

**NEONATAL SEPSIS: BACTERIAL ETIOLOGIC AGENTS AND THEIR  
ANTIBIOTIC SUSCEPTIBILITY PATTERN IN TIKUR ANBESSA  
UNIVERSITY HOSPITAL, ADDIS ABABA, ETHIOPIA.**



**BY  
DEMISSIE SHITAYE, B.Sc**

**DEPARTEMENT OF MICROBIOLOGY, IMMUNOLOGY AND  
PARASITOLOGY, FACULTY OF MEDICINE ABABA UNIVERSITY**

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## **ABBREVIATIONS**

ANC	Antenatal Care
ATCC	American Type Culture Collection
CRP	C - reactive protein
CBC	Complete Blood Count
CDC	Center for Disease Control
CoNS	Coagulase Negative Staphylococcus
EOS	Early Onset Neonatal Sepsis
FDRE	Federal Democratic Republic of Ethiopia
GBS	Group B Streptococcus
G-CSF	Granulocyte Colony- Stimulating Factor
IFN	Interferon
IL	Interleukin
IVIG	Intravenous Immunoglobulin
I:T ratio	Immature to total neutrophil ratio
LOS	Late Onset Neonatal Sepsis
LPS	Lipopolysaccharide
NCCLS	National Committee for Clinical Laboratory Standards
NICU	Neonatal Intensive Care Unit
PROM	Premature Rupture Of Membrane
TNF	Tumor Necrosis Factor
VLBW	Very Low Birth Weight
WHO	World Health Organization

## ABSTRACT

**BACKGROUND:** Neonatal sepsis is one of the most common reasons for admission to neonatal units in developing countries. It is also a major cause of mortality in both developed and developing countries. The type and pattern organisms that cause neonatal sepsis changes over time and vary from one hospital to another hospital, even in the same country. In addition the causative organisms have developed increased drug resistance for the last two decades. Maternal, neonatal and environmental risk factors have contributed for the development of sepsis.

**OBJECTIVES:** This study was undertaken to determine the pattern of bacterial agents causing neonatal sepsis and to assess their susceptibility pattern to various antimicrobial agents in the Ethiopian setting. An attempt has been also made to identify the possible maternal and neonatal risk factors responsible for neonatal septicemia.

**METHODS:** During the period of October 2006 and March 2007 a cross-sectional prospective study was conducted at the Department of Pediatrics and Child Health, Ethio-Swedish Children's Hospital, Addis Ababa, Ethiopia. Blood cultures were performed from newborn babies (n=302, age: 0-28 days) admitted to the hospital with a clinical diagnosis of neonatal sepsis. Antimicrobial susceptibility testing was performed for all blood culture isolates according to the criteria of the National Committee for Clinical Laboratory Standards by disk diffusion method.

**RESULTS:** Of the 302 patients, 46.4% were males and 53.6% were females ( $p > 0.05$ ) resulting in an overall male to female ratio of 0.8:1. The mean age of the neonates was  $1.23 \pm 8.96$  days and 70.2% of neonates were below age of 1 day. A total of 298 (98.7%) neonates presented with early-onset sepsis (EOS) and four (1.3%) presented with late-onset sepsis (LOS). Out of the 302 neonates, 57.3% were preterm and 62.7% had low birth weight. The most prevalent clinical features of sepsis were hypothermia (84.8%), respiratory distress (72.8%), failure to feed (71.5%) and lethargy (30.1%). Among the 302 neonates admitted with suspected cases of sepsis, 59 (19.5%) had abnormal white blood cell count (high and low). Immature/total neutrophil ratio  $\geq 0.2$  and  $< 0.2$  was observed in 58.0% and 42.0% peripheral blood smear examined respectively. Of the 302 neonates investigated for sepsis, 135 (44.7%) were positive for blood culture. The Gram-positive and negative bacteria accounted for 43.7% and 56.3% respectively ( $p > 0.05$ ). The most common isolated organisms were *Klebsiella spp.* (39.2%) and *Staphylococcus aureus* (22.2%). Neonatal risk factors such

as prematurity, low birth weight, abnormal WBC count (high and low) and I:T ratio  $\geq 0.2$  were strongly associated with culture proven neonatal sepsis. No maternal risk factors were identified. In general both gram positive and negative bacteria isolated from blood culture showed low resistance rates (<60%) to ciprofloxacin, doxycycline, kanamycin, streptomycin, trimethoprim-sulphamethoxazole and tetracycline. Gram-negative bacteria showed high-level resistance (>80%) to ampicillin, ceftriaxone, cephalothin, chloramphenicol, and gentamicin. Ciprofloxacin was the most effective drug against the tested gram-positive and gram-negative bacteria. Multiple resistance (resistance to two or more drugs) was observed in 45.7% and 84.2% gram positive and gram negative bacteria respectively ( $p < 0.05$ ).

**CONCLUSION AND RECOMMENDATIONS:** *Klebsiella* spp. and *S. aureus* were the most common organisms causing neonatal sepsis. Prematurity, low birth weight, abnormal WBC counts and I: T ratio  $\geq 0.2$  were strongly associated with blood culture proven neonatal sepsis. Gram-negative bacteria showed high level of resistance to commonly used antibiotics. Ciprofloxacin was the most effective drug when compared to other drugs tested against the gram-positive and gram-negative bacteria. Multi-drug resistance detected in 67.4% isolates. The detection of multi-drug resistant isolates may further limit therapeutic options. Routine bacterial surveillance and the study of their resistance patterns must be an essential component of neonatal care. A knowledge of these patterns is essential when local policies on the uses of antibiotics are being devised.

**Key words:** Neonatal sepsis, clinical features, bacterial pathogens, risk factors, antimicrobial susceptibility pattern, Ethiopia

## CHAPTER I: INTRODUCTION

### 1.1. Introduction

Neonatal sepsis remains as an important cause of morbidity and mortality among infants in developing countries accounting for 30-50% of total deaths each year (Bang *et al.*, 2005). It has been classified as either early onset sepsis (EOS) (0-7 day of age) or late onset sepsis (LOS) (7-28 days of age) (Kaftan and Kinney, 1998; Vergnano *et al.*, 2005). A few papers distinguish between very early onset (within 24 hours), EOS (24 hours to six days), and LOS (more than six days to 30days) sepsis (Tallur *et al.*, 2000; Stoll *et al.*, 2002). EOS is due to vertical transmission during labor or birth. It includes bacteremia and/or sepsis, meningitis and pneumonia. LOS is due to vertical, horizontal or nosocomial infection. Its most clinical manifestations are: meningitis (30-40%), bacteremia (40%), septic arthritis (5% to 10%), and, more rarely, omphalitis and osteomyelitis. The reported incidence of neonatal sepsis varies from 7 to 38 per 1000 live births in Asia (Lim *et al.*, 1995), from 6.5 to 23 per 1000 live births in Africa (Airede, 1992; The WHO multicentre study group, 1999), and from 3.5 to 8.9 per 1000 live births in South America and the Caribbean (Moreno *et al.*, 1994; Robilland *et al.*, 1993). By comparison, rates reported in the United States and Australasia range from 6–9 per 1000 live birth (Hyde *et al.*, 2002; Heath *et al.*, 2003) and in Europe 0.3-3 per 1000 live births (Vesikari *et al.*, 1985). In most developing countries, gram-negative bacteria remain the major cause of neonatal sepsis (Anwer *et al.*, 2000; Joshi *et al.*, 2000). These organisms have developed increased drug resistance over the last two decades (Bhutta and Yusuf, 1997) and management of neonates with sepsis has become a major problem (Musoke and Revathi, 2000). On the other hand Group B *Streptococcus* (GBS) has been the most frequent ethological agent of neonatal sepsis in developed countries, being responsible for high morbidity and mortality rates (Freedom *et al.*, 1981).

In Ethiopia previous studies showed that pneumonia, sepsis, meningitis, were common causes of admissions and deaths in Pediatrics Hospital, Addis Ababa, Ethiopia (Tafesse, 1973; Tafari *et al.*, 1976; Ghiorghis, 1991 and 1997; Muhe *et al.*, 1999). The incidence of neonatal sepsis ranged from 4.2-11 per 100 live born babies and the leading etiologic agents were gram-negative bacteria in the same hospital (Tafari *et al.*, 1976; Tafari and Ljungh, 1980; Ghiorghis, 1997). Since the spectrum of organisms that cause neonatal sepsis changes overtime and varies from region to region and hospital to hospital even in the same

city/country, it is necessary to conduct periodic surveillance to access the changing pattern of organisms causing neonatal sepsis. Based on this consideration, a cross-sectional prospective study was carried out to investigate the causative bacterial organisms of neonatal sepsis and to assess their antibiotic susceptibility pattern during the period of October 2006 and March 2007 in the neonatal unit of Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

## 1.2. Literature Review

### 1.2.1. Definition and Classifications of Neonatal Sepsis

#### a. Neonatal sepsis

Neonatal sepsis may be defined both clinically as shown in Table 1.1 and/or microbiologically, by positive blood and/or cerebrospinal fluid/urine cultures.

Table 1.1. Clinical criteria for diagnosis of neonatal sepsis (Adapted from Vergnano *et al.*, 2005)

	IMCI* Criteria for Severe Bacterial Infection**	WHO Young Infant Study group
Convulsions	X	X
Respiratory rate > 60 breaths/min	X	X (divided by age group)
Severe chest in drawing	X	X
Nasal flaring	X	
Grunting	X	
Bulging fontanel	X	
Pus draining from the ear	X	
Redness around umbilicus extending to the skin	X	
Temperature >37.7°C or <35.5°C	X	X
Lethargic or unconscious	X	X (not caused by minimal stimulus)
Reduced movements	X	X (change in activity)
Not able to feed	X	X (not able to sustain suck)
Not attaching to breast	X	
No sucking at all	X	
Crepitations		X
Cyanosis		X

\* IMCI: Integrated Management of Childhood Illness

\*\*Any of the signs of listed implies high suspicion of severe bacterial infection

#### b. Classifications of neonatal sepsis

Neonatal sepsis may be classified according to the time of onset of the disease: early onset (EOS) and late onset (LOS) (Kaftan and Kinney, 1998; Vergnano *et al.*, 2005). The

distinction has clinical relevance, as EOS disease is mainly due to bacteria acquired before and during delivery, and LOS disease to bacteria acquired after delivery (nosocomial or community sources). In the literature, however, there is little consensus as to what age limits apply, with EOS ranging from 48 hours to 6 days after delivery. This makes it difficult to compare studies where cases are grouped into EOS and LOS without further details (Haque, 2007). Those studies using longer definitions will incorporate a larger proportion of cases where the organism is acquired horizontally, from nosocomial or community sources, rather than as a result of vertical transmission. Different practices of care can therefore impact on these rates—for example; hospitals with early discharge policies may expose infants to community infections, and those with late discharge policies to nosocomial infections. Studies based in hospitals with early discharge will probably report lower rates of late-early or LOS infection, especially if infants presenting from the community are not incorporated into analyses. A few papers distinguish between very early onset (within 24 hours), EOS (24 hours to six days), and LOS (more than six days) sepsis (Tallur *et al.*, 2000; Karunasekera and [Pathirana \*et al.\*](#), 1999).

### **1.2.2. Routes of Infection**

Routes of infection and organisms causing neonatal sepsis are summarized in Table 1.2.

#### **a. Early onset sepsis (EOS)**

Transplacental, hematogenous transmission of bacteria is an uncommon route of EOS and occurs primarily with *Listeria (L. monocytogenes)* (Soman *et al.*, 1985). The most common route of EOS in preterm and term infants is via an ascending amniotic infection. Members of the maternal genital flora, such as GBS and *Escherichia coli (E. coli)*, the organisms responsible for the majority of cases of EOS, may ascend through the birth canal to the amniotic fluid either through intact amniotic membranes or, more commonly, after rupture of membranes (Kaufman and Fairchild, 2004). Once infected amniotic fluid is aspirated or swallowed by the fetus, pathogens may penetrate through immature mucosal barriers, resulting in pneumonia or bacteremia, and may penetrate the blood-brain barrier, leading to meningitis. Bacteria have been implicated as a major cause of premature rupture of membranes and, consequently, of premature labor and delivery (Klein, 1992; McGregor *et al.*, 1990). Thus, prevention and timely treatment of intra-amniotic infection are important steps in preventing preterm delivery and improving neonatal outcome. EOS includes

bacteremia and/or sepsis, meningitis and pneumonia (The WHO multicentre study group, 1999; Raad *et al.*, 1997).

**b. Late onset sepsis (LOS)**

LOS most commonly occurs via horizontal or nosocomial transmission, but it may occur via vertical transmission at birth, leading to colonization and, later, to infection (Jiang *et al.*, 2004). Skin or mucosal colonization with potential pathogens may be acquired from the hands of health care workers or family members, from water used in incubator or ventilator humidification systems, or from contaminated fomites such as stethoscopes, which may carry organisms directly from one patient to another (Benirschke, 1960). Colonizing organisms may enter the bloodstream through breaks in the skin or mucosa or by gastrointestinal translocation or may be introduced through invasive devices such as vascular catheters, endotracheal tubes, or feeding tubes. Alternately, nosocomial infection may result from infusion of contaminated intravenous solutions (especially lipid-based or high-glucose solutions) or from contaminated formula or breast milk. In LOS most common clinical manifestations are: meningitis (30-40%), bacteremia (40%), and septic arthritis (5-10%) (Baltimore, 1998; Wolach, 1997).

**1.2.3. Organisms Causing Neonatal Sepsis**

**I. Bacteria**

**a. Developing countries**

The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. Overall, Gram negative organisms are more common and are mainly represented by *Klebsiella*, *E. coli*, *Pseudomonas*, and *Salmonella* spp. (Karthikeyan and Premkumar, 2001; Tallur *et al.*, 2000). Of the Gram positive organisms, *Staphylococcus aureus* (*S. aureus*) (Karthikeyan and Premkumar, 2001; Bhutta and Yusuf, 1997), coagulase negative staphylococci (CoNS) (Malik and Pennie, 1994), *Streptococcus pneumoniae* (*S. pneumoniae*) (Muhe *et al.*, 1999) and *Streptococcus pyogenes* (*S. pyogenes*) (Muhe *et al.*, 1999; Gatchlian *et al.*, 1999) are most commonly isolated. *E. coli*, *Enterobacter*, *Enterococcus*, and *Listeria* spp. are mostly associated with EOS disease. *Klebsiella*, *Acinetobacter*, and *S. aureus* are associated with EOS and LOS. *Pseudomonas*, *Salmonella*, and *Serratia* spp. are more often associated with LOS disease. CoNS are found in both. There appears to be a wide variety of bacteria causing EOS and LOS sepsis in developing countries. Group B streptococcus (GBS) is generally rare (Kuruvilla *et al.*, 1999;

Lim *et al.*, 1997) or not seen at all (Daoud *et al.*, 1995), although maternal rectovaginal carriage rates of GBS may be similar to those recorded in developed countries (Stoll and Schuchat, 1994). In most of the African studies, the incidence of GBS is low (Mulholland *et al.*, 1999; Muhe *et al.*, 1999), with the exception of South Africa (Bomela *et al.*, 2001). In Asia, GBS is also reported to be extremely rare (Al-Harthi *et al.*, 2000; Gomaa *et al.*, 2001). In South America GBS incidence is comparable to the West (Robilland *et al.*, 1993). It is not known whether these differences reflect true differences in pathogens across the world, reflecting an epidemiological transition in some countries, or whether it reflects an epidemiological bias linked to the fact that most EOS babies in developing countries die at home before reaching the health facilities and they do not appear in the statistics.

#### **b. Developed countries**

Neonatal infection surveillance in developed countries generally identifies GBS and *E coli* as the dominant EOS pathogens and CoNS the dominant LOS pathogen followed by GBS and *S. aureus* (Hyde *et al.*, 2002; Isaacs, 2003). EOS disease is often more severe and case fatality rate is higher than it is for LOS disease. As the latter is usually caused by CoNS, the associated morbidity and mortality are low (Isaacs, 2003). This may not be the case in developing countries LOS has a higher case fatality rate, particularly when Gram negative bacteria are involved.

## **II. Fungi**

With increasing survival of smaller, more immunocompromised preterm infants, the incidence of invasive fungal infection is increasing among Neonatal Intensive Care Unit (NICU) patients, with high associated morbidity and mortality (Stoll *et al.*, 2002). The early NICU course favors colonization and proliferation of fungi since many Very Low Birth Weight (VLBW) Infants have central vascular catheters and are exposed to broad spectrum antibiotics and parenteral nutrition. The vast majority of fungal infections in preterm neonates are due to *Candida* species, with a small number being due to *Malassezia* and other rare fungi. *Candida* is an opportunistic pathogen; the major factor predisposing VLBW infants to colonization and invasive infection is the ability of *Candida* species to adhere to skin, mucosal, and catheter surfaces as commensal organisms. With damage to the skin or mucosal membranes and with the diminished immune defenses of preterm infants, *Candida* can disseminate to the bloodstream. Acute mortality from *Candida* infection may be associated with septic shock due to the production of endotoxin-like substances, hemolysin, and pyrogens by the organism (Marodi and Johnston, 2004). Finally, owing to its adherence

properties, *Candida* may seed tissues and form abscesses, which are difficult to eradicate (Hostetter, 1994, 1998, 1999).

Table 1.2. Relationship between time of onset of neonatal infection and mode of transmission of infection (Adapted from Baltimore, 2002)

<b>Time of onset</b>	<b>Age at onset of infection</b>	<b>Mode of transmission of infection</b>	<b>Major risk factors</b>	<b>Most common organisms</b>
Prenatal	Prior to birth	Transplacental or ascending	Maternal infection, usually primary infection Prolonged rupture of membrane (PROM)	Cytomegalovirus <i>Treponema palladium</i> <i>Toxoplasma gondii</i> Maternal vaginal flora HIV
Early-onset	Birth to 2-5 days	Maternal flora transmitted peripartum	Prolonged rupture of membrane Prematurity Septic or traumatic delivery Fetal anoxia Male gender Maternal infection (especially urogenital) Maternal poverty, pre-eclampsia, cardiac disease, diabetes mellitus	<i>Esherichia coli</i> Group B Streptococci <i>Klebsiella pneumoniae</i> <i>Enterococcus</i> spp. <i>Listeria monocytogens</i> Other enteric Gram-negative bacilli
Late-onset	>2-5 to 30 days	Nosocomial	Intravascular catheters Endotracheal intubation Assisted ventilation Surgery (including necrotizing entercolitis) Contact with hand of colonized personnel Contact with contaminated equipments	Those causing early onset sepsis <i>Staphylococcus aureus</i> Coagulase negative staphylococci <i>Pseudomonas aeruginosa</i> <i>Candida</i> spp.
Late, late-onset	>30 days	Nosocomial	Indwelling intravascular devices Extreme prematurity Bronchopulmonary dysplasia Short gut syndrome Complex congenital malformations Previous broad spectrum antibacterial therapy	<i>Staphylococcus aureus</i> Coagulase negative staphylococci <i>Pseudomonas aeruginosa</i> <i>Candida</i> spp. Resistant Gram-negative bacilli

#### **1.2.4. Epidemiology of Neonatal Sepsis**

World Health Organization (WHO) estimates that globally there are about 5 million neonatal deaths per year, ninety eight percent of them are occurring in developing countries in the first week of life (WHO, 1996). In developing countries, neonatal mortality (deaths in the first 28 days of life per 1000 live births) from all causes is about 34; most of these deaths occur in the first week of life, mostly on the first day (Costello *et al.*, 2001) (according WHO 2001 Estimates). In contrast, neonatal mortality for developed countries is in the region of five (Costello *et al.*, 2001). Neonatal mortality in Asia is about 34, in Africa about 42, and in Latin America and the Caribbean about 17 per 1000 live birth, although there are wide variations between different countries in these regions as well as within the countries themselves. For example, neonatal mortality for different African countries ranges from 68 in Liberia to 11 per 1000 live birth in South Africa (Costello *et al.*, 2001). Discrepancies will often be due to under-reporting: in some countries, babies, in particular those born preterm and small for dates, are not registered, because of registration fees, ignorance, or logistical difficulties. In some traditions, babies do not become part of the family until they are a few days or weeks old, therefore early deaths are not acknowledged (Bang *et al.*, 2001). It is generally assumed that neonatal mortality in developing countries is under-reported by at least 20% (WHO, 1996). The most common causes of death in the neonatal period are neonatal infections (32%), including meningitis, respiratory infections, diarrhoea, and neonatal tetanus, followed by birth asphyxia and injuries (29%), and prematurity (24%) (Costello *et al.*, 2001).

The reported incidence of neonatal sepsis varies from 7 to 38 per 1000 live births in Asia (Lim *et al.*, 1995; Tallur *et al.*, 2000), from 6.5 to 23 per 1000 live births in Africa (Airede, 1992; WHO multicentre study group, 1999), and from 3.5 to 8.9 per 1000 live births in South America and the Caribbean (Moreno *et al.*, 1994; Robilland *et al.*, 1993). By comparison, rates reported in the United States and Australasia range from 1.5 to 3.5 per 1000 for EOS sepsis and up to 6 per 1000 live births for LOS sepsis, a total of 6–9 per 1000 for neonatal sepsis (Hyde *et al.*, 2002; Heath *et al.*, 2003).

### **1.2.5. Risk Factors Associated with Neonatal sepsis**

#### **a. Intrauterine infections**

Procedures disturbing the integrity of the uterine contents, such as amniocentesis (Gibbs and Duff, 1991), cervical cerclage (Charles and Edwards, 1981), transcervical chorionic villus sampling (Fejgin *et al.*, 1993), or percutaneous blood sampling (Gibbs and Duff, 1991; Wilkins *et al.*, 1989), can permit entry of skin or vaginal organisms, causing amnionitis and secondary fetal infection. Certain bacteria, particularly *Treponema palladium* and *Listeria monocytogenes*, can reach the fetus through the maternal bloodstream despite placental protective mechanisms, causing transplacental infection (Benirschke, 1960). This process is uncommon, but it leads to either congenital infection not unlike infections caused by certain viruses or *Toxoplasma* or to stillbirth resulting from overwhelming infection.

#### **b. Intrapartum infections (Perinatal infections)**

Initial colonization of the neonate usually takes place after rupture of the maternal membranes (Klein, 1992). In most cases, the infant is colonized with the microflora of the birth canal during delivery. However, particularly if the rupture of membranes lasts longer than 24 h, vaginal bacteria may ascend and in some cases produce inflammation of the fetal membranes, umbilical cord, and placenta (St. Geme *et al.*, 1984). Fetal infection can result from aspiration of infected amniotic fluid (Yoder *et al.*, 1983), leading to stillbirth, premature delivery, or neonatal sepsis (Gibbs and Duff, 1991; St.Geme *et al.*, 1984; Hillier *et al.*, 1991). The organisms most commonly isolated from infected amniotic fluid are anaerobic bacteria, GBS, *E. coli*, and genital mycoplasmas (Gibbs and Duff, 1991; Hillier *et al.*, 1991). Infection of the mother at the time of birth, particularly genital infection, is the principal pathway of maternal transmission (Prober, 1997; Klein, 2001) and can play an important role in the development of infection in the neonate. Transplacental hematogenous infection during or shortly before delivery (including the period of separation of the placenta) is possible, although it seems more likely that the infant is infected during passage through the birth canal.

#### **c. Postnatal infection**

Finally, bacteria can be introduced after birth from the environment surrounding the baby; either in the nursery or at home. Sophisticated equipment for respiratory and nutritional support combined with invasive techniques provides life support to the ill infant. Arterial and venous umbilical catheters, central venous catheters, peripheral arterial and venous cannulas,

urinary indwelling catheters, hyperalimentation infusions, and assisted ventilation provide enormous opportunities for relatively non-virulent pathogens to establish infection and to invade the host (Sáez-Lioens and McCracken, 1998). Transient bacteremia may accompany procedures that traumatize the skin and mucosal membranes. In bacteremia identified in infants who received endotracheal suctioning; the bacteremia was present immediately after the procedure (Auriti *et al.*, 2003).

**d. Other risk factors**

Many pre-and intrapartum obstetric complications are associated with an increased risk of infection in new born infants. Among these are premature onsets of labor, prolonged rupture of the fetal membranes, uterine inertia with high forceps extraction, and maternal pyrexia (Sáez-Lioens and McCracken, 1998). Risk factors associated with neonatal sepsis in preterm infants are summarized in Table 1.3.

Table 1.3. Risk factors associated with neonatal sepsis in preterm infants (Adapted from Kaufman and Fairchild, 2004)

<b>Antimicrobial defense</b>	<b>Preterm infant compromised defense</b>
<b>Epidermal and epithelial barriers</b>	<p>Immature skin</p> <p>Insensible water loss from skin and humidification system creating moist skin that favors the growth of microorganism</p> <p>Invasive catheters and tubes</p> <p>Surface for colonization provided by intravascular catheters breaching intact epidermis</p> <p>Proliferation due to parenteral nutrition and biofilms on plastic catheters</p> <p>Colonization of endotracheal and nasogastric tube</p>
<b>Intact endothelial tissues</b>	<p>Trauma to endothelium and endocardium from central venous catheters</p> <p>Injury from hyperosmolar nutrition solutions and medication</p>
<b>Gastrointestinal mucosa</b>	<p>Decreased acid production</p> <p>Immature peristalsis and reduced absorption, favoring microorganism overgrowth</p> <p>Thin mucin layer, leading to decreased barrier function and secretory IgA binding</p> <p>Diminished number of intraepithelial lymphocytes</p>
<b>Microflora</b>	<p>Competitive bacterial flora diminished by broad-spectrum antibiotics</p> <p>More commensal gram-positive organism selected by human milk</p>
<b>Complement</b>	<p>Lower level with decreasing gestational age</p>
<b>Cytokines</b>	<p>Decreased production of IL-1, IL-8, gamma interferon, TNF-<math>\alpha</math>, G-CSF, and GM-CSF</p>
<b>Defensins</b>	<p>Diminished with decreasing gestational age</p>
<b>Neutrophils</b>	<p>Decreased bone marrow storage pool and G-CSF level</p> <p>Immature neutrophil oxidative burst</p>
<b>Monocyte</b>	<p>Diminished number and function</p> <p>Decrease adherence at site of infection</p> <p>Decreased opsonization and phagocytosis</p> <p>Diminished gamma interferon, IL-3 and G-CSF release</p>
<b>T-cells, B-cells and antibodies</b>	<p>Decreased number of lymphocytes in gastrointestinal tract</p> <p>Majority of transfer of maternal IgG after 32 weeks gestation</p> <p>Decreased production of antibodies by preterm lymphocyte</p>

### **1.2.6. Clinical Features of Neonatal sepsis**

The clinical features of neonatal sepsis are summarized in Table 1.1. Neonatal sepsis can be defined as the presence of positive cultures, whether in the blood, CSF, or urine associated with systemic clinical signs of infection such as fever, temperature instability, poor feeding and respiratory distress (Vergnano *et al.*, 2005.)

### **1.2.7. Diagnosis of Neonatal Sepsis**

The diagnosis of neonatal sepsis begins with clinical suspicion as shown in Table 1.1. The challenge for the neonatal practitioner is to decide which babies need empiric antibiotic therapy and for how long, a decision which is complicated by the common occurrence and nonspecific nature of sepsis-like symptoms in preterm infants.

#### **a. Blood culture**

The “gold standard” for diagnosing neonatal sepsis remains the blood culture, even though, in many cases, blood cultures are negative in the face of strong clinical indicators of septicemia and even in autopsy-proven disseminated bacterial or fungal infections. Maternal antibiotics given in the majority of preterm deliveries may suppress the growth of bacteria in culture, yet the neonate may have clinical symptoms and laboratory findings consistent with a diagnosis of sepsis. False-negative blood cultures in apparently septic neonates may also result from insufficient sample size. One study estimated that as many as 60% of blood cultures would be falsely negative for common neonatal pathogens if only 0.5 ml of blood is sampled in low-colony-count (4 CFU/ml) sepsis (Kellogg *et al.*, 1997). While neonates are commonly thought to have high-colony-count bacteremia compared with adults, as many as half of the neonates in one study were found to have low-colony-count bacteremia (Kellogg *et al.*, 1997). Furthermore, in a prospective study of nearly 300 blood cultures drawn from critically ill neonates, 55% of culture vials contained less than 0.5 ml of blood (Neal *et al.*, 1986). Technical difficulties associated with phlebotomy in small, sick preterm neonates often limit the volume of blood obtained and thus decrease the sensitivity of blood culture for diagnosing sepsis in this population.

#### **b. Adjunct laboratory tests used for diagnosis of neonatal sepsis**

A number of adjunctive tests, including measurements of serum interleukin-6 (IL-6) (Dollner *et al.*, 2001; Ng *et al.*, 2003), IL-8 (Franz, 2001), procalcitonin (Kordek *et al.*, 2003), and C-Reactive Protein (Chan and Ho, 1997; Gerdes, 1991) levels and heart rate characteristics

(Griffin and Moorman, 2001; Griffin *et al.*, 2003) have been studied for their ability to predict sepsis particularly in preterm neonates with clinical signs and symptoms of infection.

**c. Non-culture microbiological methods for predicting neonatal sepsis**

**1. Antigen detection**

Given the inherent problems with using blood culture as the sole method of detecting septicemia in premature neonates, other non-culture microbiologic methods have been developed. Antigen detection in the urine or CSF has been used in the past as an adjunctive test for the presence of GBS. The sensitivity of latex particle agglutination compared to culture for the detection of invasive GBS disease has been reported at 72 to 89% for CSF and slightly lower for urine (Baker and Rench, 1983; Kumar *et al.*, 1980). However, a number of studies have shown that GBS antigen is often detectable in the urine in the absence of a positive blood culture, possibly due to surface colonization (Sanchez *et al.*, 1990), absorption of GBS antigen from the gastrointestinal tract (Palmer *et al.*, 1996), or maternal treatment with antibiotics to prevent neonatal bacteremia (Harris *et al.*, 1989). Thus, while the sensitivity and negative predictive value of urine GBS latex agglutination are high, false-positive rates as high as 30% have limited its usefulness as a screening tool for sepsis in neonates, and many laboratories have discontinued testing for urine GBS antigen (Williamson *et al.*, 1995).

**2. Molecular diagnostics**

Polymerase chain reaction (PCR) has proved to be a valuable adjunct for detection of neonatal viral infections such as human immunodeficiency virus, herpes simplex virus, and hepatitis C virus when used in conjunction with other diagnostic testing such as serologic testing or culture. However, the use of PCR to detect bacteremia and fungemia is more challenging and thus is still under investigation. Detection of bacterial DNA in the blood has been accomplished by PCR amplification of the gene for 16S rRNA, a gene universally present in bacteria but absent in humans. Detection of as few as 10 organisms per ml of whole blood has been reported, and research into how to enhance the sensitivity and automate the PCR is under way (McCabe *et al.*, 1995). A major concern about bacterial PCR is possible contamination due to the widespread presence of bacterial DNA in the environment, which may be a major stumbling block to clinical applications. However, several studies with neonates have shown promising results (Kaufman and Fairchild, 2004).

### **1.2.8. Management of Neonatal Sepsis**

#### **a. Antimicrobial therapy**

The mainstay of empiric therapy for EOS for both term and preterm infants in most centers is Ampicillin and Gentamicin, pending blood culture results. The emergence of Gentamicin-resistant gram-negative bacteria in some centers may prompt the use of other aminoglycosides. In cases of known or suspected sepsis due gram-negative bacteria, and particularly if meningitis is present, addition of a broad-spectrum cephalosporin may be beneficial (Aurangbezeb and Hameed, 2003; Mokulu *et al.*, 2002).

For empiric treatment of suspected LOS presenting after 3 to 7 days of age, nafcillin or oxacillin and an aminoglycoside may provide sufficient initial coverage. Most CoNS are resistant to beta-lactam antibiotics and many centers use empiric Vancomycin for LOS (Aurangbezeb and Hameed, 2003; Lee *et al.*, 2004). When choosing treatment for LOS, consideration should also be given to the possibility of *Pseudomonas* and to non-bacterial etiologies such as *Candida* and herpes simplex virus. These infections are becoming increasingly common among preterm infants and are associated with very high morbidity and mortality. In addition, in neonates with intestinal perforation, anaerobic coverage may be appropriate pending the results of blood and peritoneal fluid cultures. The vast majority of true bacterial pathogens are detected in blood culture within 48 h, and in the absence of other strong clinical indicators of infection, empiric antibiotic use may be discontinued at this time (Kawamura and Nishida, 1995; Wasunna *et al.*, 1990). Once a pathogen is identified, antibiotic coverage should be narrowed based on susceptibility testing. Repeat culture is important for documenting adequate treatment and determining the duration of therapy. Treatment of bacteremia due to gram-positive organisms has traditionally lasted 7 to 10 days, although shorter courses may be adequate for uncomplicated CoNS bacteremia. Bacteremia due to gram-negative organisms, deep-seated infections, abscesses, meningitis, endocarditis, and osteomyelitis require longer courses of antibiotic treatment.

Resistance to commonly used antimicrobials is emerging and constitutes an important problem worldwide. Reports of multi-resistant bacteria causing neonatal sepsis in developing countries are increasing, particularly in intensive care as shown in Tables 1.4 and 1.5. *Klebsiella* and *Enterobacter* species are often reported in this context (Arias *et al.*, 2003). Spread of resistant organisms in hospitals is a recognized problem, although babies admitted from the community may also carry resistant pathogens (Bhutta, 1996). The wide availability of over the counter antibiotics and the inappropriate use of broad spectrum antibiotics in the

community may explain this. More studies are needed to compare patterns of resistance in babies born in and out of hospital.

Table 1.4. Pattern of resistance of Gram positive bacteria to the most commonly used antibiotics in developing countries (Adapted from Vergnano *et al.*, 2005)

	% of Resistance	
	<i>S. aureus</i>	<i>Streptococci</i>
Penicillin	63-93	0-100
Cloxacillin	64-90	
Ampicillin	40-98	0-100
3 <sup>rd</sup> generation cephalosporin		
Ceftizoxime	50-67	
Ceftazidime	8-63	
Ceftriaxone	5-59	
Erythromycin	12 to >90	0-30
Gentamicin	4-76	
Amikacin	13-62	
Co-trimoxazole	30-83	0-58
Ciprofloxacin	3-45	
Chloramphenicol	30-60	0-33
Methicillin	64-85	

Table 1.5. Pattern of resistance of Gram negative bacteria to the most commonly used antibiotics in developing countries (Adapted from Vergnano *et al.*, 2005)

	% of Resistance				
	<i>Klebisella spp</i>	<i>Pseudomonas spp</i>	<i>Acinetobacter</i>	<i>Citrobacter</i>	<i>E.coli</i>
Penicillin				100	100
Ampicillin	65-100	75-100	88-100	100	69-100
3 <sup>rd</sup> generation cephalosporin					
Ceftizoxime	0-86	10-100	4-84	0	0-75
Ceftazidime	66-95	56-90			0-67
Ceftriaxone	66-96	12-100			0-56
Erythromycin	87	100		100	
Gentamicin	16-85	0-79	3-73	0-40	30-93
Amikacin	0-74	23-100	14-79	0	0-67
Co-trimoxazole	22-97	33-67	30-65	17	12-62
Ciprofloxacin	0-36	0-49	0-7	5	15-56
Chloramphenicol	49-100	40-93	87-96	50	23-100
Methicillin	0-6	0-48			0

**b. Adjunct therapies**

VLBW infants may require blood pressure support with crystalloid, blood products, and vasopressors. In VLBW infants with hypotension unresponsive to conventional pressors, a short course of stress dose hydrocortisone has been shown to raise the blood pressure, although effects on the short- or long- term outcome have not been established (Seri *et al.*, 2001; Watterberg, 2002). Use of hydrocortisone plus a fluorinated steroid improved the outcome in septic adults with low cortisol levels but worsened the outcome if cortisol levels were high (Annane *et al.*, 2002). Consideration should be given to evaluating the serum cortisol level prior to initiating therapy with stress dose hydrocortisone in hypotensive preterm infants.

Several small trials and a meta-analysis of IVIG treatment for suspected neonatal sepsis have shown a small reduction in mortality among patients with subsequently culture-proven sepsis (Ohlsson and Lacy, 2001); however, the majority of patients in these studies were term neonates. A recent meta-analysis of several small randomized trials has also shown that granulocyte colony- stimulating factor (G-CSF) treatment may reduce mortality in neutropenic septic neonates (both term and preterm) (OR, 0.34, 95% CI, 0.12 to 0.92) but not

in neonates with normal neutrophil counts (Banerjea and Speer, 2002; Carr *et al.*, 2003). Recombinant activated protein C (Dalton, 2003) and PAF acetylhydrolase (Schuster *et al.*, 2003) have shown promise in reducing mortality in adults with sepsis but have not been tested in neonates. Although there is overlap in the pathophysiology of sepsis in adults and neonates, there are significant differences, and one cannot assume that therapies proven efficacious in adults will improve the outcome of septic preterm infants.

**c. Preventive measures**

Newborn infants are especially vulnerable to nosocomial infections because of their intrinsic susceptibility to infection as well as the invasive procedures to which they are subjected. This is particularly so for those born prematurely or of low birth weight. To plan effective strategies to reduce the burden of neonatal sepsis, it is essential to define the sources of infection. Therefore continuous surveillance is essential (Adams-Chapman, 2002).

Preventive measures need to be implemented as follows: -

- I. Hand washing has been shown to be effective ever since the 19<sup>th</sup> century and several guidelines are available (Boyce and Pittet, 2002). Unfortunately, across the world, implementation of correct hand washing protocols has been difficult, even in optimal conditions.
- II. Health personnel require education, continuous reminding, and feedback if compliance is to be maintained (Chandra and Milind, 2001).
- III. In developing countries, further obstacles to the implementation of hand washing include the lack of water, soap, and sink in the nurseries, low level of staffing and consequently low morale and overcrowding. Bedside dust is ubiquitous and difficult to deal with. Studies looking at early discharge policies for the low risk newborns as a means of reducing staff workload and exposure to nosocomial infection need to be undertaken.
- IV. Minimizing invasive procedures has also shown an impact in reducing nosocomial infections. Fewer venepunctures and intravenous catheters minimize the risk of infection. As Gentamicin is part of the first line antibiotics in most neonatal units across the world, a number of studies have emphasized that it can be safely and effectively administered once a day to newborn (Hansen *et al.*, 2003). This results in fewer procedures to the newborn and reduces the workload of nursing staff.

- V. Skin preparation before procedures has been shown to be effective, but studies are needed on the exact procedures and antiseptic to be used. The importance of appropriate sterilization procedures for the equipment used in neonatal units also needs to be emphasized (Adams-Chapman, 2002).
- VI. The impact of using antiseptic solution to disinfect the birth canal on the incidence of neonatal sepsis needs to be further explored (Taha *et al.*, 1997). Possible advantages deriving from changes in neonatal unit practice such as implementation of strict antibiotic policy and restriction of admissions to neonatal units also need to be investigated.
- VII. Close involvement of mothers (Mother-infant cohorting) in the care of high risk infants reduces infectious complications, improves rates of breast feeding, and allows early discharge from hospital (Bhutta *et al.*, 1998). Another important step in reducing neonatal mortality is the prompt recognition of infection and appropriate referral in suspected cases. The simplest strategy for decreasing nosocomial sepsis and the most difficult with which to achieve compliance is good hand hygiene (Vergnano *et al.*, 2005; Clark *et al.*, 2004).
- VII. The most effective intervention in reducing the risk of neonatal infection globally is exclusive breast feeding. Even in circumstances with high rates of maternal HIV, the benefits of exclusive breastfeeding may far outweigh the risk of viral transmission, and in many circumstances this may be the only life-saving measure available. In developing countries a number of studies have shown the benefit of colostrums and maternal breast milk feeds in reducing the rates of neonatal infection in both normal and high-risk LBW infants (Narayanan *et al.*, 1981, Narayanan *et al.*, 1983).

### 1.2.9. Significance of the Study

Neonatal sepsis is one of the most common reasons for admission and death in neonates in developing countries (Bang *et al.*, 2005). Gram negative organisms remain the major causes of neonatal sepsis, particularly early onset of neonatal sepsis (Anwer *et al.*, 2000; Joshi *et al.*, 2000). These organisms have developed increasing resistance to the commonly used antibiotics over the last two decades due to the indiscriminate and inappropriate use of antibiotics, over the counter sale of antibiotics and lack of antibiotic policy, poor sanitation and infective control in the maternity services (Bhutta and Yusuf, 1997; Vergnano *et al.*, 2005).

Ethiopia is one of the developing countries with an estimated infant mortality rate (IMR) of 77 per 1000 live birth (MOH, 2004/2005). The top ten leading causes of outpatient visits, admissions and deaths among infants in Ethiopia are summarized in Tables 1.6, 1.7 and 1.8, and most of these clinical conditions can be associated with sepsis.

Analysis of admissions of the Pediatrics Hospital showed that pneumonia accounts for 11% of all admissions and 7% of hospital deaths (Tafesse, 1973). Sepsis and meningitis were also common causes of admissions and deaths in this hospital (Tafesse, 1973; Tafari *et al.*, 1976; Ghiorghis, 1991 and 1997; Muhe *et al.*, 1999). The incidence of neonatal sepsis ranged from 4.2-11 per 100 live born babies and the leading etiologic agents were gram negative bacteria, mainly *Klebsiella* spp. (Tafari *et al.*, 1976; Tafari and Ljungh, 1980; Ghiorghis, 1997). Another study conducted by Muhe *et al.* (1999) in the same hospital showed that the traditionally known acute respiratory pathogens *S. pneumoniae*, *H. influenzae* and *S. pyogenes* were the commonest caused of pneumonia, sepsis and meningitis in infants younger than three month of age. Equally important was the finding that *Salmonella* group B was also a common pathogen in the same study population. These findings showed that the pattern of bacterial etiologic agents even can vary from time to time in the same hospital.

A retrospective record review conducted in Gondar University Teaching Hospital, North West Ethiopia showed that among 304 neonates admitted to the pediatrics ward from September 1994 to 31 August 1999, sepsis was diagnosed clinically in 228 (75%) neonates, while pneumonia was diagnosed in 84 (28%) neonates (Woldehanna and Idejene, 2005). The neonatal mortality seen in this hospital during the period of the study was unacceptably high. It is a well known fact that the spectrum of organisms that cause neonatal sepsis changes overtime and varies from region to region. It can even vary from hospital to hospital in the

same city. This is due to the changes in life style and changing pattern of antibiotic use. Therefore this study was undertaken to investigate bacterial agents of neonatal sepsis and their antibiotic susceptibility pattern and to assess risk factors associated with neonatal sepsis in Pediatrics Hospital, Addis Ababa, Ethiopia. Findings from study will help to assess changes in the pattern etiologic agents and their sensitivity pattern through time by comparing the results of the previous studies done in Ethiopia and elsewhere in the world. Results from this study will also provide update information for appropriate management of neonatal sepsis.

Table 1.6. Top ten leading causes of outpatient visits in infants in Ethiopia (Adapted from FDRE, MOH, 2002/2003)

Rank	Diagnosis	Cases	%
1	All types of malaria	162,711	19.03
2	Primary atypical other and unspecified pneumonia	110,034	12.87
3	Acute upper respiratory infections	75,841	8.87
4	Helminthes	55,970	6.55
5	Gastro-enteritis and colitis	55,164	6.45
6	Tuberculosis	53,929	6.31
7	Infections of skin and subcutaneous tissue	32,510	3.80
8	Dysentery	32,503	3.80
9	Inflammatory disease of eye (except trachoma)	28,569	3.34
10	Hypertrophy of tonsils and adenoids	28,368	3.32

Table  
1.7.  
Top

ten leading causes of admissions for infants in Ethiopia (Adapted from FDRE,MOH,2004/2005)

Rank	Diagnosis	Cases	%
1	All types of malaria	4655	17.79
2	Lobar pneumonia	2185	8.35
3	Gastro-enteritis and colitis	1222	4.67
4	Pyrexia of unknown origin (Fever)	1116	4.27
5	Bronchopneumonia	1030	3.94
6	Tuberculosis	515	1.97
7	Dysentery	501	1.91
8	Acute upper respiratory infections	360	1.38
9	Malnutrition	304	1.16
10	Chronic other and unspecified Nephritis	288	1.10

Table 1.8. Top ten leading causes of deaths for infants in Ethiopia (Adapted from FDRE, MOH, 2004/2005)

Rank	Diagnosis	Cases	%
1	All types of malaria	608	28.10
2	Bronchopneumonia	521	24.08
3	Gastro-enteritis and colitis	178	8.23
4	Tuberculosis	126	5.82
5	Dysentery	49	2.26
6	Other infections of newborn	34	1.57
7	Kwashiorkor	24	1.11
8	Pyrexia of unknown origin (Fever)	21	0.97
9	Tetanus	16	0.74
10	Pernicious Anaemia	12	0.55

### **1.3. OBJECTIVES OF THE STUDY**

#### **General Objective**

- To determine the pattern of bacterial agents responsible for neonatal sepsis in Pediatrics Hospital, Addis Ababa, Ethiopia.

#### **Specific Objectives**

- To isolate and identify the bacterial etiologic agents responsible for neonatal sepsis
- To compare and contrast the prevalent bacterial pathogens isolated from early onset neonatal sepsis (EOS) and late onset neonatal sepsis (LOS)
- To access risk factors associated with neonatal sepsis
- To determine the susceptibility pattern of isolates to the commonly used antimicrobial agents in the treatment of sepsis

## **CHAPTER II. MATERIALS AND METHODS**

### **2.1. Study Design and Area**

A cross-sectional prospective study was conducted at the Department of Pediatrics and Child Health Hospital, Addis Ababa, Ethiopia.

### **2.2. Study Population**

During the period of October 2006 and March 2007 a total of 302 neonates (0 to 28 days of age) admitted with suspected cases of early onset sepsis (0-7 days of age, n=298) and late onset sepsis (>7-28 days of age, n=4) were investigated. Written informed consent was obtained from their parents/guardians. Following detailed clinical examination, neonates with suspected sepsis having any one of the clinical symptoms and signs as outlined in Table 1.1 and appendix I were investigated for bacterial etiologic agents. Admitted neonates who don't fulfill the above clinical criteria were excluded from the study.

Demographic, clinical and other relevant data were obtained by attending pediatrician/s and were transferred to the questionnaire prepared for this study (see appendix I).

The sample size (n) was calculated by taking prevalence of culture proven neonatal sepsis approximately 23.9% in previous Ethiopian study (Ghiorghis, 1997). The expected margin of error (d) was 0.05 and the confidence interval ( $Z\alpha/2$ ) was 95%. Contingency for the unknown circumstance was 10%.

$$n = \frac{(Z\alpha/2)^2 * P(1-P)}{d^2} \quad n = \frac{(1.96)^2 * 0.239(0.761)}{(0.05)^2} = 279 + 10\% = 306$$

### **2.3. Sample Collection, Handling and Transport**

Using aseptic technique by applying Povidone iodine and 70% alcohol at the site of vein puncture, 2 ml venous blood was drawn from the antecubital or femoral vein by the attending nurse. One ml blood was inoculated into Tryptone Soy Broth (TSB) (Oxoid, Hampshire, England, UK) for culture and the remaining 1 ml blood was used for white blood cell count and differential. The specimens were transported within one hour to the Core Laboratory of Faculty of Medicine, Addis Ababa University.

Of the 302 admitted neonates, 91 (30.1%) received a combination of antibiotics on the day of admission before blood sample was collected. Of these, 81 (89.0%) received Ampicillin + gentamicin, 6 (6.6%) Ceftriaxone + Gentamicin and Ampicillin + Cloxacillin 4 (4.4%).

#### **2.4. Culture and Identification**

All blood culture were incubated aerobically at 37°C and inspected daily for 7 days for presence of visible microbial growth by observing any of one of the following: turbidity, haemolysis, gas production and coagulation of broth. For blood cultures that showed signs of microbial growth, subcultures were made onto blood, chocolate and MacConkey agar (Oxoid, Ltd). The blood and MacConkey agar plates were incubated in aerobic and chocolate agar in microaerophilic atmosphere using a candle jar at 37°C for 24-48 hrs. All positive blood cultures were identified by their characteristic appearance on their respective media, gram-staining reaction and confirmed by the pattern of biochemical reactions using the standard method (Cheesbrough, 2001). Members of the family enterobacteriaceae were identified by indole production, H<sub>2</sub>S production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization tests and other tests using API 20E identification kits (Biomerieux, France). For gram-positive bacteria coagulase, catalase, bacitracin and optochin susceptibility tests, and other tests were used. Blood culture broth which showed no microbial growth within 7 days was reported as culture negative, only after result of routine subculture on blood, MacConkey and chocolate agar.

#### **2.5. Antimicrobial Susceptibility Testing**

Antimicrobial susceptibility testing was performed for all blood culture isolates according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) by disk diffusion method (NCCLS, 2000).

From a pure culture 3-5 selected colonies of bacteria was taken and transferred to a tube containing 5 ml sterile normal saline and mixed gently to a homogenous suspension and incubated at 37°C until the turbidity of the suspension become adjusted to a McFarland 0.5.

A sterile cotton swab was used and the excess suspension was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller Hinton agar (Oxoid).

The inoculated plates were left at room temperature to dry for 3-5 minutes and a set of 16 antibiotic discs (Oxoid) were then placed on the surface of a Muller-Hinton plate. The drugs

for disc diffusion testing were in the following concentrations: Ampicillin (AMP) (10 µg), Amoxicillin-Clavulanic Acid (AMC) (30 µg), Ceftriaxone (CRO) (30 µg), Cephalothin (KF) (30 µg), Ciprofloxacin (CIP) (5 µg), Chloramphenicol (C) (30 µg), Doxycycline (DO) (30 µg), Erythromycin (E) (15 µg), Gentamicin (CN) (10 µg), Kanamycin (K) (30 µg), Methicillin (MET) (5 µg), Penicillin (P) (10 units), Streptomycin (S) (10 µg), Tetracycline (TE) (30 µg), Trimethoprim-Sulphamethoxazole (SXT) (25µg) and Vancomycin (30 µg). Penicillin, Vancomycin, Erythromycin and Methicillin were tested only for Gram-positive bacteria.

The plates were then incubated at 37°C for 24-48 hours. Diameters of the zone of inhibition around the disc were measured to the nearest millimeter using an electronic digital caliper, and the isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the NCCLS (2000).

High, intermediate and low level of resistance is defined when the percentage of resistance is >80%, 60-80% and < 60% respectively.

## **2.6. White Blood Cell and Immature Neutrophil Count**

Peripheral blood smears were prepared and stained by Wright's stain as described by Dacie and Lewis (1994). White Blood Cell count, differential count and immature neutrophil count were performed then Immature/Total neutrophil count (I/T) ratio was calculated.

## **2.7. Reference Strains**

*P. aeruginosa* (ATCC-27853), *S. aureus* (ATCC-25923) and *E. coli* (ATCC-25922) were used as a quality control throughout the study for culture and antimicrobial susceptibility testing.

## **2.8. Statistical Analysis**

Data entry and analysis was done using SPSS version computer 13 software. Comparisons were made using Chi-square test with Fisher exact tests. A *p*-value of <0.05 was considered indicative of a statistically significant difference. Odds ratio and chi-square tests were used to determine presence of association between risk factors and culture results. Logistic regression was used to explain the dependent variable based on the independent variable.

## **2.9. Ethical Consideration**

The M.Sc. research project proposal was approved by the Department of Microbiology, Immunology and Parasitology, ethically cleared by the Faculty Research Publication

Committee-II (FRPC-II) and endorsed by the Faculty of Medicine Academic commission. Official permission from the study site was obtained.

Written informed consent was obtained from parents/guardian's of neonates who participated in this study (see Appendix II).

## CHAPTER III. RESULTS

### 3.1. Study Population

A total of 302 admitted neonates (0 to 28 days of age) with suspected cases of sepsis were investigated for bacterial infection between October 2006 and March 2007. The age and sex distribution of neonates with suspected sepsis investigated for bacterial infection are presented in Table 3.1. Of the 302 patients, 140 (46.4%) were males and 162 (53.6%) were females ( $p > 0.05$ ) resulting in an overall male to female ratio of 0.8:1. The mean age of the neonates was  $1.23 \pm 8.96$  days and 70.2% of neonates were below age of 1 day. A total of 298 (98.7%) neonates presented with EOS and four (1.3%) presented with LOS (Table 3.1). Among the neonates with EOS, 139 (46.6%) were males and 159 (53.4%) were females ( $p > 0.05$ ). Among neonates with LOS, 1 (25.0%) was male and 3 (75%) were females ( $p > 0.05$ ).

[Table 3.1 Age and sex distribution 302 neonates with suspected sepsis investigated for bacterial infections at Pediatrics Hospital, Addis Ababa, Ethiopia \(October 2006 and March 2007\)](#)

Category			
	Neonates with EOS (0-7 days of age)	Neonates with LOS sepsis ( >7-28 days of age)	Total (0-28 days of age)
	No. (%)	No (%)	No (%)
Male	139 (46.6)	1 (25.0)	140 (46.4)
Female	159 (53.4)	3 (75.0)	162 (53.6)
<b>Total</b>	<b>298 (98.7)</b>	<b>4 (1.3)</b>	<b>302 (100.0)</b>

EOS: Early-onset sepsis

LOS: Late-onset sepsis

### **3.2. Maternal and Neonatal Characteristics**

Maternal and demographic data on 302 neonates with sepsis are shown in Table 3.2. Of the 302 neonates, 173 (57.3%) were preterm (gestational age less than 37 weeks), 299 (99.0%) were born in the health care institutions (hospital/health centers/clinics), 140 (46.3%) delivered by caesarian section/instrument. Approximately, 189 (62.6%) neonates with sepsis had low birth weight (<2500 g), out of these 54 (28.6%) had very low birth weight (< 1500 g). The mean birth weight and gestational age of the study population were  $2280 \pm 680$  gram and  $36.3 \pm 16$  weeks, respectively. In the first and fifth minutes after birth, respectively, 182/244 (74.5%) and 157/244 (64.3%) of neonates had an Apgar score less than or equal to 7.

**Table 3.2.** Maternal and Neonatal data of the 302 neonates investigated for sepsis [at Pediatrics Hospital, Addis Ababa, Ethiopia](#) (October 2006 and March 2007)

Characteristics	No. (%)
<b>I. Maternal Data</b>	
<b>Gestational age</b>	
<37 weeks (Pre term)	173 (57.3)
37-42 weeks (Term)	125 (41.4)
>42 weeks (Post term)	4 (1.3)
<b>Place of delivery</b>	
Hospital	211(69.8)
Health centre	82 (27.2)
Clinic	6 (2.0)
Home	3 (1.0)
<b>Mode of delivery</b>	
Vaginal	162 (53.6)
Caesarian section	123 (40.7)
Instrument (Forceps/Vacuum)	17 (5.7)
<b>II. Neonatal Data</b>	
<b>Weight at birth</b>	
<1500 g (VLBW)	54 (17.9)
<2500 g (LBW)	135 (44.7)
2500-4000 g (Normal weight)	112 (37.1)
> 4000g (Overweight)	1 (0.3)
<b>Apgar score at the 1<sup>st</sup> minute (n=244)</b>	
≤7	182 (74.6)
>7	62 (25.4)
<b>Apgar score at the 5<sup>th</sup> minute (n=244)</b>	
≤7	157 (64.3)
>7	87 (35.7)

VLBW: Very Low Birth Weight

LBW: Low Birth Weight

Apgar Activity, Pulse, Grimace, Appearance, and Respiration

### 3.3. Clinical Features

The clinical features of EOS and LOS are summarized in Table 3.3. The most prevalent clinical features were hypothermia (Temp <36°C) (84.8%), respiratory distress, (72.8%),

failure to feed (71.5%) and lethargy (30.1%). Common clinical features observed in both EOS and LOS, were hypothermia, respiratory distress and failure to feed ( $p > 0.05$ ).

Table 3.3. Symptoms and signs of neonates with suspected sepsis to the age at onset [investigated for bacterial infections at Pediatrics Hospital, Addis Ababa, Ethiopia](#) (October 2006 and March 2007)

Symptoms and signs	Category		
	EOS (n=298)	LOS (n=4)	Total (n=302)
	No. (%)	No. (%)	No. (%)
Hyperthermia (Temp >37.5°C)	20 (6.7)	-	20 (6.6)
Hypothermia (Temp <36.5°C)	252 (84.5)	4 (100.0)	256 (84.8)
Failure to feed	213 (71.5)	3 (75.0)	216 (71.5)
Respiratory distress	216 (72.5)	4 (100.0)	220 (72.8)
Lethargic	91 (30.5)	-	91(30.1)
Jaundice	29 (9.7)	-	29 (3.1)
Irritability	23 (7.7)	-	23 (7.6)
Vomiting	18 (6.0)	-	18 (6)
Cyanosis	18 (6.0)	-	18 (6)
Seizure	16 (5.3)	-	16 (1.7)
Bulging anterior fontanelle	11 (3.7)	-	11(3.6)
Abdominal distension	9 (3.0)	-	9 (3.0)
Hepatomegaly	3 (1.0)	-	3 (0.3)
Diarrhea	2 (0.7)	-	2 (0.7)
Shock	2 (0.7)	-	1 (0.7)
Coma	1 (0.3)	-	1(0.3)

EOS: Early-onset sepsis

LOS: Late-onset sepsis

### 3.4. Etiologic Agents

#### a. Overall prevalence

Among the 302 neonates admitted with suspected cases of sepsis, 135 (44.7%) had positive blood culture for bacteria as shown in Table 3.4. *Klebsiella spp.* (*K. pneumoniae* and *K. terrigena*) accounted for 39.2% of the total isolates followed by *S. aureus* (22.2%),

Coagulase negative staphylococci and *E. coli* each with prevalence of 10.0%, *Serratia* spp. (*S. marcescens* and *S. ficaria*) (6.6%), *Enterococcus* spp. (5.2%), *Streptococcus pneumoniae* and *Streptococcus pyogenes* each with an incidence of 4.4% and *Proteus mirabilis* (4.4%). The Gram-positive and negative bacteria accounted for 59/135 (43.7%) and 76/135 (56.3%) respectively ( $p > 0.05$ ).

**b. Early-onset sepsis**

Among the 298 neonates admitted with suspected cases of EOS, 131 (44.0%) had proven sepsis confirmed by positive blood culture. *K. pneumoniae* was the commonest isolates (38.2%) followed by *S. aureus* (23.0%) and *E. coli* (7.6 %). Other bacteria were also isolated from EOS as shown in Table 3.4. The Gram-positive and negative bacteria accounted for 55/131 (42.0%) and 76/131 (58.0%) respectively ( $p > 0.05$ ).

**c. Late onset sepsis**

All the four neonates admitted with suspected cases of LOS were positive for bacterial culture. Coagulase negative staphylococci accounted for 10.7%, followed by *Enterococcus* spp. (3.6%) (Table 3.4).

Table 3.4. Bacterial etiologic agents isolated from blood culture in neonates with suspected cases early-onset sepsis and late-onset sepsis [at Pediatrics Hospital, Addis Ababa, Ethiopia](#) (October 2006 and March 2007)

<b>Etiologic agents</b>	<b>EOS No. (%)</b>	<b>LOS No. (%)</b>	<b>Total No. (%)</b>
<i>Klebsiella pneumoniae</i>	50 (38.2)	-	50 (37.0)
<i>Staphylococcus aureus</i>	30 (23.0)	-	30 (22.2)
Coagulase negative staphylococci	7 (5.3)	3 (75.0)	10 (7.4)
<i>Escherichia coli</i>	10 (7.6)	-	10 (7.4)
<i>Enterococcus spp.</i>	6 (4.5)	1 (25.0)	7 (5.2)
<i>Streptococcus pneumoniae</i>	6 (4.5)	-	6 (4.4)
<i>Streptococcus pyogenes</i>	6 (4.5)	-	6 (4.4)
<i>Serratia marcescens</i>	5 (3.8)	-	5 (3.7)
<i>Proteus mirabilis</i>	4 (3.1)	-	4 (3.0)
<i>Serratia ficaria</i>	4 (3.7)	-	4 (3.0)
<i>Klebsiella terrigena</i>	3 (2.3)	-	3 (2.2)
<b>Total</b>	<b>131 (97.0)</b>	<b>4 (3.0)</b>	<b>135 (100%)</b>

EOS: Early-onset sepsis

LOS: Late-onset sepsis

### 3.5. Use of Antibiotics and Culture Outcome

Of the patients who received antibiotic/s, 47/91 (51.6%) had positive culture results, while those who did not receive any antibiotics 88/211 (41.7%) had positive culture results ( $p > 0.05$ ).

### 3.6. White Blood Cell Count and Immature/total neutrophils ratio

Among the 302 neonates admitted with suspected cases of sepsis, 243 (80.5%) had normal white blood cell count (5000-20,000/mm<sup>3</sup>), 30 (10%) high WBC count (>20,000/mm<sup>3</sup>) and 29 (9.5%) low WBC count (<5000/mm<sup>3</sup>) (Table 3.5). Immature/total neutrophils ratio  $\geq 0.2$  and  $< 0.2$  was observed in 175 (58.0%) and 127 (42.0%) peripheral blood smear examined respectively (Table 3.6).

Table 3.5. White blood cell counts in neonates admitted with suspected cases of sepsis [at Pediatrics Hospital, Addis Ababa, Ethiopia](#) (October 2006 and March 2007)

WBC Count/mm <sup>3</sup>	No. (%)
5000-20,000 (Normal)	243 (80.5)
>20, 000 (High)	30 (10.0)
< 5000 (Low)	29 (9.5)
Total	302 (100.0)

WBC : White Blood Cells

Table.3.6 I:T ratio of white blood cell in peripheral blood smear examined

I:T ratio	No. (%)
$\geq 0.2$	175 (58.0)
$< 0.2$	127 (42.0)
Total	302 (100.0)

I:T: Immature/total neutrophils ratio

### **3.7. Risk Factors Associated with Blood Culture Proven Neonatal Sepsis**

#### **a. Neonatal risk factors**

Neonatal risk factors associated/not-associated with culture proven sepsis are outlined in Table 3.7a. Among these, preterm neonates (OR: 3.03, 95% CI: 1.83-5.02, p= 0.00), neonates with low birth weights (OR: 3.44, 95% CI: 2.04-5.84, p=0.00), abnormal WBC (high and low) (OR: 6.12, 95% CI: 3.01-12.66, p=0.00) and I:T ratio  $\geq 0.2$  (OR: 3.5, 95% CI: 2.11-5.82, p=0.00) were at risk in developing culture proven neonatal al sepsis.

**Table 3.7a. Neonatal risk factors associated with blood culture proven neonatal sepsis**

Variables	Culture Positive (n=135) No. (%)	Culture Negative (n=167) No. (%)	OR (95% CI)	P value
<b>Gestational age</b>				
<37 weeks (Pre term)	94	72	3.03 (1.83-5.02)	0.00
37-42 weeks (Term)	41	91		
>42 weeks (Post term)	0	4		
<b>Weight at birth</b>				
<1500 g (VLBW)	27	22	3.44 (2.04-5.84)	0.00
1500-2499 g (LBW)	75	57		
2500-4000 g (Normal)	33	87		
> 4000g (Overweight)	0	1		
<b>Place of delivery</b>				
Hospital	121	90	1.32 (0.78-2.22)	0.27
Health centre	41	41		
Clinic	3	3		
Home	2	1		
<b>Mode of delivery</b>				
Vaginal	90	72	1.32 (0.81-2.15)	0.28
Instrument (Forceps/Vacuum)	14	3		
Caesarian section	63	60		
<b>White Blood Cell count</b>				
High(>20000/mm <sup>3</sup> )	24	6	6.12 (3.01-12.66)	0.00
Low(<5000/mm <sup>3</sup> )	22	7		
Normal(5000-20000/mm <sup>3</sup> )	89	154		
<b>I:T ratio</b>				
≥0.2	119	56	3.5 (2.11-5.82)	0.00
<0.2	48	79		
<b>Apgar score at 1<sup>st</sup> minute (n=244)</b>				
≤7	85	97	1.39 (0.74-2.61)	0.34
>7	24	38		
<b>Apgar score at 5<sup>th</sup> minute (n=244)</b>				
≤7	78	79	1.27 (0.73-2.23)	0.44
>7	38	49		

VLBW: Very Low Birth Weight

LBW: Low Birth Weight

Apgar: Activity, Pulse, Grimace, Appearance, and Respiration.

I:T Immature to Total neutrophil ratio

### b. Maternal risk factors

Maternal risk factors are specified in Table 3.7b. None of these had a statistically significant association with culture proven neonatal sepsis.

Table 3.7b. Maternal risk factors associated with culture proven neonatal sepsis

Variables	Culture Positive (n=135) No. (%)	Culture Negative (n=167) No. (%)	OR (95% CI)	P value
<b>Antenatal care</b>				
Yes	119	150	0.84 (0.39-1.84)	0.78
No	16	17		
<b>PROM* &gt;24 hrs</b>				
Yes	49	54	1.19 (0.72-1.98)	0.55
No	86	113		
<b>Maternal fever</b>				
Yes	15	16	1.18 (0.53-2.63)	0.80
No	120	151		
<b>Parity</b>				
Primiparous	78	94	1.06 (0.65-1.73)	0.88
Multiparous	57	73		
<b>Foul smelling liquor</b>				
Yes	7	5	1.77 (0.49-6.60)	0.50
No	128	162		
<b>Chorioamnionitis</b>				
Yes	8	7	1.44 (0.46-4.55)	0.67
No	127	160		

\*PROM: premature rupture of membrane

### 3.8. Antimicrobial Susceptibility

#### a) Gram positive bacteria

The susceptibility patterns of gram-positive bacteria (n=59) isolated from neonatal sepsis against 16 antimicrobial agents are presented in Table 3.7. Low level of resistance (<60%) were observed to most antimicrobials tested: Ampicillin, Amoxicillin-Clavulanic acid, Ceftriaxone; Cephalothin, Chloramphenicol, Ciprofloxacin, Doxycycline, Gentamicin, Kanamycin Streptomycin, Trimethoprim-sulphamethoxazole, Tetracycline and Vancomycin. All isolates showed intermediate level of resistance (60-80%) to Erythromycin, Methicillin and Penicillin.

#### b) Gram negative bacteria

The susceptibility patterns of gram-negative bacteria (n=76) isolated from neonatal sepsis against 12 antimicrobial agents are presented in Table 3.9. High level of resistance (>80%) were observed to Ampicillin, Ceftriaxone, Cephalothin, Chloramphenicol, and Gentamicin. All isolates showed low level of resistance (<60%) to Ciprofloxacin, Doxycycline, Kanamycin, Streptomycin, Trimethoprim-sulphamethoxazole and Tetracycline. Intermediate level of resistance (60-80%) was observed only for Amoxicillin-Clavulanic acid.

In general ciprofloxacin was the most effective drugs against the tested gram-positive and gram-negative bacteria (Tables 3.8 and 3.9).

**c. Multi-Drug Resistance (MDR)**

Multiple resistance (resistance to two or more drugs) was observed in 27/59 (45.7%) and 64/76 (84.2%) gram positive and gram negative bacteria respectively ( $p < 0.05$ ). Among the gram positives, MDR was observed among *Staphylococcus aureus*, coagulase negative staphylococci and *Enterococcus* spp., while among the gram negatives, MDR was observed in *Klebsiella* spp. *Serratia* spp and *Escherichia coli*.

Table 3.8. Susceptibility Patterns of Gram-Positive Bacteria Isolated from blood culture (October 2006 and March 2007)

Organisms		Antimicrobial agents (%)													
		AMP	AMC	CRO	KF	C	CIP	E	DO	CN	K	MET	P	S	SX
<i>Staphylococcus aureus</i> (n=30)	S*	60.0	46.7	53.3	43.3	30.0	96.7	16.7	80.0	80.0	80.0	23.3	6.7	46.7	86
	I*	3.3	30.0	40.0	30.0	13.3	3.3	-	6.7	3.3	6.7	10.0	13.3	36.7	3.3
	R*	36.7	23.3	6.7	26.7	56.7	-	83.3	13.3	16.7	13.3	66.7	80.0	16.4	10
CoNS (n=10)	S*	20.0	60.0	60.0	30.0	40.0	100	40.0	60.0	80.0	90.0	50.0	20.0	80.0	90
	I*	20.0	10.0	30.0	40.0	-	-	-	-	-	-	-	20.0	10.0	-
	R*	60.0	30.0	10.0	30.0	60.0	-	60.0	30.0	20.0	10.0	50.0	60.0	10.0	10
<i>Enterococcus spp</i> (n=7)	S*	57.1	14.3	100.0	29.0	57.1	100	-	71.4	57.1	71.4	-	-	52.0	85
	I*	-	28.6	-	-	-	-	-	-	-	14.3	14.3	42.9	29.0	-
	R*	42.9	57.1	-	71	42.9	-	100	28.6	52.1	14.3	85.7	57.1	29.0	14
<i>Streptococcus pneumoniae</i> (n=6)	S*	66.7	50.0	66.6	33	-	100	16.7	100	66.6	100	50.0	-	83.0	50
	I*	-	16.7	16.7	50	50	-	33.3	-	16.7	-	16.7	50.0	17.0	33
	R*	33.3	33.3	16.7	17.0	50.0	-	50.0	-	16.7	-	33.3	50.0	-	16
<i>Streptococcus pyogenes</i> (n=6)	S*	50.0	-	-	17.0	-	100.0	-	100.0	83.3	100	50.0	16.7	-	10
	I*	50.0	100.0	100	83.0	100.0	-	-	-	-	-	-	83.3	100	-
	R*	-	-	-	-	-	-	100.0	-	16.7	-	50.0	-	-	-
<b>Total (n=59)</b>	<b>S*</b>	<b>52.5</b>	<b>40.7</b>	<b>50.8</b>	<b>35.6</b>	<b>28.8</b>	<b>98.3</b>	<b>16.9</b>	<b>79.7</b>	<b>76.3</b>	<b>84.7</b>	<b>30.5</b>	<b>8.5</b>	<b>50.8</b>	<b>84</b>
	<b>I*</b>	<b>1.2</b>	<b>32.2</b>	<b>32.3</b>	<b>35.6</b>	<b>22.0</b>	<b>1.7</b>	<b>3.4</b>	<b>5.0</b>	<b>3.4</b>	<b>5.1</b>	<b>8.5</b>	<b>28.8</b>	<b>35.6</b>	<b>5.1</b>
	<b>R*</b>	<b>37.3</b>	<b>27.1</b>	<b>16.9</b>	<b>28.8</b>	<b>49.2</b>	<b>0.0</b>	<b>79.7</b>	<b>15.3</b>	<b>20.3</b>	<b>10.2</b>	<b>61.0</b>	<b>62.7</b>	<b>13.6</b>	<b>10</b>

\*S= Sensitive \*I=Intermediate \*R=Resistant CoNS

AMP: Ampicillin; AMC: Amoxicillin-Clavulanic acid; CRO: Ceftriaxone; KF: Cephalothin; C: Chloramphenicol; CIP: Ciprofloxacin; DO: Doxycycline; E: Erythromycin; CN: Gentamicin; K: Kanamycin; MET: Methicillin; P: Penicillin; S: Streptomycin; SXT: Trimethoprim-sulphamethoxazole; VA: Vancomycin

Table 3.9. Susceptibility Patterns of Gram-Negative Bacteria Isolated from Blood Culture (October 2006 and March 2007)

Organisms		Antimicrobial agents (%)										
		AMP	AMC	CRO	KF	C	CIP	DO	CN	K	S	SXT
<i>Klebsiella pneumoniae</i>	S*	-	-	-	-	-	100.0	86.0	-	68.0	80.0	26.0
(n=50)	I*	-	30.0	-	-	-	-	10.0	-	30.0	10.0	20.0
	R*	100.0	70.0	50.0	100.0	100.0	-	4.0	100.0	2.0	10.0	54.0
<i>Escherichia coli</i>	S*	30.0	30.0	20.0	50.0	40.0	90.0	40.0	20.0	70.0	40.0	70.0
(n=10)	I*	-	-	-	10.0	20.0	-	60.0	-	20.0	20.0	-
	R*	70.0	70.0	80.0	40.0	40.0	10.0	-	80.0	10.0	40.0	30.0
<i>Serratia marcescens</i>	S*	-	-	40.0	20.0	40.0	100	100	40.0	40.0	40.0	100
(n=5)	I*	-	-	60.0	20.0	-	-	-	60.0	-	-	-
	R*	100.0	100	-	60.0	60.0	-	-	-	60.0	60.0	-
<i>Proteus mirabilis</i>	S*	75.0	75.0	100	-	100	100	100	100	75.0	100.0	100.0
(n=4)	I*	-	25.0	-	25.0	-	-	-	-	-	-	-
	R*	25.0	-	-	75.0	-	-	-	-	25.0	-	-
<i>Serratia ficaria</i>	S*	-	-	-	-	-	100.0	100.0	-	25	100.0	100.0
(n=4)	I*	-	-	-	-	-	-	-	-	75.0	-	-
	R*	100.0	100.0	100.0	100.0	100.0	-	-	100.0	-	-	-
<i>Klebsiella terrigena</i>	S*	-	-	-	-	33.3	66.7	66.7	-	-	100	-
(n=3)	I*	-	33.3	-	-	-	-	-	-	66.7	-	-
	R*	100	66.7	100	100	66.7	33.3	33.3	100	33.7	-	100.0
<b>Total (n=76)</b>	<b>S*</b>	<b>7.9</b>	<b>7.9</b>	<b>10.6</b>	<b>7.9</b>	<b>14.5</b>	<b>97.4</b>	<b>81.6</b>	<b>10.5</b>	<b>61.8</b>	<b>75.0</b>	<b>43.4</b>
	<b>I*</b>	<b>-</b>	<b>22.4</b>	<b>3.9</b>	<b>3.9</b>	<b>2.6</b>	<b>-</b>	<b>14.5</b>	<b>3.9</b>	<b>29.0</b>	<b>9.2</b>	<b>13.2</b>
	<b>R*</b>	<b>92.1</b>	<b>69.7</b>	<b>85.5</b>	<b>88.2</b>	<b>82.9</b>	<b>2.6</b>	<b>3.9</b>	<b>85.6</b>	<b>9.2</b>	<b>15.8</b>	<b>43.4</b>

\*S= Sensitive \*I=Intermediate \*R=Resistant

AMP: Ampicillin; AMC: Amoxicillin-Clavulanic acid; CRO: Ceftriaxone; KF: Cephalothin; C: Chloramphenicol; CIP: Ciprofloxacin; DO: Doxycycline; CN: Gentamicin; K: Kanamycin; S: Streptomycin; SXT: Trimethoprim-sulphamethoxazole; TE: Tetracycline

## CHAPTER IV: DISCUSSION

About five million neonatal deaths occur worldwide every year, 98% of which occur in developing countries, particularly Asia and Africa. Infections such as tetanus, pneumonia, sepsis, meningitis and diarrhea account for 30-50% of neonatal deaths in developing countries (Darmstadt, 2001). Neonatal sepsis generally refers to systemic symptomatic bacterial, fungal, and viral infections that, on earliest presentation, may be associated with any gradation of symptoms, from subtle feeding disturbances to frank septic shock (Baltimore, 2003). It is a life threatening emergency and any delay in treatment may result in death (Yurdakok, 1998). The spectrum of organisms that cause neonatal sepsis changes over time and varies from region to region (Movahedian *et al.*, 2006). These organisms have also developed increasing multi-drug resistance over the last two decades (Maryam *et al.*, 2001). Therefore knowledge of the pattern of bacterial isolates and their antimicrobial susceptibility pattern is useful for prompt treatment of patients. Although an extensive research is available worldwide (Vergnano *et al.*, 2005; Bhutta, 1999; Klein *et al.*, 1990), very few reports are available on neonatal sepsis in Ethiopia (Tafesse, 1973; Tafari *et al.*, 1976; Ghiorghis, 1991 and 1997; Muhe *et al.*, 1999; Tafari and Ljungh, 1980). The present study was undertaken to highlight the pattern of bacterial isolates in neonates with clinical diagnosis of septicemia admitted at Pediatrics Hospital, Addis Ababa, Ethiopia from October 2006 and March 2007. An attempt has been also made to identify the possible risk factors responsible for neonatal septicemia.

In the present investigation, 98.7% and 1.3% neonates presented with EOS and LOS, respectively (Table 3.1). We found that early onset sepsis (EOS) was more common than late onset sepsis (LOS), which is in agreement with the reports from other developing countries e.g. in Iran (77.5% vs.22.5%) (Movahedian *et al.*, 2006) and Bangladesh (70.7 vs. 29.3%) (Rasul *et al.*, 2006), but in contrast with reports from Saudi Arabia (39% vs. 61%) (Dawodu *et al.*, 1997), Pakistan (42% vs.58%) (Aftab and Iqbal, 2006) and Libya (31 vs.69%) (Misallati *et al.*, 2000), where late onset sepsis is more common. The possible explanation for a lower frequency of LOS in this study might be the early discharge policy in the hospital.

Infections in newborn often present with sepsis, with or without focal signs of infection. Focal signs and symptoms in neonates due to localized infections may be clinically imperceptible, and thus difficult to differentiate on initial presentation from generalized or blood stream infections. Often the early signs of neonatal sepsis are non-specific, such as temperature instability, difficulty in breathing, lethargy, poor feeding, and unexplained

jaundice (Baltimore, 2003). Clinical assessment using a combination of symptoms and signs are useful guides to provisional diagnosis of neonatal sepsis (Meremikwu *et al.*, 2005). The characteristics of neonates with suspected cases of sepsis (such as preponderance of low birth weight and prematurity) (Table 3.2) and clinical features of sepsis (Table 3.3) in the present study are similar to those previously reported in Ethiopia (Tafari *et al.*, 1976; Ghiorgis, 1997) and elsewhere (Dawodu and Alausa, 1980; Bennet *et al.*, 1981; Klein and Marcy, 1990; Dawodu *et al.*, 1997; Jain *et al.*, 2003; Rasul *et al.*, 2006).

Blood culture to isolate the offending pathogen remains the mainstay of diagnosis for neonatal sepsis. The results of blood culture may take about a week, necessitating initial empirical treatment of suspected septicemia. In this study out of 302 neonates admitted with suspected cases of sepsis, 44.7% were positive for bacterial culture (Table 3.4). The isolation rate of bacteria in this study is comparable to rates reported in Nigeria (45.9%) (Meremikwu *et al.*, 2005), India (52.6%) (Murty and Gyaneshwari, 2007), Pakistan (54.0%) (Aftab and Iqbal, 2006) and Uganda (37.5%) (Mugalu *et al.*, 2006). Lower isolation rates were reported in previous studies done in Ethiopia (23.1%- 27.9%) (Tafari *et al.*, 1976; Ghiorgis *et al.*, 1997) and studies conducted in other developing countries e.g. in Iran (6.6%) (Movahedian *et al.*, 2006), Guadeloupe (French West Indies) (4.8%) (Robillard *et al.*, 1993), Libya (5.9%) (Misallati *et al.*, 2000), Bahrain (4.2%) (Bindayna *et al.*, 2006). Similar lower rates were also reported India (14.0%-25.0%) (Manucha *et al.*, 2002; Agnihotri *et al.*, 2004; Kapoor *et al.*, 2005) and Nepal (28.3%) (Jain *et al.*, 2003). Even though there was no statistically significant difference in blood culture positivity in neonates who received antibiotic/s (51.6%) and did not receive any antibiotics (41.7%), the percentage of culture positivity in patients who have taken antibiotics is high which shows the importance of culture and sensitivity in the management of neonatal sepsis.

The spectrum of organisms causing neonatal sepsis is shown in Table 3.3. The frequency of isolation of gram positive and negative bacteria from blood culture in this study was 43.7% and 56.3%, respectively ( $p > 0.05$ ). This finding is similar to that of other studies which showed that gram negative bacteria were responsible in most cases of neonatal sepsis (Manucha *et al.*, 2002; Ahmed *et al.*, 2002; Rahman *et al.*, 2002; Jain *et al.*, 2003; Waheed *et al.*, 2003; Simiyu, 2005; Agnihotri *et al.*, 2004; Kapoor *et al.*, 2005; Movahedian *et al.*, 2006; Aftab and Iqbal, 2006; Ghiorgis *et al.*, 1997 ). This was in contrast to other studies where gram positive bacteria were the commonest cause of neonatal sepsis (Robillard *et al.*, 1993; Anwer *et al.*, 2000; Bromiker *et al.*, 2001; Mokuolu *et al.*, 2002; Millege *et al.*, 2005;

Bindayna *et al.*, 2006; Mugalu *et al.*, 2006), while another study showed that the frequency of isolation of both gram positive and negatives was equal (Dawodu *et al.*, 1997).

In early onset sepsis, in the present investigation, gram positive and negative bacteria accounted for 42.0% and 58.0% respectively ( $p > 0.05$ ). Among gram negatives, most cases were due to *Klebsiella* spp. and *S. aureus* was the commonest gram positive organisms. In late onset sepsis, all (n=4) were gram positive bacteria; coagulase negative staphylococci accounted for 75%(n=3), followed by *Enterococcus* spp. 25% (n=1). Comparable findings have been reported in other studies (Trotman and Bell, 2006; Makhoul *et al.*, 2002; Mehr *et al.*, 2002).

In general, *Klebsiella* spp. were the most common isolates (38.2%) causing neonatal sepsis in this study. Similar findings have been reported in previous studies done in Ethiopia (Tafari *et al.*, 1976; Tafari and Ljungh, 1980; Ghiorgis *et al.*, 1997) and elsewhere e.g. in Pakistan (Bhutta and Yusuf, 1997), Palestine (Elamreen, 2007), Libya (Misallati *et al.*, 2000), Jamaica (Bell *et al.*, 2005; Trotman and Bell, 2006), India (Manucha *et al.*, 2002; Kapoor *et al.*, 2005) and Bangladesh (Afroza, 2006). *Klebsiella* species have often been isolated in hospital setting and are often implicated in nursery outbreaks (Nanthoo *et al.*, 1993).

*S. aureus* was the second most common organism isolated in this study. Similar finding has been reported in Pakistan (Rahman *et al.*, 2002), but in contrast to the previous study done in Ethiopia, whereby *E. coli* was the second most common isolates (Ghiorghis, 1997).

Studies from different countries report CoNS as the predominant organisms in LOS and among infants with indwelling central venous catheters from intensive care units (Munson *et al.*, 1982; Baumgart *et al.*, 1983). However, in this study, coagulase negative Staphylococci (CoNS) were recovered from 10% cases. But recovery of CoNS from blood of septicemic neonates needs to be reviewed with caution since most of them are regarded as contaminants. CoNS especially *Staphylococcus epidermidis* are the major normal flora of the skin and they can contaminate blood at the venipuncture site during collection of blood (Okada *et al.*, 2000; Klein, 1990). Although a single blood is fairly sensitive for diagnosis of neonatal sepsis, the chances of a contaminant growing mainly coagulase negative staphylococci (CoNS) in a blood culture are high enough that concordance or discordance between two cultures is helpful to determine whether the organisms was a true causes of sepsis. Concordant positive results indicate a higher probability of sepsis and discordant results indicate a higher probability that the isolate is a contaminant; therefore, ideally two

blood cultures obtained by venipuncture from separate sites are recommended if this will not delay treatment. The possibility of taking multiple blood samples for culture from a neonate is technically challenging, therefore the other option to minimize contamination is using proper antiseptics at the site of venipuncture.

Generally the spectrum of organisms causing neonatal sepsis in this study is similar to that reported from developing countries, with gram negative bacteria being responsible in most cases. But the pattern of isolated organisms in our study slightly differs from the findings in Iran (Movahedian *et al.*, 2006), India (Joshi *et al.*, 2000), Jamaica (Orrett and Shurland, 2001) and Nigeria (Ako-Nai *et al.*, 1999), where *Pseudomonas aeruginosa* was the most common cause of neonatal sepsis followed by *Klebsiella* spp. and *E. coli*. In a similar study from Bangladesh, Nepal and Pakistan, *E. coli* was the leading cause of neonatal sepsis followed by *Klebsiella* spp. (Ahmed *et al.*, 2002; Jain *et al.*, 2003; Aftab and Iqbal, 2006). In other studies gram positive bacteria such as *S. aureus* and group B streptococcus (GBS) were found to be the most common isolates in neonatal septicemia (Robillard *et al.*, 1993; Agnihotri *et al.*, 2004; Mugalu *et al.*, 2006).

The definitive diagnosis of neonatal sepsis is made by culture, which requires a minimum of 48-72 hours yields a positive result in only 30-70% of cases and may not be available in all hospitals (Janjindamai and Phetpisal, 2006). In recent years, various investigators have evaluated some markers (e.g. serum interleukin-6 (IL-6, IL-8, C-reactive protein and procalcitonin) to diagnose neonatal sepsis. Although these markers are highly sensitive and specific, they require sophisticated and expensive kits and are therefore, impractical for routine clinical work-up in community health delivery systems, particularly in developing countries. Various hematological parameters, a complete blood count, WBC count and differential, immature neutrophil count, band form count and platelet count, immature /total neutrophil count and immature/mature neutrophil count ratios individually and in combination have been evaluated for their ability to predict neonatal sepsis (Manucha *et al.*, 2002). In the present study neonates who were clinically suspected of sepsis, 80.5%, 10% and 9.5% had normal; high and low WBC counts respectively (Table 3.5). The white blood cell count (WBC) and differential count are useful for assessing a neonate who may have sepsis and for evaluating a neonate being treated for proven sepsis. The bone marrow reserves of leukocytes in a newborn are relatively small compared with those of older children and adults and leukopenia (low WBC count) occurs more frequently as sign of overwhelming infection. The normal peripheral WBC count of newborns is from 5000-

20,000/mm<sup>3</sup> ( $5-20 \times 10^9/L$ ) (Manroe *et al.*, 1979), but even values outside this range still have a poor specificity for predicting sepsis, because of the wide variation in values and overlap between normal and abnormal values.

In the present investigation, immature/total neutrophil count ratio  $\geq 0.2$  and  $<0.2$  was observed in 58.0% and 42.0% peripheral blood smear examined respectively (Table 3.6). There is some evidence that to increase the specificity parameters of WBC as an indicator of sepsis, the ratio of the concentration of immature cells of the neutrophilic series (bands, metamyelocytes and myelocytes) to total cells of the neutrophils series, known as I:T ratio has been used. An increased concentration of immature neutrophil series cells and an I:T ratio of  $\geq 0.2$  have been reported to have moderately increased specificity for sepsis. The I:T ratio takes into consideration the normative values over the first days of life (Manroe *et al.*, 1979). However the reported cut-off values I:T ratio is variable in different studies possibly due to the variation in interpretation of peripheral smear by different observers (Namedo *et al.*, 1985).

The outcome of neonates with infections is strongly related to their appropriate diagnosis and management. Diagnosing neonatal infection, however, is a challenge, since clinical signs and symptoms are often nonspecific for a particular infection. As a consequence, deciding whether to treat or not, balancing optimal patients care with aspects such as possible adverse events or antibiotic resistance, may be difficult. In line with this idea, the recognition of the risk factors for neonatal infections is extremely relevant in the clinical setting, since it contributes to the diagnostic reasoning and supports clinical decisions. Also, this knowledge offers the target to control strategies that may minimize the morbidity, mortality and, consequently, the high costs associated with hospital acquired infections. In this study pre-term neonates, neonates with low birth weight, abnormal WBC count (high and low) and I:T ratio  $\geq 0.2$  were at risk in developing culture proven neonatal sepsis (Table 3.7a). None of maternal risk factors had a statistically significant association with culture proven neonatal sepsis as outlined in Table 3.7b. Several risk factors for early and late onset neonatal infections have a very strong influence on infection rates as shown in Table 1.2 (Kaufman and Fairchild, 2004; Baltimore, 2002).

Neonatal septicemia is a life threatening emergency and rapid treatment with antibiotics is essential for a favorable outcome. Classical initial (empiric) treatment of neonatal sepsis and meningitis consists of a combination of penicillin (benzylpenicillin, ampicillin, or cloxacillin) and aminoglycoside (most commonly gentamicin) (Kaufman and

Fairchild, 2004; Klein *et al.*, 1990). With the advent of the third-generation cephalosporins, however, the empiric antimicrobial approach for neonatal sepsis has changed in many centers. Despite this, antibiotic resistance is increasing worldwide particularly in developing countries such as such as gentamicin-resistant *Klebsiella* species, third-generation cephalosporin-resistant gram-negative organisms, methicillin-resistant staphylococci (MRSA), vancomycin-resistant enterococci (VRE) and penicillin-resistant *Streptococcus pneumoniae* as shown in Tables 1.4 and 1.5. Infection with resistant organisms has been associated with treatment failure, higher morbidity and mortality and increased costs. Therefore knowledge of antimicrobial susceptibility pattern of common pathogens of neonatal sepsis in a given area helps to inform the choice of antibiotics.

The susceptibility pattern of gram positive and gram-negative organisms to the most relevant antibiotics is depicted in Tables 3.8 and 3.9. Our results have demonstrated that in general both gram positive and negative bacteria isolated from blood culture showed low resistance rates to ciprofloxacin, doxycycline, kanamycin, streptomycin, trimethoprim-sulphamethoxazole and tetracycline.

Gram-negative bacteria showed high-level resistance to ampicillin, ceftriaxone, cephalothin, chloramphenicol, and gentamicin. This observation is comparable to that of other researchers (Bhutta *et al.*, 1991; Anwer *et al.*, 2000; Joshi *et al.*, 2000; Misallati *et al.*, 2000; Orrett and Shruland, 2001; Rahman *et al.*, 2002 Bindayna *et al.*, 2006; Movahedian *et al.*, 2006). This was in contrast from a previous Ethiopian study which was conducted 32 years ago at Ethio-Swedish Pediatric Clinic, Addis Ababa, where 98-100% of gram negative bacteria, *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. were sensitive to gentamicin. On the other hand similar resistance pattern for ampicillin and chloramphenicol was observed as in the present study (Tafari *et al.*, 1976). Another study from the same hospital reported that *Klebsiella oxytoca* isolates from different clinical specimens including from blood culture were resistant to ampicillin, amoxicillin, chloramphenicol (100% each), trimethoprim-sulphamethoxazole (93%) and gentamicin (87%) (Worku, 1997). Another contrary study from Sydney Neonatal Infection Surveillance has mentioned that all gram negative bacteria were susceptible to gentamicin and third generation cephalosporin (Levine *et al.*, 1999).

In the present study ciprofloxacin was the most effective drug against the tested gram-positive and gram-negative bacteria. Similar finding has been reported elsewhere (Kapoor *et al.*, 2005). This is a relatively new class of quinolone antibiotics, the use of which has recently become very common, particularly in general practice (Rahman *et al.*, 2002). In

addition quinolones have also been found to be effective by other investigators in the treatment of multidrug resistant gram negative infections in various patients, including premature and extremely low birth infants (Khaneja *et al.*, 1999).

Multiple resistance (resistance to two or more drugs) was observed in 45.7% and 84.2% gram positive and gram negative bacteria respectively ( $p < 0.05$ ) in the present study. Among the gram positives, MDR was observed among *Staphylococcus aureus*, coagulase negative staphylococci and *Enterococcus* spp., while among the gram negatives, MDR was observed in *Klebsiella* spp. *Serratia* spp and *Escherichia coli*. Emerging multiple drug resistance has also reported in other parts of the world. Leibvoitz *et al.* (1997) reported the emergence of extremely virulent multi-resistant *Klebsiella* spp. in their neonatal intensive care unit in Kaplan Hospital, Israel. Koksai *et al.* (2001) from India reported a series of 35 cases of severe gram negative sepsis with all organisms being multi-resistant. The detection of multi-drug resistant isolates may further limit therapeutic options. The high prevalence of gram-negative septicemia associated with resistance to the commonly used antibiotics in this study and studies done elsewhere indicate that the infection was most probably nosocomial in origin. Generally it is not an easy task to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable. Few studies compare antibiotic susceptibility over time in the same unit, but where data are available they show increasing resistance to commonly used antibiotics (Bhutta and Yusuf, 1997; Bromiker *et al.*, 2001).

#### **LIMITATION OF THE STUDY**

1. It was not possible logistically to include anaerobic blood culture due to budget constraints and the laboratory setup where the research was conducted. Anaerobic bacteria such as *Bacteriodes fragilis*, *Peptostreptococcus* spp. *Clostridium* spp. and *Fusobacterium* spp. were found to play a significant role causing persistent bacteremia/septicemia in children (Brook, 2002).
2. The design of the study did not include fungi and other bacterial pathogens such as Group B-Streptococcus (GBS). Many published reports have confirmed that GBS is the most frequent cause of neonatal sepsis particularly in early neonatal septicemia (Schuchat, 2000 and 2001). Neonatal fungal infections, particular by *Candida* spp. is the most frequent cause of sepsis in preterm infants with high attributable mortality and poor outcome (Manzoni *et al.*, 2007). Neonatal fungal infections include blood stream, urine and cerebrospinal fluid infections.

3. Single blood culture was performed from each neonate for isolation and identification of bacterial etiologies of neonatal sepsis.
4. Most neonates with septicemia may have coexisting infections such as meningitis, pneumonia and others. Collection of other clinical samples, other than blood, was not done in the present studying order to investigate the occurrence of these coexisting infections.

## CONCLUSION AND RECOMMENDATIONS

*Klebsiella* spp. and *S. aureus* were the most common organisms causing neonatal sepsis. Prematurity, low birth weight, abnormal WBC counts and I:T ratio  $\geq 0.2$  were strongly associated with blood culture proven neonatal sepsis. No maternal risk factors were identified. Gram negative bacteria showed high level of resistance to commonly used antibiotics. Ciprofloxacin was the most effective drug when compared to other drugs tested against the gram-positive and gram-negative bacteria. Multi-drug resistance was detected in 67.4% isolates. The detection of multi-drug resistant isolates may further limit therapeutic options.

Based on the findings of the present study and the above mentioned limitations the following recommendations are made: -

- Empirical antibiotic regimens for gram positive and gram negative sepsis must take into consideration the high rates of ampicillin and gentamicin resistance that are now prevalent. The antibiotic sensitivity profile suggests that ciprofloxacin is the most suitable drug for the treatment of neonatal septicemia.
- Routine bacterial surveillance and the study of their resistance patterns must be an essential component of neonatal care. A knowledge of these patterns is essential when local policies on the uses of antibiotics are being devised.
- Recovery of CoNS from blood of a septicemic neonate needs to be reviewed with caution since not all of them are true bacteremic agents.
- The role of anaerobic bacteria, group B streptococcus and fungi in neonatal sepsis should be investigated.
- Investigation of coexisting infections by collection of other clinical samples in addition to blood is recommended.

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## APPENDIX I: QUESTIONNAIRE

Questionnaire for investigation of the bacterial profile and pattern of antimicrobial resistance of neonatal sepsis in Pediatrics Hospital

### A. NEONATE

#### I. General

Serial No ..... Card number.....Date of admission.....

Ward: Pediatric /others..... Address .....

Age at the time of sepsis.....Sex .....

Dx at the time of admission.....

Condition at the time of admission (critical/not critical)

Is there any other localized infection Yes..... No.....If yes, mention.....

Birth Weight.....Gestational age.....

Is the neonate on antimicrobial treatment? Yes..... No.....

If yes, name of antibiotics .....

#### II. Clinical data

Symptoms and signs of sepsis	YES	NO
Hyperthermia	-----	-----
Hypothermia	-----	-----
Irritability	-----	-----
Respiratory Distress	-----	-----
Vomiting	-----	-----
Cyanosis	-----	-----
Diarrhea	-----	-----

Abdominal distention	-----	-----
Lethargic	-----	-----
Failure in feeding	-----	-----
Bulging anterior fontanel	-----	-----
Coma	-----	-----
Seizure	-----	-----
Edema	-----	-----
Jaundice	-----	-----
Hepatomegaly	-----	-----
Shock	-----	-----
APGAR score at 1& 5 minute	-----	-----
Others	Specify: _____	

**B. MOTHER**

**I. SOCIO-DEMOGRAPHIC DATA**

**A. EDUCATIONAL BACKGROUND OF THE FAMILY**

Illiterate.....Read and write.....Primary School.....

Secondary school.....Higher Education.....

**B. FAMILY MONTHLY INCOME (in birr)**

Below 150.....151-650.....651-1400.....More than 1400.....

**II. OBSTETRIC DATA**

History of antenatal care Yes..... No.....

History of Maternal Fever Yes..... No.....

History of foul smelling liquor Yes..... No.....

History of Chorioamnionitis Yes..... No.....

History of premature rupture of membrane Yes..... No.....

If yes, Duration of rupture of membranes.....Parity.....

Mode of delivery

Vaginal.....Caesarean section.....Vacuum.....Other.....

Place of delivery

Hospital.....Health center.....Clinics.....Home.....Other.....

### III. Laboratory data

Date of specimen collection-----Total WBC count-----

per  $\mu$ l

Differential WBC count (in %)

Lymphocyte.....Neutrophile.....Band (immature).....

Polymorph nuclear cell.....Monocytes .....

Eosinophile .....Basophile.....

#### 4. Culture and identification of blood specimens

Name of bacteria isolated.....

#### 5. Antimicrobial susceptibility testing

	S	I	R
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Ampicillin	----	----	----
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Cephalothine	-----	-----	-----
--------------	-------	-------	-------

Ceftriaxone	-----	- -----	-----
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Ciprofloxaciline	-----	-----	-----
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Augmentine	-----	-----	-----
------------	-------	-------	-------

Gentamicine	-----	-----	-----
-------------	-------	-------	-------

Erythromycin	-----	-----	-----
--------------	-------	-------	-------

Streptomycin	-----	-----	-----
--------------	-------	-------	-------

Vancomycin	-----	-----	-----
------------	-------	-------	-------

Methicillin	-----	-----	-----
-------------	-------	-------	-------

Chloramphenicol	-----	-----	-----
Tetracycline	-----	-----	-----
Doxycycline	-----	-----	-----
Kanamycin	-----	-----	-----
TMP-SXT	-----	-----	-----
Penicillin G	-----	-----	-----

**IV. Comments** \_\_\_\_\_

\_\_\_\_\_

Name of investigator \_\_\_\_\_

## APPENDIX-II: CONSENT FORM

(To be translated in to the patient's language)

Name..... Card no..... Ward..... Serial no.....

Date of admission ..... Reason of admission.....

I have been informed that the objective of this study is to determine the incidence of bacterial agent and pattern of antimicrobial resistance of neonatal sepsis. The aim of the study is explained to me (the guardian accompanying the infants on admission). The results of this study have importance to treat neonates who have sepsis and according the result profile, which guide the pediatrician to manage the patient. I have also informed about the confidentiality of the questionnaires. Because, I have requested to participate in the study, which would require my response to an interview, and to provide 1-2 ml blood, if there is suspected sepsis. Therefore, with full understanding of the importance of the study, I agreed voluntarily to give my child the requested samples in the above for clinical investigation in the study and I benefit only from the free laboratory investigation result.

I \_\_\_\_\_ here by give my Consent for giving of the requested information and blood specimen as the doctors find best for my child.

Signature: \_\_\_\_\_ Date \_\_\_\_\_

ቅጽ 2

የስምምነት መግለጫ  
( ትርጉም በአማርኛ)

ተራ ቁጥር..... ካርድ ቁጥር.....

የመታከምያ ክፍል.....

በሽተኛው የተኛ በት ቀን.....

በሽተኛው የተኛ በት ምክንያት.....

የዚህ ጥናት ዋና ዓላማ ዕድገቶቻቸው ከ 28 ቀን በታች በሆኑት ህፃናት የደም ዉስጥ ኢንፌክሽን በሚሆኑት በሽታ አምጪ ተህዋስያንና ለፀረ ተህዋስያን መድሃኒት የመቋቋም ባህርያቸው ለማጥናት በመሆኑ፤ የጥናቱ ውጤቱ ደግሞ ለልጅና ለሌሎች ታከሚዎች ህክምና ጥቅም እንድሚውልና እንደሚያገለግል ተረድቻለሁ።

በተጨማሪም በጥናቱ ውስጥ ልጅ ገብቶ ዕኔም የቃል መረጃ በመስጠት ዕና የደም ናሙና ልጅ እንድሰጥ በአጥኚው ባለሙያ ፈቃደኝነቱን ተጠይቄያለሁ።

በመሆኑም የጥናቱ ዓላማና ጥቅም በሚገባ ስለተገነዘብኩ ፤ ከጥናቱ የሚገኘው ጥቅምም ነፃ የላቦራቶሪ ምርመራ ብቻ መሆኑንም ጭምር አውቄ ከላይ የተጠቀሱትን ለጥናቱ የሚያስፈልጉ ሁሉ ለመስጠት በሙሉ ፈቃደኝነት መስማማቴን በፊርማዬ አረጋግጣለሁ።

ስም .....

ቀን.....

ፊርማ .....