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EVALUATION OF CULTURE-PROVEN NEONATAL SEPSIS AT A TERTIARY CARE HOSPITAL IN SOUTH AFRICA

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree

of

Master of Medicine in the branch of Paediatrics.

Johannesburg, 2015.

DECLARATION

I, Mamaila Martha Lebea declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Paediatrics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

.....

05th day of September 2015.

To my loving parents; Tshwane and Mohlanalo Lebea

Thank you for the support, love and encouragement

ABSTRACT

Background: Organisms causing neonatal sepsis differ in different regions and also change with time in the same area. The antibiotic susceptibility of microorganisms also changes with time, with emergence of multidrug resistant organisms. A periodic survey of the causes of sepsis and their antibiotic sensitivity patterns is essential in the design of effective infection control programs and in guiding empiric antibiotic therapy.

Aim: To evaluate the epidemiology of culture-proven neonatal sepsis and to describe the clinical characteristics of patients with neonatal sepsis at a tertiary care hospital in South Africa over a one year period.

Methods: This was a retrospective descriptive study conducted in the neonatal unit at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). Clinical and laboratory data of patients, admitted to the CMJAH neonatal unit between 1 January 2012 and 31 December 2012 with positive blood cultures were reviewed.

Results: During this time there were 196 patients with blood-culture proven neonatal sepsis (NNS). This gave an incidence of 10.26 per 100 admissions. Late-onset sepsis (LOS) accounted for 83.7% of cases of NNS. Of the 196 patients with NNS, 117 (59.39%) were males. The median gestational age for patients with NNS was 30 weeks and the median birth weight was 1300g. HIV exposure was present in 30.67 % of patients.

Predominant isolates were *Klebsiella pneumioniae* (32.20%), coagulase-negative staphylococci (23.72%) and methicillin-resistant *Staphylococcus aureus* (13.13%). The majority of the isolated *K.pneumoniae* were extended beta-lactamase-producing (ESBL) with resistance to ampicillin and gentamicin.

Conclusion: Neonatal sepsis is an important cause of mortality at CMJAH neonatal unit. Compared to previous audits in the unit, the incidence of NNS in the unit is on the increase while mortality from NNS has remained relatively constant. LOS was more common than EOS at CMJAH. A changing pattern of bacteria isolated has been observed. Gram-negative microorganisms comprised the majority of the neonatal sepsis, with ESBL *Klebsiella pneumonia*e and *A. baumannii* being the most prevalent. Coagulase negative staphylococcus remains an important cause of NNS, and is the most prevalent gram-positive organism isolated. Resistance to the first-line antibiotic regimen for both EOS and LOS is significant. Due to the changing pattern of bacteria isolated and changing patterns in antibiotic sensitivity, recommendations are made regarding early empiric antibiotic therapy.

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PREFACE

Neonatal sepsis is a significant cause of morbidity and mortality in newborns. It is the cause of 1.6 million deaths per annum in developing countries. Organisms causing neonatal sepsis differ in different regions and also keep changing with time in the same area. The antibiotic susceptibility of microorganisms also changes with time, with emergence of multidrug resistant organisms. A periodic survey on the causes of sepsis and their antibiotic sensitivity patterns is essential in the design of effective infection control programs and in guiding empiric antibiotic therapy

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LIST OF ABBREVIATIONS

CHBAH- Chris Hani Baragwanath Academic Hospital

CMJAH- Charlotte Maxeke Johannesburg Academic Hospital

CoNS- Coagulase negative staphylococcus

CRP-C-reactive protein

EOS-Early onset sepsis

FBC-Full blood count

GBS-Group B streptococcus

HIV-Human Immunodeficiency Virus

IgG- Immunoglobulin

NHLS-National Health Laboratory Service

NNS-Neonatal Sepsis

LOS-Late onset sepsis

MSAF- Meconium stained amniotic fluid

MRSA-Methicillin-resistant *Staphylococcus aureus*

NICU-Neonatal Intensive Care Unit

PCT-Procalcitonin

PROM- Prolonged rupture of membranes

US-United States of America

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CHAPTER 1

1.0 LITERATURE REVIEW

1.1 Introduction

The World Health Organization estimates that there are about 5 million neonatal deaths a year globally.[1, 2] Ninety eight percent of these deaths occur in developing countries.[1, 2] Neonatal sepsis (NNS) is a significant cause of death in these countries.[1, 3, 4] It is a cause of about 1.6 million deaths per annum in developing countries.[1] In South Africa, the neonatal mortality rate is 21 per 1000 live births, with 21% of these deaths caused by severe infections.[5]

Organisms causing sepsis vary significantly between different neonatal units.[1, 4, 5] Even in the same unit, the causes of sepsis changes over time.[1, 4, 6, 7] Furthermore, the antibiotic sensitivity of the organisms changes over time.[1, 4] There has been a worldwide increase in the number of multidrug resistant organisms causing sepsis.[1, 4] Ongoing surveillance of the causes of neonatal sepsis and their antibiotic sensitivity is paramount.[1, 4] Surveillance data is useful in the design of effective infection control programs and in guiding empiric antibiotic therapy in neonatal sepsis.[3, 8]

1.2 Definition and classifications

Neonatal sepsis or *sepsis neonatorum* refers to a constellation of clinical and laboratory findings associated with systemic infection occurring within the first 28 days of life.[1, 9] Traditionally, it has been associated with bacteremia, although it may be caused by other pathogens such as viruses and fungi.[8]

Neonatal sepsis is classified into two major categories which are early and late-onset neonatal sepsis.[1, 9] This classification depends on the time of onset of symptoms of sepsis. In the literature, there are variations in the periods used to define early and late-onset sepsis.[1, 7] Some define early-onset sepsis (EOS) as sepsis occurring within 72 hours of birth and late-onset sepsis (LOS) occurring after 72 hours.[1] Other definitions use 48 hours for EOS, while some use 9 days as a cut off age.[1]

1.3 Epidemiology

The worldwide incidence of NNS ranges from 1 to 10 per 1000 live births.[10] However, the incidence of NNS varies significantly between countries, with higher rates reported in developing countries.[1] In Africa, the incidence of NNS varies from 6.5 to 23 per 1000 live births.[1] In a study at a tertiary hospital in Johannesburg South Africa, the incidence of NNS was reported as 10.5 cases per 1000 live births.[3] In another Sub-Saharan referral hospital in Zimbabwe, the incidence was much higher, reported as 21 cases per 1000 live births.[7] In developed countries such as the United States (USA) and Australia, the incidence of NNS ranges from 6 to 9 per 1000 live births.[1]

The contribution of EOS and LOS cases to the overall incidence of NNS also differs significantly between different neonatal institutions.[11] A South African study by Ballot et al, showed that LOS occurred more frequently than EOS.[4] In this study, 93.5% cases of NNS were of late onset.[4] Other studies in both developing and developed countries, also showed that LOS occurred more frequently than EOS.[11, 12] A one year prospective study of neonatal infections in eight neonatal units in Asia, showed that 90% (453/408) of the cases of NNS were of late onset.[11]

1.4 Risk factors for neonatal sepsis

Neonates can be regarded as immune compromised individuals.[4, 13, 14] They have a series of defects in their specific and nonspecific immunity, which predisposes them to infection.[13-15] Their immune dysfunction is characterized amongst others by decreased phagocytic activity of white cells, decreased cytokine production and impaired immunoglobulin production.[15] Physical barriers to infections such as the skin are weak and thin, and may be easily interrupted.[15]

Beside their inherent predisposition to sepsis, there are many other pre- and intrapartum obstetric complications associated with an increased risk of infection in newborn infants.[15-17] The following are some of the factors associated with neonatal sepsis: *a)* preterm birth and low birth weight, *b)* prolonged rupture of membranes *c)* maternal group B Streptococcus (GBS) colonization, *d)* chorioamnionitis and e*)* invasive procedures.[15-17]

1.4.1 Preterm birth and low birth weight

Preterm delivery and low birth weight are well-established risk factors for neonatal sepsis.[17] Preterm infants have a 3 to 10 fold higher incidence of infection when compared to full term infants, with sepsis more common amongst the most premature infants.[18, 19] Reports from India showed 50-60% of septic babies are premature babies and those with birth weight less than 1500g are more vulnerable.[2] In a cohort of infants admitted to 250 neonatal intensive care units (NICU) in the US, there were 374 infections for every 1000 admissions for infants < 750 g birth weight and only 7 infections per 1000 admissions in infants >2500 g birth weight.[18]

Possible explanations for the increased risk of infections in preterm infants include: *a)* immune dysfunction, *b)* maternal genital tract infection that causes preterm labour, with an increased risk of vertical transmission to the newborn, *c)* the frequency of intrauterine infection is inversely related to gestational age and *d)* the need for invasive procedures such as endotracheal intubation in premature infants.[19] The ability of the newborn infant to resist infection is highly dependent on the fetus' ability to acquire and utilize maternal immunity.[14] Prematurity is one of the factors known to interfere with materno-fetal transport of specific immunoglobulin (IgG) across the human placenta. [13] Maternal IgG transfer usually occurs in the third trimester of pregnancy.[20] Premature infants thus have limited time to acquire humoral immunity.[20]

1.4.2 Prolonged rupture of membranes

Throughout pregnancy, the fetus is relatively protected from the maternal microbial flora by the chorioamniotic membranes, the placenta and other antibacterial factors in amniotic fluid.[16] Following rupture of membranes, particularly if the rupture is prolonged, bacteria may ascend and in some cases lead to fetal infection.[9] Prolonged rupture of membranes (PROM) is defined as rupture of membranes prior to the onset of labor for more than 18 hours.[21] It is a major risk factors for chorioamnionitis and neonatal sepsis.[22] It is associated with a 10-fold increase in the risk of neonatal infection.[21] The aetiology of PROM is multifactorial and has been associated with the following maternal factors: *a)* black race, *b)* cigarette smoking, *c)* low socioeconomic status, *d)* multiparity, *e)* polyhydroamnios and *f)* a history of PROM in previous pregnancies.[21]

1.4.3 Maternal group B Streptococcus colonization

In the 1930s GBS was thought to be a normal commensal.[23] By the 1970s invasive Group B streptococcal disease emerged as the leading cause of neonatal morbidity and mortality in the US.[23] Maternal colonization with GBS was linked to neonatal morbidity and mortality.[23, 24] Maternal colonization with GBS substantially increases the risk of EOS due to GBS.[24] Over the past decades, there has been a significant decline in the incidence of EOS due to GBS.[25] A multicenter study in the US showed a decline of 65%; from 1993-1998. [25]

Clinical trials in the mid-1980s already demonstrated that antibiotic prophylaxis during labour to mothers colonized with Group B streptococci was highly effective in preventing

disease in newborns.[23] The decline demonstrated by the US multicenter study; coincided with the active public health and clinical efforts to increase the administration of prophylactic intrapartum antibiotics to mothers at risk of transmitting GBS.[25] Other measures to reduce maternal colonization such as vaginal chlorhexidine wipes have been shown not to be effective in preventing vertical transmission GBS and neonatal sepsis.[26]

1.4.4 Invasive procedures

Interventional invasive procedures aimed at providing nutritional or respiratory support for the ailing newborn may provide excellent opportunities for relatively non-virulent pathogens to establish infection and to invade the host.[16] Transient bacteraemia may accompany procedures that traumatize the skin and mucosal membranes such as endotracheal intubation and venepuncture.[16] Immunological mechanisms are then activated to eradicate the bacteraemia, but if these fail, overt sepsis can occur.[16] The following are some of the procedures that may predispose the newborn infant to sepsis:

- Arterial and venous umbilical catheterization
- Central venous catheters
- Peripheral arterial and venous cannulation
- Intubation and assisted ventilation
- Bladder catheterization
- Hyperalimentation[16]

1.4.5 Maternal HIV infection as a risk factor for neonatal sepsis

HIV uninfected infants born to HIV infected mothers represent a growing population, due to the success of current prophylaxis regimens in decreasing mother-to-child transmission of HIV.[27] HIV uninfected infants born to HIV infected mothers were thought to be at increased risk of sepsis.[17, 27, 28] Maternal HIV infection was thought to be a risk factor for neonatal sepsis because of number of factors which include immune system aberrations, impaired maternal antibody transfer and altered exposure to pathogenic bacteria.[26] A recent analysis confirmed that maternal HIV infection is not a direct risk factor for neonatal sepsis.[17, 28] A South African study done at Chris Hani Baragwanath Academic Hospital (CHBAH) by Cutland et al showed that maternal HIV infection was not associated with increased risk of maternal bacterial colonization, vertical transmission, EOS, or LOS in their newborns.[17, 28] The same study rather showed that HIV-infected neonates were at increased risk of both culture-positive EOS and LOS.[28]

1.4.6 Other factors

Meconium stained amniotic fluid (MSAF), primiparity, male gender and the use of broad spectrum antibiotics are other factors associated with NNS.[17] Although studies in industrialized countries have not commonly identified significant associations between MSAF and sepsis, two studies in Africa and one in Australia have shown a strong association between MSAF and culture-confirmed early and late-onset sepsis.[17] Meconium is thought to enhance the growth of pathogenic micro-organisms and may result in neurological damage that predisposes to late-onset sepsis.[17] In a South African study at CHBAH, MSAF was associated with twice the risk of sepsis and death.[17]

1.5 Aetiology of neonatal sepsis

Organisms causing neonatal sepsis differ significantly between different neonatal units.[1, 4, 5] Even in the same unit, the causes change over time.[4] A 10 year survey of NNS in a tertiary care neonatal unit in India, showed that in the first 5yrs of the study *Enterobacter aerogenes* was the most common cause of late-onset sepsis whereas *Staphylococcus aureus* became the main organism causing LOS in the later 5 years.[4] Another example is that of two studies at a tertiary hospital in South Africa, CMJAH, done 7 years apart.[3, 4] Between 2002 and 2003, the most common gram negative organism causing LOS was *E. coli*. Seven years later the most common cause of LOS was a *Klebsiella* species with a higher percentage of ESBL producing species compared to the previous study.[3, 4]

The table 1.1 below shows trends in the predominance of organisms causing neonatal sepsis in a neonatal facility at Yale.[29] In the last decade of the study, the overall percentage of sepsis caused by *E.coli* and *group B streptococcus* decreased.[29] There was a marked increase in the number of coagulase negative staphylococcus, with no episodes of sepsis from *Streptococcus pneumoniae* and *Streptococcus pyogenes* which were commonly observed in the early years of the survey.[29]

Table 1.1 Neonatal inborn sepsis at Yale Hospital 1928-2003

	1928-32	1933-43	1944-57	1958-65	1966-78	1979-88	1989-2003
				% per study			
Gram-positive							
Organism							
S.aureus	28	9	13	3	5	3	8
CoNS				1	1	8	29
Beta-hemolytic streptococci	38	41	18	11	36	45	21
S.viridans		2		3	1	3	1
S.pneumoniae	5	11	5	3	1	1	
L.monocytogenes		2	2		1	1	<1
Gram-negative							
Organism							
E.coli	26	25	37	45	32	20	11
Klebsiella-Enterobacter				11	12	3	11
Pseudomonas	3		21	15	2	3	3
Haemophilus				1	4	5	1
Salmonella			2		1	1	
Others					1	3	
Fungi					2	1	8
Other Organisms		9	3	5	5	1	6

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% do not add to 100 due to rounding

A retrospective study by Gwee et al. demonstrated how the change in the pattern of organisms causing NNS and their antibiotic susceptibility can occur over a short period

of time (Table 1.2).[30]The review of neonatal sepsis over a 5year period showed a significant decline in the importance of *Staphylococcus aureus* as a cause of sepsis and an increase in the predominance of gram negative organisms over a one year period (between 2006 and 2007).[30] All these studies emphasize the importance of ongoing surveillance of microbiological isolates and their sensitivity patterns.

Table 1. 2. Ten most common pathogenic bacterial isolate for all age groups, 2002-2007

	2002	2003	2004	2005	2006	2007	2008
				% per study			
Gram-positive							
Organism							
S.aureus	19	19	15	16	13	4	15.3
Group B *Streptococci*	15	13	11	18	13	6	13.5
Alpha-hemolytic *streptococci*	3	2	11	12	5	5	7.4
Group D Streptococci	1	2	4	10	3	4	4.4
S.pneumoniae	5	4	6	5	6	15	5.9
Others	7	5	9	4	10	0	6.3
Gram-negative							
Organism							
E.coli	7	13	12	7	10	19	10.5
Klebsiella sp	6	9	4	6	10	14	7.3
Enterobacter sp	2	5	5	5	5	4	4.6
Acinobacter sp	3	2	2	5	2	5	3.1
Salmonella	23	14	9	7	16	18	12.6
Others	10	12	12	6	8	5	9

"Reproduced from [Bacteraemia in Malawian neonates and young infants 2002-2007: a retrospective audit, Gwee A, Coghlan B, Everett D, Chagoma N, Phiri A, Wilson L, et al., 2(3):1-7,2012,British Medical Journal Open] with permission from BMJ Publishing Group Ltd"

Overall, gram negative organisms are a more common cause of NNS in developing countries. They are mainly represented by *Klebsiella, Escherichia coli, Pseudomonas,* and *Salmonella.*[1] Of the gram positive organisms, *Staphylococcus aureus*, *coagulase negative staphylococci*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* are most commonly isolated.[1] Group B *streptococcus* is generally rare.[1] Table 1.3 below shows the most common isolates in neonatal sepsis from studies in developing countries.[1]

The causes of sepsis also differ according to the age of the infant at the onset of sepsis. Early onset sepsis is more likely to reflect organisms acquired vertically from the mother, while late onset sepsis most likely reflects organisms acquired in the home, hospital or community.[16] Group B *streptococcus* was the leading cause of EOS in the 1980's.[23, 31] The decline in the incidence of GBS has seen gram negative enteric bacteria becoming the leading cause of EOS. [31] Enteric gram negative organisms causing EOS include *E. coli, Klebsiella* and *Pseudomonas*. *Staphylococci* and enterococci can be found in EOS, but are more commonly associated with LOS .[31]

Table 1.3 Studies of neonatal sepsis in developing countries

Country	Type of Study	Number of positive blood cultures	EOS (%)	LOS (%)	Most common pathogens
Malaysia	Prospective surveillance	136	26	74	*Acinobacter,Klebsiella*
Nigeria	Prospective surveillance	62	47	53	*Staphylococcus aureus, Pseudomonas*
India	Prospective surveillance	157	86	14	*Klebsiella, Pseudomonas*
Panama	Retrospective survey	577	44	56	*Klebsiella, Staphylococcus aureus*
Saudi Arabia	Case Control Survey	61	39	61	*Staphylococcus aureus, Klebsiella,Enterobacter, Serratia*
The Gambia, Papua new Guinea, Ethiopia, Philippines	Multicentre study (4 prospective study)	167	30	70	*Staphylococcus aureus, Klebsiella, E.coli*

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1.6 Clinical features of neonatal sepsis

In both term and preterm infants, early warning signs and symptoms of neonatal sepsis are often minimal, subtle, and nonspecific and can be easily misinterpreted as being due to other neonatal problems.[1, 2] The clinical manifestations of infection depend on the

virulence of the infecting organism and the body's inflammatory response.[19] Table 1.4 shows some of the clinical features suggestive of neonatal sepsis.[1,19]

Although the initial manifestation may involve only limited symptomatology (one or more of the symptoms above), it may be an acute catastrophic manifestation with multiorgan dysfunction.[19] Neonatal sepsis may manifest with systemic inflammatory response syndrome (SIRS).[1, 8, 19] This is a clinical syndrome characterized by the presence of two or more of the following: (*a*) fever or hypothermia, (*b*) tachycardia, (*c*) tachypnea or hyperventilation, and (*d*) abnormal white blood cells or increase in immature forms.[1, 8, 19,16] It is not specific to sepsis and maybe caused by a variety of severe insults such as trauma or surgery.[1, 8, 19,16]

Table 1. 4 Clinical features of neonatal sepsis

General	**Respiratory**
Poor feeding/ not sucking well	Respiratory distress
Irritability	
Lethargy	**Cardiovascular**
Temperature instability (>37.7 or <35.5°c)	Hypotension
	Shock
CNS	Tachycardia
Hypotonia	
Seizures	**Skin**
Bulging fontanelle	Petechia
Reduced movement	Sclerema

1.7 Diagnosing neonatal sepsis

Sepsis is defined as constellation of clinical and laboratory findings associated with systemic infection.[1, 9] Documentation of a positive microbiological culture in a patient with clinical features suggestive of sepsis is the first diagnostic criterion that must be met for a diagnosis sepsis to be made.[19] However, this has many challenges, which include delay in the availability of results.[32] Early diagnosis and treatment of neonatal sepsis are critical to improve neonatal outcomes.[16, 33] But over diagnosis is also dangerous, as it can lead to inappropriate use of antibiotics that may foster antibiotic resistance.[33] An ideal diagnostic test for neonatal sepsis should therefore have the following properties: *a)* be rapid; *b)* be sensitive and specific; *c)* be able to detect all organisms relevant to neonatal sepsis; and d*)* not be affected by maternal antibiotics.[33] Table 1.6 shows some of the characteristics of an ideal infection marker.[33]

Table 1.5 Characteristics of an ideal infection marker

Clinical characteristics	Laboratory characteristics
1. A well-defined optimal cut off	1. Requires small volume of specimen
2. Sensitivity approaching 100%	2. Quantitative measurement
3. Specificity >85%	3. Cheap
4. Positive predictive value > 85%	4. Easy to do
5. Negative predictive value approaching 100%	5. Stable compound
6. Early detection of infection	6. Results comparable between laboratories
7. Can monitor response to treatment	
8. Can guide treatment duration	

"Reproduced from [Molecular Assays in the Diagnosis of Neonatal Sepsis: A Systematic Review and Meta-analysis,Pammi M, Flores A, Leeflang M, Versalovic J, 128(4),973-88,2011,Pediatrics] with permission from BMJ Publishing Group Ltd"

A wide variety of hematological and biochemical markers have been investigated for the evaluation of clinical sepsis.[32] In recent years, some authors have suggested that the presence of one clinical sign (compatible with infection) along with a biochemical marker (C-reactive protein (CRP) value >10 mg/L) is sufficient to make the diagnosis of early- and late-onset neonatal clinical septicemia.[16] However hematological and biochemical markers are also not without limitations. [16] The use of multiple markers and serial measurements of some of the infection markers have shown to improve diagnostic

sensitivity and accuracy.[32] Table 1.7 shows some of the diagnostic markers of infection that have been evaluated for use in preterm and term infants.[32]

Table 1.6 Diagnostic markers of infection for preterm and newborn infants

Haematological markers	Acute phase reactants and other proteins
• Total white blood cell count • Total neutrophil count • Immature neutrophil count • Immature/total neutrophil ratio • Neutrophil morphology: vacuolisation, toxic granulations, Do¨hle bodies, intracellular bacteria • Platelet count • Granulocyte colony-stimulating factor (G-CSF) • D-dimer • Fibrinogen • Thrombin-antithrombin III complex (TAT) • Plasminogen activator inhibitor-1 (PAI-1) • Plasminogen tissue activator (tPA)	• Antitrypsin • C Reactive protein (CRP) • Fibronectin • Haptoglobin • Lactoferrin • Neopterin • Orosomucoid • Procalcitonin (PCT)

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1.7.1 Microbiological culture

Microbial cultures of blood or other sterile body fluids are the gold standard in the diagnosis of neonatal sepsis.[33] Microbial cultures for diagnosis of neonatal sepsis are subject to low sensitivity and reporting delay.[33] The low sensitivity of blood cultures in newborns is due to: a) a low degree of neonatal bacteremia; b) small inoculation volumes in culture bottles; and c) the use of intrapartum antibiotics.[33] The past 20 years has seen significant refinement in culture media and automated blood culture, in an attempt to shorten the time to detection of positive results and to increase the yield for a given

sample.[34] Most blood cultures are positive within 24 to 36hours of incubation if organisms are present.[31]

The most widely accepted recommendation is that 0.5 to 1ml of blood be taken for a blood culture sample.[34] Although in the past it was advised that a blood sample of at least 0.5ml volume be taken for culture, a study by Schelonka et al, demonstrated that a 0.5ml sample is inadequate for sensitive and timely detection of bacteraemia.[34]

Once an organism is isolated from a blood culture sample, the distinction of whether it is a true pathogen or a contaminant is of paramount importance.[8] Organisms such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, gram negative enteric bacteria, and *Candida* species are considered true pathogens.[8] Typical contaminants include *Bacillus*, *Corynebacterium* and *Propionibacterium* species.[8] Other common contaminants such as coagulase-negative *Staphylococci* and *Streptococcus viridans* require further evaluation, including a repeat blood culture.[8] They are more likely to be pathogens if isolated within 48hrs of incubation or if isolated from two blood cultures from different sites.[8]

1.7.2 C- Reactive Protein

C-reactive protein was first described in 1930 by Tillet and Francis at Rockefeller University.[35, 36] It is the most extensively studied and used of the acute phase reactants.[32] Acute phase reactants are proteins that are predominantly produced by the liver in

response to infection or tissue injury.[32] CRP is synthesized within 6 to 8 hours of exposure to an infective process or tissue damage.[32] It is essential for ruling out an infection and for monitoring the response to treatment.[36] The most used cut-off value for CRP is 10 mg/l, with a CRP >10 mg/l suggestive of infection.[16, 36] It is well known that CRP values undergo physiological changes during the newborn period, and that preterm infants have a lower baseline CRP and lower CRP response to infection.[36] Irrespective of the gestational and postnatal age of the infant, the cut-off value of 10 mg/l is still used.[36] Two consecutive values <10 mg/l determined more than 24 hours apart exclude the likelihood of infection.[36] However an elevated CRP is not specific to sepsis, as it may also be caused by perinatal maternal conditions or other neonatal problems such as meconium aspiration syndrome.[36] The specificity and positive predictive value of CRP range from 93% to 100%.[32] Serial measurements at 24 and 48 hours after the onset of symptoms considerably improve the sensitivity.[36, 37]

1.7.3 Full Blood Count

In the early and mid-1980s, neonatologists relied mainly on haematological indices as adjunct indicators for early diagnosis of neonatal sepsis.[32] The white blood cell counts (WBC), absolute neutrophil count (ANC), immature-to-total neutrophil ratio (I/T ratio), and platelet are widely used as potential markers of infection (CBC).[32]

A low white blood cell count, low absolute neutrophil count, low platelet count and high I/T ratio are associated with increasing likelihood of infection.[38] However the sensitivity and specificity of WCC and I/T ratio as a marker of infection varies widely across different

studies. [32] The sensitivity and specificity of WCC and I/T ratio as a markers of infection ranges from 17% to 90% and 31% to 100% respectively. A study by Hornik et.al, showed that specificity for WBC as a marker of infection was high for WBC counts <5000/mm^3 and WBC counts <1000/mm^3. The sensitivity was poor, with 60% (1315/2177) of subjects with a positive culture having a "normal" WBC count (5000/mm^3 – 19,999/mm^3).[38] Hornik et.al concluded from their study that although abnormality in WCC, platelets and I/T ratio had increased odds of infection, no complete blood cell count-derived index possesses the sensitivity to rule out reliably sepsis in neonates.[38]

1.7.4 Procalcitonin

Procalcitonin (PCT) is a 116-aminoacid peptide and one of the precursors of calcitonin.[39] It is another acute phase reactant that has emerged in recent years as a strong biomarker of bacterial sepsis.[32] It is claimed to be superior to other acute phase proteins, including CRP, with sensitivity and specificity ranging from 87% to 100%.[32] The exact site of production of PCT in sepsis is not yet known, with monocytes and hepatocytes thought to be the sources.[32] Serum concentrations of PCT begin to rise four hours after exposure to bacterial endotoxin, peak at six to eight hours, and remain raised for at least 24 hours.[32, 39]

1.8 Specific organisms causing NNS

1.8.1 *Staphylococci*

Staphyloccoci are aerobic, gram-positive bacteria that grow in pairs and clusters.[8, 19, 31] They are ubiquitous bacteria that colonize and are pathogenic for humans and animals.

[8, 19] They are resistant to heat and drying, and may be recovered from nonbiologic environment weeks to months after contamination.[19] They are categorized by their ability to produce coagulase into coagulase-positive and coagulase-negative *Staphylococci* (CoNS).[8, 19] *Staphylococcus aureus* is a coagulase-positive and is a predominant pathogen causing a variety of infections.[8, 19] CoNS include *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Staphylococcus haemolyticus*. CoNS are pathogens in neonates, compromised hosts and patients with foreign bodies.[8, 19]

CoNS has emerged as an important cause of NNS, reported in some studies as the most commonly isolated organism in cases of LOS.[3, 8, 31] *S. aureus* is carried in 20-40% of the adult population. Neonates become colonized early in life.[8, 19] In the NICHD study, CoNS (47.9%) was the most commonly isolated organism, followed by *S.aureus* (7.8%), in cases of LOS.[8]

1.8.2 *Klebsiella*

Klebsiella are gram negative enteric rods.[8, 19] They lack motility and grow as large mucoid colonies on solid media.[8] *K. pneumonia*e is the most common pathogen in this genus followed by *K.oxytoca*. *Klebsiella* species are common opportunistic nosocomial pathogens and newborn outbreaks continue to be a frequent occurrence worldwide.[8] As shown in Table 2*, Klebsiella* species are amongst the commonest isolated pathogens in cases of NNS in developing countries.[1]

1.8.3 Acinetobacter

Acinetobacter is a gram-negative rod¸oxidase-negative and lactose-nonfermenting organism.[8, 19] It belongs to a genus of coccobacillary bacteria in the family Neisseriaceae.[8, 19] The most common isolates of the genus are *A.baumannii*, *A.lwoffi*, *A.haemolyticus*, and *A.johnsonni*.[8, 19] Acinetobacter is an uncommon pathogen in healthy persons, but it is seen with increasing frequency in hospitalized and immunocompromised individuals.[8, 19]

1.8.4 *Candida*

Candidiasis is the most common fungal infection in the world.[19] Systemic candidiasis is a serious form of nosocomial infection in low birth weight (LBW) infants.[31] *C. albicans* accounts for most human infections.[31] Other candida species that have been reported with increasing frequency are *C.parapsilosis*, *C. krusei*, *C. lusitaniae and C.glabrata.* [31]

CHAPTER 2

2.1 AIM AND OBJECTIVES

The aim of this study was to evaluate the epidemiology of culture-proven neonatal sepsis and to describe the clinical characteristics of patients with neonatal sepsis at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) neonatal unit over a one year period.

The specific objectives were:

1. To identify the organisms causing early onset neonatal sepsis from 1st January 2012 to 31st December 2012
2. To identify the organisms causing late onset neonatal sepsis from 1st January 2012 to 31st December 2012
3. To review antibiotic sensitivity patterns of organisms causing neonatal sepsis
4. To describe the clinical features of patients with neonatal sepsis
5. To suggest guidelines for empiric antibiotic therapy at CMJAH neonatal unit

2.2 METHODS

This was a retrospective descriptive study conducted in the neonatal unit of CMJAH. The study involved review of clinical and laboratory data of patients with positive blood culture results from the 1st January 2012 to the 31st December 2012.

2.2.1 Charlotte Maxeke Johannesburg Academic Hospital neonatal unit

The Division of Neonatology at CMJAH provides neonatal services to 15000 newborn deliveries per annum. This includes 9132 babies born at CMJAH and a further 3000 babies born at Hillbrow and Alexandra Clinics.

In addition to the above, this unit serves as a major tertiary referral centre for problem babies from Gauteng, Northwest, Limpopo and Mpumalanga provinces. Paediatric surgical facilities are severely restricted throughout these regions and large numbers of babies with neonatal surgical problems are referred to CMJAH for surgery and ongoing neonatal care.

The unit consists of a 15-bed intensive care unit for babies requiring ventilation (combined with paediatric intensive care); and a 35 to 40-bed high care facility for low birth weight infants and other newborns with complications not requiring ventilation. In this area nasal CPAP for early onset respiratory distress and therapeutic hypothermia for hypoxic ischaemic encephalopathy are routinely employed. There is a 20-bed low care ward and

a 15-bed Kangaroo Mother Care unit for growing infants prior to discharge. A total of 1903 newborns required admission to the neonatal unit during the period from 1st January 2012 to 31st December 2012.

2.2.2 Suspected Sepsis policy at CMJAH during the study period

Blood cultures, FBC and CRP were basic investigations performed on babies with obstetric risk factors and/or clinical features of sepsis such as apnoea, respiratory distress, feeding intolerance and shock. It was recommended that a sterile 1ml sample be sent for blood culture, prior to commencing antibiotic therapy, in patients suspected of having NNS. For those suspected with EOS, blood culture and FBC with a differential count were done on admission (usually on the first day of life) and a CRP was done after 24 hrs of initial work-up. During the study period, EOS was treated empirically with penicillin and gentamicin. In those with suspected LOS, blood culture, C-reactive protein (CRP), and a full blood count (FBC) with a differential white blood count were done on the same day. Empiric treatment was started with Amikacin and Piperacillin/Tazobactam.

2.2.3 Study Population

The study population included all neonates admitted to the CMJAH neonatal unit, including NICU, between 1st January 2012 and 31st December 2012. It consisted of patients with a positive blood culture during the study period. The clinical data of patients with culture-proven neonatal sepsis were reviewed. Patients with bacteraemia but with

no other features suggestive of sepsis or those with organisms considered as contaminants were excluded. Infants who were in the neonatal unit beyond 28 days of life were also excluded from the study.

Definitions

- A neonate was defined as defined as an infant in the first 28 days of life
- Culture-proven sepsis was defined as a pathogenic organism, either bacterial or fungal isolated on blood culture with other clinical and laboratory features consistent with infection.
- Laboratory features of sepsis included an abnormal white cell count, reduced platelet count and/ or a CRP >10. Age appropriate definitions of low or high white cell count and reduced platelets count were used.
- The following organisms were considered to be contaminants:
    1. *Micrococcus species*
    2. *Bacillus species*
    3. *Corynebacterium species*
    4. *Streptococcus viridans*
- Coagulase negative Staphylococcus was considered significant if two blood cultures drawn within 72-hrs of each other grew the same organism or there was a single positive blood culture in association with other laboratory features of sepsis.
- When one significant organism was isolated from the same patient within 7 days, this was considered to be a single episode of sepsis.

- Sepsis was categorized into early-onset and late-onset sepsis. Early-onset sepsis was defined as sepsis occurring within 72-hrs of life and LOS as sepsis occurring after 72-hrs of life.

2.2.4 Data Collection

A list of positive blood cultures done on neonates admitted at CMJAH neonatal unit during the study period was obtained from National Health Laboratory Service (NHLS) Computer Data Warehouse. Information obtained from this database included patient identification, the organism(s) isolated and the antibiotic sensitivity of each organism. The NHLS database was further reviewed to obtain laboratory results, i.e. white cell count, platelets and CRP, done at the same time as the positive blood culture. Hospital records were retrieved to obtain patient demographic and clinical data. All this information was captured on a data collection sheet (Appendix A).

The demographic information captured included patients' gender, race and gestational age. Limited clinical data obtained included birth weight, gestational age, mode of delivery, place of birth, maternal booking status and HIV results. The final outcome was documented as discharged or demised. The patient was defined as being discharged if he or she left the CMJAH to go home or was transferred to another hospital facility once the sepsis episode had resolved. Death due to sepsis; was defined as any death certified due to NNS; in a patient with culture proven sepsis.

2.2.5 Statistical Analysis

Data were entered into a Microsoft Excel spreadsheet for data management and transferred to Statistica software package where further data management and statistical analysis were performed. Appropriate descriptive statistical analysis was carried out. Categorical variables were reported as proportions and percentages. Numerical variables were reported as mean and standard deviation when normally distributed, and median and interquartile ranges when not normal distribution. Comparison between categorical variables was performed using the Chi-squared or Fisher exact test. Comparison between continuous variables was performed using Mann-Whitney U test for abnormally distributed data. A significant difference was taken as a p-value of less than 0.05.

2.2.6 Ethical Considerations

The research protocol was submitted and approved by the Human Research Ethics Committee of the University of the Witwatersrand and the NHLS Ethics Committee. Permission was obtained from the CMJAH Chief Executive Officer and the Head of Paediatrics and Child Health at the hospital. (Appendixes B, C, D).

CHAPTER 3

3.0 RESULTS

3.1 Incidence of neonatal sepsis

The total number of babies born at CMJAH from 1st January 2012 to 31st December 2012 was 9132. There were 1903 admissions to neonatal unit during this one year period. This number included babies born and babies not born at the CMJAH. One hundred and ninety six (196) neonates were identified as having blood culture positive neonatal sepsis. This gave an incidence of 10.26 per 100 admissions. The majority of cases of NNS were of late onset, with the median age at the onset of NNS of 7days (IQR 4-14days). There were 33 patients identified with blood culture positive EOS. This gave an incidence of EOS of 1.73 per 100 admissions. One hundred and sixty four (164) babies were identified as a having LOS, which gave the incidence of LOS of 8.61 per 100 admissions.

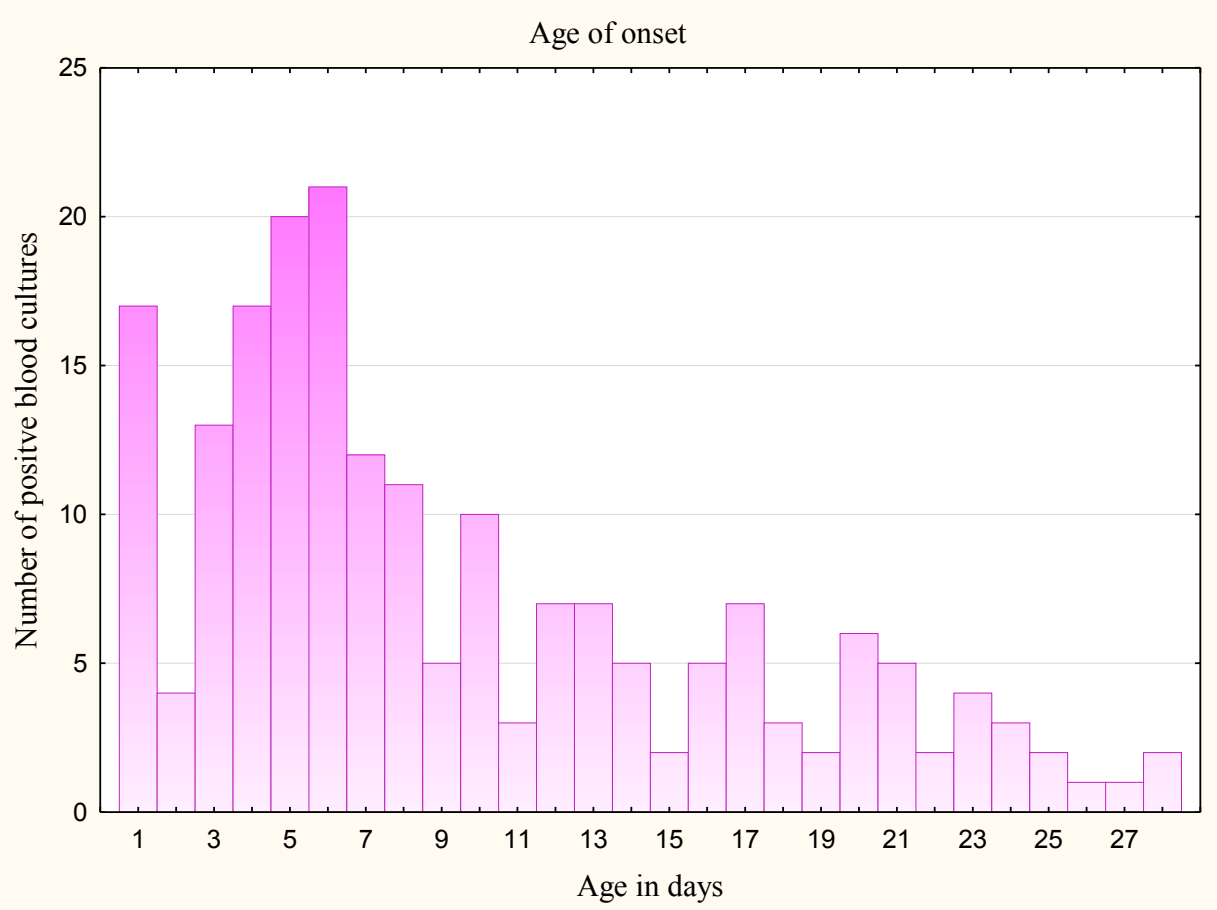

Figure 3.1 Age at onset of NNS

3.2 Patient clinical data

Of the 196 patients with NNS, 117 (59.39%) were male and 79 (40.30%) female. The median gestational age for all cases of NNS was 30 weeks (IQR 28-32) and the median birth weight was 1300g (IQR 1000-1720). The majority of the mothers received antenatal care and had documented HIV status. A total of 119 patients, which was 60.71% of patients with sepsis, received antenatal care. There were 162 mothers with a known HIV

status and 36 whose status was not documented. Thirty percent (30.67 %) of babies with known maternal status were HIV exposed.

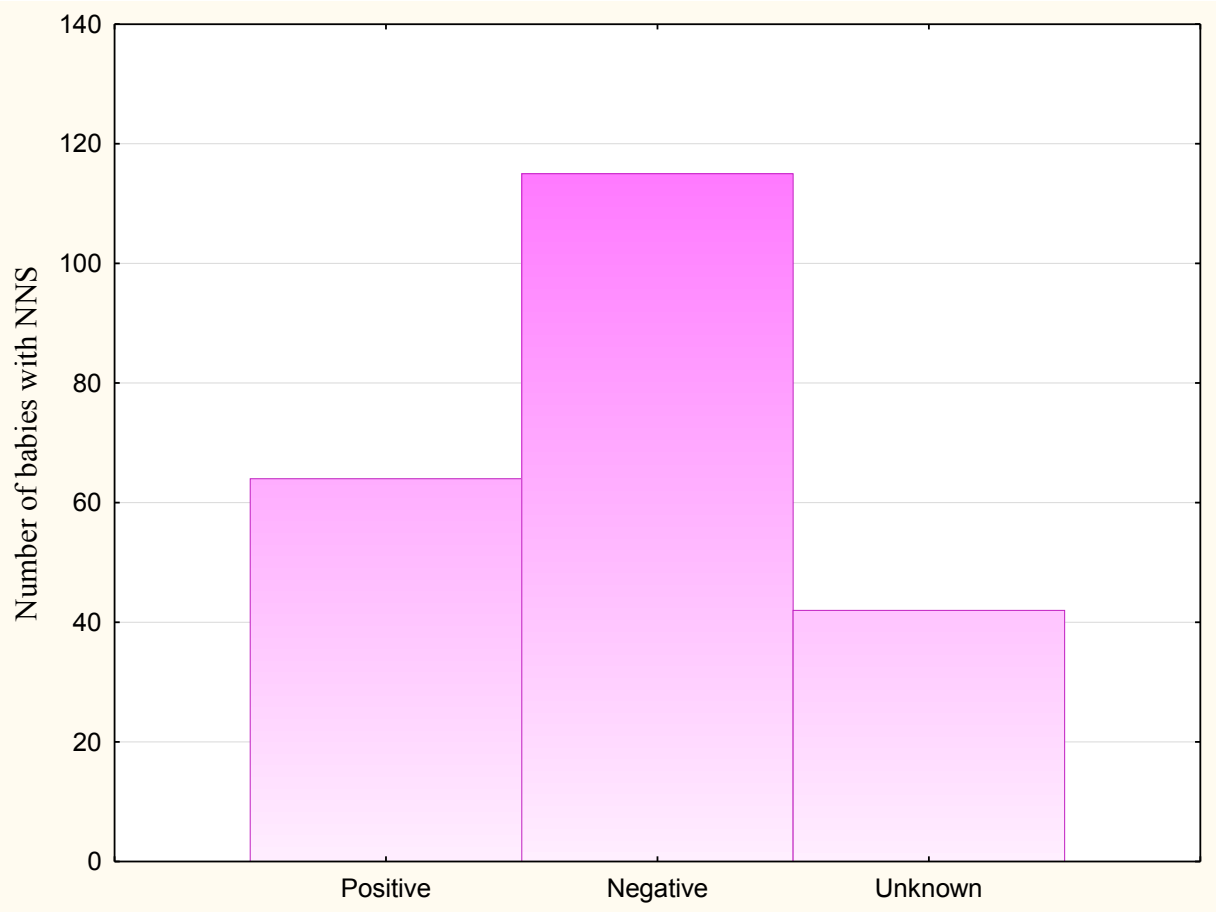

Figure 3.2 Maternal HIV Status

Sixty-nine percent (136/196) of babies with sepsis were born at CMJAH. Although the majority of patients were delivered vaginally, there was a large number of patients who were delivered by C/S (39.59%).

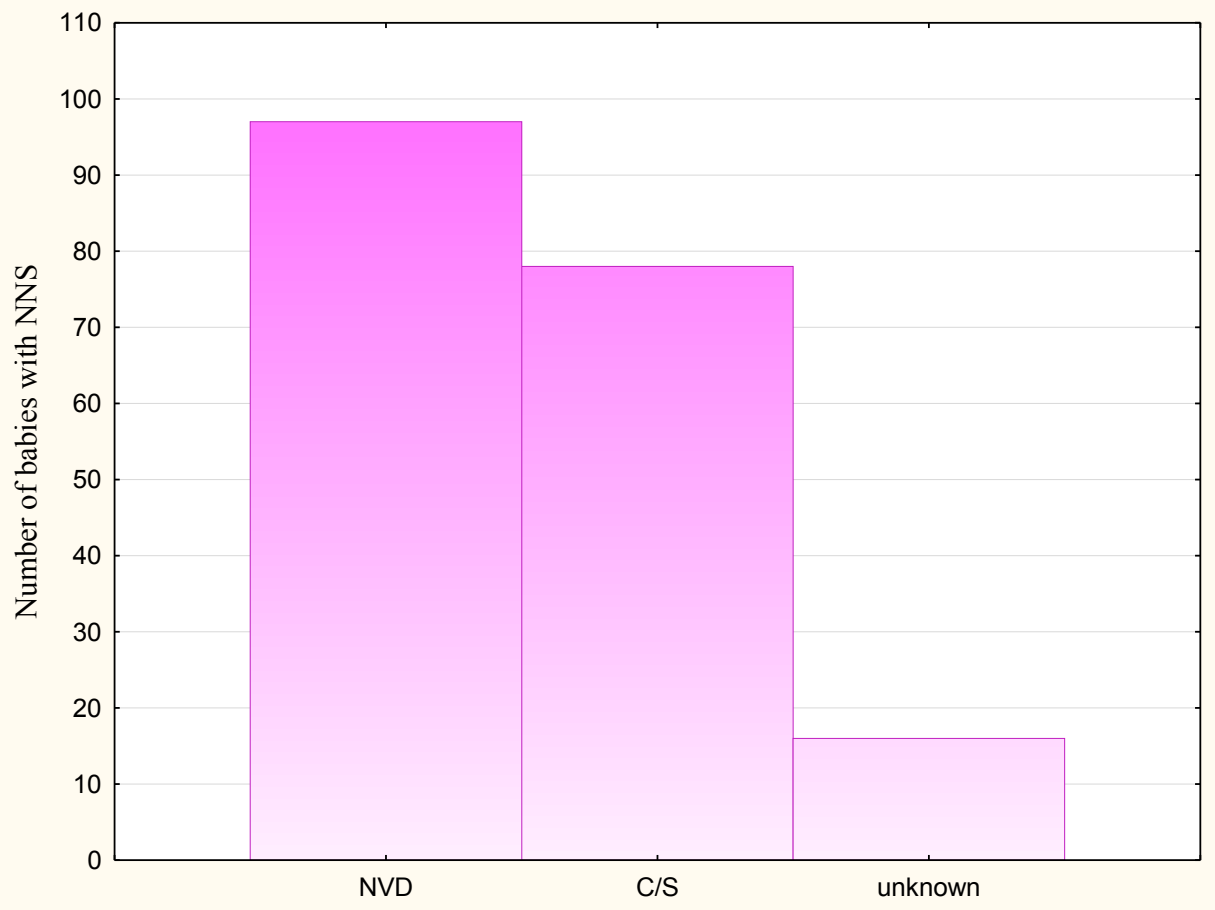

Figure 3.3 Mode of delivery

There was no statistically significant differences in the sex, maternal HIV status, place of birth and mode of delivery between cases of EOS and LOS. There was a significant difference in the birth weight and gestational age between the two groups. The LOS group weighed less with a median birth weight of 1290g vs 1460g in the EOS group (p= 0.02). The LOS group had a lower gestational age with a median gestational age of 30 weeks vs 32 weeks in the EOS group (p=0.007).

Table 3.1 Patient clinical data

Patient characteristic	All cases of NNS n=196	EOS n=33	LOS n=164	p-value
Sex				0.09
Male	117 (59.69%)	24 (72.72%)	93 (56.70%)	
Female	79 (40.30%)	9 (27.27%)	71 (43.29%)	
Place of birth				
Inborn	136 (69.38%)	27 (81.81%)	109 (66.46%)	0.17
Outborn	53 (27.04%)	6 (18.18%)	48 (29.26%)	
Unknown	7 (3.57%)	0	7 (4.26%)	
Maternal HIV				
Negative				
Positive	102 (52.04%)	17 (51.51 %)	85 (51.82%)	0.99
Unknown	60 (30.61 %)	10 (30.30%)	50 (30.48%)	
	34 (17.34%)	6 (18.18%)	29 (17.68%)	
All-cause mortality	45 (22.95%)	8 (24.24%)	37 (22.56%)	0.98
Mode of delivery				0.21
NVD	96 (48.97%)	14(42.42%)	83 (50.60%)	
C/S	78 (39.79 %)	17 (51.51%)	61 (37.19%)	
Unknown	22 (11.22%)	2 (6.06%)	20 (12.19%)	
Booking status				
Yes	119 (60.71%)	21 (63.63%)	98 (59.75%)	0.27
No	56 (28.57%)	11 (33.33%)	45 (27.43%)	
Unknown	21 (10.72%)	1 (3.04%)	20 (12.82%)	
Birth weight (g)				
Median	1300	1460	1290	0.02
L-QRT	1000	1060	980	
U-QRT	1720	2530	1590	
Gestational age				
Median	30	32	30	0.007
L-QRT	28	29	28	
U-QRT	33	38	32	

3. 3 Organisms causing neonatal sepsis

There were 236 microorganisms isolated on blood culture of 196 patients with culture proven NNS during the study period. Of the 196 patients, 7 had two episodes of NNS and 4 had three episodes. There were ten cases of polymicrobial sepsis, i.e. more than one organism isolated per episode. Seven of these cases presented with LOS.

Of the 236 microorganisms¸ gram negative organisms were the most commonly isolated organisms in cases of culture-proven NNS [116 (49%). Amongst the cases with gram negative organisms, most were due ESBL *Klebsiella pneumoniae* [76 (65.5%)], *Acinobacter baumannii* [20(17.2%)] and *E.coli* [12 (10.3%)]. Gram positive organisms constituted 42.4% (100/236) of the organisms isolated. Coagulase negative Staphylococcus [56 (56%)] and MRSA [31(31%)] were the most common gram positive organisms isolated. Yeasts accounted for 8.47% (20/236) of the isolated organisms and the following yeasts were isolated; *C. albicans* [13(65%)], *C.parapsilosis* [5(25%)] and *C.glabrata* [*2(10%)*].

Table 3.2 Organisms causing NNS

Organism	Number	Percentage
Gram positive Organisms	100	42.3
GBS	3	1.3
MRSA	31	13.1
CoNS	56	23.7
L.monocytogenese	1	0.4
E.faecalis	7	3.0
E.faecium	2	0.8
Gram negative organisms	**116**	**49**
ESBL *K.pneumoniae*	76	32.2
A.baumannii	20	8.5
E.coli	14	5.9
K.oxytoca	2	0.8
Pseudomonas aeruginosa	1	0.4
K.pneumoniae	2	0.8
E.cloacae	1	0.4
Fungi	**20**	**8.4**
C.albicans	13	5.5
C.parapsilosis	5	2.1
C.glabrata	2	0.8

3.4 Organisms causing early onset neonatal sepsis

There were 39 organisms isolated from 33 patients with EOS. The most common organisms isolated were gram-negative organisms, which accounted for 51.3% (20/39) of the organisms. The most common gram-negative organisms were *Klebsiella pneumoniae* (45%) and *A.baumanii* (35%). Gram-positive organisms accounted for the

remaining 48.5% and there were no fungi isolated. CNS was the most common isolated gram-positive organism [12/19 (63.2%)].

Table 3.3 Organisms causing early onset sepsis

Organisms	Number	Percentage
Gram negative organisms	**20**	**51,3**
ESBL *K.pneumoniae*	9	23.1
A.baumanii	7	17.9
E.coli	2	5.1
K.oxytoca	2	5.1
Gram positive Organisms	**19**	**48.7**
CNS	12	30.8
GBS	2	5.1
MRSA	3	7.7
E.faecalis	2	5.1

3.5 Organisms causing late onset neonatal sepsis

There were 197 microorganisms isolated in 164 patients with LOS (Table 3.4). Gram-negative organisms accounted for the majority of isolates in cases of LOS [103 (48.2%)]. The most common gram-negative organisms were ESBL *K.pneumoniae* [67 (70.5%)], *A.baumannii* [13 (13.7%)] and *E.coli* [10 (10.5%)]. Gram- positive organisms were isolated in 41.6% (82) of the cases. The most commonly isolated gram-positive organisms were CNS [44(53.7%)] and MRSA [28 (34.1%)]. *Streptococcus agalactiae* was only

isolated in one of the cases. There were 20 fungi isolated, with *C.albicans* [13 (65%)] being the commonest fungus isolated, followed by *C.parapsilosis* [5(25%)]. *C.glabrata* was only isolated in two of the cases with LOS.

Table 3.4 Organisms causing late onset sepsis

Organism	Number	Percentage
Gram positive organisms	**82**	**41.6**
CNS	44	22.3
MRSA	28	14.2
E.faecalis	5	2.5
E.faecium	2	1.0
GBS	1	0.5
L.monocytogenese	1	0.5
Gram negative organisms	**95**	**48.2**
ESBL K.pneumoniae	67	34.0
A.baumannii	13	6.6
E.coli	12	6.1
K.pneumoniae	2	1.0
E.cloacae	1	0.5
Fungi	**20**	**10.2**
C.glabrata	2	1.0
C.albicans	13	6.6
C.parapsilosis	5	2.5

3.6 Antibiotic susceptibility

3.6.1 Gram negative organisms

The patterns of antibiotic susceptibility for the most common gram negative organisms isolated in the study is shown in Table 3.5. Susceptibility results were not available for all the isolated organisms. Overall, there was a high resistance against Ampicillin (94.6%),

Gentamicin (86.7%) and Ceftazidime (66.1%). *K.pneumoniae* was mostly sensitive to Meropenem [63/64 (94.4%)], Amikacin [59/66 (89.4%)] and Piperacillin/Tazobactam [45/64 (70.3%)]. *K.pneumoniae* was resistant to Ampicillin, Gentamicin and Cefotaxime, with all the isolated strains resistant to Ampicillin; and 98.4% resistant to Gentamicin and Cefotaxime.

A.baumannii was mostly sensitive to Amikacin [13/20 (65%)] and Ceftazidime [15/20 (75%)], with high resistance to Gentamicin (85%) and Meropenem (80%). All of the isolated *A.baumannii* strains were resistant Tazobactam/Piperacillin. All of the isolated *E.coli* were sensitive to Meropenem and Cefotaxime, with many also sensitive to Amikacin [13/20(65%)] and Tazobactam/Piperacillin [13/14(92.8%)]. Only 57.14% of the E.coli were sensitive to Gentamicin.

Table 3.5 Pattern of resistance of gram negative organisms to common antibiotics

Bacteria / **Antibiotics**	*K. pneumoniae*			*A. baumannii*			E. coli			Overall Resistance %
	NT	RS	%	NT	RS	%	NT	RS	%	
Ampicillin	64	64	100	15	13	86	14	11	78.6	94.6
Gentamicin	64	63	98.4	20	17	85	14	5	35.7	86.7
Amikacin	66	7	10.6	20	7	35	14	2	14.3	16.0
Tazobactam/ Piperacillin	64	19	26.7	20	20	100	14	1	7.14	19.0
Meropenem	64	1	1.6	20	16	80	14	0	0	17.3
Cefotaxime	64	63	98.4	----------			14	0	0	1.3
Ceftaxidime	64	61	95.3	20	5	25	14	7	50	66.1

Note: NT=Number tested RS=Resistant strains

3.6.2 Fungi

All the isolated fungi were sensitive to Amphotericin B. The majority of the isolated *C. albicans* (84.62%) were sensitive to Fluconazole and Voriconazole. All isolated *C.glabrata* species were sensitive to Fluconazole and Voriconazole. Only 20% of the isolated *C.parapsilosis* were sensitive against to Fluconazole and Voriconazole.

Table 3.6 Pattern of resistance of fungi to common antifungals

Fungi / **Antifungal**	*C. albicans*			*C. parapsilosis*			*C. glabrata*			Overall Resistance %
	NT	RS	%	NT	RS	%	NT	RS	%	
Amphotericin B	13	0	0	5	0	0	2	0	0	0
Fluconazole	13	2	15.38	5	4	80	2	0	0	30
Voriconazole	13	2	15.38	5	4	80	2	0	0	30

Note: NT=Number tested RS=Resistant strains

3.6.3 Gram positive organisms

All the *isolated Staphylococcus aureus* were methicillin-producing and were sensitive to Vancomycin and Linezolid. All the *Streptococcus agalactiae* were sensitive to Ampicillin

and Cefotaxime. Susceptibility of CNS to commonly used antibiotics such as Ampicillin, Gentamicin and Vancomycin was not available.

Table 3.7 Pattern of resistance of gram positive organisms to common antibiotics

Bacteria / **Antibiotic**	*CNS* NT	RS	%	*MRSA* NT	RS	%	*GBS* NT	RS	%	Overall Resistance %
Ampicillin	----------			----------			3	0	0	0
Vancomycin	----------			31	0	0	----------			-------
Linezolid	34	0	0	31	0	0	----------			0
Cefotaxime	----------			----------			3	0	0	0

Note: NT=Number tested RS=Resistant strains

3.7 Mortality

Of the 196 patients with culture-positive NNS, 45 died before hospital discharge, giving an all-cause mortality of 22.95% for patients diagnosed with sepsis. The all-cause mortality for EOS was 24.24% and for LOS was 22.56%. Table 3.8 compares some of the clinical data between survivors and those who died from NNS. Mortality was significantly associated with delivery outside the CMJAH (p=0.0001) ¸ vaginal delivery (p=0.01) and lack of antenatal care (p=0.01). There was no association between maternal

HIV status and patient outcome. Sepsis caused by gram-negative organisms was more significantly associated with death than sepsis caused by gram-positive organisms (P=0.01).

Table 3.8 Patient clinical data and outcome

Clinical data	Survivors N (%)	Deaths N (%)	P-value
Maternal HIV status			
Negative	68 (58.6)	24(70.6)	0.20
Positive	8 (41.4)	10(29.4)	
Gender			
Male	75(56.4)	25(58.1)	0.67
Female	58(43.6)	18(41.9)	
Delivery			
C/S	66(51.6)	9(28.1)	0.01
Vaginal	62(48.4)	23(71.9)	
Booking status			
Booked	91(71.1)	16(48.5)	0.01
Unbooked	37(28.9)	17(51.5)	
Place of birth			
Inborn	104(82.5)	20(41.7)	0.0001
Outborn	22(17.5)	28(58.3)	
Gestational Age (weeks)			
<35	102(79.1)	25(83.3)	0.59
>35	27(20.9)	5(16.7)	
Birth weight (g)			
< 1500	88(66.2)	31(75.6)	0.25
> 1500	45(33.8)	10(24.4)	
Organisms			
Gram negative	70(48.6)	36(69.2)	0.01
Gram positive	74(51.4)	16(30.8)	

CHAPTER 4

4.0 Discussion

The aim of this study was to evaluate the epidemiology of culture-proven neonatal sepsis and to describe the clinical characteristics of patients with neonatal sepsis at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) neonatal over a one year period. The main findings of this study were: *1)* neonatal sepsis is a common problem at CMJAH neonatal unit and is associated with significant mortality *2)* Gram-negative organisms are the most commonly isolated organisms in cases of NNS *3)* resistance to the commonly used antibiotics in the unit is common,

4.1 Incidence

The finding of this study confirms that neonatal sepsis is an important cause of morbidity in newborns and that the incidence is on the increase at CMJAH neonatal unit. The incidence of NNS in the current study is 10.26 per 100 admissions. The incidence of NNS is probably higher than estimated by this study, because patients with clinical or laboratory signs suggestive of sepsis but with negative blood cultures were not included. Although microbial cultures of blood or other sterile body fluids are considered the gold standard in the diagnosis of neonatal sepsis they are subject to low sensitivity.[33]

There has been an increase in the incidence of NNS in the unit. A previous study in the same neonatal unit in 2003 showed that the incidence of NNS was 8.4 per 100

admissions.[3] The reason for this increase maybe due to overcrowding of the neonatal facility. Over the years, there has been an increase in the number of admissions to the neonatal facility. In the previous study, there were 1129 neonatal admissions over a one year period, while in this study period 1903 patients were admitted.[3] Furthermore advances in neonatal intensive care have allowed the survival of low birth weight and sick infants, and at the same time created risks for neonatal sepsis.[20] Over the years there has been important advances in the CMJAH neonatal unit, which includes the acquisition of more ICU beds.

It is difficult to compare the incidence of NNS at CMJAH to those of other neonatal facilities in the world, because many of the studies report the incidence per 1000 live births. In this study, it was difficult to calculate the incidence as per 1000 live births. CMJAH is a referral hospital which receives patients from other hospitals (including other provinces), and for the proper rate to be calculated birth rates at the referring hospital needs to be included.

Similar to the previous studies in the facility the proportion of LOS and EOS remained the same, with predominance of LOS.[3, 4] Studies by Motara et al and Ballot et al, showed that LOS accounted for 94.3 % and 93.5 % of cases of NNS respectively.[3, 4] In this study, LOS accounted for 83, 2% of cases. [4] Other studies in both developing and developed countries, also showed that LOS occurred more frequently than EOS.[11, 12]

4.2 Organisms causing neonatal sepsis

Bacterial isolates in the current study are considerably different from those obtained in a similar study in the same unit in 2002/3 audit, with some similarities to the 2009/2010 audit .[3, 4] Table 4.1 shows the trend in bacterial isolates in NNS at the CMJAH. This confirms that pathogens causing NNS changes over time, and emphasize the need for ongoing surveillance.

Table 4.1 Bacterial isolates at Charlotte Maxeke Johannesburg academic

Organism	**2002-2003** **N (%)**	**2009-2010** **N** (%)	**2012** N (%)
Gram-positive organism	**79 (68.1)**	**134(54.4)**	**100 (46.3)**
CNS	65 (56.0)	62 (22.5)	56 (25.9)
S. aureus	4 (3.4)	23 (8.3)	31 (14.3))
E. faecalis	4 (3.4)	13(4.7)	7 (3.2)
GBS	2 (1.7)	10 (3.6)	3 (1.4)
E. faecium	0	11 (4.0)	2 (0.9)
L. monocytogenes	0	0	1 (0.5)
S. viridans	4 (3.4)	15(5.4)	0
Gram-negative	**37(31.9)**	**112 (40.5)**	**116 (53.7)**
Klebsiella spp	12 (10.3)	47 (18.3)	80 (37.0)
A.baumannii	0	27 (11.0)	20(9.2)
E. coli	20 (17.2)	23 (9.3)	14 (6.5)
P.aeruginosa	2 (1.7)	4 (1.6)	1(0.5)
Others	3 (2.6)	11 (4.5)	1(0.5)

There has been a significant increase in the predominance of gram-negative microorganisms as a cause of NNS in the CMJAH neonatal unit over the years. The high proportion of gram-negative microorganisms as a cause of neonatal sepsis is similar to findings from surveillance in other developing countries. Similar to other developing countries gram-negative organisms were predominant in both EOS and LOS. [1, 40]

The most common isolate overall was *K. pneumoniae*, followed by CNS, MRSA and *A. baumanii*. The predominance of *K. pneumoniae* in the current study accords with several reports from Nigeria and other developing countries.[40] CNS, a gram-positive was the most common isolate in EOS. CNS have remained an important cause of NNS in the unit accounting for 23.7% of the isolated microorganisms in the current study. This is contrary to reports from other developing countries where CNS are usually amongst the least common organisms. [30] The reason for this might be because isolated CNS is excluded from analysis as it is often considered to be a contaminant.[30] Although CNS is often considered a contaminant, it is a pathogen in neonates, compromised hosts and patients with foreign bodies.[8, 19] Further evaluations such as a repeat blood culture are required to determine the clinical significance of CNS.[8, 41] The importance of CNS as a cause of NNS has been reported by studies from developed countries and Asian countries.[12,8] A one year prospective study in eight neonatal units in Australia, reported that CNS was the most commonly isolated organism. [8]

There has been a significant increase in the proportion of *Klebsiella spp* isolated in the unit with the emergent of ESBL producing *K. pneumoniae* isolated. A study by Ballot et al showed that 70.8% of the isolated *K. pneumoniae* were ESBL producing compared to 97.3% in the current study. [4] *S. aureus* and *A. baumannii* are two other organisms that are becoming predominant in the unit. The importance of *A. baumannii* as a cause of NNS in the unit was noted by Ballot et al, when this microorganism accounted for 10% of the bacterial isolates compared to the previous study where this organism was not isolated. .[3, 4]

4.3 Antibiotic resistance

Emergence of resistant organisms causing neonatal sepsis is now a worldwide problem. [1] Reports of multiresistant bacteria causing neonatal sepsis in developing countries are on the increase. [1] The current study also showed increasing resistance to commonly used antibiotics in the unit. Most of the isolated *K. pneumoniae* and *A. baumannii* were resistant to Ampicillin and Gentamicin; which were the first-line antibiotic agents used in EOS. Overall, 94.6% and 86.7%, of top three gram-negative causing sepsis were resistant to Ampicillin and Gentamicin respectively. These organisms also showed high resistance against the Ceftazidime with the overall resistance of 66.1%.

With regard to treatment of LOS, Amikicin and Tazobactam/Piperacillin were used as first-line agents. Most of the *Klebsiella pneumoniae* were sensitive to these drugs, while *A. baumannii* was mostly sensitive to Amikacin and Ceftazidime. All the isolated

S.aureus were methicillin-resistant, with resistance to Cloxacillin. Susceptibility testing was not routinely done in cases of coagulase negative *Staphylococci*. It was reported by the microbiologist that almost all coagulase negative *Staphylococci* isolated at CMJAH neonatal unit are methicillin resistant and are sensitive to Vancomycin. Resistance to methicillin is widespread among hospital isolates of CNS, with overall resistance ranging globally from 75–90%.[42]

Only a few of the isolated *C.albicans* showed resistance to Fluconazole, with all of the isolated fungi sensitive to Amphotericin B. *C.parapsilosis*, which was the second most common fungi isolated in the unit, showed high resistance to fluconazole. The findings of this study are similar to those by Nakwa et al, which showed that 100% of the isolated *C.albicans* were sensitive to Amphotericin B and 96% sensitive to Fluconazole. Only 58% of the isolated C. parapsilosis were sensitive to Fluconazole.[20]

4.4 Patient clinical data

Factors associated with neonatal sepsis are well described in the literature. [15-17] The current study only looked at limited patient clinical data. Limited clinical data obtained included birth weight, gestational age, mode of delivery, place of birth, maternal booking status, maternal HIV results and final outcome. The majority of the patients with sepsis were males, born premature and were of low birth weight. Most of the patients were delivered at CMJAH. Preterm delivery and low birth weight are well-established NNS risk factors in developed and developing countries, and our finding that the majority of babies

with sepsis had low birth weight and we born premature is similar to that reported in other studies. Newborns are considered to be immunocompromised, especially when premature.[4, 13, 14] There are a number of factors which predispose premature and low birth weight infants to infections.

HIV uninfected infants born to HIV infected mothers were thought to be at increased risk of sepsis.[17, 27, 28] Our finding was that fewer patients with sepsis were born to HIV infected mothers, with only thirty percent (30.67 %) of babies with known maternal status exposed to HIV. A study by Cutland et al established that maternal HIV infection is not associated with increased risk of bacterial EOS or LOS, and that HIV infected neonates are at increased risk of EOS and LOS.[26] During the study period¸ HIV testing was not routinely done in neonates and hence we did not look at the HIV status of the patients.

When comparing clinical data of babies with EOS and LOS, there was no statistically significant differences in the sex, maternal HIV status, place of birth and mode of delivery. There was a significant difference in the birth weight and gestational age between the two groups. The LOS group weighed less (p= 0.02) and had a lower gestational age (p=0.007). This is probably because the more premature and smaller babies require longer hospital stay and interventional invasive procedures aimed at providing nutritional or respiratory support, which predisposes them to infections.

4.5 Mortality

This study confirms that sepsis is a significant cause of mortality newborns. Forty-five patients with NNS died before hospital discharge, giving an all-cause mortality of 22.95%. The mortality rate for LOS in the CMJAH neonatal unit remained fairly constant compared to the previous audit in the unit which showed a mortality rate of 20.9%. All-cause mortality for EOS was 24.24% and for LOS was 22.56%. The mortality rate for EOS showed a drastic increase when compared to the 2009/2010 which had a mortality rate of 6.5%. The mortality rate from NNS at the CMJAH is relatively higher than that reported in other developing countries.[43] [11]

In keeping with findings from other studies, mortality was significantly associated with gram negative sepsis. .[3, 4] Other factors associated with mortality from NNS are delivery outside the CMJAH¸ vaginal delivery and lack of antenatal care. There was no association between maternal HIV status and patient outcome, which is in keeping with the findings that HIV exposed neonates are not at an increased risk of NNS.[28]

CHAPTER 5

5.0 Conclusion

Neonatal sepsis is still an important cause of morbidity and mortality at CMJAH neonatal unit. Although mortality from LOS has remained relatively constant, the incidence of NNS in the unit is on the increase. A changing pattern of bacteria isolated has been observed. Gram-negative microorganisms comprised the majority of the neonatal sepsis, with ESBL *Klebsiella pneumonia*e and *A. baumannii* being the most prevalent. CoNS remains an important cause of NNS, and is the most prevalent gram-positive organism isolated. Resistance to the first-line antibiotic regimen for EOS is significant. It seems from the findings of our study, Ampicillin and Gentamicin do not provide adequate cover for EOS, given that highly resistant *K. pneumoniae, A. baumannii* and CoNS which is mostly sensitive to Vancomycin are responsible for 71.8% of cases of EOS. The first-line regimen for nosocomial LOS, also seems inadequate as it does not cover MRSA and CoNS.

5.1 Recommendations

- A change in the first-line regimen for LOS, to include CoNS and MRSA cover with Vancomycin should be considered. We recommend changing first-line regimen for nosocomial LOS to be Meropenem, Ceftazidime and Vancomycin. Amikacin would have also been a good first-line, however to avoid the shared side-effects with Vancomycin, Ceftazidime is instead recommended.

- Few organisms were isolated in EOS, hence this study is not adequate to recommend changes to the first-line regimen that consists of Ampicillin and Gentamicin. However we recommend that clinicians should have a low thresh hold for changing antibiotics in cases of clinical deterioration on these antibiotics. Clinicians should strongly consider the possibility of multidrug resistant organisms.

- A periodic survey on the causes of sepsis and their antibiotic sensitivity patterns is recommended.

- Infection control measures such as hand washing must be emphasized, especially because most cases of NNS were nosocomially acquired.

CHAPTER 6

6.0 Limitations

There were some limitations which resulted from the retrospective nature of the study. Study participants were identified based on blood culture results. Due to the retrospective nature of the study, researchers had no control on how the blood cultures were collected. If strict sterility was not observed while taking blood cultures and inadequate volumes were taken, these could have influenced sensitivity and specificity of the blood cultures, and lead to sampling error.

Some of the study patients had incomplete database information or missing files, which might have resulted in reporting bias. Our study only looked at limited clinical data. Important data such as apgars scores, presence of MSAF, multiple gestation were not looked at.

The susceptibility to antimicrobial agents, only looked at commonly used agents and not all microorganisms had susceptibility results.

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APPENDIX A: ETHICS APPROVAL

R14/49 Dr Mamaila Martha Lebea

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130350

NAME: (Principal Investigator)	Dr Mamaila Martha Lebea
DEPARTMENT:	Department of Paediatrics and Child Health Charlotte Maxeke Johannesburg Academic
PROJECT TITLE:	Evaluation of Culture-Proven Neonatal Sepsis at a Tertiary Care Hospital in South Africa
DATE CONSIDERED:	05/04/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof VA Davies
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	05/04/2013

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature

Date 06 May 2013

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX B: NHLS APPROVAL

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs Research and Quality Assurance
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16 May 2013

Applicant : Dr Mamaila Lebea; Paediatric Registrar
Institution: University of the Witwatersrand
Department : Department of Paediatrics and Child Health
Contact : 0795701670
Email : lbxmam001@yahoo.com

Re : Approval to access National Health Laboratory Service (NHLS) data

Your application to undertake a research project titled "*Evaluation of culture proven neonatal sepsis at a tertiary hospital in South Africa*" using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you to conduct the proposed study as outlined in the submitted application.

Please note that the approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met:

- Ethics clearance have been obtained from an approved local Ethics Committee
- Informed consent is obtained from all participants
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted to the NHLS Academic Affairs and Research Office and the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs, Research and Quality Assurance. Please complete and sign the attached data request form. This should be submitted to Helpdesk4@nhls.ac.za for processing by the Corporate Data Warehouse. Any data related queries may be directed to Sue Candy, manager NHLS Corporate Data Warehouse, Tel: (011) 386 6036. Email: sue.candy@nhls.ac.za.

Yours sincerely,

Dr Johan van Heerden
Executive Manager: NHLS Academic Affairs, Research and Quality Assurance

Chairperson: Prof Algonda Perez Deputy Chairperson: Dr Mahamed Randera CEO: Sagie Pillay
Physical Address: 1 Modderfontein Road, Sandringham, Johannesburg, South Africa Postal Address: Private Bag X8, Sandringham, 2131, South Africa
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Fixed Number: 6200236

APPENDIX C: DATA COLLECTION SHEET

DATA COLLECTION SHEET

Demographic Information

Name:……
……

File number:……

Gender:
……

Birth history

Birth weight (g):

</=1000 □

>1000-1499 □

1500-2499 □

>/=2500 □

Gestational Age (weeks):

<28 □

28-35□

35-37□

>37□

Maternal booking blood result

HIV status:

Positive□

Negative □

Unknown□

Blood cultures

<72hrs of life

Total blood culture done:……

No. Positive blood culture:……

No. Negative cultures:……

Organisms identified and antibiotic sensitivity

1…… ……

2…… ……

3…… ……

4…… ……

5…… ……

Blood culture

>72hrs of life

Total blood culture done:……

No. Positive blood culture:……

No. Negative cultures:……

Organisms identified and antibiotic sensitivity

1…… ……

2…… ……

3…… ……

4…… ……

5…… ……

Final outcome

Discharged home □

Transfer to other wards□

Transfer to other hospitals□

Demised□