assistance with laboratory work and reviewing it with me.
- Tracy Pondo from Center for Disease Control and Prevention (CDC), Atlanta, Georgia in USA for her support and assistance with the statistical analysis
- Thomas Taylor, Jr. from CDC for teaching me SAS statistical software so that I could do statistical analysis independently
- The CDC, Atlanta Georgia staff, especially Matthew Westercamp, and Stephanie Schrag for hosting me during my sabbatical leave to analyze the data and writing up the thesis
- Clare Cutland for her friendship and encouragement over the years and for reviewing draft of this thesis
- The Respiratory and Meningeal Pathogens Research Unit staff, especially Naseem Ebrahim and Shiraz Ismail for their assistance with solving recurrent computer problems on loading and transfer of data
- The National Institute of Communicable Disease for making this study possible through funding and giving technical support for the project via the South African Global Disease Detecting Center Cooperative Agreements with CDC, Atlanta, USA
- The South African Medical Research Council for giving me a grant so that I could take sabbatical leave to work on my research and write up this thesis

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LIST OF ABBREVIATIONS AND SYMBOLS

AMP	Antimicrobial peptides
ANC	Antenatal Care
aRRR	adjusted Relative Risk Ratio
CHBAH	Chris Hani Baragwanath Academic Hospital
CAS	Community acquired sepsis
CFR	Case fatality rate
Cfu	colony forming unit
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DNA	Deoxyribose nucleic acid
dL	deciliter
EDTA	Ethylaminedimethyltetraamine
EO-pSBI	Early-onset possible serious bacterial infection
EOS	Early onset sepsis
FBC	Full blood count
GBS	Group B streptococcus
GNB	Gram negative bacilli
GPC	Gram positive cocci
HIV	Human immunodeficiency virus
HEU	HIV exposed uninfected
IFN	Interferon
IL6	Interleukin 6

LOS	Late onset sepsis
LRTI	Lower respiratory tract infection
mg	milligrams
ml	millilitres
nmol	nanomoles
NICU	Neonatal intensive care unit
NPOP	Naso-/oro-pharyngeal
OR	Odds ratio
PAMPs	Pathogen associated molecular patterns
PCR	Polymerase chain reaction
pLCM	Partially latent class model
pSBI	Possible serious bacterial infection
PTH	Parathyroid hormone
qPCR	quantitative polymerase chain reaction
RNA	Ribose nucleic acid
TAC	Taqman Array Card
TLDA	Taqman low density array
TLR	Toll-like receptors
UTM	Universal Transport Media
UVB	Ultraviolet B
VDR	Vitamin D receptors
WCC	White cell count
WHO	World Health Organization

α	alpha
ºC	degrees Celcius
%	percent
<	less than
>	greater than
25(OH)D	25-hydroxyvitamin D

DEFINITIONS OF SEPSIS

1. **Possible Serious Bacterial Infection (pSBI)** – neonate has clinical signs suggestive of infection and attending physician has taken blood for culture and started on empiric antibiotics

2. **Protocol Define Sepsis**- neonate has clinical signs with abnormality in any one of the ancillary laboratory tests and/or positive blood/ cerebrospinal fluid culture due to an organism considered to be a pathogen

3. **Culture-Confirmed Sepsis** - neonate has clinical signs with a positive blood/ cerebrospinal fluid culture due to an organism considered to be a pathogen

4. **Early-Onset Sepsis** – any type of sepsis listed in 1, 2 and 3 above, diagnosed in a neonate at <3 days of life.

5. **Community-Acquired Sepsis** – any type of sepsis listed in 1, 2 and 3 above, diagnosed in a neonate at 3-27 days of life who has not previously been ill and/ or admitted in hospital since birth except for the days in hospital with mother post-delivery

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1 INTRODUCTION

1.1 General Introduction

Globally in 2013, 44% of all under -5 childhood deaths occurred during the neonatal period (first 28 days of life), of which approximately 20% were due to sepsis.[1] In sub-Saharan Africa, neonatal sepsis is the third most common cause of deaths in the first month of life, after prematurity and intrapartum-related complications.[1] Many deaths among premature infants might also be due to sepsis, so the current estimates of sepsis are most likely an underestimate. A study from Uganda reported neonatal sepsis as the leading cause of neonatal deaths, ahead of prematurity and asphyxia.[2] The number of deaths due to neonatal sepsis has remained relatively unchanged over the past 14 years, despite a 40% reduction in under-5 childhood mortality, emphasizing the need to focus on neonatal deaths. Reducing neonatal deaths due to sepsis requires an understanding of its epidemiology, including identification of the major risk-factors. Early diagnosis and treatment of neonatal sepsis is critical in improving outcomes. Treatment requires starting empiric management promptly while awaiting confirmatory microbiology results.

There are many factors that may contribute to the high incidence of neonatal sepsis in low-income settings, including maternal factors, and quality of health-care at delivery and during the neonatal period. Maternal factors associated with sepsis include maternal illness, poor nutritional status, and inadequate quality of care related to infection control at delivery. Both maternal human immunodeficiency virus (HIV) infection and vitamin D deficiency have been associated with increased incidence of neonatal sepsis.[3-6] Possible mechanisms for the association of both conditions with neonatal infection relate to impaired immunity in the fetus. Not only does HIV infection in the fetus impair immunity, but *in-utero* HIV exposure to viral products even in the absence of HIV-infection in the neonate causes immune dysregulation.[7-12] Therefore, even if vertical transmission of HIV is prevented, a sizeable number of neonates who are born to HIV-infected mothers remain at risk of immune dysregulation. Maternal vitamin D deficiency and HIV infection are both prevalent in developing countries. Therefore determining the association of vitamin D deficiency and HIV exposure on the epidemiology of neonatal sepsis is important, and might assist in developing strategies to reduce neonatal sepsis.

The incidence of neonatal sepsis is often underestimated, as most studies involve passive surveillance of hospitalizations. This often excludes neonates who succumb to sepsis prior to reaching health-care facilities. Another potential reason for underestimating the incidence of neonatal sepsis relates to difficulties in diagnosing sepsis. Among very low birth weight (<1500g) newborns, the contribution of sepsis towards neonatal mortality in developing countries is often underestimated, as mortality in these neonates may be attributed to complications of prematurity rather than specifically attributed to sepsis, since many of these patients may die without workup for sepsis because of limited resources. In settings where resources are available for managing VLBW or preterm infants, infections have been reported to be the third common cause of death in these infants.[13, 14] Similarly, newborns might present with non-specific signs such as neonatal encephalopathy, which too could be caused but not recognized as sepsis. This is pertinent in the context of the high incidence of birth-asphyxia in developing countries, where newborns with neonatal encephalopathy might be misdiagnosed as having birth-asphyxia rather than sepsis. The odds of an early neonatal death due to intrapartum asphyxia in newborns exposed to maternal infection are increased compared to unexposed newborn highlighting the role of sepsis in these deaths.[15] A combination of maternal fever and prematurity results in a 7-fold increase in risk of birth asphyxia mortality compared to term infants born to afebrile mothers.[16] Among term infants who present with neonatal encephalopathy after birth, 25% have been reported to be having infections.[17] Therefore sepsis is likely the leading cause of neonatal deaths, as deaths due to sepsis might be inappropriately attributed to prematurity and birth asphyxia. In order to delineate the epidemiology of neonatal sepsis, reliable diagnostic tests are required.

The current available test used as the gold standard in diagnosing sepsis is blood culture, which has low sensitivity (24-30%).[18, 19] The blood culture sensitivity might be further reduced in newborns of mothers who received intrapartum antibiotics.[20, 21] In countries with a high incidence of HIV infection, there is likely to be a higher number of pregnant women treated with intrapartum antibiotics because of preterm labour and non-pregnancy related infections associated with underlying immunosuppression in the women.[22-24] Thus the incidence of sepsis might be further underestimated in countries with a high incidence of maternal HIV infection. Consequently, other diagnostic tests with improved sensitivity compared to blood culture are required to better determine the aetiology and incidence of neonatal sepsis.

This study involved three components. Firstly it focused on the epidemiology of culture-confirmed neonatal sepsis in Soweto, South Africa; specifically evaluating the incidence of early-onset neonatal sepsis for both facility and home births and community acquired late-onset neonatal sepsis. Further, this was stratified by the maternal HIV-infection status and neonates categorized as either HIV-exposed and HIV-unexposed. This study component also evaluated the antibiotic susceptibility of pathogenic bacteria. Secondly, I investigated the utility of polymerase chain reaction (PCR) based technology, i.e. Taqman array card (TAC) molecular diagnostic to supplement blood culture to diagnose and estimate the incidence of pathogen specific causes of neonatal sepsis. Lastly, I investigated the role of maternal vitamin D status on risk of clinically-defined (clinical signs and abnormal laboratory findings) early-onset neonatal sepsis.

1.2 Epidemiology of Neonatal Sepsis

This section reviews the epidemiology of neonatal sepsis, including clinical algorithms for diagnosing sepsis, inflammatory markers of sepsis, incidence of disease and causative pathogens and their antimicrobial susceptibilities, sepsis related case fatality rates, and the utility of molecular assays for diagnosing pathogen-specific causes of neonatal sepsis.

1.2.1 Definition of sepsis

Sepsis is traditionally defined as bacteraemia accompanied by clinical signs suggestive of infection. The International Sepsis Definition Conference defined sepsis as a clinical syndrome, which requires the presence of both infection and systemic inflammatory response.[25] This definition recognizes that sepsis has two components, the presence of an organism which may manifest as a positive blood culture (infection) and an inflammatory response evidenced by clinical signs or changes in physiological parameters. Bone et al.[26] states that sepsis is a continuum of phases from bacteraemia to clinical manifestations and ultimately to organ failure and death. Thus he classifies infection into five phases, bacteraemia, systemic inflammatory response syndrome, sepsis, severe sepsis and septic shock. Systemic inflammatory response syndrome results in development of clinical signs of sepsis.

This definition of sepsis has some limitations when used during the neonatal period. While reliance might be placed on positive blood cultures in adults and children where large

volumes or multiple blood samples can be taken, in neonates it is not always possible to draw large volumes of blood, thus reducing the sensitivity of a blood culture.[27-29] Secondly neonates are relatively immunodeficient compared to older children, and therefore may not always develop an inflammatory response syndrome resulting in some patients having infection without clinical signs.[30] Thus while normal physical examination is strongly associated with a lower risk of having a positive blood culture and the presence of clinical signs is associated with high risk for infection,[31-33] not all cases of neonatal sepsis manifest with obvious signs and symptoms of sepsis. The presence of risk factors for bacterial infection such as maternal fever, chorioamnionitis, low birth weight and prematurity could be associated with sepsis, despite the absence of clinical signs due to an attenuated systemic inflammatory response in neonates.[34] Conversely, many neonates with clinical signs suggestive of infection may not have bacteraemia.

1.2.2 Classification of sepsis

Common localized infective syndromes that are classified separately in older children are often accompanied by septicaemia in neonates due to an immature immune function, which is unable to contain the infection to a single organ. This results in diagnoses like pneumonia and meningitis during the neonatal period being included under the broad umbrella of sepsis. Another term that is used to be more inclusive when sepsis is suspected is 'possible serious bacterial infections" (pSBI), including suspected cases of sepsis, meningitis, pneumonia and other neonatal bacterial infections.

Neonatal sepsis is stratified into early-onset (EOS) and late-onset sepsis (LOS), partly based on identifying the most likely source and pathogen causing the infection. This also informs appropriate empiric antibiotic treatment. EOS is commonly considered to be due to bacterial infections acquired from the maternal recto-vaginal tracts. LOS is generally regarded as originating from the care-giving environment within both the healthcare and community settings; therefore it is further stratified into hospital-acquired or healthcare-associated sepsis and community-acquired sepsis, based on the environment to which the neonate was exposed at the time of developing sepsis. The definition of EOS varies from study to study.[35-48] Some studies have defined EOS as sepsis occurring within the first three days of life,[35-43] while others have defined it as sepsis occurring within the first seven days of life.[44-48] Zaidi et al. described EOS occurring within the first three days of life as very-early onset sepsis.[49] There are also some studies that have defined EOS as infection with onset within 48 hours of

birth.[41, 50] Using a definition of EOS as within three days of birth rather than seven days is more biologically useful, in identifying the recto-vagina as being the likely source of the infection than cases which occur beyond three days of age, among whom infection could also have been acquired through healthcare or community environment sources.

1.2.3 Diagnosing sepsis

Clinical signs

Early diagnosis of sepsis depends on the detection of clinical signs, as this allows for early initiation of empiric antibiotic treatment before infection proceeds to severe sepsis and death. In infants less than 60 days of age, signs associated with sepsis, meningitis and pneumonia include reduced feeding ability, decrease in spontaneous movement (lethargy), fever (temperature $\geq 38^0$C) or hypothermia (temperature <35.5°C), being drowsy/ unconscious, being agitated (irritability), the presence of lower chest wall in drawing, respiratory rate >60 breaths per minute, grunting, cyanosis, convulsions, a bulging fontanelle, and slow digital capillary refill time.[51] The presence of any one of these signs has been reported to have sensitivity for severe disease (including sepsis, meningitis, hypoxaemia and radiologically proven pneumonia) of 87% and a specificity of 54%. A combination of signs from this list resulted in lower sensitivity. The Young Infant Study Group assessed sensitivity and specificity of a number of signs during the neonatal period, which included history of difficulty with feeding, movement only when stimulated (lethargy), temperature $<35.5^0$ or $>37.5^0$ C, respiratory rate ≥60 breaths per minute, prolonged capillary refill time, grunting, cyanosis, severe chest in-drawing, convulsions and stiff limbs. These individual signs were reported to have a sensitivity of 87% and specificity of 74%.[52] The shortcoming of using these signs is the lack for specificity due to them also manifesting in other illnesses; for example lethargy, poor feeding and convulsions are common findings in neonates with hypoxic ischaemic encephalopathy or asphyxia. Furthermore, some healthy neonates may have respiratory rate of >60 breaths per minute which has been used to define tachypnoea.[30, 53-55] Thus, clinical signs are non-specific and therefore a diagnosis of sepsis based on clinical signs alone will result in overestimation of the true burden of sepsis. This is highlighted by a study, which estimated that 30 non-infected neonates will be treated for every patient with confirmed infection.[56] Stoll et al.[35] reported that 50% of all very low birth weight newborns receive empiric antibiotic therapy based on clinical signs and risk factors for sepsis, yet only 1.9% had culture-proven sepsis.

There is no consensus on the predictive value of the various clinical signs and symptoms in diagnosing sepsis. In the absence of a positive blood culture result and with the recognition of the low specificity of clinical signs, a combination of clinical signs and other ancillary laboratory tests has been proposed to diagnose sepsis in neonates.[28, 37, 57] The laboratory tests that have been used and that are fairly accessible include white cell count, neutrophil count, platelet count, C-reactive protein (CRP), and cerebrospinal fluid white cell count in those who had a lumbar puncture.

Ancillary laboratory tests

One of the commonly used laboratory tests in assessing infants with suspected neonatal sepsis is the full blood count (FBC). When newborn infants are symptomatic, white cell count, absolute neutrophil count and platelet count are commonly used to assess the likelihood of infection, and the consequent need for antibiotics. The major limitation of using these parameters is that they have low sensitivity (0.3% - 54.5%)[58] and are affected by factors other than infection including maternal hypertension,[59-61] mode of delivery,[62-64] gender,[63] age[31, 59, 65-67] and method of blood sampling.[68-70] Leucopenia and neutropenia are also associated with increasing odds of infection. For example leucopenia of <5500/mm^3 and neutropenia of <1540/mm^3 are associated with increasing odds of EOS with odds ratios (OR) of 8.80 (95% CI 6.29-11.20) and 10.74 (95% CI 7.76-15.06), respectively.[58] However, the sensitivities of leucopenia, neutropenia and thrombocytopenia in diagnosing sepsis are only 18%, 19% and 14%, respectively.[58] A number of studies have shown that a high (>0.2) immature (I) to total (T) neutrophil ratio (I/T ratio) is associated with increasing odds of EOS, thus can be used as a predictor for sepsis.[31, 67, 71, 72] Though I/T ratio can predict those neonates with EOS, with high specificity and negative predictive value of >90%, it has a low sensitivity with studies reporting sensitivities <60% and a positive predictive value of <10%.[71-73]

A high C-reactive protein (CRP) (>10 mg/L) is associated with infection and/or inflammation. In neonates in whom the incidence of non-infective inflammatory conditions is rare, a raised CRP is most likely related to infection. It takes at least 6-12 hours for CRP levels to be raised following antigenic stimulation,[74, 75] therefore it is important that the CRP measurement is delayed compared to time of onset of infection or it should be done at presentation and repeated 12-24 hours later. However, CRP levels in neonates must be interpreted with caution, as it might be influenced by gestational age, fetal distress and

maternal fever, and therefore cannot be used on its own.[76-79] It has also been reported to have poor sensitivity (35%-47%) for diagnosing EOS.[80-82] Another biomarker that has been recommended in the diagnosis of sepsis is procalcitonin (PCT). It is a prohormone of calcitonin secreted from the liver and circulating macrophages in response to endotoxins.[83] Its serum levels increase within 2-3 hours of the infection, peaking up by 6-12 hours and returning to normal levels within 2 days.[84] In children, the normal concentration has been reported to be <0.5 ng/mL, with a slight increase to levels of 0.5-2 ng/mL during viral infections and non-infectious inflammation or stress situations and an increase to above 2 ng/mL in bacterial and fungal infections.[85, 86] It rises earlier than CRP and correlates with the severity of the disease.[87, 88] A systematic review of 18 studies that assessed PCT diagnostic accuracy in neonatal EOS reported that it had sensitivity ranging from 47.4% (95% CI 27.3-68.3) to 100% (95% CI 67.6-100) and specificity from 35.3% (95% CI 17.3-58.7) and 100% (95% CI 96.8-100).[89] The possible reasons for this wide variation in sensitivity and specificity of PCT from these studies include different cutoff levels used to define high PCT which ranged from 0.253 to 100 ng/mL, and different postnatal ages ranging from birth to three days of life. In neonates normal PCT levels have been shown to be affected by postnatal age with one study reporting that PCT start at levels of <1 ng/mL at birth, peaking up to levels of 10ng/mL at about 28 hours and returning to <1 ng/mL at 72-96 hours.[90] Therefore when one is using PCT to diagnose EOS, cutoff levels should be assessed against postnatal age.

Cerebrospinal fluid biochemistry and cytology results are often difficult to interpret in neonates, since unlike adults, CSF of neonates may normally contain white cells,[91-94] and secondly lumbar punctures in neonates are often contaminated with blood, making it difficult to interpret the CSF results to diagnose meningitis.[95] Values of CSF white cell count>20 cells/ mm^3 have been reported to have a sensitivity of 79% and specificity of 81% for diagnosing bacterial meningitis.[92]

A number of studies have used these ancillary laboratory tests in addition to clinical signs as criteria to diagnose neonatal sepsis.[37, 57] Since the definitive diagnosis of sepsis requires isolation of bacteria from blood, patients with clinical signs and abnormal ancillary tests but negative blood cultures are categorized as having clinical sepsis or probable sepsis.

Culture

The gold standard for diagnosing sepsis historically has been a positive blood or cerebrospinal fluid culture. The limitation of using blood culture as a gold standard is that the yield from culture varies based on volume of blood sent for culture and degree of bacteraemia, previous exposure to antibiotics and whether or not the causative pathogen grows on standard blood culture media. In adults it has been reported that a greater volume of blood improves the yield of culture, with the yield increasing by 3% per milliliter of blood cultured.[96, 97] In neonates, the probability of bacterial growth on blood culture is affected by both the sample volume and the degree of bacteraemia. As an example the probability of having a positive culture if there is one colony forming unit per milliliter (cfu/ml) increases from 0.39 with 0.5 ml of blood to 0.63, 0.87 and 0.98 with 1 ml, 2 ml and 4 ml of inoculated blood, respectively. When the colony count is 4 cfu/ml, the probability of positivity is 0.87 with 0.5 ml and increases to 0.98, 0.99 and 0.99 with 1ml, 2 ml and 4 ml of inoculated blood, respectively.[98] In patients with a high bacterial load in blood (≥10 cfu/ml), as little as 0.25 ml of blood might be required to consistently detect the pathogen in blood.[27] The challenge in young children, including neonates, is that a large proportion of patients with infection (60-64%) have low bacterial loads (<10 cfu/ml), including 23-42% with levels as low as 1 cfu/ml.[99, 100] Therefore, blood volumes >0.25 ml will be needed to detect such low levels of bacteraemia. The suggested minimum blood volume to send for blood culture in neonates is between 0.5 to 1 ml.[101] In neonates who have been exposed to maternal intrapartum antibiotics the sensitivity of culture is questionable because if maternal antibiotics do cross the placenta they might lead to suppression of bacterial growth in a neonate, thus the recommendation that well neonates exposed to maternal intrapartum antibiotics should be observed in hospital for 48 hours. Yield of blood cultures in patients exposed to antibiotics is lower than those who have not been exposed with one study reporting that pathogens were detected in 14.3% of blood cultures obtained before the institution of antibiotic treatment compared to 7% in those under antibiotic treatment.[102] This effect of antibiotics suppressing bacterial growth is supported by improvement in detection of pathogens when resin that adsorbs antibiotics is added before performing culture.[103]

The positive blood culture rate also varies depending on age of neonate at onset of sepsis and the setting from which the neonate is presenting at time of culture. The blood culture from neonates with suspected EOS (<3days age) has low positivity yield of 0.7% to 3.3%[104-106] in developed countries, compared to yield of 33% to 47% in developing countries.[39, 40] This

difference could be due to clinicians in developed countries having a low threshold to diagnose or investigate for sepsis or patients presenting late in developing countries with fulminant sepsis and high bacterial counts. In those suspected with EOS (<seven days of life) the yield is high at 46-67%.[107, 108] The high yield in this group of patients with EOS defined as less than seven days is probably because the group includes cases that might have developed healthcare-associated infections (onset of infection after being in hospital for >72 hours) and therefore more likely to have higher colony counts.[109] Furthermore, possible contaminants are not excluded. The blood culture yield from community acquired neonatal sepsis is 3.4% to 13.5% (**Table 1.1**).[44, 48, 110-112] Many studies reporting on community acquired neonatal sepsis have excluded possible contaminants, and this would explain the relative lower rates of positive culture when compared to studies based in healthcare facilities.

Table 1.1: Positivity of blood cultures according to category of neonatal sepsis

	Year	Country	Number enrolled	Sepsis Criteria	Number with Positive Culture	Positive culture Excluding Contaminants
Early-onset sepsis (<3 days), healthcare facility						
- Jardine, L et al, 2006[104]	2000-4	Australia	2640	Risk factors or Clinical Signs	56 (2.1%)	21 (0.8%)
- Ogunlesi, T. et al, 2011[39]	2006-8	Nigeria	360	Risk factors or Clinical signs	119 (33%)	
- Kayange, et, al, 2010[40]	2009	Tanzania	121	Clinical signs	57 (47%)	
- Sgro, M. et al, 2011[105]	2003-8	Canada	62453	NICU admissions	405 (0.7%)	
- Guerti, K. et al, 2011[106]	2001-7	Belgium	1783	NICU admissions and suspected sepsis	58 (3.3%)	
Early-onset Sepsis (<7 days), healthcare facility						
- Tallur, S. et al, 2000[107]	1996-7	India	202	Clinical signs	135 (67%)	
- Mugalu, J. et al, 2006[108]	2002	Uganda	119	Clinical signs	55 (46%)	
Community acquired Sepsis						
- English, M. et al. 2003[44]	1999-2001 1998-2002	Kenya	432 (0-6 days)	Admissions	67 (15.8%)	41 (9%)
- Berkley, J. et al. 2005[112]	1994-2000 2004-6	Kenya	867 (<7 days)	Admissions		117 (13.5%)
- Quiambao, B. et al. 2007[110]	Not stated	Philippines	767 (0-59 days)	Clinical signs		26 (3.4%)
- Darmstadt, G. et al. 2009[111]		Bangladesh	500 (0-27 days)	Clinical signs		29 (5.8%)
- Hamer, DH, et al. 2015[48]		Bangladesh Bolivia Ghana India Pakistan South Africa	424 (0-7days) 358 (7-59 days)	Clinical signs Clinical signs		44 (10.4%) 39 (10.9%)

*- NICU – neonatal intensive care unit

Polymerase chain reaction (PCR) based molecular technology

The typical isolation media and incubation conditions do not allow the growth of all bacterial strains, thus a large number of microbial species remain unidentified in blood culture of infants with infection. Even among those pathogens which can be cultured, some might be difficult to culture. Therefore, in order to identify all possible causes of infection in neonates, tests other than blood culture that can identify all forms of microbes are required.

Microbes are reported to exist in three macroscopic physiological states, viable, viable but dormant and non-viable state.[113] Microbes in the viable state can reproduce and replicate and are detectable using blood culture. In the dormant state the organism is neither obviously viable, nor immediately culturable but has the potential to reproduce. Identification of dormant organisms is important as even if they are not the cause of infection at the time of detection they could become viable and therefore cause infection. An organism in a non-viable state is unable to reproduce under any conditions, and therefore it is possible that such microbes might be present in a human body or blood without causing illness. It has been reported that even blood from "healthy" individuals might contain bacterial 16S ribosomal RNA, which can be detected and allows for the identification of bacterial phyla.[114-116]

While blood culture only identifies viable organisms, molecular assays may detect viable and non-viable microbes including viruses and dormant organisms through identification of 16S ribosomal RNA gene sequence. The new molecular pathogen detection methods are based on amplification of specific target regions in the microbial genome using polymerase chain reaction (PCR). The PCR targets the 16S rRNA gene, a ubiquitous gene preserved in all bacteria and comprises both conserved and variable regions. The conserved regions are targeted by universal primers for identification of bacteria while identification of the variable regions allow for genus or species-specific detection. Amplified target regions may then be subjected to downstream applications such as sequencing or microarray/probe hybridization. A number of amplification methods have been evaluated in neonates to diagnose of sepsis.[117] These include broad-range conventional PCR assays, real-time PCR in which amplification is monitored in real time, PCR followed by post-PCR processing such as sequencing or hybridization, multiplex PCR in which amplification is directed against multiple organisms in the same assays and species-specific and genus-specific assays. These techniques rely on PCR amplification of the 16S rRNA gene, a highly conserved gene which is absent in

humans. These assays have been used successfully to identify a wide range of organisms including bacteria and viruses.[118-121] Molecular assays have an advantage over blood culture as they can detect pathogens more rapidly, use smaller volumes of blood and have ability to detect small amounts of bacteria, including dormant and non-viable ones and have better sensitivity than cultures.[122] The turn-around time for TAC is less than 4 hours including sample preparation and real-time PCR compared with 1 to 2 days to detect growth in culture or 5 days to finalize no growth culture results.[123]

A number of studies have compared PCR methodology with cultures as the gold standard and have reported variable results with some suggesting that PCR has low sensitivity (41% - 67%),[122, 124, 125] while others report sensitivity of 100% and specificity >85% (89-91).[126-128] In studies where these PCR assays were compared head–to-head with blood culture, PCR had a higher detection rate (8.3% - 38.5% vs. 3.8% - 15.4%).[126 129-131] Though PCR tests may have a significant impact on early diagnosis and treatment of neonatal sepsis, currently they do not provide information about antibiotic susceptibility, which is important in antibiotic stewardship and patient outcomes. Thus in studies to assess the epidemiology and burden of sepsis in neonates, PCR techniques should always be used in combination with blood culture.

Detecting pathogens using Taqman Array Card

One of the molecular methods used in detecting pathogen is a multiplex PCR. This allows for detection of multiple pathogens from a single specimen, which is advantageous in neonates, in whom volumes of blood available are often small and many different infectious agents may contribute to the illness. This allows for more comprehensive evaluation of specimens in a relatively short period of time. Although the multiplex PCR detect multiple pathogens, a number of bacteria and virus antigen targets will still need to be included in these PCR assays, as sequence data on known and emerging pathogens become available. The disadvantage of multiplex PCR based technology is that any change to existing primers or addition of new primers and probes requires re-evaluation of the sensitivity and specificity of the entire assay and this is costly. An alternative to this approach, is the use of a singleplex quantitative PCR which allows for simultaneously detection of multiple pathogens. The format that allows for this is the Taqman low-density array (TLDA), which has been used for multiple gene expression and micro-RNA expression analyses in cancer research.[132, 133] Compared to multiplex PCR, the TLDA platform allows for the flexibility of adding new

primers and probes without recalibration of others already incorporated in the assay. In the TLDA card, also known as Taqman array card (TAC), reagents for each assay are pre-allocated to the reaction wells making it easy to use and the samples are added only once.[134] It has a 384-well microfluidic array consisting of dried-down individual singleplex qPCR reactions.

One of the advantages of using TAC is that total nucleic acids from one clinical specimen or control can be combined with a PCR mix, loaded into a TAC port, and separated by microfluidics into 48 separate reactions capable of amplifying either DNA or RNA.[135] The TAC technology has been evaluated for detection of respiratory pathogens and enteropathogens and has been used to determine the aetiology of respiratory outbreaks in the United States.[135, 136] The role of TAC for detection of pathogens in respiratory secretions, blood or cerebrospinal fluid of neonates with suspected infection has not previously been assessed in sub-Saharan Africa. TAC tests for both DNA and RNA, which include panels containing 21 specific primers and probes for simultaneous detection of bacteria and/ or viruses and two human genome controls, all in duplicate in each lane. There are 48 wells in each of the 8 lanes (384 wells in total). This allows testing of 7 different specimens for up to 21 pathogens with a positive control for each pathogen. All these 384 reactions can be completed within 3 hours. The reagents for each assay are pre-allocated to the reaction wells making it easy to use, thus reducing the chance of operator-error. There are lyophilized pathogen specific primers and probes in each well.

The pathogens tested for in blood and CSF using TAC are *Escherichia coli/Shigella sp.,* Group A *streptococcus*, Group B *streptococcus, Haemophilus influenzae, Klebsiella pneumoniae, Neisseria meningitides, Pseudomonas aeruginosa, Salmonella species, Staphylococcus aureus, Streptococcus pneumoniae, Ureaplasma urealyticum/ parvum* and *Enterovirus.* In respiratory secretions or nasopharyngeal/oropharyngeal (NPOP) swabs, the pathogens tested for are *Bordetella pertussis, Chlamydia pneumoniae, Chlamydia trachomatis, Escherichia coli/Shigella,* Group B *streptococcus, Klebsiella pneumoniae, Mycoplasma pneumoniae, Streptococcus pneumoniae and Ureaplasma urealyticum/ parvum,* Adenovirus, Cytomegalovirus, Enterovirus, Human metapneumovirus, Human Parechovirus, Influenza A and B viruses, Parainfluenza 1, 2 and 3 viruses, Rhinovirus and Rubella. Once specific nucleic acids are added to the wells, reactions are completely contained within each

well. This is of particular benefit in resource-poor settings where there are generally not dedicated facilities for different standard PCR steps. The TAC system allows for internal positive and two nucleic acid quality controls. In conclusion, TAC allows for simultaneous detection of multiple pathogens, eliminating the need for processing of clinical specimens for different pathogens sequentially. It reduces the amount of handling and chances of human error and contamination. Other advantages of using TAC in detection of pathogens, are the abilities to customize a panel of pathogen targets without the need for revalidation, testing for multiple pathogens including both bacteria and viruses, the requirement for small volume of specimens, short turn-around time, reduced potential for contamination due to the closed system format of TAC and ease of use, including minimal hands-on setup.[134]

Detecting organisms with molecular technology in addition to culture

The clinical suspicion of sepsis is based on the presence and interpretation of clinical signs. The major limitation of using clinical signs only during the neonatal period is that they are non-specific. The likelihood of detecting infection is augmented by laboratory investigations. The most readily available laboratory investigations when investigating for sepsis include white cell count with differential and platelet count and CRP. Although abnormalities in any of the above improve the specificity for diagnosing neonatal sepsis, they have low individual sensitivity. The confirmatory test is positive culture from a sterile site, usually either blood and/or CSF. The major limitations of blood culture are that it is often negative, despite strong clinical suspicion of sepsis and that some of the bacteria grown on blood culture might be contaminants and not the causative pathogens for sepsis. Among the bacteria that are grown on blood culture, there are those that are always considered pathogens namely Group B *streptococcus*, and those that are always considered contaminants namely *Corynebacteria* sp.; whereas others could be either pathogens or contaminants; e.g. Coagulase negative *Staphylococcus*. Consequently, although blood culture is considered as a "gold standard" for diagnosing sepsis, it does not satisfy the criteria of an appropriate gold standard because it has a high proportion of false negatives and positives (contaminants), thus the need for use of complementary tests like TAC to identify the pathogens involved in sepsis.

1.2.4 Incidence of neonatal sepsis

The incidence of culture confirmed neonatal sepsis varies between countries and regions. In developing countries the incidence (per 1000 live births) of neonatal sepsis varies from 7.1 to

40 in Asia[107, 137, 138] and 6.5 to 23 in Africa,[52, 139, 140] compared to 6 to 9 in United States and Autralasia.[34, 141-143] These rates include healthcare associated infections, which are influenced by other factors such as infection control and overcrowding and therefore do not reflect the burden of community associated sepsis. To assess the burden of community-associated sepsis, one needs to specifically study EOS defined as occurring within 3 days of birth and community acquired sepsis (CAS), hence limiting the chances of including healthcare-associated sepsis.

Early-onset sepsis

The incidence (per 1000 live births) of clinical diagnosed EOS in developing countries is reportedly 20.7 to 35.6 using a definition of <3 days [37, 144] and as high as 44.8 using a definition of <7 days,[145] while in developed countries it is much lower at 11.3.[146] The facility based incidence of culture confirmed EOS defined as within the first three days of life is reported to be 0.6 to 3.5 in developed countries.[34, 38, 41, 147-152] The facility-based incidence of culture confirmed EOS in developing countries varies widely amongst the different studies from as low as 0.72-0.93,[43, 153] to as high as 8.6-9.8.[144, 154]

Community acquired sepsis (CAS)

Most studies investigating CAS often do not differentiate between early and late onset CAS, thus in this review CAS includes both categories. Community-based studies from developing countries have reported an incidence (per 1000 live births) of clinically diagnosed neonatal sepsis ranging between 21 to 170.[155-158] The wide range in these studies is possible due to the different methods used to collect the data, the studies with high incidence of clinical sepsis were associated with active household surveillance of newborns by village health workers, therefore identifying more infants with clinical signs who might have been missed by those studies collecting data only from patients who present to facilities.[157, 158] It could also be due to different sensitivities of algorithms used to diagnose clinical sepsis and skills of the healthcare providers to recognize signs suggestive of sepsis. Population-based surveillance studies from developed countries have reported incidence (per 1 000 live births) of culture confirmed CAS to be from 1.1 to 1.7,[141, 146, 159] while in developing countries it varies from as low of 2.9[111] to as high of 5.5.[112]

1.2.5 Bacterial causes of sepsis

Early-onset sepsis

Knowledge of the pathogens most likely to cause neonatal sepsis assists in guiding the choice of empiric antibiotic treatment while awaiting culture and antibiotic susceptibility results. Pathogens isolated from neonates with sepsis vary from region to region and country to country. In order to exclude pathogens that might have been acquired from hospital, studies which are discussed in the following section are those reporting on EOS defined as sepsis occurring within the first 3 days of life.

In developed countries, the pathogen most commonly isolated from neonates with EOS sepsis is Group B streptococcus (GBS) which account for 30-50% of cases of EOS in the neonatal period in these countries.[38, 41, 160] In contrast, pathogens most commonly isolated from neonates in developing countries are Gram-negative bacteria (GNB), with a few countries reporting Gram-positive bacteria (GPB) as common pathogens (**Table 1.2**). In many countries in Asia, the common pathogens isolated from neonates with EOS (within 3 days) are GNB, namely *Klebsiella species*, *Pseudomonas aeruginosa*, *Serratia* and *Escherichia coli* (*E. coli*).[138, 144, 161] Among the GPB, the common pathogen isolated in Asia is *Staphylococcus aureus*.[138, 144] Only one study from countries in Asia during the first decade of this century reported GBS as a common pathogen.[153] In sub-Saharan Africa, the pathogens isolated in neonates with EOS also vary from country to country, for example in Nigeria the common pathogens isolated are GNB, namely *Klebsiella species* (29-65%), *E. coli* (7-8%), *Proteus* (5-7%) and *Pseudomonas* (3%) and the common GPB is *Staphylococcus aureus* (15-40%) as in Asia.[39, 162] In Kenya, Kohli-Kochlar et al.[45] reported, that GPB were more common than GNB, with *Staphylococcus aureus* (27%), *Streptococcus species* other than GBS (17%), *Enterococcus* (12%) and *Staphylococcus epidermidis* (27%) accounting for more than two thirds of isolates. Studies from South Africa reported GBS as a common pathogen isolated in neonates with EOS accounting for between 40% and 60% of all pathogens.[37, 43] In summary it would appear that the pathogen most commonly isolated from neonates with EOS is uniformly GBS in most developed countries, while in developing countries the bacteria causing EOS vary from country to country with GNB more common in Asia than in other countries. These differences, however, could be due to which neonates are enrolled in these studies, e.g. preterm infants are more likely to be enrolled in developed than developing countries, therefore pathogens might differ. Secondly in developing countries where most

deliveries occur outside healthcare facilities, it is possible that some neonates might die before they reach the healthcare facility and are never investigated. This could mitigate against detecting bacteria, which can be acquired *in utero* and present clinically at the time of birth, e.g. early-onset disease due to GBS commonly presents within 24 hours of delivery (and at birth) and therefore cannot be excluded as a possible cause of sepsis in these early newborn deaths. Thus its incidence is possibly underestimated in some African and Asian countries where most of the births occur outside healthcare facilities or even in health facilities which do not have the capabilities of undertaking blood culture.

Community acquired sepsis

A number of studies that assessed the pathogens causing CAS have included neonates and infants under the age of 60 days, which makes the interpretation and comparison of data difficult.[46, 48, 112] A systematic review of studies from developing countries including 2066 infants with CAS, aged from birth to 59 days of life, reported that the common pathogens isolated were *Escherichia coli* (16-18%), *Staphylococcus aureus* (12-15%), *Klebsiella sp.* (8-13%), *Pseudomonas* sp. (8-9%), *Acinetobacter* sp (6-7%) and Group B Streptococcus 1.7-7%.[46] A systematic review of studies with 2594 neonatal (0-28 days) sepsis cases, reported similar findings with *E.coli* (17%), *Klebsiella species* (14%) and *Staphylococcus aureus* (13%) being the common pathogens.[49] A more recent systematic review and meta-analysis of 3077 sepsis cases <1 month age from developing countries, reported that *Staphylococcus aureus* (26%), *Klebsiella species* (21%), and *E. coli* (8%) were the common pathogens.[163] A multicenter study including sites from six countries (Bangladesh, Bolivia, Ghana, India, Pakistan and South Africa) reported that the common pathogen for infants <60 days age was *Staphylococcus aureus* (43%).[48] Pathogens causing CAS in developing countries also vary from region to region and country to country. In a systematic review, Waters et al.[46] reported that in Africa the common pathogens were *Staphylococcus aureus* (14.6%), *Streptococcus pneumoniae* (13.9%), *E. coli* (11.0%) and GBS (6.9%). In South East Asia, the common pathogens were *Klebsiella species* (33.5%), *Staphylococcus aureus* (10.0%), *E. coli* (9.0%) and *Pseudomonas species* (9.0%). Focusing only on studies from developing countries conducted since 2000s, it is also clear that pathogens isolated from neonates with CAS vary by country (**Table 1.3**). Quiambao et al reported that the common pathogens causing CAS in Philippines were GNB with *Enterobacter species* (27%) and *Klebsiella species (*17%) being the commonest.[110] A study from Bangladesh reported that the common pathogens found in

neonates (0-27 days) with CAS were *Staphylococcus aureus* (33%) and *Pseudomonas aeruginosa* (17%).[111] Studies from Kenya and Malawi reported that the commonest pathogen causing sepsis in neonates (<30 days age) admitted to a district hospital was GBS (17-19%).[44, 164] Two years later another study from Kenya reported that the common pathogens identified to be causing CAS in the <7 day age group were *E. coli* (19%), *Acinetobacter species* (12%) and *Klebsiella species* (10%), while in those infants between 7-59 days of age the common pathogens were *Group A streptococcus* (15%), GBS (13%), *Streptococcus pneumoniae* (12%) and *Staphylococcus aureus* (12%).[112] In summary in developing countries the common pathogens causing CAS are *Staphylococcus aureus* (33-70%), *E. coli* (14-19%) and *Klebsiella species* (10-16%), but the sequence hierarchy of these bacteria varies from country to country and age at onset. Unlike the situation in developed countries, in developing countries GBS has not consistently been identified as a dominant pathogen associated with sepsis except in the southern part of sub-Saharan Africa. These studies also suggest that the relative contribution of different pathogens causing CAS may vary over time, highlighting the importance of ongoing auditing of pathogens isolated in neonates with sepsis.

Table 1.2: Bacteria isolated in neonates with early-onset (<3 days of life) in studies from 2000 onward

Study	Years Studied, Region	Countries	Number with positive culture	Pathogens	Percent
Chacko, et al. 2005[144]	2000-1, Asia	India	N = 15	*Pseudomonas species* *Klebsiella pneumoniae* *Staphylococcus aureus* *Escherichia coli* *Viridans streptococcus*	70% 13% 13% 7% 7%
Sundaram, et al. 2009[138]	2001-2006, Asia	India	N = 527	Non fermenting GNB *Staphylococcus aureus* *Klebsiella pneumoniae* *Escherichia coli* *Enterococcus faecalis*	30% 20% 12% 9% 7%
Tiskumara, et al. 2009[153]	2005, Asia	China, Hong-Kong, India, Iran, Kuwait, Malaysia, Thailand	N = 47	Group B *streptococcus* CONS *Escherichia coli* *Staphylococcus aureus*	38% 17% 13% 4%
Tosson, et al. 2011[161]	2003-5, Middle East	Jordan,	N = 100	*Klebsiella species* *Serratia species* *Escherichia coli* *Enterobacter species* *Pseudomonas species*	49% 11% 10% 9% 5%
Ogunlesi, et al. 2011[39]	2006-8, Sub-Saharan Africa	Nigeria	N = 119	*Staphylococcus aureus* *Klebsiella species* Unclassified coliforms *Escherichia coli*	34% 29% 10% 8%
Cutland, et al. 2009[37]	2004-7, Sub-Saharan Africa	South Africa	N = 28	Group B *streptococcus* *Enterococcus species* *Acinetobacter species* *Viridans streptococcus*	57% 11% 11% 7%
Ballot, et al. 2012[43]	2009-10, Sub-Saharan Africa	South Africa	N = 16	Group B *streptococcus* CONS *Viridans streptococcus* Other	44% 25% 19% 12%
Vergnano, et al. 2011[41]	2006-9, Europe	England	N = 124	Group B *streptococcus* *Escherichia coli* Other Streptococci *Listeria monocytogenes* *Staphylococcus aureus*	50% 18% 6% 6% 5%
Simonsen, et al. 2014[165]	2005-8, Americas	USA	N = 739	Group B *streptococcus* *Escherichia coli* *Viridans streptococcus* *Staphylococcus aureus* *Haemophilus influenzae*	34% 32% 16% 4% 4%
Perez, et al. 2015[166]	2013-14, Americas	Mexico	N = 68	*Escherichia coli* *Klebsiella pneumoniae* *Enterobacter cloacae* *Streptococcus bovis*	25% 21% 10% 9%

GNB – Gram negative bacteria, CONS – Coagulase negative staphylococcus

Table 1.3: Pathogens isolated in neonates (<28 days of age) with community acquired sepsis

Study	Year, region	Country	Number culture positive	Pathogens	Percent
Quiambao, et al. 2007[110]	1994-2000, Asia	Philippines	N = 22	*Enterobacter species* *Klebsiella species* *Streptococcus pneumoniae* *Pseudomonas aeruginosa* *Staphylococcus aureus*	27% 14% 14% 9% 9%
Darmstadt, et al. 2009[111]	2004-6, Asia	Bangladesh	N = 30	*Staphylococcus aureus* *Pseudomonas species* *Klebsiella species* *Acinetobacter species* *Streptococcus pneumoniae*	33% 17% 10% 10% 10%
English, et al. 2003[44]	1999-2001, Africa	Kenya	N = 64	Group B *streptococcus* *Klebsiella species* *Escherichia coli* *Streptococcus pneumoniae* *Pseudomonas species*	19% 16% 14% 11% 9%
Berkley, et al. 2005[112]	1998-2002, Africa	Kenya	N = 129	*Escherichia coli* *Acinetobacter species* *Klebsiella species* Group B *streptococcus* *Staphylococcus aureus*	19% 12% 10% 8% 5%
Milledge, et al. 2005[164]	1996-2001, Africa	Malawi	N = 784	Group B *streptococcus* *Salmonella non-typhoidal* *Staphylococcus aureus* *Streptococcus pneumoniae* *Escherichia coli*	17% 14% 11% 10% 8%
Blomberg, et al. 2007[167]	2001-2, Africa	Tanzania	N = 155	*Salmonella species* *Escherichia coli* *Enterococci species* *Klebsiella species* *Candida species*	17% 16% 16% 12% 10%
Hamer, et al. 2015[48]	Asia	Bangladesh, India, Pakistan	N = 55	*Staphylococcus aureus* *Acinetobacter species* *Escherichia coli* *Enterobacter species*	31% 16% 11% 9%
Hamer, et al. 2015[48]	Africa	Ghana, South Africa	N = 27	*Staphylococcus aureus* *Klebsiella species* Other	70% 15% 15%

1.2.6 Susceptibility of bacterial pathogens to antimicrobials

Antibiotics recommended in empiric treatment of EOS and CAS include intravenous ampicillin or penicillin combined with an aminoglycoside targeted at covering both GPB and GNB. Cloxacillin is an alternative if *Staphylococcal* infection is suspected. The effectiveness of this treatment depends on whether or not the pathogens causing sepsis are susceptible to these antibiotics. Definitive therapy should be chosen based on antibiotic susceptibility. In developed countries the two most common pathogens, GBS and *E. coli*, are sensitive to antibiotics recommended by WHO for empiric treatment of neonates with EOS.[38] GBS is reported to be 100% sensitive to ampicillin or penicillin and although *E. coli* is 78% resistant to ampicillin, only 4% of strains are resistant to gentamicin.[38] Studies reporting on antibiotic susceptibilities in facilities from developing countries often do not differentiate between pathogens isolated from neonates with EOS and those with healthcare associated infections.[39, 40, 45, 108, 168-170]

A number of studies have reported on antibiotic susceptibilities of common pathogens causing CAS. Darmstadt et al reported that among the 10 isolates of *Staphylococcus aureus* cultured from neonates with CAS in Bangladesh, none were sensitive to ampicillin, 90% were sensitive to gentamicin and seven of nine (78%) were sensitive to cloxacillin.[111] A multicenter observational study from six developing countries (Bangladesh, Bolivia, Ghana, India, Pakistan and South Africa) reported that 83% and 29% of *E coli* were resistant to ampicillin/ amoxicillin and gentamicin, respectively.[48] All *Klebsiella species* were resistant to ampicillin and only 38% were sensitive to gentamicin.

A systematic review and meta-analysis of studies reporting on susceptibilities of different pathogens to WHO currently recommended antibiotics of penicillin/ampicillin and gentamicin for CAS, found that only 14% (95% CI 8-20%) and 80% (95% CI 71-89%) of *Staphylococcus aureus*; 3% (95% CI 1-5%) and 28% (95% CI 17-38%) of *Klebsiella species*; and 22% (95% CI 9-35%) and 52% (95% CI 29-76%) of *E. coli* were sensitive to ampicillin and gentamicin, respectively.[163] This meta-analysis showed a wide variation in susceptibilities of the common pathogens from different studies, and this is supported by findings from another systematic review, which reported variation in percentage sensitivity patterns of most prevalent pathogens causing CAS, where susceptibility of *E. coli* to

ampicillin varied from 29% to 100% and susceptibility of *Klebsiella species* to gentamicin varied from 43% to 100%.[46]

1.2.7 Case fatality rate

Globally, 6.3 million children under the age of 5 years died in 2013, and 52% of these deaths globally and 61% in sub-Saharan Africa were due to infectious causes.[1] Most of deaths under the age of 5 years occur in developing countries. In developing countries, neonatal deaths account for 34-56% of deaths in children under the age of 5 years.[1] In developed countries 15% of neonatal deaths are due to infections, whereas in developing countries 32-34% of deaths are due to infections.[2, 171]

Two-thirds to three-quarters of neonatal deaths occur within the first week of life.[2, 172] It is estimated that as many as 42% of neonatal deaths in the first week of life are due to infectious causes.[173] Thus EOS is the major contributor to mortality. Studies reporting on case fatality rates (CFR) in neonates with culture-confirmed EOS (defined as sepsis occurring <3 days) have reported rates of 3-16% in developed countries,[38, 105, 151, 174] compared to rates of 6-28% in developing countries.[42, 43, 152, 153] Many other studies from developing countries have reported on sepsis CFR occurring in neonates <7 days age, to be between 47-65%.[164, 175] These high rates could be due to inclusion of neonates who might have had healthcare-associated sepsis.

Many studies that have looked at CFR in CAS in developing countries have included neonates and infants between 0 and 91 days of age and reported CFR of 26-70%.[110, 176-179] Some studies have reported on neonates and infants between 0-59 days, stratifying them into early onset (0-6 days of age) and late-onset (7-59 days age) and reported higher CFR among the EOS (27-56%) than late-onset (5-26%) cases.[44, 112] Factors like prematurity, low birth weight, very low birth weight and type of organism have been associated with increased risk for mortality in neonates with sepsis in both developed and developing countries.[35, 38, 40, 42, 137, 151, 174, 180] Case fatality rates in preterm infants have been reported to be 21.5%-24.4% compared to 1.6-1.7% in term infants in the USA.[151] A study from South Africa reported that preterm delivery was associated with strongest risk of death in neonates with EOS with adjusted relative risk ratio (aRRR) of 5.9 (95% CI: 3.1-11.2).[42] Also both low birth weight and very low birth weight are associated with a high mortality in neonates with EOS.[35, 42, 181]

Mortality has also been reported to vary according to the type of organism causing the infection. GNB are associated with high CFR (41%) compared to GPB (26%), for example the CFR for *E. coli* (33%) is higher than that for GBS (9%) in the USA.[35, 38] However, Tiskumara et al from Asia reported the opposite with GBS having a higher CFR (22%) than GNB (12%).[153] This difference could be due to differences in virulence of pathogens in the different regions or differences in the groups of patients who are infected by these pathogens, the time of presentation and the presenting clinical syndromes.

1.3 Human immunodeficiency virus exposure as a risk factor for neonatal sepsis

Intact innate and adaptive immunity are important in the neonate's defense against infections, therefore, any factors that affect the qualitative and/or quantitative function of the immune system could increase the risk for sepsis. In sub-Saharan countries, including South Africa, the high incidence of sepsis and its associated mortality in neonates and infants occurs on the background of a high prevalence of maternal HIV infection. Both HIV-infection and HIV-exposure may contribute to this high incidence of neonatal infections. The heightened susceptibility of HIV-infected children to infections is well documented and relates to immunosuppression.[182, 183] Some studies have reported that children who are HIV exposed but HIV-uninfected (HEU) are also at increased risk of infectious morbidity and mortality compared to HIV unexposed infants.[184, 185] As many as 60% of infants born to mothers with HIV infection experience infectious disease morbidity during their first 6 months of life.[186] HIV exposed but uninfected children with pneumonia have been reported to have more treatment failures than unexposed children.[187] In addition, among children hospitalized with pneumonia, those who were HIV infected or HEU were more likely to have a worse outcome than HIV unexposed children.[188] It would appear that the high infectious morbidity seen in infants born to mothers who are HIV infected starts early in the neonatal period. Neonates, especially very low birth weight infants born to mothers who are HIV positive might have a higher incidence of sepsis, possibly due to exposure to the vaginal flora of the HIV-infected mother, which might be different, both in terms of density and type of organism to that of HIV-non-infected mothers.[189, 190] In addition newborns of HIV-infected mothers could have increased susceptibility to bacterial infection because of lower transplacental acquired antibody, including due to lower maternal antibody levels and impaired transplacental

transfer.[191-194] The transfer of transplacental antibodies, which primarily occurs after 34 weeks of gestational age, may be further reduced in newborns that are very low birth weight or born preterm. Therefore maternal HIV-infection may contribute to the high rate of neonatal sepsis in high-HIV burdened countries. A study from Latin America and the Caribbean reported a high incidence of neonatal infections (26/1000 live births) among infants born to mothers who were HIV infected, and that those with one or more episodes of infections were more likely to be born to mothers with advanced HIV disease.[186] Cutland et al, reported that neonates born to severely immunocompromised mothers (CD4+ count <200 cells/mm^3) were at higher risk of developing clinically diagnosed EOS with an incidence of 79/1000 live births compared to 19/1000 live births in those born to mothers with CD4+ count >350 cells/mm^3.[195] This study also reported that the proportion of infants colonized with *E. coli* tended to be higher among HIV exposed than HIV unexposed infants (60% vs 53%, p = 0.066) and that the incidence of clinical EOS was greater among HIV-exposed at 33.7/1000 live births compared to 20.6/1000 live births in HIV unexposed infants. A study from Belgium also reported that 1.55% of HIV exposed infants developed invasive GBS disease compared to 0.08% in HIV unexposed infants.[196] In a setting with high prevalence of HIV infection similar findings were noted, where the incidence of invasive GBS disease was reported to be 4.46 /1000 live births in HIV exposed infants compared to 1.98/1000 live births in HIV unexposed infants.[3] This difference in incidence of invasive GBS disease between HIV exposed and unexposed infants was noted for both early-onset disease (2.10 vs 1.24/ 1000 live births, risk ratio of 1.69) and late-onset disease (2.36 vs 0.74/ 1000 live births, risk ratio of 3.18).[3] Neonates who are HIV-infected have also been reported to have diseases such as tuberculosis (TB), syphilis and cytomegalovirus, most likely due to immunosuppression.[197]

While the reasons for the high incidence of infections in HIV-infected infants are well understood to be due to immune-suppression, the reasons for differences in infectious morbidity between the HEU and HIV unexposed (HU) neonates are less clear. This could be due to an increase in exposure to infective agents from the infected mother or due to disturbances in the infant's innate immunity. Mothers who are HIV infected are likely to be immunosuppressed, which could be associated with increased shedding of opportunistic agents such as cytomegalovirus, and reactivation of dormant infections such as TB and herpes infections, thereby increasing the risk of neonates being infected by these

organisms.[198, 199] Immune suppression may also result in high rates of bacterial vaginal colonization.[200-202] These suggestions are supported by studies, which have reported that HEU infants born to mothers with advanced HIV disease or with low CD4+ counts are more likely to have neonatal or post-neonatal infections and associated increased mortality.[9, 203] Mothers with advanced disease might have lower antibody titers to common pathogens and might also have impaired transplacental transfer of antibodies to the fetus.[9, 191-193] Recently two studies reported lower levels of GBS antibodies in HIV-infected mothers and lower transplacental transfer or reduced maternal-cord ratio of GBS antibodies in their newborns.[194, 204] Dangor et al. reported that median capsular antibody concentrations were lower in HIV-infected than -uninfected women and that the maternal-cord ratios were lower in HIV-infected mothers[194]. Similar findings were reported by Le Doare et al. who found that both maternal mean concentration of surface-binding GBS antibody and antibody-mediated complement deposition onto GBS were reduced in HIV-infected women compared to HIV-uninfected women; and that median transplacental transfer of antibody from HIV-infected women to their infants was reduced compared to HIV-uninfected women.[204]

A number of studies have shown aberrations in the immune system of HEU infants.[205-208] These abnormalities include changes in the proportion of T-cell populations, cytokine production and dendritic or antigen presenting cells. Among HEU, it is unclear whether the abnormalities are due to fetal exposure to HIV products, antiretroviral drugs or maternal responses to the HIV. The abnormalities in the immune system associated with HIV exposure have included reduced CD4+/ CD8+ lymphocyte ratios due to fewer total and naive CD4+ cell counts.[205, 206] Plasma and mononuclear were also functionally affected, including being unable to produce Th1 phenotype cytokines namely interleukin (IL)-2 and gamma-interferon (IFN-γ). Low Th1 cytokine production results in impaired antigen presentation and increased susceptibility to intracellular pathogens.[209] Another identified abnormality has been an imbalance in cytokine production between pro-inflammatory and anti-inflammatory cytokines. The production of pro-inflammatory cytokines like IFN-γ and TNF-α is significantly higher in HEU than HIV-unexposed infants, whereas the anti-inflammatory cytokines like IL-4 and IL-10 have been noted to be dominant in HIV-unexposed infants.[206] The circulating myeloid dendritic cells have also been found to be lower in HEU infants.[209] Some of the immune abnormalities have been related to maternal viral loads. Interleukin-10 which is an anti-inflammatory cytokine, is higher in HIV-unexposed and HEU infants born to

mothers with lower HIV-1 viral loads or who are on anti-retroviral treatment. The reverse is seen with pro-inflammatory cytokines like IFN-γ and TNF-α, which were significantly higher in a group of HEU infants born to mothers with high HIV-1 viral loads.[206] The abnormalities in the immune system are expected to be associated with increased risk of developing different types of infections. Therefore one would expect that infants who are HIV-exposed even if not infected will have a higher incidence of sepsis than HIV-unexposed infants. Recently guidelines for prevention of mother to child transmission of HIV have been changed from a single drug to highly active antiretroviral therapy, and the effects of this combination on the impaired immunity associated with HIV exposure are not known.

1.4 Vitamin D status as a risk factor for neonatal sepsis

Sepsis is more common in developing countries where problems of malnutrition are also common, than in developed countries. A number of micronutrients like vitamin A and zinc have been shown to play a role in reducing infections or their complications in children.[210-214] The role of micronutrient deficiencies in sepsis during the neonatal period has not been fully established. One of the micronutrient deficiencies reported to be associated with increased risk of developing infections in both neonates and children is vitamin D.[6, 178, 215-217] This is thought to be due to its immunoregulatory effect on both innate and adaptive immune systems. Vitamin D is a pro-hormone which is either ingested or produced in the skin from 7-dehydrocholesterol through absorption of ultraviolet B from the sun to form cholecalciferol (Vitamin D_3).

Cholecalciferol and dietary vitamin D are transported to the liver where they are metabolized to form 25-hydroxyvitamin D [25(OH)D] which is the major circulating vitamin D metabolite and is the best marker of vitamin D nutritional status. 25(OH)D is converted in the kidney to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] which is the active form of vitamin D. $1,25(OH)_2D$ reacts with a single nuclear type 2 receptor to facilitate the activation or suppression of target genes.[218] The proteins produced from activation of target genes carry out the functions of vitamin D. In addition to its well-known functions of increasing absorption of calcium and phosphorus in the intestines and regulating parathyroid hormone (PTH) production, vitamin D also plays a role in the functioning of the immune system.[219]

1.4.1 Serum 25-hydroxyvitamin D Levels

Serum 25(OH)D levels provide an excellent measure of overall vitamin D status. They reflect both intake from the diet and the amount contributed by skin synthesis. Adequate circulating 25(OH)D levels have been defined as levels where PTH, calcium absorption and bone mineral density (BMD) are maintained within normal limits.[220, 221] There is an inverse relationship between circulating 25(OH)D and PTH, whereas it has direct relationship with calcium absorption and therefore levels of serum calcium.[221-223] Thus one definition of 25(OH)D deficiency includes the level at which PTH start to increase or where there is a decrease in intestinal calcium absorption.[224, 225]

Currently there is no agreement as to what cut-off level of serum 25(OH)D should be used to define vitamin D deficiency. The lack of agreement on the definition of 25(OH)D deficiency and its optimal levels has resulted in a range of terminology and values to describe vitamin D status, namely deficiency, insufficiency and adequacy or optimal. A number of systematic reviews of the literature looking at 25(OH)D levels have come up with different cut-off levels to define deficiency, insufficiency and optimal concentrations.[226-228] The Institute of Medicine in the United States has defined deficiency as serum 25(OH)D less than 30 nmol/L, insufficiency as levels of 30-50 nmol/L and sufficiency or adequacy as levels of >50 nmol/L.[226] The WHO has defined insufficiency as a serum 25(OH)D 25-50 nmol/L and deficiency as levels below 25 nmol/L.[227] The Endocrine Society Task Force has defined deficiency as a serum 25(OH)D <50 nmol/L with optimal concentrations being greater than 75 nmol/L.[228] The International Osteoporosis Foundation has recommended using levels >75 nmol/L as desirable, levels 50-74 nmol/L as inadequate, 25-49 nmol/L as insufficient and <25 nmol/L as deficient.[229, 230] The reasons for these different cutoff levels are most likely related to studies included in the different systematic reviews and interpretation of results from the different studies.[231] Individual studies will report different levels to define deficiency or adequacy possibly due to vitamin D assay methodology used, population studied, geographic latitude, season in which blood samples were taken, age range of study participants, ethnicity and sex.[230]

25(OH)D levels are strongly associated with the degree of skin pigmentation and season of the year.[232-235] Individuals with lighter pigmentation exposed to similar ultra-violet B (UVB) doses produce higher serum 25(OH)D concentrations compared with individuals with darker

pigmentation.[234] This difference is due to the effect of melanin pigment absorbing the UVB photons responsible for the photolysis of 7-dehydrocholesterol. Another strong determinant of serum 25(OH)D levels is the season of the year. Serum 25(OH)D levels, are higher during summer than winter months.[232, 233, 235] However this response is also influenced by genetic factors, duration and dose of UVB exposure and baseline serum 25(OH)D levels.[236, 237] Therefore it is important to report levels of 25(OH)D for different communities based on skin pigmentation and seasons of the year.

1.4.2 Serum vitamin D levels in pregnant women and in cord blood

During pregnancy a mother provides large amounts of calcium to the developing foetus with more than 150 mg/kg/day actively transferred across the placenta from the mother to the foetus.[238, 239] Therefore pregnancy is a time when there is an increased calcium requirement and the need for higher concentrations of the active metabolite, 1, 25$(OH)_2$D are required to increase calcium absorption by the intestine to meet the increased demands. With this increased need for vitamin D, it is important to assess the levels of 25(OH)D during the pregnancy, especially in those women who are at risk of vitamin D deficiency, such as those with dark pigmented skin, living at high latitude, or with social and religious customs which reduce skin exposure to UVB.

A number of studies have reported on the prevalence of vitamin D deficiency in pregnant women using different serum levels of 25(OH)D for the definition of deficiency. Studies assessing the prevalence of vitamin D deficiency defined as serum levels <25 nmol/L in white near-term or term pregnant women or soon after delivery reported a prevalence of 5.9% – 19.5%.[240-246] The prevalence of vitamin D deficiency in a similar group of women was 5 – 50% using a cut-off level of 25 – 50 nmol/L to define vitamin D deficiency.[246-249] Studies in pregnant women of non-Western descent reported a higher percentage of women with vitamin D deficiency using a similar definition as those studies reporting from women of western origin. Using a cut-off level of 25 nmol/L, a study from the Netherlands reported a prevalence of 81% and 84% in Moroccan and Turkish pregnant women respectively,[243] a study from Iran reported a prevalence of 46% and 89% in pregnant women in summer and winter respectively,[250] while another study from the same country that defined deficiency as levels <35 nmol/L reported a prevalence of 66.8% .[251] Sachan et al. from North India reported a prevalence of 42.5% and 66.7% using levels of 25 and 37.5 nmol/L respectively for

definition of vitamin D deficiency.[252] In summary there is a high prevalence of vitamin D deficiency during pregnancy especially in black women and in those who wear long dresses and veils. The prevalence varies depending on cut-off level of 25(OH)D used to define vitamin D deficiency.

25(OH)D readily crosses the human placenta, thus the vitamin D status in the foetus and newborn depends on maternal 25(OH)D. The half-life of serum 25(OH)D is two to three weeks, therefore cord serum levels reflect the maternal-foetal status during the final months of pregnancy and first months of life post-delivery.[253] There is a linear positive association between the cord 25(OH)D and maternal 25(OH)D,[252, 254-256] with a correlation of approximately 0.8 (218).[252] Therefore the high prevalence of vitamin D deficiency seen in pregnant women at delivery has a major influence on the umbilical cord blood 25(OH)D levels. Using a serum level of 25(OH)D of 25-37.5 nmol/L to define vitamin D deficiency, the prevalence of vitamin D deficiency was 4-11% in cord blood of white newborns [254, 257-259] compared to 45.6% to 65.5% in black patients in the USA.[258, 259] The effect of the poor vitamin D status in newborns has not been well studied. In children vitamin D deficiency has been associated with increased risk for different types of infection including upper respiratory tract infections, pneumonia and bronchiolitis.[178, 215-217] and recently a number of studies have reported an association with EOS.[6, 260] Vitamin D status in pregnant women and newborns in South African is not well known. Only one report, presented in abstract form has reported on vitamin D status in this population and found that 3.4% and 4.6% of pregnant women and their newborns respectively were vitamin D deficient [25(OH)D <30 nmol/L].[261] As far as I know, no studies from South Africa or sub-Saharan Africa have evaluated association of vitamin D status with EOS in neonates.

1.4.3 Vitamin D and Immune System

A basic understanding of the components of the immune system is important to contextualizing the role of vitamin D in immunity. The immune system is divided into innate and acquired or adaptive immune systems. The innate immune system is important in protecting the neonate against infection as it is present at birth. The main components of the innate immune system are physical epithelial barriers, macrophages, monocytes, neutrophils, dendritic cells, natural killer cells and circulating plasma proteins. The system is non-specific and reacts the same way to the same organism at each encounter. The acquired immune

system is activated once the pathogen has overcome or evaded the innate immune system. It is not fully functional at the time of birth. It has two main components, humoral immunity which mediates its function by antibodies produced by B lymphocytes, and cell mediated immunity, which mediates its function through T lymphocytes, of which there are a number of different types (such as suppressor T cells, helper T cells, and killer T cells). There are two types of T helper (Th) cells, Th1 cells which potentiate cellular immunity and Th2 cells, which potentiate the humoral immunity.

The first barrier to infection is the mechanical barrier of the skin and other epithelial cells. In epithelial cells antimicrobial peptides are produced constitutively but can also be induced by infection or inflammation. These antimicrobial peptides are cathelicidin and defensins. They have a broad spectrum activity against gram positive and negative bacteria, viruses, fungi and protozoa.[262, 263] They protect against infection through damaging lipoprotein membranes, increasing chemotactic activity of macrophages and monocytes and increasing release of cytokines.[264-266] Cathelicidin stimulates chemotaxis of neutrophils, monocytes and T cells, neutralizes the effects of lipopolysaccharide and stimulates angiogenesis.[267-270] The innate immune system distinguishes pathogens by identifying pathogen molecular associated patterns with pattern recognition receptors. One of the groups of these pattern recognition receptors is called the Toll like-receptors (TLR), which are found on cell membranes of many cells of the innate immune system.[271] TLRs enable the host to sense the microbial components within minutes, allowing invading foreign micro-organisms such as bacteria, viruses and fungi to be dealt with quickly and efficiently.[272, 273] For example TLRs bind peptidoglycans from gram positive bacteria such as *Streptococci*[274] and *Staphylococci* [275] and TLR4 is activated by lipopolysaccharides found in gram negative bacteria like *Salmonella* and *E. coli.* [276] Activation of TLRs results in release of pro-inflammatory mediators. Monocytes and macrophages constitutively express high levels of TLR2 and TLR4 and thus are in the forefront of defence against pathogens.[277] Once the pathogen is identified by these receptors, phagocytosis and opsonisation are induced and the pathogen gets presented to the cells of the acquired immune system.

The biological action of vitamin D is achieved by $1,25(OH)_2D$ binding to the vitamin D receptors (VDR) found in the nucleus of various cells of the body including monocytes, macrophages, dendritic cells, natural killer cells, B and T lymphocytes.[278, 279] Activated VDR

binds to vitamin D response elements in genes resulting in the formation of different proteins.[280] Some of the proteins encoded by the genes activated by $1,25(OH)_2D$ are proteins required for formation of tight junctions, gap junctions and adherens junctions.[281-283] These junctions are important in maintaining the integrity of epithelial cells which are barriers against infection. Vitamin D also induces genetic expression of the antimicrobial peptides (AMPs) cathelicidins and defensins in epithelial cells, macrophages, monocytes and neutrophils.[284-286] The AMPs have a broad spectrum of antimicrobial and antiviral activities.[262, 287] Cathelicidin releases a molecule LL37 which attaches to phospholipid head groups of capsular polysaccharides on membrane surfaces of pathogens leading to disruption of the membrane.[288, 289] LL37 also stimulates the production of chemokines and cytokines. $1,25(OH)_2D$ also regulates the expression of TLR2 and TLR4 and its co-receptor CD14.[290] Treatment of human monocytes with $1,25(OH)_2D$ has a dose dependent effect on the expression of TLR2 and TLR4, with higher doses inhibiting their expression and therefore inducing a state of hyporesponsiveness to pathogen associated molecular patterns (PAMPs), while low doses increase the expression of both TLR2 and TLR4.[291, 292] This negative feedback is thought to prevent excessive TLR activation and therefore limit inflammation and the severity of sepsis at a later stage of the infection. The attachment of PAMPs to TLR also results in the induction of the 1α-hydroxylase enzyme which catalyses the production of $1,25(OH)_2D$ and potentiates the effect of activation of TLRs. Vitamin D also modulates the adaptive immune system via its effects on T-cell activation. It is associated with a dose dependent reduction in the transcription of Th-1 cytokines such as IL-2, GM-CSF and interferon gamma as well as the expression of the Th-2 cytokines, IL-4, IL-5 and IL-10.[293]

An essential element of the innate immune response is its capacity to recognize microbial invasion and stimulate production of antimicrobial peptides. Innate immunity is mediated by the Toll family of pattern-recognition receptors, whose activation induces expression of anti-microbial peptides. [294] Activation of Toll-like receptors (TLRs) results in a direct antimicrobial response in monocytes and macrophages *in vitro*. Activation of monocytes with a TLR ligand triggers a vitamin D-dependent pathway leading to the induction of the antimicrobial peptide cathelicidin.[267, 295] TLR stimulation of human macrophages up-regulates expression of the vitamin D receptor and induces the enzyme CYP27B1 which catalyses the conversion of 25(OH)D to $1,25(OH)_2D$. In the presence of adequate 25(OH)D, activation of the upregulated vitamin D receptors leads to induction of cathelicidin. Therefore

the TLR antimicrobial pathway is dependent on the presence of 25(OH)D which is converted in monocytes and macrophages to $1,25(OH)_2D$. These immunomodulatory properties of vitamin D may result in a reduction in incidence of infections in infants with adequate vitamin D status compared to those with vitamin D deficiency. Neonates with low 25(OH)D levels at birth may thus be at risk of developing early neonatal sepsis.

1.4.4 Vitamin D and Infections

A number of studies have reported on association between vitamin D status and the incidence or severity of infections in different age groups. In-vitro, TLR activation of human macrophages up-regulates expression of the VDR and 1α-hydroxylase genes leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular *Mycobacterium tuberculosis*. Furthermore, sera from individuals known to have increased susceptibility to TB had low 25(OH)D and were inefficient in supporting cathelicidin messenger RNA induction.[267] It is also reported that treatment of epithelial cells with $1,25(OH)_2D$ induced antimicrobial activity against the airway pathogens *Bordetella bronchiseptica* and *Pseudomonas aeruginosa* in normal human bronchial epithelial cells.[286] An inverse association between 25(OH)D levels and prevalence of both bacterial and viral infections has also been reported .[178, 215, 216, 296-302] Children with nutritional rickets have a higher incidence of lower respiratory tract infections (LRTI) compared to patients without clinical rickets,[215] and in an Ethiopian study, children presenting with pneumonia have been reported to have a 13-fold higher incidence of rickets compared to those without pneumonia.[178]

In children aged 2-60 months the odds of having severe acute respiratory infections were significantly lower if children had levels of 25(OH)D levels >22.5 nmol/L (OR- 0.09, 95% CI 0.03-0.24, $p<0.001$).[216] Among children with active TB, 86% were either vitamin D deficient (serum 25(OH)D <20 nmol/L) or insufficient (serum 25(OH)D <75 nmol/L).[296] A prospective study of 50 patients presenting with culture positive tuberculosis (TB) found that they had lower serum 25(OH)D concentrations than healthy matched controls.[297] Serum 25(OH)D levels have also been reported to be inversely associated with a recent episode of upper respiratory infection.[300] It is postulated that the high incidence of viral infections during the winter months is related to 25(OH)D levels as these are frequently low during the winter months.[301] This hypothesis is supported by a study by Urashima et al who reported that school children supplemented with 1200 IU of vitamin D_3, had fewer episodes of infection

due to Influenza A compared to those who received a placebo with relative risk (RR) -0.58, 95% CI 0.34-0.99).[302]

The association between vitamin D status and infection has also been reported in neonates.[6, 217, 260, 303] In a study from Turkey, newborns with acute respiratory tract infections who were admitted to neonatal intensive care unit, had lower serum 25(OH)D concentrations than healthy newborns of similar gestation, weight and gender (9.12 ± 8.8 ng/ml vs 16.3 ± 13.4 ng/ml, p = 0.011).[217] Similar findings were noted in their mothers, with mothers of neonates with acute respiratory tract infections having 25(OH)D levels lower than mothers of controls. Neonates with congenital pneumonia have also been shown to have lower serum 25(OH)D compared to controls.[303] Two studies have reported an association between serum 25(OH)D levels and risk of EOS.[6, 260] Cord blood 25(OH)D levels of neonates with EOS were reported to be significantly lower than those of healthy controls with median and ranges of 12.6 (3.1-78.9) ng/ml compared to 21 (5-118) ng/ml, p=0.038.[260] Both maternal and neonatal 25(OH)D levels in those with EOS have been reported to be lower at 22.2 and 8.6 ng/ml compared to 36.2 and 19 ng/ml respectively in controls.[6] One study did not find an association between neonatal 25(OH)D levels and EOS.[304] The levels of 25(OH)D in cord blood or neonates is not only associated with infection during neonatal period but also during childhood.[305-307] Concentrations of cord 25(OH)D were reported to be lower in neonates who developed Respiratory syncytial virus (RSV) LRTI within the first year of life compared to those who did not (65 vs 84 nmol/L, p=0.009); and neonates who had cord 25(OH)D concentrations <50 nmol/L had a six-fold increase in risk of RSV infection in the first year of life compared with those with 25(OH)D ≥75 nmol/L.[305] Mohamed et al.[307] also reported similar findings in the first two years of life when low cord blood 25(OH)D levels were associated with increased risk of acute LRTI. Camargo et al.[306] reported that cord blood level of 25 (OH)D had an inverse association with risk of respiratory infection at 3 months and risk of wheezing at 15 months, 3 years and 5 years of age but no association with incidence of asthma.

A number of clinical studies have also reported an association between vitamin D status and severity or prevalence of infection in both children and adults. In adults admitted to the intensive care unit, the prevalence of vitamin D insufficiency defined as 25(OH)D <30 ng/ml was 100% in critically ill patients with sepsis compared to 92% in critically ill patients

without sepsis and 66.5% in healthy controls.[308] In critical ill children lower levels of 25(OH)D were associated with septic shock.[309] McNally et al.[310] reported that though they found no significant difference in 25(OH)D levels between patients who had acute respiratory tract infections and controls, significantly more children admitted to the paediatric intensive care unit with acute respiratory tract infections were vitamin D deficient. In children with severe pneumonia the presence of rickets was significantly associated with high treatment failure rate.[311]

1.5 Justification for the study

Knowledge of the causative pathogens of sepsis is essential for appropriate and effective treatment of neonatal infections. The marked difference in the types of bacteria causing sepsis in different regions and countries noted in the above-sections suggests a regional or local approach rather than a global one in developing treatment guidelines for neonatal sepsis. One of the major causes of neonatal deaths is sepsis; therefore it is important that in the efforts to reduce these deaths we have a clear understanding of the burden of sepsis in our facilities and regions. In order to accurately identify patients who are infected, reliable diagnostic tests are required. The current available test used as the gold standard in diagnosing sepsis is the blood culture, which however, has low sensitivity and is prone to many limitations. The sensitivity of this test might be further reduced in newborns of mothers who received intrapartum antibiotics. For these reasons, tests other than the blood culture, with improved sensitivity and reasonable specificity, need to be investigated to better delineate the epidemiology of neonatal sepsis. This could include investigating the utility of new molecular based assays, which are less likely to be affected by the use of intrapartum antibiotics and are also able to detect non-culturable pathogens.

There are many factors that may contribute to the high incidence of neonatal sepsis in developing countries, including maternal factors and the quality of health-care at delivery and during the neonatal period. Maternal factors associated with sepsis include maternal illness, nutritional status, and quality of care related to infection control at time of delivery. Both maternal HIV infection and vitamin D deficiency have been associated with increased incidence of sepsis in neonates.[3-6] Some of the possible mechanisms for the association of both conditions with neonatal infection relate to impaired immunity in the fetus. It would

appear that it is not only HIV infection of the fetus that impairs the immunity, but also the exposure of the fetus to the effects of maternal HIV infection[205, 209]. Therefore even if vertical transmission of HIV can be reduced, a significant number of neonates will still be born to mothers who are HIV infected. Maternal vitamin D deficiency and HIV infection are both prevalent in developing countries. Therefore determining the roles of vitamin D status and HIV exposure in neonatal sepsis could assist in developing strategies to reduce neonatal sepsis.

In the United States poorer vitamin D status has been implicated in the disparities in perinatal outcomes between black and white populations.[312] It is possible that poor perinatal outcomes observed in countries in Africa might be related to vitamin D deficiency. Rich sources of dietary vitamin D like fish oil and organ meats are often not affordable to women from poor communities. Therefore in the absence of these dietary sources of vitamin D, sunlight becomes the only source of vitamin D. In blacks skin melanin decreases the penetration of ultraviolet B through the skin therefore limiting the production of vitamin D. Thus the high melanin content of the skin and an inadequate intake of vitamin D could put black women at risk of developing vitamin D deficiency, therefore putting their infants at risk of infection. Pregnant women have been reported to generally have poor vitamin D status around the world.[243, 252, 313-315] The extent and the effect of these low levels on infant infectious morbidities have not been well studied in developing countries including South Africa.

1.6 Aims and Objectives

Aims

i). To determine the epidemiology of early-onset sepsis (EOS) (<3 days of age) and community acquired sepsis (CAS) using standard blood and cerebrospinal fluid culture;

ii). To evaluate the role of a molecular diagnostic assay in improving the sensitivity of methods used to detect sepsis.

iii). To delineate the role of respiratory viruses in early onset and community acquired sepsis.

iv). To assess the role of maternal HIV infection on the incidence of EOS and CAS in neonates born in Soweto, South Africa.

iv). To assess vitamin D status in pregnant mothers and their offspring and its role on EOS in neonates.

Objectives

1. To determine the incidence of early-onset pSBI (EO-pSBI), community acquired pSBI (CA-pSBI), and laboratory confirmed EOS and CAS in neonates, stratified according to HIV exposure status;
2. To determine the aetiology and incidence of culture-confirmed EOS and CAS;
3. To determine antibiotic susceptibility of bacteria isolated from neonates with EOS and CAS;
4. To determine case fatality rates and predictors of mortality in neonates with EOS and CAS;
5. To determine the utility of molecular diagnostic assay using the Taqman array card to identify putative pathogens associated with protocol-defined sepsis among neonates with pSBI
6. To determine sensitivity and specificity of Taqman Array Card in detecting pathogens in neonates;
7. To determine maternal vitamin D status according to HIV status;
8. To determine the relationship between maternal and cord blood 25(OH)D levels ;
9. To determine the seasonal variation in maternal and newborn 25(OH)D levels.
10. To evaluate the association of 25(OH)D levels in maternal and cord blood serum and risk for EOS.

2 METHODS AND MATERIALS

2.1 Epidemiology of Sepsis and Detection of Pathogens Using Blood Culture and the Taqman Array Card

2.1.1 Study Setting

This study was undertaken at Chris Hani Baragwanath Academic Hospital (CHBAH), in Soweto, Johannesburg, South Africa. Though CHBAH is a secondary-tertiary level hospital, about a third of women delivering at this hospital are low risk in terms of adverse pregnancy outcomes. It is also the referral centre for the Soweto Community Health Clinics and provides free maternal and child care to an urban, low-middle income population. Approximately 22,000 of the 32,000 annual births in Soweto occur at CHBAH, with the remaining births conducted by midwives in the public community health centres or clinics. There are very few births conducted outside healthcare facilities in Soweto, thus the majority of births are attended by skilled birth attendants. At the time of the study (12 August 2013 to 30 September 2014), CHBAH was the only public facility that admitted neonates requiring hospital care in the Soweto region.

All pregnant women attending antenatal care are routinely counselled and offered HIV testing. During the study period, pregnant women who were HIV positive were started on full antiretroviral treatment (tenofovir, emtricitabine, lamivudine and efavirenz) either for their own health or for prevention of mother to child transmission of HIV. All neonates were put on nevirapine for a period of six weeks and had HIV PCR done at six weeks. The HIV prevalence has been approximately 29% among pregnant women in Soweto since 2006.[316] Management of women in labour includes recommended administration of intrapartum antibiotics to women with chorioamnionitis, prolonged rupture of membranes (≥18 hours), preterm labour (<34 weeks) and previous birth with GBS infection. There is no systematic screening of pregnant women for recto-vaginal GBS colonization during pregnancy in Soweto, nor any systematic provision of intrapartum antibiotics for this indication to colonized women.

Newborns with respiratory depression or asphyxia are resuscitated according to the South African Paediatric Guidelines that were adapted from the American Academy of Paediatric

Neonatal Resuscitation Program.[317] Infants who need respiratory support are admitted to the neonatal unit for non-invasive assisted ventilation if birth weight is between 799 and 1000 grams; and are eligible for both non-invasive and invasive mechanical ventilation if birth weight is greater than or equal to 1000 grams. All newborns born at or referred to CHBAH with clinical signs suggestive of sepsis have a FBC with a differential count and blood culture done prior to antibiotic administration. Though the local haematology laboratory uses the automated cell counter which generates leukocyte related abnormal cell flags, this is often not followed up routinely with identification of abnormal cell types in neonatal bloods, thus is often reported as left shift being present or not which makes it difficult to calculate immature to total neutrophil ratio. Serum CRP is measured 24-48 hours later in newborns whose clinical signs are considered to be significant and/ or persistent beyond 24 hours. Serum PCT is not part of protocol for workup for sepsis because of its high cost. Lumbar puncture is not done routinely as part of workup for sepsis in neonates with suspected EOS, it is only done if the blood culture is positive for an organism considered a pathogen. Neonates born outside the hospital and who are admitted beyond 24 hours of life are admitted to the general paediatric wards and managed in a similar manner to those born in the hospital, except that the investigation might include CRP on admission and a lumbar puncture if there are signs suggestive of neonatal sepsis. The empiric antibiotic regimen for both EOS and CAS is a combination of ampicillin and gentamicin.

2.1.2 Study Population

All neonates admitted at CHBAH who had blood cultures done at the discretion of the attending physician for early-onset or community acquired pSBI were eligible for enrolment to the study.

***Inclusion criteria for epidemiology of sepsis*:**

Possible serious bacterial infection (pSBI) (early-onset or community-acquired):

- Any neonate aged between 0-27 days of life hospitalized at CHBAH who had blood and/or cerebrospinal fluid taken for culture according to the standard hospital protocol for early-onset (<3 days age) or community acquired (onset of sepsis at 3-27 days of life) pSBI,
- Had not been hospitalized during the preceding 27 days, except for postnatal hospital observation for facility-related births.

Inclusion criteria for detection of pathogens using TAC

Cases

Clinical EOS, was defined by clinical presentation with signs suggestive of sepsis (requiring the presence of respiratory distress or at least two other clinical criteria in the absence of another recognizable congenital infection) between days 0-2 of life, and at least one laboratory criterion [37] (**Table 2.1**).

Culture confirmed EOS, was defined by isolation of a putative pathogen that is not a common contaminant from a normally sterile body site between 0-2 days of life. Bacteria that were considered to be contaminants included coagulase negative *Staphylococcus* (CONS), *Corynebacteria species* and *Bacillus species*. Further, bacteria that are generally normal commensals but which could also be pathogens, namely *Viridans streptococcus,* were classified as "possible pathogens". Other bacteria such as *Group B Streptococcus* (GBS), *Staphylococcus aureus* and *E. coli* were categorized as "definite pathogens".

Clinical CAS was defined as being present if a neonate was hospitalized from home between the ages of 3-27 days with signs suggestive of sepsis, as defined by presence of respiratory distress and one other clinical criterion or at least two other clinical criteria, and at least one laboratory criterion (**Table 2.1**). The neonate also should not have been admitted to hospital within the preceding 27 days, excluding postnatal hospital observation for facility-related births.

Culture confirmed CAS, was defined as being present in a neonate admitted from home and on the isolation of a microorganism (as defined in the section of EOS) that is not a common contaminant from a normally sterile body site between 3-27 days of life. The categorization of identified bacteria for CAS was the same as for EOS detailed above.

Severe infection was defined as disease resulting in death, or the presence of lethargy, or the need for admission to NICU for mechanical ventilation [51, 318].

Controls

Using TAC, increases the likelihood of being able to detect the genome of organisms that may not viable and therefore not a cause of sepsis, making interpretation of results from molecular assays such as TAC difficult. Thus, in order to evaluate the epidemiological association between the identification of putative pathogens using TAC in children and

suspected sepsis, there was a need to estimate the background prevalence of positivity for the epitopes included in TAC among healthy neonates. To address this, we enrolled healthy controls from whom we collected blood and nasopharyngeal/ oropharyngeal (NPOP) swabs for TAC.

The controls for the EOS cases were age-group matched healthy newborns <3 days of age, born at CHBAH. They represented the general population as they were enrolled from maternal wards where well neonates weighing more than 1800 grams at birth were registered for rooming-in with their mothers, to establish breastfeeding before discharge. They were selected from the ward register using computer generated random numbers stratified according to mode of delivery; and had all study procedures (following maternal consent) performed before they were discharged home post-delivery.

Controls for the CAS cases were healthy neonates with ages between 3-27 days, born at CHBAH and subsequently discharged home without having been admitted for any reason, and as such were considered as representative of the general population. They were selected from the birth register using computer generated random numbers. After random selection, parents were phoned and were informed about the study and were asked about the condition of the infant. If the infant was reported to be well and the mother consented, the mother was given a date to bring the neonate to CHBAH. If the parent refused participation at any stage, the next eligible participant on the list was phoned. On arrival the mother was reconsented and the neonate had a clinical assessment, and if the neonate was well the study procedures (blood and NPOP swab taken) were performed.

Exclusion criteria for enrolment into the study evaluating the TAC assay

Case for EOS and CAS: Infants with major congenital abnormalities, whose care was being redirected because of poor prognosis (expected to demise within hours), born to mothers aged less than 18 years and those who had a previous hospital admission other than at the time of delivery were excluded.

Controls for EOS and CAS: Well infants with congenital abnormalities or born to mothers less than 18 years old, or who had a subsequent illness after being discharged following birth were excluded.

Table 2.1: **Clinical signs and laboratory tests used in the definition of clinical sepsis**[37]

Clinical Criteria	**Definition**
Respiratory distress	- Respiratory rate >60 breaths per minute, and/or - Cyanosis, and/or - Chest wall in-drawing, and/ or - Grunting on expiration, and/or - Respiratory distress noted in medical records, and/or - Requirement for ventilation support
Cardiovascular instability	- Hypotension defined as mean arterial pressure < 2 standard deviations from mean for weight/ age; and/or - Mottled skin
Pyrexia or Hypothermia	- Axillary temperature >38.0ºC, not attributable to external warming or - Axillary temperature <36.0ºC
Abdominal/ feeding problems	- Abdominal distension or - feeding intolerance (>20% residual over 24 hours) or - poor feeding after have been feeding well, or - > 2 episodes of emesis
Bleeding diathesis	- Defined as petechiae, ecchymosis, mucous membrane bleeding, pulmonary haemorrhage or excessive oozing from venipuncture sites
Lethargy or irritability	- 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 apnoea
Metabolic abnormalities	- Hyperglycaemia (blood glucose >180mg/dL or 10 mmol/L) or - Hypoglycaemia (blood glucose <45 mg/dL or 2.5 mmol/L) or - Metabolic acidosis: base excess (BE) <-10mEq/L
Laboratory Test	**Definition**
White blood cell count (WCC)	- WCC <5 x 10^9 cells/L or WCC > 25 x 10^9cells/L
Absolute neutrophil count (ANC)	- ANC < 1.75 x 10^9/L or > 15 x 10^9/L
Platelet count	- < 150 x 10^9cells/L
C – reactive protein	- >10 mg/L
Elevate cerebrospinal fluid (CSF) white blood cell count (WCC)	- >30 cells/ mm^3 in absence of significant red blood cells

Footnote: 1. Protocol defined early-onset sepsis – one respiratory clinical criterion or two other clinical criteria with at least one laboratory criterion and/ or positive blood or CSF culture due to an organism (not a common contaminant) from a normally sterile body site (blood or CSF) in a neonate with age between 0-2 days. 2. Protocol defined community-acquired sepsis - presence of respiratory distress (two criteria required) or one feature of respiratory distress and one other clinical criterion or at least two other clinical criteria and at least one laboratory criterion and/ or positive culture due to an organisms that is not a common contaminant from a normally sterile body site in a neonates with age 3-27 days.

2.1.3 Study Procedures

Enrolment

Neonates diagnosed by the attending physician as having pSBI were identified through a daily review of all neonatal medical records and hospital admission registries from Monday through Friday. Clinical data were collected by research nurses from maternal and neonatal medical records using a standardized data entry form. Collected information included maternal and neonatal demographics, sepsis risk factors, clinical presentation, care received, pertinent laboratory findings, and outcome. Results of blood culture, FBC with differential count, CRP and CSF parameters (culture, cell count and chemistry) were obtained from the National Health Laboratory Service (NHLS) electronic laboratory system. Data collection used paper based digitized forms using a digital pen, with information transmitted electronically, and central data storage and management using the Xcallibre system (Xcallibre, Durban, South Africa).

Specimen collection from cases and controls

Clinical specimens for the study included approximately 0.5 -1.0 ml of whole blood and naso- and oro-pharyngeal (NPOP) swabs. Attending physicians were requested, when they took blood for routine tests which included CSF and blood culture, to take an extra 0.5 - 1 ml of blood in a ethylenediaminetetraacetic acid (EDTA) containing sterile tube, and an extra 0.5 - 1 ml of cerebrospinal fluid (CSF) in a plain sterile tube for TAC concurrently. The blood and CSF for TAC were only taken if sufficient volume (0.5 - 1ml) had been obtained for blood and CSF culture. In order to reduce contamination during venipuncture, the study provided gloves, cotton wool and chlorhexidine solution for cleaning of the skin. Extra blood taken for TAC was labeled with a laboratory code similar to that used for blood cultures for each patient and immediately stored in a -2°C to 8^0C fridge until informed consent was obtained. The extra blood and CSF were taken before consenting to avoid a second venipuncture within a short period of time, thus minimizing the risks associated with venipuncture. Collection of the required blood and CSF samples prior to consenting was approved by the Human Research Ethics Committee. After reviewing the records and assessing the condition of the neonate, mothers were approached for consent to allow their infants to be enrolled in the study. NPOP swabs were taken from all neonates who were consented using flocculated swabs. The exceptions were those who were intubated or on

nasal continuous positive airway pressure, who only had an oropharyngeal swab taken. Both swabs from each case were immediately placed in a specimen bottle with Universal Transport Media (UTM) (Copan Diagnostics, Murrieta, CA, USA). Blood from controls was collected by a study doctor. NPOP swabs were collected by a study nurse from both cases and controls.

Blood culture processing

Blood culture bottles (BacT/ALERT® PF bottle) and other specimens for routine tests (FBC, CRP and CSF) were collected from the wards hourly and taken to the CHBAH NHLS laboratory. The microbiology laboratory used an automated continuous monitoring blood culture system (BacT/Alert system, BioMerieux, Marcy l'Etoile, France). If bacterial growth was detected, a Gram stain was performed and the sample sub-cultured onto appropriate media and incubated overnight. Further identification and antimicrobial susceptibility was performed with the automated Vitek system (BioMerieux, Marcy l'Etoile, France) using breakpoints annually published by the Clinical and Laboratory Standards Institute.[319]

Storage of specimens for TAC

The NPOP swabs were collected and placed in 1 mL Universal Transport Media (UTM, Copan Diagnostics, Murrieta, CA, USA). Blood and CSF specimens were collected in standard EDTA blood collection tubes and plain tubes respectively and were placed in a -2 to 8^0C fridge for not more than 72 hours, after which they were placed in a -70^0C freezer. Only samples from cases identified to fulfil the protocol-defined criteria for sepsis had samples tested by TAC, which was undertaken at the National Institute for Communicable Diseases (NICD). Blood, CSF and NPOP swabs in UTM were transported to the NICD laboratory on dry ice and stored at -70°C until processing.

Molecular testing of specimens using TAC

The nucleic acid extraction, procedures and analysis for TAC were performed at NICD as described in previous studies[134, 320]. To determine if the positive amplification of the *E. coli/ Shigella* spp. target in study specimens was due to the presence of residual *E. coli* DNA in the enzyme mastermix, the assay was repeated as an individual single-plex real-time PCR reaction using an ultra-purified DNA enzyme (Phoenix Hot Start Taq DNA Polymerase, Enzymatics, Beverly, MA, USA). A nuclease free water negative control was used as template in reactions using this ultra-purified polymerase. A negative signal in this negative

control indicates no residual *E coli* DNA in the reaction mix. For this study, original specimen or extracted nucleic acid from specimens in which amplification was observed for the *E. coli/Shigella* spp. assay on TAC with a threshold cycle (Ct) value ≥ 30 were shipped to the CDC for further testing. If no extracted nucleic acid was available from the first extraction, the original specimen was re-extracted at CDC using the MagNA Pure Compact instrument (Roche).[134, 320] Each extract was tested in triplicate 25 µl reactions on the Applied Biosystems 7500 real-time PCR instrument (Life Technologies). All specimens in which amplification was observed in at least one reaction, regardless of the Ct value, were assigned a positive final result, whereas specimens exhibiting no amplification in any reactions were considered negative for *E.coli/Shigella* spp.

2.1.4 Sample size considerations

The study was planned to be conducted over a 12 month period, during which we estimated that 22 000 live births would occur at CHBAH and an additional 10 000 in the surrounding midwife obstetric units (MOUs). Based on a previous study[37] conducted on women delivering at CHBAH, we estimated an incidence for protocol defined EOS of 35 per 1000 live births. We therefore anticipated that there would be approximately 800 neonates (cases) eligible for testing using TAC over a 12 month period. Furthermore, we estimated that 500 children would be admitted from home to the general paediatric wards monthly, approximately 10% of whom would be neonates (0-27 days of age) with protocol defined CAS, resulting in approximately 600 neonates with CAS over the study period. Controls were enrolled at a ratio of 1 control per 2-3 cases. Based on the estimate of 800 cases with protocol defined EOS and 600 cases of protocol defined CAS, we planned to enroll 250-400 and 200-300 controls for EOS and CAS cases respectively. Cases and controls were not matched for any characteristics.

2.1.5 Data analysis

Data were entered into Excel 2010 and statistical analyses were carried out using SAS version 9.2 (Carey, NC; USA). Analyses were performed with cases stratified according to HIV exposure. The chi-square test was used to compare proportions with p-values of <0.05 indicating significance. The Student's t-test was used to compare means from data with normal distribution. The Wilcoxon Mann Whitney U test was used to compare medians from

non-parametric data. Odds ratios with 95% confidence intervals were determined to compare cases and controls. A multivariate analysis was performed to determine predictors of culture-proven sepsis, and mortality utilizing those variables which on univariate analysis had a p-value of <0.2.

The incidence of pSBI, protocol defined sepsis, culture- confirmed sepsis and specific pathogens was expressed per 1000 live births for both EOS and CAS. Since most neonates who are admitted to CHBAH are born within Soweto, the number of live births was estimated as those born in CHBAH, and the seven midwifery obstetric units which in Soweto and surrounding areas namely Lenasia South, Lillian Ngoyi, Mofolo, Zola, Chiawelo, Itireleng and Stretford Community Health Centres. In estimating the incidence for the birth cohort, adjustment was made for the days when enrolment did not occur and for non-enrolment due to non-eligibility and refusal of consent. The adjustment factor used was calculated from the following equation:
(Number presenting with pSBI/ Number screened) x (Number eligible for study/ Number enrolled).

Further statistical analysis was performed by a statistician from CDC, and is as detailed below. A non-parametric Bayesian latent class regression approach was developed to estimate the pathogen proportions for each pathogen class. This approach was based on the Partially Latent Class Model (pLCM) developed by Wu.[321] The relationship between observed binary test results and modeled population-level pathogen proportions can be summarized by a basic linear mixture model of pathogen classes. We extended the basic aetiologic model to include date of enrolment, maternal HIV status, and severity of infection as covariates. Parameters in the model were estimated under a Bayesian analysis framework with Gibbs sampling. A non-parametric Bayesian approach was applied to model the covariates. Gibbs sampling generates a chain of samples each of which is correlated with nearby samples. Samples from the beginning of the chain are called the burn-in period as they may not accurately represent the desired distribution. The burn-in period is the set of initial values that start off the estimations. The first few estimations are usually not representative of the distribution that we want to generate. Over time, the estimates get closer to the "best" answer and we used several thousand of the last iterations from the Gibbs sampler to report the mean and confidence intervals of the value that we were trying to estimate.

The complete pathogen class list included the pathogen targets tested by PCR plus an "other/none" class for episodes that could not be attributed to one of the evaluated pathogen classes. Modelled pathogens varied across covariate strata and were selected by a stepwise procedure. Due to low the overall prevalence, pathogens not selected for modelling in any strata were incorporated into the "other/none" class. For pathogens included in some strata but not others, the pathogen proportion in excluded strata was set to zero.

In addition to estimated pathogen proportions, the proportion of cases attributed to pathogens identified by blood culture but not included in our etiologic models were estimated indirectly by calculating the product of the number of blood culture isolates in this "other blood culture" class and the averaged etiologic proportion attributed to modeled pathogens that included blood culture (*Escherichia coli*, Group A *streptococcus,* Group B *streptococcus, Haemophilus influenzae, Klebsiella pneumoniae, Neisseria meningitides, Staphylococcus aureus,* and *Streptococcus pneumoniae*). The final estimate was calculated as the sum of these pathogen-specific etiologic proportions divided by the total blood culture isolates for these pathogens. Estimated incidence was calculated as the product of the pathogen proportion and total number of cases divided by the number of live births in the catchment population during the study period.

The burn-in period for the Gibbs sampler was 50,000 iterations with samples taken from every 50 iterations post burn-in to generate 3,000 samples for the calculation of posterior distributions of model parameters. Posterior means and the corresponding 95% credible intervals were generated from the corresponding samples. Model convergence was assessed through trace and other diagnosis plots. Model assumptions were examined using interim model outputs by stopping the Gibbs Sampler at random cycles. Model fit to input data was evaluated by comparing the fitted and observed number of positives for each PCR test and blood culture. Programming and analysis were conducted using the R platform version 3.2.3.

2.2 Vitamin D Status and Neonatal Sepsis

2.2.1 Study design and setting

We undertook a prospective cohort study to determine the role of vitamin D status in the pathogenesis of neonatal sepsis at CHBAH in Soweto, Johannesburg, South Africa between March 2013 and November 2014. Soweto has latitude of 26°S, an average elevation of 1632m and a temperate climate. The average daily sunshine hours vary between 7.7 and 9.4 hours per month over the year. The setting is as described under section 2.1.1 above.

2.2.2 Study population

All mothers and their infants who were born in the hospital during weekdays between 08h00 and 14h00 were eligible for inclusion in the study, provided they did not have severe congenital abnormalities. Infants were stratified into well and sick neonates based on whether or not they were admitted to a neonatal ward and had a diagnosis of suspected sepsis assigned by the attending physicians.

2.2.3 Study Procedures

The study was conducted over an 18 month period to cover all seasons of the year, enrolling 8-12 neonates a month. Selection of patients was based on a convenience sample, enrolling the first two consenting participants screened between 08h00 and 14h00 on weekdays who had delivered newborns meeting the inclusion criteria. Post-delivery, placentas were kept until the mother was approached for consent and the neonate had been assessed for eligibility. After the neonate had been weighed and assessed by the attending physician, mothers were approached for consent for enrolment into the study following which blood was collected into EDTA tubes from the mother and placental side of the umbilical cord from both healthy newborns and those suspected to have sepsis. Blood samples were promptly refrigerated at 2 to 8^0C and centrifuged at 2000g for 5 minutes within 6 hours of collection. Processed serum was then refrigerated at -70^0C until the assays were performed on all samples at the end of the study. 25(OH)D levels were measured in both maternal and cord blood samples. C-reactive protein and interleukin 6 (IL6) were measured in the cord blood samples only.

Clinical data

The maternal data collected included race, age, gravidity, HIV status, and mode and month of delivery and the neonatal data included birth weight, gestational age, Apgar score, and placement of the infant post-delivery (admitted or not). If the neonate was admitted, medical records were reviewed and data on clinical signs, white cell and platelet counts, blood culture and CRP were collected.

Laboratory assays for 25(OH)D, C-reactive protein, and interleukin 6

These tests were conducted in the research laboratory of the South African Medical Research Council (SAMRC)/ Developmental Pathways for Health Research Unit (DPHRU), University of the Witwatersrand, South Africa. This laboratory participates in the external vitamin D quality assurance system, DEQAS.

25(OH)D assay

25(OH)D levels were measured in both maternal and cord blood samples using the Liaison chemiluminescent immunoassay, (DiaSorin, Saluggia, Italy). The inter-assay CV for lower and higher controls was 10% and 9%, respectively, the intra-assay CV was 8% and 6%, respectively. Vitamin D deficiency was defined as a 25(OH)D concentration of <30 nmol/L.[226] To assess seasonal variation in 25(OH)D concentrations, the seasons were divided as follows: summer (December to February), autumn (March to May), winter (June to August) and spring (September to November).

C-reactive protein and interleukin 6

C-reactive protein and IL-6 assays were performed using an Immulite 1000 automated immunoassay system. The detectable ranges for CRP were 0.3 mg/L for lower level and 100 mg/L for upper level; and for IL-6 the lower detectable level was 2 pg/mL and upper level was 1000 pg/mL. CRP >10 mg/L or IL-6 >70 pg/mL were considered to be suggestive of probable clinical sepsis.[28]

Identification of infants with sepsis

Infants were identified as having suspected sepsis if they had clinical signs suggestive of sepsis as documented by the attending physician, had blood taken for full blood count and culture, and were started on antibiotics. C-reactive protein was done as part of standard

hospital practice in newborns with suspected sepsis only if their clinical signs had not resolved by 12-24 hours as assessed by the attending physicians, or if they had abnormal full blood count. Because of low sensitivity of full blood count, CRP and blood culture in diagnosing sepsis, serum IL-6 at birth was also assessed in all neonates who had clinically suspected neonatal sepsis. Therefore for this study, a laboratory based definition of sepsis was defined as the presence of clinical signs with CRP>10 mg/L and/or IL6>70 pg/mL and/or a positive blood culture due to an organism not considered to be a contaminant. Serum PCT was considered for inclusion as one of the biomarkers for defining sepsis but it was not included because of its high cost.The main focus of the study was a comparison of cord 25(OH)D levels between newborns with and without protocol defined sepsis.

2.2.4 Sample size estimation

Sample size estimation for pregnant mothers: We estimated that we needed to enrol 320 pregnant women, based on an estimated prevalence of vitamin D deficiency (25(OH)D <30 nmol/L) during pregnancy of 3.4% at CHBAH,[261] within 2% confidence limits, that is 2% on either side of 3.4% and a 95% confidence level.

Sample size for infants with early-onset sepsis: Using a significance of 0.05 and a power of 90% and assuming that about 5% of well neonates at birth will have vitamin D deficiency (<30 nmol/L)[261] and that the odds of neonates with clinical EOS having vitamin D deficiency is 1.5 fold greater, and that 27% of neonates with clinical signs suggestive of sepsis will have clinical EOS,[57] 320 neonates with suspected EOS and 320 well neonates needed to be enrolled.

2.2.5 Data analysis

Frequencies and percentages were used to report dichotomous or categorical variables, while means with standard deviations were used to report continuous variables. In order to assess the relationship between 25(OH)D levels and HIV status, mothers were stratified into HIV negative and HIV positive, and newborns into HIV-unexposed and -exposed. Neonates were grouped according to gestational age (<30, 30-34, 35-37 and >37 weeks) and birth weight (1000-1499, 1500-1999, 2000-2499 and ≥2500 grams). Comparisons of 25(OH)D levels between newborns with and without sepsis; and between HIV infected and non-infected mothers were performed using Student's t-test and Mann-Whitney U tests for normally and

not normally distributed data respectively. Differences were considered to be significant if the p-value was <0.05. Correlations between cord blood and maternal 25(OH)D concentrations were assessed using the Pearson test. Multivariate logistic regression analyses were performed to determine factors associated with vitamin D deficiency or sepsis, using variables that had a correlation with a p-value of <0.2 on univariate analysis.

2.2.6 Ethical considerations

Ethical approval to conduct the study was obtained from the University of the Witwatersrand Human Research Ethics Committee (M120651), and permission to conduct the study at CHBAH was obtained from the hospital Chief Executive Officer. Written, informed consent was obtained from the mothers for themselves and their newborns for study inclusion.

3 RESULTS

3.1 Epidemiology of Neonatal Sepsis and HIV Exposure as a Risk Factor

3.1.1 Early-onset Sepsis

Number of neonates screened and enrolled with early-onset sepsis

There were 34 808 live births over the study period of 13 months throughout the study area in the Soweto clinics and CHBAH. A total of 4 045 neonates with ages between 0-2 days were admitted to the neonatal unit for suspected EO-pSBI by the attending physicians. Of these neonates, 3 323 were screened for study eligibility, 3013 (90.7%) were assessed as being eligible. The reasons for non-eligibility (n=310) among the others included mother being <18 years of age and therefore unable to consent independently (n = 147; 47.4%), anticipated immediate demise (n = 76, 24.5%) and major congenital abnormalities (n = 49, 15.8%). Of the 3 013 eligible neonates with EO-pSBI, a further 389 (12.9%) were not enrolled, the commonest reason being a refusal to give consent (n=208/389; 53.5%). Hence a total of 2624 (79.0%) of the original 3323 neonates with EO-pSBI who had been screened for eligibility were finally enrolled in the study (**Figure 3.1**). All patients had blood cultures done but lumbar puncture was not done unless a neonate subsequently had a positive blood culture. This was done as part of standard hospital protocol for working up neonates with EO-pSBI

Maternal and Infant Characteristics

All enrolled (EO-pSBI)

Maternal and neonatal characteristics of enrolled newborns with early-onset pSBI stratified according to maternal HIV-status are shown in (**Table 3.1)**. Most of the women of these pSBI cases had attended antenatal care (94%), the proportion being slightly higher among HIV-infected (97%) than HIV-uninfected women (95%) (p=0.012). Ninety eight percent of the mothers delivered either in hospital or at the clinic, and 55% were delivered by Caesarean section, and the percentage being slightly lower among HIV-infected (52%) than HIV-uninfected women (57%; p=0.019). Seven percent of mothers had prolonged rupture of membranes (PROM), which was also more common among HIV-infected (9%) than HIV-uninfected women (6%; p= 0.002). Nine percent of mothers received intrapartum antibiotics, (12% and 8% in HIV-infected and HIV-uninfected mothers respectively (p=0.004)). The

amniotic fluid was meconium-stained in 20% of women with no differences by maternal HIV status. Infants born to HIV-infected women were more likely to have a lower birth weight and to be born preterm than those born to HIV-uninfected women. Eighteen percent of infants had an Apgar score <7 at 5 minutes with no differences between HIV-exposed and -unexposed newborns. Eighty two percent of neonates with EO-pSBI were enrolled on the day of birth with only 1 percent of neonates being enrolled on day 2 of life.

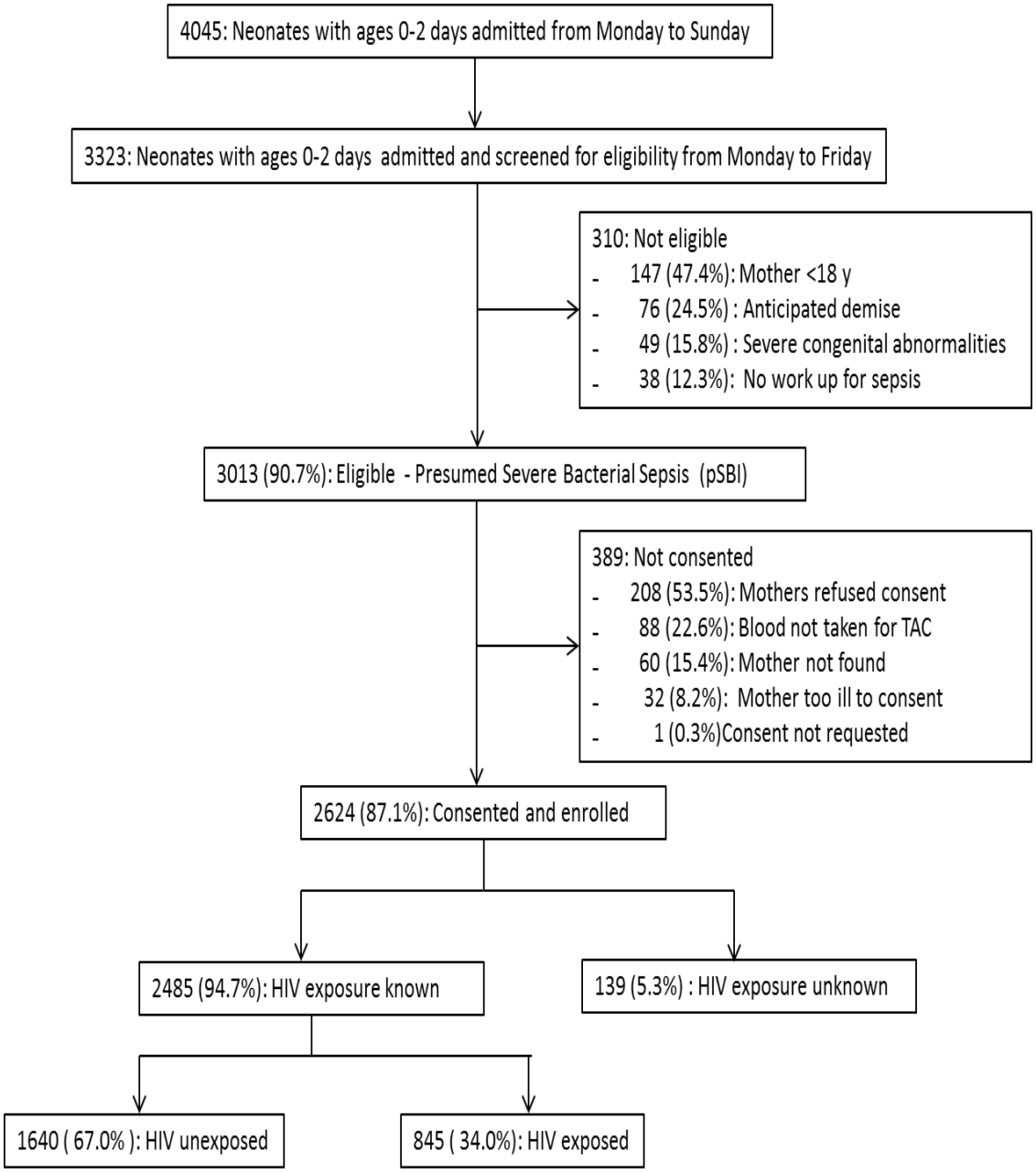

Figure 3.1: Number of neonates who were screened for and enrolled with early-onset presumed serious bacterial infection

Protocol defined EOS

Among the 2624 neonates enrolled with early-onset pSBI, 1231 (46.9%) met the criteria for protocol defined EOS. Their maternal and infant characteristics are listed in **Table 3.2**. Maternal HIV status was unknown for 90 infants (7.3%); and of those with known maternal HIV status, 359 (31.5%) were HIV exposed. Maternal characteristics of the protocol defined

EOS cases were similar to those of the overall group with EO-pSBI, with 93% of mothers having attended antenatal care, 96% born in a healthcare facility, and 47% delivered by Caesarean section. Twenty-three percent of protocol-defined EOS cases were exposed to meconium stained amniotic fluid and 5% were born to mothers with PROM. Only 9% of women who gave birth to neonates with protocol defined EOS received intrapartum antibiotics. Forty eight percent of cases with protocol defined EOS were born preterm and 56% were low birth weight (birth weight <2500 grams). About a fifth of cases with EOS (21%) had an Apgar score <7 at 5 minutes. A higher proportion of HIV-exposed newborns with protocol defined EOS were born to mothers who did not receive antenatal care (6% vs 2%, p=0.001), had PROM (7% vs 4%, p=0.023), or had received intrapartum antibiotics (12% vs 7%, p=0.008) than those who were HIV unexposed. The HIV exposed compared to HIV unexposed protocol defined EOS cases were also more likely to be born preterm (gestation age <37 weeks) (56% vs 41%, p<0.001) and be of low birth weight (<2500 grams; 67% vs 48%, p<0.001) (**Table 3.2)**.

Table 3.1: Maternal and infant characteristics of neonates admitted with early-onset possible serious bacterial infection in Soweto, 2013-14

Maternal characteristics	**All enrolled n = 2624***	**HIV-infected n = 845** n/N (%)	**HIV-uninfected n = 1640** n/N (%)	**HIV-infected vs HIV-uninfected** OR (95% CI)	p-value
Antenatal care	2442/2612 (93.5)	801/839 (95.5)	1592/1636 (97.4)	0.57 (0.36-0.89)	**0.012**
Healthcare facility births	2523/ 2617 (96.4)	819/843 (97.3)	1600/1637 (97.7)	0.82 (0.49-1.39)	0.469
Vaginal delivery	1224/2624 (46.6)	407/845 (48.2)	709/1640 (43.2)	1.22 (1.03-1.44)	**0.019**
Prolonged rupture of membranes	174/2589 (6.7)	77/837 (9.2)	94/1615 (5.8)	1.64 (1.20-2.24)	**0.002**
Meconium stained amniotic fluid	516/2591 (19.9)	169/740 (20.1)	331/1616 (20.5)	0.98 (0.79-1.20)	0.832
Intrapartum antibiotics	243/2592 (9.4)	101/841 (12.0)	136/1614 (8.4)	1.48 (1.13-1.95)	**0.004**
Infant Characteristics	**All enrolled n = 2624**	**HIV-exposed n = 845** n/N (%)	**HIV-unexposed n = 1640** n/N (%)	**HIV-exposed vs HIV-unexposed** OR (95% CI)	p-value
Male	1400/2619 (53.5)	469/845 (55.5)	862/1636 (52.7)	1.12 (0.95-1.32)	0.183
Gestational Age					**<0.001**
< 30 weeks	344 (13.1)	111 (13.2)	177 (10.8)	1.58 (1.21-2.06)	
30-34 weeks	688 (26.2)	271 (32.1)	383 (23.4)	1.78 (1.47-2.16)	
35-37 weeks	185 (7.1)	74 (8.7)	101 (6.2)	1.84 (1.34-2.55)	
>37 weeks	1404 (53.6)	388 (46.0)	977 (59.6)	Ref	
Median Gestational Age (weeks) (25^{th} -75^{th}centile)	40 (32-40)	36 (32-40)	40 (33-40)		**<0.001**
Birth weight					**<0.001**
<1000 grams	172 (6.6)	47 (5.6)	100 (6.1)	1.30 (0.90-1.88)	
1000-1499 grams	381 (14.5)	134 (15.8)	208 (12.7)	1.78 (1.38-2.29)	
1500-1999 grams	489 (18.6)	207 (24.5)	255 (15.6)	2.24 (1.78-2.80)	
2000-2499 grams	378 (14.4)	145 (17.2)	217 (13.2)	1.84 (1.44-2.36)	
≥2500 grams	1204 (45.9)	312 (36.9)	860 (52.4)	Ref	
Median Birth Weight (grams) (25^{th} -75^{th}centile)	2375 (1600-3070)	2115 (1550-2890)	2600 (1680-3185)		**<0.001**
Apgar score at 5 min. <7	480/2624 (18.3)	140/845 (16.6)	282/1640 (17.2)	0.96 (0.77-1.19)	0.693
Age at enrolment (days)					0.277
0	2160 (82.3)	707 (83.7)	1342 (81.8)	1.14 (0.91-1.42)	
1 - 2 days	464 (17.7)	138 (16.3)	298 (18.2)	Ref	
Median age at enrolment (25th -75th centile)	0 (0-0)	0 (0-0)	0 (0-0)		0.569

*In 139 HIV status was not recorded.

Table 3.2: Maternal and infant characteristics of neonates admitted with early-onset defined sepsis

Maternal Characteristics	**All enrolled N = 1231***	**HIV-infected N = 389**	**HIV-uninfected N = 786**	**HIV-infected vs HIV-uninfected**	
		n (%)	n (%)	OR (95% CI)	p-value
Antenatal care visit	1136/1221 (93.0)	359/383 (93.7)	763/782 (97.6)	0.37 (0.20-0.69)	**0.002**
Healthcare facility births	1184/1228 (96.4)	372/387 (96.1)	770/786 (98.0)	0.52 (0.25-1.05)	0.069
Vaginal delivery	558/1231 (45.3)	178/389 (45.8)	337/ 786 (42.9)	1.12 (0.88-1.44)	0.349
Prolonged rupture of membranes	60/1231 (4.9)	28/387 (7.2)	32/779 (4.1)	1.82 (1.08-3.07)	**0.023**
Meconium stained amniotic fluid	277/ 1231 (22.5)	86/388 (22.2)	185/780 (23.7)	0.92 (0.68-1.22)	0.554
Intrapartum antibiotics	103/1120 (8.4)	46/388 (11.9)	56/ 779 (7.2)	1.74 (1.15-2.62)	**0.008**
Infant Factors	**All enrolled N = 1231**	**HIV-exposed N = 389**	**HIV-unexposed N = 786**	**HIV-exposed vs HIV-unexposed**	
		n (%)	n (%)	OR (95% CI)	p-value
Male	675/1231 (45.2)	213/389 (45.2)	429/784 (45.3)	1.00 (0.78-1.28)	0.991
Apgar score <7 at 5 minutes	254/1231 (20.6)	79/389 (20.3)	153/786 (19.5)	1.05 (0.78-1.43)	0.730
Gestational age					**<0.001**
<30 weeks	200 (16.2)	67 (17.3)	109 (13.9)	1.66 (1.17-2.36)	
30 - 34 weeks	307 (25.0)	123 (31.7)	168 (21.4)	1.98 (1.48-2.65)	
35 - 37 weeks	80 (6.5)	28 (7.2)	49 (6.2)	1.55 (0.94-2.54)	
>37 weeks	643 (52.3)	170 (43.8)	460 (58.5)	Ref	
Median Gestational age (weeks) ($25^{th} – 75^{th}$)	40 (31-46)	35 (31-40)	40 (32-40)		**<0.001**
Birth weight					**<0.001**
<1000 grams	115 (9.4)	37 (9.5)	69 (8.8)	1.67 (1.07-2.61)	
1000 - 1499 grams	222 (18.0)	81 (20.8)	120 (15.3)	2.10 (1.49-2.97)	
1500 - 1999 grams	195 (15.8)	85 (21.9)	103 (13.1)	2.57 (1.81-3.64)	
2000 - 2499 grams	155 (12.6)	56 (14.4)	89 (11.3)	1.96 (1.33-2.89)	
≥2500 grams	544 (44.2)	130 (33.4)	405 (51.5)	Ref	
Median Birth weight (grams) ($25^{th} – 75^{th}$)	2294 (1440-3065)	1940 (1375-2780)	2585 (1530-3200)		**<0.001**
Age at onset of sepsis					0.561
Day 0	1035 (84.1)	324 (83.3)	665 (84.6)	0.91 (0.65-1.26)	
Day 1-2	196 (15.7)	65 (16.7)	121 (15.4)	Ref	
Median age (25th-75th centile)	0 (0-0)	0 (0-0)	0 (0-0)		0.569

*In 56 patients HIV status not recorded

Clinical signs

The common clinical signs in neonates with EO-pSBI included chest wall retractions (52%) and tachypnoea (28%) (**Table 3.3**). Twenty four percent of cases required ventilator support, with 80% of these receiving nasal continuous positive airway pressures (nCPAP) and the rest intermittent positive pressure ventilation (IPPV). Other common clinical signs were hypothermia (19%), hypotonia (15%), jaundice (14%), apnoea (13%) and lethargy (11%). Common bedside abnormal laboratory results were metabolic acidosis (29%) and hypoglycaemia (12%). Clinical signs and symptoms were generally similar between HIV-exposed and -unexposed neonates, except for retractions (57% vs. 51%, p=0.007) and the need for assisted ventilation (28% vs. 21%, p<0.001) which were more common among the HIV-exposed neonates.

Clinical signs of those with protocol defined EOS were similar to those with EO-pSBI and the common clinical signs were chest wall retractions or indrawing, and metabolic acidosis found in 60% and 36% of cases, respectively (**Table 3.4**). There was a higher proportion of neonates who had chest retractions (68% vs 56%, p<0.001), needed mechanical ventilation (35% vs 25%, p<0.001), and had metabolic acidosis (40% vs 33%, p=0.011) among the HIV-exposed compared to -unexposed neonates, while seizures/ bulging fontanelle was seen more commonly in HIV-unexposed infants (3% vs 6%, p=0.033) with protocol defined EOS.

Table 3.3: Clinical signs and management of neonates with early-onset possible serious bacterial infection

	All N = 2624*	**HIV exposed N = 845** n/N (%)	**HIV unexposed N = 1640** n/N (%)	**HIV exposed vs unexposed** OR (95% CI)	p-value
General Signs					
Lethargy	310/2606 (11.9)	91/840 (10.8)	192/1629 (11.8)	0.91 (0.70-1.18)	0.481
Irritability	41/2606 (1.6)	12/838 (1.4)	28/1631 (1.7)	0.83 (0.42-1.64)	0.596
Jaundice	356/2563 (13.9)	103/832 (12.4)	234/1598 (14.6)	0.82 (0.64-1.06)	0.125
Fever	22/2624 (0.8)	6/845 (0.7)	14/1640 (0.9)	0.83 (0.32-2.17)	0.704
Hypothermia	509/2624 (19.4)	157/845 (18.6)	319/1640 (19.5)	0.94 (0.76-1.17)	0.601
Gastrointestinal Tract					
Poor feeding	18/977 (1.8)	4/292 (1.4)	13/657 (2.0)	0.69 (0.22-2.13)	0.514
Distension	104/2605 (4.0)	37/ 840 (4.4)	61/1629 (3.7)	1.18 (0.78-1.80	0.426
Central nervous system					
Apnoea	333/2539 (14.1)	104/816 (12.7)	200/1590 (12.6)	1.01 (0.79-1.31)	0.970
Seizures or bulging fontanelle	121/2602 (4.7)	31/836 (3.7)	85/1630 (5.2)	0.70 (0.46-1.07)	0.094
Hypotonia	401/2602 (15.4)	126/839 (15.0)	241/1626 (14.8)	1.01 (0.80-1.30)	0.897
Cardiorespiratory system					
Tachypnoea (RR>60/min)	749/2624 (28.5)	224/845 (26.5)	484/1640 (29.5)	0.86 (0.72-1.04)	0.116
Chest wall indrawing	1379/2597 (53.1)	475/836 (56.8)	829/1624 (51.0)	**1.26 (1.07-1.49)**	**0.007**
Required ventilation	625/2517 (24.8)	227/810 (28.0)	336/1576 (21.3)	**1.44 (1.18-1.75)**	**<0.001**
Non-invasive ventilation (CPAP)	472/583 (81.0)	187/472 (39.6)	285/472 (60.4)		
Invasive ventilation (CMV)	111/583 (19.0)	42/111 (37.8)	69/111 (62.2)		
Hypotension requiring inotropes	65/2529 (2.6)	21/813 (2.6)	37/1586 (2.3)	1.11 (0.64-1.91)	0.706
Metabolic					
Hypoglycaemia	315/2597 (12.1)	89/837 (10.6)	206/1623 (12.7)	0.82 (0.63-1.07)	0.136
Hyperglycaemia	193/2597 (7.4)	57/836 (6.8)	114/1624 (7.0)	0.97 (0.70-1.35)	0.852
Metabolic acidosis	770/2598 (29.6)	257/839 (30.6)	456/1623 (28.1)	1.13 (0.94-1.36)	0.189
Antibiotics use				Not estimable	N/A
Ampicillin	2624/2624 (100)	845/845 (100)	1640/1640 (100)		
Gentamicin	2624/2624 (100)	845/845 (100)	1640/1640 (100)		

*-In 139 neonates HIV exposure status was not recorded

Required ventilation includes nasal continuous positive airway pressure (CPAP) and intermittent positive pressure ventilation (IPPV)

Hypoglycaemia = glucose <2.6 mmol/L, Hyperglycaemia = glucose >10 mmol/L; Metabolic acidosis = base deficit >10 mmol/L

Table 3.4: Clinical signs and management of neonates with protocol-defined early-onset sepsis

	All enrolled N = 1231	**HIV exposed N = 389** n /N (%)	**HIV unexposed N = 786** n/ N (%)	**HIV exposed vs unexposed** OR (95% CI)	p-value
General Signs					
Lethargy	183/1216 (15.0)	57/381 (15.0)	106/774 (13.7)	1.07 (0.76-1.52)	0.686
Irritability	20/1227 (1.6)	5/387 (1.3)	15/776 (1.9)	0.67 (0.24-1.86)	0.444
Jaundice	175/1208 (14.5)	51/378 (13.5)	116/769 (15.1)	0.88 (0.62-1.26)	0.484
Fever	13/1231 (1.1)	4/382 (1.0)	8/778 (1.0)	0.90 (0.27-2.93)	0.857
Hypothermia	267/1160 (23.0)	83/382 (21.7)	184/778 (23.7)	0.91 (0.68-1.22)	0.518
Gastrointestinal Tract					
Poor feeding	7/413 (1.7)	1/114 (0.9)	5/289 (1.7)	0.49 (0.06-4.28)	0.511
Abdominal distension	48/1227 (3.9)	17/381 (4.5)	28/776 (3.6)	1.24 (0.67-2.29)	0.497
Central nervous system					
Apnoea	191/1201 (15.9)	64/375 (17.1)	111/760 (14.6)	1.20 (0.86-1.67)	0.284
Seizures or bulging fontanelle	60/1226 (4.9)	12/380 (3.2)	47/776 (6.1)	0.50 (0.26-0.95	0.033
Hypotonia	234/990 (23.6)	77/380 (20.3)	136/773 (17.6)	1.20 (0.88-1.63)	0.256
Cardiorespiratory system					
Tachypnoea (RR>60/min)	398/1231 (32.3)	108/382 (28.3)	262/778 (33.7)	0.77 (0.59-1.01)	0.064
Chest wall indrawing	734/1222 (60.1)	256/379 (67.5)	433/773 (56.0)	1.63 (1.26-2.10)	**<0.001**
Required ventilation	354/1185 (29.9)	129/367 (35.1)	185/750 (24.7)	1.64 (1.25-2.15)	**<0.001**
Hypotension requiring inotropes	37/1179 (3.1)	9/369 (2.4)	22/750 (2.9)	0.76(0.35-1.66)	0.497
Metabolic					
Hypoglycaemia	163/1224 13.3)	45/380 (11.8)	107/775 (13.8)	0.83 (0.58-1.20)	0.326
Hyperglycaemia	115/1223 (9.4)	36/369 (9.8)	67/774 (8.7)	1.12 (0.74-1.71)	0.574
Metabolic acidosis	441/1223 (36.1)	153/380 (40.3)	254/774 (32.8)	1.38 (1.08-1.78)	**0.011**
Empiric antibiotics					
Ampicillin	1231/1231 (100)	382/382 (100)	778/778 (100)	Not estimatable	N/A
Gentamicin	1231/1231 (100)	382/382 (100)	778/778 (100)	Not estimatable	N/A

*- In 56 neonates, HIV exposure status was not recorded

Assisted ventilation included nasal continuous positive airway pressure (nCPAP) and intermittent positive pressure ventilation (IPPV).

Hypoglycaemia = glucose <2.6 nmol/L, Hyperglycaemia = glucose >10 nmol/L; Metabolic acidosis = base deficit >10 nmol/L

Ancillary Laboratory Findings

Fourteen percent of patients with early-onset pSBI had an abnormal full blood count (FBC) with 9% having leukopenia, 13% neutropenia, and 38% thrombocytopenia. Twenty-four percent also had CRP >10 mg/L, and 16% CRP >20 mg/L (**Table 3.5**). There were more children with leukocytosis (6.4% vs 4.1%; p=0.042) and neutrophilia (7.8% vs 4.2%; p=0.005) among the HIV-unexposed than -exposed cases. Among the protocol-defined EOS cases, 21% had an abnormal FBC, and 38% had CRP >10mg/L and 25% with CRP >20 mg/L (**Table 3.6**). The HIV-unexposed cases were more likely to present with neutrophilia compared to HIV-exposed protocol-defined EOS cases.

Table 3.5: Laboratory findings in neonates with early-onset possible serious bacterial infection

	All enrolled N = 2624* n/N (%)	**HIV-exposed N = 845** n/N (%)	**HIV-unexposed N = 1640** n/N (%)	**HIV-exposed vs HIV-unexposed** p-value
Number with white cell count (cells/10^9)	***N = 2598***	***N = 838***	***N = 1621***	**0.042**
<5 x 10^9/L	225 (8.7)	79 (9.4)	135 (8.3)	
5-25 x 10^9/L	2228 (85.8)	725 (86.5)	1382 (85.3)	
>25 x 10^9/L	145 (5.5)	34 (4.1)	104 (6.4)	
Median white cell count (25^{th} -75^{th} centile)	12.1 (8.41-16.6)	11.3 (8.04-15.2)	12.7 (8.94-17.5)	**<0.001**
Number with absolute neutrophil count	***N = 2306***	***N = 742***	***N = 1437***	**0.005**
<1.75 x 10^9/L	306 (13.3)	102 (13.7)	186 (12.9)	
1.75 - 15.0 x 10^9/L	1851 (80.3)	609 (82.1)	1139 (79.3)	
>15 x 10^9/L	149 (6.4)	31 (4.2)	112 (7.8)	
Median absolute neutrophil count (25^{th} – 75^{th})	6.31 (3.35-9.64)	5.67 (3.15-9.00)	6.89 (3.81-10.2)	**<0.001**
Number with platelet count	***N = 2603***	***N = 837***	***N = 1627***	0.641
<100 x 10^9/L	443 (17.0)	154 (18.4)	278 (17.1)	
100 - 150 x 10^9/L	548 (21.1)	170 (20.3)	349 (21.4)	
>150 x 10^9/L	1612 (61.9)	513 (61.3)	1000 (61.5)	
Median platelet count (25^{th} – 75^{th})	177 (129-227)	177 (126-227)	176 (128-227)	0.842
Number with c-reactive protein	***N = 1622***	***N = 528***	***N = 994***	0.056
<10 mg/L	1227 (75.6)	415 (78.6)	732 (73.6)	
10 - 20 mg/L	154 (9.5)	48 (9.1)	95 (9.6)	
>20 mg/L	241 (14.9)	65 (12.3)	167 (16.8)	
Median c-reactive protein	2 (1-9)	6 (1-6)	3 (1-11)	**<0.001**

*-In 139 neonates, the HIV exposure status was not recorded

Table 3.6: Laboratory findings in neonates with early-onset protocol-defined sepsis

	All enrolled N = 1231*	**HIV-exposed N=389**	**HIV-unexposed N=786**	**HIV-exposed vs HIV-unexposed**
	n%	n (%)	n (%)	p-value
Number with white cell count	***N = 1214***	***N = 383***	***N = 775***	0.106
<5 x 10^9/L	124 (10.2)	41 (10.7)	74 (9.6)	
5-25 x 10^9/L	958 (78.9)	311 (81.2)	607 (78.3)	
>25 x 10^9/L	132 (10.9)	31 (8.1)	94 (12.1)	
Median white cell count (x10^9)	11.7	10.8	12.3	
(25^{th} – 75^{th})	(7.50-17.8)	(7.15-15.9)	(7.94-18.7)	**<0.001**
Number with absolute neutrophil count	***N = 1102***	***N = 349***	***N = 702***	**0.019**
<1.75 x 10^9/L	219 (19.9)	74 (21.3)	130 (18.5)	
1.75 - 15.0 x 10^9/L	755 (68.5)	248 (71.1)	477 (68.0)	
>15 x 10^9/L	128 (11.6)	27 (7.7)	95 (13.6)	
Median absolute neutrophil count (x10^9)	6.15	5.15	6.99	
(25^{th} – 75^{th})	(2.62-10.3)	(2.30-9.21)	(3.06-11.4)	**<0.001**
Number with platelet count	***N = 1220***	***N = 383***	***N = 781***	0.440
<100 x 10^9/L	326 (26.7)	112 (29.2)	204 (26.1)	
100 - 150 x 10^9/L	493 (40.4)	151 (39.4)	319 (40.8)	
>150 x 10^9/L	401 (32.8)	119 (31.3)	258 (33.0)	
Median platelet count (x10^9)	131	130	131	
(25^{th} – 75^{th})	(97-179)	(95-180)	(98-177)	0.603
Number with c-reactive protein	***N = 882***	***N = 271***	***N = 567***	0.247
<10 mg/L	544 (61.7)	177 (65.3)	341 (60.1)	
10 - 20 mg/L	122 (13.8)	37 (13.7)	76 (13.4)	
>20 mg/L	216 (24.5)	57 (21.0)	150 (26.5)	
Median c-reactive protein (25^{th}-75^{th})	5 (1-20)	3.5 (1-18)	5.0 (1-22)	**0.013**

*-In 56 neonates, the HIV exposure was not recorded

Organisms detected on blood culture of neonates with EO-pSBI

Blood cultures were positive in 228 (8.7%) of 2624 EO-pSBI cases, 132 (5.0%) of which grew presumed contaminants, whilst 96 (3.7%) yielded an organism considered to be a putative pathogen. There were no statistical significant differences in blood culture contamination rates (5.1% vs 4.6%, p=0.650) or pathogen-confirmed positivity between HIV-unexposed (3.8%) and HIV-exposed neonates (3.6%; p=0.773) (**Table 3.7**). The cultured organisms considered to be contaminants were *Corynebacterium species* (n=59; 45%), Coagulase negative *staphylococcus* (n=57; 43%), and *Bacillus species* (n=15; 12%). Among the 96 neonates with putative pathogens, eight had more than one pathogen cultured concurrently. Among the 105 putative pathogens cultured from blood in neonates with EO-pSBI, 77 (73.3%) were considered as definite pathogens and 28 (26.7%) as possible pathogens. Overall, 73 (69.5%) of all putative pathogens were Gram positives. The

commonest bacteria among the 77 definite pathogens were GBS (45.4%), *Enterococcus* and *E. coli* both accounting for 13.0% each. Among the organisms that were considered to be possible pathogens, *Viridans streptococcus* was the most common (82.1%). Among the neonates with positive blood cultures only one had a positive CSF culture and the organism was GBS, the same as that grown on blood culture.

Table 3.7: Positive culture results and organisms isolated from neonates with early-onset possible serious bacterial infection

	All enrolled N = 2624*	**HIV-Exposed N = 845**	**HIV-Unexposed N = 1640**	**HIV- exposed vs HIV-unexposed**
	n (%)	n (%)	n (%)	p-value
All patients with positive blood cultures	228* (8.7)	69 (8.2)	146 (8.9)	0.536
Patients with positive blood culture due to contaminants	132* (5.0)	39 (4.6)	84 (5.1)	0.650
Patients with positive blood culture due to pathogens	96* (3.7)	30 (3.6)	63 (3.8)	0.773
Definite Pathogens	77* (2.9)	25 (3.0)	48 (2.9)	0.933
Group B Streptococcus	35* (1.3)	6 (0.7)	25 (1.5)	0.106
Enterococcus sp.	10 (0.4)	3 (0.4)	7 (0.4)	0.829
Escherichia coli	10 (0.4)	3 (0.4)	7 (0.4)	0.828
Acinetobacter species	3 (0.1)	1 (0.1)	2 (0.1)	0.829
Staphylococcus aureus	3 (0.1)	1 (0.1)	2 (0.1)	0.829
Haemophilus influenza	3 (0.1)	3 (0.4)	0	N/A
Candida species	3 (0.1)	1 (0.1)	2 (0.1)	0.829
Enterobacter species	2 (0.1)	0	2 (0.1)	N/A
Sphingomonas paucimobilis	2 (0.1)	1 (0.1)	1 (0.1)	0.615
Citrobacter koseri	1 (0.0)	1 (0.1)	0	N/A
Klebsiella pneumoniae.	1 (0.0)	0	1 (0.1)	N/A
Morganella morganii	1 (0.0)	1 (0.1)	0	N/A
Proteus mirabilis	1 (0.0	1 (0.1)	0	N/A
Pseudomonas species	1 (0.0)	1 (0.1)	0	N/A
Salmonella species	1 (0.0)	1 (0.1)	0	N/A
Possible Pathogens	28 (1.1)	7 (0.8)	21 (1.3)	0.273
Viridans streptococcus	23 (0.9)	6 (0.7)	17 (1.0)	0.612
Neisseria species	2 (0.1)	0	2 (0.1)	N/A
Mucor fungi	1 (0.0)	0	1 (0.1)	N/A
Other streptococcus species (mitis& bovis)	2 (0.1)	1 (0.1)	1 (0.1)	0.829
ALL PATHOGENS	105* (4.0)	31 (3.7)	70 (4.2)	0.542
- Gram positives†	73* (69.5)	17 (54.8)	52 (74.3)	
- Gram negatives†	28 (26.7)	13 (41.9)	15 (21.4)	
- Fungi†	4 (3.8)	1 (3.2)	3 (4.3)	
Contaminants	131* (5.0)	39 (4.6)	83 (5.1)	0.650
Coagulase Negative Staphylococcus	59* (2.2)	18 (2.1)	36 (2.2)	0.883
Corynebacteria species	57* (2.2)	17 (2.0)	36 (2.2)	0.280
Bacillus species	15 (0.6)	4 (0.5)	11 (0.7)	0.741

* -The sum of HIV exposed and unexposed is not adding up to total because not all had HIV exposure recorded

†- percentages calculated out of the total of all pathogens

Footnote*:* Eight patients grew more than one bacteria (polymicrobial), 1 *Citrobacter koseri+Enterococcus*, 1 *Enterobacter cloacae+Enterococcus*, 1 *Escherichia coli+Enterococcus*, 1 *Salmonella sp + Enterococcus*, 1 *Staphylococcus aureus* + Group B *streptococcus*, 1 *Viridans streptococcus + Neisseria sp*. and 2 *Escherichia coli* + Group B *streptococcus*

Factors associated with culture confirmed sepsis in neonates with early-onset pSBI

On univariate analysis of all EO-pSBI cases, factors/ signs associated with culture-confirmed EOS were lethargy (OR: 1.81; 95% CI 1.05 – 3.11), fever (OR: 4.56; 95% CI 1.32 – 15.76), metabolic acidosis (OR: 1.92; 95% CI 1.29 – 3.04), severe infection (OR: 1.65; 95% CI 1.04-2.61) and CRP >10mg/L (OR: 3.96; 95% CI 2.36 – 6.65). On multivariate analysis the predictors of culture confirmed sepsis among neonates with EO-pSBI were vaginal delivery (OR: 2.82; 95% CI 1.55 – 5.13) and CRP >10 mg/L (OR: 3.00; 95% CI 1.70 – 5.30), with 47% of culture-confirmed cases having CRP>10 mg/L (**Table 3.8**). The only organism that was found to be isolated more commonly in vaginal deliveries than caesarean sections was group B *streptococcus* (1.90% vs 0.64%, p = 0.007).

Table 3.8: Predictors of culture-confirmed sepsis in neonates with early-onset possible serious bacterial infection

	Culture-Positive sepsis N = 96	**Culture-Negative sepsis N = 2528**	**Univariate analysis**		**Multivariate analysis**	
	n/N (%)	n/N (%)	**OR (95%CI)**	**p-value**	**aOR (95% CI)**	**p-value**
Characteristics						
HIV Exposure	30/92 (32.6)	815/2393 (34.1)	0.94 (0.60-1.46)	0.773	N/A	N/A
PROM	8/95 (8.4)	166/2499 (6.7)	1.29 (0.62-2.71)	0.500	N/A	N/A
Maternal Antibiotics	12/93 (12.9)	231/2499 (9.2)	1.45 (0.82-2.86)	0.235	N/A	N/A
Meconium stained liqour	25/95 (26.3)	491/2496 (19.7)	1.45 (0.91-2.33)	0.112	1.49 (0.79-2.83)	0.220
Vaginal delivery	64/96 (66.7)	1160/2528 (45.9)	**2.34 (1.53-3.63)**	**<0.001**	**2.82 (1.55-5.13)**	**<0.001**
Low birth weight	44/96 (45.8)	1376/2528 (54.4)	0.71 (0.47-1.07)	0.097	1.01 (0.56-1.84)	0.963
Male sex	52/96 (54.2)	1348/2523 (53.4)	1.03 (0.68-1.55)	0.887	N/A	N/A
Apgar at 5 min <7	18/96 (18.8)	462/2528 (18.3)	1.03 (0.61-1.74)	0.906	N/A	N/A
General Signs						
Lethargy	19/94 (20.2)	291/2512 (11.6)	**1.93 (1.15-3.24)**	**0.011**	1.47 (0.56-1.84)	0.491
Irritability	1/94 (1.1)	40/2512 (1.6)	0.66 (0.09-4.88)	0.686	N/A	N/A
Jaundice	17/94 (18.1)	339/2469 (13.7)	1.39 (0.81-2.37)	0.238	N/A	N/A
Fever	3/96 (3.1)	19/2528 (0.75)	**4.26 (1.24-14.6)**	**0.012**	<0.01 (<0.01->99)	0.990
Hypothermia	25/96 (25.8)	485/2528 (19.2)	1.40 (0.88-2.25)	0.157	0.78 (0.37-1.62)	0.500
Central nervous system						
Apnoea	12/93 (12.9)	321/2448 (13.1)	0.98 (0.53-1.82)	0.951	N/A	N/A
Seizures	9/94 (9.6)	112/2508 (4.5)	**2.26 (1.11-4.62)**	**0.027**	0.83 (0.23-3.03)	0.774
Hypotonia	21/94 (22.3)	380/2508 (15.2)	1.61 (0.98-2.65)	0.058	0.61 (0.26-1.46)	0.265
Gastrointestinal tract						
Poor feeding	1/35 (2.9)	17/942 (1.8)	1.60 (0.21-12.4)	0.649	N/A	N/A
Abdominal distension	2/94 (2.1)	102/2511 (4.1)	0.51 (0.13-2.11)	0.347	N/A	N/A

Table 3.8 (continued): Predictors of culture-confirmed sepsis in neonates with earl-onset possible serious bacterial infection

	Culture-Positive sepsis N = 96	**Culture-Negative sepsis N = 2528**	**Univariate analysis**		**Multivariate analysis**	
	n/N (%)	n/N (%)	**OR (95%CI)**	**p-value**	**aOR (95% CI)**	**p-value**
Cardiorespiratory						
Tachypnoea (RR>60/min)	25/96 (26.0)	724/2528 (28.6)	0.88 (0.55-1.40)	0.580	N/A	N/A
Chest wall indrawing	53/94 (56.4)	1326/2503 (53.0)	1.15 (0.76-1.74)	0.516	N/A	N/A
Required ventilation	20/89 (22.5)	605/2492 (24.9)	0.87 (0.53-1.45)	0.600	N/A	N/A
Hypotension	1/91 (1.1)	64/2438 (2.6)	0.41 (0.06-3.00)	0.366	N/A	N/A
Metabolic						
Hypoglycaemia	11/93 (11.8)	304/2504 (12.1)	0.97 (0.51-1.84)	0.928	N/A	N/A
Hyperglycaemia	7/94 (7.4)	186/2503 (7.4)	1.00 (0.46-2.20)	0.995	N/A	N/A
Metabolic acidosis	42/94 (44.7)	728/2504 (29.1)	**1.97 (1.30-2.99)**	**<0.001**	1.51 (0.85-2.69)	0.156
Severe infection*	31/96 (32.3)	527/2528 (20.8)	**1.81 (1.17-2.81)**	**0.007**	0.09 (0.39-2.16)	0.840
Laboratory findings						
Leukopenia (WCC<5.0x10^9)	9/91 (9.9)	216/2507 (8.6)	1.16 (0.58-2.35)	0.671	N/A	N/A
Leukocytosis (WCC>25.0x10^9)	5/91 (5.5)	140/2507 (5.6)	0.98 (0.39-2.46)	0.971	N/A	N/A
Neutropenia	14/82 (17.1)	292/2224 (13.1)	1.36 (0.76-2.45)	0.301	N/A	N/A
Thrombocytopenia	34/93 (36.6)	957/2510 (38.1)	0.94 (0.61-1.44)	0.760	N/A	N/A
High CRP (>10 mg/L)	31/61 (50.8)	343/1561 (22.0)	**3.67 (2.19-6.15)**	**<0.001**	**3.00 (1.70-5.30)**	**<0.001**

* - Severe infection was defined as a combination of lethargy, poor feeding, admission to NICU and deaths; HIV - human immunodeficiency virus; PROM- prolonged rupture of membranes; RR- respiratory rate; WCC- white cell count; CRP- c-reactive protein

Incidence

In assessing the incidence of sepsis, patients were stratified into three categories, including EO-pSBI, protocol defined EOS, and culture confirmed EOS. The incidence was expressed per 1000 live births, (34 808 live births over the study period). The measured incidence was adjusted by a factor of 1.4, to include accounting for non-enrolment of pSBI cases that did not occur over weekends (n=722) and for refusal to consent among the eligible neonates (n=389). The adjusted incidence (per 1000 live births) of early-onset pSBI was 106, of protocol defined EOS 49.5 and of for culture-confirmed sepsis 3.9. (**Table 3.9**). The odds of having early-onset pSBI (OR: 1.45; 95% CI 1.34-1.56), or protocol defined EOS (OR: 1.36; 95% CI 1.22-1.51) were higher for HIV exposed compared to HIV-unexposed newborns, with a similar trend observed for culture-confirmed EOS (OR: 1.29; 95% CI 0.0.89-1.87, although not statistical significant.

The adjusted incidence (per 1000 live births) of early-onset culture confirmed GBS infection was 1.41 (95% CI 1.06 – 1.86); while that of *Viridans streptococcus*, *Enterococcus species*, and *E. coli* were 0.92 (95% CI 0.65- 1.30); 0.40 (95%CI 0.20-0.61) and 0.40 (95%CI 0.20-0.61), respectively (**Table 3.10**). There was no statistical significant difference in incidence of culture confirmed EOS due to any of these organisms between HIV exposed and unexposed neonates.

Table 3.9: Incidence of early-onset possible bacterial, protocol-confined and culture-confirmed sepsis

	Number	Numbers after adjusting for Non-enrolment	Live births	Incidence* (95% CI)	HIV-exposed vs. HIV-unexposed OR (95% CI)	Adjusted Incidence* (95% CI)	HIV-exposed vs HIV-unexposed aOR (95% CI)
Early-onset possible serious bacterial sepsis							
All	2624	3674	34808	75.4 (72.7-78.2)		105.6 (102.5-108.9)	
HIV exposed	845	1183	9400	89.9 (84.3-95.9)		125.8 (119.3-132.7)	
HIV unexposed	1640	2296	25408	64.6 (61.6-67.6)	**1.43 (1.31-1.56)**	90.4 (86.9-94.0)	**1.45 (1.34-1.56)**
Protocol define early-onset sepsis							
All	1231	1723	34808	35.4 (33.5-37.4)		49.5 (47.3-51.8)	
HIV exposed	389	545	9400	41.4 (37.5-45.6)		58.0 (53.4-62.9)	
HIV unexposed	786	1100	25408	30.9 (28.9-33.1)	**1.35 (1.20-1.53)**	43.3 (40.9-45.9)	**1.36 (1.22-1.51)**
Culture-confirmed early-onset sepsis							
All	96	136	34808	2.79 (2.28-3.40)		3.91 (3.30-4.62)	
HIV exposed	30	42	9400	3.19 (2.23-4.56)		4.47 (3.30-6.04)	
HIV unexposed	63	88	25408	2.48 (1.94-3.17)	1.29 (0.83-1.99)	3.46 (2.81-4.27)	1.29 (0.89-1.87)

* - incidence per 1000 live births

Footnote:

Adjustment for non-enrolment was calculated by multiplying numbers enrolled by factor 1.4 derived from: (Number admitted with possible serious bacterial infection/ Number screened) x (Number Eligible/Number enrolled)

Table 3.10: Incidence of *Group B streptococcus, Viridans streptococcus, Enterococcus species, Escherichia coli* and *Staphylococcus aureus* in neonates with early-onset sepsis

	Numbers enrolled	Numbers after adjusting for Non-enrolment	Live births	Incidence* (95% CI)	HIV-exposed vs. HIV-unexposed OR (95% CI)	Adjusted Incidence* (95% CI)	HIV-exposed vs HIV-unexposed aOR (95% CI)
Group B *Streptococcus*							
Total	35	49	34808	1.01 (0.72-1.40)		1.41 (1.06-1.86)	
HIV exposed	6	8	9400	0.64 (0.29-1.42)		0.85 (0.43-1.70)	
HIV unexposed	25	35	25408	0.98 (0.66-1.46)	0.65 (0.27-1.58)	1.38 (0.99-1.92)	0.62 (0.29-1.33)
Viridans Streptococcus							
Total	23	32	34808	0.66 (0.44-0.99)		0.92 (0.65-1.30)	
HIV exposed	6	8	9400	0.64 (0.29-1.42)		0.85 (0.43-1.70)	
HIV unexposed	17	24	25408	0.67 (0.42-1.08)	0.95 (0.38-2.42)	0.94 (0.63-1.41)	0.90 (0.41-2.01)
Enterococcus species							
Total	10	14	34808	0.29 (0.16-0.54)		0.40 (0.20-0.61)	
HIV exposed	3	4	9400	0.32 (0.10-0.99)		0.42 (0.16-1.13)	
HIV unexposed	7	10	25408	0.28 (0.13-0.58)	1.16 (0.30-4.48)	0.39 (0.21-0.73)	1.08 (0.34-3.45)
Escherichia coli							
Total	10	14	34808	0.29 (0.16-0.54)		0.40 (0.20-0.61)	
HIV exposed	3	4	9400	0.32 (0.10-0.99)		0.42 (0.16-1.13)	
HIV unexposed	7	10	25408	0.28 (0.13-0.58)	1.16 (0.30-4.48)	0.39 (0.21-0.73)	1.08 (0.34-3.45)

Table 3.10 (continued) : Incidence of Group B streptococcus, Viridans streptococcus, Enterococcus species, Escherichia coli and Staphylococcus aureus in neonates with early-onset sepsis

	Numbers enrolled	Numbers after adjusting for Non-enrolment	Live births	Incidence* (95% CI)	HIV-exposed vs. HIV-unexposed OR (95% CI)	Adjusted Incidence* (95% CI)	HIV-exposed vs HIV-unexposed aOR (95% CI)
Staphylococcus aureus							
Total	3	4	34808	0.09 (0.03-0.27)		0.12 (0.04-0.31)	
HIV unexposed	2	3	25408	0.08 (0.02-0.32)		0.12 (0.04-0.37)	
HIV exposed	1	1	9400	0.11 (0.02-0.76)	1.35 (0.12-14.9)	0.11 (0.02-0.76)	0.90 (0.09-8.66)

*- incidence per 1000 live births

Footnote:

Adjustment for non-enrolment was calculated by multiplying numbers enrolled by factor 1.4 derived from: (Number admitted with early-onset possible serious bacterial infection / Number screened) x (Number eligible/Number enrolled)

Susceptibility of bacteria isolated from neonates with EO-pSBI

The median time for bacterial growth detection on BactTAlert system was 7 hours. The median times to positive results for GBS and E. *coli* were shorter than for Enterococcus *species*, *Staphylococcus aureus* or Viridans s*treptococcus* (**Table 3.11**). All GBS isolates were susceptible to penicillin, ampicillin, clindamycin and cefotaxime; and 91.2% were sensitive to macrolides (**Table 3.12**). Only 14% of *E. coli* were sensitive to ampicillin, but all were sensitive to gentamicin and cefotaxime. *Staphylococcus aureus* were all sensitive to cloxacillin, gentamicin and clindamycin.

Table 3.11: Time to growth for the common pathogens isolated in neonates with early-onset culture-confirmed sepsis

		Time to growth (hours)	
	Number*	Mean ± SD	Median (25-75th Centile)
Escherichia coli	7	5.0 ± 2.9	5.0 (2.0 - 7.0)
Group B Streptococcus	34	7.8 ± 7.2	5.0 (3.0 - 9.0)
Viridans streptococcus	21	15.6 ± 18.7	10.0 (7.0 - 13.0)
Enterococcus sp.	8	7.4 ± 6.8	11.0 (5.0 - 17.0)
Staphylococcus aureus	3	11.0 ± 6.0	11.0 (5.0 - 17.0)
All	73	9.8 ± 11.5	7.0 (4.0 - 11.0)

*- Missing results for time to growth for *Escherichia coli* (n=2), Group B Streptococcus (n=2), *Viridans streptococcus* (n=3) and *Enterococcus* (n=2).

Table 3.12: Antimicrobial susceptibility of pathogens isolated from neonates with early-onset possible serious bacterial sepsis

	Number*	Pen n (%)	Ampi n (%)	Cloxa n (%)	Erythro n (%)	Clinda n (%)	Genta n (%)	Cefotax n (%)
Group B *streptococcus*	34	34 (100)	34 (100)	NT	31 (91.2)	34 (100)	31 (91.2)	34 (100)
Viridans streptococcus	21	NT	14/15 (93.3)	NT	7/12 (63.2)	17/19 (89.5)	NT	19 (90.5)
Escherichia coli	7	NT	1 (14.3)	NT	NT	NT	7 (100)	7 (100)
Enterococcus faecalis	7	NT	7 (100)	NT	NT	NT	NT	NT
Staphylococcus aureus	3	NT	0	3 (100)	2 (66.7)	3 (100)	3 (100)	NT
Haemophilus influenzae	2	NT	1 (50)	NT	NT	NT	NT	2 (100)
Enterococcus faecium	1	NT	0	NT	NT	NT	NT	NT
Klebsiella species	1	NT	0	NT	NT	NT	1 (100)	1 (100)
Enterobacter species	1	NT	0	NT	NT	NT	1 (100)	1 (100)

Pen= penicillin, Ampi= ampicillin, Cloxa= cloxacillin, Erythro= erythromycin, Genta=gentamicin, Cefotax= cefotaxime; NT – Not tested

*- Missing results for antimicrobial susceptibility for *Escherichia coli* (n=2), Group B Streptococcus (n=2), *Viridans streptococcus* (n=3) and *Enterococcus* (n=2).

Predictors of mortality in neonates with early-onset presumed serious bacterial infection or protocol defined early-onset sepsis

Overall the CFR for EO-pSBI cases was 9%. The CFR ranged from 6.2% among those who did not meet the protocol defined sepsis criteria to 17.7% among those with culture-confirmed EOS (**Table 3.13**). The odds ratios for mortality were high in protocol-defined culture negative EOS (OR: 1.98; 95% CI 1.49 –2.62), and culture positive EOS (OR= 3.23, 95% CI: 1.83 – 5.70) compared to mortality in neonates who did not meet the criteria for protocol defined nor culture confirmed sepsis.

Table 3.13: Case fatality ratio among neonates with early-onset sepsis

Sepsis category	Total	Died	Survived	OR (95% CI)
All neonates with early-onset possible serious bacterial infection	2624	236 (9.0%)	2388 (81.0%)	_
Neonates with possible serious bacterial infection NOT meeting protocol defined sepsis criteria	1393	87 (6.2%)	1306 (93.80%)	REF
Neonates with protocol-defined, culture negative sepsis	1134	132 (11.6%)	1003 (88.4%)	1.98 (1.49 - 2.62)
Neonates with culture positive sepsis	97	17 (17.5%)	79 (83.3%)	3.23 (1.83 – 5.70)

On multivariate logistic regression analysis, factors which were found to be predictors of death in neonates with pSBI were vaginal delivery (aOR: 2.08; 95% CI 1.39-3.10), low birth weight (aOR: 3.16; 95% CI 1.51-6.60), prematurity (aOR: 2.23; 95%CI 1.14-4.36), hypothermia (aOR: 1.79; 95%CI 1.19-2.69), apnoea (aOR: 2.95; 95% CI 1.89 –4.60), hypotonia (aOR: 2.89; 95%CI 1.78-4.68), need for ventilation (aOR: 2.04; 95% CI 1.32-3.14), hyperglycaemia (aOR: 3.13; 95% CI 1.93-5.07), metabolic acidosis (aOR: 1.87; 95% CI 1.25-2.82), leukocytosis (aOR: 3.43; 95% CI 1.71-6.90) and positive blood culture (aOR: 3.30; 95%CI 1.43-7.61) (**Table 3.14**). Predictors of mortality in those with protocol-defined sepsis were similar to those observed with possible serious bacterial infection except for not being significant for metabolic acidosis (**Table 3.15**).

Table 3.14: Predictors of mortality in neonates with early-onset possible serious bacterial sepsis

	Died N = 236	**Survived N = 2388**	**Univariate analysis**		**Multivariate analysis**	
	n/N (%)	n/N (%)	**OR (95%CI)**	**p-value**	**aOR (95% CI)**	**p-value**
Characteristics						
Antenatal care	197/235 (83.8)	2245/2377 (94.5)	0.31(0.21-0.45)	<0.001	1.07 (0.58-2.00)	0.824
HIV Exposure	76/207 (36.7)	769/2278 (33.8)	1.14 (0.85-1.53)	0.390	N/A	N/A
PROM	11/232 (4.7)	163/2362 (6.9)	0.67 (0.36-1.25)	0.207	N/A	N/A
Maternal Antibiotics	20/233 (8.6)	223/2359 (9.4)	0.90 (0.56-1.45)	0.664	N/A	N/A
Meconium stained liquor	20/233 (8.6)	496/2358 (21.0)	0.35 (0.22-0.56)	<0.001	0.60 (0.30-1.22)	0.162
Vaginal delivery	147/236 (62.3)	1077/2388 (45.1)	2.01 (1.53-2.65)	<0.001	**2.08 (1.39-3.10)**	**<0.001**
Low birth weight	200/236 (84.8)	1220/2388 (51.1)	5.32 (3.70-7.65)	<0.001	**3.16 (1.51-6.60)**	**0.002**
Prematurity	190/234 (81.2)	1003/2377 (42.2)	5.92 (4.22-8.29)	<0.001	ND	ND
Male sex	120/235 (51.1)	1280/2384 (53.7)	0.90 (0.69-1.18)	0.441	N/A	N/A
Apgar at 5 min <7	89/236 (37.7)	391/2388 (16.4)	3.09 (2.33-4.11)	<0.001	1.11 (0.69-1.78)	0.663
General Signs						
Lethargy	71/233 (30.5)	239/2373 (10.1)	3.92 (2.87-5.33)	<0.001	1.37 (0.46-4.05)	0.572
Jaundice	51/227 (22.5)	305/2236 (13.1)	1.93 (1.38-2.70)	<0.001	1.10 (0.67-1.81)	0.706
Hypothermia	93/236 (39.4)	416/2388 (17.4)	3.08 (2.33-4.09)	<0.001	**1.79 (1.19-2.69)**	**0.005**
Central nervous system						
Apnoea	89/229 (38.9)	244/2310 (10.6)	5.38 (4.00-7.24)	<0.001	**2.95 (1.89-4.60)**	**<0.001**
Seizures	14/233 (6.0)	107/2369 (4.5)	1.35 (0.76-2.40)	0.302	N/A	N/A
Hypotonia	92/230 (40.0)	309/2372 (13.0)	4.45 (3.33-5.95)	<0.001	**2.89 (1.78-4.68)**	**<0.001**
Cardiorespiratory system						
Tachypnoea (RR>60/min)	63/236 (26.7)	686/2388 (28.7)	0.90 (0.67-1.22)	0.510	N/A	N/A
Chest wall indrawing	166/231 (71.9)	1213/2366 (51.3)	2.43 (1.80-3.27)	<0.001	1.38 (0.89-2.12)	0.152
Required invasive ventilation	111/224 (63.0)	484/2293 (21.1)	6.35 (4.76-8.48)	<0.001	**2.04 (1.32-3.14)**	**0.001**
Hypotension requiring inotrope	29/221 (13.1)	36/2308 (1.56)	9.53 (5.72-15.89)	<0.001	1.66 (0.69-1.78)	0.256

Table 3.14 (continued): Predictors of mortality in neonates with early-onset possible serious bacterial sepsis

	Died N = 236	**Survived N = 2388**	**Univariate analysis**		**Multivariate analysis**	
	n/N (%)	n/N (%)	**OR (95%CI)**	**p-value**	**aOR (95% CI)**	**p-value**
Hypoglycaemia	39/230 (17.0)	276/2367 (11.7)	1.55 (1.07-2.23)	0.018	1.43 (0.84-2.41)	0.186
Hyperglycaemia	70/233 (30.0)	123/2364 (5.2)	7.82 (5.60-10.9)	<0.001	**3.13 (1.93-5.07)**	**<0.001**
Metabolic acidosis	139/232 (59.9)	631/2366 (26.7)	4.11 (3.11-5.42)	<0.001	**1.87 (1.25-2.82)**	**0.002**
Laboratory findings						
Leukopenia (WCC<5.0x10^9)	31/234 (13.2)	194/2364 (8.2)	1.71 (1.14-2.56)	0.009	0.97 (0.50-1.90)	0.932
Leucocytosis (WCC>25.0x10^9)	28/234 (12.0)	117/2364 (4.95)	2.61 (1.69-4.04)	<0.001	**3.43 (1.71-6.90)**	**<0.001**
Neutropenia	56/208 (26.9)	250/2098 (11.9)	2.72 (1.95-3.80)	<0.001	1.41 (0.86-2.32)	0.170
Thrombocytopenia	99/235 (42.10	892/2368 (37.7)	1.21 (0.92-1.58)	0.179	1.06 (0.72-1.57)	0.769
High CRP (>10 mg/L)	33/161 (20.5)	341/1461 (23.3)	0.85 (0.57-1.26)	0.416	N/A	N/A
Positive culture	16/236 (6.8)	26/2388 (3.2)	2.21 (1.27-3.86)	0.004	**3.30 (1.43-7.61)**	**0.005**

*- HIV- immunodeficiency virus, WCC- white cells count, CRP- c-reactive protein

Table 3.15: Predictors of mortality in neonates with early-onset protocol-defined sepsis

	Died N = 149	**Survived N = 1082**	**Univariate analysis**		**Multivariate analysis**	
	n/N (%)	n/N (%)	**OR (95%CI)**	**p-value**	**aOR (95% CI)**	**p-value**
Characteristics						
Antenatal care	128/149 (86.5)	1008/1082 (93.9)	0.41 (0.24-0.70)	<0.001	1.98 (0.54-7.28)	0.306
HIV Exposure	55/135 (40.7)	334/1040 (32.1)	1.45 (1.01-2.10)	0.045	1.05 (0.57-1.95)	0.876
PROM	4/145 (2.8)	56/1077 (5.20)	0.52 (0.18-1.45)	0.202	N/A	N/A
Maternal Antibiotics	10/146 (6.9)	93/1077 (5.20)	0.78 (0.40-1.53)	0.466	N/A	N/A
Meconium stained liqour	16/146 (11.0)	261/1078 (24.2)	0.38 (0.22-0.66)	<0.001	0.68 (0.26-1.78)	0.436
Vaginal delivery	88/149 (59.1)	470/1082 (43.4)	1.88 (1.33-2.66)	<0.001	**2.13 (1.17-3.86)**	**0.013**
Low birth weight	126/149 (84.6)	561/1082 (51.9)	5.09 (3.21-8.06)	<0.001	**7.26 (2.89-18.2)**	**<0.001**
Prematurity	120/148 (81.1)	459/1078 (42.6)	5.78 (3.76-8.87)	<0.001	ND	ND
Male sex	74/148 (50.0)	598/1080 (55.4)	0.81 (0.57-1.14)	0.218	N/A	N/A
Apgar at 5 min <7	55/149 (36.9)	199/1082 (18.4)	2.60 (1.80-3.75)	<0.001	1.63 (0.82-3.24)	0.162
General Signs						
Lethargy	48/148 (32.4)	135/1078 (12.5)	3.35 (2.27-4.94)	<0.001	0.78 (0.34-1.82)	0.572
Jaundice	30/145 (20.7)	145/1063 (13.6)	1.65 (1.07-2.56)	0.024	1.28 (0.63-2.60)	0.503
Hypothermia	59/149 (39.6)	222/1082 (20.5)	2.54 (1.77-3.64)	<0.001	1.36 (0.72-2.58)	0.351
Central nervous system						
Apnoea	57/146 (39.0)	134/1055 (12.7)	4.40 (3.01-6.43)	<0.001	**2.01 (1.02-3.99)**	**0.045**
Seizures	10/148 (6.8)	50/1078 (4.64)	1.49 (0.74-3.01)	0.263	N/A	N/A
Hypotonia	57/146 (39.0)	177/1078 (16.4)	3.26 (2.25-4.72)	<0.001	**2.52 (1.19-5.30)**	**0.015**
Cardiorespiratory system						
Tachypnoea (RR>60/min)	44/149 (29.5)	354/1082	0.86 (0.59-1.25)	0.436	N/A	N/A
Chest wall indrawing	112/146 (76.7)	622/1076 (57.8)	2.40 (1.61-3.60)	<0.001	2.36 (1.14-4.86)	0.021
Required ventilation	93/143 (65.0)	261/1042 (25.1)	5.57 (3.84-8.07)	<0.001	**3.83 (1.97-7.47)**	**0.031**
Hypotension	19/140 (13.6)	18/1039 (1.73)	8.91 (4.55-17.4)	<0.001	1.54 (0.42-5.67)	0.514

Table 3.15 (continued): Predictors of mortality in neonates with early-onset protocol-defined sepsis

	Died N = 149	Survived N = 1082	Univariate analysis		Multivariate analysis	
	n/N (%)	n/N (%)	**OR (95%CI)**	**p-value**	**aOR (95% CI)**	**p-value**
Metabolic						
Hypoglycaemia	27/147 (18.4)	136/1077 (12.6)	1.55 (0.99-2.45)	0.055	1.26 (0.60-2.66)	0.546
Hyperglycaemia	43/148 (29.1)	72/1075 (6.7)	5.71 (3.72-8.75)	<0.001	**2.36 (1.14-4.86)**	**0.022**
Metabolic acidosis	85/147 (57.8)	356/1076 (33.1)	2.77 (1.95-3.94)	<0.001	1.38 (0.74-2.54)	0.311
Laboratory findings						
Leukopenia (WCC<5.0x10^9)	28/147 (19.0)	96/1067 (9.0)	2.38 (1.50-3.78)	<0.001	1.39 (0.61-3.21)	0.436
Leucocytosis (WCC>25.0x10^9)	26/147 (17.7)	106/1067 (9.9)	1.95 (1.22-3.11)	0.005	**3.76 (1.40-10.1)**	**0.009**
Neutropenia	49/131 (37.4)	170/971 (17.5)	2.82 (1.91-4.16)	<0.001	1.41 (0.71-2.82)	0.325
Thrombocytopenia	94/148 (63.5)	715/1072 (66.7)	0.87 (0.61-1.24)	0.442	N/A	N/A
High CRP (>10 mg/L)	31/106 (29.3)	307/776 (39.6)	0.63 (0.41-0.98)	0.040	0.80 (0.40-1.59)	0.521
Positive culture	16/146 (11.0)	77/1027 (7.5)	1.52 (0.86-2.68)	0.148	**3.19 (1.11-9.19)**	**0.031**

HIV- human immunodeficiency virus, WCC- white cell count, CRP- c-reactive protein, N/A- Not applicable, ND – Not done

3.1.2 Community Acquired Sepsis

Neonates enrolled with possible serious bacterial infection

There were 1371 neonates 3-27 days of age admitted during the study period, 919 (67.0%) of whom were screened for eligibility to the study. Of the 919 screened, 137 (14.9%) were not eligible for enrolment, mainly due to previous hospital admission (84/137; 61.3%). Among the 782 eligible neonates with community-acquired pSBI (CA-pSBI), consent was not obtained for 146 (18.7%) neonates, the most common reason being refusal of the mother to participate (n=89; 61.0%). Of the 636 neonates enrolled, 11 (1.7%) were born to mothers whose HIV infection status was unavailable. Among the 625 neonates in whom maternal HIV infection status was known, 174 (27.4%) were HIV-exposed (**Figure 3.2**).

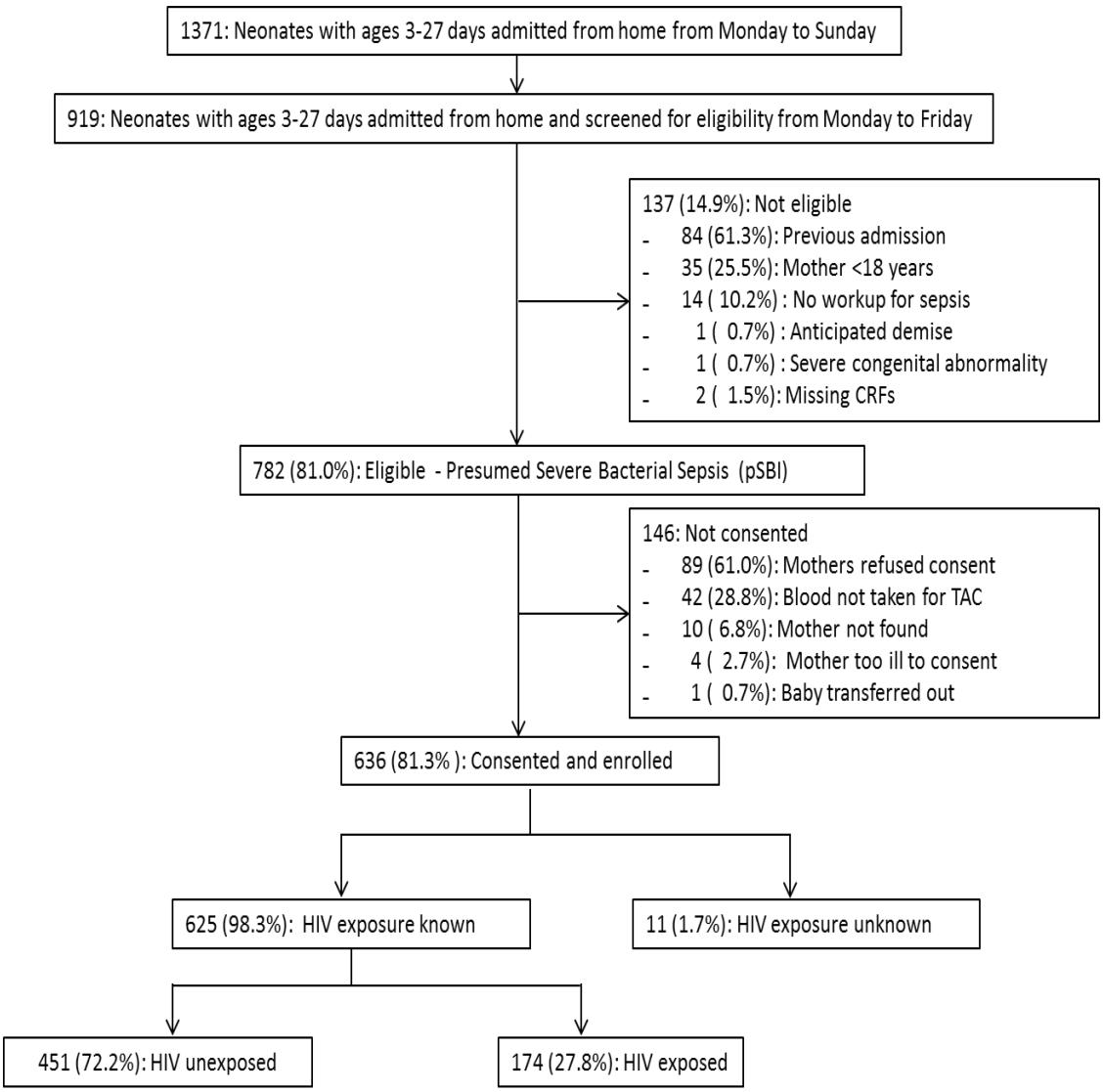

Figure 3.2: Number of neonates with community acquired presumed serious bacterial infection who were screened and enrolled in the study

Maternal and infant characteristics

All enrolled CA-pSBI

Almost all mothers of neonates with CA-pSBI had attended antenatal care (98%) and were delivered either at a clinic or in hospital (98%), with 80% born by vaginal delivery. There were no differences between HIV-uninfected and HIV-infected women with regard to attending antenatal care, and place and mode of delivery. Only seven percent of CA-pSBI cases were born preterm, and 14% had birth weight <2500 grams. The odds of being low birth weight were greater in HIV-exposed than -unexposed (OR=1.72, 95% CI 1.07-2.76) CA-pSBI cases. Approximately two-thirds (68%) of CA-pSBI presented after the first seven days of life, at median age of 10 days. A higher proportion of HIV-unexposed (36%) presented at between ages 3-7 days than HIV-exposed (20%, p<0.001) neonates (**Table 3.16)**.

Protocol defined CAS

Among the 636 neonates enrolled with CA-pSBI, 137 (22%) met the criteria for protocol defined CAS. Maternal HIV status was unknown for 3 infants (2.20%); and among those with known maternal HIV status, 57 (42.0%) were HIV exposed. Maternal and infant characteristics of neonates with protocol-defined CAS were similar to those observed in CA-pSBI. Compared to the CA-pSBI, the protocol defined CAS group had fewer neonates who were males among the HIV-exposed neonates than in those who were HIV-unexposed (49.1% vs 68.4%, p = 0.024). A higher percentage of HIV-unexposed (36%) presented within 3-7 days with protocol defined CAS than HIV-exposed neonates (17%, p<0.001) (**Table 3.17**).

Table 3.16: Maternal and infant characteristics of neonates hospitalized for community acquired possible serious bacterial infection

Maternal Factors	**All enrolled N = 636***	**HIV-infected N = 174**	**HIV-uninfected N = 451**	**HIV-infected vs HIV-uninfected**	
		n (%)	n (%)	OR (95% CI)	p-value
Antenatal care visit	623/633 (98.4)	169/172 (98.3)	446/450 (99.1)	0.51 (0.11-2.28)	0.367
Vaginal delivery	521/636 (81.9)	136/174 (78.2)	376/451 (83.4)	0.71 (0.46-1.10)	0.129
Healthcare facility births	621/633 (98.1)	170/173 (98.3)	441/450 (98.0)	1.16 (0.31-4.32)	0.828
Infant Factors	**All enrolled N = 636**	**HIV-exposed N = 174**	**HIV-unexposed N = 451**	**HIV-exposed vs HIV-unexposed**	
		n (%)	n (%)	OR (95% CI)	p-value
Low birth weight (<2500 g)	90/636 (14.2)	33/174 (19.0)	54/451 (12.0)	1.72 (1.07-2.76)	**0.024**
Prematurity (<37 weeks)	43/632 (6.8)	14/172 (8.1)	29/449 (6.5)	1.28 (0.66-2.49)	0.460
Male	378/636 (59.4)	99/174 (56.9)	270/451 (59.9)	0.88 (0.63-1.26)	0.499
Apgar score<7 at 5 minutes	71/636 (11.2)	26/174 (14.9)	45/451 (10.0)	1.58 (0.94-2.66)	0.080
Age at enrolment					**<0.001**
3 - 7 days	201 (31.6)	34 (19.5)	164 (36.4)	2.35 (1.54-3.58)	
8 - 27 days	435 (68.4)	140 (80.5)	287 (63.6)	Ref	
Median age at onset (25^{th} -75^{th}) days	10 (5-17)	14 (8-20)	10 (5 -15)		**<0.001**

*-In 11 neonates, the maternal HIV status was not recorded

Table 3.17: Maternal and infant characteristics of neonates with community acquired protocol-defined sepsis

Maternal Factors	**All enrolled N = 137**	**HIV-infected N = 57**	**HIV-uninfected N = 76**	**HIV-infected vs HIV-uninfected**	
		n/N (%)	n/N (%)	OR (95% CI)	p-value
Antenatal care visit	134/136 (98.5)	55/56 (98.2)	76/76 (100)	Not estimable	
Vaginal delivery	116/137 (84.7)	52/57 (91.2)	61/76 (80.3)	2.53 (0.87-7.51)	0.08
Healthcare facility births	136/136 (100)	56/56 (100)	76/76 (100)	Undefined	
Infant Factors	**All enrolled N = 137**	**HIV-exposed N = 57**	**HIV-unexposed N = 76**	**HIV-exposed vs HIV-unexposed**	
		n/N (%)	n/N (%)	OR (95% CI)	p-value
Low birth weight	24/137 (17.5)	11/57 (19.3)	11/66 (14.5)	1.41 (0.57-3.53)	0.459
Preterm	12/137 (8.8)	5/57 (8.8)	7/76 (9.2)	0.95 (0.28-3.15)	0.930
Male	83/137 (60.5)	28/57 (49.1)	52/67 (68.4)	0.44 (0.21-0.91)	**0.024**
Apgar score <7 at 5 minutes	17/137 (12.4)	8/57 (14.0)	9/76 (11.8)	1.21 (0.44-3.37)	0.708
Age at onset of sepsis					0.047
3-7 days	36 (26.3)	10 (17.5)	25 (32.9)	2.30 (1.00-5.30)	
7 - 27 days	101 (73.7)	47 (82.5)	51 (67.1)	Ref	
Median age at onset (25^{th} -75^{th}) days	10 (5-17)	14 (8-20)	10 (5 -15)		**<0.001**

Clinical signs

The common clinical signs in neonates with CA-pSBI included jaundice (47%), irritability (22%), tachypnoea (21%), chest wall retractions (21%) and abdominal distension (16%) (**Table 3.18**). The odds of presenting with the following signs, lethargy (OR: 2.48; 95% CI 1.49-4.13), irritability (OR:2.04; 95% CI 1.37-3.03), fever (OR:2.36; 95% CI 1.41-3.92), poor feeding (OR:1.96; 95% CI 1.12-3.44), abdominal distension (OR:1.80; 95% CI 1.15-2.82), seizures or bulging fontanelle (OR:2.60; 95% CI 1.25-5.38), hypotonia (OR:2.54; 95% CI 1.17-5.53), tachypnoea (OR:1.87; 1.24-2.81), retractions (OR:1.86; 95% CI 1.24-2.81), need for ventilation (OR:3.26; 95% CI 0.98-10.8) and metabolic acidosis (OR:5.06; 95% CI 1.84-13.9) were greater, while those of presenting with jaundice (OR:0.12; 95% CI 0.08-0.19) were lower in HIV-exposed compared to HIV-unexposed neonates with CA-pSBI. The common clinical signs among the neonates with protocol defined CAS were similar to those with CA-pSBI (**Table 3.19**). Differences between HIV-exposed and -unexposed neonates with pSBI included odds being high for fever (OR: 3.11; 95% CI 1.37-7.05) and tachypnoea (OR: 2.90; 95% CI 1.37-6.40), but lower for jaundice (OR: 0.18 95% CI 0.08-0.48) in HIV-exposed neonates.

Table 3.18: Clinical presentation of neonates with community acquired possible serious bacterial infection

Clinical Signs	All Enrolled N = 636	HIV-exposed N = 174 n/N (%)	HIV-unexposed N = 451 n/N (%)	HIV-exposed vs HIV-unexposed OR (95% CI)	p-value
General Signs					
Lethargy	71/630 (11.3)	32/171 (18.7)	38/448 (8.5)	2.48 (1.49-4.13)	**<0.001**
Irritability	140/631 (22.2)	55/172 (32.0)	84/448 (18.8)	2.04 (1.37-3.03)	**<0.001**
Jaundice	302/624 (48.4)	27/169 (15.0)	270/444 (60.8)	0.12 (0.08-0.19)	**<0.001**
Fever	70/636 (11.0)	31/174 (17.8)	38/451 (8.4)	2.36 (1.41-3.92)	**<0.001**
Hypothermia	40/636 (6.3)	7/174 (4.0)	33/451 (7.3)	0.53 (0.23-1.22)	0.131
Gastrointestinal Tract					
Poor feeding	60/586 (10.2)	23/153 (15.0)	35/423 (8.3)	1.96 (1.12-3.44)	**0.017**
Distension	101/631 (16.0)	38/172 (22.1)	61/448 (13.6)	1.80 (1.15-2.82)	**0.010**
Central nervous system					
Apnoea	33/630 (5.2)	14/171 (8.2)	19/448 (4.2)	2.01 (0.99-4.11)	0.051
Seizures or bulging fontanelle	32/630 (5.1)	15/171 (8.8)	16/448 (3.6)	2.60 (1.25-5.38)	**0.008**
Hypotonia	27/629 (4.3)	13/171 (7.6)	14/447 (3.1)	2.54 (1.17-5.53)	**0.015**
Cardiorespiratory system					
Tachypnoea (RR>60/min)	131/636 (20.6)	50/174 (28.7)	80/451 (17.7)	1.87 (1.24-2.81)	**0.002**
Chest wall indrawing	135/628 (21.5)	50/171 (29.2)	81/446 (18.2)	1.86 (1.24-2.80)	**0.003**
Required ventilation	11/ 628 (1.8)	6/169 (3.6)	5/448 (1.1)	3.26 (0.98-10.8)	**0.042**
Hypotension requiring inotropes	9/601 (1.5)	3/166 (1.8)	6/424 (1.4)	1.28 (0.32-5.19)	0.727
Metabolic					
Hypoglycaemia	9/629 (1.4)	3/171 (1.8)	6/447 (1.3)	1.31 (0.32-5.31)	0.702
Hyperglycaemia	40/636 (6.3)	12/171 (7.0)	26/447 (5.8)	1.22 (0.60-2.48)	0.578
Metabolic acidosis	17/625 (2.7)	11/170 (6.5)	6/445 (1.3)	5.06 (1.84-13.9)	**<0.001**
Empiric antibiotics used					
Ampicillin	636/636 (100)	176 (100)	451/451 (100)	Not estimatable	N/A
Gentamicin	636/636 (100)	176 (100)	451/451 (100)		

*-In 11 neonates, the maternal HIV status was not recorded

Table 3.19: Clinical presentation of neonates with community acquired protocol-defined sepsis

	All Enrolled N = 137*	**HIV-exposed N = 57** n/N (%)	**HIV-unexposed N = 76** n/N (%)	**HIV-exposed vs HIV-unexposed** OR (95% CI)	p-value
General Signs					
Lethargy	29/135 (21.5)	16/54 (29.6)	12/76 (15.8)	2.19 (0.94-5.11)	0.067
Irritability	54/136 (39.7)	25/56 (44.6)	28/76 (36.8)	1.38 (0.68-2.79)	0.366
Jaundice	84/134 (62.7)	9/34 (26.5)	36/76 (47.4)	0.18 (0.08-0.48)	**<0.001**
Fever	33/137 (24.1)	21/57 (36.8)	12/76 (15.8)	3.11 (1.37-7.05)	**0.005**
Hypothermia	10/137 (7.3)	4/57 (7.0)	6/76 (7.9)	0.88 (0.24-3.28)	0.849
Gastrointestinal Tract					
Poor feeding	21/122 (17.2)	9/46 (19.6)	11/67 (16.4)	1.35 (0.51-3.56)	0.545
Distension	33/136 (24.3)	16/56 (28.6)	16/76 (21.1)	1.50 (0.67-3.39)	0.319
Central nervous system					
Apnoea	12/135 (8.9)	5/55 (9.1)	7/76 (9.2)	0.99 (0.30-3.29)	0.981
Seizures or bulging fontanelle	12/136 (8.8)	7/56 (12.5)	4/76 (5.3)	2.57 (0.71-9.25)	0.137
Hypotonia	11/136 (8.1)	7/56 (12.5)	4/76 (5.3)	2.57 (0.71-9.25)	0.137
Cardiorespiratory system					
Tachypnoea (RR>60/min)	40/137 (29.2)	24/57 (42.1)	15/76 (19.7)	2.90 (1.37-6.40)	**0.005**
Chest wall indrawing	47/136 (34.6)	23/56 (41.1)	22/76 (28.9)	1.71 (0.83-3.54)	0.146
Required invasive ventilation	8/134 (6.0)	5/54 (9.3)	3/76 (3.9)	2.48 (0.57-10.87)	0.214
Hypotension requiring inotrope	6/134 (4.5)	3/54 (5.6)	3/76 (3.9)	1.43 (0.28-7.38)	0.667
Metabolic					
Hypoglycaemia	3/136 (2.2)	2/56 (3.6)	1/76 (1.3)	2.78 (0.25-31.4)	0.390
Hyperglycaemia	9/136 (6.6)	4/56 (7.1)	5/76 (6.6)	1.09 (0.28-4.27)	0.898
Metabolic acidosis	7/136 (5.1)	5/56 (8.9)	2/76 (2.6)	3.63 (0.68-19.42)	0.111
Empiric antibiotics					
Ampicillin	137/137 (100)	57/57 (100)	76/76 (100)	Not estimatable	N/A
Gentamicin	137/137 (100)	57/57 (100)	76/76 (100)		

*-In 4 neonates maternal HIV status was not recorded

Ancillary laboratory findings

Six percent of neonates with CA-pSBI had an abnormal total white cell count (WCC), including 4.5% with leukopenia and 1.8% with leukocytosis (**Table 3.20**). Among those who had white cell differential count, 19% had neutropenia. There were no significant differences in prevalence of white cell count abnormalities and thrombocytopenia between the HIV-exposed and -unexposed neonates. A higher proportion of HIV-exposed neonates had high CRP>10 mg/L (37.0%) compared to HIV- unexposed neonates (10.9%, p<0.001). Among the neonates with protocol defined CAS, 18% had abnormal WCC, including 31.8% with neutropenia and 38.4% with CRP >10 mg/L (**Table 3.21**). There were no differences in proportion of HIV-exposed and -unexposed neonates with abnormal WCC, platelet count or elevated CRP among those with protocol-defined CAS.

Table 3.20: Laboratory findings in neonates with community acquired presumed serious bacterial infection

	All **N = 636*** n (%)	**HIV- exposed** **N = 174** n (%)	**HIV- unexposed** **N = 451** n (%)	**HIV-exposed vs HIV-unexposed** p- value
Number with white cell count	N = 615	N = 168	N = 436	0.152
<5 x 10^9/L	28 (4.5)	11 (6.5)	17 (3.9)	
5-25 x 10^9/L	576 (93.7)	152 (90.5)	413 (94.7)	
>25 x 10^9/L	11 (1.8)	5 (3.0)	6 (1.4)	
Median white cell count (x10^9) (25^{th} – 75^{th} centile)	10.8 (8.75-13.4)	11.2 (8.73-14.9)	10.7 (8.76-13.0)	0.125
Number with absolute neutrophil count	N = 582	N = 155	N = 416	0.205
<1.75 x 10^9/L	111 (19.1)	26 (16.8)	82 (19.7)	
1.75 - 15.0 x 10^9/L	460 (79.0)	124 (80.0)	329 (79.1)	
>15 x 10^9/L	11 (1.9)	5 (3.2)	5 (1.2)	
Median absolute neutrophil count (x10^9) (25^{th} -75^{th} centile)	3.34 (2.16-4.97)	3.59 (2.16-6.29)	3.25 (2.16-4.46)	**0.014**
Number with platelet count	N = 614	N = 168	N = 436	0.823
<100 x 10^9/L	23 (3.7)	7 (4.2)	16 (3.7)	
100 - 150 x 10^9/L	30 (4.8)	7 (4.2)	23 (5.3)	
>150 x 10^9/L	561 (91.5)	154 (91.6)	397 (91.0)	
Median platelet count (x10^9) (25^{th}-75^{th} centile)	324 (233-422)	367 (276-472)	310 (220-404)	**<0.001**
C-reactive protein	N = 335	N = 81	N = 248	**<0.001**
<10 mg/L	278 (83.0)	51 (63.0)	221 (89.1)	
10 - 20 mg/L	22 (6.6)	14 (17.3)	8 (3.2)	
>20 mg/L	35 (10.4)	16 (19.7)	19 (7.7)	
Median c-reactive protein (x10^9) (25^{th} – 75^{th} centile)	1 (0-6)	3 (0-14)	1 (0-3)	**<0.001**

*-In 11 neonates, the maternal HIV status was not recorded

Table 3.21: Laboratory findings in neonates with community acquired protocol-defined sepsis

	All N = 137* n (%)	**HIV- exposed N=57** n (%)	**HIV- unexposed N=76** n (%)	**HIV-exposed vs HIV-unexposed** p- value
Number with white cell count	N = 134	N = 56	N = 74	0.450
<5 x 10^9/L	14 (10.4)	8 (14.3)	6 (8.1)	
5-25 x 10^9/L	110 (82.1)	43 (76.8)	63 (85.1)	
>25 x 10^9/L	10 (7.5)	5 (8.9)	5 (6.8)	
Median white cell count (x10^9) (25^{th} – 75^{th} centile)	10.1 (7.54-14.6)	10.5 (7.54-16.5)	9.51 (7.54-13.3)	0.278
Number with absolute neutrophil count	N = 129	N = 50	N = 74	0.511
<1.75 x 10^9/L	41 (31.8)	14 (28.0)	26 (35.1)	
1.75 - 15.0 x 10^9/L	78 (60.5)	31 (62.0)	44 (59.5)	
>15 x 10^9/L	10 (7.8)	5 (10.0)	4 (5.4)	
Median absolute neutrophil count (x10^9) (25^{th} – 75^{th} centile)	3.04 (1.63-6.38)	3.93 (1.66-7.91)	2.77 (1.60-5.64)	0.084
Number with platelet count	N = 134	N = 56	N = 74	0.662
<100 x 10^9/L	4 (3.0)	2 (3.6)	2 (2.7)	
100 - 150 x 10^9/L	18 (13.4)	6 (10.7)	12 (16.2)	
>150 x 10^9/L	112 (83.6)	48 (85.7)	60 (81.1)	
Median platelet count (x10^9) (25^{th} – 75^{th} centile)	311 (197-418)	342 (245-423)	274 (194-387)	0.051
Number with c-reactive protein	N = 73	N = 29	N = 42	0.115
<10 mg/L	45 (61.6)	14 (48.3)	29 (69.0)	
10 - 20 mg/L	7 (9.6)	5 (17.2)	2 (4.8)	
>20 mg/L	21 (28.8)	10 (34.5)	11 (26.2)	
Median c-reactive protein (mg/L) (25^{th} – 75^{th} centile)	3 (1-40)	10 (1-58)	1.5 (0-22)	0.054

*-In 4 neonates maternal HIV status was not recorded

Culture

Blood culture was positive in 160 (25.2%) of 636 the CA-pSBI, including 105/636 (16.5%) with presumed contaminants, whilst 55/636 (8.6%) yielded a pathogen considered to be significant. This yielded a culture-confirmed sepsis rate of 8.6% among the CA-pSBI cases and 40.1% (55/137) among those with protocol-defined community acquired sepsis. Among the 636 CA-pSBI cases, 540 (85%) had a lumbar puncture done, 47 (8.7%) of which had a positive bacterial culture. Of these, 24/540 (4.4%) were classified as presumed contaminants, and 23/540 (4.3%) were considered pathogenic (**Table 3.22**). Among the neonates who had both blood and lumbar puncture done (n = 540), 45 had positive blood cultures due to pathogenic bacteria, of whom 11 (24.4%) also had a positive CSF culture, 8 of which (72.7%) had the same pathogen cultured on blood. The HIV-exposed CA-pSBI cases were more likely

to have a positive blood or CSF culture due to pathogenic bacteria (27/174; 15.5%) than those who were HIV-unexposed (38/451; 8.4%; OR:2.00; 95% CI 1.18 – 3.38).

Table 3.22: Blood or cerebrospinal fluid culture results in neonates with community acquired possible serious bacterial infection

Blood and CSF culture results	**All N = 636**	**HIV-exposed N = 174**	**HIV-Unexposed N = 451**	**HIV-exposed vs HIV-unexposed**
	n /N (%)	n/N (%)	n/N (%)	OR (95% CI)
Neonates with positive blood and/or CSF culture	187/636 (29.4)	54/174 (31.0)	129/451 (28.6)	1.12 (0.77 - 1.64)
Positive blood culture (blood culture done in all)	160/636 (25.2)	48/174 (27.6)	108/451 (24.0)	1.21 (0.81 - 1.60)
Positive CSF culture (lumbar puncture done in 540 patients)	47/540 (8.7)	14/153 (9.1)	32/377 (8.5)	1.08 (0.56 - 2.10)
Neonates with positive culture due to pathogens				
Blood and/or CSF	67/636 (10.5)	27/174 (15.5)	38/451 (8.4)	**2.00 (1.18 – 3.38)**
Blood	55/636 (8.7)	23/174 (13.2)	30/451 (6.6)	**2.14 (1.20 - 3.80)**
CSF	23/540 (4.3)	10/153 (6.5)	12/377 (3.2)	2.13 (0.90 - 5.03)
Neonates with positive culture due to presumed contaminants				
Blood and/or CSF	120/636 (18.9)	27/174 (15.5)	91/451 (20.2)	0.73 (0.45 – 1.16)
Blood	105/636 (16.5)	25/174 (14.4)	78/451 (17.3)	0.80 (0.49 - 1.31)
CSF	24/540 (4.4)	4/153 (2.6)	20/377 (5.3)	0.48 (0.16 - 1.43)

*-In 11 neonates maternal HIV results were not recorded

Organisms detected in blood of neonates with CA- pSBI

Among the 55 neonates with positive blood cultures, 1 had 3 pathogens and 3 had 2 pathogens cultures, giving a total of 60 pathogens. Forty of these bacteria were considered definite pathogens, with GBS (35.0%), *Staphylococcus aureus* (30.0%), and E. *coli* (22.5%) being the most common definite pathogens, whilst *Viridans streptococcus* was the most common among the "possible pathogen" (85%) category. Gram positive bacteria constituted 76.7% of all pathogens. There was a higher proportion of HIV exposed neonates with GBS on blood culture (5.7%) compared to HIV-unexposed neonates (0.7%; OR: 6.81, 95% CI 2.11-22.0), whilst this did not differ for other bacteria (**Table 3.23**).

Table 3.23: Organism isolated from neonates with community acquired possible serious bacterial infection

	All enrolled	**HIV-exposed**	**HIV-unexposed**	**HIV-exposed vs HIV-unexposed**
	N = 636* n (%)	**N = 174** n (%)	**N = 451** n (%)	OR (95% CI)
ALL PATHOGENS	**60 (9.4)**	**27 (15.5)**	**32 (6.9)**	**2.41 (1.39-4.15)**
- Gram positive bacteria†	46 (76.7)	20 (74.1)	25 (78.1)	
- Gram negative bacteria†	14 (23.3)	7 (25.9)	7 (21.9)	
Definite Pathogens	**40 (6.3)**	**21 (12.1)**	**18 (4.0)**	**3.30 (1.71-6.36)**
Group B Streptococcus	14 (2.2)	10 (5.7)	4 (0.9)	**6.81 (2.11-22.0)**
Staphylococcus aureus	12 (1.9)	5 (2.9)	7 (1.5)	1.88 (0.59-5.99)
Escherichia coli	9 (1.4)	5 (2.9)	4 (0.9)	3.31 (0.88-12.5)
Klebsiella sp.	2 (0.3)	2 (1.2)	0	n/a
Enterococcus sp.	1 (0.2)	0	1 (0.2)	n/a
Enterobacter species	1 (0.2)	0	1 (0.2)	n/a
Proteus mirabilis	1 (0.2)	0	1 (0.2)	n/a
Possible Pathogens	**20 (3.1)**	**5 (2.9)**	**14 (3.1)**	0.92 (0.33-2.60)
Viridans streptococcus	17 (2.7)	5 (2.9)	11 (2.4)	1.18 (0.41-3.46)
Other *Streptococcus species*	2 (0.3)	0	2 (0.4)	n/a
Lactobacillus species	1 (0.2)	0	1 (0.2)	n/a
CONTAMINANTS	**105 (16.5)**	**24 (13.8)**	**79 (17.5)**	0.75 (0.46-1.24)
Coagulase Negative Staphylococcus	102 (16.0)	24 (13.8)	76 (16.8)	0.79 (0.48-1.30)
Bacillus species	2 (0.3)	0	2 (0.4)	n/a
Corynebacteria	1 (0.2)	0	1 (0.2)	n/a

*-In 11 neonates, maternal HIV results were not recorded

†-percentages calculated out of a total of all pathogens

Footnote: 4 patients grew more than 1 organism in blood culture; 1 Group B *streptococcus* + *Klebsiella pneumoniae* + *Staphylococcus aureus*, 1 *Enterobacter cloacae* +*Proteus mirabilis*, 1 *Klebsiella pneumoniae* + *Escherichia coli* and 1 *Lactobacillus* + *Neisseria species*

Organisms detected in cerebrospinal fluid of neonates with CA- pSBI

Among the 540 CA-pSBI cases investigated by lumbar puncture, 23 (4.3%) cultured a pathogenic bacteria on CSF, including one neonate with 3 different pathogenic bacteria, resulting in 25 bacteria overall. Among these 25 pathogenic bacteria, 21 (84.0%) were considered as definite pathogen due to (GBS (9/21; 42.9%), *Staphylococcus aureus* (5/21; 23.8%), *Enterococcus species* (4/21; 19.0%) and *E. coli* (2/21; 9.5%). Further, among the 9 cases from whom GBS was cultured from CSF, seven (77.8%) also had a positive culture from blood. The HIV-exposed neonates with CA-pSBI were more likely to have GBS cultured from CSF (3.9%) than HIV-unexposed neonates (0.8%; OR: 5.09; 95%CI 1.26-20.6) (**Table 3.24**).

Factors associated with blood or CSF culture confirmed sepsis in neonates with CA-pSBI

Among the neonates with CA-pSBI, we did not identify any maternal or infant characteristics that were predictive of positive blood or CSF bacterial culture (**Table 3.25**).

Table 3.24: Bacteria isolated in cerebrospinal fluid culture of neonates with community acquired possible serious bacterial infection

	All enrolled and CSF taken N = 540 n (%)	**HIV-exposed** N = 153 n (%)	**HIV-unexposed** N = 377 n (%)	**HIV-exposed vs HIV-unexposed** OR (95% CI)
ALL PATHOGENS	25 (4.6)	11 (6.5)	13 (3.4)	2.17 (0.95-4.96)
- Gram positives bacteria*	22 (88.0)	11 (100)	11 (84.6)	
- Gram negatives bacteria*	3 (12.0)	0	2 (15.4)	
Definite Pathogens	21 (3.9)	9 (5.9)	11 (2.9)	2.08 (0.84-5.12)
Group B Streptococcus	9 (1.7)	6 (3.9)	3 (0.8)	**5.09 (1.26-20.6)**
Staphylococcus aureus	5 (0.9)	3 (2.0)	2 (0.5)	3.75 (0.62-22.7)
Enterococcus sp.	4 (0.7)	0	4 (1.1)	n/a
Escherichia coli	2 (0.4)	0	1 (0.3)	n/a
Klebsiella sp.	1 (0.2)	0	1 (0.3)	n/a
Possible Pathogens	4 (0.7)	2 (1.3)	2 (0.5)	2.48 (0.35-17.8)
Viridans streptococcus	4 (0.7)	2 (1.3)	2 (0.5)	2.48 (0.35-17.8)
CONTAMINANTS	24	2 (1.3)	20 (5.3)	0.24 (0.06-1.02)
Coagulase Negative Staphylococcus	22 (4.1)	1 (0.6)	19 (5.6)	**0.12 (0.02-0.93)**
Bacillus	2 (0.4)	1 (0.6)	1 (0.3)	2.47 (0.15-39.8)

*- percentages calculated out of a total of all pathogens

Footnote: 1 patient grew *Enterococcus faecalis+ Staphylococcus aureus + Klebsiella pneumoniae*

Table 3.25: Predictors of culture-confirmed sepsis in neonates with community acquired serious bacterial infection

	Culture-Positive sepsis N = 67	Culture-Negative sepsis N = 569	Univariate analysis		Multivariate analysis	
	n/N (%)	n/N (%)	OR (95%CI)	p-value	aOR (95% CI)	p-value
Characteristics						
HIV Exposure	27/65 (41.5)	147/560 (26.2)	2.00 (1.18-3.38)	0.009	1.38 (0.50-3.83)	0.538
Vaginal delivery	59/67 (88.1)	462/569 (81.2)	1.71 (0.79-3.68	0.167	2.10 (0.57-7.75)	0.264
Low birth weight	13/67 (19.4)	77/569 (10.7)	1.54 (0.80-2.95)	0.192	1.24 (0.40-3.86)	0.709
Male sex	35/67 (52.2)	343/569 (60.3)	0.72 (0.43-1.20)	0.205	N/A	N/A
Apgar at 5 min <7	10/67 (14.9)	61/569 (10.7)	1.46 (0.71-3.01)	0.301	N/A	N/A
General Signs						
Lethargy	11/65 (16.9)	60/565 (10.6)	1.71 (0.85-3.46)	0.128	3.72 (0.29-47.7)	0.787
Irritability	21/66 (31.8)	119/565 (21.1)	1.75 (1.00-3.05)	0.047	0.79 (0.25-2.50)	0.685
Jaundice	27/65 (41.5)	275/559 (49.2)	0.73 (0.44-1.23)	0.242	N/A	N/A
Fever	11/67 (16.4)	59/569 (10.4)	1.69 (0.84-3.42)	0.135	0.63 (0.14-2.75)	0.537
Hypothermia	5/67 (7.5)	35/569 (6.2)	1.23 (0.46-3.25)	0.676	N/A	N/A
Central nervous system						
Apnoea	5/66 (7.6)	28/564 (5.0)	1.57 (0.58-4.21)	0.368	N/A	N/A
Seizures	10/66 (15.2)	22/564 (3.9)	4.40 (1.98-9.76)	<0.001	1.06 (0.16-7.03)	N/A
Hypotonia	8/66 (12.1)	19/563 (3.4)	3.95 (1.66-9.42)	<0.001	1.01 (0.09-11.74)	0.993
Gastrointestinal tract						
Poor feeding	12/59 (20.3)	48/527 (9.1)	2.55 (1.26-5.13)	0.007	2.71 (0.72-10.26)	0.141
Abdominal distension	11/66 (16.7)	90/565 (15.9)	1.06 (0.53-2.09)	0.877	N/A	N/A
Cardiorespiratory system						
Tachypnoea (RR>60/min)	16/67 (23.9)	115/569 (20.2)	1.24 (0.68-2.25)	0.482	N/A	N/A
Chest wall indrawing	13/66 (19.7)	122/562 (21.7)	0.88 (0.47-1.68)	0.707	N/A	N/A
Required ventilation	5/65 (7.7)	5/563 (1.07)	7.74 (2.29-26.1)	0.001	>99 (<0.01->99.9)	0.979
Hypotension	4/65 (6.2)	5/536 (0.93)	6.96 (1.82-26.6)	0.001	>99 (<0.01->99.9)	0.980

Table 3.25 (continued): Predictors of culture-confirmed sepsis in neonates with community acquired serious bacterial infection

	Culture-Positive sepsis N = 67	**Culture-Negative sepsis N = 569**	**Univariate analysis**		**Multivariate analysis**	
	n/N (%)	n/N (%)	**OR (95%CI)**	**p-value**	**aOR (95% CI)**	**p-value**
Metabolic						
Hypoglycaemia	1/66 (1.5)	8/563 (1.4)	1.07 (0.13-8.67)	0.951	N/A	N/A
Hyperglycaemia	6/66 (9.1)	34/563 (6.0)	1.56 (0.63-3.86)	0.336	N/A	N/A
Metabolic acidosis	3/66 (4.6)	14/559 (2.5)	1.85 (0.52-6.62)	0.335	N/A	N/A
Laboratory findings						
Leukopenia (WCC<5.0x10^9)	6/66 (9.1)	22/549 (1.8)	2.40 (0.93-6.14)	0.061	1.27 (0.17-9.61)	0.820
Leukocytosis (WCC>25.0x10^9)	1/66 (1.5)	10/549 (1.8)	0.83 (0.11-6.58)	0.859	N/A	N/A
Neutropenia	16/64 (25.0)	95/518 (18.3)	1.48 (0.81-2.73)	0.201	N/A	N/A
Thrombocytopenia	9/67 (13.40	44/553 (8.00	1.80 (0.83-3.86)	0.130	1.26 (0.29-5.43)	0.758
High CRP (>10)	10/33 (30.3)	44/302 (14.6)	2.54 (1.14-5.72)	0.020	2.45 (0.73-8.27)	0.150

Incidence of community acquired sepsis

We evaluated the incidence (per 1000 live births) of CA-pSBI, protocol defined CAS, and culture confirmed CAS. The live birth cohort for the study area during this period was 34 808. The incidence estimate was adjusted by a factor of 1.8, to adjust for non-enrolment over weekends and refusal of consent by mothers of some eligible neonates (**Figure 3.2**). The adjusted incidence of CA-pSBI was 33.4, that of protocol defined CAS 7.21, and that of blood culture-confirmed sepsis 2.90 and that of culture-confirmed meningitis 0.92 (**Table 3.26**). HIV-exposed compared to HIV-unexposed neonates were more likely to have protocol defined CAS (OR: 2.01; 95% CI 1.55-2.59), blood-culture confirmed CAS (OR: 2.07; 95% CI 1.39-3.09) and culture-confirmed meningitis (OR: 2.26; 95% CI 1.19-4.13).

Table 3.26: Incidence of community acquired possible serious bacterial, protocol-defined and culture-confirmed sepsis

	Number Enrolled	Numbers after Adjusting for Non-enrolment	Live births	Incidence* (95% CI)	HIV- exposed vs HIV-unexposed OR (95% CI)	Adjusted Incidence* (95% CI)	HIV-exposed vs HIV-unexposed aOR (95% CI)
Community acquired possible bacterial infection							
All	636	1164	34808	18.2 (16.8-19.6)		33.4 (31.6-35.4)	
HIV exposed	174	318	9400	18.5 (16.0-21.4)		33.8 (30.3-37.7)	
HIV unexposed	451	825	25408	17.6 (16.2-19.4)	1.04 (0.87-1.24)	32.5 (30.8-34.7)	1.04 (0.92-1.19)
Protocol defined community acquired sepsis							
All	137	251	34808	3.94 (3.33-4.65)		7.21 (6.38-8.16)	
HIV exposed	57	104	9400	6.06 (4.68-7.86)		11.1 (9.14-13.4)	
HIV unexposed	77	141	25408	3.03 (2.42-3.79)	**2.01 (1.42-2.83)**	5.55 (4.71-6.54)	**2.01 (1.55-2.59)**
Blood and/or CSF culture-confirmed community acquired sepsis							
All	67	123	34808	1.92 (1.52-2.44)		3.53 (2.96-4.22)	
HIV exposed	27	49	9400	2.87 (1.97-4.19)		5.21 (3.94-6.89)	
HIV unexposed	38	70	25408	1.50 (1.09-2.06)	**1.92 (1.17-3.15)**	2.76 (2.18-3.48)	**1.90 (1.32-2.74)**
Blood culture-confirmed community acquired sepsis							
All	55	101	34808	1.52 (1.21-2.06)		2.90 (2.39-3.53)	
HIV exposed	23	42	9400	2.45 (1.63-3.68)		4.47 (3.30-6.04)	
HIV unexposed	30	55	25408	1.18 (0.83-1.69)	**2.08 (1.21-3.57)**	2.17 (1.66-2.82)	**2.07 (1.39-3.09)**

Table 3.26 (continued): Incidence of community acquired possible serious bacterial, protocol-defined and culture-confirmed sepsis

	Number Enrolled	Numbers after Adjusting for Non-enrolment	Live births	Incidence* (95% CI)	HIV-exposed vs HIV-unexposed OR (95% CI)	Adjusted Incidence* (95% CI)	HIV-exposed vs HIV-unexposed aOR (95% CI)
CSF culture-confirmed community acquired sepsis							
All	23	42	34808	0.67 (0.44-0.99)		0.92 (0.65-1.30)	
HIV exposed	10	18	9400	1.06 (0.57-1.97)		1.60 (0.96-2.65)	
HIV unexposed	12	22	25408	0.47 (0.27-0.83)	2.25 (0.97-5.22)	0.63 (0.39-1.03)	**2.21 (1.19-4.13)**

* - per 1000 live births

Footnote:

Adjustment for non-enrolment was calculated by multiplying numbers enrolled by factor 1.8 derived from: (Number admitted with possible serious bacterial infection/ Number screened) x (Number eligible/Number enrolled)

Incidence of common bacterial infection in neonates with CAS

The incidences of the most common bacterial infections are illustrated in **Table 3.27**. The adjusted incidence (per 1000 live births) of *Staphylococcus aureus*, *Viridans streptococcus*, GBS and *E. coli* CAS were 0.95, 0.90, 0.75 and 0.58, respectively. HIV-exposed neonates had a higher incidence of *Staphylococcus aureus* (OR: 2.71; 95% CI 1.35-5.41), *Viridans streptococcus* (OR: 2.41; 95% CI 1.39-4.15), GBS (OR: 4.81; 95% CI 2.13-10.9) and *E. coli* (OR: 3.16; 95% CI 1.06-9.39) identified on blood and/or CSF culture than HIV-unexposed neonates.

Table 3.27: Incidence of *Staphylococcus aureus*, *Viridans streptococcus*, *Group B streptococcus* and *Escherichia coli* in neonates with culture confirmed (blood and/or cerebrospinal fluid) community acquired infection

	Numbers Enrolled	Numbers after Adjusting for Non-enrolment	Live births	Incidence* (95% CI)	HIV-exposed vs HIV-unexposed OR (95% CI)	Adjusted Incidence (95% CI)	HIV- exposed vs HIV-unexposed aOR (95% CI)
Staphylococcus aureus							
All	18	33	34808	0.52 (0.33-0.82)		0.95 (0.67-1.33)	
HIV exposed	9	16	9400	0.96 (0.50-1.84)		1.70 (1.04-2.78)	
HIV unexposed	9	16	25408	0.35 (0.18-0.68)	**2.71 (1.07-6.81)**	0.63 (0.39-1.03)	**2.71 (1.35-5.41)**
Viridans streptococcus							
All	17	31	34808	0.49 (0.30-0.79)		0.90 (0.63-1.27)	
HIV exposed	5	9	9400	1.02 (0.42-2.45)		1.84 (0.96-3.53)	
HIV unexposed	11	20	25408	0.43 (0.24-0.78)	2.36 (0.82-6.79)	0.79 (0.51-1.22)	**2.41 (1.39-4.15)**
Group B *streptococcus*							
All	14	26	34808	0.40 (0.24-0.68)		0.75 (0.51-1.10)	
HIV exposed	9	16	9400	0.96 (0.50-1.84)		1.70 (1.04-2.78)	
HIV unexposed	5	9	25408	0.20 (0.08-0.47)	**4.87 (1.63-14.5)**	0.35 (0.18-0.68)	**4.81 (2.13-10.9)**
Escherichia coli							
All	11	20	34808	0.32 (0.18-0.57)		0.58 (0.37-0.89)	
HIV exposed	5	9	9400	0.53 (0.22-1.28)		2.71 (1.07-6.82)	
HIV unexposed	5	9	25408	0.20 (0.08-0.47)	2.71 (0.78-9.34)	0.35 (0.18-0.68)	**2.71 (1.07-6.81)**

* - per 1000 live births

Footnote: Adjustment for non-enrolment was calculated by multiplying numbers enrolled by factor 1.8 derived from: (Number admitted with possible serious bacterial infection/ Number screened) x (Number eligible/Number enrolled)

Susceptibility of bacteria isolated in neonates with CA culture-confirmed sepsis

Among CA-pSBI in whom putative bacteria were cultured, the median time for detecting growth on the BactT-Alert system was 7 hours. In isolates from CA-pSBI, the median times to growth were shorter for *Enterococcus sp., GBS and Staphylococcus aureus* when compared to those of *E. Coli* or *Viridans streptococcus* (**Table 3.28**). All GBS isolates were susceptible to penicillin, ampicillin, clindamycin and cefotaxime; and 93.3% were sensitive to macrolides (**Table 3.29**). Only 11% of *E. coli* were susceptible to ampicillin but all were susceptible to gentamicin and cefotaxime. *Staphylococcus aureus* were all susceptible to cloxacillin and 83% were susceptible to gentamicin.

Table 3.28: Time to growth for common pathogens isolated from neonates with culture-confirmed community acquired sepsis

		Time to growth (hours)	
	Number	Mean ± SD	Median (25-75th Centile)
Enterococcus species	3	4.6 ± 1.7	2.0 (2.0 - 10.0)
Group B streptococcus	15	5.5 ± 2.9	5.0 (3.0 - 8.0)
Staphylococcus aureus	12	9.3 ± 2.9	6.0 (4.0 - 8.0)
Escherichia coli	9	6.8 ± 4.1	9.5 (7.0 - 11.0)
Viridans streptococcus	12	14.1 ± 6.8	13.0 (7.0 - 19.0)
All	49	8.6 ± 5.4	7.0 (5.0 - 11.0)

Table 3.29: Antimicrobial susceptibility of pathogens isolated from blood and/or cerebrospinal fluid in neonates with community acquired sepsis

	Number	Pen N (%)	Ampi N (%)	Cloxa N (%)	Erythro N (%)	Clinda N (%)	Genta N (%)	Cefotax N (%)
Group B Streptococcus	15	15 (100)	15 (100)	NT	14 (93.3)	15 (100)	NT	14/14 (100)
Escherichia coli	9	NT	1 (11.1)	NT	NT	NT	9 (100)	9 (100)
Staphylococcus aureus	12	NT	2 (16.7)	12 (100)	10 (83.3)	NT	10 (83.3)	NT
Enterococcus faecalis	2	NT	2 (100)	NT	NT	NT	NT	NT
Enterococcus faecium	1	NT	1 (100)	NT	0	NT	NT	NT
Klebsiella species	2	NT	0	NT	NT	NT	2 (100)	2 (100)
Enterobacter species	1	NT	0	NT	NT	NT	1 (100)	1 (100)
Viridans streptococcus	12	NT	8/10 (80)	NT	6/10 (60)	NT	NT	NT

Pen=penicillin; Ampi-Ampicillin; Cloxa- Cloxacillin; Erythro- Erythromycin; Clinda-Clindamycin; Genta- Gentamicin; Cefotx- Cefotaxime; NT- Not tested

Mortality in neonates with community acquired sepsis

Overall, the case fatality rate for CA-pSBI was 1.4% (9/636). The case fatality rate was 10.5-fold (95% CI 2.30-48.0) higher among culture-confirmed CAS (6.0%) than pSBI-CAS cases who did not fulfil the protocol-defined criteria for sepsis (0.6%) (**Table 3.30)**. We did not undertake a risk factor analysis for predictors of mortality among pSBI-CAS cases due to the small number of deaths observed in neonates with CAS as noted above.

Table 3.30: Case fatality rate among neonates with community acquired sepsis

Sepsis category	Total	Died n (%)	Survived n (%)	OR (95% CI)
Possible serious bacterial infection NOT fulfilling protocol defined sepsis criteria	499	3 (0.6)	496 (99.4)	REF
Protocol-defined community acquired sepsis but culture negative	70	2 (2.9)	68 (97.1)	4.86 (0.80 – 29.6)
Culture confirmed community acquired sepsis	67	4 (6.0)	63 (94.0)	10.5 (2.30 – 48.0)

3.2 Examining Strength of Attribution of Causality of Detected Putative Pathogens Using Taqman Array Card on Blood, Nasopharyngeal and Oropharyngeal Samples

3.2.1 Early-onset sepsis

A total of 4045 newborn infants with ages less than 3 days were hospitalized for EO-pSBI during the study period, 3323 of whom were screened for eligibility from Mondays to Fridays. Among the screened neonates, 310 (9.3%) were ineligible for enrolment. Reasons included mother being <18 years age (n=147/310; 47.4%) and therefore unable to consent for her child. Among the 3013 eligible neonates, 389 (12.9%) were not enrolled, the commonest reason being mother's unwillingness to participate in the study (n=208; 53.5%) (**Figure 3.3**). Consequently, 2624 neonates with EO-pSBI who had blood cultures done as part of standard care were consented and enrolled into the study. Of these, 1231 (47%) met the protocol-defined criteria for EOS, of whom 1223 had blood and/or NPOP taken, 914 had both blood and NPOP samples, 19 only blood sample and 290 had only NPOP swabs taken for molecular testing using TAC. Overall, among those who fulfilled protocol-defined criteria for EOS, 933 (75.9%) had blood samples and 1204 (97.9%) had NPOP swabs taken for testing using TAC (**Figure 3.3**). Maternal HIV status was known in 96% of those who had blood taken and 95.4% of those who had NPOP swabs taken for TAC, among whom the prevalence of HIV-exposure was 33.0% and 32.5%, respectively .

Among the 23 228 well neonates born in hospital, 383 were screened for eligibility as controls, 15 (3.92%) of whom were excluded due to the mother being <18 years age. Furthermore, an additional 56 were not enrolled, 46 (82.3%) of whom refused consent. Consequently, a total of 312 well neonates were enrolled as controls. Among the 312 enrolled controls, 298 had both blood and NPOP swab samples, 6 only blood sample and 5 only NPOP swabs (**Figure 3.4**). Maternal HIV status was known in 99% of neonates, of whom 32.0% were HIV exposed.

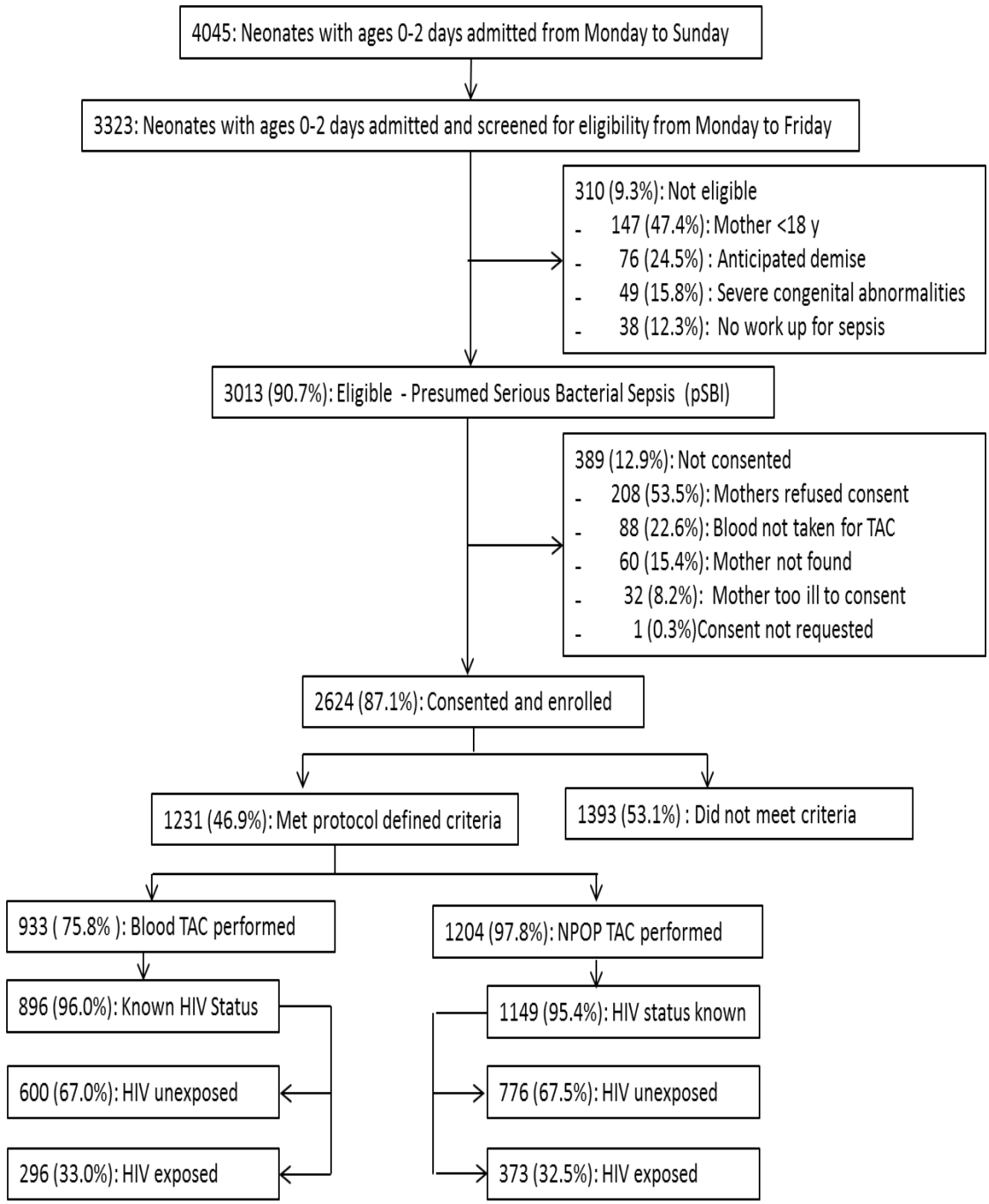

Figure 3.3: Neonates with early-onset presumed serious bacterial infection who met criteria for protocol defined sepsis and had specimens for Taqman array card collected (cases)

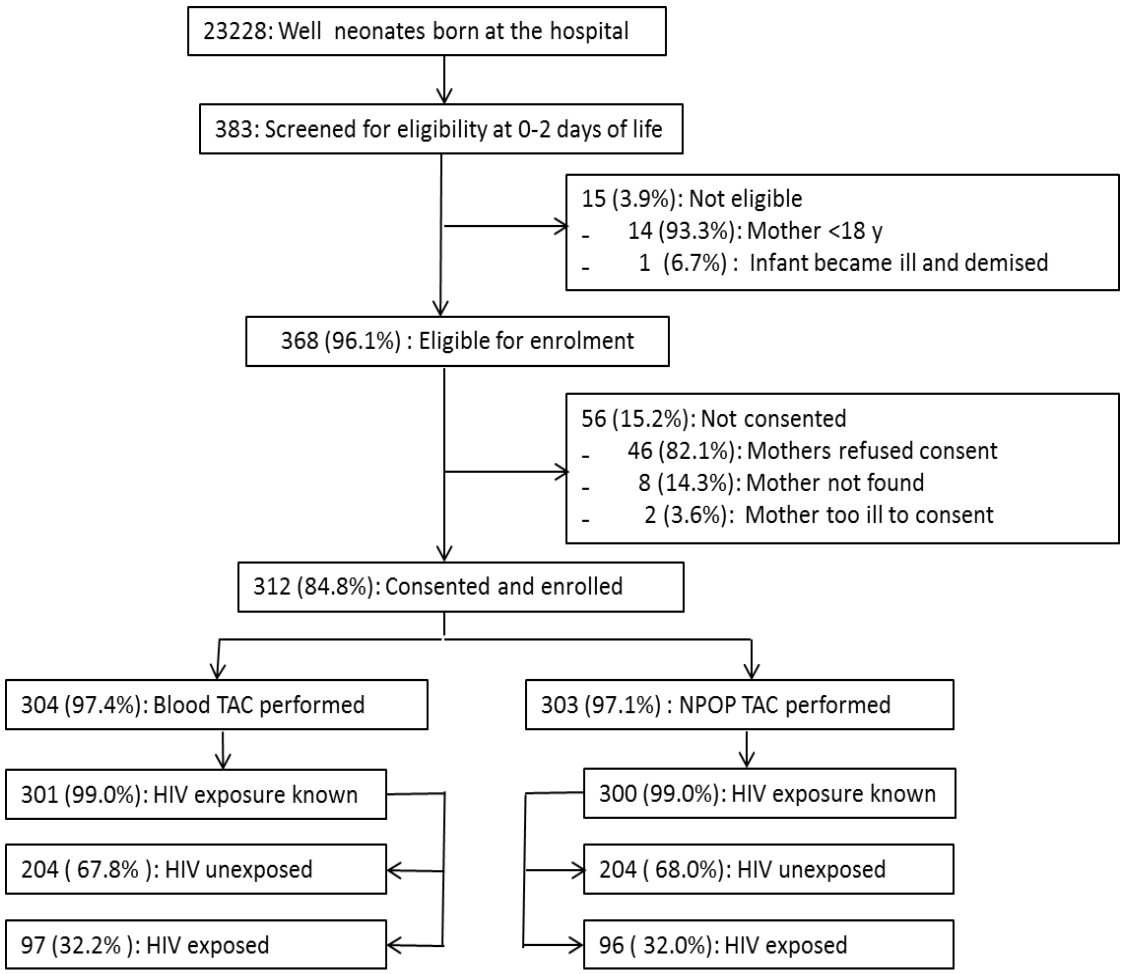

Figure 3.4: Healthy neonates with ages <3 days who were enrolled as controls

Comparing cases with protocol defined early-onset sepsis and controls aged <3 days

a. Characteristics of cases and controls

Maternal and infant characteristics of cases with protocol-defined EOS, and controls with ages <3 days, tested using TAC in blood and/or NPOP swabs is shown in **Table 3.31.** More than 90% of mothers of both cases and controls attended antenatal care, and delivered in a healthcare facility, although a lower percentage of mothers of cases than controls had attended antenatal care (93.4% vs 98.3%, p<0.001), delivered vaginally (43.9% vs 69.1%, p<0.001) and delivered in a healthcare facility (96.9% vs 99.3%, p=0.021). There were more cases born through meconium stained amniotic fluid than controls (22.6% vs 11.4%, p<0.001). Conversely, cases compared to controls were more likely to be male (55.3% vs 48.5%, p=0.042), born preterm (45.4% vs 6.7%, p<0.001), were of low birth weight (54.6 % vs 9.40%, p<0.001), and had an Apgar score <7 at 5 minutes (18.4% vs 5.4%, p<0.001). Approximately one-third of mothers from both cases and controls were HIV infected. A

higher percentage of cases were enrolled on day 0 of life than controls (84.4% vs 19.5%) ($p<0.001$).

Table 3.31: Characteristics of neonates with early-onset protocol-defined sepsis (cases) and healthy neonates with ages <3 days (controls) who had blood and/or pharyngeal swabs tested using the Taqman array card

	Cases n = 1223	**Controls n = 309**	**OR (95% CI)**	**p-value**
Maternal Factors				
Antenatal care visit	1130/1214 (93.1)	302/307 (98.4)	0.22 (0.09-0.55)	**<0.001**
Vaginal delivery	553/1223 (45.2)	214/309 (69.3)	0.37 (0.28-0.48)	**<0.001**
Healthcare facility birth	1176/1220 (96.4)	306/308 (99.4)	0.18 (0.04-0.72)	**0.007**
HIV infected	384/1168 (32.9)	99/306 (32.4)	1.02 (0.78-1.34)	0.862
Prolonged rupture of membranes	59/1214 (4.9)	22/308 (7.1)	0.66 (0.40-1.10)	0.111
Meconium stained amniotic fluid	275/1216 (22.6)	35/307 (11.4)	2.27 (1.56-3.31)	**<0.001**
Intrapartum antibiotics	101/1215 (8.3)	27/307 (8.8)	0.94 (0.60-1.47)	0.786
Infant Factors				
Preterm (<37 weeks)	574/1218 (47.1)	20/308 (6.5)	12.8 (8.05-20.5)	**<0.001**
Male	668/1222 (54.7)	151 (49.0)	0.78 (0.62-1.02)	0.072
Low birth weight	682/1223 (55.8)	28/309 (9.1)	12.6 (8.44-19.0)	**<0.001**
Apgar score<7 at 5 minutes	251/1223 (20.5)	16/309 (5.2)	4.75 (2.82-8.01)	**<0.001**
Age at enrolment				
Day 0	1027 (84.0)	60 (19.4)		**<0.001**
Day 1-2	196 (16.0)	249 (80.6)		
Median age at enrolment	0 (0-0)	1 (1 - 1)		**<0.001**

* - Missing data accounted for <5% for each variable and for both cases and controls

b. Detection of organisms in blood and pharyngeal swabs in cases with early-onset sepsis and controls of ages <3 days using the Taqman Array Card

Blood Taqman analysis

Among the 933 neonates with protocol defined EOS with available blood sample for testing by TAC, 346 (37.1%) had at least one organism detected, which was 2.35 fold (95% CI 1.72-3.21) greater compared to than in controls (19.8%; p<0.001) (**Table 3.32**). The positivity rate among cases were similar for those investigated on day 0 (37.9%) and day 1-2 (32.6%), both of which were greater than observed among controls at those time-periods. Although the majority of cases (70.5%) and controls (86.9%) had only one organism detected per sample, concurrent detection of >1 organism per sample was more common in cases (29.5%) than controls (13.1%; p=0.028). There were no differences in HIV exposure prevalence between cases and controls overall, or among those with a TAC positive result.

Naso/oro-pharyngeal swab Taqman analysis

A total of 531 (44.1%) of 1204 cases with protocol defined EOS with NPOP swabs tested had at least one organism detected on the NPOP swab TAC assay, which was however lower than observed among controls (53.4%; OR: 0.67, 95% CI 0.52-0.88) (**Table 3.32**). There was no difference in positivity rates between cases (43.4%) and controls (48.3%) on day 0 of life, while fewer cases had a positive NPOP TAC result on days 1-2 (44.1%) than controls (54.6%; OR: 0.66, 95% CI 0.43-0.99; p=0.046). There was no difference in HIV exposure prevalence between cases and controls overall, or among those with a TAC positive result.

Table 3.32: Detection of organisms using Taqman array card in blood and pharyngeal swabs in neonates with protocol defined sepsis (cases) and healthy neonates <3 days (controls)

	Cases n (%)	Controls n (%)	OR (95% CI)	p-value
Total number of neonates with protocol defined early-onset sepsis	1231	312		
Blood				
Total number with blood taken for Taqman Array Card	933	304		
Total number with positive Taqman Array Card in blood	346/933 (37.1)	61/304 (19.5)	2.35 (1.72-3.21)	
Number with blood collected according to age at enrolment				<0.001
Day 0	789 (84.6)	58 (19.1)		
Day 1-2	144 (15.4)	246 (80.9)		
Number with positive Taqman Array Card according to age of enrolment				
Day 0	297/789 (37.6)	14/58 (24.1)	1.90 (1.02-3.52)	
Day 1-2	49/144 (34.0)	47/246 (19.1)	2.18 (1.37-3.49)	
Number of organism per sample in those with positive Taqman Array Card				0.028
1 organism	244 (70.5)	53 (86.9)		
2 organisms	83 (24.0)	7 (11.5)		
≥3 organisms	19 (5.5)	1 (1.64)		
Exposure to human immunodeficiency virus				
Number exposed amongst those with blood collected	296/896 (33.0)	97/301 (32.2)	1.04 (0.79-1.37)	
Number exposed among those positive for Taqman Array Card	109/332 (32.8)	22/61 (36.1)	0.87 (0.49-1.53)	
Pharyngeal Swabs				
Total number with swabs taken for Taqman Array Card	1204	303		
Total number with positive Taqman Array Card in pharyngeal swabs	531/1204 (44.1)	161/303 (53.1)	0.69 (0.54-0.90)	
Number with swabs collected according to age at enrolment				<0.001
Day 0	1009 (83.8)	60 (19.8)		
Day 1-2	195 (16.2)	243 (80.2)		
Number with positive Taqman Array Card according to age of enrolment				
Day 0	444/1009 (44.0)	30/60 (50.0)	0.79 (0.47-1.32)	
Day 1-2	87/195 (44.6)	134/243 (55.1)	0.66 (0.45-0.96)	

Table 3.32 (continued): Detection of organisms using Taqman array card in blood and pharyngeal swabs in neonates with protocol defined sepsis (cases) and healthy neonates <3 days (controls)

	Cases n (%)	**Controls n (%)**	**OR (95% CI)**	**p-value**
Number of organism per sample in those with positive Taqman Array Card				0.696
1 organism	354 (66.7)	107 (65.2)		
2 organisms	142 (26.7)	43 (26.2)		
≥3 organisms	35 (6.6)	14 (8.5)		
Exposure to human immunodeficiency virus (HIV)				
Number exposed among those with swabs taken and tested for HIV	373/1149 (32.5)	96/300 (32.0)	1.02 (0.78-1.34)	
Number exposed among those with positive Taqman Array Card	175/507 (34.5)	51/161 (31.7)	1.14 (0.78-1.66)	

c. *Organisms detected in blood of cases with protocol defined early-onset sepsis and controls with ages <3 days using Taqman Array Card*

The common organisms detected in the blood of protocol-defined EOS cases using TAC were *Streptococcus pneumoniae* (14.2%), *Ureaplasma urealyticum/ parvum* (9.2%); *Pseudomonas aeruginosa* (8.2%), GBS (7.0%) and *Klebsiella pneumoniae* (3.7%) (**Figure 3.5**). In controls, similar organisms were detected by TAC, but the prevalence differed for some compared to in cases. Organisms with a higher prevalence in cases than controls included Ureaplasma *urealyticum/ parvum* (9.2% vs 2.0%; OR: 4.03, CI 95% 1.72-9.43), *Pseudomon*as *aeruginosa* (8.2% vs 2.0%; OR: 3.81; 95% CI 1.63-8.95), and GBS (7.0% vs 1.6%; OR: 3.63, 95% CI 1.42-9.23). There were, however, no statistical significant differences between cases and controls in the prevalences of detection of *Streptococcus pneumoniae* (14.2% vs 9.9%, p=0.177), *Staphylococcus aureus* (1.3% vs 0.3%, p=0.298); *E. coli* (2.2% vs 1.3%, p=0.907); *Klebsiella species* (3.8% vs 2.6%, p=0.769); *Salmonella species* (3.1% vs 2.3%, p=0.511) and *Neisseria meningitides* (1.1% vs 1.0%, p=0.882).

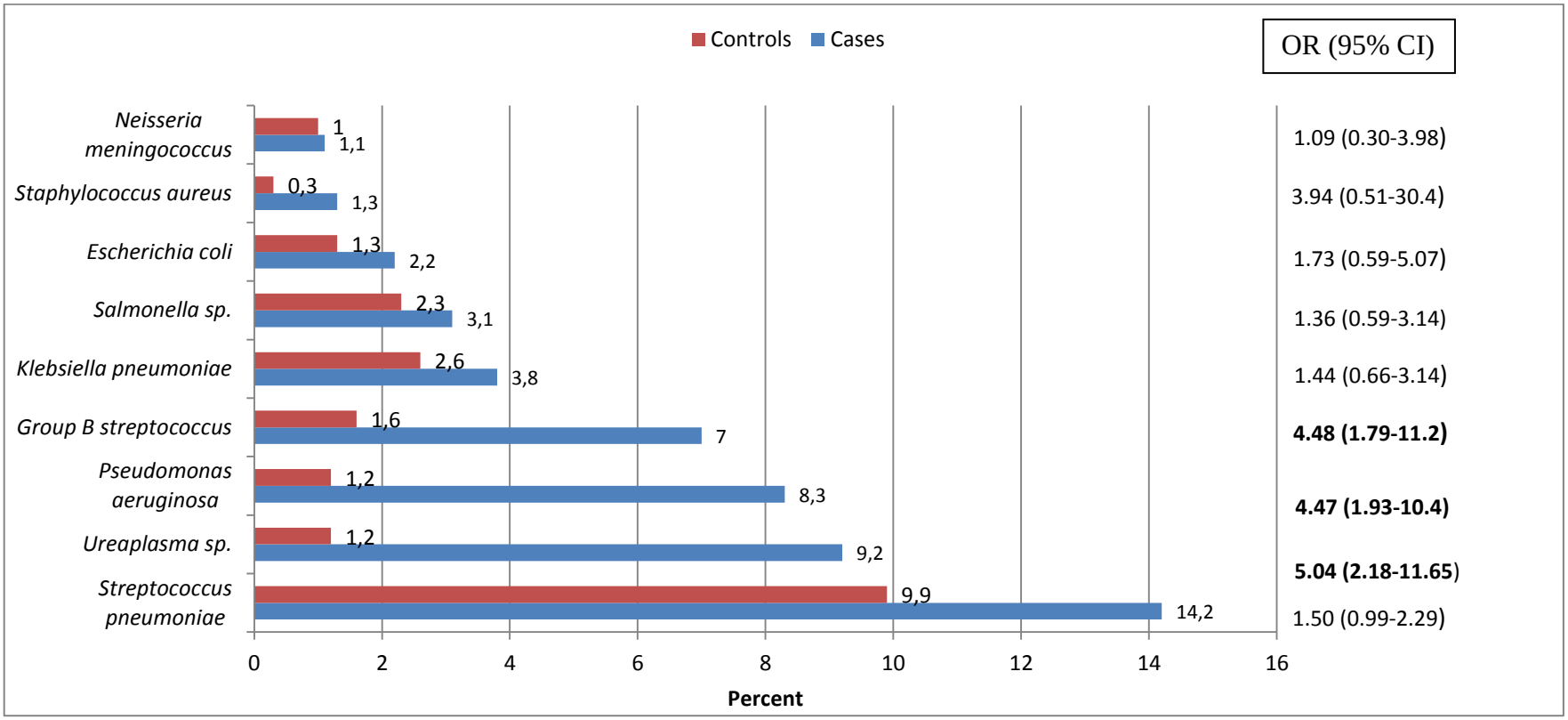

Figure 3.5: Organisms detected using Taqman array card in blood in neonates with early-onset protocol-defined sepsis (cases) and healthy neonates of ages <3 days (controls)

d. Organisms detected in naso/oro-pharyngeal swabs in neonates with early onset sepsis and controls aged <3 days using the Taqman Array Card

The common bacterial pathogens detected on NPOP swabs of cases were *Ureaplasma urealyticum/ parvum* (19.9%), Klebsiella *pneumoniae* (11.9%), GBS (8.5%) and *E. coli* (8.3%) (**Figure 3.6**); whilst Cytomegalovirus (5.7%) and Enterovirus (1.4%) were the most commonly detected viruses. A number of organisms were, however, detected less commonly in cases than controls, including *E. coli* (8.3% vs 18.2%; OR: 0.37, 95% CI 0.25-0.55), *Streptococcus pneumoniae* (2.2% vs 5.0%; OR: 0.40, 95% CI 0.20-0.79), GBS (8.5 % vs 13.2%; OR: 0.54; 95% CI 0.35-0.81) and *Enterovirus* (1.4% vs 8.6%; OR: 0.13, 95% CI 0.06-0.25). *Ureaplasma urealyticum/ parvum* was the only organism detected with a higher frequency among cases (19.9%) than controls (14.5%; OR: 1.69, 95% CI 1.17-2.44). Organisms detected among cases with similar prevalence compared to controls included *Bordetella pertussis* (0.7% vs 0.30%, p = 0.226), *Klebsiella pneumoniae* (11.9% vs 10.2%, p = 0.059), Rhinovirus (0.7% vs 1.0%, p = 0.353), Cytomegalovirus (5.7% vs 5.9%, p = 0.523), Human parechovirus (0.3% vs 0.3%, p = 0.800), Human metapneumovirus (0.4% vs 0.3%, p = 0.574) and RSV (0.2% vs 1.0%, p = 0.165*). Rubella virus, Chlamydia pneumoniae*, *Chlamydia trachomatis* and Parainfluenza A virus were not detected among controls, and had a low prevalence (<1.0%) among cases, **Figure 3.6**.

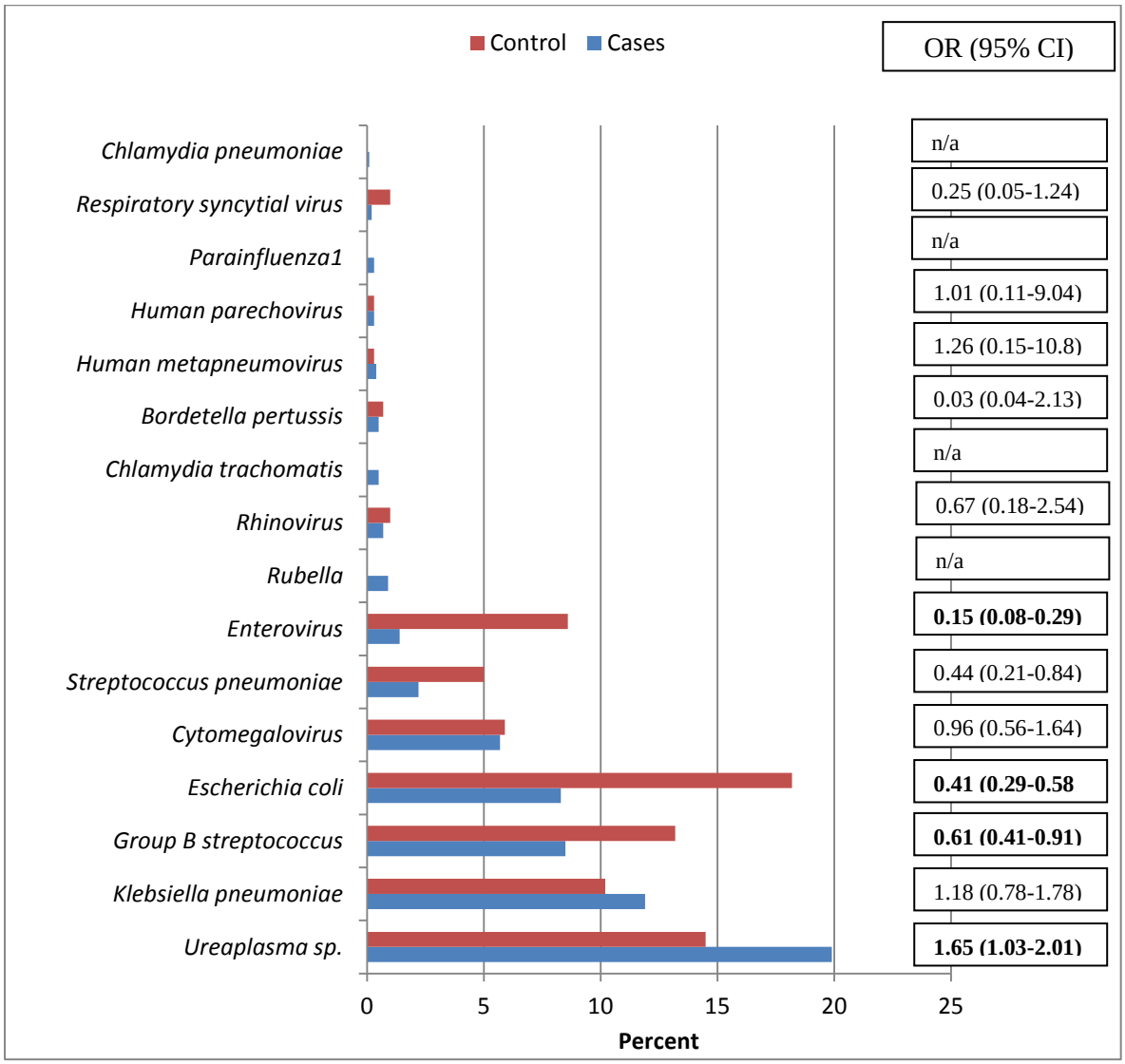

Figure 3.6: Organisms detected in pharyngeal swabs using the Taqman array card in neonates with early-onset protocol-defined sepsis (cases) and healthy neonates aged <3 days (controls)

e. Detection of organisms in blood and pharyngeal swabs from neonates with early-onset sepsis and controls with ages <3 days stratified according to HIV exposure

Blood

Similarly to the overall group, positivity prevalence on blood TAC was higher among cases than controls in both HIV-exposed (37.2% vs 19.1%; OR 2.50, 95% CI 1.70-3.68) and HIV-unexposed (36.8% vs 22.7%; OR 1.99, 95% CI 1.17-3.38) neonates. This also remained significantly so for Pseudomonas *aeruginosa* (8.0% vs 1.0%; OR: 8.30, CI 1.98-34.9), *Ureaplasma* species (8.2% vs 1.5%, OR: 5.19, 95% CI 1.56-17.2) and GBS (7.3% vs 2.0%; OR: 95% CI 1.56-17.2) among HIV-unexposed newborns, with similar trends observed in HIV-exposed neonates, though not statistically significant (**Table 3.33**). There were no significant differences in neonates with positive TAC in blood between HIV exposed and unexposed cases or controls.

Table 3.33: Organisms detected using Taqman array card in blood in neonates with early-onset protocol-defined sepsis (cases) and healthy neonates <3 days (controls) stratified according to HIV exposure

	HIV Unexposed			**HIV exposed**		
	Cases N = 600	**Controls N = 204**	**OR (95%CI)**	**Cases N = 296**	**Controls N = 97**	**OR (95%CI)**
	n (%)	n (%)		n (%)	n (%)	
Any organism detected	223 (37.2)	39 (19.1)	**2.50 (1.70-3.68)**	109 (36.8)	22 (22.7)	**1.99 (1.17-3.38)**
Pseudomonas aeruginosa	48 (8.0)	2 (1.0)	**8.30 (1.98-34.9)**	25 (8.4)	4 (4.1)	1.73 (0.56-5.29)
Ureaplasma species	49 (8.2)	3 (1.47)	**5.19 (1.56-17.2)**	30 (10.5)	3 (3.1)	3.04 (0.88-10.5)
Group B streptococcus	44 (7.3)	4 (2.0)	**3.13 (1.08-9.05)**	17 (5.7)	1 (1.0)	4.38 (0.56-34.2)
Neisseria meningitides	6 (1.0)	1 (0.5)	2.31 (0.27-20.2)	4 (1.4)	2 (2.1)	0.64 (0.11-3.70)
Staphylococcus aureus	9 (1.4)	1 (0.5)	2.16 (0.25-18.5)	2 (0.7)	0	n/a
Salmonella species	18 (3.0)	3 (1.5)	1.91 (0.55-6.69)	11 (3.7)	4 (4.1)	0.92 (0.28-3.04)
Streptococcus pneumoniae	89 (14.8)	20 (9.8)	1.39 (0.82-2.67)	38 (12.8)	10 (10.3)	1.22 (0.57-2.61)
Escherichia coli/ Shigella	16 (2.7)	4 (2.0)	1.33 (0.40-4.41)	4 (1.4)	0	n/a
Klebsiella pneumoniae	24 (4.0)	5 (2.4)	1.30 (0.47-3.61)	10 (3.4)	3 (3.1)	1.11 (0.29-4.22)
Enterovirus	2 (0.3)	1 (0.5)	0.44 (0.03-7.03)	0	0	N/A
Group A streptococcus	2 (0.3)	0	n/a	0	0	N/A
Haemophilus influenzae	2 (0.3)	0	n/a	3 (1.03)	0	0.75 (0.71 - 0.80)

Naso/oro-pharyngeal swabs

Overall there were fewer cases with positive TAC in NPOP swabs than in controls among the HIV unexposed neonates (42.8% vs 53.9%; OR: 0.64, 95% CI 0.46-0.87), and a similar trend was observed in HIV exposed neonates. For specific organisms, *E. coli* (8.2 % vs 15.2%; OR: 0.46, 95% CI 0.28-0.75), *Streptococcus pneumoniae* (2.2% vs 5.9%; OR: 0.32, 95% CI 0.15-0.71) and Enterovirus (1.2% vs 7.8%; OR: 0.11, 95% CI 0.05-0.28) were detected less commonly in cases than controls among HIV-unexposed neonates. Among HIV-exposed neonates, *Escherichia coli* (8.8% vs 24.0 %; OR: 0.26, 95% CI 0.14-0.51), GBS (6.4% vs 15.6%; OR: 0.34, 95% CI 0.16-0.73) and *Enterovirus* (2.1 % vs 10.4%; OR: 0.17, 95% CI 0.06-0.50) were also detected less frequently in cases than in controls. The only organism identified more frequently in cases than controls was *Ureaplasma urealyticum/ parvum* (19.0% vs 11.5%; OR: 2.08 (1.02-4.26), albeit only significant among HIV exposed neonates (**Table 3.34**).

Table 3.34: Organisms detected using Taqman array card in naso/oro-pharyngeal swabs in neonates with early-onset protocol-defined sepsis (cases) and healthy neonates with ages <3 days (controls) stratified according to HIV exposure

	HIV Unexposed			HIV Exposed		
	Cases N = 776	**Controls N = 204**	**OR (95%CI)**	**Cases N = 373**	**Controls N = 96**	**OR (95%CI)**
	n (%)	n (%)		n (%)	n (%)	
Positive TAC for any organism	332 (42.8)	110 (53.9)	**0.64 (0.46-0.87)**	175 (46.9)	51 (53.1)	0.78 (0.50-1.22)
Bacteria						
Escherichia coli/ Shigella	64 (8.2)	31 (15.2)	**0.46 (0.28-0.75)**	33 (8.8)	23 (24.0)	**0.26 (0.14-0.51)**
Streptococcus pneumoniae	17 (2.2)	12 (5.9)	**0.32 (0.15-0.71)**	8 (2.1)	3 (3.4)	0.61 (0.14-2.55)
Group B Streptococcus	75 (9.7)	24 (11.8)	0.67 (0.40-1.13)	24 (6.4)	15 (15.6)	**0.34 (0.16-0.73)**
Klebsiella pneumoniae	93 (12.0)	21 (10.3)	1.40 (0.82-2.38)	44 (11.8)	10 (0.4)	1.83 (0.80-4.19)
Ureaplasma sp.	157 (20.2)	33 (16.2)	1.46 (0.94-2.25)	71 (19.0)	11 (11.5)	**2.08 (1.02-4.26)**
Bordetella pertussis	6 (0.8)	0	n/a	0	2 (2.1)	n/a
Chlamydia pneumoniae	1 (0.1)	0	n/a	1 (0.3)	0	n/a
Chlamydia trachomatis	4 (0.5)	0	n/a	2 (0.5)	0	n/a
Mycoplasma pneumoniae	0	0	N/A	0	0	N/A
Viruses						
Enterovirus	9 (1.2)	16 (7.8)	**0.11 (0.05-0.28)**	8 (2.1)	10 (10.4)	**0.17 (0.06-0.50)**
Rhinovirus	3 (0.4)	2 (1.0)	0.84 (0.09-7.75)	3 (0.8)	1 (1.0)	0.26 (0.02-4.68)
Cytomegalovirus	30 (3.9)	9 (4.4)	0.90 (0.41-2.01)	37 (9.9)	8 (8.3)	2.16 (0.85-5.54)
Human parechovirus	1 (0.1)	1 (0.5)	0.23 (0.01-3.73)	3 (0.8)	0	n/a
Human metapneumovirus	3 (0.4)	1 (0.5)	0.80 (0.08-8.11)	1 (0.3)	0	n/a
Respiratory syncytial virus	3 (0.4)	1 (0.5)	0.86 (0.08-9.21)	0	2 (2.1)	n/a
Rubella virus	9 (1.2)	0	n/a	2 (0.5)	0	n/a
Parainfluenza virus 1	4 (0.5)	0	n/a	0	0	N/A
Parainfluenza virus 2	0	0	N/A	0	0	N/A
Parainfluenza virus 3	0	0	N/A	0	0	N/A
Adenovirus	0	0	N/A	0	0	N/A
Influenza A	0	0	_	0	0	N/A
Influenza B	0	0	_	0	0	N/A

3.2.2 Community acquired sepsis

Enrolment of neonates with community acquired sepsis (cases) and healthy neonates (controls) aged between 3 and 27 days tested with TAC

A total of 1371 neonates 3-27 days of age were hospitalized with physician-diagnosed pSBI, 919 (67.0%) of whom were screened for eligibility. One hundred and thirty seven of the screened neonates (14.9%) were ineligible for enrolment, common reasons being previous admission (n = 84; 61.3%), or the mother being <18 years of age (n=35, 25.6%). Among the 782 eligible neonates, 146 (18.7%) were not enrolled, with refusal to provide consent (n = 89; 61.0%) being the most common reason. Consequently, 636 neonates with CA-pSBI who had a blood culture done as part of standard care were enrolled into the study. Of these, only 137 (21.5%) fulfilled the protocol-defined criteria for CAS, among whom 135 had blood and/ or NPOP swab taken, 80 had both blood and NPOP samples collected, 3 had only blood sample and 52 only NPOP swabs collected. Overall, among those who fulfilled protocol-defined criteria for CAS, 83 (60.6%) had blood samples and 132 (96.4%) had NPOP swabs taken for TAC testing. Maternal HIV status was known for 80 (96.4%) of those who had blood and 128 (97.0%) of those with NPOP swabs taken for TAC. Among neonates whose maternal HIV status was known, 46.2% (37/80) of those with blood and 43.8% (56/128) of those with NPOP swabs available for TAC analysis were HIV exposed (**Figure 3.7**).

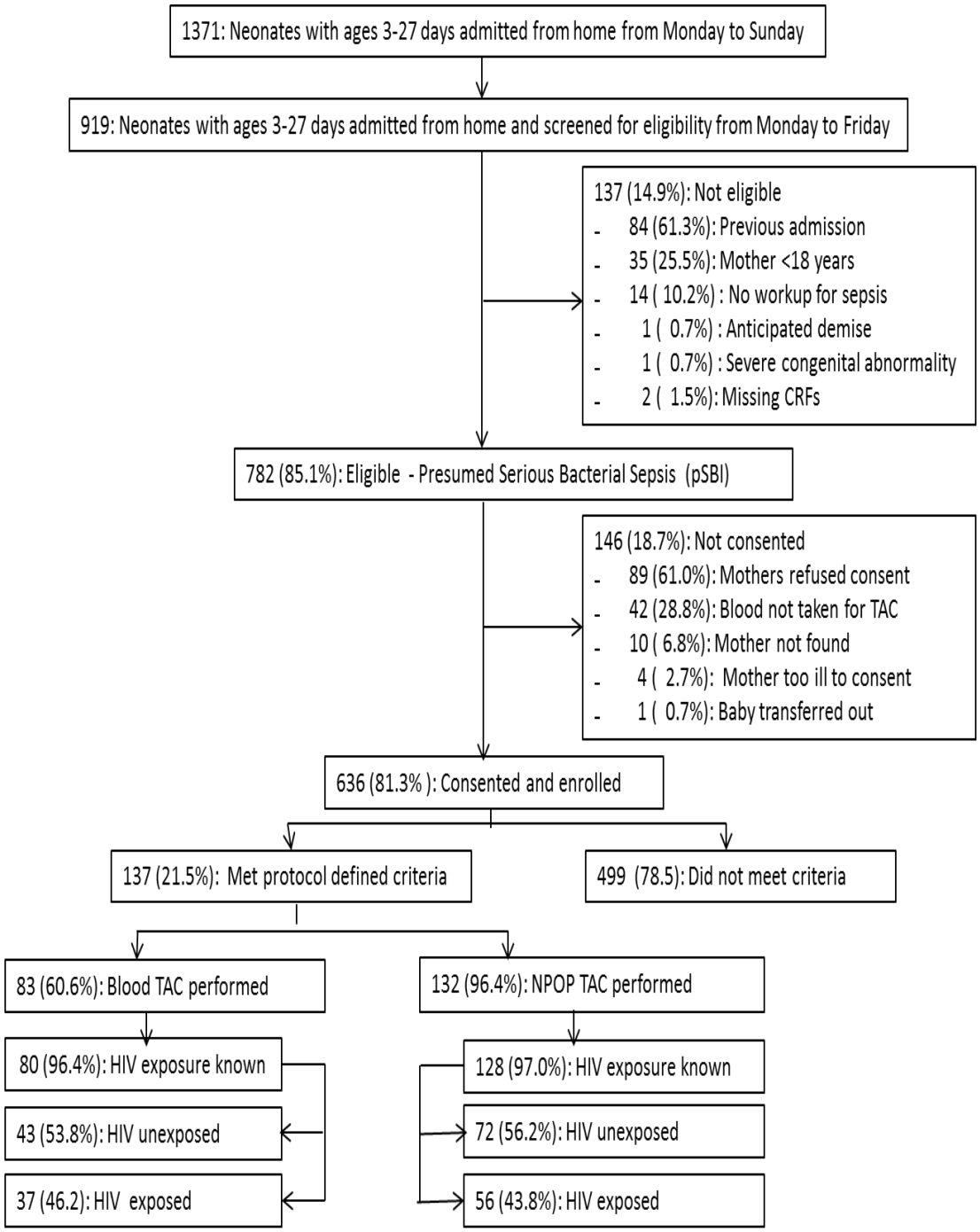

Figure 3.7: Neonates with community acquired possible serious bacterial infection and those who met criteria for testing with the Taqman array card

Among the 23,228 healthy neonates born in hospital, 195 were screened for eligibility as controls for the community acquired sepsis cases, of whom 192 were enrolled as controls at 3-27 days of age. Of these 192 enrolled controls, 185 had blood and/ or NPOP swabs taken, 178 had both blood and NPOP swab samples taken, 1 only had blood sample and 6 only had NPOP swab taken. Thus overall 179 (93.2%) healthy neonates had blood samples and 184 (95.8) had NPOP swabs taken for testing using TAC. Maternal HIV status was known for 177 (98.9%) of those who had blood and 182 (98.9%) of those with NPOP swabs taken for TAC. Among those infants with known maternal HIV status, 81 (45.8%) of those with blood and 86 (47.2%) of those with NPOP swabs taken for TAC were HIV-exposed (**Figure 3.8**).

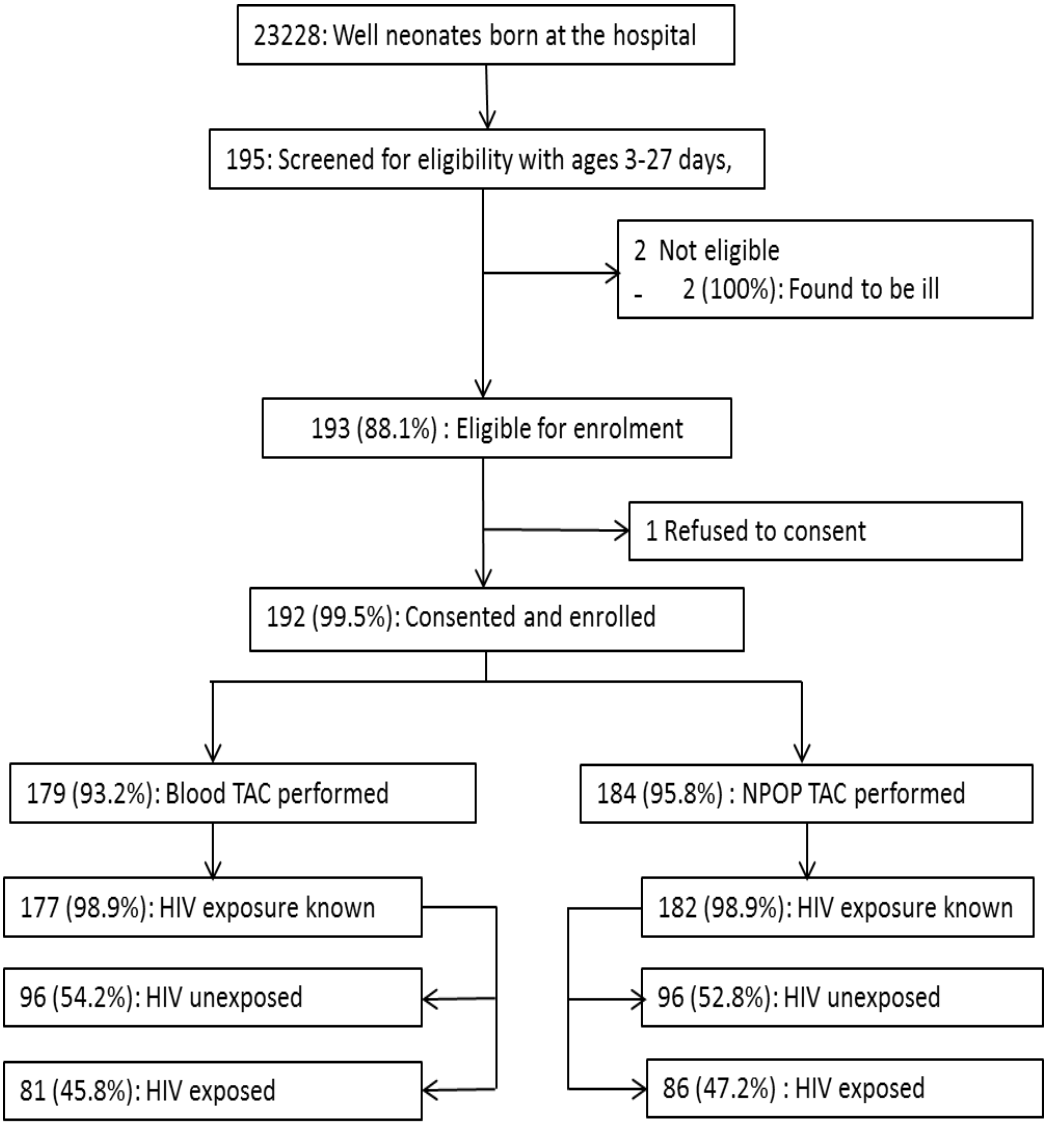

Figure 3.8: Healthy neonates of ages 3-27 days who were enrolled as controls

Comparing cases with community-acquired sepsis and controls of ages 3-27 days

a. Characteristic of cases and controls

There were no statistical significant differences in maternal characteristics between cases and controls. Cases compared to controls were, however, younger at enrolment (median age 12 vs 16 days, p<0.001), more likely to be male (60.7% vs 46.4%; p=0.012) and to be born with low birth weight (17.9% vs 9.7%; p=0.035). In the cases, the neonates were fairly evenly spread throughout the 25 days period of enrolment, but in the controls, only one was enrolled in the first week of life (**Table 3.35**)**.**

Table 3.35: Characteristics of neonates with community acquired protocol-defined sepsis (cases) and healthy neonates (controls) with ages 3-27 days who had blood and/or pharyngeal swabs tested using the Taqman array card

	Cases	Controls	OR (95% CI)	p-value
Total number of neonates enrolled	137	192		
Total number of neonates tested with Taqman array card in blood and/ or pharyngeal swabs	135	185		
Maternal Factors				
Antenatal care visit	132/134 (98.5)	183/184 (99.5)	0.36 (0.03-4.02)	0.387
Vaginal delivery	114/135 (84.4)	151/185 (81.6)	1.22 (0.67-2.22)	0.509
Healthcare facility birth	134/134 (100)	184/185 (99.5)	n/a	0.394
HIV infected	57/131 (43.5)	86/186 (47.0)	0.90 (0.57-1.40)	0.541
Infant Factors				
Preterm (<37 weeks)	12/135 (8.89)	8/185 (4.32)	2.16 (0.85-5.44)	0.096
Male	82/135 (60.7)	85/183 (46.4)	1.78 (1.14-2.80)	**0.012**
Low birth weight	24/135 (17.9)	18/185 (9.73)	2.00 (1.04-3.87)	**0.035**
Apgar score<7 at 5 minutes	17/135 (12.6)	18/185 (10.3)	1.26 (0.63-2.52)	0.516
Age at enrolment				**<0.001**
Day 3-6	36 (26.7)	1 (0.54)		
Day 7-13	38 (28.2)	36 (19.5)		
Day 14-20	38 (28.2)	91 (49.2)		
Day 21-27	23 (17.0)	57 (30.8)		
Median age at enrolment in days (25-75th)	12 (6-19)	16 (14-22)		**<0.001**

* - Missing data accounted for <5% for each variable and for both cases and controls

b. Detection of organisms using TAC in blood and NPOP of cases with protocol defined CAS and controls among 3-27 day old neonates

Blood TAC results

Identification of bacteria on the blood TAC assay was 2.24-fold (95%CI 1.30-3.86) more common in cases (45.8%) than controls (27.4%); (**Table 3.36**). This difference in blood TAC positivity between cases and controls was significant among age-groups 7-13 days (60.2% vs 32.3%; OR: 3.14, 95% CI 1.00-9.88) and 14-20 days (53.8% vs 31.5%; OR: 2.54, 95% CI 1.04-6.20). There was limited power, due to only a single control in the 3-6 day age group for such an analysis to be undertaken. There was no statistically significant difference in the number of organisms identified per sample between cases and controls. Furthermore, the prevalence of HIV exposure was similar between cases and controls in the overall group, as well as among those with positive TAC results.

Naso/oro pharyngeal swabs

Ninety-nine (75.0%) neonates with CAS had at least one organism identified by TAC on NPOP swabs, which was similar to that observed among controls (70.1%, p = 0.407), even after stratifying by age groups (**Table 3.36**). A higher percentage of cases had ≥3 organisms detected per sample (30.3%) than controls (14.7%; p = 0.018). There was no difference in HIV-exposure overall or those with positive TAC results on NPOP swabs between cases and controls.

Table 3.36: Detection of organisms using the Taqman array card in blood and pharyngeal swabs from neonates with community acquired protocol-defined sepsis (cases) and healthy neonates (controls) with ages 3-27 days

	Cases n (%)	**Controls n (%)**	**OR (95% CI)**	**p-value**
Total number of neonates with ages 3-27 days old	137	192		
Blood				
Total number with blood taken for Taqman array card	83	179		
Total number with positive Taqman array card in blood	38/83 (45.8)	49/179 (27.4)	2.24 (1.30-3.86)	**0.005**
Number with positive Taqman array card according to age of enrolment				
Day 3-6	7/25 (28.0)	0/1 (0)	n/a	0.535
Day 7-13	12/20 (60.0)	11/34 (32.3)	3.14 (1.00-9.88)	**0.045**
Day 14-20	14/26 (53.8)	28/89 (31.5)	2.54 (1.04-6.20)	**0.034**
Day 21-27	5/12 (41.7)	10/55 (18.2)	3.21 (0.84-12.2)	0.165
Number of organism per sample in those with positive Taqman array card				0.132
1 organism	31 (81.6)	36 (73.5)		
2 organisms	4 (10.5)	12 (24.5)		
≥3 organisms	3 (7.9)	1 (2.0)		
Exposure to human immunodeficiency virus				
Number exposed amongst those with blood collected	57/80 (46.2)	81/177 (45.8)	1.02 (0.60-1.73)	0.950
Number exposed among those positive Taqman array card	19/38 (50.0)	16/48 (33.3)	2.00 (0.83-4.80)	0.180
Pharyngeal swabs				
Total number with swabs taken for Taqman array card	132	184		
Total number with positive Taqman array card in pharyngeal swabs	99 (75.0)	129 (70.1)	1.28 (0.77-2.12)	0.407
Number with positive Taqman array card according to age of enrolment				
Day 3-6	22/35 (62.9)	0/1 (0)	n/a	0.407
Day 7-13	25/37 (67.6)	20/35 (57.1)	1.56 (0.60-4.08)	0.503
Day 14-20	31/38 (81.8)	67/91 (76.6)	1.59 (0.62-4.08)	0.461
Day 21-27	21/22 (95.5)	42/57 (73.7)	7.50 (0.93-60.7)	0.065

Table 3.36 (continued): Detection of organisms using the Taqman array card in blood and pharyngeal swabs from neonates with community acquired protocol-defined sepsis (cases) and healthy neonates (controls) with ages 3-27 days

	Cases n (%)	**Controls n (%)**	**OR (95% CI)**	**p-value**
Number of organism per sample in those with positive Taqman array card				**0.018**
1 organism	41 (41.4)	67 (51.9)		
2 organisms	28 (28.3)	43 (33.3)		
≥3 organisms	30 (30.3)	19 (14.7)		
Exposure to human immunodeficiency virus				
Number exposed among those with swabs taken	56/128 (43.8)	86/182 (47.3)	0.87 (0.65-1.37)	0.542
Number exposed among those with positive Taqman array card	48/97 (49.5)	72/128 (56.2)	0.76 (0.45-1.29)	0.313

c. Organisms detected in blood of cases with community acquired sepsis and controls, in neonates 3-27 days of age, using the Taqman array card

The common bacteria detected on blood by TAC assay among cases were *Streptococcus pneumoniae* (15.7%), GBS (14.5%), *Escherichia coli* (8.4%), *Staphylococcus aureus* (6.0%) and *Klebsiella pneumoniae* (4.8%) (**Figure 3.9).** Similar organisms were, however, also detected on blood among controls, with a higher prevalence in cases observed for E. *coli* (8.4 % vs 0.6%; OR: 16.4, 95% CI 1.98-135.6) and GBS (14.5% vs 1.7%; OR: 9.12, 95% CI 2.71-36.2). There were no statistically significant differences in prevalence of detection by TAC on blood samples between cases and controls for *Salmonella species* (2.4% vs 0.6%, p=0.493), *Staphylococcus aureus* (6.0% vs 1.7%, p=0.129), *Haemophilus influenzae* (2.4% vs 1.1%, p=0.801), *Pseudomonas aeruginosa* (2.4% vs 1.1%, p=0.801), *Ureaplasma species* (1.2% vs 0.6%, p = 0.838), *Klebsiella pneumoniae* (4.8% vs 2.8%, p=0.636), *Neisseria meningitides* (1.2% vs 1.1%, p = 0.574) and *Streptococcus pneumoniae* (15.7% vs 19.0%, p=0.630).

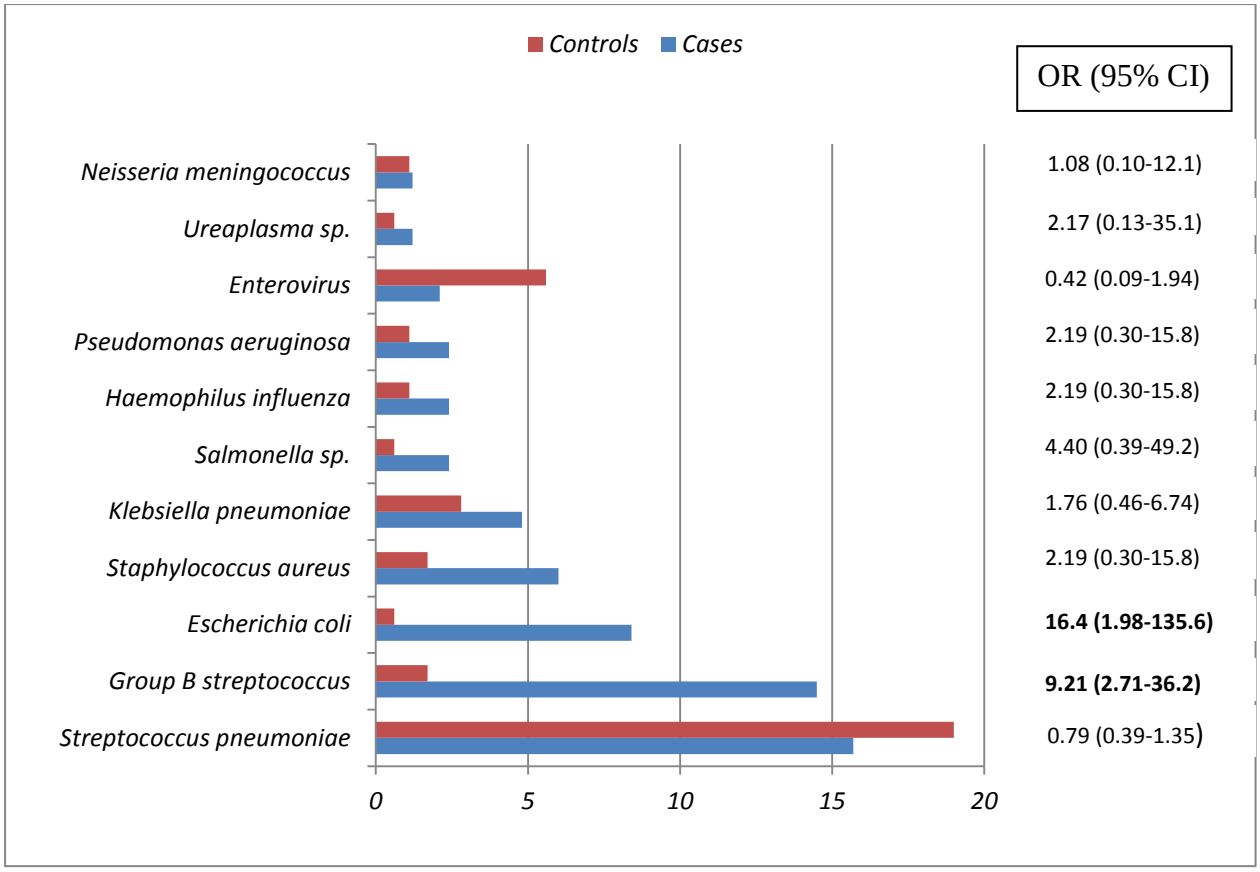

Figure 3.9: Organisms detected using Taqman array card in blood in neonates with community acquired protocol-defined sepsis (cases) and healthy neonates with ages 3-27 days (controls)

d. Organisms detected in nasopharyngeal swabs of cases with community acquired sepsis and controls in neonates 3-27 days of age using the Taqman array card

The common bacterial organisms detected on NPOP swabs in cases were GBS *(*28.0%), *Klebsiella species (*24.2%), *Escherichia coli* (13.6%), *Streptococcus pneumoniae (*13.6%) *and Ureaplasma urealyticum/ parvum* (13.6%). The common viruses detected among cases were cytomegalovirus (9.9%), rhinovirus (11.4%), enterovirus (7.6%) and RSV (7.6%). The prevalence of most bacteria and viruses was similar between cases and controls, except for higher positivity in cases than controls for GBS (28.0% vs 14.7%; OR: 2.26, 95% CI 1.30-3.96), and RSV *(*7.6% vs 2.2%; OR: 3.69, 95% CI 1.13-12.0), (**Figure 3.10**).

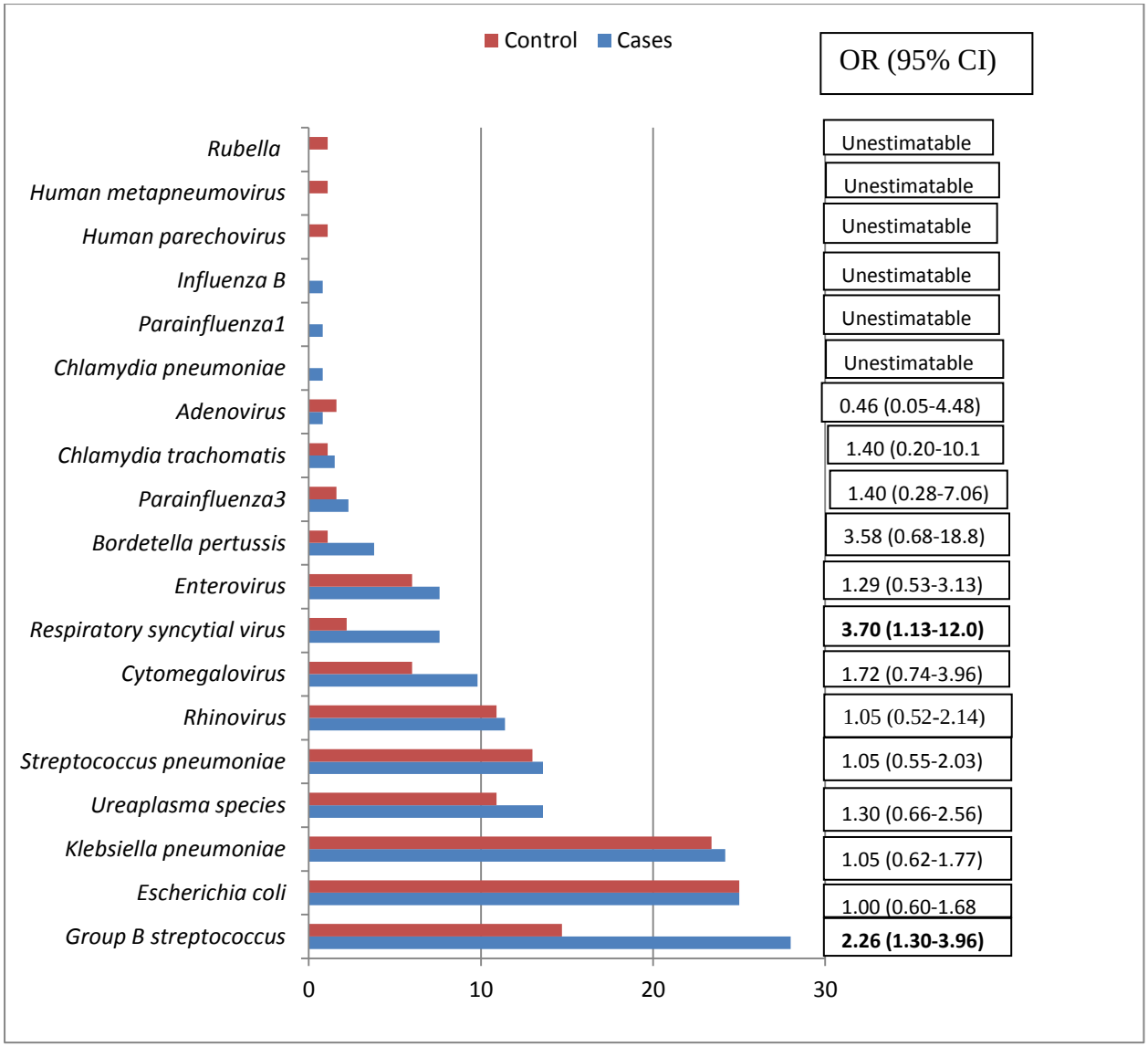

Figure 3.10: Organisms detected using Taqman array card in pharyngeal swabs in neonates with community acquired protocol-defined sepsis (cases) and healthy neonates with ages 3-27 days (controls)

e. Detection of organisms in blood and pharyngeal swabs from neonates with community acquired sepsis and controls with ages 3-27 days stratified according to HIV exposure

Blood

There were no differences in the percentage of cases and controls with positive TAC results on blood among HIV-unexposed neonates (44.2% vs 33.3%, p=0.184), although cases had a 4.3 fold higher positivity rate than controls among HIV-exposed infants (51.4% vs 19.8%; OR: 4.29, 95% CI 1.84-9.99) (**Table 3.37)**. There were, however, no differences in prevalences of individual organisms between cases and controls when stratified by HIV exposure status.

Naso/oro-pharyngeal swabs

On stratifying the neonates according to HIV exposure, there were no statistically significant differences in percentage with positive TAC results between cases and controls for all those with ages 3-27 days. For specific organisms, cases were more likely than controls to be positive for Klebsiella *pneumoniae* (26.4% vs 13.5%; OR: 2.29, 95% CI 1.04-5.02) and RSV (12.5% vs 2.1%; OR: 6.71, 95% CI 1.40-32.1) in HIV unexposed neonates, and for GBS (39.3% vs 16.3%; OR: 3.33, 95% CI 1.52-7.29) among HIV exposed neonates (**Table 3.38)**.

Table 3.37: Organisms detected using the Taqman array card in blood in neonates with community acquired protocol-defined sepsis (cases) and healthy neonates with ages 3-27 days (controls) stratified according to HIV exposure

	HIV Unexposed			**HIV exposed**		
	Cases N = 43	**Controls N = 96**	**OR (95%CI)**	**Cases N = 37**	**Controls N = 81**	**OR (95%CI)**
	n (%)	n (%)		n (%)	n (%)	
Any positive Taqman array card	19 (44.2)	32 (33.3)	1.58 (0.76-3.31)	19 (51.4)	16 (19.8)	**4.29 (1.84-9.99)**
Escherichia coli/ Shigella	2 (4.6)	1 (1.0)	4.63 (0.41-52.5)	5 (13.5)	0	n/a
Group B streptococcus	5 (11.6)	3 (3.1)	4.08 (0.93-17.9)	7 (18.9)	0	n/a
Salmonella species	0	1 (1.0)	n/a	1 (2.7)	0	n/a
Staphylococcus aureus	2 (4.6)	1 (1.0)	4.63 (0.41-52.5)	3 (8.6)	2 (2.5)	3.48 (0.56-21.8)
Haemophilus influenzae	1 (2.3)	1 (1.0)	2.26 (0.14-37.0)	1 (2.7)	1 (1.2)	2.22 (0.14-36.5)
Pseudomonas aeruginosa	1 (2.3)	1 (1.0)	2.26 (0.14-37.0)	1 (2.7)	1 (1.2)	2.22 (0.14-36.5)
Ureaplasma urealyticum/ parvum	0	1 (1.0)	n/a	1 (2.7)	0	n/a
Klebsiella pneumoniae	3 (7.0)	3 (3.1)	2.32 (0.45-12.0)	1 (2.7)	1 (1.2)	2.22 (0.14-36.5)
Neisseria meningitides	0	2 (2.1)	n/a	1 (2.7)	0	n/a
Streptococcus pneumoniae	8 (18.6)	23 (24.0)	0.73 (0.30-1.78)	5 (13.5)	10 (12.4)	1.15 (0.36-3.63)
Enterovirus	2 (4.6)	5 (5.2)	0.89 (0.17-4.76)	0	5 (6.2)	n/a
Group A streptococcus	0	0	N/A	0	0	N/A

Table 3.38: Organisms detected using Taqman array card in pharyngeal swabs in neonates with community acquired protocol-defined sepsis (cases) and healthy neonates with ages 3-27 days (controls) stratified according to HIV exposure

	HIV Unexposed			HIV Exposed		
	Cases N = 72 n (%)	Controls N = 96 n (%)	OR (95% CI)	Cases N = 56 n (%)	Controls N = 86 n (%)	OR (95% CI)
Any positive Taqman array card	49 (68.1)	56 (58.3)	1.52 (0.80-2.89)	48 (85.7)	72 (83.7)	1.17 (0.56-2.99)
Bacteria						
Bordetella pertussis	2 (2.8)	1 (1.0)	2.71 (0.24-30.5)	3 (5.4)	1 (1.2)	4.81 (0.49-47.4)
Group B Streptococcus	14 (19.4)	13 (13.5)	1.54 (0.67-3.54)	21 (39.3)	14 (16.3)	**3.33 (1.52-7.29)**
Chlamydia trachomatis	0	1 (1.0)	n/a	1 (1.8)	1 (1.16)	1.55 (0.09-25.2)
Ureaplasma species	7 (9.7)	11 (11.5)	0.83 (0..31-2.27)	10 (17.9)	8 (9.3)	2.12 (0.78-5.75)
Klebsiella pneumoniae	19 (26.4)	13 (13.5)	**2.29 (1.04-5.02)**	13 (23.2)	30 (34.9)	0.56 (0.26-1.21)
Streptococcus pneumoniae	7 (9.7)	7 (7.3)	1.37 (0.46-4.09)	11 (19.6)	17 (19.8)	0.99 (0.43-2.31)
Escherichia coli/ Shigella	10 (13.8)	16 (16.7)	0.81 (0.34-1.90)	22 (39.3)	30 (34.9)	1.18 (0.53-2.65)
Mycoplasma pneumoniae	1 (1.4)	0	n/a	0	0	N/A
Chlamydia pneumoniae	0	0	N/A	1 (1.8)	0	n/a
Virus						
Respiratory syncytial virus	9 (12.5)	2 (2.1)	**6.71 (1.40-32.1)**	0	2 (2.3)	n/a
Cytomegalovirus	5 (6.9)	4 (4.2)	1.72 (0.44-6.63)	8 (14.3)	7 (8.1)	1.88 (0.64-5.52)
Parainfluenza virus 3	0	0	N/A	3 (5.4)	3 (3.5)	1.57 (0.31-8.05)
Enterovirus	5 (6.9)	1 (1.0)	7.09 (0.81-62.1)	5 (8.9)	10 (11.6)	0.74 (0.24-2.31)
Rhinovirus	7 (8.3)	7 (7.3)	1.17 ((0.37-3.60)	9 (16.1)	13 (15.1))	1.08 (0.43-2.71)
Adenovirus	1 (1.4)	1 (1.0)	1.34 (0.08-21.8)	0	2 (2.3)	n/a
Influenza B	1 (1.4)	0	n/a	0	0	N/A
Parainfluenza virus 1	0	0	N/A	1 (1.8))	0	n/a
Human metapneumovirus	0	1 (1.0)	n/a	0	1 (1.2)	n/a
Human parechovirus	0	0	N/A	0	2 (2.3)	n/a
Rubella	0	1 (1.0)	n/a	0	1 (1.2)	n/a
Influenza A	0	0	N/A	0	0	N/A
Parainfluenza virus 2	0	0	N/A	0	0	N/A

3.2.3 Detection rate of organisms using both Taqman array card and culture in blood of neonates with protocol defined early-onset or community acquired sepsis

Among the 933 neonates with protocol-defined EOS who had blood samples sent for culture and TAC assay analysis, 3.6% (34) were positive for pathogens on blood culture, whilst 32.8% (n=306) were positive for culturable organisms on TAC. After correcting for positive controls for culturable organisms on TAC in neonates with protocol-defined EOS, only 135 (14.5%) were positive on TAC. The odds of detecting a culturable pathogen in neonates with EOS using TAC was 4.5- fold (95%CI: 3.03-6.59) greater than by blood culture. The common organism detected in neonates with EOS on blood culture was GBS (23/46; 67.6% of positive blood cultures), while it accounted for 36.6% (49/134) of bacteria detected on TAC after adjusting for controls. *Pseudomonas aeruginosa* accounted for 43.3% of all culturable organisms identified by TAC (**Table 3.39)**. Detection of culturable bacteria by TAC was higher compared to blood culture for all putative pathogens, except for *Neisseria meningitides.*

Among the 83 neonates with protocol-defined CAS who had blood analyzed by conventional culture and on TAC, 25 (30.1%) yielded a putative pathogen on culture and 36 (43.4%) on TAC. After correcting for controls with positive TAC, the number positive on TAC (n=16; 19.2%) for culturable putative pathogens was similar to culture positivity (n= 25; 30.1%; OR: 0.27; 95%CI 0.27-1.14). There were no differences in detection for specific culturable bacteria by culture compared to TAC (**Table 3.40)**. Because of limited number of neonates with ages between 3 and 27 days of age, we did not undertake any further modelling to assess which pathogens attribute to CAS.

In assessing the value of TAC in diagnosing sepsis in neonates using culture as the gold standard, overall the sensitivity of TAC was 60.9%, specificity 69.7% and positive and negative predictive value of 12.8% and 96.1% respectively (**Table 3.41)**. The diagnostic utility of TAC appeared to differ for specific bacteria. For GBS, the sensitivity and specificity were of 84.4% and 94.9%, respectively; whilst its specificity was 85.7% for *S. pneumoniae* for which the positive predictive value was zero.

Table 3.39: Comparing detection of culturable bacteria using Taqman array card (TAC) versus culture in blood of neonates with early-onset protocol-defined sepsis who had both tests done

	Number detected in blood in cases using TAC N=933	Number detected in blood in controls using TAC N = 304	Number detected in blood in cases using TAC adjusted for positive controls N = 933	Number detected on blood culture N = 933	Comparing detection of culturable organism using TAC (adjusted) vs culture OR (95% CI)
Group B streptococcus	65 (6.97)	5 (1.64)	49 (5.25)	23 (2.46)	**2.19 (1.32-3.63)**
Escherichia coli	21 (2.25)	4 (1.34)	8 (0.86)	4 (0.43)	2.00 (0.60-6.69)
Haemophilus influenzae	6 (0.64)	0	6 (0.64)	2 (0.21)	3.01 (0.61-15.0)
Neisseria meningitides	10 (1.07)	3 (0.99)	1 (0.11)	2 (0.21)	0.50 (0.04-5.52)
Pseudomonas aeruginosa	77 (8.25)	6 (1.97)	58 (6.20)	1 (0.11)	**61.8 (8.54-446)**
Klebsiella pneumoniae	35 (3.75)	8 (2.63)	11 (1.18)	1 (0.11)	**11.1 (1.43-86.3)**
Staphylococcus aureus	12 (1.29)	1 (0.33)	9 (0.96)	1 (0.11)	**9.08 (1.14-71.8)**
Streptococcus pneumoniae	132 (14.2)	29 (9.87)	40 (4.29)	0 (0)	n/a
Salmonella species	29 (3.11)	7 (2.30)	29 (3.11)	0 (0)	n/a
Group A *streptococcus*	2 (0.21)	0	2 (0.21)	0 (0)	n/a
All	**306 (32.8)**	**56 (18.4)**	**134 (14.4)**	**34 (3.64)**	**4.47 (3.03-6.59)**

Footnote: Correction for positive controls in TAC was calculated by multiplying number of cases with positive TAC by a factor derived from the following equation: (Relative risk for cases minus Relative risk for controls)/Relative risk for cases for each and for all culturable organisms.

Table 3.40: Comparing detection of culturable pathogens using Taqman array card (TAC) versus culture in blood of neonates with protocol-defined community acquired sepsis

	Number detected in blood in cases using TAC N = 83	Number detected in blood in controls using TAC N = 179	Number detected in blood in cases using TAC adjusted for positive controls N = 83	Number detected in blood in cases using culture N = 83	Comparing detection of culturable organism using adjusted TAC vs culture OR (95% CI)
Group B *streptococcus*	12 (14.5)	3 (1.68)	11 (13.2)	9 (10.8)	1.26 (0.49-3.21)
Staphylococcus aureus	5 (6.02)	3 (1.68)	4 (4.82)	8 (9.64)	0.45 (0.14-1.64)
Escherichia coli	7 (8.43)	1 (0.56)	7 (8.43)	6 (7.23)	1.18 (0.38-3.68)
Klebsiella pneumoniae	4 (4.82)	5 (2.79)	2 (2.41)	2 (2.41)	1.00 (0.14-7.27)
Streptococcus pneumoniae	13 (15.7)	34 (19.0)	3 (3.61)	0	n/a
Haemophilus influenzae	6 (7.23)	2 (1.12)	1 (1.21)	0	n/a
Pseudomonas aeruginosa	2 (2.41)	2 (1.12)	1 (1.21)	0	n/a
Salmonella species	2 (2.41)	1 (0.56)	1 (1.21)	0	n/a
Neisseria meningitis	1 (1.20)	2 (1.12)	0	0	n/a
Group A *streptococcus*	0	0	0	0	n/a
All	**36 (43.4)**	**42 (23.5)**	**16 (19.2)**	**25 (30.1)**	**0.55 (0.27-1.14)**

Footnote: Correction for positive controls in TAC was calculated by multiplying number of cases with positive TAC by a factor derived from the following equation: (Relative risk for cases minus Relative risk for controls)/Relative risk for cases for each and for all culturable organisms.

Table 3.41: Sensitivity, specificity, positive and negative predictive values of Taqman array card in neonates with early-onset culture-confirmed sepsis

	Sensitivity	**Specificity**	**Positive predictive value**	**Negative predictive value**
Group B *streptococcus*	82.6%	94.9%	29.2%	99.5%
Escherichia coli	50.0%	98.0%	9.5%	99.8%
Haemophilus influenzae	50.0%	99.5%	16.7%	99.9%
Neisseria meningitis	0	98.9%	0	99.8
Pseudomonas aeruginosa	0	91.7%	0	99.9%
Klebsiella pneumoniae	0	96.2%	0	99.9%
Staphylococcus aureus	100%	98.8%	8.3%	0
Streptococcus pneumoniae	N/A	85.8%	0	0
Salmonella species	N/A	96.9%	0	0
Group A *streptococcus*	N/A	99.8%	0	0
All	78.8%	68.9%	8.5%	98.9%

3.2.4 Aetiologic attribution to neonates with early-onset sepsis using statistical modelling factoring type of case, test used and type of specimen

Of the 28 pathogens considered for aetiologic attribution, 10 were identified at a rate sufficient for aetiologic modeling (**Table 3.42**). Overall, one was able to attribute aetiology to a specific pathogen for 26.7% of cases using blood culture or TAC, and therefore 73.3% of cases did not have an identifiable aetiology from the pathogens tested in culture or TAC (**Figure 3.11**). The three most common pathogens among those included in the TAC panel attributed to the sepsis episodes were *Ureaplasma* (5.4%), GBS (4.8%), and *Klebsiella pneumoniae* (1.8%). Putative pathogens in the "other blood culture" group (i.e. bacteria cultured from blood, but not included on the TAC panel) accounted for an additional 8.7% of cases, with *Viridans streptococci* (4.2%), *Enterococcus faecalis* (1.4%), and *Acinetobacter baumannii* (0.7%) being the most common. Among the neonates with severe infection the most commonly attributed pathogens were GBS (7.6%), *Ureaplasma* (5.2%) and *Viridans streptococcus* (4.1%) (**Figure 3.12)**, and in 68.8% of cases with severe infection no pathogen was identified as the possible cause of the sepsis.

Table 3.42: List of organisms from culture, blood and respiratory Taqman array card included in the aetiologic modelling

Organism	Culture	Blood TAC	Resp. TAC	Modeled
Adenovirus			X	
Bordetella pertussis I			X	
Chlamydia pneumoniae			X	
Chlamydia trachomatis			X	
Cytomegalovirus			X	X
E. coli/Shigella	X	X	X	X
Rhinovirus/Enterovirus		X	X	X
Group A *Streptococcus*	X	X		
Group B *Streptococcus*	X	X	X	X
Human metapneumovirus			X	
Human parechovirus			X	
Influenza A			X	
Influenza B			X	
Klebsiella pneumoniae	X	X	X	X
Mycoplasma pneumoniae			X	
Neisseria meningitides	X	X		X
pan-Haemophilus influenzae	X	X		
pan-*Salmonella*	X	X		X
Parainfluenza virus 1			X	
Parainfluenza virus 2			X	
Parainfluenza virus 3			X	
Pseudomonas aeruginosa	X	X		
Respiratory syncytial virus			X	
Rubella			X	
Staphylococcus aureus	X	X		X
Streptococcus pneumoniae	X	X	X	X
Ureaplasma sp.		X	X	X
Acinetobacter Baumannii	X			X*
Viridans streptococci	X			X*
Enterococcus faecalis	X			X*

Culture: Culturable using the BACTEC automated blood culture system

Blood TAC: Included on whole blood Taqman array card panel

Resp. TAC: included on respiratory (NP/OP) Taqman array card panel

Modeled: Included in etiologic models based on the Partially Latent Class Model developed by Wu, et al[321].

** Estimated indirectly from the "other blood culture" class*

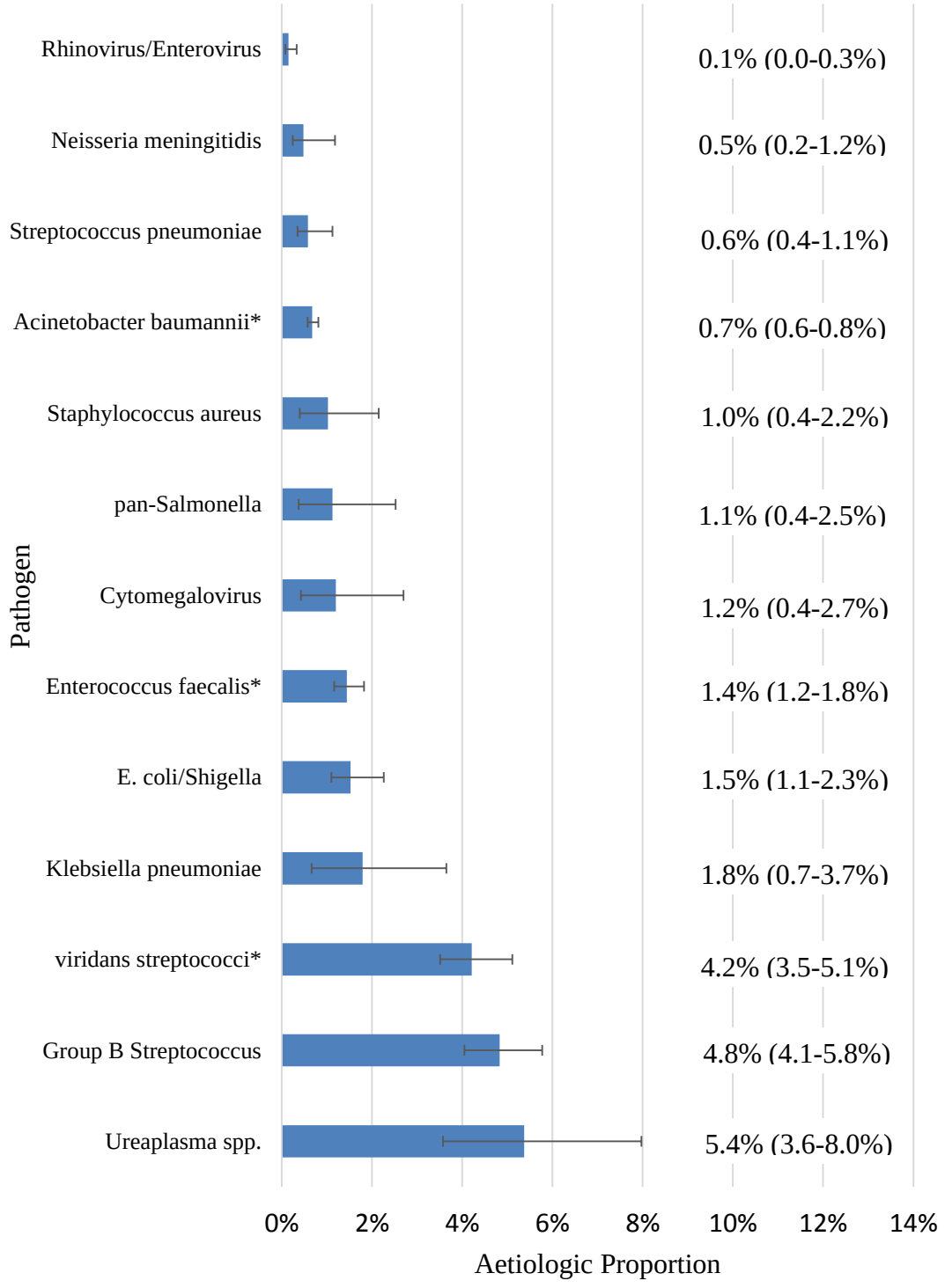

Figure 3.11: Estimated proportions with 95% confidence intervals attributable to specific pathogens among cases with early-onset sepsis

* Pathogen proportion estimated indirectly from the "other blood culture" class.

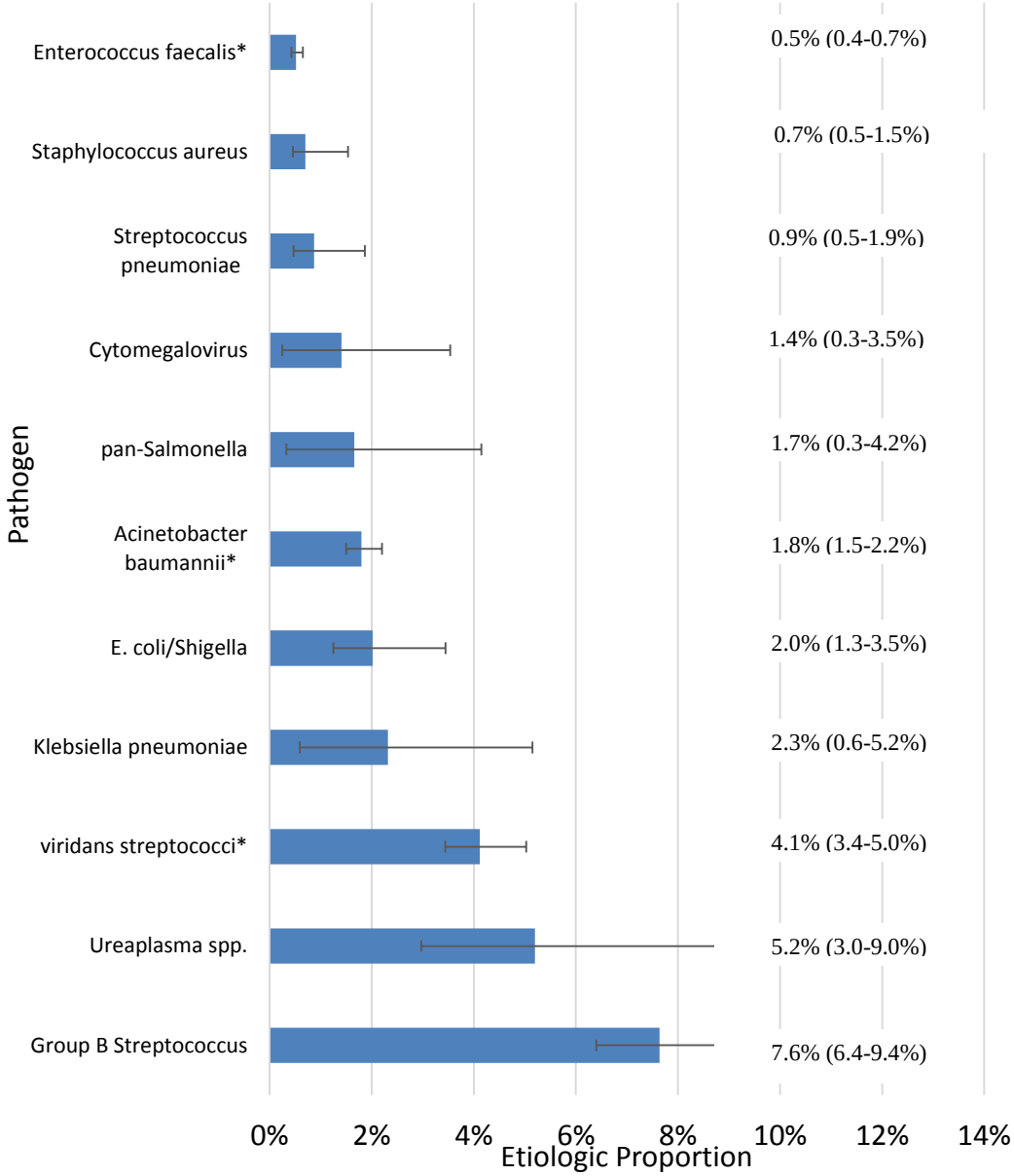

Figure 3.12: Estimated proportions with 95% confidence intervals attributable to specific organism among cases with severe infections (i.e. presence of lethargy, NICU admissions or died)

* Pathogen proportion estimated indirectly from the “other blood culture” class.

While the adjusted incidence (per 1000 live births) of culture-confirmed EOS among neonates with EO-pSBI was 3.8, using the modelling output which included positive blood culture, and positive TAC in blood and NPOP swabs and adjusting for positive controls, the incidence for TAC or culture confirmed EOS sepsis was 9.7. Among those cases with TAC or cultured confirmed bacterial sepsis, the incidence of Gram-positive bacteria was 4.7 higher than that of 2.9 observed for Gram-negative bacteria. *Ureaplasma sp.*, GBS, and *Viridans streptococcus* all had estimated incidence of >1 per 1,000 live births (**Table 3.43).**

Table 3.43: Observed and estimated pathogen specific incidence (per 1000 live births) of early-onset protocol-defined sepsis

Pathogen	Observed Incidence	Estimated Incidence	Estimated LCL	Estimated UCL
Ureaplasma spp.	NA	2.11	1.40	3.13
Group B *Streptococcus*	1.01	1.90	1.59	2.27
Viridans streptococci	0.66	1.65	1.38	2.01
K. pneumoniae	0.03	0.70	0.26	1.43
E. coli/Shigella	0.29	0.60	0.43	0.89
E. faecalis	0.29	0.57	0.46	0.71
Cytomegalovirus	NA	0.47	0.16	1.06
Pan-*Salmonella*	0.03	0.44	0.15	0.99
A. baumannii	0.13	0.26	0.22	0.32
S. pneumoniae	--	0.23	0.14	0.44
N. meningitides	0.06	0.19	0.09	0.46
Staphylococcus aureus	0.09	0.07	0.03	0.19
Rhinovirus/Enterovirus	NA	0.06	0.03	0.13
Bacterial Sepsis	3.16	9.71	8.67	10.88
Gram-negative	0.80	2.86	2.31	3.53
Gram-positive	2.23	4.74	4.02	5.58

Observed incidence based on pathogen isolation from blood culture specimens
Estimated incidence based on the Partially Latent Class Model developed by Wu, et al.[321]
LCL (lower confidence limit) and UCL (upper confidence limit) based on 95% credible intervals

3.3 Maternal and Neonatal Vitamin D Status and its Association with Early-Onset Neonatal Sepsis in Black South Africans

3.3.1 Enrolled pregnant women and their offspring

Vitamin D deficiency (VDD) has been associated with increased risk for early-onset sepsis (EOS) in neonates; however, there is a paucity of data on vitamin D status in South African pregnant women and its association with EOS. Therefore in this part of the project I aimed to assess vitamin D status in pregnant South African women and their offspring, and its association with EOS. Pregnant women at time of delivery and their offspring living in Johannesburg were prospectively enrolled over a period of 18 months.

Of 696 pregnant women who were approached to participate in the vitamin D status study 75 (10.8%) refused consent, resulting in 621 mothers and 653 neonates (including 32 sets of twins) being enrolled (**Figure 3.13**). Among the 621 women, 254 (41%) were HIV positive and 98% were black African. The mean maternal age was 28 years and 30.8% were primigravida. The average duration of pregnancy at time of delivery was 37 weeks (**Table 3.44)**. The 653 enrolled neonates, included 293 (44.8%) with suspected sepsis, including 83 (12.7%) with protocol defined EOS. Infants with protocol defined sepsis were more likely to be born by caesarean section (32.6% vs 6.4%%; $p<0.001$), to be of lower birth weight (2405±874 vs 2875±551 g; $p<0.001$), lower gestation age (36±4 vs 38±2 weeks; $p<0.001$), to have a lower median Apgar score at 5 minutes (9 vs 10, $p=0.001$) and to have been born in winter and spring; (42.2% & 36.1% vs 23.1% & 16.1%, $p<0.001$) compared to well neonates (**Table 3.44**).

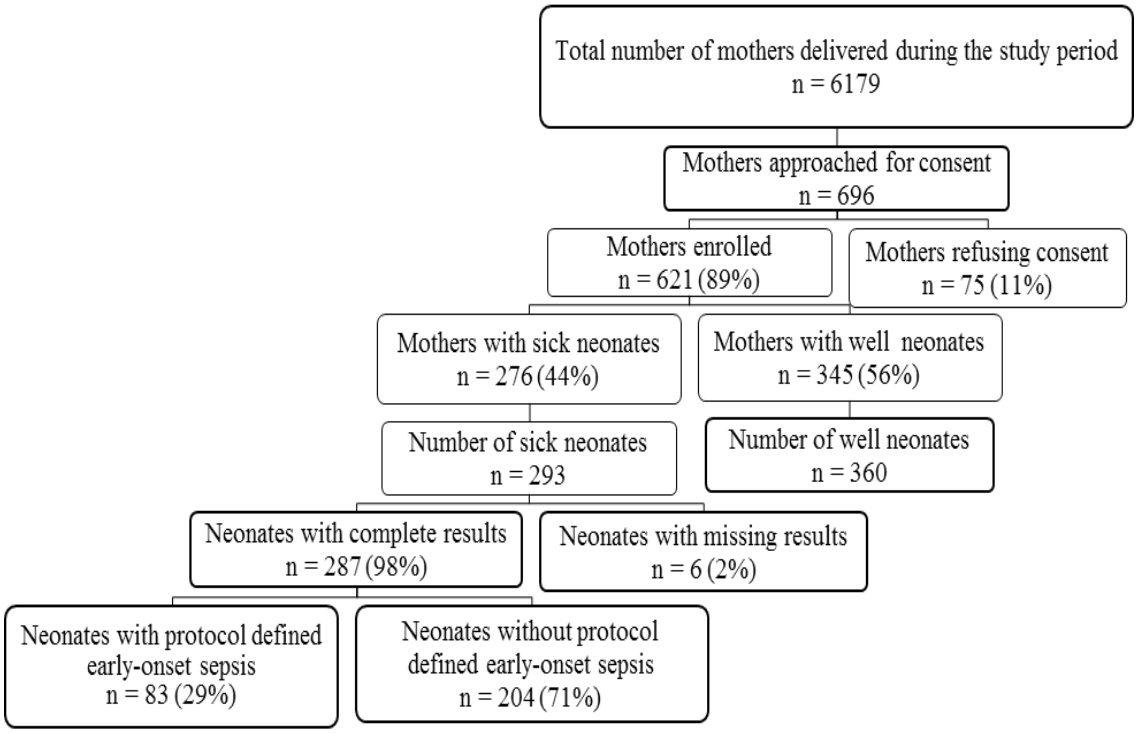

Figure 3.13: Number of mothers and neonates enrolled in the study

Table 3.44: Characteristics of mother-newborn dyads enrolled in the vitamin D study

	All n = 653‡	**Well infants (n = 360)**	**Suspected Sepsis (n = 204)**	**Protocol Defined Sepsis (n = 83)**	**Well vs Suspected Sepsis p-value**	**Well vs. Protocol Defined Sepsis p-value**
	n (%)	n (%)	n (%)	n (%)		
Maternal Characteristics						
Mother black African	636 (97.4)	352 (97.8)	197 (96.6)	81 (97.6)	0.558	>0.999
Average maternal age*	28± 6	28± 6	28± 6	26±5	0.548	0.032
Gravidity					0.552	0.071
1	200 (30.7)	108 (30.1)	59 (28.9)	33 (39.8)		
2 – 4	422 (64.7)	230 (64.1)	137(67.2)	49 (59)		
>4	30 (4.6)	21 (5.8)	8 (3.9)	1 (1.2)		
Maternal HIV results**					0.01	0.109
Positive	265(40.9)	163(45.5)	69(34.2)	29(35.4)		
Negative	383(59.1)	195(54.5)	133(65.8)	53(64.6)		
Mode of delivery					<0.001	<0.001
Vaginal	526(80.6)	337(93.6)	131(64.2)	56(67.5)		
Caesarean section	127(19.4)	23(6.4)	73(35.8)	27(32.5)		
Season of the year at birth						<0.001
Autumn	230(35.2)	169(46.9)	45(22.1)	13(15.7)		
Winter	179(27.4)	83(23.1)	61(29.9)	35(42.2)		
Spring	171(26.2)	58(16.1)	82(40.2)	30(36.1)		
Summer	73(11.2)	50(13.9)	16(7.8)	5(6)		

Table 3.44 (continued): Characteristics of mother-newborn dyads enrolled in the vitamin D study

	All n = 653‡	**Well infants (n = 360)**	**Suspected Sepsis (n = 204)**	**Protocol Defined Sepsis (n = 83)**	**Well vs Suspected Sepsis p-value**	**Well vs. Protocol Defined Sepsis p-value**
	n (%)	n (%)	n (%)	n (%)		
Infant characteristics						
Average birth weight*	2690±724	2875±551	2468±805	2405±874	<0.001	<0.001
Birth weight categories (g)						<0.001
1000-1499	39(6)	0(0)	24(11.8)	14(16.9)		
1500-1999	83(12.7)	15(4.2)	45(22.2)	22(26.5)		
2000-2499	137(21)	93(25.8)	35(17.2)	8(9.6)		
≥2500	393(60.3)	252(70)	99(48.8)	39(47)		
Average gestational age*	37±3	38±2	36±4	36±4	<0.001	<0.001
Gestational age categories						<0.001
<30 weeks	26(4)	2(0.6)	18(8.8)	6(7.2)		
30 - 34 weeks	119(18.2)	40(11.1)	47(23)	30(36.1)		
35 - 37 weeks	161(24.7)	105(29.2)	44(21.6)	12(14.5)		
>37 weeks	347(53.1)	213(59.2)	95(46.6)	35(42.2)		
Median Apgar score						
at 1 minute†	9(7,9)	9(9,9)	8(5,9)	8(6,9)	<0.001	<0.001
at 5 minutes†	10(9,10)	10(10,10)	9(8,10)	9(8,10)	<0.001	0.001

‡- 6 patients had missing results defining sepsis; * - Mean ± standard deviation; ** - 5 had missing HIV results;
† - Median (25th, 27th centiles)

3.3.2 25-hydroxyvitamin D in pregnant women

The mean 25(OH)D concentration in black pregnant women was 54.7±30.1 nmol/L, with no statistically significant difference between HIV-infected and HIV-uninfected women (54.4 ±29.3 vs 55.0 ± 31.3 nmol/L, p=0.798) nor between mothers of healthy newborns and those who developed EOS; **Table 3.45**. Overall, vitamin D deficiency (serum 25(OH)D <30 nmol/L) was noted in 18.8% of mothers, with a similar prevalence by HIV-infection status. Although the mean 25(OH)D concentration did not differ between mothers' of healthy newborns compared to EOS cases, a higher percentage of mothers whose children had protocol defined sepsis (27.7%) were vitamin D deficient compared to mothers of healthy newborns (15.9%, p=0.003). There were no significant differences in 25(OH)D levels or prevalence of vitamin D deficiency among the women delivering at different gestational ages.

Table 3.45: Maternal serum 25(OH)D concentrations and proportion with vitamin D deficiency

	Serum 25(OH)D concentrations (nmol/L)			Proportion with Vitamin D Deficiency (<30 nmol/L)		
	HIV- Infected	**HIV- Uninfected**	**All***	**HIV- Infected**	**HIV- Uninfected**	**All***
	Mean ± SD	Mean ± SD	Mean ± SD	n (%)	n (%)	n (%)
Mothers to all neonates	n = 254	n = 362	n = 621	n =254	n = 362	n = 621
Gestational age						
<30 weeks (n=21)	61.6±40.6	50.1±20.8	57.7±32.3	1/10 (10.0)	2/10 /(20.0)	3/21 (14.3)
30 - 34 weeks (n=113)	53.2±35.3	58.9±36.4	56.2±35.5	12/52 (23.1)	10/59 (17.0)	22/113 (19.5)
35 - 37 weeks (n=147)	50.8±31.3	52.8±27.2	51.9±29.0	16/64 (25.0)	15/83 (18.1)	31/147 (21.1)
>37 weeks (n=340)	57.3±28.8	53.8±28.3	55.2±28.5	25/128 (19.5)	35/210 (16.7)	61/340 (17.9)
All (n=621)	55.0±31.3	54.4±29.3	**54.7±30.1**	54/254 (21.2)	62/362 (17.1)	**117/621 (18.8)**
Mothers to well neonates	n = 158	n = 185	n = 345	n = 158	n = 185	n = 345
Gestational age						
<30 weeks (n=2)	31.7	71.5	51.6±28.1	0/1	0/1	0/2 (0)
30 - 34 weeks (n=40)	51.4±34.2	46.2±21.8	48.6±27.9	4/19 (21.1)	4/20 (20.0)	8/40 (20.0)
35 - 37 weeks (n=96)	53.3±31.2	52.7±26.8	53.0±28.8	9/46 (19.6)	10/50 (20.0)	19/96 (19.8)
>37 weeks (n=207)	61.8±28.7	55.3±27.2	58.1±28.0	12/92 (13.0)	15/114 (13.2)	28/207 (13.5)
All (n=345)	55.0±28.3	57.9±30.2	**53.7±26.5**	25/158 (15.8)	29/185 (15.7)	**55/345 (15.9)**
Mothers to neonates with suspected sepsis but laboratory tests negative for sepsis	n = 70	n = 137	n = 210	n = 70	n = 137	n = 210
Gestational age						
<30 weeks (n=17)	86.4±44.8	52.2±16.2	62.2±30.5	0/5 (0)	1/11 (8.33)	1/17 (5.88)
30 - 34 weeks (n=48)	63.9±44.1	68.1±34.9	66.4±38.0	4/19 (21.1)	4/28 (14.3)	8/48 (16.7)
35 - 37 weeks (n=45)	42.1±22.9	55.2±29.7	50.3±27.8	6/17 (35.3)	4/28 (14.3)	10/45 (22.0)
>37 weeks (n=100)	46.7±26.7	54.1±31.6	52.3±30.4	9/29 (31.0)	13/70 (18.6)	22/100 (22.0)
All (n=210)	53.1±35.5	57.0±31.1	**55.9±32.2**	19/70 (27.1)	22/137 (15.9)	**41/210 (19.5)**

Table 3.45 (continued): Maternal serum 25(OH)D concentrations and proportion with vitamin D deficiency

	Serum 25(OH)D concentrations (nmol/L)			Proportion with Vitamin D Deficiency		
	HIV- Infected	**HIV- Uninfected**	**All***	**HIV- Infected**	**HIV- Uninfected**	**All***
	Mean ± SD	Mean ± SD	Mean ± SD	n (%)	n (%)	n (%)
Mothers to neonates with suspected sepsis and laboratory tests positive for sepsis	n = 32	n = 50	n = 83	n = 32	n = 50	n = 83
Gestational age						
<30 weeks (n=7)	58.9±47.7	17.9	58.3±44.9	1/5 (20)	1/1 (100)	2/7 (28.6)
30 - 34 weeks (n=31)	45.0±26.4	76.9±63.8	60.5±50.1	5/16 (31.2)	2/15 (13.3)	7/31 (22.6)
35 - 37 weeks (n=11)	72.7±61.1	44.4±19.7	52.1±34.6	1/3 (33.3)	2/8 (25.0)	3/11 (27.3)
>37 weeks (n=34)	40.2±22.4	47.1±23.7	45.4±23.2	4/8 (50.0)	7/26 (26.9)	11/34 (32.4)
All (n=83)	48.6±32.9	55.0±41.6	**53.0±38.4**	11/32 (34.4)	12/50 (24.0)	**23/83 (27.7)**

* - A total of 5 mothers had missing HIV results and of these 2 were mothers of well neonates

3.3.3 25-hydroxyvitamin D in cord blood

The overall mean cord blood 25(OH)D among the 653 infants was 39.0±21.3 nmol/L. There were no differences in cord blood mean 25(OH)D levels between HIV-exposed and -unexposed neonates (37.5 ±20.4 vs 39.8 ± 21.9 nmol/L, p=0.172). Vitamin D deficiency was identified in 39.8% of neonates overall, which did not differ between HIV-exposed and -unexposed newborns (42.6% vs 38.1%, p=0.248) (**Table 3.46**). There were also no significant differences in 25(OH)D levels or prevalence of vitamin D deficiency among neonates of differing gestational ages or birth weights (data not shown). The transplacental transfer ratio of 25(OH)D was 0.79±0.45, which did not differ by gestational age or birth weight. There was modest correlation between maternal and cord serum 25(OH)D levels for both healthy neonates (r=0.47) (**Figure 3.14**) and neonates with protocol defined sepsis (r=0.50) (**Figure 3.15**).

Table 3.46: Cord blood concentrations, cord to maternal ratio of 25-hydroxyvitamin D and proportion with vitamin D deficiency according to gestational age

	25(OH)D concentration (nmol/l)			25(OH)D Cord to Mother Ratio	Proportion with Vitamin D Deficiency (<30 nmol/L)		
	HIV-exposed	HIV-unexposed	All	All	HIV-exposed	HIV-unexposed	All*
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	n (%)	n (%)	n (%)
All Neonates	n=265	n=381	n=651	n=649	n=265	n=381	n=651
Gestational Age							
<30 weeks	34.9±14.6	39.8±24.4	39.1±21.3	0.69±0.38	4/11 (36.4)	5/13 (38.5)	9/25 (36)
30 - 34 weeks	39.1±22.4	42.6±23.5	41.1±22.9	0.81±0.45	21/54 (38.9)	20/63 (31.7)	41/119 (34.5)
35 - 37 weeks	33.3±17.5	37.1±22.6	35.5±20.6	0.74±0.34	34/70 (48.6)	45/91 (49.5)	79/161 (49.1)
>37 weeks	39.4±21.2	40.2±20.9	39.9±21	0.81±0.5	54/130 (41.5)	74/214 (34.6)	129/346 (37.3)
All	37.5±20.4	39.8±21.9	**39±21.3**	**0.79±0.45**	113/265 (42.6)	144/381 (37.8)	**258/651 (39.6)**
Well Neonates	n=163	n=194	n=359	n=358	n=163	n=194	n=359
Gestational Age							
<30 weeks	13.8±NA	27.8±NA	20.8±9.8	0.41±0.03	1/1 (100)	1/1 (100)	2/2 (100)
30 - 34 weeks	47.2±29.6	34±17.2	41.1±24.8	0.86±0.49	7/19 (36.8)	9/20 (45)	16/40 (40)
35 - 37 weeks	36.4±18.3	41±23.1	38.8±21	0.8±0.36	20/50 (40)	22/55 (40)	42/105 (40)
>37 weeks	43±21.8	44.7±20.9	43.9±21.2	0.83±0.44	32/93 (34.4)	28/118 (23.7)	60/212 (28.3)
All	41.3±22	42.4±21.3	**42±21.6**	**0.82±0.42**	60/163 (36.8)	60/194 (30.9)	**120/359 (33.4)**

Table 3.46 (continued): Cord blood concentrations, cord to maternal ratio of 25-hydroxyvitamin D and proportion with vitamin D deficiency according to gestational age

	25(OH)D concentration (nmol/l)			25(OH)D Cord to Mother Ratio	Proportion with Vitamin D Deficiency (<30 nmol/L)		
	HIV-exposed Mean ± SD	**HIV-unexposed** Mean ± SD	**All** Mean ± SD	**All** Mean ± SD	**HIV-exposed** n (%)	**HIV-unexposed** n (%)	**All*** n (%)
Neonates with suspected sepsis but negative laboratory tests for sepsis	n=69	n=132	n=203	n=202	n=69	n=132	n=203
Gestational Age							
<30 weeks	38.9±17.1	43.9±24	42.1±21.4	0.73±0.42	2/6 (33.3)	3/11 (27.3)	5/17 (29.4)
30 - 34 weeks	34.6±18.4	42.6±21.1	39.4±20.1	0.73±0.41	7/18 (38.9)	8/28 (28.6)	15/47 (31.9)
35 - 37 weeks	27.8±12.4	32.7±21.4	30.8±18.4	0.65±0.27	11/17 (64.7)	17/27 (63)	28/44 (63.6)
>37 weeks	31±17.6	33.8±18.7	32.9±18.3	0.74±0.46	16/28 (57.1)	32/66 (48.5)	49/95 (51.6)
All	31.8±16.6	36.3±20.5	**34.7±19.2**	**0.72±0.41**	36/69 (52.2)	60/132 (45.5)	**97/203 (47.8)**
Neonates with suspected sepsis and positive laboratory tests for sepsis	n=29	n=53	n=83	n=83	n=29	n=53	n=83
Gestational Age							
<30 weeks	34.2±6.3	7.5±NA	36.8±22.6	0.66±0.31	1/4 (25)	1/1 (100)	2/6 (33.3)
30 - 34 weeks	33.8±14.8	53.9±30.5	43.9±25.7	0.89±0.44	7/15 (46.7)	3/15 (20)	10/30 (33.3)
35 - 37 weeks	13.4±7.9	26.9±19.1	23.5±17.8	0.52±0.24	3/3 (100)	6/9 (66.7)	9/12 (75)
>37 weeks	29.8±15.6	37.1±22.3	35.6±21.2	0.95±0.83	4/7 (57.1)	13/28 (46.4)	17/35 (48.6)
All	30.8±14.5	39.5±26	**36.9±23.2**	**0.85±0.62**	15/29 (51.7)	23/53 (43.4)	**38/83 (45.8)**

* - A total of 5 neonates had missing maternal HIV results, and of these 4 were well neonates,
1 neonate with suspected had missing 25 (OH)D results

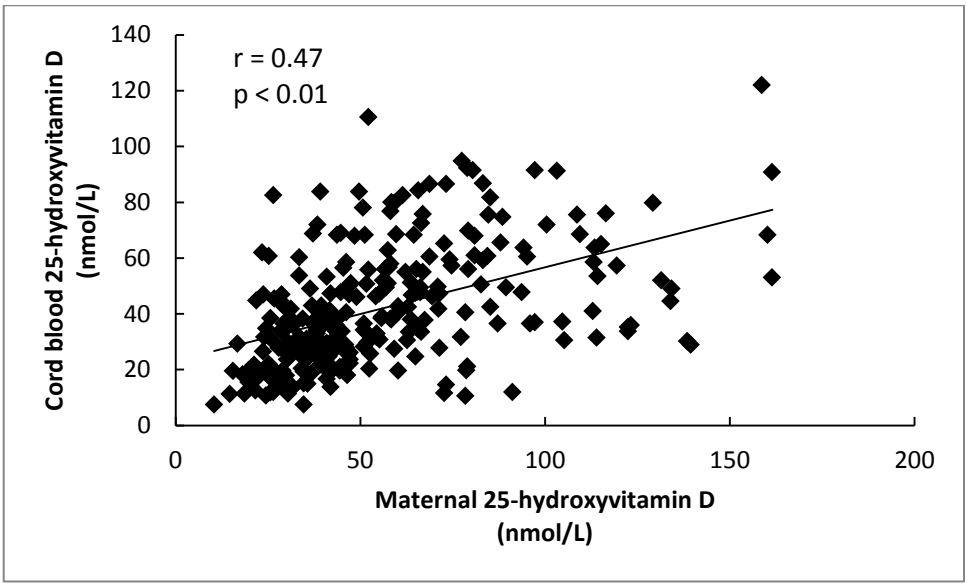

Figure 3.14: Correlation between maternal and cord blood 25-hydroxyvitamin D concentrations in well neonates

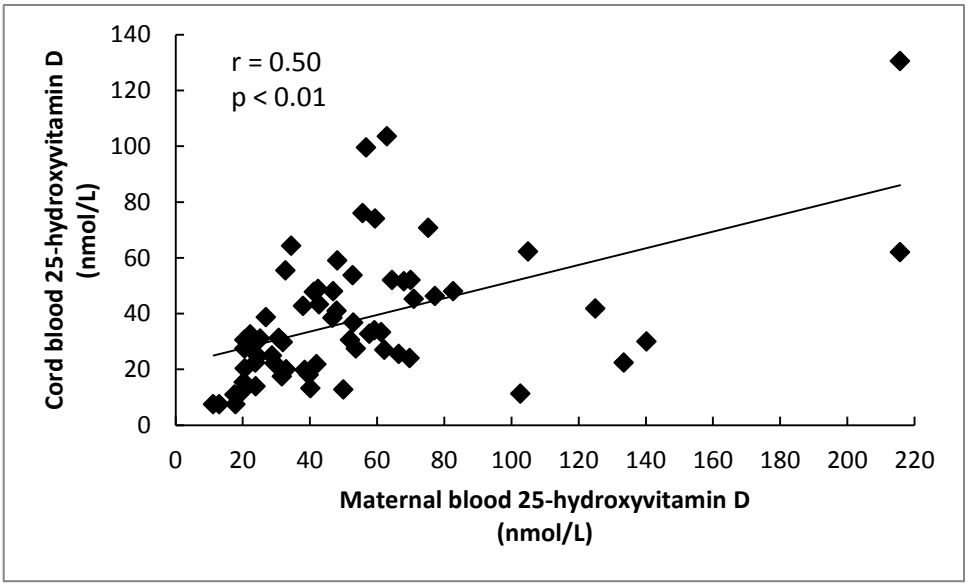

Figure 3.15: Correlation between maternal and cord blood 25-hydroxyvitamin D concentrations in neonates with protocol defined sepsis

3.3.4 25(OH)D levels in relation to seasons of the year and mother-infant pairs

25(OH)D levels in women were highest in summer, and lowest in winter and spring for mothers and their newborns (**Figure 3.16**). The percentage of women with vitamin D deficiency increased from 7% in summer to 27% in winter, while newborn vitamin D deficiency increased from 22% in summer to 60% in winter.

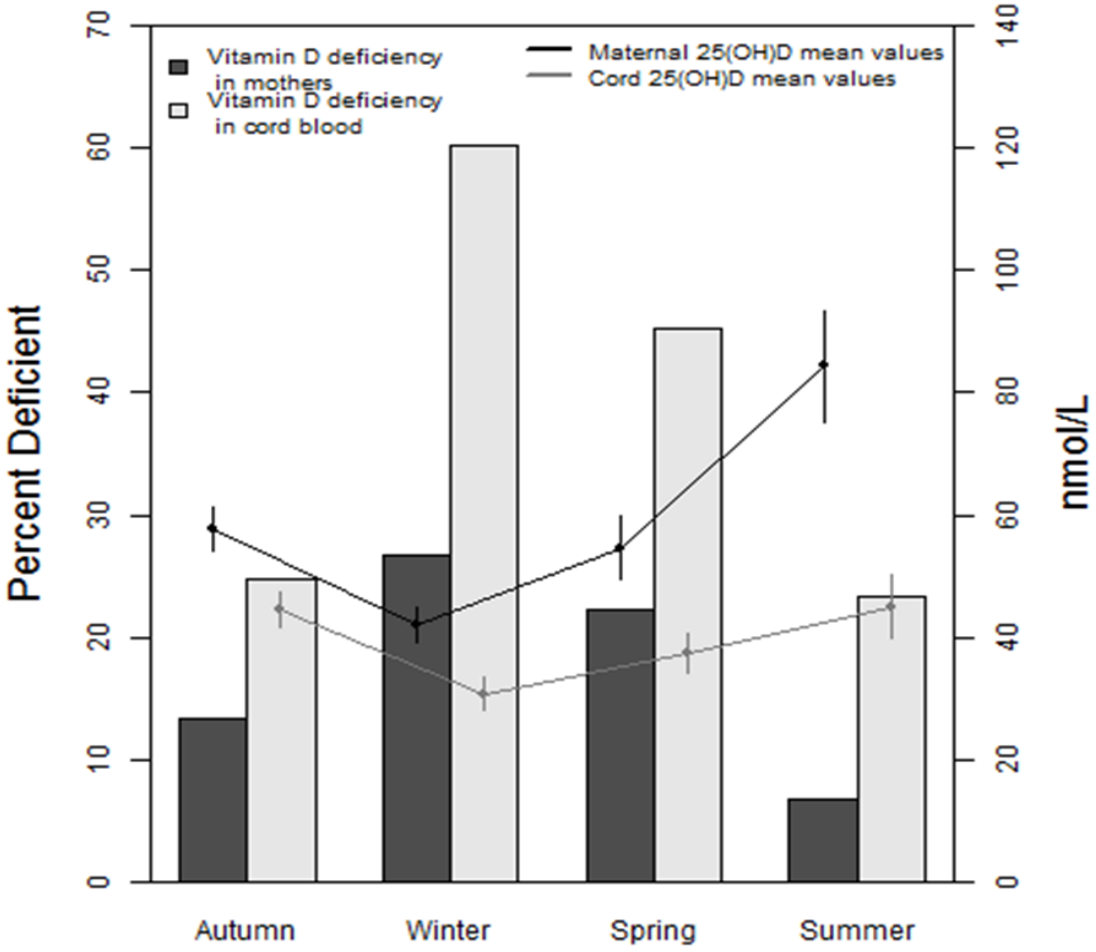

Figure 3.16: Vitamin D deficiency and 25-hydroxyvitamin D concentrations in maternal and cord blood according to seasons of the year

Factors associated with vitamin D deficiency in newborns

Neonates, who were born with vitamin D deficiency, were by univariate analysis more likely to be born by caesarean section, be of low birth weight, and their mothers also had vitamin D deficiency. Using multiple logistic regression, the predictors of vitamin D deficiency in cord blood included being born with low birth weight (aOR: 2.39, 95%CI 1.26-4.55), delivery by caesarean section (aOR:5.07, 95% CI 1.92-13.37; p<0.001), being born in winter (aOR: 4.76 (2.73-8.29; p<0.001) and being born to mothers with low 25(OH)D concentrations (aOR: 3.54, 95% CI 1.87-6.69; p<0.001) (**Table 3.47**).

Table 3.47: Univariate and multivariate analysis for factors associated with vitamin D deficiency in cord blood

	Vitamin D Deficient N = 121 n (%)	**Vitamin D Sufficient** N = 239 n (%)	**Univariate** p-value	**Multivariate** aOR (95% CI)	p-value
Maternal age in years					
<20	4 (3)	28 (12)		ref	ref
20-35	102 (85)	174 (73)	0.01	3.73 (1.16-12.02)	0.027
>35	14 (12)	36 (15)	0.106	2.42 (0.64-9.12)	0.191
Mother black African	115 (95.8)	236 (98.7)	0.096	0.75 (0.15-3.79)	0.731
Mother primigravida	36 (30)	72 (30.3)	0.961	N/A	N/A
Mother HIV infected	60 (50)	103 (43.5)	0.242	N/A	N/A
Mother HIV infected and on HAART	46 (37.8)	92 (38.5)	0.906	N/A	N/A
Preterm (<37 weeks)	40 (33.3)	64 (26.8)	0.197	0.62 (0.32-1.21)	0.161
Low birth weight	50 (41.7)	57 (23.8)	0.001	2.39 (1.26-4.55)	0.008
Caesarean section	15 (12.5)	8 (3.3)	0.002	5.07 (1.92-13.37)	0.001
Median Apgar at 1 minute*	9 (9,9)	9 (9,9)	0.221	N/A	N/A
Median Apgar at 5 minutes*	10 (10,10)	10 (10,10)	0.269	N/A	N/A
Delivered in winter months	50 (41.7)	33 (13.8)	<0.001	4.76 (2.73-8.29)	<0.001
Mother vitamin D deficient	35 (28.9)	23 (9.6)	<0.001	3.54 (1.87-6.69)	<0.001

HIV - Human immunodeficiency virus; SD - Standard deviation

* - Numbers in parenthesis are 25th and 75th centiles

- Number in parenthesis is standard deviation

3.3.5 Association between 25-hydroxyvitamin D levels and protocol defined early neonatal sepsis

Neonates who had protocol-defined EOS were more likely to be born in winter (42.2% vs 23.1%, p<0.001), had vitamin D deficiency in cord blood (45.8% vs 33.4%, p=0.035), were born preterm (49.4% vs 28.9%, p<0.001), had lower birth weight (53.0% vs 30.0%, p<0.001), were born by Caesarean section (32.5% vs 6.4%, p<0.001) and had lower Apgar scores at 1 and 5 minutes (p<0.001) (**Table 3.48**). On multivariate analysis the factors that were shown to be predictors of protocol-defined EOS were being born in winter (aOR: 2.91; 95% CI 1.47-5.76), being born preterm (aOR: 2.92, 95% CI 1.29-6.61), and being delivered by caesarean section (aOR: 6.06, 95% CI 2.63-14.0).

Table 3.48: Univariate and multivariate analysis for factors associated with early-onset protocol-defined sepsis in neonates

	Neonates with Protocol Defined Sepsis N = 83 n (%)	**Well Neonates** N = 360 n (%)	**Univariate** p - value	**Multivariate** OR (95% CI)	p-value
Maternal age in years					
<20	10 (12)	32 (9)		ref	ref
20-35	68 (82)	277 (77)	0.01	1.54 (0.51-4.59)	0.442
>35	5 (6)	50 (14)	0.106	0.94 (0.2-4.43)	0.939
Mother black African	81 (97.6)	352 (97.8)	0.917	N/A	N/A
Mother primigravida	33 (39.8)	108 (30.1)	0.09	1.73 (0.86-3.47)	0.123
Mother HIV-infected	29 (35.4)	163 (45.5)	0.096	0.71 (0.36-1.38)	0.31
Delivered in winter months	35 (42.2)	83 (23.1)	<0.001	2.91 (1.47-5.76)	0.002
Maternal vitamin D deficiency	21 (25.3)	58 (16.1)	0.051	1.59 (0.72-3.51)	0.252
Newborn vitamin D deficiency	38 (45.8)	120 (33.4)	0.035	0.82 (0.41-1.64)	0.567
Preterm (<37 weeks)	41 (49.4)	104 (28.9)	<0.001	2.92 (1.29-6.61)	0.01
Low birth weight (<2500 g)	44 (53)	108 (30)	<0.001	1.36 (0.61-3.05)	0.449
Birth by Caesarean section	27 (32.5)	23 (6.4)	<0.001	6.06 (2.63-13.97)	<0.001

*-HIV - Human immunodeficiency virus

4 DISCUSSION

4.1 Burden of Clinical Sepsis

Sepsis in neonates is an important contributor to the global burden of under-5 morbidity and mortality, including being responsible for 27% of neonatal deaths in sub-Saharan Africa in 2013.[1] Furthermore, neonatal sepsis in very low birth weight infants has been associated with increased risk for neurodevelopmental impairment (OR: 2.09; 95% CI 1.65-2.65) including cerebral palsy (OR: 2.09; 95% CI 1.78-2.45).[322] Preventing mortality and morbidity related to bacterial sepsis requires early diagnosis and treatment. Because of the high mortality rate associated with untreated or delayed treatment of bacterial sepsis, treatment needs to be started early, as soon as the diagnosis is made based on clinical signs before availability of laboratory confirmation of sepsis. Therefore neonates are often managed as having pSBI based on clinical signs.

One of the objectives of this thesis was to determine the incidence of sepsis, including pSBI, in neonates presenting within the first three days of life (EO-pSBI), and in those presenting from home within the first 28 days of life (CA-pSBI). The clinical diagnosis of neonatal sepsis is challenging due to the non-specificity of signs for sepsis in this age group. The WHO Young Infants Clinical Signs Study listed a number of clinical signs that if present are suggestive of severe bacterial infection and have a sensitivity and specificity of 85% and 75%, respectively.[52] In the present study the common clinical signs among the neonates with EO-pSBI were chest wall retractions and tachypnoea, while among neonates with CA-pSBI the commonest presenting sign was jaundice. This difference in type of presenting signs between the EO-pSBI and CA-pSBI is most likely related to the fact that these signs are general common signs for conditions including sepsis that commonly present during the time periods that define these 2 groups. Neonates soon after delivery might present with signs suggestive of sepsis even though they might not have sepsis, for example they might present with tachypnoea because they still have fluid in the alveolar space (wet lung syndrome) which might affect compliance and therefore results in tachypnoea, and secondly those who are born preterm are likely to have tachypnoea and chest retractions because of alveolar collapse from surfactant deficiency (respiratory distress syndrome). Because of difficulties of differentiating patients with tachypnoea or retractions due to pneumonia from those with wet lung or respiratory distress syndrome, these neonates are often admitted with a diagnosis of suspected

sepsis and have blood cultures done, thus providing a rationale for why respiratory symptoms are a common sign in EO-pSBI. Similarly patients who present with different signs of CA-pSBI are also more likely to have jaundice as it commonly present after the first 48 hours of life and peaks during the second week of life in well neonates. One study looking at clinical signs of sepsis in neonates with CAS reported that jaundice was the common reason for neonates seeking help at outpatients department.[48] Thus the significance of signs of respiratory distress and jaundice in this study as common signs found in patients with pSBI should be interpreted with caution, a finding which is confirmed by the lack of association with culture-proven sepsis on univariate and multivariate analysis. This finding is not supported by Kayange et al., who reported both chest indrawing and jaundice to be associated with sepsis.[40] The signs we found to be associated with culture-proven EOS were lethargy and fever; while for CAS irritability, lethargy, poor feeding, seizures, hypotonia and hypotension were associated with culture-proven sepsis. These signs are similar to those reported to be found in infants with pSBI in other studies.[51, 52, 318]

The incidence (per 1000 live births) of EO-pSBI and CA-pSBI in our study was 105.6 and 33.4 cases, respectively; with an overall incidence of pSBI among neonates in Soweto of 139 in the years 2013 to 2014. This is higher than that reported in India and Nepal, where incidences of 105 and 90/1000 live births, respectively are given.[323, 324] A possible reason for these differences is that majority of neonates in our study were born in hospital and therefore were most likely to be examined by skilled healthcare providers who are more likely to have a low index of suspicion for sepsis while in the studies from Asia neonates with pSBI were most likely diagnosed by community healthcare workers. The other possible explanation is the high prevalence of HIV exposure in our population, which in this study was shown to be positively associated with higher incidence of pSBI, with HIV-exposed having about 1.5 fold increase in incidence of CA-pSBI compared to HIV-unexposed neonates.

Neonates with pSBI were further categorized as having protocol-defined sepsis, based on presence of clinical signs and abnormality in any one of the ancillary tests (white cell counts, platelet counts and c-reactive protein) (clinical sepsis) or having positive blood culture due to an organism considered a pathogen (culture-confirmed sepsis). The overall incidence of protocol-defined EOS (clinical or culture confirmed) in this study was 49.5 per 1000 live births. This was higher than previously reported in the same population in 2004-2008 by Cutland et al., who reported an incidence of 35/ 1000 live births.[37] This difference is most

likely due to the study by Cutland et al. having fewer neonates who were HIV exposed at 26% compared to 34% in this study, and secondly it was designed to minimize enrolment of preterm newborns enrolling 4% infants were preterm compared to 46% in this study. Prematurity has been shown to be a risk factor for early-onset neonatal sepsis in a number of studies[34, 325, 326] with one study reporting that odds of being preterm among the neonates with sepsis was increased ten-fold compared to controls.[326]

4.2 Incidence of culture confirmed sepsis

The blood culture positivity rate due to putative bacterial pathogens was 4% and 7.7% for EOS and CAS, respectively in this study. The positive culture rate of 4% due to pathogens in EOS in this study was higher than that reported in studies from developed countries where positive culture rate was reported to be 0.7 – 3.3%,[104, 105, 107] but lower than that reported in other developing countries which reported positive culture rates of 33-47% .[39, 40] The high rates from developing countries are most likely due to number of births occurring in the community where births might be conducted in a non-sterile environment. In developed countries births are usually conducted inside healthcare facilities where infection control measures are closely observed. In this study most births occurred in a healthcare facility therefore rates were closer to those in developed countries. The positive culture rate of 7.7% due to pathogens in CAS in this study is similar to that in studies conducted in developing countries where culture positive rates of 3.4 to 13.5% are reported.[48 44, 110-112]

The incidence of culture confirmed EOS was 2.97/ 1000 live births, which is higher than that of CAS at 1.98 /1000 live births. The incidence of culture confirmed CAS is much lower than that reported in Kenya at 5.4/ 1000 live births[112] and from Bangladesh at 2.9 / 1000 live births.[111] The reason for this difference is most likely due to early care-seeking behavior in this study, thus many patients who presented with possible signs of sepsis were actually not infected. This is supported by the high incidence of pSBI compared to culture-confirmed sepsis in this study, which is more than ten times lower. The incidence of culture-confirmed sepsis was similar to that reported in developed countries.[34, 141]

The common organisms considered to be definite pathogens isolated in blood culture were GBS, *Enterococcus species* and *E. coli* in those with EOS and GBS, *Staphylococcus aureus* and *Enterococcus species* in those with CAS. Therefore overall GBS was the common

pathogen causing EOS or CAS during the neonatal period. The GBS being a common pathogen causing neonatal sepsis is commonly reported from developed countries.[41, 165] The finding in this study is different from many studies in developing countries where the common pathogens are reported to be Gram negatives and *Staphylococcus aureus* infection.[46, 49, 163] It is not clear why studies from the developing countries have different results from developed countries, as GBS has been reported to be one of the colonizers of the female genital tract from both developed and developing countries.[327] A possibility is that as many of the births in developing countries occur in the community, those infected with GBS may die before reaching the healthcare facility as many cases of GBS present within the first 24 hours of life. There are some studies from developing countries that reported GBS as a common pathogen and most of these studies were from Africa,[37, 43, 44, 164] except for one from the Asia.[153] The incidence of GBS in EOS was 1.41/ 1000 live births and that for CAS was 0.97/ 1000 live births giving an overall incidence of 2.38/ 1000 live births. The incidence of GBS EOS was more than 2 fold higher than the worldwide incidence of 0.43/ 1000 live births[328] or 0.41 and 0.50/1000 live births from developed countries like USA[38] and UK[41] respectively. It is also higher than that seen in developing countries like Brazil[329] and India[138, 330] which have reported incidences of 0.39/1000 and 0.15/1000 live births respectively. The incidence of GBS in EOS from this study is similar to that of 1.37/1000 live births reported by Dangor et al. in the same region and province,[331] and lower than that reported in the same institution in 2003 (2.07) and 2009 (1.97) per 1000 live births.[37, 180] This reduction is possibly due to an increase in the use of intrapartum antibiotics for patients with risk-factors for GBS. The incidence of GBS in neonates with CAS is higher that reported in Pakistan [111] at 0.10/1000 live births. In summary the incidence of GBS in this study was higher than that reported in developed countries, and other countries in sub-Saharan Africa, highlighting the importance of each country having its own surveillance in identifying common pathogens causing neonatal sepsis. This is supported by a number of systematic reviews that have reported on the incidence of GBS in different countries in the world, which have shown a wide variation among these countries.[328, 332, 333] The lower incidence of GBS in India and Brazil compared to this study could also be related to the relative lower incidence of colonization of the mother's genital tract with GBS of 12% and 14% in India/ Pakistan and Americas compared to 19% in sub-Saharan Africa.[327] Vaginal delivery was a predictor for positive blood culture in EOS, supporting that one of the mechanism of acquisition of infection peripartum is through colonization of the neonate during delivery, and through ascending infection. This difference was more prominent with GBS confirming the role of colonization on acquisition of GBS.

4.3 Antimicrobial Susceptibility

The first line antibiotics recommended by WHO for EOS and CAS are ampicillin and gentamicin. Overall at least 80% of pathogens including both Gram positives and Gram negatives isolated from both EOS and CAS were susceptible to at least one of the two empiric antibiotics used in the unit and recommended by WHO. This number is similar to that reported by Talbert et al in Kenya which had 84% of bacteria isolated in neonates with community acquired infection being susceptible to ampicillin and gentamicin.[334] But it is higher than that reported from Bangladesh where only 70% of pathogens isolated from neonates with community acquired sepsis were sensitive to ampicillin and gentamicin with 53% and 46.7% of Gram negatives being resistant to gentamicin and cephalosporins respectively.[111] The differences in susceptibilities of organisms between Bangladesh and those in this study are most likely due to high proportion of Gram negatives in neonates with sepsis, whereas in South Africa the predominant organisms are Gram positives

In focusing on susceptibility of specific organisms to these antibiotics, the finding from this study is that all GBS isolates were susceptible to ampicillin for both EOS and CAS. Among the *E. coli* isolates only 11.1% and 14.3% were susceptible to ampicillin for EOS and CAS respectively, but were all susceptible to gentamicin and cephalosporins. All *Staphylococcus aureus* were susceptible to cloxacillin from both EOS and CAS, and all those isolated from neonates with EOS were susceptible to gentamicin and 83% of the isolates from CAS were susceptible. With *Staphylococcus aureus* being the third common bacteria isolated in neonates, it would be appropriate to consider cloxacillin as part of empiric antibiotics used in neonates with community acquired sepsis, but this would mean giving three empiric antibiotics which might encourage selection of resistant bacteria. While this change might be appropriate, for now one would suggest waiting with cloxacillin as it would appear that gentamicin might be adequate as cover for *Staphylococcus aureus* since there were no deaths among the neonates with positive culture due to this organism and majority were sensitive to gentamicin. None of the *Staphylococcus aureus* were methicillin or cloxacillin resistant which is different from a study conducted in Nigeria which reported that 30.7% of community acquired *Staphylococcus aureus* were methicillin resistant (MRSA). [335] Based on these findings, it remains appropriate to use ampicillin and gentamicin for EOS and CAS in the setting where this study was conducted. Recently a number of studies from developing countries have reported on antimicrobial susceptibilities for different pathogens but they did

not differentiate between EOS and late-onset sepsis, which is important as the common pathogens causing EOS in these studies were different from those found in this study.[336, 337] The antimicrobial susceptibilities of both GBS and *E. coli* are similar to those reported from countries where these organisms are common causes of EOS.[38, 338]

4.4 Case fatality rates and predictors of mortality

The case fatality rate (CFR) in EOS was 12% and 18% in neonates with clinical and culture confirmed sepsis respectively. These are much higher than those observed in neonates with CAS which were 6% and 3% for clinical and culture confirmed sepsis respectively. The higher CFR in EOS compared to CAS is similar to the reports from other studies in which neonates aged 0-7 days were reported to have a mortality rate of 27-56% compared to 5-26% in the age group 8-59 days.[44, 112] The CFR of 18% in culture confirmed EOS is higher than the rate of 3-16% reported from developed countries [38, 105, 151, 160] but similar or lower than that of 6-28% from developing countries.[39, 42, 43, 152, 153, 174] The factors that were identified as predictors of mortality in this study were vaginal delivery, very low birth weight, clinical presentation with apnoea and need the for mechanical ventilation. While the other three factors were expected because they reflect the vulnerability or maturity of organs and severity of illness, the finding of vaginal delivery was unexpected. The possible mechanism for this association could be due to possible increased risk of intraventricular haemorrhage which has been reported to be associated with vaginal delivery in preterm infants.[339] Intraventricular haemorrhage is associated with sepsis and high mortality if severe. This is only a hypothesis, as we did not collect information on intraventricular haemorrhage. The other reason could be that vaginally delivered neonates were exposed to a high load of bacteria and therefore severe disease as confirmed by its association with culture-confirmed sepsis. This association could also be explained by the corollary, that caesarean section provided survival advantage especially in the preterm infants.[340] Vaginal delivery might have other complications that were not recorded in this study, namely prolonged second stage which is associated with morbidity and mortality. Prolonged second stage especially in nulliparous women is associated with neonatal sepsis (OR: 2.34; 95%CI 1.28-4.27), asphyxia (OR: 2.39; 95%CI 1.22-4.66) and perinatal mortality (OR 5.92; 95%CI 1.43-24.5).[341]

4.5 HIV Exposure and Neonatal Sepsis

Exposure to HIV is more likely to predispose neonates to sepsis as it is associated with abnormalities in the humoral and cellular immune systems in the neonate [205, 206, 208, 209] and an increases in bacterial vaginosis and diversity of vaginal microbiota in the infected mother.[342, 343] Bacterial vaginosis is associated with premature rupture of and prematurity,[344, 345] thus the possible reason for high prevalence of PROM and use of intrapartum antibiotics in HIV-infected women. Therefore it was important that patients were stratified according to HIV exposure in this study. Though there were no differences in most of the clinical signs between HIV exposed and unexposed neonates with EOS, more patients in the HIV exposed neonates required mechanical ventilation. This is most likely due to the fact that HIV exposed neonates were of lower gestation than unexposed neonates and it is well know that HIV exposed infants tend to be of lower gestation and birth weight than unexposed neonates.[346, 347] Among the neonates with CAS more HIV exposed neonates presented with signs other than jaundice than unexposed infants. This finding might suggest a greater degree of severity of their illness than in unexposed infants who commonly presented with jaundice which is a common physiological condition during the neonatal period. Thus signs considered to be suggestive of sepsis were more common in HIV exposed infants than unexposed. The findings are also supported by the fact that HIV positive mothers were more likely to present with prolonged rupture of membranes which increases the risk of infection to the foetus. The high prevalence of metabolic acidosis in HIV-exposed neonates in this study could be explained by perinatal exposure to antiretroviral therapy as all HIV-positive mothers were put on three drugs including nucleoside reverse transcriptase inhibitors either as part of their treatment or PMTCT. It has been reported that in-utero exposure to antiretroviral therapy is associated with transient lactic acidaemia in neonates.[348, 349]

The incidence of EO-pSBI among those who were HIV exposed was significantly higher than those who were not exposed, but this difference was not observed in those with CA-pSBI. The incidence of laboratory-diagnosed sepsis was about 2 fold higher in HIV exposed than in unexposed neonates with both early-onset and community-acquired sepsis. The high incidence of EO-pSBI in HIV exposed could be explained by a number of factors, namely a higher proportion of neonates who were HIV exposed were preterm, therefore more likely to present with signs of respiratory distress, a sign used in the diagnosis of pSBI. Secondly HIV exposed neonates might have impairment in their immune system therefore be at greater risk

of sepsis or they may be exposed to high load of pathogens in their mothers' genital tract at the time of delivery. The possibility of an impaired immunity is further supported by the higher incidence of protocol defined and culture-proven CAS, which was 2-3 folds higher in HIV exposed than unexposed neonates. The high incidence of sepsis observed in HIV exposed neonates in this study has been observed in other studies.[195, 196] A study from Belgium reported that the incidence of neonatal GBS sepsis was greater in HIV exposed than unexposed newborns.[196] Similarly a study from South Africa reported that the vertical transmission of *E. coli* was higher in the HIV exposed than unexposed neonates.[195] Therefore it appears that HIV exposure is a risk factor for development of sepsis during the neonatal period.

This study highlights the burden of sepsis, which is partly due to the high prevalence of maternal HIV infection in the region and Southern Africa in general. Therefore strategies introduced to reduce neonatal sepsis should also include those to reduce maternal HIV infection.

4.6 Use of the Taqman array card in the diagnosis of sepsis

4.6.1 Blood Taqman array card

In this study it was found that TAC detected organisms in the blood of 37% and 46% neonates with clinical EOS and CAS respectively. These detection rates are much higher than those observed using blood culture which were 4% and 7% in EOS and CAS respectively. These differences might be explained partially by the fact that TAC detects bacteria that are not detectable on microbiological cultures, namely *Ureaplasma*, *Mycoplasma* and *Bordetella pertussis* and viruses. It is therefore important that when one compares the detection rate between the two tests one should only include pathogens that can be detected by both tests. Using this methodology and correcting the positive results obtained using TAC in healthy neonates, TAC still had a higher detection rate at 14.5% and 19% for EOS and CAS respectively, compared to 4% and 7% using blood culture. The other reason for the differences in detection rate between TAC and culture relates to the methods used in detection of organisms. The PCR-based assays, including the TAC system detect the 16S ribosomal RNA in blood, thus are able to detect viable and therefore culturable organisms, non-culturable but viable dormant organisms and non-viable organisms. Blood culture detects only culturable organisms and culturability of an organism refers to its viability at the time or

circumstances under which the culture is done. Therefore a negative culture does not necessarily mean that there is no organism as the isolation media and incubation conditions might not allow for the growth of all strains. Some culturable organisms might be dormant but not dead, and thus have a potential to return to a viable state of being culturable.
In assessing the role of TAC in diagnosing neonatal sepsis, using blood culture as a gold standard test, overall TAC had a sensitivity of 79% but a specificity of only 70%. These numbers seem to vary for different organisms, for example, the sensitivity and specificity for GBS was 83% and 95% respectively compared to sensitivity of 50% and specificity of 98% for *E. coli.* This suggest that while TAC might be useful in diagnosis of neonatal sepsis, it needs to be developed further for it to be used to detect a broader range of pathogens.

Finding evidence of *Pseudomonas aeruginosa* and *Streptococcus pneumonia* being so common was unexpected. These are not organisms commonly cultured in neonates. In this study, we did not have a single culture positive for *Streptococcus pneumoniae* and there was only one positive culture for *Pseudomonas aeruginosa*. The possible explanation for the high detection of these organisms in TAC but not in blood culture could be due either to contamination of the specimen after the blood had been injected into the blood culture bottle but before testing the blood by TAC or to there being other organisms with similar genetic make-up but not detectable on blood culture. The high positivity rate for *Pseudomonas aeruginosa* was most likely due to contamination as the positive results were clustered over two months. Previously contamination of blood with *Pseudomonas aeruginosa* had been observed in Chris Hani Baragwanath Academic Hospital, there had been a cluster of positive blood cultures without other markers of sepsis being suggestive and babies recovered without the use of appropriate antibiotics covering *Pseudomonas aeruginosa*. This was subsequently found to be due to health care providers putting the required blood specimen in the blood gas analyzer first before putting the remaining specimen into the blood culture bottle. The nozzle of the blood gas analyzer was found to be colonized with *Pseudomonas aeruginosa*. In order to exclude the second possibility that the positive result was due to a different organism with similar TAC profile, the specimens that were positive for *Streptococcus pneumonia* on TAC were selected randomly to be tested with LytA and were found to be positive suggesting that TAC correctly detected *Streptococcus pneumoniae*. The source of *Streptococcus pneumoniae* is unknown as vaginal swabs from women in labour did not culture *Streptococcus pneumoniae* (unpublished data) making it unlikely that the organisms were from maternal

genital tract. The significance of finding *Streptococcus pneumoniae* in blood using TAC is unknown as its prevalence was similar in cases and controls.

4.6.2 Organisms from Naso/oro-pharyngeal swabs using the Taqman array card

One of the entry points for infection into the blood is the mucosa of the respiratory tract system, therefore one would expect that colonization of the oropharyngeal and nasopharyngeal airway might be associated with the development of clinical sepsis. In this study, neonates with clinical sepsis and well neonates (controls) had NPOP taken for detection of organisms by TAC. Three-quarters of the cases between ages 3-27 days and less than half of cases aged between 0-2 days had organisms detected in their NPOP swabs, suggesting that the colonization of the airways increases with postnatal age. This was confirmed by the proportion of colonized cases increasing from 44% at day 0 to 95% at day 21-27 days; and similarly among controls the percentage increased from 54% at day 0 to 74% at day 21-27 days. The colonization of the airways did not appear to be a factor in the pathogenesis of in neonatal sepsis as the numbers of colonized infants were not significantly different between cases and controls with rates 44% vs 54% in 0-2 days age group and 75 vs 70% in the age group 3-27 days. The acquisition of the organisms in the nasopharynx appears to start in utero or at the time of birth as 44% and 54% of controls was colonized as early as day 0. This is the first study reporting on colonization of naso-oropharyngeal airways from birth through the neonatal period.

4.7 Attributable Proportion

In considering the fact that TAC can detect 16S ribosomal RNA of organisms that might not be viable and therefore not causative of sepsis, we also performed TAC assay in blood of neonates considered to be well in order to assess the significance of finding an organisms in neonates with clinical sepsis. In this study we found that among the healthy neonates (controls) 20% in the first 3 days of life and 27% in the age group 3-27 days had organism detected in blood. These findings brought into question whether blood is a true sterile site as it is commonly referred to or not, and secondly whether the finding of an organism in blood of a sick neonate means that the organism detected is a causative organism or not. The detection of viruses and bacteria in blood by TAC in healthy neonates in the current study challenges the notion that blood is a sterile site. Other studies have also reported that blood from healthy individuals can contain bacterial 16S ribosomal DNA.[114-116]

The positive results in blood of healthy neonates based on TAC challenge the notion that healthy humans have sterile blood, as it suggests that non-culturable or dormant forms of organisms are present.[350-354] Secondly finding non-culturable organisms such as Ureaplasma *species* and a statistical modelling using TAC suggests that the presence of a negative blood culture does not mean an absence of a causative organism. Therefore detection of organisms using PCR-based technology like TAC in patients with clinical sepsis should be interpreted with caution as some of the organisms might be dormant or non-viable and therefore not the cause of the current illness, although they have potential for causing disease when reactivated or resuscitated. The finding of organisms in the blood of healthy neonates makes it difficult to implicate an organism found in a neonate with clinical sepsis as being the causative organism. Therefore in order to assess which detected organisms contribute to illness in cases and what the odds are of detecting the pathogenetic organism, a statistically modelling was used for EOS and included positive blood cultures in cases and positive blood and nasopharyngeal results from TAC in both cases and controls. Using the statistical modelling only 27% of cases could be attributed to have aetiological organisms from the organisms which could be detected in blood culture or in TAC, thus 73% of cases could not be attributed a causative organism. Among those neonates with an attributed aetiology, the common pathogens were *Ureaplasma species*, GBS and *Klebsiella pneumoniae*. These three organisms are commonly found in the urogenital tract of pregnant women, suggesting acquisition from the mother. This concept is further supported by the finding that vaginal delivery is a predictor of culture-confirmed sepsis.

Though GBS and *Klebsiella pneumoniae* are commonly considered causes of neonatal sepsis, *Ureaplasma species* has not been considered a common pathogen in neonatal sepsis. The neonatal disease that has been reported to be associated with *Ureaplasma* colonization/ infection is bronchopulmonary dysplasia, a condition commonly seen in neonates born preterm.[355] *Ureaplasma species* are detected in 67% of sexually active women of reproductive age, compared to 40% of sexually inactive and 25% of postmenopausal women.[356] The prevalence of amniotic fluid infection with *Ureaplasma* is seen 6-9% in those with preterm and intact membranes compared to 22% in those with preterm labour with ruptured membranes.[357-359] The rate of *Ureaplasam species* respiratory tract colonization increases with duration of rupture of membranes.[360, 361] Therefore this could explain the difference between cases and controls in the prevalence of *Ureaplasma* in this study as more cases had prolonged rupture of membranes than controls. However, 45% of neonates with *Ureaplasma* on TAC

were born preterm, leaving 55% being full-term infants suggesting that this infection is not limited only to those born preterm.

The finding in this study that organisms were identified in both NPOP and blood samples suggests possible translocation of the organism from the mucosa of the respiratory tract. This translocation can occur with or without inflammation.[362] and therefore present with or without clinical signs of infection making it difficult to attribute the illness to the presence of the organism. One study reported that 26% of neonates who had positive *Ureaplasma* in the lower respiratory tract also had bacteraemia[363] and another study reported that 23% of neonates had positive culture for *Ureaplasma* in cord blood.[364] Therefore it appears that there is a relationship between the presence of *Ureaplasma* in blood and pharyngeal secretions, supporting translocation from the respiratory tract through the mucosa to the blood stream. A number of studies that looked at treatment of patients with positive tracheal aspirate cultures for *Ureaplasma* reported that macrolides led to clearance of the organism but no reduction in neonatal morbidity.[365] The lack of effect on morbidity in these studies could be related to not having adequate sample size, secondly studying different types of macrolides or that *Ureaplasma* does not cause diseases in the neonate. Therefore there is a need to conduct larger randomized clinical trials with clear enrolment criteria to assess the efficacy and safety of available macrolides. .

4.8 Vitamin D and Sepsis

The increasing number of reports on vitamin D deficiency being associated with adverse obstetric and neonatal outcomes suggests that the extent of this problem should be assessed for each and every community or population in which vitamin D deficiency might be prevalent. Serum 25(OH)D reflect the adequacy of vitamin D intake and cutaneous production and is therefore used for the assessment of vitamin D status. In this study we assessed serum 25(OH)D in pregnant women and their offspring in Johannesburg, South Africa (latitude 26°S) in order to assess the prevalence of vitamin D deficiency, the factors associated with this deficiency and the role of vitamin D status in early-onset neonatal sepsis. In a cohort of 621 pregnant women in this study the mean 25(OH)D was 54.7 ± 30.1 nmol/L and nearly one in five (19%) pregnant women at delivery were vitamin D deficient [25(OH)D <30nmol/L]. These results are very similar to another study conducted in adult black African females in Johannesburg, who were not pregnant, in whom the mean 25(OH)D was 58.3 nmol/L.[366] The

mean 25(OH)D observed in pregnant women in the present study is much higher than those reported from Asian countries which have reported means of <40 nmol/L,[367] but similar to levels reported in African Americans from the United States.[258, 368, 369] Our mean is lower than those reported from other sub-Saharan countries.[370-372] In Nigeria the mean 25(OH)D was reported to be 90 nmol/L in pregnant women not practicing purdah (use of veils by women).[370] In East Africa the serum 25(OH)D concentrations in pregnant women from the ethnic groups of Maasai and Sengerema were 147.7 nmol/L and 141.9 nmol/L.[371] The higher levels of serum 25(OH)D in blacks from East Africa are thought to be related to sun exposure as they tend to spend more time outside.[371] The role of sunlight in maintaining 25(OH)D levels is supported by the findings that women practicing purdah in Nigeria had lower serum 25(OH)D than those not practicing purdah (53 vs 90 nmol/L).[370] In the present study the levels of 25(OH)D varied by season, with levels during summer being nearly double those during winter (82.9 vs 41.8 nmol/L). The prevalence of vitamin deficiency among mothers followed a similar pattern (27% and 7% during winter and summer respectively). Numerous other studies have also reported similar seasonal variations.[244, 373-375] This study and these other studies confirm that sun exposure is a major determinant of vitamin D status in humans.[243-245, 249, 254, 258]

Mean 25(OH)D concentrations in cord blood was 39 nmol/L and it correlated with maternal serum 25(OH)D concentrations (r=0.47). This finding is similar to other studies that have consistently reported a correlation between cord blood 25(OH)D and maternal concentrations.[244, 256, 258, 369, 376, 377] The main factors influencing cord blood concentrations of 25(OH)D in the present study were maternal serum 25(OH)D and season of the year. The seasonal variation in cord blood concentrations reflect seasonal changes seen in maternal serum concentrations. The prevalence of vitamin D deficiency in neonates was 40%. This prevalence is lower than that reported in black neonates from other studies, in which vitamin D deficiency varied from 46% -65%,[258, 259, 378, 379] while in white neonates the prevalence is much lower at 4-11%.[254, 257-259] A number of studies have reported an association between HIV-infection and vitamin D deficiency.[380-384] This association has been related to the degree of immunosuppression/severity of infection and thus vitamin D status has been reported to improve with the use of antiretroviral drugs.[385, 386] It is possible that the absence of association between HIV infection and vitamin D deficiency in this study could be due to most patients being on antiretroviral agents and well, and their activities not being restricted by ill-health.

Even though neonates with early-onset sepsis (EOS) had lower mean cord blood 25(OH)D than healthy neonates (36.3 vs 42.0 nmol/L, p=0.033), and a higher proportion of neonates with vitamin D deficiency were found in the group with EOS than in healthy neonates (45.8% vs 33.6%, p = 0.037), vitamin D deficiency was not a predictor of neonatal sepsis based on multivariate analysis. This finding is contrary to that reported from Turkey where vitamin D deficiency was associated with sepsis.[6, 260] Therefore it remains unclear whether low 25(OH)D concentrations predispose to EOS or sepsis results in low levels of 25(OH)D. The finding of similar concentrations in both groups of mothers suggests that sepsis in the neonate might play a role in lowering circulating concentrations in the neonate.

A systematic review of studies conducted in adults reported that patients with vitamin D deficiency before or during hospitalization had higher odds of developing sepsis compared to individuals without vitamin D deficiency, suggesting that vitamin D deficiency predisposes to infection.[387] Despite this proposed mechanism, supplementation with vitamin D to normal levels in vitamin D deficient patients has not resulted in a reduction in the incidence of sepsis.[388] Another suggested mechanism is that sepsis has an effect on vitamin D binding protein reducing serum concentrations. Serum levels of vitamin D-binding protein, the carrier protein of 25(OH)D, have been reported to be lower in patients with sepsis compared to healthy controls, thus resulting to lower serum concentrations of 25(OH)D without affecting the biologically active free levels.[308, 389] It is also possible that the association observed between low 25(OH)D and EOS might be related to high c-reactive protein (CRP) in neonates with sepsis. Recently a study in neonates reported an inverse relationship between 25(OH)D concentrations and CRP.[390] In this study there were few neonates with culture proven sepsis, therefore the diagnosis was primarily made on abnormal biomarkers, c-reactive protein and interleukin-6. High c-reactive protein on its own has been associated with low vitamin D. Therefore the next step will be to enrol more patients with culture proven sepsis with or without raised biomarkers. The second step is to assess levels of vitamin D binding protein between those with or without sepsis as levels of this protein have also been associated with neonatal sepsis. The third step is to assess whether prevention of vitamin D deficiency is associated with reduction in neonatal sepsis through randomizing pregnant women to vitamin D supplementation or placebo. Lastly it would be appropriate to determine genotype associated with low 25(OH)D levels, and see if there is a difference in neonates with or without this genotype in terms of developing neonatal sepsis.

4.9 Strengths of the study

A large number of neonates were enrolled in this study allowing one to determine the incidence of both clinical and culture-confirmed sepsis per 1000 live births for both HIV exposed and unexposed neonates. Most patients suspected to have either EOS or CAS were born inside a healthcare facility consistent with the current statistics from Statistics South Africa that most (95.6%) births in Gauteng, South Africa take place in healthcare facilities.[391] This is in contrast to reports from many developing countries, where more than 50% of births are reported to take place at home.[392] The majority of neonates enrolled with EOS had a blood culture done within the first 24 hours of life. The fact that most neonates were born within a healthcare facility and developed their illness soon after birth, they had blood culture taken at presentation generally within the first 24 hours, enables one to conclude that the most likely source of infection is from the mother rather than from the community environment. Another strength of the study was the design of the research protocol, which included controls in the assessment of TAC, thus enabling us to determine the true positive rate of this test, which could otherwise have been overestimated if controls had not been included. A number of studies previously have assessed the use of PCR-based tests without including controls therefore potentially overestimating the incidence of sepsis. For the study on vitamin D levels, as far as one is aware, this is the first study that has measured 25(OH)D concentrations in black pregnant women and their offspring and its association with sepsis in the South African context. The strengths of this part of the study include the large number of patients enrolled and the fact that the study was conducted throughout the different seasons of the year.

4.10 Limitations

One of the limitations of the study was that we were unable to enroll over weekends due to research staff constraints, therefore not all sick neonates were enrolled into the study, which could have resulted in an underestimation of incidence of sepsis. However, this is unlikely as one would not expect that neonates born over the weekend would be sicker or grow more pathogens than those being born on weekdays. Secondly in calculating for incidence for sepsis we corrected for non-enrolment over the weekends. Blood cultures were taken by doctors as part of routine care, and thus blood volumes available and put in the culture bottle might have been lower than the minimum required volume of 0.5-1 ml, resulting in an underestimation of culture-confirmed sepsis. Another limitation was not having HIV-PCR at

birth to further elucidate the role of HIV infection in neonatal sepsis and placental histopathology with TAC to assess if the positive TAC results in blood and NPOP swabs reflects or is affected by integrity if the placenta.

Furthermore, blood from cases was taken by the attending doctor at the time of the blood culture, resulting in not all cases having bloods taken for TAC. This could have resulted in error in the estimation of TAC-confirmed sepsis through sample selection bias. Regarding the study on vitamin D the major limitation relates to the small number of positive cultures in neonates with suspected EOS resulting in a small number of infants being confirmed with definite EOS. A further limitation was the inability to track the changes in maternal vitamin D status through the three trimesters of pregnancy, which might have influenced immune status and susceptibility of the neonate to infection, and not recording intake of diet or supplements that might affect vitamin D levels.

5 CONCLUSIONS

Sepsis is a major burden in neonates in Soweto with 139/1000 and 7/1000 live births having possible serious bacterial and culture-confirmed early-onset or community acquired sepsis respectively. The incidence of neonatal sepsis is much higher than that observed in developed countries but is similar to that reported in other developing countries. The common pathogens causing culture-confirmed sepsis are GBS, *E. coli* and *Staphylococcus aureus* in neonates with EOS and CAS. The vast majority of these common pathogens are susceptible to the recommended first line antibiotics, ampicillin and gentamicin with 100% of GBS and *E. coli* being susceptible to ampicillin and gentamicin respectively, and 83% of *Staphylococcus aureus* being susceptible to gentamicin. Neonatal sepsis is associated with high case fatality rate. Neonates born to mothers who are HIV infected have high incidence of sepsis compared to those born to uninfected mothers.

TAC detects pathogens in neonates with sepsis but results from this test must be interpreted with caution as the pathogen detected might not be the cause of sepsis as the test might detect components of the pathogen that might not be viable to cause sepsis. Using TAC in its current form as part of routine care in diagnosing bacterial neonatal sepsis still has major limitations, because of its low specificity, high false positive rate and inability to test for antimicrobial susceptibility. However, it has a number of advantages over blood culture because of its ability to detect non-culturable pathogens namely atypical bacteria like Ureaplasma, Mycoplasma and viruses, and the short turnaround time of less than 4 hours in getting results. In its current form, the strategy to use TAC will first require that more probes are added in the current list. In the setting where this study was conducted these probes will need to include *Acinetobacter species, Candida* species, *Enterococcus* species, *Viridans streptococci* and coagulase negative *staphylococcus*. Once these probes are added it will need to be used in supplementation to blood culture. Blood culture will still need to be part of work up because of TAC's low specificity and not being able to assess antimicrobial susceptibility. The approach that one would need to adopt will be as follows, 1. if TAC is positive with a common pathogen known to cause sepsis, then a patient is changed to a targeted antibiotic, while awaiting blood culture results. If blood cultures subsequently come back positive with the same pathogen as in TAC, then the patient completes the full course on the targeted antibiotic. If blood culture is negative, then assess clinical and ancillary laboratory test responses and if the responses are positive then continue targeted antibiotics for organisms

identified on TAC. 2. If both TAC and blood culture are negative for culturable organisms, then consider non-culturable organism isolated in TAC as a possible causes and start on targeted treatment if available, e.g. use chlarithromycin or azithromycin for Ureaplasma.

The major current limitation of TAC is that it does not differentiate between viable and non-viable organisms. However there are advances that are starting to differentiate DNA from a viable to that from non-viable organism. There are techniques that can eliminate PCR signals from dead organisms or cells and able to quantify viable cells.[393, 394] Incorporating these techniques into TAC technology will revolutionize the use of PCR-based technology in diagnosing neonatal sepsis. In getting TAC to fully takeover the role of blood culture a technique similar to gene-X-pert used in diagnosing rifampicin sensitive *Mycobacterium tuberculosis* will also need to be adopted for TAC in order to detect resistant organism.

One in five black pregnant women delivering at Chris Hani Baragwanath Academic Hospital has vitamin D deficiency. The concentrations of 25(OH)D in maternal blood correlate with those in cord blood of their offspring and are related to season of the year. The prevalence of vitamin D deficiency in infants was double that seen in mothers. Even though neonates with EOS were more likely to have lower levels of 25(OH)D or to be vitamin D deficient on univariate analysis, these were not predictors of sepsis on multivariate analysis, suggesting that there could be another explanation for the low levels of 25(OH)D in neonates with EOS. One possibility includes that there were more neonates enrolled in winter months than summer months. Future studies should look at including a larger number of neonates with definite culture-confirmed sepsis and spread out through the different seasons of the year. They should also assess the effect of vitamin D supplementation during pregnancy on the incidence and severity of neonatal sepsis and on the role of sepsis on vitamin D binding protein concentrations.

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7 APPENDICES

7.1 Appendix 1: Ethics clearance certificates

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

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Prof Sithembiso Velaphi

CLEARANCE CERTIFICATE	**M120552**
PROJECT	Use of New Molecular Technology in Diagnosing Early-Onset and Community Acquired Neonatal Sepsis
INVESTIGATORS	Prof Sithembiso Velaphi.
DEPARTMENT	Department of Paediatrics
DATE CONSIDERED	25/05/2012
+DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 25/05/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

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

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...

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

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Professor Sithembiso Velaphi

CLEARANCE CERTIFICATE	M120651
PROJECT	Vitamin D tatus and Early Neonatal Sepsis
INVESTIGATORS	Professor Sithembiso Velaphi.
DEPARTMENT	Department of Paediatrics/Neonatology
DATE CONSIDERED	29/06/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

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

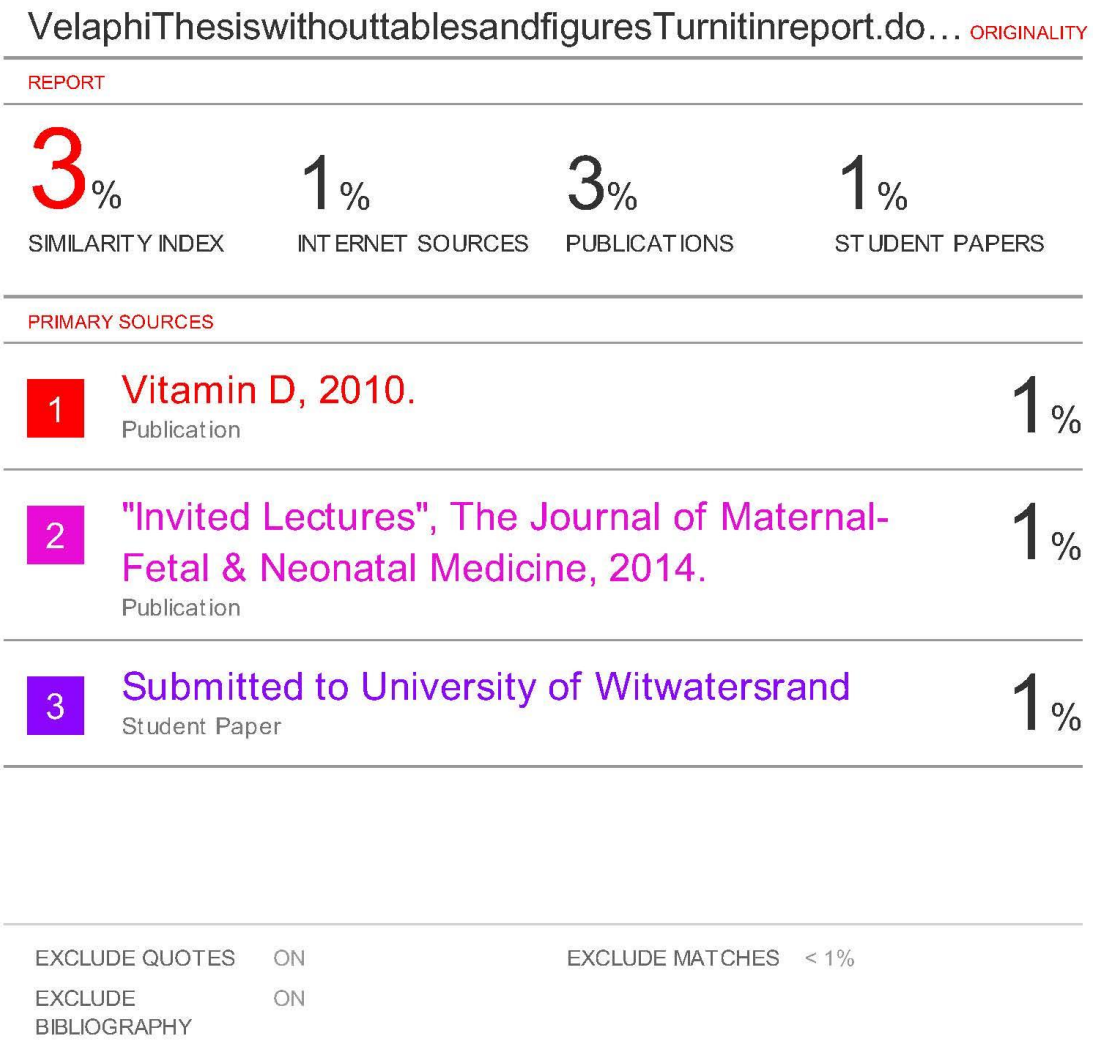
DATE 29/06/2012 CHAIRPERSON .....
(Professor PE Cleaton-Jones)

*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...

7.2 Appendix 2: TurnItIn® plagiarism report summary



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7.3 Appendix 3: Case report forms

7.3.1 Case report form 1

1. Inclusion Criteria for Early-Onset Sepsis

Sepsis Aetiology in Neonates in South Africa (SANISA): CASE REPORT FORM
INCLUSION CRITERIA

A. Participant Identifiers

Barcode with ID No

1. Participant Number: ___ ___ ___ ___ ___ ___
2. Initials: ____ ____ ____ ____ - ___
3. Date of Birth: ___ ___ / ___ ___ / ___ ___
4. Date of Screening.for the study: ___ ___ / ___ ___ / ___ ___

B. Inclusion Criteria

5. Is the baby older than 28 days old? ☐ Yes ☐ No
6. Does the baby have severe congenital abnormalities? ☐ Yes ☐ No
7. Has the baby been admitted in hospital in the last 28 days other than time of birth? ☐ Yes ☐ No
8. Is the baby's medical care redirected because of anticipated demise/ death ☐ Yes ☐ No
9. Is the mother less than 18 years old? ☐ Yes ☐ No
10. Is this participant eligible for enrolment? ☐ Yes ☐ No

(Participants are eligible if the answer is "No" to questions 5 through 9 OR if they are enrolled through Dr. Ziyaad Dangor's study),

(Participants are NOT eligible if any one of the answers in questions 5 through 9 is "Yes"

(If not eligible do not obtain consent and do not enroll)

C. Informed consent

1. Consent obtained from the mother ☐ Yes ☐ No
2. If not, state reason: 1. Unable to find the mother ☐; 2. Mother did not wish to participate ☐
   3. Study blood not taken ☐; 4. Mother too ill to give consent (e.g. in ICU) ☐

(If no consent obtained do not enroll)

D. Patient enrolled ☐ Yes ☐ No

If Yes, patient enrolled as ☐ Case ☐ Control

Date of enrolment into the study: ___ ___/ ___ ___/ ___ ___; Time of enrolment: ___ ___h ___ ___

Version 2
24 April 2013

Form completed by: _____
Date completed: __ __/ __ __/ __ __

7.3.2 Case report form 2

2. Demographic, Admission, Birth and Risk Factors for Early-onset Sepsis

Sepsis Aetiology in Neonates in South Africa (SANISA): CASE REPORT FORM
DEMOGRAPHIC, BIRTH AND ADMISSION DETAILS AND RISK FACTORS

A. PARTICIPANT IDENTIFIERS

Barcode with ID No

1. Participant Number: : ___ ___ ___ ___ ___ ___
2. Initials: ____ ____ ____ ____

B. DEMOGRAPHICS AND BIRTH DETAILS:

3. Child's date of birth (dd/mm/yyyy): ___ ___ / ___ ___ / 20 ___ ___
4. Time of birth (hh/min): __ __h __ __
5. Race: ☐ African ☐ Coloured ☐ Indian ☐ White ☐ Oriental
6. Sex: ☐ M ☐ F
7. Place of birth: ☐ CHBH ☐ Clinic ☐ Other hospital ☐ Home
8. Mother attended antenatal clinic ☐ Yes ☐ No
9. Birth weight or admission weight if no birth weight: ___ ___ ___ ___ grams
10. Prematurity ☐ Yes ☐ No
    10.1. If yes, state gestational age: _____ weeks
11. Apgar scores: 1 minute: ___ ___ 5 minute: ___ ___
12. Arterial blood gas done within 1 hr of birth: ☐ Yes ☐ No ☐ Not recorded
    12.1. If yes, state the pH: ____; BE: ____; HCO_3: _____; Lactate: _____
13. Is infant HIV- exposed ☐ Yes ☐ No ☐ Not recorded
14. Maternal CD4 count done: ☐ Yes ☐ No ☐ Not applicable
    14.1. If yes, state value: _____
15. Mode of delivery Vaginal ☐ Caesarean ☐

C. ADMISSION DETAILS

16. Date of admission: ___ ___ / ___ ___ / 20 ___ ___ (dd/mm/yyyy)
17. Time of admission: __ ___/ ___ ___ (hh/min)
18. Admitting ward: ☐ NICU ☐ TC ☐ Ward 66 ☐ Ward 17 ☐ Ward 18 ☐ Ward 19 ☐ Ward 33

Version 2
24 April 2013

Form completed by : _____
Date completed: __ __/ __ __/ __ __

D. RISK FACTORS ***(Only for babies ≤3 days)***

19. Prolonged rupture of membranes (>18 hours) □ Yes □ No □ Not recorded

19.1. If yes state number of hours: _____

20. Meconium stained liquor □ Yes □ No □ Not recorded

21. Offensive liquor □ Yes □ No □ Not recorded

22. Fever during labour (Any temperature >38ºC) □ Yes □ No □ Not recorded

23. Did mother receive antibiotics during labour and delivery? □ Yes □ No □ Not recorded

(If yes complete sections 23.1 to 23.3, using the numbers provided in the list of antibiotics below)

23.1. If yes, state antibiotic code*: ___

23.1.1. Number doses given: _____

23.1.2. Interval of dosing: _____

23.1.3. Date and time of last dose: __ __/ __ __/ 20 __ __ __ __ h __ __

23.2. If yes, state antibiotic code*: ___

23.2.1. Number doses given: _____

23.2.2. Interval of dosing: _____

23.2.3. Date and time of last dose: __ __/ __ __/ 20 __ __ __ __ h __ __

23.3. If yes, state antibiotic code*: ___

23.3.1. Number doses given: _____

23.3.2. Interval of dosing: _____

23.3.3. Date and time of last dose: __ __/ __ __/ 20 __ __ __ __ h __ __

24. Was antibiotic given at caesarian section? □ Yes □ No □ Not recorded □ N/A

24.1. If yes, state antibiotic code* for all antibiotics she received: ___; ___; ___

***Antibiotic codes:**

1=Amikacin; 2=Ampicillin; 3=Amphotericin B (Ambisome); 4=Cefepime; 5=Cefotaxime; 6=Ceftriaxone (Rocephin); 7=Cefuroxime; 8=Clindamycin; 9=Cloxacillin; 10=Co-Amoxiclav; 11=Erythromycin; 12=Gentamicin; 13=Meropenem; 14=Metronidazole (Flagyl); 15=Penicillin; 16=Pip-Taz; 17=Sulfamethoxazole/Trimethoprim (Bactrim); 18=Tobramycin; 19=Vancomycin

7.3.3 Case report form 3

3. Clinical presentation for Early-Onset Sepsis

Sepsis Aetiology in Neonates in South Africa (SANISA): CASE REPORT FORM
CLINICAL PRESENTATION

A. Participant Identifiers

Barcode with ID No

1. Participant Number: ___ ___ ___ ___ ___ ___

2. Initials: ____ ____ ____ ____

B. Clinical presentation (signs and symptoms) within 24 hours of onset of symptoms or admission:

1. Date of earliest symptom onset: ___ ___ / ___ ___ / 20 ___ ___

2. Non-specific signs

2.1.	Lethargy	□ Yes	□ No	□ Not recorded
2.2.	Irritability	□ Yes	□ No	□ Not recorded
2.3.	Hypotonia	□ Yes	□ No	□ Not recorded
2.4.	Conjunctivitis	□ Yes	□ No	□ Not recorded
2.5.	Jaundice	□ Yes	□ No	□ Not recorded

3. Skin

3.1.	Petechial rash	□ Yes	□ No	□ Not recorded
3.2.	Sclerema	□ Yes	□ No	□ Not recorded
3.3.	Bleeding from puncture sites	□ Yes	□ No	□ Not recorded

4. Temperature Abnormalities

4.1.	Record lowest body temperature	___ ___, ___ °C
4.2.	Record highest body temperature	___ ___, ___ °C

Version 2
24 April 2013

Form completed by : _____
Date completed: __ __/ __ __/ __ __

7.3.4 Case report form 4

4. Samples taken for Early-onset and Community Acquired Sepsis

Sepsis Aetiology in Neonates in South Africa (SANISA): CASE REPORT FORM
BLOOD CULTURE AND STUDY SAMPLES

A. Participant Identifiers

Barcode with ID No

1. Participant Number: ___ ___ ___ ___ ___ ___
2. Initials: ____ ____ ____ ____

B. Samples Taken

3. **Blood taken for culture** .....☐ Yes ☐ No
   - 3.1. If yes, state volume: _____ mls;
   - 3.2. Bottle weight before blood draw: __ __ . __ g
   - 3.3. Bottle weight after blood draw (with the cap): __ __ . __ g
   - 3.4. Date taken __ __/ __ __/ __ __
   - 3.5. Time taken : __ __h__ __
   - 3.6. Did the baby receive antibiotics before the blood culture was taken? ☐ Yes ☐ No
4. **Blood taken for TAC (in EDTA tube)** .....☐ Yes ☐ No
   - 4.1. If yes, state volume: _____ mls;
   - 4.2. Date taken :__ __/ __ __/ __ __
   - 4.3. Time taken: __ __ h __ __

Barcode with ID No (Blood)

5. **Oro-pharyngeal swab taken for TAC** .....☐ Yes ☐ No
6. **Nasopharyngeal swab taken for TAC** .....☐ Yes ☐ No
   - 6.1. If yes, state date : __ __/ __ __/ __ __ ;
   - 6.2. Time taken: __ __ h __ __

Barcode with ID No (Swab)

7. **Cerebrospinal Fluid (CSF) taken for TAC** .....☐ Yes ☐ No
   - 7.1. If yes, state date: __ __/ __ __/ __ __;
   - 7.2. Time: __ __h __ __

Barcode with ID No (CSF)

Version 2
24 April 2013

Form completed by : _____
Date completed: __ __/ __ __/ __ __

7.3.5 Case report form 5

5. Hospital Laboratory Results for Early-onset and Community Acquired Sepsis

Sepsis Aetiology in Neonates in South Africa (SANISA): CASE REPORT FORM
HOSPITAL LABORATORY RESULTS

A. PARTICIPANT IDENTIFIERS

Barcode with ID No

1. Participant Number: ___ ___ ___ ___ ___ ___
2. Initials: ____ ____ ____ ____

B. HOSPITAL LABORATORY RESULTS

3. FBC (Full blood count) ***(Latest results within 72 hours of sepsis event if more than 1 FBC done):***

3.1. Date of testing: __ __ / __ __ / 20 __ __ (dd/mm/yyyy)

3.2. White cell count (WCC): ___ ___ , __ __ x 10^9 / l

3.2.1. Neutrophil absolute: ___ ___, __ __ x 10^9 / l

3.2.2. Lymphocyte absolute: ___ ___ , __ __ x 10^9 / l

3.2.3. Left shift on the smear .....☐ Yes ☐ No

3.2.4. Platelet count: ___ ___ ___ x 10^9 / l

4. CRP (C-reactive protein)*(Latest results within 72 hours of sepsis event if more than 1 CRP done)*:

4.1. Date of testing: __ __ / __ __ / 20 __ __

4.2. Results: ___ ___ ___, ___ mg/l

5. Blood Culture results

5.1. Culture Positive: .....☐ Yes ☐ No

5.1.1. If Yes, state code of Organism*: __ if other organism, state name: _____

5.1.1.1. State time it took to grow: _____hours

5.2. Was a second organism identified in the blood culture: .....☐ Yes ☐ No

5.2.1. If Yes, state code of Organism*: __ if other organism, state name: _____

5.2.1.1. State time it took to grow: _____hours

*Organism codes:
1= No growth
2= GBS
3= *E. coli*
4= *Klebsiella pneumoniae*
5= *Staphylococcus. aureus*
6= *Enterobacter*
7= *Serratia*
8= *Pseudomonas aeruginosa*
9= *S. epidermidis*
10= *Strep* viridans
11= Other

6. Cerebrospinal Fluid (CSF) results

6.1. PMN: __ __ __ __ cells/ mm^3

6.2. Lymphocytes: __ __ __ __ cells/ mm^3

6.3. Red cell count (RBC): __ __ __ __ __ __ cells / mm^3

6.4. Protein: __ __ __ mg/dL

6.5. CSF gucose: __ __ __ mmols/L

6.6. Blood glucose: __ __ __ mmols/L

6.7. Culture Results Positive: .....☐ Yes ☐ No

6.7.1. If Yes, state code of organism*: __ if other organism, state name: _____

6.7.2. State time it took to grow: _____hours

Version 2
24 April 2013

Form completed by : _____
Date completed: __ __/ __ __/ __ __

7.3.6 Case report form 6

6. Final Diagnosis and Outcomes of Infants with Early-Onset and Community Acquired Sepsis

Sepsis Aetiology in Neonates in South Africa (SANISA): CASE REPORT FORM
FINAL DIAGNOSES AND OUTCOME

A. PARTICIPANT IDENTIFIERS

Barcode with ID No

1. Participant Number: ___ ___ ___ ___ ___ ___
2. Initials: ____ ____ ____ ____

B. FINAL DIAGNOSES AND OUTCOMES

3. List diagnoses recorded in the hospital file until the time of discharge

	Date Diagnosis Listed	Diagnosis	ICD 10 Code
3.1.	_____	_____	_____
3.2.	_____	_____	_____
3.3.	_____	_____	_____
3.4.	_____	_____	_____
3.5.	_____	_____	_____

C. ANTIMICROBIAL SUSCEPTIBILITY

4. Blood culture pathogen number 1 is resistant to:

Amikacin ☐
Ampicillin ☐
Cefazolin ☐
Cefepime ☐
Cefotaxime ☐
Cefoxitin ☐
Ceftazidime ☐
Cefuroxime ☐
Chloramphenicol ☐
Ciprofloxacin ☐
Clindamycin ☐
Co-amoxiclav ☐
Colistin ☐
Cotrimoxazole ☐
Ertapenem ☐
Erythromycin ☐
Fusidic acid ☐
Gentamicin ☐
Imipenem ☐
Linezolid ☐
Meropenem ☐
Minocycline ☐
Moxifloxacin ☐
Nalidixic acid ☐
Oxacillin ☐
Pen/ampicillin ☐
Pip-taz ☐
Rifampicin ☐
Streptomycin ☐
Synercid ☐
Telithromycin ☐
Tetracycline ☐
Tobramycin ☐
Vancomycin ☐
None ☐

5. Blood culture pathogen number 2 is resistant to:

Amikacin ☐
Ampicillin ☐
Cefazolin ☐
Cefepime ☐
Cefotaxime ☐
Cefoxitin ☐
Ceftazidime ☐
Cefuroxime ☐
Chloramphenicol ☐
Ciprofloxacin ☐
Clindamycin ☐
Co-amoxiclav ☐
Colistin ☐
Cotrimoxazole ☐
Ertapenem ☐
Erythromycin ☐
Fusidic acid ☐
Gentamicin ☐
Imipenem ☐
Linezolid ☐
Meropenem ☐

Version 2
24 April 2013

Form completed by : _____
Date completed: __ __/ __ __/ __ __

Minocycline ☐
Moxifloxacin ☐
Nalidixic acid ☐
Oxacillin ☐
Pen/ampicillin ☐
Pip-taz ☐
Rifampicin ☐
Streptomycin ☐
Synercid ☐
Telithromycin ☐
Tetracycline ☐
Tobramycin ☐
Vancomycin ☐
None ☐

6. CSF culture pathogen is resistant to:

Amikacin ☐
Ampicillin ☐
Cefazolin ☐
Cefepime ☐
Cefotaxime ☐
Cefoxitin ☐
Ceftazidime ☐
Cefuroxime ☐
Chloramphenicol ☐
Ciprofloxacin ☐
Clindamycin ☐
Co-amoxiclav ☐
Colistin ☐
Cotrimoxazole ☐
Ertapenem ☐
Erythromycin ☐
Fusidic acid ☐
Gentamicin ☐
Imipenem ☐
Linezolid ☐
Meropenem ☐
Minocycline ☐
Moxifloxacin ☐
Nalidixic acid ☐
Oxacillin ☐
Pen/ampicillin ☐
Pip-taz ☐
Rifampicin ☐
Streptomycin ☐
Synercid ☐
Telithromycin ☐
Tetracycline ☐
Tobramycin ☐
Vancomycin ☐
None ☐

D. ANTIBIOTIC USE:

7. Did the infant receive any antibiotics during this septic event (admission)? ☐ Yes ☐ No

7.1. *If yes: Enter codes of all antibiotic(s)* the patient received:* ___ ; ___; ___; ____

7.1.1. Start date of antibiotics: ___ ___/___ ___/___ ___,

7.1.2. Time 1st earliest dose: __ __h__ __

7.1.3. Stop date of all antibiotics started on admission or after positive culture: __ __/ __ __/ __ __ or

☐ baby was discharged on antibiotics

E. Outcome

8. ☐ Discharged alive ☐ Died in hospital ☐ Transferred ☐ Refused hospital treatment

9. Outcome Date: __ __ / __ __ / __ __

***Antibiotic codes:**

1=Amikacin; 2=Ampicillin; 3=Amphotericin B (Ambisome); 4=Cefepime; 5=Cefotaxime; 6=Ceftriaxone (Rocephin); 7=Cefuroxime; 8=Clindamycin; 9=Cloxacillin; 10=Co-Amoxiclav; 11=Erythromycin; 12=Gentamicin; 13=Meropenem; 14=Metronidazole (Flagyl); 15=Penicillin; 16=Pip-Taz; 17=Sulfamethoxazole/Trimethoprim (Bactrim); 18=Tobramycin; 19=Vancomycin

Form completed by : _____
Date completed: __ __/ __ __/ __ __

7.3.7 Case report form 7

VITAMIN D LEVELS IN PREGNANT WOMEN AND IN CORD BLOOD STUDY

DATA COLLECTION SHEET

Participant's Number: □ □ □ □

Mother's initials: __ __ __ __

Date of enrolment: __ __/ __ __ /__ __

1. Cord blood taken: □ Yes □ No

1.1. If yes, please label blood with : I- □□□□ (Participant's number)

1.2. If yes, state date and time taken: __ __ / __ __ / __ __; Time: __ __h__ __

2. Mother's blood taken: □ Yes □ No

2.1. If yes, please label blood with : M- □□□□ (Participant's number)

2.2. If yes, state date and time taken: __ __ / __ __ / __ __; Time: __ __h__ __

3. **Maternal details**

3.1. Maternal age (years): _____

3.2. Parity: _____

3.3. Gravidity: _____

3.4. Race: □ African, □ Indian, □ Coloured, □ Oriental, □ White

3.5. WR results: 1 = Positive; 2 = Negative; 3 = Unknown

If positive what was the mother's titres: _____

3.6. HIV Elisa results: 1 = Positive; 2 = Negative; 3 = Unknown

3.6.1. If HIV positive was the viral load done: □ Yes □ No □ Not applicable

3.6.1.1. If viral load done, state results: _____, date done: ___/ ___/ ___

3.6.2. If HIV positive was the CD4 count done: □ Yes □ No □ Not applicable

3.6.2.1. If CD4 count done, state results: _____, date done: : ___/ ___/ ___

3.6.3. If HIV positive is she on HAART: □ Yes □ No □ Not applicable

3.6.3.1. If yes, state when HAART was started: __ __/ __ __ /__ __ □ Not applicable

3.6.3.2. If on HAART, state names of drugs: _____
_____, □ Not applicable

3.7. Calcium supplements during pregnancy: □ Yes □ No

3.7.1. If yes, name of supplement: _____; and dose per day: _____

3.8. Temperature ≥38°C during labour: □ Yes □ No

3.9. Offensive amniotic fluid: □ Yes □ No

3.10. Meconium stained amniotic fluid: □ Yes □ No

3.11. Duration of rupture of membranes (hours): _____

3.12. Antenatal steroids: □ Yes □ No

3.13. Multivitamins during pregnancy: □ Yes □ No

3.14. Intrapartum antibiotics: □ Yes □ No

3.15. If yes, state name of antibiotics:
_____

Infant details

3.16. Birthweight (grams): __ __ __ __

3.17. Gestational Age (weeks): _____

3.17.1. Gestational age based on: 1 = LMP/Exam; 2= Early Sonar; 3 = Late Sonar; 4= Ballard

3.18. Date of Birth (dd/mm/yyyy): ___ // ___//___

3.19. Time of Birth: ___ ___

3.20. Mode of Delivery: 1 – Vaginal Delivery; 2 –Caesarian Section

3.21. Apgar Score at 1 min: ___ ___

3.22. Apgar Score at 5 min:___ ___

3.23. Resuscitation

3.23.1. Bag Mask Ventilation □ Yes □ No

3.23.2. Intubated □ Yes □ No

3.23.3. Chest Compressions □ Yes □ No

3.23.4. Adrenalin □ Yes □ No

3.24. Is baby well at birth: □ Yes □ No

3.25. If not well, state ward were the baby is admitted: □ Ward 66 □ TC □ NICU

4. **25-Hydroxyvitamin D Levels**

4.1. Mother's Results: _____

4.2. Cord blood Results: _____

IF BABY SICK AT BIRTH OR ADMITTED PLEASE COMPLETE CASE REPORT 2

7.3.8 Case report form 8

VITAMIN D LEVELS IN PREGNANT WOMEN AND IN CORD BLOOD STUDY

DATA COLLECTION SHEET

Participant's Number: □ □ □ □

Mother's initials: __ __ __ __

Date of enrolment: __ __/ __ __ /__ __

DATA FOR SICK OR ADMITTED INFANTS

Ward of Admission: □ Ward 66; □ TC □ NICU

Mother's Hospital Number: _____

Baby's Hospital Number: __ _____

1. Clinical signs at admission/ birth and up to 24 hours after birth

Date of admission: ___ // ___// ___

1.1. **General**

1.1.1. Hypothermia (Temperature <36.5)

1.1.2. Fever (Temperature >38)

1.1.3. Lethargy

1.2. **Respiratory system**

Are the following signs recorded in the doctors or nurses' notes

1.2.1. Respiratory rate> 60/min for 3 consecutive readings in nurses notes□ Yes □ No

1.2.2. Tachypnoea □ Yes □ No

1.2.3. Recession or retraction □ Yes □ No

1.2.4. Respiratory distress recorded in chart □ Yes □ No

If yes, state severity: 1 = mild; 2 = moderate; 3 = severe

1.2.5. On supplemental oxygen □ Yes □ No

1.2.6. On a ventilator or CPAP □ Yes □ No

1.3. **Cardiovascular system**

Are the following signs recorded in doctors or nurses' notes

1.3.1. Heart rate more than 180/ minute for 3 consecutive readings □ Yes □ No

1.3.2. Heart rate less than 110/ minute for 3 consecutive readings □ Yes □ No

1.3.3. State lowest Mean arterial BP (MAP): _____

1.3.4. On inotropes (Dopamine or dobutamine or adrenalin) □ Yes □ No

1.4. **Abdominal findings**

1.4.1. Vomiting □ Yes □ No

1.4.2. Large aspirates (>20% of previous feed) □ Yes □ No

1.4.3. Distension □ Yes □ No

1.4.4. Feeding intolerance □ Yes □ No

1.4.5. Poor feeding □ Yes □ No

1.5. **Central nervous system**

1.5.1. Irritability □ Yes □ No

1.5.2. Apnoea □ Yes □ No

1.5.3. Seizures/ Convulsions □ Yes □ No

1.6. **Metabolic**

1.6.1. Hypoglycaemia (HGT < 2.6 mmol/ L) □ Yes □ No

1.6.2. Metabolic acidosis (Base deficit >10) □ Yes □ No

1.6.3. Lactate levels (Levels >2) □ Yes □ No

2. **Laboratory Findings on Tests done on Admission**

2.1. ***Full/ Complete Blood Count***

Date of FBC (dd/mm/yyyy): ___ // ___ // ___

2.1.1. Record the white cell count (wcc):____x10^9/L from FBC done at time sepsis work-up

2.1.2. Record the Neutrophils: _____ % from FBC done at time of sepsis work-up

2.1.3. Record the platelet count: _____ x10^9/L from FBC done at time of sepsis work-up

2.2. ***C-reactive protein (CRP)***

Date CRP done (dd/mm/yyyy): ___// ___// ___

2.2.1. CRP results: ____ mg/L

2.3. ***Cerebrospinal fluid results***

Lumbar puncture (LP) done Yes No

If yes, date of lumbar puncture (dd/mm/yyyy): ___// ___// ___

2.3.1. Polys/ PMN: _____ cells/ mm^3

2.3.2. Lymphocytes: _____ cells/ mm^3

2.3.3. Red blood cells: _____ cells/ mm^3

2.3.4. Protein: _____

2.3.5. Glucose: _____

3. **Antibiotics**

Were antibiotics started/ changed in light of this event Yes No

If yes, complete the following

3.1. Antibiotic:_____;Dose:_____; Start date:_____;Stop date:_____

3.2. Antibiotic: _____;Dose: _____; Start date:_____;Stop date: _____

3.3. Antibiotic: _____;Dose: _____; Start date: _____;Stop date:_____

4. **Culture Results**

4.1. Culture positive: : Yes No

4.1.1. If yes, select site of the culture from below:

1 = blood 2 = CSF 3 = Urine 4 = Other, specify

Date culture done: ___ // ___ // ___

4.1.2. Organisms isolated: Yes No

If yes; circle name of organism as below

1 =Group B Strep/ Strep. Agalactiae 2 = Staph. Aureus (MSSA) 3 = Staph. Aureus (MRSA)

4 = Enterococcus faecalis 5 = Enterococcus faecium 6 = Strep. Viridans 7 = CONS/ CNS

8 = E. coli 9 = Klebsiella species 10 = Enterobacter species 11 = Acinetobacter baumanni

12 = Pseudomonas species 13 = Salmonella 14 = Serratia

15= Candida albicans 16 = Candida parapsillosis 17 = Candida glabrata 18 = Candida krusei

19 = Candida tropicalis 20 = Other, state name of organism: _____

If organisms is isolated complete the susceptibility table below

Name of Organisms: Time organism took to grow:

Names of antibiotics Sensitive to: _____

Names of antibiotics Resistant to: _____

5. **Sepsis Diagnosis on Admission**

5.1. Pneumonia□ Yes □ No

5.2. Septicaemia□ Yes □ No

5.3. Meningitis□ Yes □ No

6. **Other diagnosis and outcome**

6.1. Hyaline Membrane Disease□ Yes □ No

6.2. Meconium aspiration syndrome□ Yes □ No

6.3. Asphyxia with Hypoxic ischaemic encephalopathy □ Yes □ No

6.4. Asphyxia without Hypoxic ischaemic encephalopathy□ Yes □ No

6.5. Congenital abnormality□ Yes □ No

If yes, state the diagnosis: _____

6.6. Died □ Yes □ No

If died, state date of death: _____

If survived, state date of discharge:_____