

## Neonatal Sepsis: An Update

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### ABSTRACT

Sepsis is the most common cause of neonatal mortality. As per National Neonatal-Perinatal Database (NNPD), 2002-2003, the incidence of neonatal sepsis in India was 30 per 1000 live births. Signs and symptoms of sepsis are nonspecific; therefore empirical antimicrobial therapy is promptly initiated after obtaining appropriate cultures. The early manifestations of neonatal sepsis are vague and ill-defined. Novel approaches in the diagnosis of neonatal sepsis include heart rate analysis on ECG, and colorimetric analysis of skin color. Although blood culture is the gold standard for the diagnosis of sepsis, culture reports are available only after 48-72 hours. In this era of multidrug resistance, it is mandatory to avoid unnecessary use of antibiotics to treat non-infected infants. Thus, rapid diagnostic test(s) that include Interleukin-6 (IL-6), neutrophil CD64 index, procalcitonin and nucleated RBC count— and differentiate the infected infants from the non-infected, particularly in the early neonatal period— have the potential to make a significant impact on neonatal care. The aim of this review is to specify the diagnostic criteria, treatment guidelines, and a summary of recent diagnostic tests of sepsis, along with the preventive measures.

**Keywords:** Clinical Features, Diagnosis, MDR Neonatal Sepsis, Neonatal Sepsis, Prevention

### Introduction

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection, and accompanied by bacteremia in the first month of life (1). Sepsis is the most common cause of neonatal mortality, and is responsible for 30-50% of total neonatal deaths, each year in developing countries (2-4). The term neonatal sepsis, refers to the systemic infection of neonates including septicemia, pneumonia, meningitis, arthritis, osteomyelitis, and urinary- tract infection. As per National Neonatal-Perinatal Database (NNPD) (2002-2003), infection is the primary cause of mortality in 18.6% of intramural neonates, among which *Klebsiella pneumoniae* is the most frequent bacterial isolate (32.5%), followed by *Staphylococcus aureus* (13.6%). Sepsis is 12 times more common in extramural admissions (39.7%). In extramural admissions, *Klebsiella* is the most prevalent bacteria responsible (27.5%), preceding *S. aureus* (14.9%). Sepsis is responsible for deaths in 38.0% of these extramural babies (4, 5).

Knowledge of all the common pathogens causing septicemia, along with multidrug-resistance organism in neonates, like antibiotic-resistant nosocomial pathogens— such as vancomycin-resistant Enterococcus, carbapen-

emas eproducing Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (6) - and their antimicrobial susceptibility is essential in order to select the appropriate antimicrobial treatment, along with a multidrug resistance organism.

### Definition

National Neonatal Forum of India has defined neonatal sepsis as follows: (4)

1. *Probable (clinical) sepsis*: It is found in an infant having a clinical picture suggestive of septicemia if any one of the following criteria are present:

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

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2. **Culture Positive Sepsis:** In an infant having a clinical picture suggestive of septicemia, pneumonia or meningitis, if either of the following criteria are found:

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

## Classification

### Early Onset Sepsis (EOS)

Presence of foul smelling liquor or three of the aforementioned risk factors should be considered as having EOS, and be treated with antibiotics. Presence of  $\geq 2$  risk factors should be investigated with sepsis screens, and be treated, accordingly (9).

### Late Onset Sepsis (LOS)

LOS usually appears after 72 hr of age. The source of infection is either nosocomial or community-acquired, and is present in neonates with septicemia, pneumonia or meningitis (10, 11). Risk factors concerning the development of LOS include:

- NICU admission
- Poor hygiene
- Low birth weight (LBW)
- Poor cord care
- Prematurity
- Bottle feeding
- Invasive procedure
- Superficial infection (pyoderma, umbilical sepsis)
- Prelacteal feeding
- Ventilation
- Aspiration of feeds

## Clinical features

Manifestation of neonatal sepsis is vague and ill-defined. Alteration in the established feeding behavior is an early and common symptom, though nonspecific. Other symptoms are hypothermia or fever (former is more common in LBW babies), lethargy, poor cry, poor perfusion i.e. prolonged capillary refill time ( $>2$  seconds), hypotonic or absent neonatal reflexes, bradycardia or tachycardia, respiratory distress i.e. apnea or gasping respiration, hypoglycemia or hyperglycemia, and metabolic acidosis. Specific system-wise features are:

### Central nervous system

These are bulging anterior fontanel, blank look, high-pitched cry, excessive irritability, coma,

seizures, and neck retraction. Presence of these signs should raise clinical suspicion of meningitis.

### Cardiac

The cardiac signs are mainly hypotension and poor perfusion. A recent study emphasized the value of early diagnosis of sepsis by analyzing heart rate characteristics by ECG monitoring. Griffin *et al* found that abnormal heart rate characteristics such as reduced variability, and transient decelerations occurred 24 hours prior to the onset of symptoms in sepsis and sepsis-like diseases (12). Another study found that sample asymmetry of RR intervals increased in 3-4 days prior to sepsis, with the steepest increase in the last 24 hours. These tests may prove helpful in starting the therapy long before the baby shows signs of deterioration (13).

### Gastrointestina

The symptoms present in this system are feed intolerance, vomiting, diarrhea, abdominal distension paralytic ileus, and necrotising enterocolitis.

### Hepatic

The common hepatic signs are hepatomegaly and direct hyperbilirubinemia. (Infants with the onset of jaundice after 8 days of age, or with direct hyperbilirubinemia, were more likely to have urinary tract infection)(14).

### Renal

There may be acute renal failure.

### Hematological

Hematological signs are bleeding, petechiae, and purpura.

### Skin signs

There may be multiple pustules, sclerema, mottling, umbilical redness and discharge. De Felice *et al* used colorimetric analysis of skin color to analyze the severity of sepsis. Color readings were taken from 10 different body sites using a portable tristimulus (15).

## Investigation

### HRC monitoring [heart rate characteristics]

Research about cardiac electrical patterns has revealed that reduced variability and transient decelerations in heart rate may be early indicators of clinical instability, and are hypothesized to be mediated by the cholinergic anti-inflammatory pathway (16). The HRC index is a statistically-derived interpretation of the beat-to-beat

variation in a patient (17). A low index indicates normal variation, but as normal variation is lost, the index rises, and so does the risk of clinical deterioration.

A recent randomized controlled trial of >3,000 very-low-birth-weight infants revealed that the use of HRC monitoring significantly decreased the 30-day mortality rate after a septic-like event, without a significant increase in antibiotic days (16). The mechanism by which mortality decreases in the monitored cohort remains unclear. In a separate study, neonates with culture-proven sepsis had a statistically higher HRC during the 24 hours leading to the septic episode, compared with the healthy control group (17). However, neonates with culture-negative, septic-like events had a statistically similar rise in HRC. Therefore, HRC is able to serve as an early warning sign of impending clinical instability. Additional research is needed to determine if it can differentiate between true sepsis and a culture-negative, septic-like event.

### Blood culture

Blood culture is the gold standard for diagnosis of septicemia. It should be done in all cases before starting antibiotics. One-ml sample of blood should be adequate for a blood-culture bottle containing 5-10 ml of culture media. Blood culture should be observed for 72 hours before labeling it sterile. Although it is time-consuming, empirical antibiotics are administered during this period.

### Sepsis screen (18, 19)

This is a panel of tests consisting of:

Component	Abnormal value
TLC	< 5000/mm <sup>3</sup>
ANC	< as per Manroe chart for term (12) and Mouzinho's chart for very LBW (VLBW) infants (13)
Immature/total neutrophil	> 0.2
Micro-ESR	> 15mm in 1st hr
CRP	> 1mg/dl

All neonates, suspected to have sepsis, should have a septic screen to corroborate the diagnosis. However, the decision to start antibiotics need not be conditional to the sepsis screen result if there is a strong clinical suspicion of sepsis. Sepsis screen is considered positive if two of these are positive. If the screen is negative but clinical suspicion persists, it should be repeated within 12 hours. If the screen is still negative, sepsis can be excluded with reasonable certainty. The absolute neutro-

phil count varies considerably in the immediate neonatal period, and normal reference ranges are available from Manroe's chart (20). For very-low-birth-weight infants, the reference ranges are available from Mouzinho's charts (21). Presence of two abnormal parameters in the screen is associated with 93-100% sensitivity, 83% specificity, and positive and negative predictive values of 27% and 100%, respectively in detecting sepsis.

In a recently published paper, the authors have evaluated the SNAP-II score for the assessment of illness severity which consists of 6 physiological parameters, namely lowest mean arterial pressure (MAP), worst ratio of partial pressure of oxygen (PaO<sub>2</sub>) to fraction of inspired oxygen (FiO<sub>2</sub>), lowest temperature (in °F), lowest serum pH, occurrence of multiple seizures, and urine output (<1mL/kg/hr). They found that SNAP-II can predict mortality as well as organ dysfunction in severely-septic neonates; individual components of the score do not have equal predictive abilities (22).

### Lumbar puncture (LP)

The incidence of meningitis in neonatal sepsis varies from 0.3-3%, in various studies (4, 10). In EOS, lumbar puncture is indicated in the presence of a positive blood culture, or when the clinical picture is consistent with septicemia. In case of LOS, LP should be done in all infants, prior to starting antibiotics. LP should not be done in the following cases: (23)

- Asymptomatic babies being investigated for maternal risk factors; However, LP should be performed in these cases as well, if blood culture becomes positive, subsequently.
- Premature neonates afflicted with respiratory distress syndrome (RDS); In this case, LP should be postponed in critically-ill and haemodynamically-unstable babies; if traumatic, it should be repeated within 12-72 hours. The cerebrospinal fluid characteristics are unique in the neonatal period, and normal values are presented in Table 1 (24).

### Urine culture

The rate of positive urine culture in infants with EOS is low. Given the low yield of positive

**Table 1.** Normal values of CSF in newborn period

Tests	Term	Preterm
Cells		
WBCs	7 (0-32)	9 (0-29)
Polymorphonuclear Cells	61 %	57 %
Protein (mg /dl)	90 (20-170)	115 (65-150)
Glucose (mg / dl)	52 (34-119)	50 (24-63)
CSF glucose : blood glucose	81 (44-248)	74 (55-105)

urine culture results, and costs of processing the specimens, urine culture should not be part of the traditional sepsis evaluation in the first 72 hours of life (24). Urine cultures obtained by suprapubic puncture or bladder catheterization have been recommended in all cases of LOS. However, neonates at risk of fungal sepsis, and very-low-birth-weight infants with poor weight gain, should have a urine examination to exclude urinary tract infection (UTI). UTI may be diagnosed in the presence of one of the following:

- (a) >10 WBC/mm in a 10 ml centrifuged sample.
- (b) >10 organisms /ml in urine obtained by catheterization, and
- (c) any organism in urine obtained by suprapubic aspiration.

## Radiology

Chest X-ray is done in cases of respiratory distress or apnea. Abdominal X-ray should be done for diagnosis of necrotizing enterocolitis.

### The Most Recent Diagnostic Tests of Neonatal Sepsis

Isolation of bacteria from blood is the most specific and standard method used to diagnose neonatal sepsis. The drawback of culture-based diagnosis is the 24–48 hour assay time. Newer diagnostic tests can be grouped into:

1. Acute phase reactants
2. Cell surface markers
3. Granulocyte colony-stimulating factor
4. Cytokines
5. Molecular genetics
6. Mol cell proteomics

#### Acute phase reactants

These groups of endogenous peptides are produced by the liver as part of an immediate response to the infection or tissue injury. These reactants are *C-reactive protein*, *procalcitonin*, *fibronectin*, *haptoglobin*, *lactoferrin*, *neopterin* and *oromucosoid*.

#### C-reactive protein (CRP)

CRP is synthesized within six to eight hours of exposure to an infective process or tissue damage, with a half life of 19 hours, and can increase more than 1000-fold during an acute phase response. In a study, it was concluded that CRP, IL-6 and IgM are helpful in the early diagnosis of Gram-negative neonatal sepsis, although CRP continues to be the best single test. A CRP value of 5 mg/l was the best among the three parameters with 95% sensitivity and 98% negative predictive value. The best combination was CRP  $\geq$  5 mg/dl and/or IgM of >

or = 20 mg/dl. The use of both CRP and IgM in combination was the most helpful method in predicting Gram-negative neonatal sepsis which had a significant role in making decisions regarding antibiotic treatments (25).

Another recent study was carried out in order to compare the efficiency of Serum Amyloid A (SAA) with that of C-reactive protein (CRP) and procalcitonin (PCT), in diagnosis and follow-up of neonatal sepsis in pre-term infants. The results showed that SAA is an accurate and reliable marker for the diagnosis and follow-up of neonatal sepsis. It is especially useful at the onset of inflammation for the rapid diagnosis of neonatal sepsis, and can be safely and accurately used in combination with other sepsis markers, such as CRP and PCT in diagnosis and follow-up of neonatal sepsis in pre-term infants (26).

#### Procalcitonin

Procalcitonin (PCT) which is produced by monocytes and hepatocytes, begins to rise four hours after exposure to bacterial endotoxin, reaches its peak after six to eight hours, and remains raised for at least 24 hours, with a half life of 25–30 hours. Both procalcitonin (2.3 ng/ml) and CRP (30 mg/l) had high specificity and positive predictive values (97%, 91% and 96%, 87%, respectively), though with low sensitivity (48% and 41%, respectively) for sepsis diagnosis. The conclusion was that procalcitonin >2.3 ng/ml or CRP > 30 mg/l indicates a high likelihood for neonatal sepsis, and antibiotic therapy should be continued even in the presence of sterile cultures. However, it is not a readily available diagnostic assay in most institutions (27).

#### Cell surface markers

Neutrophil CD11b and CD64 appear to be promising markers for the diagnosis of early- and late-onset infections. For culture-positive sepsis episodes, the CD64 index had the highest area under the curve (0.852) of all hematological variables, with a sensitivity of 80% , a specificity of 79%, and a cutoff value of 4.02. Therefore, neutrophil CD64 is a highly sensitive marker for neonatal sepsis. Prospective studies incorporating CD64 into a sepsis scoring system are warranted (28).

CD11b is a subunit of the  $\beta$ 2 integrin adhesion molecule, normally expressed at a very low concentration on the surface of non-activated neutrophils. There is a 2–4 fold increase in neutrophil CD11b expression in infants with blood culture positive sepsis. The sensitivity and specificity of CD11b for diagnosing EOS are 96–



100% and 81-100%, respectively. Nevertheless, in pre-term infants with RDS, significant activation of circulating phagocytes occurs within 1 to 3 hours of the onset of mechanical ventilation, independent of surfactant administration, which indicates that mechanical ventilation may be the inducer of this systemic inflammatory response. Therefore, CD11b is not a good marker for neonatal sepsis (29).

### **Granulocyte colony-stimulating factor**

Granulocyte colony-stimulating factor (GCSF), a mediator produced by bone marrow, facilitates proliferation and differentiation of neutrophils, and has been proposed to be a reliable infection marker for early diagnosis of neonatal sepsis. A concentration  $\geq 200$  pg/ml has a high sensitivity (95%), and negative predictive value (99%) for predicting early onset neonatal bacterial and fungal infections (30).

### **Cytokines**

As antigen-specific immunity develops later in infant's life, e.g. at 2 years of age in case of encapsulated bacteria, neonates initially depend on their natural (innate) immunity. This includes phagocytes (by monocytes, tissue macrophages, and neutrophils), natural killer cells, and humoral mediators (CRP, complements, and transplacentally-acquired maternal antibodies). In response to antigens such as bacterial endotoxins, activated tissue macrophages produce tumor necrosis factors (TNF) and interleukins (IL). These proinflammatory cytokines stimulate endothelial cells to express receptors for intercellular adhesion molecules on white blood cells. This initiates the cytokine cascade towards the increased production of IL6, IL8, and chemokines. Newborn infants display a higher percentage of IL6 and IL8 positive cells than adults do. There is a sharp rise in IL6 concentration on exposure to bacterial products, which precedes the increase in CRP. Umbilical cord blood IL6 has consistently been shown to be a sensitive marker for diagnosing early-onset neonatal sepsis at the onset of infection, compared with other biochemical markers, including CRP, IL1 $\beta$ , TNF $\alpha$ , and Eselectin, although sensitivity is reduced at 24 and 48 hours, since IL6 concentrations fall rapidly and become undetectable after 24 hours. The measurement of IL6 (early and sensitive) along with CRP (late and specific) in the first 48 hours of presumed septic episodes, improves the sensitivity compared with either of them alone (31).

IL-6 levels may be useful in the initiation, as well as early termination of antibiotic therapy in

late-onset neonatal sepsis (32). IL8 is a proinflammatory cytokine that is predominantly produced by monocytes, macrophages, and endothelial cells, with similar kinetics to IL6. IL8 is considered to be a highly accurate marker with its sensitivity ranging from 80% to 91%, and specificity from 76% to 100%.

TNF- $\alpha$  is a proinflammatory cytokine that stimulates IL6 production and has a broad spectrum of biological actions on several types of target cells, both immune and non-immune. Newborns developing early-onset infections are born with higher TNF- $\alpha$  concentrations than non-infected infants. Other markers studied over the last few years include adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, Eselectin, L-selectin), complement activation products (C3a-desArg, C3bBbP, SC5b-9), and IL-1 $\alpha$ , IL-1 $\beta$ , and IL-receptor antagonist (IL1RA), which have been found to significantly increase during sepsis, though these findings require further evaluation for clinical application in the diagnosis of newborns' infections. It has been demonstrated that median IL-6 and TNF- $\alpha$  levels were significantly higher in groups of patients with a diagnosis of clinical sepsis than in the controls. The optimal cutoff point was 32 pg/ml for IL-6 and 12 pg/ml for TNF- $\alpha$ . The combination of TNF- $\alpha$  and IL-6 provided a sensitivity of 98.5%, and it is a highly sensitive marker of sepsis in the immediate postnatal period (33).

In a recent study, it was demonstrated that the cytokines released in sepsis have an important role in stimulating nucleated RBC (NRBC) production, independent of hypoxia. In this study, significantly elevated NRBC was observed in EOS infants (no Early Onset Neonatal Sepsis (EOS) (n=49)) 1330 cells/cmm (665-2630), EONS (n=19) 3020 cells/cmm (1388-4558),  $p=0.011$ ), along with significantly elevated IL-6 in EONS; although no increase in the level of umbilical cortisol or erythropoietin was noticed. Increased NRBC count immediately after the birth could be an interesting marker of EONS in the absence of hypoxia and it awaits further evaluation (34).

### **Molecular genetics (35-38)**

Polymerase chain reaction (PCR) analysis relies on the fact that the bacteria specific 16S rRNA gene is highly conserved in all bacterial genomes, and so it can be useful for identification of bacteria in clinical samples. Amplification targeting of this 16S rRNA gene is a potentially valuable clinical tool in samples with low copy numbers of bacterial DNA, as this gene is present

**Table 2.** Accuracy of diagnostic tests or combinations of tests for early-onset neonatal sepsis

Diagnostic test with cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Antenatal				
Amniotic TNF 41 pg/ml (41)	82	79	47	95
PCR for genomic DNA in amniotic fluid (35)	100	100	100	100
Postnatal				
CRP (5 mg/dl) (23)	95			98
CRP (high cutoff 30mg/dl)(42)	41	91	87	
Procalcitonin (high cutoff 2.3ng/ml) (42)	48	97	96	
CD64 index plus ANC	95	57	63	93
CD64 index (4.02) (25)	70	62	59	73
IL-6 (32 pg/ml)(29)	98.5			
GCSF 200 pg/m (27)	95	73	40	99
Umbilical cord IL6	87-90	93	93	93-100
IL6 & IL10 & RANTES (31)	100	97		
IL6 (32 pg/ml) and TNF- $\alpha$ (12pg/ml)	98.5			
IL8 (not a good marker)	-	-	-	-
Real time PCR (43)	100	100	100	100

in 1 to more than 10 copies in all bacterial genomes. The gene also has a number of divergent regions nested within it, so PCR can be targeted for species-specific detection of bacteria in clinical samples. This technology has also been reported to be a very sensitive (100%), and rapid method for detecting potential pathogens in amniotic fluid, commonly involved in the pathogenesis of pre-term labour and adverse neonatal outcomes (35).

However, the performance of broad-range PCR analysis at a level of high analytical sensitivity is complex, and remains one of the most challenging PCR applications in the diagnostic laboratories. For instance, as 16S rRNA gene amplification targets all bacterial species, small amounts of inherent residual DNA present in the reagents may be coamplified, resulting in false positivity. Methods for the removal of potential background contaminations include long wave UV light gamma irradiation DNase, restriction endonuclease digestion, ultra filtration, and low DNA polymerases. However, many of these methods result in a reduced sensitivity in detecting target DNA, with a detection limit range of 10<sup>3</sup>-10<sup>4</sup> copies/ml, which is not ideal for diagnosing sepsis in clinical settings. It was found that a combination of pre-PCR culture with the use of AmpliTaq Low DNA achieves an acceptable level of sensitivity (5-50 copies/ml in a turn around time of eight hours) for the real time amplification of bacteria in blood samples, without the need to remove any inherent DNA contamination. Detection by PCR does not yield the antimicrobial sensitivity pattern of the pathogen. Early exclusion of bacterial infection could help to reduce overuse of antibiotics. It is predicted that eventually real time PCR combined with DNA Micro Array technology will allow not only identification of the organism but also the antimicrobial sensitivity pattern, which is so

critical to clinical care. It has been revealed from an Indian study that PCR is useful, and superior to blood culture for early diagnosis of sepsis in neonates with 100% sensitivity and 100% specificity. Once available in most tertiary centers, PCR can help in early and accurate diagnosis (38).

#### **Role of proteomics for diagnosis of neonatal infection (39, 40)**

In a study, it was found that significant alterations in levels of eight serum proteins in infected pre-term neonates (specifically P- and E-selectins, interleukin-2, soluble receptor  $\alpha$ , interleukin-18, neutrophil elastase, urokinase plasminogenactivator, its cognate receptor, and C-reactive protein) were observed at statistically significant increased levels.(39) Molecular tools (16S-rRNA) demonstrate that the diversity of microbial agents of intra-amniotic infection exceeds what is suspected clinically or is documented by cultures. The resulting inflammatory process has the potential to damage the fetus *in utero*. Stepwise algorithms (mass restricted score) have been developed to extract proteomic profile characteristics of amniotic fluid inflammation. The mass restricted score includes four proteomic biomarkers: defensin-2, defensin-1, S100A12, and S100A8 proteins. Other amniotic fluid biomarkers relevant for pre-term birth are S100A9 and insulin-like growth factor-binding protein 1. S100A12 - ligand for the receptor of advanced glycation end products - which have the strongest association with histological chorioamnionitis and funisitis. So the conclusion was that the presence of S100A12 and S100A8 in amniotic fluid is predictive of early-onset neonatal sepsis and poor neurodevelopmental outcome (40).

#### **Accuracy of Diagnostic Tests**

The accuracy of diagnostic tests or combina-

tions of tests for early onset neonatal sepsis has been summarized in Table 2.

## Management

### Supportive Treatment

Attention should be given to basic supportive care of a sick child. The infant should be nursed in a thermoneutral environment to avoid hypothermia and hyperthermia. Oxygen saturation should be maintained in the normal range, and ventilation should be initiated, as required. The infant should be regularly monitored for hypoglycemia/ hyperglycemia. Colloids and inotropes are used for maintaining normal blood pressure and tissue perfusion. Enteral feeding should be avoided till the baby is haemodynamically stable. Packed cells and fresh frozen plasma should be used appropriately for the management of anemia and bleeding diathesis.

### Antimicrobial Treatment

The choice of antibiotics for an infant with suspected sepsis will depend upon the predominant pathogen and antibiotic sensitivity pattern of a given region. For the neonates suspected to have community-acquired sepsis, and in whom resistant strains are unlikely, the combination of ampicillin and gentamicin is advised for septicemia and pneumonia; third-generation cephalosporin e.g. cefotaxime, is added if meningitis is present in these cases. Cefotaxime, a broad-spectrum cephalosporin, should be used for its reduced toxicity. Ceftriaxone is not commonly used due to its potential for displacement of bilirubin from albumin, and causing hypoprothrombinemia and bleeding. When infection is hospital-acquired, and there is a high probability of resistant strain, cefotaxime in combination with an aminoglycoside, should be used for septicemia and pneumonia, as well as meningitis. Third-generation cephalosporins have very good CSF penetration and are traditionally thought to have excellent antimicrobial activity against Gram-negative organisms. Hence they were considered to be a good choice for the treatment of nosocomial infections and meningitis. However, recent reports suggest that at least 60-70% of the Gram-negative organisms are resistant to them. Moreover, the routine use of these antibiotics might increase the risk of infections with extended-spectrum beta-lactamase (ESBL) positive organisms. Therefore it is preferable to use antibiotics, such as piperacillin-tazobactam or methicillin/vancomycin in units with high incidence of resistant strains. A combination of piperacillin/tazobactam with

amikacin should be considered if *Pseudomonas* sepsis is suspected.

Penicillin-resistant staphylococcus aureus (PRSA) should be treated with cloxacillin, nafcillin or methicillin. Addition of an aminoglycoside is useful in the therapy against staphylococcus. Methicillin-resistant *Staphylococcus aureus* (MRSA) should be treated with a combination of ciprofloxacin or vancomycin with amikacin.

### Treating Multidrug Resistance (MDR) Infections

Many clinicians are now finding that their infant patients are infected with antibiotic-resistant nosocomial pathogens, such as vancomycin-resistant *Enterococcus*, carbapenemase-producing *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (44). Described here are several antibiotic agents, not routinely used in the NICU, which are capable of treating infections, caused by resistant organisms. As with older antibiotics, the emergence of microbial resistance to these newer antibiotics is inevitable, and, in many cases, has already occurred. Thus, continued judicious use of antibiotics is necessary to maintain the effectiveness of these new antimicrobials.

### Gram-Positive Infections

Recent studies have reported that linezolid and daptomycin are used in children and neonates for the treatment of infections, caused by Gram-positive organisms resistant to  $\beta$ -lactams and vancomycin. Linezolid is an oxazolidinone antimicrobial which was licensed by the U.S. Food and Drug Administration for use in pediatrics, in 2002. The antimicrobial effect of the drug is mediated through the disruption of bacterial protein synthesis, induced by linezolid binding to ribosomal RNA (45). Although linezolid is bacteriostatic against all *Staphylococcal* and *Enterococcal* species, it has been successfully used to treat neonatal methicillin-resistant *Staphylococcal*, and vancomycin-resistant *Enterococcus* infections (45). A subset analysis of 63 neonatal patients in a pediatric Phase III study, compared the use of linezolid with vancomycin in the treatment of resistant Gram-positive infections, and revealed equal cure rates and lower adverse events in the linezolid group (46). Linezolid has good penetration into the skin and lungs and is approved for the treatment of pediatric skin and soft-tissue infections, and nosocomial pneumonias. Linezolid has been used successfully to treat pediatric bloodstream infections and endocarditis, caused by vancomycin-resistant organisms (47). Adverse

effects of linezolid treatment are seen more commonly in therapy courses lasting >2 weeks, and include reversible myelosuppression and lactic acidosis. During the treatment with linezolid, patients should have their serum lactate and blood cell counts checked, periodically.

Daptomycin is a cyclic lipopeptide antimicrobial, derived from *Streptomyces roseosporus* and is bactericidal against resistant Gram-positive organisms. The mechanism of action is poorly understood, but daptomycin is theorized to form pores in the cell membranes of Gram-positive organisms (48). Although daptomycin has had great success in treating adult bloodstream infections and endocarditis, it is not currently approved by the U.S. Food and Drug Administration for use in pediatric patients (49). A small number of reports describe the successful off-label use of daptomycin for the treatment of resistant infections that occurred in neonates, while receiving vancomycin therapy (49). Daptomycin is inactivated by pulmonary surfactant, rendering it ineffective in treating pulmonary infections. Until appropriate pharmacokinetic studies are performed in the pediatric population, daptomycin should be used only as a second-line drug for treatment of neonatal vancomycin-resistant infections, including bloodstream infections and endocarditis.

### **Gram-Negative Infections**

Gram-negative organisms are isolated less frequently than Gram-positive organisms. However, in some developing nations, Gram-negative organisms are more common in both EOS and LOS (50). Unfortunately, the global epidemic of antibiotic resistance has created a major problem in treating Gram-negative sepsis everywhere. Therefore, the evaluation of antimicrobial agents which are effective against Gram-negative organisms, and have not been previously used in the pediatric population, has become critically important. Here we review the use of ciprofloxacin and colistin in neonates. Ciprofloxacin is a fluoroquinolone that inhibits bacterial DNA topoisomerases (51). Ciprofloxacin is bactericidal against most Gram-negative organisms, including *P. aeruginosa*. The use of fluoroquinolones in pediatrics has been limited by reports of increased musculoskeletal adverse events in young animals. A recently published systematic review suggests that there is increased risk of arthropathy in ciprofloxacin-treated pediatric patients, but this risk is relatively low, and the arthropathy is reversible (52). Despite the

concerns over possible adverse effects, ciprofloxacin has been approved by the U.S. Food and Drug Administration for the treatment of complicated urinary tract infections in children.

A systematic review of five cohort studies evaluated the use of ciprofloxacin in the treatment of neonatal sepsis, caused by antibiotic-resistant Gram-negative organisms. Two of the five cohorts assessed the clinical responses, with one showing a significantly higher survival rate in the ciprofloxacin-treated group versus the treatment with ampicillin, gentamicin, and/or cefotaxime. (53). Two significant limitations to the use of ciprofloxacin in neonates are the paucity of pharmacokinetic data in the neonatal population, and the very high doses of ciprofloxacin needed to successfully treat infections caused by *Acinetobacter* and *Pseudomonas* organisms (53).

Colistin is a polymyxin initially discovered in 1947, and it functions as a detergent, dissolving the cellular membranes of Gram-negative organisms (54). Systemic polymyxins fell into disuse in the early 1980s, in favor of less toxic aminoglycosides and broad-spectrum  $\beta$ -lactams. However, with the continued emergence of MDR organisms, polymyxins have again found use as the only antibiotic left to treat some Gram-negative infections. In a retrospective study of neonates with MDR Gram-negative infections, susceptible to only colistin, 12 neonates were bacteremic; two had concomitant meningitis (54). Of the colistin-treated cohort, two-thirds of the patients survived, including one with meningitis. Two infants developed renal impairment—the main toxicity associated with colistin use – but both infants had multiorgan dysfunction before starting colistin therapy. In view of the situations requiring its use, which are often desperate, the rate of colistin-related nephrotoxicity is tolerable; however, regular monitoring of patients' renal function during colistin therapy is recommended (55). Colistin dosing guidelines have not been well-established in the neonatal population. Finally, although rare, resistance to colistin has emerged, leaving patients mortally infected by organisms resistant to all commercially available antibiotics. It should be emphasized that none of the antibiotics discussed here are superior for treatment of infections sensitive to older, more commonly used antibiotics. The use of these recent antibiotics should remain restricted to the definitive therapy of infections caused by resistant organisms. None of these drugs should be used for empirical therapies. The recommended doses of antibiotics mentioned here, and in the two previous articles are listed in Table 3.



**Table 3.** Dosing of Antimicrobial Agents

Agent	Dosing
Amphotericin B (conventional)	1 mg/kg per dose q24h
Ampicillin	50 mg/kg per dose <7 d 2 kg: q12h 2 kg: q8h GBS meningitis: 300 mg/kg per d divided q8h >7 d <1.2 kg: q12h 1.2-2 kg: q8h >2 kg: q6h GBS meningitis: 300 mg/kg per d divided q≥6h
Caspofungin	25 mg/m <sup>2</sup> per dose q24h
Cefotaxime	50 mg/kg per dose <7 d, <2 kg: q12h <7 d, ≥2 kg: q8h >7 d <1.2 kg: q12h 1.2-2 kg: q8h >2 kg: q6h
Ciprofloxacin	10 mg/kg per dose q12h
Colistimethate	2.55 mg/kg per d (expressed as colistin base) divided q6h–q12h
Daptomycin	Limited data regarding neonatal dosing <sup>b</sup>
Fluconazole	12 mg/kg per dose q24h
Gentamicin	2.5-3.5 mg/kg per dose q8h–q24h based on postnatal age, weight, and serum concentrations (varies according to institution)
Linezolid	10 mg/kg per dose GA ≤34 wk PNA <7 d: q12h PNA ≥7 d: q8h GA ≥35 wk: q8h
Meropenem	GA ≤30 wk: 20 mg/kg per dose q12h GA >30 wk PNA 0–7 d: 20 mg/kg per dose q12h PNA >7 d: 20-40 mg/kg per dose q8h
Vancomycin	10–15 mg/kg per dose q6h–q24h based on postnatal age, weight, and serum concentrations (varies by institution)

GA, gestational age; PNA, postnatal age.

Dosing regimens from Lexicomp online (<http://online.lexi.com/>).

<sup>a</sup> Higher doses may be necessary to treat organisms such as *Pseudomonas* and *Acinetobacter*.

<sup>b</sup> Use of 10 to 15 mg/kg q24h has been reported.

More recent antibiotics like aztreonam, meropenem and imipenem are also now available in the market. Aztreonam has an excellent activity against Gram-negative organisms, while meropenem is effective against most bacterial pathogens, except methicillin-resistant *Staphylococcus aureus* (MRSA) and enterococcus. Imipenem is generally avoided in neonates because of the reported increase in the incidence of seizures following its use. Empirical use of these antibiotics should be avoided; they should be reserved for situations where sensitivity of the isolated organism warrants its use. A simple approach for the management of sepsis is given in Figure 1.

#### **Duration of antibiotic therapy**

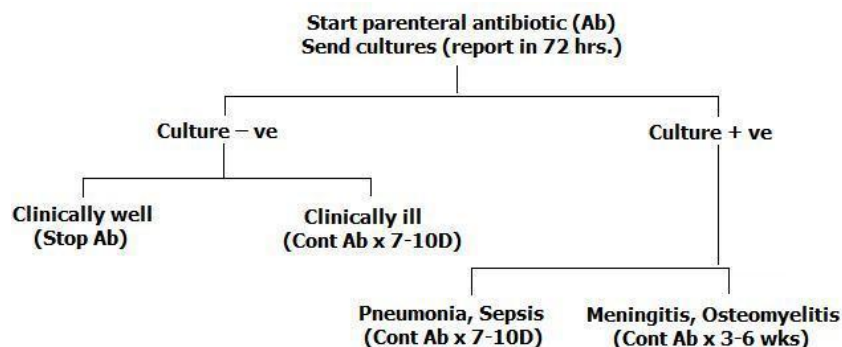
- Clinical sepsis (Based on clinical suspicion and/or positive sepsis screen) -7-10 days
- Culture-positive sepsis (not meningitis), UTI- 14 days

- Meningitis-2 weeks after sterilization of CSF culture or for a minimum of 2 weeks for Gram-positive meningitis, and 3 weeks for Gram-negative meningitis, whichever is longer.
- Bone and joint infection-4-6 weeks

### **Adjunctive therapy**

#### ***Intravenous Immune Globulin (IVIG)***

Immunotherapy used as an adjuvant holds promise for the prevention and treatment of neonatal sepsis. In a recent paper, the authors have reviewed immunotherapies that modulate the immune system of the neonate, including intravenous immunoglobulins, and myeloid haematopoietic growth factors. Future studies should focus on investigating other abnormalities of neonatal host defense and/or combined immunotherapy approaches, in an attempt to circumvent the immaturity of host defense, and



**Figure 1.** Suspected Neonatal Sepsis

potentially reduce both the incidence and severity of neonatal sepsis (56).

### **Granulocyte colony-stimulating factor (G-CSF)**

Carr and colleagues reported a randomized trial (PROGRAMS) of Granulocyte-monocyte colony-stimulating factor (GM-CSF) for the prevention of sepsis in small-for-gestational-age, pre-term neonates. This increased the neutrophil count, but had no effect on the primary end point of sepsis-free survival to 14 days from trial entry (57). According to the Cochrane Database of Systemic Review, there is currently insufficient evidence to support the introduction of either G-CSF or GM-CSF into neonatal practice, either as the treatment of established systemic infection to reduce resulting mortality, or as prophylaxis to prevent systemic infection in high-risk neonates. The limited data suggesting that G-CSF treatment may reduce mortality rate, when systemic infection is accompanied by severe neutropenia, should be investigated further in adequately powered trials, which recruit sufficient infants infected with organisms associated with a significant mortality risk (57).

### **Exchange transfusion**

Exchange transfusion in neonatal sepsis has not been extensively studied. It may be used with caution in neonatal sepsis associated with neutropenia, sclerema, earliest evidence of disseminated intravascular coagulation, and metabolic acidosis ( $\text{pH} < 7.2$ ) (58).

### **Pentoxifylline**

Pentoxifylline is a methylxanthine that has been postulated to improve outcomes in sepsis through modulating the activity of the reticulo-endothelial system and decreasing the neutrophil activation that contributes to acute tissue injury. Large-scale clinical trials have not yet been performed (59).

### **Advances in Prevention**

### **Before Delivery**

Maternal immunisation is an important method of providing neonates with appropriate antibodies, as soon as they are born (60). This approach, in comparison with other approaches, is less sensitive to obstacles in accessing the health care system. Examples of successful interventions include maternal tetanus toxoid, and influenza immunisations. Studies of maternal immunisation with *S. agalactiae* type III conjugate vaccine have demonstrated excellent placental transfer and persistence of protective levels in 2-month-old infants (60). Encouraging results are also emerging from studies of maternal immunisation with pneumococcal polysaccharide and conjugate vaccines (50). The vaccines all have excellent safety profiles. However, barriers to maternal immunisation include: liability issues for vaccine manufacturers in developed countries; education of the public and health care providers regarding the benefits of maternal immunisation; and poor ascertainment of data from low-income countries (60). The development of group B streptococcal (GBS) vaccines has been promising. The vaccines target conserved surface antigenic proteins, such as the Sip protein located on the cell surface, or immunogenic proteins from GBS pili. If a protective immune response is achieved, it would inhibit GBS adhesion to host tissue and prevent transepithelial migration. Although they are not yet commercially available, several vaccines are close to being released and will hopefully prove to be efficacious in decreasing the rates of EOS and LOS, caused by GBS (61).

### **During Labour and Delivery**

There is strong evidence that clean delivery practices and handwashing during delivery reduces rates of neonatal sepsis both at home and in health facility settings (62). Interventions to improve hand washing rates have been remarkably successful in research settings (62). New studies from Malawi and Nepal indicate that

maternal antiseptic interventions such as vaginal chlorhexidine during labour may have a significant impact on rates of neonatal mortality and sepsis in developing countries (63). Intrapartum antibiotic prophylaxis has been highly effective in reducing both early-onset neonatal bacterial and maternal sepsis in developed countries (64). Risk factors for early-onset neonatal bacterial sepsis in low-income settings are probably similar to resource-rich settings, but have not been evaluated in the context of high rates of maternal undernutrition, anaemia, HIV, and malaria.

#### **After Delivery**

There is also strong evidence that hand washing by health care providers after delivery can reduce neonatal sepsis and infection rates, especially in hospitals (62). Umbilical stump chlorhexidine cleansing has recently been shown to substantially reduce neonatal deaths in Nepal (65). There is emerging evidence that neonatal skin antiseptic preparations, such as sunflower seed oil provides cheap, safe, and effective protection against nosocomial infections in hospitalized pre-term neonates, and infants in studies in South Asia. Application of chlorhexidine to neonatal skin has also been shown to be effective in reducing neonatal sepsis in studies from South Asia (66). Neonatal immunisation has long been considered an important method of reducing neonatal infections. However, the response varies according to the antigen (67). BCG, polio, and hepatitis B vaccines are highly immunogenic when given at birth (68). However, maternal antibodies interfere with a neonate's response to measles vaccine when administered under six months of age. Protein antigen vaccines (e.g. pertussis and tetanus toxoid) given at birth, have been shown to produce poor responses compared to the same antigen given at two months of age, and are associated with later tolerance (68). Studies also indicate that *S. agalactiae* and *Streptococcus pneumoniae* vaccines are both likely to be ineffective when given in the neonatal period (67). Breast milk contains secretory IgA, lysozymes, white blood cells, and lactoferrin, and has been shown to encourage the growth of healthy lactobacilli and reduce the growth of *E. coli* and other Gram-negative pathogenic bacteria (67). RCTs that focused on increasing early initiation and exclusive breastfeeding rates demonstrated significant reductions in diarrhoea and acute respiratory infections in neonates and older infants in India (69). Neonatal micronutrient supplementation trials have focused on vitamin A

supplementation. Older studies have shown significant reductions in respiratory disease in low-birth-weight infants after the administration of parenteral vitamin A (70). More recently, trials of vitamin A supplementation in newborns have shown encouraging reductions in neonatal mortality, and more trials are underway (71).

#### **Conclusion**

The diagnosis of neonatal sepsis continues to be a major clinical challenge. In an effort to improve the outcomes of infected infants, research is being conducted to improve our ability to detect sepsis earlier, and identify the organisms more quickly. The use of biomarkers and the HRC index will hopefully provide additional tools for neonatologists to recognize and treat potentially infected infants. New technologies being introduced into the microbiology laboratory will be able to confirm the presence of a pathogen by using faster and more reliable methods. Another important challenge in neonatal sepsis is the increasing rates of antimicrobial resistance, requiring the use of unfamiliar antimicrobial agents to treat MDR organisms. Initial studies of recently-found antibiotics are promising, but large-scale studies are needed to better evaluate these agents, and provide better neonatal pharmacokinetic data. Lastly, because novel treatment agents will only take us so far, more research is needed to determine how to prevent these infections.

#### **Acknowledgement**

The authors would like to thank from all coworkers that help us to this Research.

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