CULTURE PROVEN SEPSIS IN NEONATES:

EPIDEMIOLOGICAL SURVEILLANCE AND CLINICAL SIGNIFICANCE

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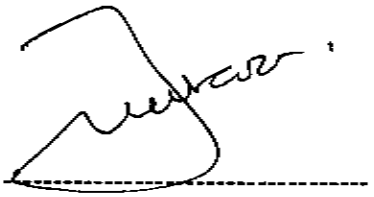
DECLARATION

I, Firoza Motara declare that this research report is my own work. It is being submitted for the degree 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.



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TO MY FAMILY

For your patience, support and love

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

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ABSTRACT

This study evaluated sepsis in the neonatal unit at Johannesburg Hospital with respect to the time of onset, clinical features, pathogens isolated, outcome of the treatment of patients, and antimicrobial sensitivity to provide site-specific guidelines for empiric antibiotic therapy. Neonates in the neonatal unit of the Johannesburg Hospital between 1st July 2002 and 30th June 2003 with growth on blood cultures had demographic, clinical; laboratory and microbiological data reviewed retrospectively. There were 132 significant isolates in 96 patients: five neonates presented with early onset sepsis (EOS) and 91 with late onset sepsis (LOS). Gram-negative bacilli were the predominant isolates in EOS. Coagulase-negative staphylococci (CoNS) were the predominant isolates in LOS followed by Gram-negative organisms. The case fatality rate was 40% for EOS and 19.7% for LOS. Multi drug resistant (MDR) patterns (resistance to>2 classes of antibiotics) were identified in 43 organisms. LOS was more common than EOS in the Johannesburg Hospital neonatal unit but had a lower case fatality rate.

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LIST OF ABBREVIATIONS

CFR	Case Fatality rate
CoNS	Coagulase Negative Staphylococci
CRP	C-reactive protein
CSF	Cerebral Spinal Fluid
EOS	Early Onset Sepsis
ESBL	Extended spectrum beta-lactamases
FS	Fungal sepsis
GBS	Group B Streptococcus
GNB	Gram Negative Bacteria
GPB	Gram Positive Bacteria
LCU	Low Care Unit
LOS	Late Onset Sepsis
MDR	Multi Drug Resistant
MRSA	Methicillin Resistant *Staphylococcus aureus*
MRSE	Methicillin Resistant *Staphylococcus epidermidis*
NEC	Necrotising Enterocolitis
NU	Neonatal Unit
NICU	Neonatal intensive care unit
PROM	Prolonged Rupture of Membranes
TCU	Transitional care unit
VLBW	Very low birth weight

CHAPTER 1 INTRODUCTION

The neonatal period is a highly vulnerable time for an infant who is completing many of the physiological adjustments required for extrauterine existence. The high neonatal morbidity and mortality rates reflect the fragility of life during this period. Global figures indicate that there are about 5 million neonatal deaths a year, 98% occurring in developing countries (Stoll, 1997).

Infections are a leading cause of morbidity and mortality in the neonatal period. Up to 2% of fetuses are infected in utero, and up to 10% of infants are infected in the first month of life (Gotoff, 2000). Neonatal infections including neonatal sepsis involves bacterial disease of infants 30 days of age or younger, primarily as bloodstream infection, although spread to other organs occurs in a substantial portion of affected infants.

Estimates of the incidence of neonatal sepsis vary from less than 1-4/1000 live births in high-income countries to 2-21/1000 live births in low-income countries (Yeung, 2005). However there has been little apparent change in the overall incidence of systemic bacterial infection in newborn infants in the past 40 years, although rates of disease differ among institutions and countries and shifts in organisms causing infections have been noted (Siegel, 1990). The incidence of meningitis is usually a fraction (approximately one third) of the number of infants with sepsis. Urinary tract infections occur in approximately 1% of all live born infants. The incidence of bacterial infections of the respiratory and gastrointestinal tracts, the skin and soft tissues, and bones and joints is substantial but not documented adequately.

Clinically and epidemiologically severe bacterial infections (i.e. sepsis or meningitis) during the neonatal period typical are divided into two distinct entities based on the age of onset: early-and-late-onset syndromes. These definitions have contributed greatly to diagnosis and treatment by identifying which microorganisms are likely to be responsible for sepsis during these periods and the expected outcomes of infection.

Early-onset sepsis (EOS) becomes clinically apparent within the first few days of life, and has been defined in most reports as occurring within the first 72 hours of life. Early-onset neonatal sepsis is acquired through vertical transmission by ascending spread from the lower genital tract, through transplacental transmission after maternal bacteremia (e.g, *Listeria monocytogenes*), or through neonatal acquisition during passage through the birth canal. The specific prenatal risk factors associated with EOS are the following:

- Prolonged rupture of membranes (> 18 hours)
- Foetal distress
- Maternal pyrexia (> 38° C) or overt infection e.g. urinary tract infection,
- Multiple obstetric procedures, including cervical sutures
- Preterm delivery, low birth weight or gestation
- History of *Streptococcus agalactiae*, Group B streptococcus (GBS), infection in previous infant, GBS bacteruria in the present pregnancy.

Late-onset sepsis (LOS) presents thereafter, with an outer limit of 30 or 90 days in various reports (Scharg, 2005). Late-onset infections may be acquired intrapartum, through horizontal spread within the hospital setting, or from maternal or other sources in the home or community.

The specific risk factors associated with LOS are:

- Prolonged hospitalisation e.g. preterm infants in a NICU
- Presence of foreign bodies e.g. intravenous catheters, endotracheal tubes, etc
- Cross infection by staff and parents.

The characteristics of neonatal infections are complex and their occurrence is a result of a number of factors:- 1-4 (Gotoff, 2000).

1) Transmission of infectious agents from mother to fetus or newborn infant may occur at various times. Transplacental hematogenous spread may occur at different times during gestation, with manifestations present at birth or delayed for months or years. Vertical transmission of infection from mother to infant may take place in utero, just before delivery, or during the process of delivery. After birth, neonates may be exposed to infectious diseases in the nursery or in the community. With the advances in neonatal intensive care, gestationally younger and lower birthweight newborns are surviving and remaining for a longer time in an environment with a higher risk of infection.

2) Newborn infants may be less capable of responding to infection due to one or more immunologic deficiencies involving the reticuloendothelial system, complement, polymorphonuclear leukocytes, cytokines, antibody, or cell mediated immunity.

3) Coexisting disease of newborns often complicate the diagnosis and management of neonatal infections. Respiratory disorders such as hyaline membrane disease

may coexist with bacterial pneumonia. Acidosis impairs functions of polymorphonuclear leukocytes.

4) The extremely variable manifestations of infectious disease in newborn infants include subclinical infection, congenital malformations resulting from infection in the first trimester present at birth or appearing later in life and mild to severe manifestation or systemic infection. Moreover the timing of exposure in utero, inoculum size, immune status, and the aetiologic agent all can interplay and influence the expression of disease in a newborn infant.

1.1 AETIOLOGY OF NEONATAL INFECTIONS

The spectrum of aetiologic agents causing neonatal infections is evolving. Over the past 7 decades, there have been several shifts in the predominant bacterial organisms responsible for neonatal septicaemia as illustrated by the database of the Yale-New Haven Hospital (Bizarro, et al., 2005). This centre has maintained the longest running, single-centre, longitudinal database of neonatal sepsis, started in 1928 and is updated periodically. As illustrated in Tables 1 and 2 it is useful to identify longitudinal trends in pathogens causing neonatal sepsis.

In the 1930s, the group A hemolytic streptococci were the most frequent cause of perinatal infections, which came under control with the introduction of penicillin (Baley, 2001). In the 1940s, the incidence of gram-negative infections, particularly *Escherichia coli*, increased and replaced the streptococci as the most common cause of infection, but by the

1950s, the penicillinase-producing staphylococci (*Staphylococcus aureus*) became predominant (Baley, 2001). As an understanding of neonatal colonization developed, new cord and skin care practices evolved, which helped to bring staphylococcal infections under control. The Gram-negative infections again became prominent in the 1960s, giving way to the GBS in the 1970s. At the same time, since the 1980s, nosocomial infections have become predominant in the intensive care and nursery. These are more likely to be *Staphylococcus epidermidis* and other gram-positive organisms. In the 1990s *E. coli* and *S. aureus* seem to be the recurring organisms.

Table 1: Pathogens Most Frequently Associated with Sepsis Neonatorum at Yale-Newhaven Hospital from 1928-1988 (Siegel, 1990)

Years	Most frequent organism	Other organisms
1928-1932	ß streptococcus	*Staphylococcus aureus, E. coli*
1933-1943	Group A streptococcus	*E. coli*
1944-1957	*E. coli*	*Pseudomonas aeruginosa*
1958-1965	*E. coli (S. aureus*)*	*Pseudomonas* spp., *Klebsiella, Enterobacter*
1966-1978	Group B streptococcus	*E. coli, Klebsiella-Enterobacter*
1979-1988	Group B streptococcus, *E .coli*	Coagulase-negative staphylococci, MRSA*, enterococcus, Candida
Nosocomial outbreaks in some nurseries.		
+Methicillin-resistant *S. aureus*		

Table 2: Organisms responsible for Neonatal Sepsis at Yale-New haven Hospital from 1928–2003 (Bizzarro, et al., 2005)

	% in Each Study (totals not equal to 100%)						
	1928–1932	1933–1943	1944–1957	1958–1965	1966–1978	1979–1988	1989-2003
Gram-positive aerobic bacteria							
S. aureus	28	9	13	3	5	3	8
Coagulase-negative staphylococcus				1	1	8	29
ß-hemolytic streptococci							
Group B		5	6	1	32	37	12
Group D			2	10	4	8	9
Nongrouped and other	38	36	10				
Viridans streptococci		2		3	1	3	1
S. pneumoniae	5	11	5	3	1	1	
Listeria monocytogenes		2	2		1	1	<1
Gram-negative aerobic bacteria							
E coli	26	25	37	45	32	20	11
Klebsiella-Enterobacter spp				11	12	3	11
Pseudomonas	3		21	15	2	3	3
Haemophilus				1	4	5	1
Salmonella			2		1	1	
Gram-negative anaerobic bacteria					1	3	
Fungi					2	1	8
Other		9	3	5	5	1	6
n	39	44	62	73	239	147	520

The changing distribution of etiological agents not only varies over time but also from one geographic area to another and this reinforces the need for local microbiological surveillance. The bacterial causes of neonatal sepsis in the developed countries of Western Europe and United States are similar, but these differ from countries in the developing world, such as Saudi Arabia, Panama and countries in Africa. A literature search for studies of neonatal sepsis is depicted in tables 3 and 4 and illustrates this geographical variation.

Table 3: Causes of bacterial sepsis in newborns from three different areas of the world. (Saez-Llorens, et al., 2004)

	Number and Percentage of Isolates		
	Dallas (U.S) (1969-1989)	Mallorca (Spain) (1977-1991)	Panama (Panama) (1975-1992)
Organisms	n=744	n=332	n=577
Gram-negative	229 (30%)	151 (45%)	361 (63%)
Escherichia coli	17%	10%	1
Klebsiella spp	7%	15%	20%
Other enteric rods	3%	10%	20%
Pseudomonas spp	2%	6%	6%
Haemophilus influenzae	1%	1%	<1%
Others	<1%	3%	2%
Gram-positive	515 (70%)	181 (55%)	216 (37%)
Group B Streptococcus	37%	22%	2%
Coagulase-negative staphylococci	9%	15%	20%
S. aureus	12%	8%	12%
Enterococci	10%	7%	1%
Listeria monocytogenes	1%	2%	<1%
S. pneumoniae	<1%	<1%	<1
Others	<1%	<1%	<1%

Table 4: Selective studies of neonatal sepsis

Study Location and year	Predominant organisms		Incidence	Comment
	Early Onet	Late onset		
1976-1980 Finland (Vesikari, et al. 1985)	GBS, *E. coli* *S. aureus*	*S. aureus* GBS. *E. coli*	3/1000	3 categories of patients
1979 – 1982 London (Placzek, et al.,1983)	*E. coli* GBS	*S .epidermidis* *S. aureus*	Not available	
1983 – 1988 Riyadh (Dawodu, et al., 1997)	*S. epidermidis* *Enterobacteriaceae*	*Enterbacteriaceae* *P. aeruginosa* *S. epidermidis*	4.9/1000	
1991–1992 Australia (Isaacs, et al., 1995)	GBS, *E. coli*	CoNS	2.2-4.4/1000 for EOS and LOS	
1994-1995 Nigeria (Ako-Nai,et al., 1999)	*S. aureus, klebsiella* *Pseudomonas*		Not available	No separation of EOS/LOS
1996-2001 Malawi (Milledge, et al., 2005)	GBS, *S aureus*	GBS, Non- typhoidal Salmonelli, *S. aureus,*	Not available	Defined EOS 0-7 days LOS 8-3days
1994-2000 Spain (Lopez, et al., 2003)	GBS	*E. coli.* GBS *K. pneumoniae*	Not available	

Currently, the usual pathogens responsible for early-onset disease include GBS, *E. coli* and other gram-negative bacilli, *Listeria monocytogenes,* and enterococci (Guerina, 1998). The three organisms that account for approximately 65 to 70 percent of all systemic neonatal bacterial disease are GBS, *E. coli* and *L. monocytogenes* (Seaz-Llorens, et al., 2004). The latter is due to contaminated food products, is isolated sporadically and occasionally in point-source epidemic form (Gerdes, 2004).

LOS can be caused by some of the organisms associated with early-onset disease, such as GBS and *L. monocytogenes,* in which case meningitis is the usual clinical manifestation. Nosocomially acquired bacteria account for the larger number of the late-onset infections. Thus, *S. aureus,* CoNS, *Pseudomonas aeruginosa,* and other gram-negative bacilli, such as *E. coli, Klebsiella pneumoniae, Enterobacter cloacae and Salmonella* spp, are the usual causative organisms (Speck, 1986; Isaacs, et al.,1999b).

Although accounting for only a small proportion of neonatal sepsis, *Enterococcus* species deserve special mention because of the increasing incidence of neonatal enterococcal sepsis in several studies (Dobson and Baker 1990, McNelley, et al., 1996) and the emergence of vancomycin resistance among enterococci (McNelley, et al., 1998). Both *Enterococcus fecalis* and *E. faecium* cause sepsis in neonates, with *E. fecalis* accounting for over 80% of cases (Kaufman, 2004). A study by Luginbuhl (1987) identified that bowel resection, non-umbilical central line placement and duration of that placement were factors associated with enterococcal sepsis. In addition it was found by Dobson and Baker (1990) that neonates <1500g were particularly prone to this infection and focal infections notably scalp abscesses were more common. The latter investigators also noted that the complications of

continued bacteraemia, and particularly meningitis make it imperative that central catheters are removed and foci of infection and abscesses are promptly and adequately drained.

A variety of other bacteria have been isolated from neonates with sepsis. Of these, *Citrobacter diversus* is an uncommon neonatal pathogen that deserves mention. It may be isolated as part of the adult intenstinal flora and can be vertically transmitted intrapartum from colonized mothers or by nosocomial spread. Species of streptococci other than GBS are infrequent agents in EOS in premature neonates and even less common in LOS.

In addition to aerobic organisms, anaerobic bacteria are recognized as causative agents of neonatal septicaemia. Four categories of anaerobic infection have been identified: transient bacteremia following premature rupture of membranes and maternal amnionitis, neonatal sepsis following postoperative complications, fulminant septicemia in the case of clostridial infection (Harris and Polin, 1983). The overall incidence of anaerobic infection is estimated to be 1.8 per 1000 live births (vs. 1-10 per 1000 live births for aerobic infections). Although the clinical presentation of infants with anaerobic infection is similar to that of other septic neonates, the overall mortality is significantly lower (4 %) (Harris and Polin, 1983).

There has also been a concomitant increase in the rate of fungal sepsis or candidaemia of up to 2.6% to 10% especially in premature neonates (Makhoul,et al., 2001). In the 1980s, *Candida albicans* was the leading causative organism in, but the rate of non-albicans *Candida* has recently increased (Fridkin, et al., 2006). Known risk factors for candidaemia include prematurity especially in VLBW neonates (Kaufman, 2004), the use of broad-spectrum antibacterial medications, notably third generation cephalosporins (Benjamin, et

al., 2000), prolonged mechanical ventilation, catheterization of central and peripheral vessels, prolonged parenteral nutrition, prolonged hospitalization in a neonatal intensive care unit, and glucocorticosteroid therapy (Saiman, et al., 2001; Benjamin, 2000). The immaturity of the neonatal immune defence system enhances Candidal proliferation and invasion. Candidaemia is a serious complication in the modern NICU, which can cause severe morbidity, mandates extensive laboratory and imaging workup for ruling out fungal dissemination, and requires prolonged antifungal therapy and hospitalization, with close follow-up and management of complications of both the fungal infection as well as the antifungal therapy.

Overall, microbiological surveillance is important to identify new pathogens, to recognize outbreaks, to monitor seasonality and geographic distribution and importantly to guide clinicians for starting empiric therapy.

1.2 EPIDEMIOLOGY OF SPECIFIC NEONATAL INFECTIONS

Knowledge of the epidemiology of neonatal sepsis is based on studies of the most common organisms causing EOS and LOS currently the GBS, followed by the enteric microorganisms and nosocomial pathogens.

1.2.1 Group B Streptococcal infections

Epidemiologic studies have shown the worldwide prevalence of GBS genital colonization among pregnant women varies from 5% to 30%, but GBS are reported more frequently as a cause of neonatal sepsis in developed countries than in developing areas (Saez –Llorens, 2004, WHO, 1999a).

Group B streptococcal disease can be caused by any type I to VIII. Vertical transmission from mother to infant occurs in 40% to 70% of women colonized with this organism. Infants born to heavily colonized women are more likely to harbor the organism, frequently at multiple sites, than are those born to lightly colonized women. Some mothers of infants who are infected with GBS are at high risk of having babies in the future who are infected similarly. A low titre of serum antibodies to the type of infecting GBS and persistence of the organism in the mother has been demonstrated. Although intrapartum mother-to-infant transfer is the initial mode of acquisition of GBS for the newborn, it is not the sole way in which the baby becomes colonized. In a Houston nursery, infant colonization rates increased from 20% – 25% at 1 day of age to 60% to 65% at 3 to 5 days of age without a concomitant increase in parturient colonization rates (Parades, et al., 1977). Thus nosocomial spread of organisms probably from the hands of nursery personnel occurs. The major sites of colonization in infants are the skin, nasopharynx, and rectum. The GBS persists in the nasophyarynx for weeks to months, whereas its cutaneous location usualty is lost by several weeks of age. For every 100 infants colonized with GBS, an estimated one or two infants will develop disease caused by this organism. Almost exclusively the type III organism causes meningitis. Clusters of three or four cases of GBS meningitis have occurred in nurseries during short intervals, suggesting nosocomial acquisition.

1.2.2 Gram Negative infections

E. coli is another common agent implicated in neonatal bacterial disease, with an annual incidence of approximately 1 case per 1000 live births. The *Escherichia* genus is antigenically complex, comprising at least 160 somatic (0), 100 capsular (K), and 50 flagellar (H) antigens. The epidemiology of this agent in relation to newborn infection was

defined more clearly with the discovery of the association between the K1 capsular polysaccharide antigen and invasive disease. Strains with K1 antigen are responsible for approximately 75 percent of neonatal meningitis cases caused by *E. coli* and 40 percent of sepsis cases. K1 strains are associated with more severe disease than are non-K1 strains. Animal studies have demonstrated that *E. coli* strains with K1 are highly virulent for mice with the minute amounts of specific K1 antibody (Robbins, 1974). The highest prevalence rates for rectal colonization with *E. coli* K1 strains are found in pregnant and non-pregnant women who are 16 to 31 years of age. [Approximately 45% to 50% percent of this population has K1 organisms on rectal culture. Studies of paediatric population have disclosed colonization rates of 20-30% for newborns on the second day of life, 40% for infants 4 weeks to 1 year of age, and 35% for children 1 to 16 years of age. As expected, the organism is dispersed widely among hospital personnel, who have rectal carriage rates of approximately 40%] (Saez-Llorens, et al., 2004). Vertical (mother-to-infant) and horizontal (nursery staff-to-infant, infant-to-infant) modes of transmission have been documented for *E. coli* K1 infections. Approximately 70% of infants born to culture-positive women acquire *E. coli* K1 strains during the first 48 hours of life, in these instances of vertical transmission, serologic concordance exists for O and H types of the *E. coli* cultured from mother and baby. Approximately 10% to 15% of infants colonized with K1 strains are born to K1-negative mothers. For this group of babies, *E. coli* is acquired at a later age (3 to 4 days), presumably from horizontal transmission. Vertical acquisition of K1 organisms has been documented in approximately three fourths of neonates with *E. coli* K1 meningitis.

1.2.3 Other Gram Positive infections

1.2.3.1 *S. aureus* infections

Since the 1980s, epidemic and endemic colonization and disease of the newborn infant with methicillin-resistant *Staphylococcus aureus* (MRSA) have been reported with increasing frequency in the United States and Europe. Risk factors associated with the development of MRSA infections include lengthy hospitalization, previous antibiotic administration, overcrowding and understaffing, and the presence of predisposing factors, such as indwelling central venous catheters, cerebrospinal shunts, mechanical ventilation, and prematurity. Potential reservoirs of MRSA in the hospital environment include colonized or infected neonates, hospital personnel, and the hospital inanimate environment. Although chronic nasal carriage of MRSA by hospital personnel has been implicated in several hospital outbreaks, it generally is an uncommon event and is not necessary for initiation or propagation of hospital outbreaks. Limited data suggest that the hospital inanimate environment may become contaminated with MRSA, possibly sustaining outbreaks of infection. Colonized patients without clinical disease contribute substantially to the inpatient reservoir of MRSA. In the past decade new isolates of MRSA that emerge in the community i.e. Community- acquired MRSA as opposed to nosocomial MRSA have emerged and are responsible for causing suppurative skin infections and severe necrotizing pneumonias and awareness of these isolates is vital for all health care clinicians (Jeyaratnam, et al., 2006).

1.2.3.2 Coagulase-negative staphylococcal infections

Coagulase-negative staphylococci have been recognized as neonatal pathogens. They are the most common species of the normal flora on the skin, nasal mucosa, and umbilicus of the newborn. Of the 31 species of CoNS 13 are known to colonize human skin. Species reported to cause disease in infants include *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and others. The major species involved in neonatal infection is *S. epidermidis*, which accounts

for approximately 50 to 80% of CoNS colonization and 60 to 93% of CoNS bloodstream infection (Kaufman, 2004).

With sensitive culture techniques, high colonization rates with CoNS of the nose, umbilicus, gastrointestinal tract, and cutaneous areas of the neonates have been found (D' Angio et al., 1989). The ubiquitous presence of the organisms and their tolerance to drying and temperature changes contribute to the increased presence of CoNS in neonates. Some factors that account for CoNS becoming important nosocomial pathogens are: prematurity, high rates of colonization, and aggressive treatment of the newborn infants in intensive care units (e.g. with the placement of umbilical catheters, central venous catheters, intravenous parenteral nutrition, mechanical ventilation) (St Geme etal., 1991).

It is unclear whether there has actually been an increase in the incidence of CoNS bacteramia, or the realization that positive blood cultures represent true infection, not culture contamination. Coagulase-negative staphylococci can account for more than 50% of bacteremia in the NICU. Resistance to antibiotics such as penicillin, semisynthetic penicillins, and gentamicin is common among clinical isolates of CoNS.

Despite plausible evidence of their increasing prevalence as neonatal pathogens, distinguishing between infection and contamination of blood cultures by these organisms often is difficult. Ideally, therefore, to distinguish true infection from blood culture contamination, the evaluation for sepsis in the newborn infant should involve collection of multiple blood cultures prior to the institution of antibiotics. In the critically ill neonate, it may not always be possible to postpone the initiation of antimicrobial therapy until multiple cultures are obtained. In this situation, additional clinical and laboratory criteria

may be needed to support the diagnosis of neonatal infection with CoNS. Schmidt et al (1987) recently demonstrated that clinical signs are more frequently abnormal in infants infected with CoNS in comparison with those whose blood cultures are negative or uninfected controls. Therefore, ones threshold for obtaining a blood culture should be low in any hospitalized premature infant who exhibits a change in behaviour.

Once faced with a positive systemic culture for CoNS, the clinician must decide whether this isolate represents contamination or true disease. Several authors have examined microbiologic methods to distinguish pathogenic from contaminant isolates. In patients with prosthetic devices, Davenport et al. (1986) demonstrated that 81% of significant infections were caused by slime-positive isolates whereas 50% of contaminants produced slime. Dunne et al. (1987) reported similar results in paediatric patients. These studies suggest that species identification and knowledge of its ability to produce slime may be helpful in evaluating the clinical significance of CoNS isolates.

St. Geme et al. (1990) investigated the value of quantitative cultures to distinguish sepsis from contamination in young infants with peripheral blood cultures growing CoNS. In that study, peripheral blood cultures from newborn infants that grew greater than 50 colony-forming units per millilitre (cfu/ml) occurred exclusively in infants with proven sepsis. Infants with sepsis, including those with colony counts <50 cfu/ml, were significantly more likely to have a central catheter or an abnormal haematologic value (white blood cell count) or both. Infants who lacked these clinical features were more likely to have contamination. Although this study was partially retrospective in its design, it suggests that quantitative blood cultures in conjunction with specific clinical information may distinguish sepsis from culture contamination with CoNs in newborn infants.

In addition, Christensen et al. (1990) recently demonstrated that staphylococcal species possess a high frequency of phase variation with regard to slime production, antibiotic resistance and virulence. This reversible expression of phenotypic characteristics within strains may explain partially the conflicting results obtained in previous studies. Clarification of the role of these proposed virulence factors would require further intensive investigation.

Unfortunately, there is disagreement in the literature regarding the value of the above factors as markers for significant infection. Clinical assessment should aid the clinician about the significance of this organism.

1.2.4 Nosocomial infections

Nosocomial bacterial infections are significant problems for the neonate requiring extended care in the special care nursery. This is particularly true for very-low birth-weight infants. The overall incidence of nosocomial infections in neonates is difficult to quantify because rates of infection vary depending on the prevalence of risk factors (Nelson, 1990). Beyond the first 1 to 2 weeks of life, the neonate who has remained in the special care nursery is likely to be colonized with perinatally (endogenous) and nosocomialy acquired flora. This places the neonate at risk of infection due to CoNS; enterococci; *S. aureus*, including methacillin-resistant strains, and gram-negative bacteria, including multiply resistant enteric strains. In addition, late-onset disease caused by GBS and *L. monocytogenes* must be considered. The most frequently identified factors contributing to nosocomial infections are postnatal age, length of stay in nursery, low birth weight, foreign bodies (e.g., intravascular catheters, chest tubes, endotracheal tubes), nursery crowding, surgery and prolonged treatment with broad-spectrum antibiotics. Strict

isolation procedures should be enforced for all infants colonized or infected with multiply resistant bacteria.

1.3 PATHOGENESIS AND RISK FACTORS OF NEONATAL INFECTIONS

Risk factors for the development of invasive bacterial disease may be classified into four general categories. The presence of any of these risk factors is associated with an increased risk of developing systemic infection.

The major risk factors for neonatal sepsis are listed below and summarized in table 6.

1.3.1 Maternal risk factors

1.3.1.1 Prolonged rupture of membranes

Once the membranes have been ruptured for >18 hours, the risk of sepsis in the neonate increases approximately 10 fold over baseline, to a rate of 1% for proven and 2% for suspected sepsis. The risk of proven sepsis in the preterm infant increases 4% - 6% if there is PROM in the mum. A 5 minute Apgar score <6 also raises the sepsis risk to 3% - 4% (St Geme, 1984; Gerdes, 2004).

1.3.1.2 Chorioamnionitis

Chorioamnionitis is generally defined as the presence of maternal fever > 38 C (100.4°F) with two or more of the following findings: fetal tachycardia, uterine tenderness, foul vaginal discharge, or maternal leukocytosis. The range of neonatal sepsis when chorioamnionitis is present is 3% - 20% (Benitz, 1999).

1.3.1.3 Maternal colonization with GBS

Maternal colonization with GBS carries a neonatal sepsis risk of 1% (Baker, 1977); the risk rises to 4% - 7% in the presence of clinical complications such as PROM, maternal fever, or prematurity (Boyer,1983); and as high as 20% in the presence of chorioamnionitis (Guerina, 1998; Benitz,1999). GBS bacteruria and having a twin with GBS disease also raises the neonatal risk. Although specific data is lacking, there is anecdotal evidence that the risk of GBS sepsis is also higher in pregnancies subsequent to one in which the neonate developed GBS sepsis. Further, there is an additive risk in the presence of multiple risk factors (Tables 5 and 6).

1.3.1.4 Maternal urinary tract infection

Urinary tract infection of any cause including GBS as stated above raises the risk of sepsis in the neonate, in part due to raising the risk of prematurity and chorioamnionitis.

1.3.1.5 Other

Socioeconomic factors appear to be important in determining whether infants are at risk of developing infection. Premature infants and infants with low birth weight are born more frequently to mothers of low socioeconomic class than those of average or high socioeconomic class.

Table 5: Summary of very high risk for early-onset GBS sepsis (Gerdes, 2004)

Maternal GBS colonization plus:	Attack rate:
PPROM (preterm)	35%-50%
Chorioamnionitis	6%-20%
Maternal GBS bacteruria	8%
Twin with GBS disease	40%
Previous sibling with GBS	Anecdotal increase in risk

Table 6: Risk factors for neonatal sepsis (Gerdes, 2004)

Condition	Incidence of proven sepsis
PROM >18 hours	1%
Maternal + GBS (preprophylaxis era)	0.5%-1%
Maternal + GBS (in prophylaxis era)	0.2%-0.4%
Maternal + GBS and PROM, fever or preterm	4%-7%
Chorioamnionitis	3%-8%
+GBS and chorioamnionitis	6%-20%
PROM + preterm	4%-6%
PROM + low Apgars score	3%-4%

1.3.2 Neonatal risk factors

1.3.2.1 Birth weight and gestational age

The most important risk factor for neonatal sepsis is low birth weight, (Guerina, 1998) with the rate of neonatal infection inversely related to birth weight and gestational age. Stoll et al. (2002b) showed that in as many as 25% of very low birth weight infants surviving beyond 3 days of life, one or more episodes of blood culture-proven sepsis developed and that these infants were significantly more likely to die (18% mortality rate) than uninfected infants of the same weight (7% mortality rate).

1.3.2.2 Twin pregnancies

Twin pregnancy remains an independent risk factor for acquisition of GBS infection and other organisms after correction for low birth weight. The first-born twin is at a higher risk of contracting ascending intrauterine infection than is the second-born. The basis for increased risk of acquisition of infection in twins includes the common features of virulent organisms, absence of protective antibody and similar genetic heritage.

1.3.2.3 Sex differences

Although no noticeable sex prediction has been observed for infants with intrauterine infections a male predominance is reported in almost all studies. The greater susceptibility of male infants is more evident in cases of sepsis caused by gram-negative enteric bacilli. The reasons behind this male predominance are unknown but may be related to sex-linked factors in host susceptibility.

1.3.2.4 Immune factors

Neonates have qualitative and quantitative deficiencies of both cellular and humoral immunity compared to that of the older child or adult, and this difference predisposes them to infections of a wide variety of pathogens. The deficiencies in the neonatal immune system are highlighted in table 7. (Table 7 modified from Orlando Regional Healthcare, Education & Development, 2004). In summary, the defective antibody response and the abnormalities in circulating immunoglobulins fail to protect against *Enterobacteriaceae* especially *E. coli*. Deficiencies in complement contribute to diminished complement-derived chemotactic activity and to diminished ability to opsonize certain organisms in the absence of antibody. Neutrophils are much more likely to be depleted in neonatal infections; this depletion is the major host factor contributing to poor outcome in bacterial sepsis. The impaired chemotaxis of monocytes affects the inflammatory response in tissues and the results of delayed hypersensitivity skin tests.

Because of the interdependence of the immune response, these individual deficiencies of the various components of immune activity in the neonate conspire to create a hazardous situation for the neonate exposed to infectious threats.

Table 7: The Immune Response in Full and Preterm Infants

Immune System Component	Full Term Infant	Preterm Infant
Immunoglobulin G	Complete placental transfer, concentrations comparable to mother	Incomplete placental transfer, concentrations decreased proportional to gestational age
Lymphocytes	Concentrations of T and B cells comparable to those in adults with normal response to antigens	Concentrations of T and B cells comparable to those in adults with normal response to antigens
Complement	50%-75% of concentration in adult	Decreased concentration
Neutrophils	Elevated numbers at birth, with impaired functional ability; i.e. decreased adherence and aggregation	Elevated numbers at birth, with impaired functional ability; i.e. decreased adherence and aggregation
Monocytes	Normal number at birth but have impaired chemotaxis	Elevated number at birth but have impaired chemotaxis
Macrophages	Normal number at birth but decreased function	Normal number at birth but decreased function
Natural Killer Cells	Concentration similar to adult level, but have diminished cytotoxic effects	Concentration similar to adult level, but have diminished cytotoxic effects
Cytokines	Decreased synthesis of interferon	Decreased synthesis of interferon, interleukin 1 and8
Physical and chemical barriers	Functionally deficient. Trauma from interventions that breach skin	Functionally deficient Immature skin, thin keratin layer. Trauma from interventions. Decreased gastric acid production

1.3.2.5 Nutritional factors

Nutritional history has been shown to be important in its relation to infection. Infants on parenteral hyperalimenatation, those with delayed initiation of enteral feedings and those with a prolonged period to reach full enteral feeding or to regain their birth weight are all at substantially increased risk of late-onset sepsis (Stoll, 1996). Weese-Mayer et al. (1987) demonstrated a 13% to 30% chance of developing a fungal infection if on total parenteral nutrition than if not. And Stoll et al. (2002b) showed that the risk of LOS increased as the duration of parenteral hyperalimemtation increased. These data suggest that efforts to initiate enteral feeding as early as possible and to reduce the number of catheter days and days on parenteral hyperalimentation/intralipids would not only improve nutritional status but also decrease the risk of infection.

1.3.2.6 Miscellaneous factors in sepsis

Perinatal asphyxia in the presence of PROM and not readily explained by an obstetric cause such as placental abruption raises the risk of neonatal sepsis (Gerdes 1991); the reasons for this finding are unknown.

Another commonly accepted risk factor is the presence of a foul smell to the amniotic fluid, or "a smelly baby". It is thought that this sign may be due to the presence of anaerobic bacteria, but there is no evidence that this finding constitutes an independent risk factor for sepsis.

Infants with galactosemia particularly are susceptible to developing sepsis by gram-negative enteric bacilli. *E. coli* is by far the organism most commonly encountered as a cause of sepsis and meningitis in these infants.

1.3.3 Environmental Factors

All newborns become contaminated with vaginal flora during labor and delivery except when delivery follows cesarean section. Under normal circumstances, the skin and mucous membranes of the neonate permit postnatal colonization and resist bacterial invasion. Infection takes place when a compromised newborn (asphyxia, prematurity, etc.) is heavily colonized with virulent microorganisms (group B streptococci) for extended periods of time (premature rupture of membranes) and when alterations of the normal mucocutaneous barriers of the newborn are compromised (congenital anomalies, fetal monitoring, obstetric manipulation, and vigorous resuscitative efforts), thereby favouring tissue invasion.

Following delivery, the newborn infant is transported from the microbial world of the mother to the hostile microbial world of the nursery or intensive care unit. Bacterial contamination with nursery pathogens begins after contact with the contaminated and/or colonized hands of nursing attendants. Accordingly, within 3 days the anterior nares, throat, and skin of the neonate are colonized with gram-positive microorganism (alpha-hemolytic streptococci, *S. aureus*, and *S. epidermidis)*, within 1week anaerobic microorganisms and the *Enterobacteriaceae* family colonizes the gastrointestinal tract. The bacterial flora of infants maintained in an intensive care unit differs from that observed in the normal newborn infant. Overcrowding and poor hand washing in the NICU, as well as contaminated equipment all add to the risk for the infant. Prolonged endotracheal intubation and central catheters provides a portal of entry for microorganisms through an intact skin barrier. More specifically, the skin, respiratory tracts, and gastrointestinal tract of intensive care infants, many of whom receive treatment with broad-spectrum antibiotics, are colonized with multiple drug-resistant gram-positive (e.g. *S. epidermidis*) and gram-

negative (e.g. *E. coli, Klebsiella* spp., *Pseudomonas* spp.) microorganisms. Opportunities for bacterial invasion and resultant infection increase in direct proportion to the number of invasive diagnostic and therapeutic procedures performed during hospitalization.

1.3.4 Microbial virulence

Of the various microbial virulence factors, the polysaccharide capsule has been studied most thoroughly. In infants, mortality and long term sequelae have been increased in cases of meningitis caused by *E. coli* K1 strains compared with those caused by non K1 strains. K1 capsular polysaccharide has been detected in cerebrospinal fluid (CSF) by counterimmunoelectrophoresis in higher concentrations and for longer durations in patients who died or were impaired neurologically compared with those who were normal survivors. Concentrations and persistence of K1 capsular polysaccharide in the CSF of neonates with *E. coli* meningitis have been correlated with concentration and persistence of endotoxin and interleukin 1B in CSF (McCracken et al.,1989). *E. coli* strains also have been found to resist phagocytosis by normal adult polymorphonuclear leukocytes, resulting in delayed clearance of bacteria from the bloodstream. This delay allows the organism to multiply and achieve the concentration of 1000 colony-forming units per milliliter of blood or more, an inoculum that generally is considered essential for invasion of the meninges (Saez-Llorens, et al., 2004). The presence of K1 antigen alone, however, does not appear to account fully for an organism's virulence. Because clones of pyelonephritis strains in which specific K types are associated with O antigens, pili, and an alpha haemolysin have been identified, the virulence of the organism appears to be multifactorial, and certain infections, specific K types, in conjunction with other bacterial properties determine pathogenicity.

Studies in children and adults have demonstrated clearly that protection from disease caused by bacteria (i.e. *Haemophilus influenzae* type b, *Neisseria meningitidis* and *Streptococcus pneumoniae)* possessing polysaccharide capsules is afforded by specific antibody directed against these structures. Resistance to bloodstream clearance probably relates in part to relative complement resistance of the encapsulated organisms. The capsule may protect the deep somatic antigen structures capable of activating the alternative complement pathway. Opsonization is essential for phagocytosis and intracellular killing of these organisms and depend primary on anticapsular antibody. The lack of type –specific maternal opsonising antibody is a significant risk factor for the development of systemic disease caused by group B streptococcal organisms in the mother and infant. Most pregnant women colonized with GBS organisms have increased concentrations of antibody in the circulation that pass transplacentally to the fetus. That specific B type antibody protects the baby against invasive disease, compared to infants born to mothers with undetectable concentrations of antibody who are susceptible to invasion by the group B streptococci.

The increased prevalence of CoNS is related to its preference for the plastic mediums found in cannulas and shunts, which increases its introduction via umbilical catheters and other indwelling lines. The bacterial capsule polysaccharide adheres well to the plastic polymers of the catheters. The adherence creates a capsule between microbe and catheter, which prevents C3 deposition and phagocytosis. Also, proteins found in the organism [AtlE and SSP-1] enhance attachment to the surface of the catheter. Biofilms are formed at the site from the aggregation of organisms that have multiplied with the protection provided by the adherence to the catheter. (St Geme, et al., 1991). Slimes are produced at the site from the extracellular material formed by the organism, which provides a barrier to

the host defense, as well as antibiotic action. Therefore, it can be seen that slime production increases the difficulty to treat coagulase-negative staphylococcal septicemia.

1.4 CLINICAL MANIFESTATIONS OF NEONATAL INFECTIONS

There is a marked variability in the symptomatic presentation of sepsis in neonates. Infection may be local on systemic: acute, sub acute or chronic. Most frequently the early signs of sepsis are subtle or minimal, but rapidly progressive. There may be temperature instability; respiratory symptoms; haemodynamic changes; abdominal distension; metabolic derangements; e.g. hyper-hypo glycaemia. Focal infections may manifest as conjunctivitis osteomyelitis, omphallitis before or during the sepsis episodes.

The signs are distinctly non-specific and may be associated with viral infections as with non-infectious disorders. Because the neonate is an impaired host, the clinical cause is unpredictable and usually rapidly progressive. The presence of any of the signs alone or in combination is an indication for complete evaluation to rule out sepsis.

The clinical signs of bacterial infections in the neonate are presented in Table.8

Table 8: Clinical Features of Neonatal Sepsis

General Poor Feeding Irritability Lethargy Temperature instability	**Gastrointestinal** Diarrhoea Hematochezia Abdominal distention
Respiratory Grunting Nasal flaring Intercostal retractions Tachypnoe / apnoea	**Hematopoeitic** Thrombocytopaenia Leukocytosis Leukopaenia
CNS Hypotonia Seizures Poor spontaneous movement	**Cardiovascular** Bradycardia/tachycardia Hypotension Cyanosis
Skin Petechiae Pustulosis Sclerema Hyperemia	

1.5 DIAGNOSING NEONATAL INFECTIONS

The early signs of sepsis in the newborn are non-specific; therefore, many newborns undergo diagnostic studies and the initiation of treatment before the diagnosis has been determined. Many episodes of suspected clinical sepsis in newborns are not confirmed through isolation of pathogens from blood cultures. Although some clinical episodes may have falsely negative cultures as a result of limitations in sample collection, processing, or the interference of antimicrobial agents, other culture- negative sepsis episodes may reflect an inflammatory response that mimics sepsis but without active bacterial involvement. (Schrag, 2005).

Thus documentation of infection is the first diagnostic criterion that must be met for sepsis. Specific diagnostic tests are performed to confirm the presence of a specific pathogen in body fluids. The recovery of an organism from a meaningful site, such as blood, CSF, urine, abscesses, substantiates the clinical impression of systemic bacterial disease. Nonspecific sepsis screen tests are used to evaluate the likelihood of infection.

1.5.1 Blood Cultures

1.5.2

Several sites for sampling blood for culture give reliable results: peripheral vein, umbilical artery and capillary blood. The preferred site is the peripheral vein venipuncture should be performed after the skin has been prepared properly by cleansing with an iodine-containing solution. The theoretical minimal amount of blood needed for detecting bacteremia is a function of the number of organisms circulating at any given time.

The problem with the blood culture in neonates is that the sensitivity for identifying sepsis is only 50% to 80% at best (Gerdes, 2004). Currently, the increase use of maternal antibiotics has further reduced the rate of positive blood culture in early-onset sepsis, as few as 2.7% of neonates with clinical sepsis may have positive blood cultures (Gerdes, 2004). In addition to the partial treatment from maternal antibiotics, bacteria may be transient in the early stages of disease, and the small blood volume typically taken from infants for culture may be insufficient to detect low bacterial-density sepsis in infants. In summary, a positive blood culture with a pathogenic organism is diagnostic of neonatal sepsis, however, a negative blood culture in no way rules out the presence of disease.

1.5.2 CSF cultures

Neonates with sepsis as the primary diagnosis should have a lumbar puncture. Not all infants with meningitis exhibit specific symptoms. Furthermore, up to 15% of neonates with positive CSF cultures may have negative blood cultures (Gerdes, 1991). CSF cultures are useful because CSF infection tends to have a high bacterial density and there are fewer interfering proteins than are found in blood.

1.5.3 Other tests

The difficulties in accurately identifying the septic neonate have prompted evaluation of many other adjunctive tests that may indicate infection, but which do not identify the inciting organism. The most frequently determined adjunctive test is the white blood cell count, differential count and related indices. In recent years, the search for diagnostic tests that may be useful in the diagnosis of neonatal sepsis and in following the course of the infection has turned to cytokines as well as to other substances associated with the inflammatory response, in some cases induced by cytokines, as possible indicators of infection.

Table 9 describes the selective diagnostic tests used in aiding diagnosis of neonatal sepsis.

Table 9: Selective diagnostic tests used in aiding diagnosis of neonatal sepsis

Diagnostic test	Comment
Bacterial Antigens	- Immunologic methods e.g. latex particle agglutination employed to detect bacterial antigens in urine, or CSF but is not sensitive. Used for GBS and *E. coli* infections.
Tracheal Cultures	- Useful when obtained within the first 12 hours of life and isolation of bacteria on tracheal aspirate and gram stain correlates with clinical or pathological pneumonia with or without bacteremia.
Urine cultures	- Urine cultures from bag specimens are unreliable and are open to contamination while sterile bladder tap or catheterization specimens minimize false-positive cultures but are difficult to obtain, have a low yield in the first 72 hours of life and are not suggested as part of the work-up for early onset disease.
C- Reactive Protein CRP	- Synthesized by the liver within 6 to 8 hours of inflammatory stimulus and infection is the most likely cause of inflammation in the neonate. - CRP concentrations decrease rapidly in infected neonates who respond to therapy and are persistently elevated in neonates whose infections fail to respond to therapy. - Serial determinations are helpful, especially in combination with other hematological tests when making a decision to stop antimicrobial therapy safely.

Diagnostic test	Comment
Blood Count	- The normal range for blood count indices is very broad, and dependent on the timing of the sample. - Neutropenia is the best predictor of sepsis, whereas neutrophilia does not correlate well.
Cytokines	- Interleukin (IL)-6 is the most intensively studied. - Procalcitonin (PCT) use probably limited in neonates
Other	- Radiographic studies, e.g. chest xrays for respiratory distress. Sonography, computed tomography, and magnetic resonance as indicated.

1.6 ANTIBIOTIC USE IN NEONATES

Selection of antibiotic therapy in newborn infants must depend on (1) the historical experience with infections in the nursery, and therefore determination of in vitro susceptibilities of frequently occurring pathogens in nurseries is critical to planning optimal therapy. (2) the susceptibility of commonly encountered bacterial pathogens, and (3) familiarity with antibiotic pharmacokinetics in neonates. It is important for paediatricians to know which organisms cause disease most commonly in the nursery and intensive care unit and the current antimicrobial susceptibilities of these organisms. Moreover, it has been shown in many instances that the judicious use of antibiotics in the nursery setting can limit the emergence and spread of resistant bacteria.

The changing physiologic processes characteristic of the neonatal period may affect the pharmacology of antimicrobial agents. Absorption, distribution, metabolism, and excretion of drugs depend in part on the maturity and age of the infant. The dosage and frequency of administration schedule must be determined for infants of different gestational and chronological ages and cannot be extrapolated from studies of normal adults. Failure to take these physiologic and metabolic changes into account when administering antibiotics to neonates may result in either ineffective or toxic drug dosages.

The principal goal of antimicrobial therapy is to cure infection, to avoid persistence of bacteria in body fluids that is directly correlated with an increased incidence of complications during therapy and of neurologic sequelae when meningitis is present.
The treatment of infants with suspected sepsis or meningitis should begin immediately after blood, cerebrospinal fluid, and urine specimens are obtained for microscopy culture and sensitivity. Lumbar puncture should be postponed in the unstable infant (thrombocytopenia or cardiovascular instability); however, this should not delay initiation of therapy.

After culture and susceptibility testing are completed, treatment should be escalated or de-escalated accordingly. The duration of therapy will depend upon (1) the portal of entry and the organ system involved; (2) the initial response to treatment; and (3) the clinical status of the infant. The duration of therapy for documented septicemia without focal infection is 10 to 14 days. Gram-negative meningitis should be treated for a minimum of 21 days, and meningitis due to GBS or *L. monocytogenes* should be treated for 14-21 days.

Because late-onset sepsis is more heterogeneous in its epidemiology than is early-onset disease and may reflect maternal, family, community, or nosocomial sources for the infecting pathogen, the organisms involved cover a broad microbial spectrum. As a result, empirical antimicrobial regimens vary. In the previously healthy infant who already has been discharged from the hospital, ampicillin and an aminoglycoside are recommended unless meningitis or a staphylococcal infection is suspected, in which case a third generation cephalosporins or an antistaphylococcal agent (e.g. oxacillin, nafcillin) should replace ampicillin. Selection of empirical antibiotic regimens can be more difficult for a septic premature infant who has had a prolonged hospitalization, previous antibiotic administration, possible prolonged tracheal intubation, and placement of a central or peripheral intravascular catheter.

Considering the predominance of CoNS as the principal cause of LOS, vancomycin has become the principal agent for presumptive gram-positive bacterial coverage. Presumptive gram-negative coverage is provided with an aminoglycoside. The aminoglycoside of choice is usually gentamicin, but resistant organisms may be prevalent in many nurseries. An alternative aminoglycoside is amikacin, resistance to this antibiotic has been reported to be low, despite long-term use in the nursery. (Guerina, 1998). The final choice of aminoglycoside i.e. gentamicin, amikacin, tobramycin is guided by the prevalence of resistant strains within an individual nursery.

The frequent use of antibiotics in a neonate unit leads to an increased incidence of resistant strains (Gould, 1999). The resistant flora can colonize untreated infants in the nursery and, as mentioned above, the colonizing flora often constitutes the etiological organisms if nosocomial septicemia occurs. It has been shown how neonates, even after a

short period of antibiotic treatment, have an almost total suppression of anaerobic flora. This might be important for the decrease of the so-called colonization resistance (Bennet,1982).

In addition to appropriate antimicrobial therapy optimal supportive care is frequently required in septic neonates. This includes ventilatory support, careful fluid management, circulatory and blood product support and parenteral nutrition. Despite these measures mortality resulting from neonatal sepsis remains distressingly high. With the hope of improving survival and decreasing the severity of sequelae in survivors, some investigators have turned their attention to studies of adjunctive modes of treatment, which addresses themselves to demonstrated deficits in the host defenses of the infected neonate and utilize immunomodulatory agents such as haemopoietic growth factors and intravenous immunoglobulin. The results of large trials are awaited before these can be recommended (Yeung, 2005).

CHAPTER 2 METHODS

2.1 SETTING OF THE NEONATAL UNIT AT JOHANNESBURG HOSPITAL

The Neonatal Unit at Johannesburg Hospital admits approximately 1000 patients per year.

These neonates come from varied sources:

1) The Johannesburg Hospital maternity unit delivers about 7000 babies per year. Approximately 1900 neonates are assessed by the paediatric medical staff (registrars and medical officers) in the unit and admitted to the unit if required.

2) The Alexander Health Centre and University Clinic is situated 10km from JHB Hospital and delivers 3600 per year. Approximately 100 neonates per year are referred to the neonatal unit for assessment.

3) The primary and secondary referral units e.g. Midwife Obstetric Units and peripheral hospitals for which JHB hospital serves as the tertiary referral center, refers about 60 neonates per year.

The neonates are assessed in the labour ward nursery that has 6 beds and after initial resuscitation, stabilization are transferred as follows:

- Well neonates with birth weight ≥1800g are transferred to the postnatal wards in care of their mothers and the postnatal staff.

- Depending on the nature and severity of their pathology, babies with birth weight ≤1800g are admitted to the Neonatal Unit i.e.:

  -ICU – if they require ventilation/ inotropes;

  -Transitional care unit (TCU) if not ventilated but require specialized care;

  -Low care unit (LCU) if neonate is stable and requires monitoring for weight.

The ICU has 8 designated neonatal ventilators. The TCU has 20 beds. The LCU has 15 beds. The neonatal unit runs at an occupancy of 100% most of the time, and overflow patients being admitted to the labour ward nursery. Approximately 250 babies per year are transferred to the postnatal wards where they are still assessed by the paediatric neonatal medical staff.

2.2 ANTIMICROBIAL POLICY IN THE UNIT

- The first choice of antibiotics to be used on all neonates in the NU at Johannesburg hospital is penicillin and amikacin. These antibiotics are discontinued as soon as sepsis is ruled out (negative CRP at 24 hours with a clinically well baby or negative blood cultures at 72 hours if the CRP is rasied) or unless otherwise indicated; e.g. GBS meningitis.

- Neonates who have been in the unit and develop an episode of suspected sepsis are treated with tazocin and amikacin.

- The other antibiotics used are;

- Meropenem if there is suspected meningitis or if the neonate was previously treated with the above combinations;
- Vancomycin if a neonate is suspected of having significant infection with CoNS;
- Fluconazole if fungal infection is suspected.

2.3 AIMS OF THE STUDY

The study aims are:

- To document the incidence of culture proven sepsis amongst neonates admitted to the Neonatal Unit at Johannesburg Hospital.

- To analyse positive blood cultures in neonates at the Neonatal Unit and to document the causative organism of the septicaemia.

- To analyse sensitivity patterns of the bacterial isolates and to tailor appropriate antibiotic guidance.

- To document the outcome of the bacteraemia in infected neonates.

2.4 METHOD OF DATA COLLECTION

The study was conducted at the neonatal unit in the department of paediatrics at the Johannesburg Hospital. This was a retrospective analytical study. All neonates with positive blood cultures admitted to the neonatal unit between 1st July 2002 and 30th June 2003 were studied. Positive blood cultures in the neonatal unit were identified from the laboratory information system of the National Health Laboratory Service.

The blood culture was taken on admission or at any time thereafter at the discretion of the consulting doctor. The blood cultures were processed using the BacT/Alert automated system (Omnimed), and identification of organisms was performed as per standard operating procedure. Anaerobic culture techniques were not undertaken. Antimicrobial susceptibility was assessed by using the Kirby-Bauer disc diffusion assay method and interpreted using the Clinical and Laboratory Standards Institute (CLSI) criteria for disc susceptibility testing. Susceptibility tests were not routinely performed on fungal isolates but were performed if requested.

All patients admitted to the NU who had positive blood cultures were identified and recorded. This list of patients was cross-referenced with the admissions register in the neonatal unit to confirm the presence or absence of individual neonates. Patients who were transferred to the NU from the designated referral centers were included but neonates who were admitted from the wards after being discharged from the neonatal unit or neonates transferred from non-designated hospitals were excluded.

All neonates had a review of the medical records and laboratory results. The following data were obtained from the medical records:

1) Demographic profile: date of birth, birth weight, sex, inborn or out born, mode of delivery, gestational age, Apgars, age at presentation when blood culture was positive.

2) Clinical data: pathology, diagnosis, haemodynamics, invasive procedures and parenteral nutrition use.

3) Treatment data: antibiotics used, duration of treatment.

4) Outcome data: death or survived and discharged.

5) Deaths: cause of death.

6) Maternal data: signs of infection, PROM, antibiotic treatment.

7) Laboratory results analysed were FBC, differential counts, platelets, CRP, and other culture results e.g. urine, cerebrospinal fluid, tracheal aspirates if available

8) Antibiotic sensitivity and resistance data analysed.

All data were recorded on data capture sheets for analysis.

2.5 DEFINITION OF TERMS

Septicaemia

Septicaemia was defined as a systemic inflammatory response with bacterial or fungal organisms isolated from blood. CoNS were considered to be a significant cause of septicaemia when two blood cultures drawn within two days of each other grew CoNS or CoNS was isolated from one blood culture in association with clinical and/or laboratory signs of sepsis.

Clinical features of sepsis included at least one of the following: lethargy; respiratory distress; apnoea; bradycardia; cyanosis; temperature instability; feeding intolerance and/or abdominal distension; impaired perfusion and blood glucose instability. The clinical course of the baby was also noted.

Laboratory features of sepsis included an abnormal white cell count according to age-related values, a reduced platelet count or a C reactive protein> 10mg/l.

Contamination was defined as:

- A positive isolate for the following organisms: *Corynebacterium spp, Fusobacterium, Micrococcus spp, Propionibacterium* and *Bacillus* spp.
- A single positive culture for other organisms, especially CoNS, in a patient whose clinical condition improved without any laboratory features of sepsis.
- Positive growth on a blood cultures after 72 hours in a baby whose presenting symptoms had resolved and who had no laboratory features of sepsis.

Early onset sepsis (EOS) was defined as:

Was defined as a positive blood culture taken within the first 72 hours of life.

Late onset sepsis (LOS) was defined as:

Was defined as a positive blood culture taken after the first 72 hours of life.

Episode

A neonate was classified as having an episode of sepsis if he/she had a positive blood culture and was treated with antibiotics for 5 days or more or treated for a shorter period if the patient died. A neonate was considered to have more than 1 episode of infection if the previous episode was treated with antibiotics for 5 days or more and the blood culture was still positive for that organism, or a different organism was cultured from a subsequent culture.

Death due to sepsis

Death was attributed to septicaemia if it occurred within 5 days of a positive culture with a significant organism.

Clinical outcome

The outcome of each episode was categorized as follows:

-Discharged alive.

-Died from causes attributable to the septicaemia episode.

-Died from causes unrelated to septicaemia.

2.6 STATISTICAL DATA ANALYSIS

Descriptive statistics were calculated using Microsoft Office Excel 2003. Continuous data were expressed as mean, standard deviation and range, while categorical variables were expressed as proportions and percentages.

2.7 ETHICS COMMITTEE APPROVAL

Application to the committee for research on human subjects (medical) for clearance on the use of patient records was made. Detail of the decision of the committee is as follows: "approved unconditionally/protocol NoM03-08-29/Ref:R14/49 Motara.

CHAPTER 3 RESULTS

3.1 PATIENT POPULATION

During the study period 1 July 2002 to 30 June 2003 a total of 1129 babies were admitted to the neonatal unit. Fig 1 illustrates the monthly admissions to the NICU and to the other neonatal wards.

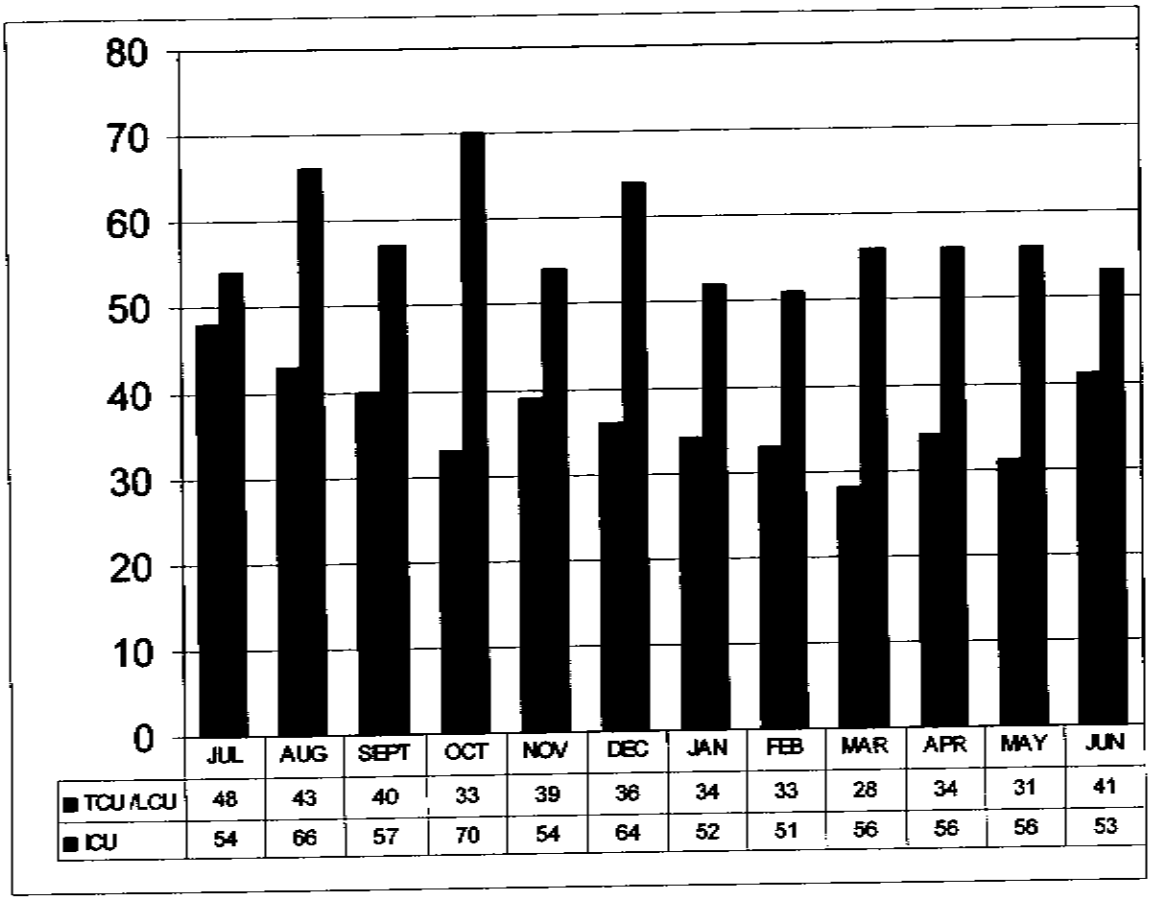
The mean birth weight of all the neonates with sepsis was 1434grams, the standard deviation 626g and range 780 to 3800grams. The mean gestation was 32 weeks and the range of 24 to 40 weeks.

The average age at presentation was 19.1 days with a standard deviation of 16.8 and a range of 0-42. As illustrated in fig 2 the majority of neonates presented with LOS and the data were further analysed separating EOS and LOS.

Any individual baby may be admitted to one ward and transferred to another; for e.g. a baby requiring ventilation initially would be admitted to NICU but subsequently discharged to TCU and finally to the low care unit. Equally a baby in the TCU or LCU whose medical condition deteriorated would be transferred to the NICU for ventilation, inotropic support.

3.2 ORGANISMS CAUSING SEPSIS

During the study period 140 positive blood cultures from 103 patients were identified from the microbiological database. Eight blood cultures were positive for organisms regarded to be definite contaminants and were excluded from analysis, leaving 132 significant isolates from 96 patients. No anaerobes were isolated. A complete list of pathogens isolated from the patients is listed in Table 10.



	JUL	AUG	SEPT	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN
TCU /LCU	48	43	40	33	39	36	34	33	28	34	31	41
ICU	54	66	57	70	54	64	52	51	56	56	56	53

Fig 1: Number of patients admitted to the neonatal unit by month and by ward

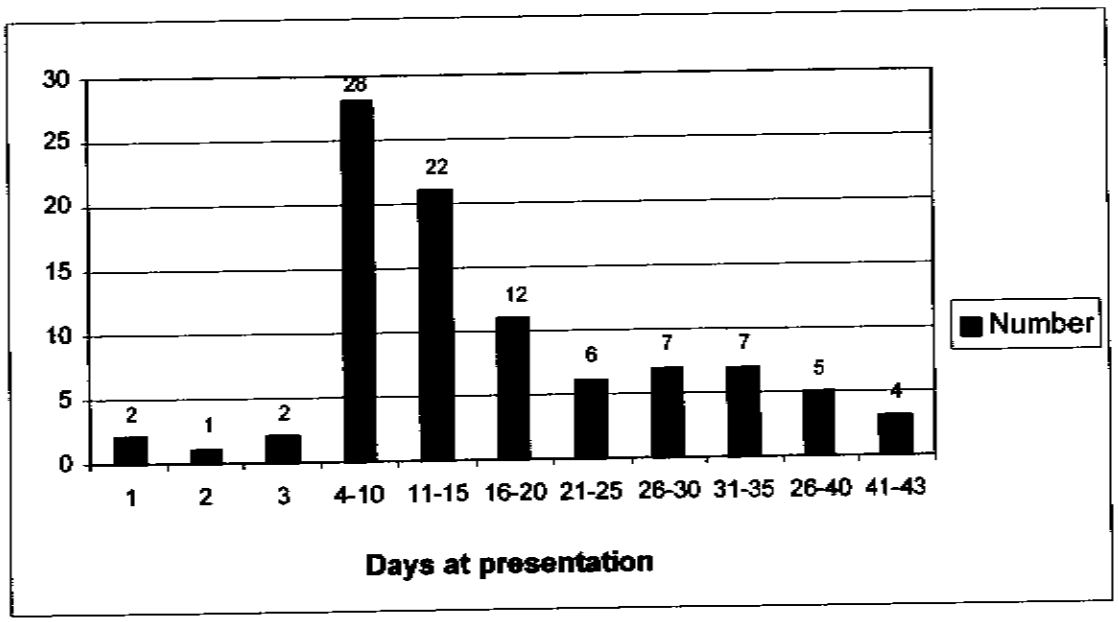


Fig 2 : Number and age at presentation of neonates with positive blood culture

Table 10 : Blood culture isolates from neonates with sepsis

Organism	EOS total (%)	LOS total (%)
Gram-positive		
CoNS	2(33)	63(49.6)
Group B Streptococcus		2(1.5)
Enterococcus faecalis		2(1.6)
Virdans streptococci		2(1.6)
S. aureus		4(3.2)
Gram-negative		
E. coli	2(33)	18 (14.2)
Klebsiela spp		12 (9.5)
Enterobacter spp	1(16.6)*	9 (7.1)
Pseudomonas aeruginosa	1(16.6)*	1(0.79)
Haemophilus influenza		1(0.79)
Proteus mirabilis		1(0.79)
Citrobacter spp.		1(0.79)
Fungi		
Candida albicans		8(6.3)
Candida parapsilosis		2(1.5)

Both isolates from one patient

**CFR-case fatality rate

% do not add up to 100 because of rounding

3.3 INCIDENCE OF CULTURE PROVEN SEPSIS

The overall incidence of culture proven sepsis per number of neonatal admissions at Johannesburg hospital in 2002/2003 was 96 /1129=8.5%.

The number of deliveries at Johannesburg hospital during the study period was 7150

The overall rate of proven sepsis was 76/ 7150* =10.6 per 1000 live births.

* The denominator used is obtained from hospital records and statistics at Johannesburg hospital only but the proper rate is difficult to obtain because birth rates at the referral centers need to be accounted for as well. Because 20 patients were out born they were excluded from the total of 96 patients.

3.4 DIFFERENTIATING BETWEEN EOS AND LOS

3.4.1EARLY ONSET SEPSIS

3.4.1.1 Incidence of EOS

There were 5 babies or 5.2% (5/96) of neonates with sepsis or 0.4% of total neonatal admissions with culture proven EOS during the study period. The incidence of EOS was thus 3/7150 = 0.4 per 1000 live births.

3.4.1.2 Demographic, microbiological and clinical data on neonates with EOS

Of the neonates with EOS 3 were delivered at Johannesburg hospital and 2 were referrals from the designated referral centers. The male female ratio was 3:2.

The demographic details of the patients with EOS are summarized in Table 11.

Table 11: Demographic and clinical features of 5 patients with EOS

	Birth weight in grams	Gestation in weeks	Age at presentation
Mean	1636 g	31.25	1.8 days
Standard deviation	1216g	5.96	1.30 days
Range	908-3800g	27-40	0-3 days

The organisms causing EOS are depicted in Table 10. Analysing the blood cultures of the two patients with CoNS revealed that one patient had definite infection while the second isolate was a contaminant. The mother of 1 neonate had proven chorioamnionitis.

One neonate had cultured 2 isolates namely *Pseudomonas aeruginosa* and *Enterobacter* spp.

3.4.1.3 Outcome in neonates with EOS

Two patients of the 5 with EOS died. The CFR of EOS was 40%. One patient was infected with *E. coli* and the other had a mixed growth of *P. aeruginosa* and *E. cloacae*.

3.4.2 LATE ONSET SEPSIS

3.4.2.1 Incidence of LOS

The majority of patients i.e. 91/96, 94.7% presented with LOS, 8.1% of total neonatal admissions. The incidence of LOS was thus 73/7150= 10.2 per 1000 live births.

3.4.2.2 Demographic, microbiological and clinical data on neonates with LOS

Of the neonates with LOS 73 were delivered at Johannesburg hospital and 12 were referrals from the designated referral centers, and there was no data available for 6 neonates. The male female ratio was 52:39

The demographic details of the patients with LOS are summarized in Table 12.

Table 12: Demographic and clinical features of 91 patients with LOS

	Birthweight in grams	Gestation in weeks	Age at presentation
Mean	1431g	31.5	17.6 days
Standard deviation	533g	3.8	10.6 days
Range	780-3098g	26-40	4-42 days

Twenty-one patients with LOS had classical features of necrotising enterocolitis.

Eleven of the 53 patients from whom CoNS was isolated had indwelling catheters. Eighteen of the patients with LOS had received total parenteral nutrition.

The most common organisms isolated in LOS were CoNS, *E. coli, Klebsiella* and Fungi.

3.4.2.2.1 CoNS isolates

There were 63 isolates of CoNS in 53 patients with LOS i.e. 53/91= 58% of patients with LOS. According to the mentioned definitions 42/63 isolates of CoNS were regarded as significant and 20 were possible contaminants, while 1 isolate could not be classified because of insufficient information.

3.4.2.2.2 Fungal isolates

There were 10 fungal isolates cultured from 7 patients. The mean birth weight of these neonates was 1374g with a range of 974g-2475g. The mean gestation was 31.5 weeks with a range of 28-40. The mean age at culture was 23 days with a range of 11-36 days. Six of the 7 neonates had NEC; one of who had a sealed perforation and one neonate required surgical intervention for the NEC. All but two of the neonates had associated cultures: 4 had CoNS and the remaining neonate had an *E. coli*. One of the seven babies died.

There were 9 episodes of bacteraemia where 2 microbes were isolated.

3.4.2.3 Outcome in neonates with LOS

Eighteen babies with LOS died CFR 19.7%

3.5. ANTIMICROBIAL SENSITIVITY AND RESISTANCE PATTERNS OF ORGANISMS CAUSING SEPSIS

3.5.1. Antimicrobial sensitivity and resistance of gram positive isolates

Table 13. Antibiotic Susceptibility, Sensitivity(S) and Resistance (R) in % of gram-positive bacterial isolates causing EOS & LOS in Neonates at JHB Hospital in 2002-2003

Organism	Number of Isolates			PEN	AMP	ERY	TET	CRO	GEN	SXT	RIF	VAN	Fuc	OX
	EOS	LOS	Total	S R	S R	S R	S R	S R	S R	S R	S R	S R	S R	S R
GBS		2	2	100 0	100 0									
Enterococcus Faecalis		2	2	100 0	75 25				100 0					
Viridans streptococci		2	2	100 0	100 0									
CoNS	2	63	65	0 100		13 87				0 100	70 30	100 0	70 30	3 97
S. aureus		4	4	0 100	0 100	50 50					100 0	100 0	50 50	33 66

PEN=penicillin
AMP=ampicillin
AUG=augmentin
ERY=erythromycin
RIF=rifampicin
OX= cloxicillin
Fuc=fucidin
VAN=vancomycin
CRO=ceftriaxone
GEN=gentamicin
SXT=trimethoprim/sulphamethoxazole
VAN=vancomycin

3.5.2. Antimicrobial sensitivity and resistance of gram-negative isolates

Table 14. Antibiotic Susceptibility, Sensitivity(S) and Resistance (R) in % of gram-negative bacterial isolates causing EOS & LOS in Neonates at JHB Hospital in 2002-2003

Organism	No. of isolates			PEN		AMP		AUG		TZP		SXT		CZ		CXM		CRO		FEP		Col		IMI		MEM		CIP		AMK		GEN		TOB	
	EOS	LOS	Total	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
E. coli	2	18	20	28	72	28	72	94	6	100	0	0	100	100	0	100	0	100	0									100	0	100	0	100	0		
Klebsiela spp		12	12	0	100	0	100	27	73	22	78	0	100	14	86	20	80	70	30			0	100	100	0	100	0	100	0			63	37		
Enterobactria spp	1	9	10	25	75	11	89	11	89	14	86			0	100	0	100	0	100					100	0	100	0	100	0	86	14	71	29		
Pseudomanas spp	1	1	2							50	50							50	50	100	0			0	100	0	100	0	100	100	0			100	0
Haemophilus spp		1	1			100	0																												
Proteus spp		1	1			100	0							100	0							100	0									100	0		
Citrobacter spp						0	100			100	0																			100		100			

PEN=penicillin
AMP=ampicillin
AUG=augmentin
PIP=pipricillin
TZP=Pipricillin-Tazobactam
OX=oxacillin
SXT=trimethoprim-sulphamethoxazole
CZ=Cefazolin
CXM=Cefuroxime
CRO=Ceftriaxone
FEP=Cefipime
IMI=imipenem
MEM=meropenem
CIP=Ciprofloxacin
AMK=amikacin
GEN=gentamicin
TOB=tobramycin

3.5.3 Antimicrobial resistance profile of organisms with MDR pattern

Table 15: Antimicrobial resistance profile of 43 organisms with MDR pattern

Organisms	Resistance pattern	Number
Coagulase-negative staphylococci	MRSE*	35
Klebsiella spp	ESBL**	3
Enterobacter cloacae	AmpC de-repressed mutants	2
	ESBL	1
Staphylococcus aureus	MRSA***	2

*Methicillin-resistant *Staphylococcus epidermidis*

**Extended spectrum beta-lactamases

***Methicillin-resitant *Staphylococcus aureus*

3.5.4 Sensitivity of fungal isolates

Fungal isolates were not routinely tested and susceptibility was available for 50% of the fungal isolates.

All the isolates i.e. 5/5 were sensitive to Amphotericin B.

Two out of the five were sensitive to5 flurocytosine.

One out of the five was resistant to ketoconazole.

3.6 OUTCOME OF SEPTIC NEONATES

Of the total of 96 patients there were a total of 27 deaths recorded.

A total of 7 babies with positive cultures died from causes not attributable to sepsis.

There were a total of 20 deaths attributed to sepsis.

The overall mortality rate for neonatal sepsis was thus 20/1129 =1.77%

Two babies with EOS and 18 babies with LOS died indicating a CFR of 40% and 19.7% respectively. The overall CFR is 20/96=20.8%.

The majority of babies i.e. 19 of the 20 that died due to sepsis were of low birth weight.

Table 16 depicts a Comparison of the most common bacterial isolates and case fatality in EOS and LOS

Table 16: Comparison of the most common bacterial isolates and case fatality in EOS and LOS

Pathogen	**EOS**		**LOS**	
	Number of isolates	**Deaths(%)#**	**Number of isolates**	**Death(%)#**
Gram positive bacteria				
CoNS	2		63	1 (1.09)
GBS			2	1 (1.09)
Gram negative bacteria				
E. coli	2	1 (20)	18	6 (6.5)
Klebsiella spp			12	2 (2.2)
Enterobacter spp	1*	1* (20)	9	5 (5.5)
Pseudomonas spp	1*	1* (20)	1	1(1.09)
Proteus mirabilis			1	1(1.09)
Candida spp			10	1(1.09)
Other			13	0
TOTALS	6	2	126	18
CASE FATALITY RATE%		**40%**		**19.7%**

***Both pathogens in 1 patient**

 # CFR-case fatality rate

Table 17 shows the Outcome of neonatal septicaemia in relation to birth weight and causative organisms.

Table 17: Outcome of neonatal septicaemia in relation to birth weight and causative organism

Pathogen	Birth Weight					
	<1500g		1500 – 2500g		>2500g	
	Number	Died	Number	Died	Number	Died
CoNS	38	0	7	1	5	0
GBS	1	1	1	0	0	0
E. coli	8	4	4	2	3	1
Enterobacter spp	5	4*	3	2	0	0
Klebsiella spp	4	1	3	1	1	0
Pseudomonas spp	2	2*	0	0	0	0
Proteus spp	0	0	1	1	0	0
Candida	6	0	1	1	0	0

* same patient

Weights not available for 3 neonates

CHAPTER 4 DISCUSSION

The NU at Johannesburg Hospital is responsible for the care of a large number of babies born to mothers who are often unbooked and of lower socioeconomic status. A large number of babies require mechanical ventilation and/or nursing in a high care area and can thus spend prolonged periods in the neonatal unit. The neonates develop a complex bacterial flora in the context of their hospital experience with microbiologic, host and environmental factors interplaying.

4.1 INCIDENCE OF CULTURE PROVEN SEPSIS

The incidence of neonatal sepsis varies from 7.1 to 38 per 1000 births in Asia, (Vergano, 2005), from 6.5 to 23 per 1000 live births in Africa (Airede, 1992; WHO, 1999), and from 3.5 to 8.9 per 1000 live births in South America (Vergano, 2005), compared to the United States and Australia with rates that range from 6 to 9 per 1000 for neonatal sepsis (Vergano, 2005). The incidence of sepsis at Johannesburg hospital as documented in this study is comparable with the incidence in developing countries with a rate of 10.6 per 1000 live births.

The rate of sepsis varies from country to country, nursery to nursery and within the same nursery at different times. The variation in the incidence of neonatal sepsis reflects differences in the population characteristics and the prevalence of predisposing factors such as obstetric and nursery practices, prenatal care, the health and nutrition of the mother and the incidence of prematurity.

Gestation and birth weight are two proven variables that reflect on the incidence of neonatal sepsis with gestational age and weight being inversely associated with the risk of

universally recorded in all infants, reporting birth weight specific sepsis rates would permit more meaningful comparison of data from other studies. Some studies group infants according to gestational age, a more accurate determinant of immune function but a more subjective criterion than birth weight. While the two are usually directly related, factors such as intrauterine growth restriction may lead to a small-for-gestational-age (SGA) VLBW infant whose immune competence and risk for infection are more in line with gestational age rather than with birth weight.

4.2 EARLY ONSET SEPSIS

Blood culture-proven early-onset sepsis remains a potentially fatal but uncommon problem among neonates receiving neonatal intensive care.

In this cohort 5 neonates or 0.44% of total neonatal admissions presented with EOS. This value is consistent with data from other studies (Stoll, 1996, 2002a).

A possible reason for this low incidence in EOS can be attributed to the marked increase in the intrapartum use of antibiotics in recent years. Recent studies have shown that a significant proportion of mothers receive antibiotics before delivery, which may reduce the incidence of vertical transmission of infection or affect the ability to detect organisms in blood cultures (Isaacs 1999a; Stoll 1996)

Eighty-five percent of newborns with early-onset infection present within 24 hours, 5% present at 24-48 hours, and a smaller percentage of patients present after this period (Gerdes,2004). Thus 90% of EOS presents within 48 hours and the study data corroborate these findings with the mean age of onset being 1.8 days.

Of the risk factors cited previously (pg 2) that contributes to EOS, this study cohort had four of the five neonates with EOS of low birth weight and low gestation and there was definite chorioamnionitis documented in one maternal case.

The pathogens responsible for early-onset sepsis generally reflect the predominant vaginal flora of the pregnant woman viz. GBS and *E. coli*. There was not a single isolate of GBS amongst the study group in patients who presented with EOS. Previous studies (Bomela, 2001) have found a much higher incidence of GBS at this institution and the findings of this study may merely reflect natural fluctuation in the annual occurrences. A fluctuating incidence of GBS has been noted in other studies previously (Moses, 1998). This absence of GBS in neonates with EOS is consistent with studies in developing countries but contrasts with findings in developed countries as described in table 4.

The low rates of invasive GBS disease in developing countries may be due to one or several factors such as low rates of maternal colonization, exposure to less virulent strains, genetic differences in susceptibility to disease, or high levels of transplacentally acquired protective antibody in serum (Stoll, 1997), or socio-economic differences. (Bhutta, 1991). To answer this question further studies in developing countries are required.

Moreover, in sequential studies at the centres of the Neonatal Research network of the National Institute of Child Health and Human Development between 1991 and 1993 and between 1998 and 2000 it was found that there has been a change in predominant pathogen causing EOS over time from predominantly GBS to *E. coli* (Stoll 1996, 2002a.). The increasing prevalence of Gram-negative enteric organisms in EOS is further supported by other workers (Bhutta, 1991; Airede, 1992). It has been found that prolonged and

complicated deliveries may be a risk factor for this occurrence (Bhutta, 1991). This study also reflects a relatively high preponderance of Gram-negative enteric organisms in EOS (although the numbers are small). Further surveillance is warranted to determine whether the observed prevalence in the incidence of early-onset gram-negative infections will be mirrored by a continued increase in the risk of infection with more virulent gram-negative organisms.

Some studies have found an increase in early-onset *H. influenzae* infections (Stoll, 2002a). In contrast in this study cohort there were no isolates of *Heamophilus* nor were early-onset *Listeria* infections identified.

CoNS although rare, seem to be emerging as an additional cause of early-onset sepsis in Neonat (es. Stoll et al (1995) have documented that 50% of the neonates from who CoNS was isolated as an early onset pathogen had true EOS with CoNS. In this study cohort one patient had definite infection while the second isolate was a contaminant. Infection should be considered in the severely ill neonate and can be established by obtaining blood cultures from different sites and testing for surrogate markers for infection – e.g., C-reactive protein. The possible emergence of CoNS as early-onset pathogens should be noted, especially in light of increased intrapartum treatment with antibiotics (primarily ampicillin) to which these organisms are usually resistant.

EOS is associated with an increased risk of several complications of prematurity, including respiratory distress syndrome, bronchopulmonary dysplasia, and severe intraventricular haemorrhage. Further study is required to assess the effect of EOS and the cytokine response to infection on these adverse outcomes of prematurity and on long-term neurodevelopmental outcome (Stoll, 2002a).

In conclusion, early-onset sepsis remains a relatively uncommon but important problem among neonates especially VLBW preterm infants, because EOS is an important cause of neonatal death especially Gram-negative sepsis which can be particularly lethal. Of almost equal importance is the need to develop better diagnostic tests to determine which neonates with symptoms are not infected, so that use of antibiotic therapy among neonates may be reduced.

4.3 LATE ONSET SEPSIS

Late-onset sepsis remains an important and potentially lethal complication among neonates. These infections are particularly poignant for parents and physicians because this complication affects infants who have survived the early causes of mortality but remain at ongoing risk for infection. With increasing survival of VLBW preterm infants, late-onset sepsis will continue to be a challenging complication that affects other morbidities, length of hospitalization, cost of care, and mortality rates(Stoll et al , 2002b).

4.3.1 Incidence of LOS

There is considerable variability in the incidence of late-onset sepsis. In this study cohort the majority of patients i.e. 95% presented with LOS as were the finding in other studies (Dawodu, 1997; Isaacs, 1995; Milledge, 2005).

Rates of LOS tend to be reported as percentage of babies admitted to a neonatal unit rather than as a proportion of live births, so it is difficult to compare results between centers. As the incidence of LOS increases with decreasing gestational age, and is also increased in babies requiring mechanical ventilation and other invasive procedures, rates of LOS will depend largely on the selection of the population studied. (Isaacs, 1995).

The incidence of infection increases with decreasing birth weight and gestational age as has been confirmed by other studies (Stoll, 1996, 2002b; Isaacs, 1999b), and this has also has been observed in this study cohort of neonates with LOS. Twenty neonates had a birth weight between 1500-2500g and 64 had a birthweight <1.500g. The decreased immunocompetence described previously (in 1.3.2.4) in these LBW babies might be the mechanism for this increased risk.

4.3.2 Gram positive bacteria causing LOS

The distribution of organisms causing LOS among neonates in this study illustrates that Gram-positive organisms were the most frequently isolated organisms. Other workers have reported similar findings (Płaczek, 1983; Vesikari, 1985; Stoll 2002b).

4.3.2.1 CoNS causing LOS

In this cohort CoNS was the predominant Gram-positive organism causing LOS. This finding concurs with that of a previous study done in this unit (Mopeli, 1998). Many other studies too have reported that CoNS are the most common organisms associated with late-onset sepsis. (Isaacs, 1995; Stoll 1996, 2002b).

There are several reasons postulated for the current prevalence of CoNS as the predominant organisms causing nosocomial infections and LOS.

- Most of the CoNS relevant to humans are common inhabitants of the skin and mucous membranes. In virtually all infants, these organisms are acquired early in life, either during passage through a colonized birth canal, or as a result of exposure to nursery personnel or other caregivers. From the studies cited previously, it appears that colonization of the skin, the upper respiratory tract, and the

gastrointestinal tract is common in newborn infants, particularly among those in an intensive care environment. Colonization is a necessary prerequisite for neonatal disease due to CoNS (Benjamin, 2000). In most cases, systemic disease develops when organisms colonizing the skin enter the bloodstream following a break in skin or mucous membrane integrity. Similarly, epidemiologic studies of disease in newborn infants noted an association between CoNS bacteremia and breech in local host defense mechanisms (St Geme, 1991). A high percentage of neonates with disease have a central intravascular catheter, thus providing a portal of entry into the bloodstream for organisms resident on the skin.

- As stated previously CoNS also elaborate adherence factors that enable them to adhere to and form biofilms on the surfaces of catheters, shunts, and prosthetic devices. Once adherence has occurred, these organisms become enmeshed in a protective layer of slime, which inhibits phagocytosis and antimicrobial activity.
- Other potential factors responsible for the organism's prevalence include the organism's meager nutritional requirements.
- In addition, CoNS may be selected as resistance organisms following the widespread use of broad-spectrum antibiotics in intensive care settings. In a study, D'Angio et al. (1989) demonstrated that the incidence of strains resistant to multiple antibiotics rose from 32% to 82% by the end of the first week of life. All infants in that study received a minimum of 3 days of parenteral antibiotics pending culture.
- As opsonization is a key process in host resistance to staphylococcal infection the decreased opsonic activity observed in premature neonates would predispose them to infections due to these ubiquitous organisms.

The presence of CoNS in the bloodstream of immunocompromised patients can no longer be regarded as culture contamination. Indeed, the interpretation of blood cultures growing CoNS is a major challenge facing clinicians, particularly in the high-risk intensive care setting.

If CoNS are the pathogens responsible for more than 50% of late-onset episodes, it would be appropriate to review empiric antibiotic therapy for suspected late-onset sepsis. The majority of these organisms are resistant to the routine antibiotics used to treat newborn infants (ampicillin, aminoglycosides, cefotaxime), and vancomycin is often required for adequate therapy. However, if many of these isolates represent contaminants rather than true infecting strains, the potential overuse of vancomycin is clearly dangerous (i.e., unnecessary use of a nephrotoxic drug, increased risk of antibiotic resistance, and possible colonization with multiply resistant organisms, fungi, or both). Because late-onset sepsis is diagnosed in so many neonates especially VLBW infants and treated with broad-spectrum antibiotics (often including vancomycin), the importance of distinguishing true infection from a possible contamination cannot be overemphasized. Furthermore, improved diagnostic strategies are needed that will allow the clinician to distinguish between the infected and the uninfected infant and to target antibiotic use to those who are truly infected.

4.3.2.2 GBS causing LOS

GBS has been intensely studied and massive efforts have been made to eradicate perinatal infection. In contrast to GBS incidence in other countries and indeed in this unit previously (Bomela, 1991) the present study showed a small proportion of neonatal infections attributed to GBS of late onset. In a previous study conducted by Mopeli (1998) in the NU

4.3.2.4 Miscellaneous Gram positive organisms causing LOS

The non- group B streptococci i.e. *E. faecalis* and viridans streptococci are cited as infrequent causative agents in reviews of neonatal sepsis (Siegal 1981, 1990) but they can be involved in relatively benign infections or cause invasive disease. In most instances 80 percent of enterococci infections occur in infants older than 1 week of age. The non-group B streptococci that were isolated in this study were a small proportion of the Gram-positive isolates and the proportion of these isolates has decreased compared to previous data from the NU (Mopeli, 1998). All patients weighed <1500 grams and 1 neonate had bowel surgery; both these factors predominate in Enterococal sepsis as cited previously. Outcome in all infants was favourable.

4.3.3 Gram Negative bacteria causing LOS

Gram-negative enteric organisms of the *Enterobacteriaceae* family, notably *E. coli Enterobacter, Klebsiella* spp and *Serratia* spp are common inhabitants of the neonatal intestine that may cause nosocomial sepsis.

4.3.3.1 *E. coli* causing LOS

Of the gram-negative organisms causing sepsis, *E. coli* strains are the most common. Unlike illnesses caused by GBS, *E. coli* infections do not fit into distinct clinical syndromes of early and late onset disease. Epidemiological studies have established that vertical transmission from mother to infant is the principal means of neonatal acquisition; however nosocomial transmission has been documented. The finding of *E. coli* being the most prevalent GNB in this study is in keeping with other studies and the previous study conducted in this unit in 1995-1996 (Placzek, 1983; Stoll, 2002b; Mopeli, 1998).

With late onset disease neonates may become colonized with *E. coli* at birth or through contact with colonized caregivers while in the NICU and then develop infection later. Environmental sources usually include ventilation systems (Isaacs, 1999b). Outbreaks of both enteropathogenic and nonenteropathogenic strains of *E. coli* have been described in the NICU.

4.3.3.2 *Klebsiella* spp and *Enterobacter* spp causing LOS

Hospital neonates develop colonization with *Klebsiella, Enterobacter,*and *Citrobacter* species (KEC species) at high rates compared with well babies at home in whom *E. coli* is the predominant bowel flora. In humans, they may colonize the skin, pharynx, or gastrointestinal tract. The risk of stool colonization with KEC species is increased with over three days of antibiotic use and with duration of hospital stay, 60% being colonized by day 15 and over 90% by day 30.17 (Royle, 1999).

Two members of the genus *Klebsiella* that are responsible for most human infections are *K. pneumoniae* and *K. oxytoca*. Like the other well-known member of the family, *E. coli*, a capsule and fimbriae that contribute to their virulence surround these organisms. This capsular polysaccharide prevents activation of the alternative complement system protecting the bacteria from opsonization, phagocytosis, and bacteriolysis.

The principal pathogenic reservoirs of infection are the gastrointestinal tract of patients and the hands of hospital personnel. Organisms can spread rapidly, often leading to nosocomial outbreaks. Common sites include the urinary tract, lower respiratory tract, biliary tract, and surgical wound sites. The spectrum of clinical syndromes includes pneumonia, bacteremia, urinary tract infection, diarrhoea, osteomyelitis, and meningitis. The presence of invasive

devices, contamination of respiratory support equipment, use of urinary catheters, and use of antibiotics are factors that increase the likelihood of nosocomial infection with *Klebsiella* species. Sepsis and septic shock may follow entry of organisms into the blood from a focal source.

Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and, subsequently, the development of multidrug-resistant strains that produce extended-spectrum beta-lactamase (ESBL). These strains are highly virulent, and have an extraordinary ability to spread. Most outbreaks are due to a single clone or single gene; the bowel is the major site of colonization with infection of the urinary tract, respiratory tract, and wounds. Bacteremia and significant increased mortality have resulted from infection with these species. Outbreaks have been reported in the literature and studies from Greece, USA and Australia indicate that multiple antibiotic resistant strains and ESBL strains are implicated in outbreaks in the NICU (Lebessi, 2002; Hill 1974; Royle 1999). More recently in South Africa an outbreak of ESBL *Klebsiella* infection occurred at a hospital in KwaZulu-Natal and claimed the lives of 80% of infected subjects. The outbreak was due to contamination of one of the intravenous medications and inadequate hand washing practices (Sturm, 2005).

These reports of recent outbreaks highlight the problem of mulitresistant strains of *Klebsiella*. Colonized babies are the commonest reported source of infection, although many primary environmental reservoirs of Gram-negative bacteria have been reported in epidemics, (Hart, 1993), including nutritional preparations.

Intensive efforts at reducing nosocomial transmission of *Klebsiella* have successfully eradicated colonization and disease with virulent enteric strains, although in some cases closing a NICU to new admissions has been deemed necessary to prevent life-threatening infections during outbreaks.

In this study this organism was the second commonest cause of LOS but only 3 isolates were ESBL producers and overall this organism caused only 2.2% of deaths.

A study by Singh et al (2002) identified two groups of neonates who were at significant risk of *Enterobacter* infections: Those of very low birth weight (<1000g) and those who have received prolonged antimicrobial therapy. Falkiner (1992) found that Enterobacter infection is commonly recognized as a hospital acquired infection and alterations in the gastrointestinal bacterial flora of hospitalized patients lead to the selection of virulent and frequently resistant strains of *Enterobacter* species, with a subsequent risk of infection.

Intestinal colonization of the newborn with multiresistant organisms could pose a risk of *Enterobacter* sepsis even in the absence of invasive procedures. In this study *Enterobacter* was the third commonest gram-negative organism causing LOS and more significantly its incidence increased. In the study by Mopeli (1989) there were only 8 isolates of this organism over a 2-year period compared to 9 in one year in this study.

4.3.3.3 *Pseudomonas* species causing LOS

Pseudomonas infection is rarely perinatally acquired; however, it is among the more common gram-negative organisms causing nosocomial sepsis in NICU patients (Isaacs, 1999b; Karlowicz, 2000). Most *Pseudomonas* infections are due to *P. aeruginosa*. *P. aeruginosa* is commonly thought of as a "water bug," thriving in moist environments such as humidified incubators , sinks , and ventilator circuits . Hands of health care workers have

also been identified as an important reservoir (Isaacs, 1999b). *Pseudomonas* can cause fulminant sepsis and a high mortality, with between 40 to 75% of patients dying (Karlowicz, 2000; Kaufman, 2004). Nosocomial septicaemia with *Pseudomonas* species was relatively rare in this study with only one patient developing late onset infection but the infection was fatal. Previously as documented by Mopeli this organism was a significant isolate in this unit (1989).

4.3.3.4 Miscellaneous organisms causing LOS

Haemophilus and *Proteus* were rare causes of sepsis with only 1 patient each developing infection.

Disseminated fungal infections, previously considered a rarity, are now frequently diagnosed in NICUS. This increasing incidence correlates with the improved survival of the VLBW infant. In this study fungal sepsis occurred at a rate of 7.2%- 7.7% of all neonatal admissions and of the organisms causing LOS, respectively. The mean birth weight in babies with AFS was 1347g with a range of 974g-2475g. These are similar rates compared to other researchers (Kaufman, 2004)

The neonates with candidaemia in this study had several of the risk factors cited previously for developing infection. Candida infections are thought to arise from invasion or disruption of colonized mucosal or epithelial surfaces. Six of the seven neonates in this study had bowel pathology in the form of NEC. Infants with mucocutaneous candidiasis are at an increased risk of developing invasive disease especially if of low birth weight. Five of the seven neonates in this study were on antibiotics at the time of the positive fungal culture. Four of the neonates in this cohort had received total parenteral nutrition at the time when the fungal cultures were positive.

Consideration of fungal sepsis is particularly necessary when neonates deteriorate whilst receiving antibiotics. Empirical treatment with an appropriate antifungal agent is necessary until cultures are reported as clear of the offending agent. Suprapubic aspirates of urine must be performed prior to starting anti -fungals, as bag specimens will often be contaminated with *Candida* colonising the skin. Duration of treatment depends upon the site of infection but generally ranges from 3 to 6 weeks. Ultrasound of the kidneys and formal fundoscopy should be performed.

Prospective surveillance in the neonatal intensive care units for nosocomial infections needs to be conducted periodically to determine baseline rates of infection, to identify new organisms of importance to neonates, to recognize epidemics, and to monitor time trends. The review of late-onset sepsis among the majority of neonates in this study underscores the importance of this serious complication and suggests that strategies to reduce late infections in neonates especially VLBW neonates and their medical, social and economic toll are needed urgently.

4.3.4 Factors associated with late onset sepsis

Infants with prolonged duration of central catheters insertion and exposure to respiratory therapy equipment and humidifiers are at an increased risk for LOS. (Isaacs, 1999b). Whenever possible, the following measures may minimize infection-associated morbidity and mortality in neonates: avoidance of central venous catheter use, care during insertion and continued surveillance for proper positioning, and prompt removal of catheters when they are no longer essential or when fungaemia or persistent bacteremia develops.

In this study 11/91 =12% of neonates with LOS had indwelling catheters and analyzing the data showed that all the neonates who had catheters also had CoNS isolated on blood

culture. In neonates with septicemia secondary to an infected indwelling catheter, removal of the catheter may be necessary, particularly if the isolate of CoNS is slime producing.

Necrotising enterocolitis is a multifactorial disease, and infection may be a predisposing factor or a secondary consequence. However, a role for bacteria in the development of NEC is suggested by the following: (a) NEC does not occur in the sterile in utero environment; (b) intestinal necrosis is absent in bacterium-free animal models (c) organisms that commonly colonize the gastrointestinal tract have been associated with temporal and geographic epidemics of NEC (Kliegman, 1984). Bacteremia may be present in neonates with NEC and in this cohort of patients 23% of neonates with LOS with isolates on blood culture had clinical features of NEC.

In the LOS group there were 9 instances of two isolates recovered in the same patient. Most cases of mixed-species septicaemia occur in neonates with central venous catheters or those with NEC, abdominal surgery, or other conditions compromising the gastrointestinal mucosa. Formation of mixed-species biofilms on vascular catheters, particularly with *Candida* and CoNS, may account in part for the relatively high incidence of mixed-species septicaemia associated with these organisms. Both these organisms form biofilms that impede the penetration of antibiotic and antifungal agents. In this study, of the seven patients with fungal isolates, four also had CoNS isolated.

In analyzing the rest of the cultures it was found that the association of two different organisms did not exhibit any particular pattern or trend. A neonate already infected with one microbe may have acquired the second one from the hospital environment, or both the bacteria could be nosocomial in origin. There is a need to correlate the occurrence of polymicrobial sepsis with clinical outcome in neonatal septicaemia.

In this study 18/91 =20% of neonates with LOS had received parenteral nutrition. The data on total parenteral nutrition and LOS cited previously highlight the need for limiting total parenteral use. Initiation of early enteral feeding especially feeding with human milk that contains a number of immune modulators has been shown to reduce the risk of sepsis and NEC in preterm infants. Thus, strategies to promote breast milk feeding in all neonates remain mandatory.

4.4 SENSITIVITY AND RESISTANCE PATTERNS OF ORGANISMS CAUSING SEPSIS

The suspicion of sepsis is almost universal in neonates especially VLBW preterm infants, and initiation of antibiotic therapy at birth is a common practice. The decision to discontinue antibiotic therapy in neonates is often difficult. There are limitations to blood culture methods, and it is possible for a single blood culture result to be negative when a neonate has bacterial sepsis. However, prolonged antibiotic therapy in VLBW neonates increases both risk and cost. Antibiotic therapy for longer than 72 hours has been associated with increased colonization by pathogenic gram-negative organisms and with emergence of drug-resistant strains (Isaacs, et al.1987). Improved diagnostic tests are needed to help the clinician determine which neonates are not infected and therefore may have antibiotic therapy safely discontinued.

The susceptibility patterns of the bacteria that cause neonatal sepsis provide the basis for the initial selection of antibiotic treatment.

4.4.1 Gram positive bacteria

Penicillin G provides effective therapy for Group B streptococci as well as the viridans streptococci. This was demonstrated in this study. When other gram-positive bacteria such as the enterococci cause serious infections, therapy with ampicillin plus an aminoglycoside is recommended. The addition of an aminoglycoside to penicillin G or ampicillin causes in vitro synergy with rapid bacteriacidal activity (Dobson, et al., 1990). Most neonates respond rapidly to the appropriate therapy with clinical improvement and sterilization of blood cultures within 24 hours. Some enterococcal strains may be highly resistant to ampicillin or an aminoglycoside making vancomycin the mainstay of therapy. There were no enterococci that displayed resistance in this study and thus penicillin and/or an aminoglycoside are the mainstays of therapy. Over the past years vancomycin-resistant Enterococci strains have been described and have been associated with hospital outbreaks especially in the United States and Europe (McNelley, et al., 1998). With longer hospitalization of extremely premature neonates, particularly those with surgical complications and prolonged central venous access, it is likely that vancomycin-resistant Enterococci will become a more significant burden in the NICU in the future. Restricting the use of vancomycin should be a high priority for those caring for these patients.

Methicillin-resistant *S. aureus* has become an increasing problem. The vast majority of *S. aureus* clinical isolates produce ß-lactamases and are resistant to penicillin and extended-spectrum penicillins. The resistance is acquired by genes that code for a penicillin-binding protein that prevents the action of ß-lactam antibiotics on cell wall synthesis. They are usually chromosomally mediated, rather than plasmid mediated. The gene alters the bacterial cell wall and appears to render the organisms more stable (Carbon, 2000.) To date, there are no published reports of vancomycin-intermediate or vancomycin-resistant *S.*

aureus isolated in neonates. With increasing use of vancomycin for treating documented MRSA and CoNS infection and as empiric therapy for suspected LOS, it is possible that vancomycin resistance among *S. aureus* strains will be of concern. In this study it was found that MRSA do not pose a major problem in the NICU. Two of the four isolates were methicillin resistant and displayed a multidrug resistant pattern i.e. resistant to more than 2 classes of antibiotics but all the isolates were fully sensitive to vancomycin.

In view of the observation that CoNS as the predominant isolates in LOS, recommend initial empiric therapy for hospitalized infants at risk for CoNS disease should be reviewed. Vancomycin is the choice most clinicians opt for but the treatment regimen should then be modified, as necessary once antimicrobial susceptibility testing is available and if the clinical isolate is truly susceptible to beta-lactam antibiotics, these agents should be used in an attempt to prevent the development of vancomycin resistance.

In a study by Karlowicz (2000) it was observed that the practice of starting vancomycin therapy only after CoNS were identified did not prolong the duration of sepsis caused by these organisms and avoidance of vancomycin as an empiric antibiotic had no impact on the rate of fulminant sepsis for coagulase-negative staphylococcal sepsis. Moreover Isaacs (2000) re-iterates that because CoNS very rarely cause fulminant sepsis, there is almost always time to commence vancomycin when the cultures are positive in neonates and that empiric vancomycin is advisable only when severe CoNS sepsis is suspected. In the present study all of the CoNS isolates were resistant to penicillin, and 97% were resistant to oxacillin and 35 isolate displayed an MDR pattern. Although there has been a worldwide effort to decrease the use of vancomycin in neonates to minimize antibiotic

resistance, the level of oxacillin resistance in this study justifies the use of vancomycin as the first-line antibiotic when CoNS is implicated in infection.

Several authors have suggested the adjunctive use of rifampicin in clearing persistent CoNS bacteraemia, sometimes even in the presence of indwelling cathters. (Shama, 2002) The success of this combination may be attributable to rifampicin's potent serum bacteriacidal activity as well as its capacity to exert intraleukocytic killing.

With respect to both MRSA and MRSE the effectiveness of infection containment policies such as the implementation of rigorous hygiene measures have a dramatic effect on the transmission of this pathogen and this vital form of prevention should be emphasized.

4.4.2 Gram Negative Bacteria

In this study the GNB displayed variable resistances to the ß-lactam antibiotics:

The penicillins i.e. ampicillin, amoxicillin/clavulanic acid and piperacillin/tazobactam were tested against *E. coli*: Ampicillins with a sensitivity was 28% are not a good choice for treatment but amoxicillin/ clavulanic acid and the piperacillin/tazobactam combinations are good choices with sensitivities of 94% and 100% respectively. The cephalosporins, aminoglycosides, and quinolones all display 100% coverage of *E. coli.*

Klebsiella spp and *Enterobacter* spp displayed fluctuating patterns and varying rates of resistance to the antibiotics tested. The only antibiotics that remained completely sensitive to both groups of organisms were the fluoroquinolones and the carbepenem ß-lactams.

There were isolates of both *Klebsiella* and *Enterobacter* that produced ESBL and that also displayed an MDR pattern of resistance. The methods of resistance to the penicillins and to

the aminoglycosides are due to the mechanisms described below. In this cohort one case of amikacin resistant Enterobacter sepsis posed a major problem for therapy necessitating the use of carbepenems.

Gram-negative organisms have both innate resistance to antibiotics and the ability to acquire resistance through new mechanisms that may be transferred from other pathogens. ß-lactamases continue to be the leading cause of resistance to -lactam antibiotics in Gram-negative bacteria. ESBLs represent a major group of -lactamases currently being identified worldwide in large numbers and are now found in a significant percentage of *E. coli* and *K.. pneumoniae* strains. They have also been found in *Pseudomonas.aeruginosa* and other *Enterobacteriacae* strains like *Enterobacter* spp, *Citrobacter* spp, *Proteus* spp, *Morganella morganii, Serratia marsescens, Pseudomonas aeruginosa, Burkholderia cepacia.* Production of these enzymes is either chromosomally mediated or plasmid mediated.

The chromosomally mediated -lactamase production is mainly through expression of ampC inducible gene. The gene encodes an enzyme more active in hydrolyzing cephalosporins than penicillins. The amount of AmpC ß-lactamase that is produced is dependant on various genetic factors as well as by exposure to a ß-lactamase inducer. Both the second and some of the third generation cephalosporins are inducers of ß-lactamase activities in the genera *Pseudomonas* and *Enterobacter.* ß-lactamase resistance also results from a plasmid-mediated ß-lactamase. Many varieties of this enzyme can be distinguished on the basis of biochemical criteria. Point amino acid substitution of the classical plasmid mediated -lactamases like TEM-1, TEM-2 and SHV-1 increases the spectrum of activity from earlier generation -lactams to 3rd generation cephalosporins and monobactams.

However, they retain their stability against cephamycins and carbapenems and are inhibited to an extent by -lactamase inhibitors in vitro.

Being plasmid mediated these enzymes spread fast amongst various bacteria and are important by infection control, clinical and therapeutic implication

Aminoglycoside resistance results from aminoglycoside modifying enzymes that are involved in acetylation, adenylation, and phosphorylation of the drug thereby preventing it from binding with the microbial ribosomes. (Neu, 1992) However the majority of aminoglycoside modifying enzymes modifies amikacin inefficiently or not at all and it therefore remains active against a number of otherwise aminoglycoside-resistant bacteria.

Imipenem has a broad antibacterial spectrum covering both Gram positive and Gram negative and may act synergistically with aminoglycosides. Extended spectrum ß-lactamases are unable to confer resistance to imipenem, but a specialized imipenem hydrolyzing ß-lactamase enzyme is produced by some organisms by a chromosomally mediated mechanism that can confer resistance; altered penicillin binding proteins have also been associated with resistance. *P. aeruginosa* and *E. aerogenes* can also become resistant to imipenem through the loss of an outer membrane protein that provides a channel for the entry of imipenem. One of the two *P. aeruginosa* isolates in this study was imipenem resistant.

Emergence of antimicrobial resistance in other perinatal pathogens also is a concern in the context of increasing GBS prophylaxis. To date the incidence of ampicillin resistance in *E. coli* populations seem to be increasing. In this study, 72 percent of *E. coli* isolates were resistant to ampicillin. Data were not collected to assess whether the high rate of resistance

reflects antibiotic-resistance patterns in the neonatal intensive care unit or resistance patterns of genital flora in the mothers in this study or in ambulatory populations more generally. Furthermore, no data on maternal antibiotic use during pregnancy but before the hospitalization for delivery are available. In terms of neonatal disease, the prevalence of ampicillin-resistant *E. coli* infections seems to have increased among cases of sepsis that occur in preterm infants, but not among infections in term infants. The benefit-to-risk ratio for the use of antimicrobial prophylaxis in the case of preterm delivery still seems to be favourable, but requires continued monitoring. (Schrag, 2005)

4.4.3 Fungal isolates

In vitro sensitivities of fungi to antifungals are notoriously unreliable. The antifungals routinely tested for and used in neonates with candidaemia in this study are reviewed.

Amphotericin B is a polyene antibiotic that acts by binding to ergosterol in the fungal cell membranes, promoting cellular leakage, and is active against both yeast and mycelial forms. *C. albicans* is universally sensitive to amphotericin B, but some *Candida* species e.g. *C. lusitaniae* may not respond clinically to amphotericin B. Limited data are available on the pharmakokinetics, efficacy, and toxicity of amphotericin B in VLBW neonates. All the pharmacokinetic studies emphasize the remarkable interindividual variability among neonates (Bayley, 1991). Nephrotoxicity is the major side effect among neonates and children.

Of the azoles, fluconazole has undergone the most extensive study in neonates. Fluconazole is a potent inhibitor of the fungal cytochrome p450 and sterol C-14 α-demethylation. It alters cell membranes, resulting in increased membrane permeability and

impairment of purine and pyrimidine precusor uptake for DNA synthesis. Fluconazole is an excellent drug for prophylaxis due to its long half-life, high CSF (70 to 90%) and pulmonary (120%) penetration, low lipophilicity, and low protein binding.

The concurrent use of 5-fluorocytosine is frequently but not consistently recommended. The drug is a fluorinated pyrimidine antimetabolite that competitively inhibits nucleic acid synthesis in fungi. Fungi rapidly acquire resistance when the drug is used alone. There is some synergy of 5-fluorocytosine in vitro against *Candida* when used with amphotericin B. Some centers routinely use both drugs, whereas others use amphotericin B alone. Overall treatment should be aggressive and prompt to optimize a favourable outcome.

Factors to consider in deciding the duration of therapy include the species of fungus involved, whether a central line was present and was removed, number of positive blood cultures, CSF indices, and findings of the evaluation for end-organ dissemination, including echocardiogram, renal ultrasound, ophthalmologic examination and neuroimaging studies. Generally, 2 to 4 weeks of intravenous therapy after negative cultures is recommended for treatment of candidemia in preterm neonates, with longer courses being appropriate for meningitis and deep-seated infections.

4.4 OUTCOME IN INFECTED NEONATES

In spite of all the advances in prevention and treatment, sepsis continues to be a significant cause of death in the neonatal period, with mortality rates as high as 15% (Gerdes, 2004). It is also difficult to be absolutely certain as to the cause of mortality in LBW neonates as most of them can have multisystem disease.

The overall CFR of 20.8 % in this study is comparable to recent published results (Stoll,1997; Vergano, 2005). Higher mortality rates have been reported from other units suggesting that neonatal septicaemia is still associated with significant mortality.

There were two deaths due to sepsis in the EOS group.

The one neonate had overwhelming infection with 2 organisms. It seems that the outcome of this neonate with very early onset form of neonatal sepsis may be determined by the intrauterine progression of the infection rather than the nature of the pathogens. Generally these infections seem to originate from the birth canal, and the causative organism may have colonised the infant before delivery or, alternatively, may be introduced with obstetric examinations or procedures. It seems that in many cases intrauterine infection must take place well before delivery to allow the infection to reach an overwhelming magnitude by the time of delivery. Thus the infant may be born with septicaemia and shock, either already present or developing shortly thereafter. The management of these patients creates a problem far beyond antimicrobial treatment. Once again early and rapid diagnosis is crucial.

It has been demonstrated that infants who develop LOS are significantly more likely to die than those without infection (Stoll, 2002b). Those with late-onset Gram-negative or fungal sepsis were at greatest risk of death. In a study by Karlowicz et al. (2000) it was found that the infecting pathogen influences mortality risk. Death rates were highest for infants infected with *Pseudomonas, E. coli,, Enterbacter* and *Klebsiella.* Moreover infants with gram-negative infections were more likely to suffer a fulminant illness with acute death. In this study, a similar trend was noted. Of the neonates with LOS the highest mortality was

found amongst babies with *E. coli* and *Enteorbacter* infections and the lowest amongst those with Gram positive infections.

The mortality rate from systemic candidiasis is high, in part because of the high-risk population with compounding problems. The mortality rate from untreated disease is nearly 80% and 25-30% for treated disease. Of the 7 neonates with candidaemia, only 1 death occurred.

The mortality rate from neonatal sepsis is dependant on a number of related factors. More specifically, mortality is increased in the presence of noninfectious complications of prematurity (intraventricular hemorrhage, NEC) with simultaneous infection of the CNS, and if the infection is fulminant.

Low birth weight is associated with an increased risk of infant mortality in both developed and developing countries. There are several demographic, socioeconomic and environmental factors that may confound this association. However in a study by Victora et al. (1988) it was found that low birth weight infants (i.e. those weighing <2500g) were 2.3 times more likely to die of an infection than those of higher birth weight, and this association persisted after allowing for confounding variables. In this study the data were not analysed for confounding variables but it was found that there was only one death among the neonates who weighed >2500g, and majority of neonates who died were of low birth weight.

Neurologic morbidity has been attributed to both EOS and LOS in preterm infants. Intra-amniotic fluid infection often leads to overproduction of inflammatory cytokines that may be cytotoxic to the developing brain and chorioamnionitis has been linked to cerebral palsy in term and near-term infants (Wu, et al.). The 2002 Neonatal Network survey reported a higher rate of severe IVH and periventricular leukomalacia in VLBW infants with EOS (Stoll, 2002a).

CHAPTER 5 CONCLUSIONS

This study confirms that neonatal sepsis remains a problem in neonatology and paediatric infectious disease today.

The bacteriological profile of neonatal septicaemia is constantly under change with advances in early diagnosis and treatment. The uncertainty surrounding the clinical approach to treatment of neonatal septicaemia can be minimized by epidemiological surveys of aetiological agents and their antibiotic sensitivity patterns (such as this study), leading to recognition of the most frequently encountered pathogens in a particular setting. Knowledge of the commonest causative organisms in the unit is important for selecting initial antibiotic treatment when sepsis is suspected but initial antibiotic selection must be constantly refined until final bacterial identification and antibiotic susceptibility are known.

Thus a rational protocol for sepsis management must be based on adequate knowledge of the causative organisms and their antibiotic sensitivity pattern and bearing the following points in mind:

- Use narrow spectrum antibiotics whenever possible.
- Keep potent broad-spectrum antibiotics in reserve.
- Treat sepsis not colonization.

Approach to EOS

On the basis of findings of this study the organisms associated with EOS are predominantly GNB thus the traditional choice of antibiotics for EOS remains suitable i.e. a broad-spectrum semisynthetic penicillin and an aminoglycoside.

Penicillin G and amikacin are recommended as this combination will treat any early onset GBS as well as the encountered GNB.

Approach to LOS

When selecting empiric antibiotics for late-onset sepsis in the NICU, neonatologists should be especially concerned about fulminant late-onset sepsis, in which infants die in <48 hours of onset of illness, often before pathogens and antibiotic susceptibilities have been identified. The study data suggest that empiric antibiotics selected for treatment of suspected sepsis in infants older than 3 days of age need to effectively treat Gram-negative pathogens, as these organisms, although less frequent, are strongly associated with fulminant late-onset sepsis in the NICU. Although CoNS were the most prevalent pathogens in late-onset sepsis, the very low frequency of fulminant sepsis suggests that avoiding empiric vancomycin therapy until culture results and susceptibilities are known is a reasonable approach to management of late-onset sepsis.

The choice of antibiotic for GNB is either an extended penicillin such as tazocin and an aminoglycoside i.e. amikacin or a carbepenem. Vancomycin is the drug of choice for clinically significant CoNS infections.

The study also showed epidemiological features of neonatal sepsis that may help direct future preventative measures. LOS was predominant in this study and this illustrates the contribution of the hospital setting and the hospital staff to the burden of these infections.

This highlights the need for an effective programme of infection control for the prevention of hospital-acquired infection and the containment of infections brought into the NICU by patients, staff or visitors. Such a policy would include specific principles of infection control like: source isolation, protective isolation, disinfection policy, disposal policy for

sharps soiled linen and waste, wearing masks and gowns as appropriate, staff health surveillance and visitor access. A single simple infection control measure like meticulous and correct hand washing cannot be emphasized enough in decreasing nosocomial spread of organisms.

The above is a short-term proposal for decreasing neonatal infections. The greatest impact on the burden of neonatal bacterial infections would be achieved through strategies that can reduce preterm delivery. Research in this area is a critical priority, but is likely to be slow to bear fruit in terms of broad implementation. However public health and clinical researchers should continue to carry out research and surveillance of the incidence, causes and health service burden that are associated with neonatal sepsis as this data allow appropriate policies for immediate management and therapy to be implemented.

The challenge for the next decade is to link science and medicine with social, cultural and economic solutions through global commitment to a long-term, long-lasting change and simple, low-cost sustainable strategies so that improvements in both maternal and newborn health can be achieved and sustained and that neonatal sepsis is highlighted as a global concern.

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CLEARANCE CERTIFICATES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Motara

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M03-08-29

PROJECT Bacteramia in Neonates Epidemiological Surveillance and Clinical Significance

INVESTIGATORS Dr F Motara

DEPARTMENT School of Clinical Medicine, Johannesburg Hospital

DATE CONSIDERED 03-08-29

DECISION OF THE COMMITTEE Approved unconditionally

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

DATE 03-09-01 CHAIRMAN.....(Professor P E Cleaton-Jones)

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

c c Supervisor: Dr DE Ballot
Dept of School of Clinical Medicine Johannesburg Hospital
Works2\lain0015\HumEth97.wdb\M 03-08-29

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DECLARATION OF INVESTIGATOR(S)

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

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

DATE .....SIGNATURE .....

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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Gauteng Department of Health

PERMISSION FOR RESEARCH

NAME OF RESEARCHER DR. FIROZA MOTARA

TITLE OF RESEARCH PROJECT BACTERAEMIA IN NEONATES :- SURVEILLANCE | SIGNIFICANCE, SENSITIVITY PATERNS.

METHODOLOGY (briefly or include a protocol) Retrospective review of positive cultures in neonatal subjects.

CONFIDENTIALITY OF PATIENTS MAINTAINED YES- CODING OF NAMES.

COST TO THE HOSPITAL NIL

APPROVAL OF HEAD OF DEPARTMENT PROFESSOR P.A. COOPER
HEAD: DEPT. OF PAEDIATRICS & CHILD HEALTH
JOHANNESBURG HOSPITAL

APPROVAL OF CRHS OF WITS UNIVERSITY APPLICATION SUBMITTED

CLINICAL EXECUTIVE PERMISSIONS

Signature Dr. D. Wojciechowska Clinical Executive **Date** 07/08/2003

Subject to any restrictions