Introduction

Neonatal sepsis or septicaemia is a clinical syndrome characterized by systemic signs of circulatory compromise (e.g., poor peripheral perfusion, pallor, hypotonia, poor responsiveness) caused by invasion of the blood stream by bacteria in the first month of life. In the pre-antibiotic era neonatal sepsis was usually fatal. Case fatality rates in antibiotic treated infants now range between 5% and 60% with the highest rates reported from the lowest income countries **(Thaver and Zaidi, 2009).**

The World Health Organization (WHO) estimates that 1 million deaths per year (10% of all under-five mortality) are due to neonatal sepsis and that 42% of these deaths occur in the first week of life **(Lawn et al., 2005).** In particular, neonates with low birth weight show relatively high morbidity and mortality **(Fujimori et al., 2010).**

There are risk factors for neonatal sepsis including poverty and poor environmental conditions. Also, prolonged rupture of membranes, preterm labour, maternal pyrexia, unhygienic intrapartum and postnatal care, low birth weight, and prelacteal feeding of contaminated foods and fluids **(Bahl et al., 2009).**

The bacteria that cause neonatal sepsis are acquired shortly before, during, and after delivery. They can be obtained

directly from mother's blood, skin, or vaginal tract before or during delivery or from the environment during and after delivery. *Streptococcus agalactiae* (Group B streptococcus, GBS) is the most common cause of neonatal sepsis in many countries; gram negative bacilli (*Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter* spp.) and gram-positive cocci (such as *Staphylococcus aureus* and *Staphylococcus epidermidis*) are other important causes **(Zaidi et al., 2005)**

Sepsis is a serious disease. A fast and correct diagnosis, followed by rapid treatment, plays an important role in the reduction of infant mortality resulting from sepsis **(Kaufman and Fairchild, 2004).** However, diagnosing neonatal sepsis is difficult since being exposed to known risk factors for sepsis is not a necessity, clinical signs are often vague, and laboratory parameters are unspecific **(Reier-Nilsen et al., 2009).**

Elevation of C–reactive protein (CRP) has been a useful marker of sepsis in many studies. Initiation of broad-spectrum systemic antibiotic treatment is based only on the suspicion of sepsis since no early definitive diagnostic test is yet available **(Gerdes, 1991)**.

Conventional blood culture is considered the gold standard in the etiological diagnosis of neonatal bacterial sepsis **(Gerdes, 1991).** However, obtaining sufficiently large amounts of blood for culture from neonates are often difficult, and it

often takes 48–72 hours to obtain a preliminary positive result **(Connel et al., 2007)**.

Antigen detection techniques allow rapid detection and identification of microorganisms without culturing. The most commonly used commercially available test is the latex agglutination assay, which is based on specific agglutination by bacterial cell wall antigens of antibody coated latex particles. However, these tests can only detect specific organisms such as *Streptococcus agalactiae* and are associated with high false positive and negative rates **(Peters et al., 2004).** New urinary antigen tests for pneumococcus are more encouraging but are also associated with false positives from pneumococcal carriage **(Moisi et al., 2009).**

Detection of bacterial DNA in blood samples of neonates is suggested to represent a rapid and sensitive supplement to blood culture in diagnosing bacterial sepsis in neonates **(Reier-Nilsen et al., 2009).** Polymerase chain reaction (PCR) amplification of highly conserved DNA sequences found in all bacteria would permit fast and sensitive determination of the presence of bacteria in clinical specimens **(McCabe et al., 1995**). There are a few studies on the use of universal primer PCR on blood samples of neonates with suspected sepsis, and they have shown promising results. Unlike blood culture, PCR does not depend on the viability of bacteria. They hypothesized that PCR results may remain positive in septicemic neonates even after antibiotic drug therapy. There are no clinical data on

this issue, but animal data suggest that a positive PCR result persists after starting antibiotic therapy **(Dutta et al., 2009).**

The rapid and accurate detection of bacteremia in newborn infants might have a significant impact in shortening hospital stays within the neonatal intensive care unit (NICU) as well as reducing the costs to the health care system (**Ruppenthal et al., 2005).**

Aim of the Work

The aim of this study was to compare a broad range 16S rDNA PCR done on whole blood samples without prior enrichment and conventional blood culture for detecting bacterial DNA in blood samples from infants with suspected sepsis.

Neonatal Sepsis

Neonatal sepsis is one of the major health problems throughout the world. Infections are a frequent and important cause of morbidity and mortality in the neonatal period. As many as 2% of fetuses are infected in utero and up to 10% of neonates are infected in first month of life. Data suggests that among four main causes of neonatal death, infection topped the list. Every year an estimated 30 million newborns acquire infection and 1-2 million of these die ***(Afroza and Begum, 2008).***

If it is diagnosed early and treated aggressively with antibiotics and good supportive care, it may be possible to save most cases of neonatal sepsis. Surviving infants can have significant neurological sequelae as a consequence of centeral nervous system involvement, septic shock or hypoxemia secondary to severe parenchymal lung disease ***(Khinchi et al., 2010).***

Definitions:

Neonatal sepsis or septicaemia is a clinical syndrome characterized by systemic signs of circulatory compromise (e.g., poor peripheral perfusion, pallor, hypotonia, poor responsiveness) caused by invasion of the bloodstream by

bacteria in the first month of life. In the pre-antibiotic era neonatal sepsis was usually fatal. Case fatality rates in antibiotic treated infants now range between 5% and 60% with the highest rates reported from the lowest income countries ***(Thaver and Zaidi, 2009).***

Probable clinical sepsis is an infant having clinical picture suggestive of septicemia, if there is the presence of any one of the following criteria as:

- Existence of predisposing factors (maternal fever or foul smelling liquor or prolonged rupture of membranes (>24 hrs) or gastric polymorphs (>5 per high power field).
- Positive septic screen - presence of two of the four parameters namely, TLC (< 5000/mm), band to total polymorphonuclear cells ratio of >0.2, absolute neutrophil count < 1800/mm^3.
- C reactive protein (CRP) >1mg/dl and micro ESR > 10 mm-first hour.
- Radiological evidence of pneumonia.

(Tripathi and Malik, 2010).

Culture positive sepsis is an infant having clinical picture suggestive of septicemia, pneumonia or meningitis, if there is presence of either of the following: ***(Tripathi and Malik, 2010)***

- Isolation of pathogens from blood or CSF or urine or abscess (es).
- Pathological evidence of sepsis on autopsy.

Incidence:

According to the World Health Organization (WHO), 130 million children are born yearly, and about 4 millions die every year, while infection causes 36% of these deaths; this is even worse in countries where critical patient resources are limited ***(Lawn et al., 2005).*** WHO estimates that 1million deaths per year (10% of all under-five mortality) are due to neonatal sepsis and that 42% of these deaths occur in the first week of life ***(Edmond and Zaidi, 2010).*** Globally, the main direct causes of neonatal mortality are thought to be preterm birth (28%), severe infections (26%) and asphyxia (23%) ***(Lawn et al., 2005).***

The incidence of neonatal sepsis varies from 1-4/1000 live births in developed countries to 10-50/1000 live births in developing countries ***(Kardana, 2011). Stoll (2000)*** put the global incidence of neonatal sepsis at 5-6 per 1000 live births,

similar to that in South and South-East Asia, 6-21 per 1000 live births in sub-Saharan Africa, 1.8-12 per 1000 live births in the Middle East and North Africa, and 2.9 per 1000 live births in the Americas/Caribbean**.** 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).*** As per National Neonatal Perinatal Database (NNPD) 2002-2003, the incidence of neonatal sepsis in India was 30 per 1000 live birth ***(Tripathi and Malik, 2010).*** In Mexico, the National Institute for Statistics and Geography (INEGI) the incidence of neonatal sepsis in 2003 was reported as between 4 and 14 -15.4/1,000 live newborns ***(Leal et al., 2012).***

In developing countries, neonatal mortality resulting from all causes of neonatal sepsis is about 34 per 1000 live births, occurring mainly in the first week of life. In developed countries, it is 5 per 1000 live births ***(Ayoniyi et al., 2009).*** In Malaysia, the incidence of neonatal sepsis was 5-10% and mortality rate was 23-50%. In the Republic of Georgia, the neonatal mortality rate in year 2000 was 25 per 1,000 live births ***(Macharashvili et al., 2009).*** 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).***

Classification:

Neonatal sepsis is still a major problem worldwide. Classical neonatal infection has been divided into late or early onset infection, depending on the time of onset of infection. Traditionally, early onset sepsis (EOS) is defined as onset of sepsis within 48–72 hours of age and late onset sepsis (LOS) as

onset beyond 48–72 hours ***(Sundaram et al., 2009).*** The cut-off point has been varied among authors from 24 hours to 7 days but 48 hours is a widely accepted choice. This classification of neonatal sepsis has important implications for the origin of the causative organisms, the type of causative organisms and the antibiotic choice ***(Van Rostenberghe, 2009).***

Early onset septicemia is more common compared to late onset septicemia. Gram negative organisms are the predominant causative agents in neonatal septicemia ***(Sriram, 2011).***

In the most recent National Institute of Child Health and Development (NICHD) surveys, LONS is over 10 times more common than EONS in very low birth weight (VLBW) infants. The NICHD reported a 21% incidence of blood-culture-proven LONS among VLBW infants .The incidence is higher among infants of <25-week gestation, with 46% of these infants suffering from LONS. Infants who develop late onset sepsis have a significantly prolonged hospital stay. They are significantly more likely to die than those who are uninfected, especially if they are infected with Gram-negative organisms (36%) or fungi (32%) ***(Paolucci et al., 2012).***

Risk factors:

The susceptibility of a neonate to sepsis is multifactorial, and can be related to immaturity of humoral, phagocytic and

cellular immunity (usually appearing in preterm and low birth weight infants), hypoxia, acidosis, and metabolic derangements ***(Puopolo, 2008).*** Various maternal, fetal and environmental factors also contribute to sepsis in newborns ***(Kardana, 2011).***

Fetal risk factors include: prematurity, low birth weight, asphyxia, resuscitation during delivery, invasive procedure, congenital anomaly, long hospital stay in neonatal intensive care unit ***(Utomo, 2010),*** parenteral nutrition ***(Rohsiswatmo, 2005)***, bottle feeding, aspiration of feeds, poor cord care, superficial infection (pyoderma and umbilical sepsis) ***(Tripathi and Malik, 2010)***, central venous catheters, use of steroids or drugs that decrease gastric acidity, and prolonged duration of mechanical ventilation ***(Wynn and Wong, 2010)*** and low Apgar score ***(Klinger et al., 2010).***

While the maternal risk factors are premature rupture of membranes especially more than 18 hours, maternal infection and fever within 2 weeks prior to delivery, meconium-stained amniotic fluid, foul smelling amniotic fluid, turbidity, instrumental delivery and multiple gestation ***(Rohsiswatmo, 2005).*** Also, more than 3 vaginal examinations during labor ***(Tripathi and Malik, 2010)***.

In many cases the infant may acquire infection postnatally from environmental sources, such as nursery

personnel, respiratory equipment, contaminated total parenteral solutions or medication vials, and incubators. Central venous lines or catheters are also risk factors for late-onset sepsis in preterm infants ***(Klein et al., 2006).***

Other risk factors including gender as it is more frequent in male than female, race as it is more frequent in black neonates, socio-economic status as it is more frequent in low socio economy neonates ***(Rohsiswatmo, 2005).***

There are wide disparities in neonatal care between high and low-income countries. In high-income countries the major concern is the increasing numbers of extremely premature infants with high nosocomial infection rates due to multi resistant organisms in intensive care units. Health facility infections are also a major problem in low income countries, but the more pressing issues are the high proportion of home deliveries in unclean environments predisposing to sepsis and ensuring that all neonates have access to effective interventions from health care providers in the first days of life ***(Lawn et al., 2005).*** Risk factors associated with neonatal sepsis in preterm infants are shown in table (1).

Table (1): Risk factors associated with neonatal sepsis in preterm infants **(Adapted from *Kaufman and Fairchild, 2004*).**

Antimicrobial defense	Preterm infant compromised defense
Epidermal and epithelial barriers	▪ Immature skin. ▪ Insensible water loss from skin and humidification system creating moist skin that favors the growth of microorganism. ▪ Invasive catheters and tubes. ▪ Surface for colonization provided by intravascular catheters breaching intact epidermis. ▪ Proliferation due to parenteral nutrition and biofilms on plastic catheters. ▪ Colonization of endotracheal and nasogastric tube.
Intact endothelial tissues	▪ Trauma to endothelium and endocardium from central venous catheters. ▪ Injury from hyperosmolar nutrition solutions and medication.
Gastrointestinal mucosa	▪ Decreased acid production. ▪ Immature peristalsis and reduced absorption, favoring microorganism overgrowth. ▪ Thin mucin layer, leading to decreased barrier function and secretary IgA binding. ▪ Diminished number of intraepithelial lymphocytes.
Microflora	▪ Competitive bacterial flora diminished by broad-spectrum antibiotics. ▪ More commensal gram-positive organism selected by human milk.
Complement	▪ Lower level with decreasing gestational age.
Cytokines	▪ Decreased production of IL-1, IL-8, gamma interferon, TNF- α, G-CSF, and GM-CSF.
Defensins	▪ Diminished with decreasing gestational age.

Neutrophils	▪ Decreased bone marrow storage pool and G-CSF level. ▪ Immature neutrophil oxidative burst.
Monocyte	▪ Diminished number and function. ▪ Decrease adherence at site of infection. ▪ Decreased opsonization and phagocytosis. ▪ Diminished gamma interferon, IL-3 and G-CSF release.
T-cells, B-cells and antibodies	▪ Decreased number of lymphocytes in gastrointestinal tract. ▪ Majority of transfer of maternal IgG after 32 weeks gestation. ▪ Decreased production of antibodies by preterm lymphocyte.

Etiology:

I. ***Bacterial causes*:**

Bacterial infections are the commonest cause of morbidity and mortality during the neonatal period. Fulminant and fatal course of infection may result from complications such as shock, disseminated intravascular coagulation and multi-system organ failure, mandating early diagnosis of this life-threatening condition for a timely treatment and a favourable outcome ***(Sriram, 2011).***

The pattern of bacterial pathogen responsible for neonatal sepsis has changed with time and varies from place to place. There is a difference in the causative organisms for neonatal sepsis between the developed and developing countries ***(Zaidi et al., 2009).***