CORRELATIONAL STUDY OF CRP AND BLOOD CULTURE IN EVALUTION OF NEONATAL SEPSIS

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INTRODUCTION

Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the first month of life. Neonatal sepsis encompasses systemic infection of the newborn including septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infection of the newborn [(1)].

Globally of the 130 million babies born every year, about 4 million die in the first 4 weeks of life, i.e. neonatal period. The main direct causes of neonatal deaths are Estimated to be preterm birth (28%), severe infection (26%), and birth asphyxia (23%).[(2)]

According to National Neonatal Perinatal Database (NNPD) 2002-03 collected from 18 centers from various parts of India, incidence of neonatal sepsis has been reported to be 29.9 per 1000 live births. Early onset sepsis contributed 67% of all sepsis. Meningitis contributed to 10.6% of all cases of sepsis. Neonatal sepsis was one of the common causes of neonatal mortality contributing to 16% of all intramural deaths . [(3)].

Neonatal septicemia with its high incidence and its grave prognosis, in spite of adequate treatment with modern antibiotics, has been a challenge for all times. Optimal diagnosis and treatment strategies are difficult to define. The signs and symptoms are protean with a high mortality and thus there is urgent need to know whether the baby has sepsis, to institute treatment as quickly as possible, Confirmation of the diagnosis by definitive culture is not possible rapidly.[(4)]

The concentration of many serum proteins raises in response to inflammation, associated with infection, trauma or tissue damage. Among these proteins important being CRP, haptoglobin and fibrinogen. These can be used as non-specific indicators of bacterial sepsis.

Sepsis screen tests involving WBC indices and CRP form simple, cheap, rapid and early easily available parameters with reasonable diagnostic accuracy especially when they are used in combination. On this basis early and rational antibiotics therapy can be started in critical septicemic infants.

AIMS AND OBJECTIVES

To determine

- Sensitivity
- Specificity
- Predictive values of CRP, as an indicator of neonatal sepsis in comparison with blood culture.

REVIEW OF LITERATURE

Epidemiology

Neonatal sepsis ranks among the three commonest illnesses affecting babies and ranks as the top most illness for neonatal mortality especially amongst low birth weight and premature babies. [7].

Neonatal sepsis may be categorized as early or late onset sepsis. Early onset sepsis syndrome is associated with acquisition of microorganism from mother. Transplacental infection or an ascending infection from cervix may be caused by organisms that colonize in the mother's genitourinary tract. The infant may acquire the microbe by passage through a colonized birth canal during delivery [7].

Late onset sepsis syndrome is acquired from environment. The infant's skin, respiratory tract, conjunctiva, gastrointestinal tract and umbilicus may become colonized from the environment, leading to the possibility of late onset sepsis from invasive microorganisms. Vector for such colonization include vascular or urinary catheters, other indwelling lines or contact from caregivers with bacterial colonization [7].

Incidence

The reported incidence in United states and Australia range from 1.5 to 3.5 per 1000 live births for early onset sepsis and up to 6 per 1000 live births for late onset sepsis, a total of 6-9 per 1000 live births for neonatal sepsis. The reported incidence of neonatal sepsis varies from 7.1 to 38 per 1000 live births in Asia and from 6.5 to 23 per 1000 live births in Africa. [8]

According to recent data published from National Neonatal Perinatal Database (NNPD) 2002-03, collected from 18 centers from various parts of India incidence of neonatal sepsis has been reported to be 29.9 per 1000 intramural live births. Early onset sepsis contributed 67% of all cases of sepsis. [9]

Risk Factors for Neonatal Sepsis

The pathogenesis of neonatal septicemia is resolved around interplay of host factors such as gestational age and birth weight of neonate, environmental factors such as intrapartum risk

factors or nosocomial risk factors and the agent factors such as virulence and load of organism causing sepsis.

- Neonatal risk factors
- Perinatal risk factors mainly for early onset sepsis.
- Nosocomial risk factors for late onset sepsis.
- Agent factors.
- Other factors.

1. Host factors

A. Immunological aspects of Newborn

An individual becomes immune or protected from re-infection in response to antigens encounter during an initial infection. Mature immunological competence is ultimately achieved through cumulative adaptive changes stimulated by exposure to large repertoire of foreign antigenic material. Since the in utero fetal environment is sequestrated from frequent encounters with microorganisms, the host defense system of the human newborn infant is inexperienced. Furthermore, although many components of the immune system of the fetus are present early in gestation, some are immature and do not become fully functional until some time after birth. This accounts for the newborn's vulnerability to microbial attack. [10]

a. Natural immunity:

The natural barriers such as the skin and mucous membranes in neonates, especially in preterm neonates may not be as efficient in older children and adults. Skin is vulnerable to damage and infection particularly so in preterm, due to lack of integrity, lack of complete cornification and antigenic inexperience of langerhan's cells. Mucosal integrity is also weak. MALT (mucosal associated lymphatic tissue) is not well formed. Lack of gastric juice and enzymes in intestinal secretions leads to colonization of pathogenic bacteria and mucosal invasion.[10]

b. Phagocytic System in Newborn

Quantitative and qualitative deficiencies of the phagocytic system are important factors contributing to increased susceptibility to infection. Neonatal neutrophils have decreased adhesion and aggregation due to abnormal expression of cell membrane adhesion molecules

(the beta 2 integrins and selectins). Abnormalities in cytoskeleton contribute to defective chemotaxis. Phagocytosis and microbial activity is usually normal except in acute stress and high bacterial load where there is increased oxidative burst and self-damage due to relative lack of detoxifying enzymes and antioxidants in neonate. (10)

The number of circulating neutrophils is elevated after birth in term and preterm infants, peaking at 12 hrs and then starts decreasing till 72 hrs. After 72 hrs it returns to normal and remains the same. Rapid depletion of circulating pool and bone marrow storage pool polymorphs due to deficiency and/or delayed response GM-CSF and G-CSF and increased margination will lead to frequent occurrence of neutropenia in preterm babies and thus increasing the risk of acquiring infection (10)

c. Immunoglobins

The impaired humoral defense of the newborn plays a significant role in the pathogenesis of neonatal septicemia. IgG is actively transported across the placenta which starts around 22 weeks of gestation, with concentration in a full term infant compatible to mother serum levels. In premature infants cord IgG levels are directly proportional to gestational age. Levels of maternally derived IgG fall rapidly after birth hence preterm babies become significantly hypogammaglobulenemic. (11) Other classes of Immunoglobulines such as IgA and IgM are not transferred across placenta. The presence of passively transferred specific IgG antibody in adequate concentration provides neonates, the protection against infections mediated by antibody (e.g. Tetanus, encapsulated bacteria such as GBS). Specific bactericidal and opsonic antibodies against enteric gram-negative bacteria are predominantly is IgM class.In general, newborns lack antibody mediated protection against Escherichia coli and other Enterobacteriaceae.(11)

d. Complement System

The complement system mediates bactericidal activity against certain organisms such as Escherichia coli and function as opsonin with antibody in the phagocytosis of bacteria such as GBS. Essentially no transplacental passage of complement from the maternal circulation takes place. Neonates in general, preterm babies in specific have slight diminished classical complement pathway activity and moderately diminished alternative pathway activity. These deficiencies contribute to diminished complement derived chemotactic activity and to a

diminished ability to opsonize certain organism like Escherichia Coli and GBS.[11]

Fibronectin is a glycoprotein found in the plasma and tissues that contribute to adhesion of neutrophils and monocytes and binds to certain bacteria with or without antibody and complement. Plasma fibronectin levels are decreased in neonates and further diminished in premature newborns and newborns with septicemia.[10]

B. Neonatal Risk Factors

Important predisposing risk factors in the host for neonatal septicemia include prematurity, low birth weight, male sex, birth asphyxia, difficult resuscitation and birth injuries.

a) Prematurity:is the single most significant factor in neonatal septicemia. Premature infants have a 3-10 fold higher risk compared to term neonates. Prematures are extremely vulnerable to infection because of their inherent compromised immunity, vulnerable skin and mucosal barrier, prolonged in hospital stay and extensive interventions for other complication of prematurity. [5]

Prematurity as a major risk factor for sepsis was reported by Khatua et al[12], Joshi et al[13] and Nawshad et al[14] in range of 50% of culture proven sepsis being prematures.

b) Low birth weight: babies with birth weight < 2500 grams are more likely to develop septicemia due to inappropriate immunological response. They have low levels of various complement system and poor mucosal defenses. Birth weight < 1000grams increases the neonatal infection rate by 26 folds when compared to term infants. The incidence of septicemia inversely proportional to birth weight and gestational age of the neonates.[14]

Higher proportion of culture proven sepsis was reported in low birth weight babies in range of 50 to 63 % by Joshi et al[13], Tallur et al[15], I Roy et al[16]

c) Birth asphyxia: is defined as one minute APGAR score 0-6. Perinatal asphyxia depresses the immune functions. Additional interventions frequent suction, intubation, prolonged ventilator care to manage asphyxia may impact extra risk of contracting infections in neonates and birth injuries may further complicate the issue.

Birth asphyxia as one of the perinatal risk factor for sepsis was reported by Tallur et al[15], I Roy et al[16] and Dawodu et al[17].

d)Male gender: Male infants are 2-6 times more at risk of neonatal septicemia than females. This may be linked to the X – linked immunoregulatory genes. [12]

Higher proportion of male babies had cultured proven sepsis as reported by Khatua et al[12], Tallur et al[15], and Kuruvilla et al[18].

C. Perinatal risk factors:

Mothers can acquire acute infections during pregnancy or just before delivery and in turn transmit the infections to the fetus transplacentally**.** Jeffery S. Gerdes[5] in his article on diagnosis and management of bacterial infection in the neonate quotes the risk factors for neonatal sepsis and incidence of proven sepsis in each of them.

Risk factors	**Incidence of proven Sepsis (%)**
PROM>18 hours	1%
Maternal + GBS(pre-prophylaxis era)	0.5%-1
Maternal + GBS(in prophylaxis era)	0.2-0.4
Maternal + GBS+PROM/fever/preterm	4-7
Chorioamnionitis	3-8
Chorioamnionitis + GBS	6-20
PROM + preterm	4-6
PROM + low APGAR score	3-4

a) PROM: The risk of neonatal septicemia increases to approximately 10 folds in neonates born to mothers with rupture of membranes for more than 18 hrs before delivery. PROM is associated with approximately 1% incidence of culture proven sepsis and 2% for suspected sepsis.[5]

b) Chorioamnionitis: The generally accepted clinical definition of chorioamnionitis is the

presence of maternal fever >100.4 deg.F (38 deg C) with two or more of the following findings – fetal tachycardia, Uterine tenderness, foul vaginal discharge or maternal leucocytosis. The reported range of neonatal sepsis when present is 3-20%.[5] Study conducted by Kuruvilla et al[18] showed that the newborn was at a 1-5% risk of acquiring infection when the gestation age was < 34 weeks and when prolonged rupture of membranes for > 24 hrs was associated with amniotic fluid infection.

c) Foul smelling liquor: The presence of foul-smelling liquor has been considered to be an indicator of chorioamnionitis. Takkar et al. [19] observed that the presence of foul smelling liquor was associated with a 10% incidence of septicemia in their study.

d) Unclean vaginal examination: History of unclean vaginal examination is associated with 10% risk of infections. Multiple vaginal examination (>3 vaginal examination after onset of labor) are associated with 20% of early onset sepsis. It is an independent risk factor causing neonatal septicemia [13]

e) Prolonged labor: Labor lasting more than 24 hrs with prolonged duration of second stage of labor with ruptured membranes, increases the chances of invasion of microorganisms into the fetus. Takkar et al[19], observed that prolonged labor was associated with a 7.3% incidence of septicemia in their study.

f) Maternal colonization with Group B streptococcus (GBS): Maternal colonization with GBS without clinical complications and without antibiotic prophylaxis carries a neonatal risk of 1%.[5].

Boyer and Gotoff's[20] comprehensive studies have identified the three high risk situations that increase the likelihood of neonatal GBS disease when the mother is colonized. They are:-

a. PROM > 18 hours (risk increased seven-fold)
b. Maternal fever (risk increased four fold)
c. Prematurity (risk increased seven fold)

Vidya Ayengar et al[21] in their study on neonatal sepsis due to vertical transmission from maternal genital tract. high vaginal swabs of 1792 expectant mothers were sent for culture at the time of delivery prior to first vaginal examination. Appropriate cultures of the babies who developed infections were sent. Bacterial growth of predominantly gram-negative organisms. Infection developed in 48(27%) babies in 1st 72 hours of life of which 28 had deep infection

while the rest had superficial infection.

g. Mode of presentation, type and place of delivery: Abnormal presentation, difficult labor and instrumental vaginal delivery are associated with increased risk of infection. Higher incidence of septicemia is also noted in babies delivered at home or at a tertiary hospital. Unclean delivery practices in home deliveries and prolonged hospital stay of babies, delivered by operative means predispose them to infections acquired from the environment[9]

Sepsis Score/Predictive Perinatal Infection Risk Score:

The earliest scoring system was evolved in 1975 by Takkar[19] VP who assigned numerical values to six adverse perinatal factors depending upon incidence deep infection in association with particular risk factor

Table 1.PREDICTIVE PERINATAL RISK SCORE

SL.NO.	Perinatal factor	Risk Score
1	Foul smelling liquor	2
2	Unclean vaginal examination done before delivery	2
3	Duration of labor exceeding 24 hours	2
4	One minute APGAR score of 0 - 6	2
5	Duration of rupture of membrane before delivery > 24 hours	1
6	Birth weight 2 kg or less and / or gestation less than 37 wks	1
	Total Score	10

Risk Group category with suggested intervention

Total sepsis score	Risk Group	Intervention Suggested
(0-3)	Low Risk	Withhold antibiotics
(4-5)	Moderate Risk	Investigate for presence of infection; give antibiotics if circumstantial evidence of infection is present.
(6 - 10)	High Risk	Start Antibiotics immediately

This scoring system assumes significance in developing countries like India where a battery of laboratory screening tests is an additional financial burden.

Gupte et al[22] modified the Septic score by adding maternal pyrexia as additional perinatal risk factor and assigned higher relative score to low birth weight and prematurity, which was widely accepted at Asia Oceanic Perinatalogy conference. This septic score is adopted as neonatal sepsis protocol in PGI, Chandigarh[23].

SEPSIS SCORE

SL.NO	Perinatal factor	Risk Score
1	Foul smelling liquor	2
2	Unclean vaginal examination done before delivery	2
3	Duration of labor exceeding 24 hours	2
4	Birth asphyxia (APGAR < 6 at 1 min.)	2
5	Birth weight 2.5 kg or less and / or gestation age< 37 wks	3
6	Duration of rupture of membrane before delivery > 24 hours	1
7	Maternal pyrexia	1
	Total Score	13

Action based on score:

Score 0-3 : observe clinically

Score >/= 4: investigate

D. Nosocomial risk factors

Nosocomial or hospital acquired infection is not uniformly defined. Many define it as infections occurring after 3 days of life those are not directly acquired from the mother's genital tract. The Centers for Disease Control and Prevention (CDC) defines a nosocomial infection as any infection occurring after admission to the NICU that was not transplacentally acquired. The risk factors associated are prematurity, LBW, prolonged length of hospital stay, crowding in nursery with no proper cohorting. invasive procedures, indwelling vascular catheters, parenteral nutrition with lipid infusions, endotracheal tubes, ventricular shunts, alteration in skin and mucous membrane barrier, lack of adequate hand washing by hospital personnel and indiscriminate use of prophylactic antibiotics that alter the indigenous flora of the neonate eliminating sensitive strains and leads to colonization and proliferation of more virulent drug resistant strains of microorganisms[(11)].

Stoll BJ in NICHD(National institute of child health and human development) Neonatal Research Network studied large cohort of VLBW babies and reported nosocomial infection rates of 20-25% which is inversely proportional to birth weight. [(27)]

National Nosocomial Infection Surveillance System monitoring device associated nosocomial infection rates has reported 11.4 infections /1000 device days for infants under l000gms and 3.8 infections/1000 device days for those over 2500gms[(11)].

Anil Kumar Pawa et al[(11)] in study on neonatal nosocomial infection profile and risk factors reported that neonates who developed nosocomial infection were significantly lower in their birth weight and gestational age. The risk of nosocomial infection was more in birth weight< 1500gms compared to > 1500gms. The other significant factor associated increased risk was mechanical ventilation >72hrs. There were no differences regarding resuscitation procedure at birth, maternal chorioamnionitis, birth asphyxia, sex of baby and malformation.

2. AGENT FACTOR

The dose and virulence of the microorganisms has great impact on the outcome of neonatal septicemia. The heavier the colonization of microorganisms in the neonate, the greater is the

risk of invasion and subsequent septicemia. Organisms such as Escherichia coli, Proteus, Klebsiella, Staphylococci and Psuedomonas are very virulent organisms causing fulminant infection in the newborn. The invasive nature of these organisms is associated with the specific virulence factors produced by them.

Bacteriology in Neonatal Sepsis

Aerobic Gram negative bacteria	**Aerobic Gram positive bacteria**
Members of Enterobacteriaceae	Staphylococcus aureus
• Escherichia coli	Coagulase negative Staphylococci
• Klebsiella pneumoniae • Citrobacter species	Group B Streptococci
• Proteus species	Streptococcus pneumoniae
• Enterobacter species • Salmonella species	Enterococcus species
Psuedomonas aeruginosa	Listeria monocytogenes
Acinetobacter species Haemophilus influenza	
Anaerobic Bacteria Bacteroides, Peptococcus, Peptostreptococcus, Clostridium tetani, Clostridium welchi Veillonella species	

Clinico Bacteriological Aspects of Common Bacteria Causing Neonatal Septicemia

Staphylococcus Aureus

They are gram-positive cocci, usually arranged in grape-like irregular clusters. They are non-motile, non-sporing and usually non-capsulated. Staphylococcus elaborates multiple adhesions, virulence associated enzymes and toxins to cause a wide range of serious disease including bacteremia, meningitis, cellulitis, omphalitis, osteomyelitis and arthritis. Staphylococcus is distinguished from Coagulase negative Staphylococci (CONS) by production of coagulase and protein A (a component of the cell) all that contributes to virulence by binding to Fc portion of immunoglobulin G (IgG) and blocking opsonization. The emergence of Methicillin resistant strains which has become a global problem in all ICU setups. The MRSA strains can spread within NICU in epidemic and endemic fashion if there is lapse in cohorting or isolation of colonized neonates and if routine surveillance programme fails[(26)].

These are skin commensals that cause opportunistic infections. Two species of CONS are pathogenic to humans - Staphylococcus epidermidis and Staphylococcus saprophyticus. These are the Staphylococci which are coagulase negative are primarily associated with nosocomial infections. They universally colonize the skin of NICU babies and cause bacteremia by first colonizing the surface of central catheters. A polysaccharide surface adhesin (PSA) is being implicated in adherence to catheter surface and subsequent biofilm and slime production that inhibit the host to eliminate the organism. Most CONS are resistant to penicillin group and gentamycin and may need vancomycin.[(26)].

Group B Streptococci (Streptococcus agalactiae)

These organisms are the most common etiological agents of neonatal septicemia in the Western countries. Infection is acquired in utero or during passage through the birth canal. Maternal colonization with GBS is strongest predictor of early onset sepsis. Maternal colonization with GBS without clinical complication and without antibiotic prophylaxis carries a neonatal sepsis risk of 1%. The risk rises to a 4-7% in the presence of clinical complication like PROM, maternal fever or prematurity and rises to as high as 20% in the presence of chorioamnionitis. GBS bacteruria and having a twin with GBS disease also raises the neonatal risk for sepsis[(5)].

These are gram positive cocci, spherical or oval in shape, arranged in chains, non- motile and non-sporing. Human pathogenic Group B strains possess a polysaccharide capsule which confers virulence. Lack of maternally derived, protective capsular polysaccharide-specific antibody is associated with the developing invasive GBS disease. Relative deficiencies of complement and neutrophil function also contribute[25].

They hydrolyze sodium hippurate and show a positive CAMP(Christie, Atkins and Munch-Peterson) reaction. These two tests help in presumptive identification of streptococci[26].

The use of intrapartum antibiotic prophylaxis (ampicillin) according to Center for Disease Control guidelines, for prevention of neonatal GBS disease has significantly reduced the incidence early onset GBS which fell from 1.5cases/1000 births to 0.5 cases/1000 births [5].

Enterococcus

These are present in the intestine, genital tract and saliva. The genus Enterococcus has three species, namely, Enterococcus faecalis, Enterococcus faecium, Enterococcus durans, Enterococcus faecalis is the most commonly isolated Enterococcus. They typically appear as oval gram positive cocci arranged in pairs and short chains. In pairs they are arranged at an angle to each other giving a typical spectacle appearance.. Enterococci are notorious causative agents of nosocomial infections. These encapsulated organisms like CONS colonize indwelling catheters by producing biofilm and slime and adhere to catheter surface. They are associated with septicemia, meningitis and also complicate NEC. Septicemia with multiple antibiotic resistant strains of Enterococci is associated with a high mortality rate of 75%. They even develop resistance to vancomycin[26].

Escherichia coli

Escherichia coli are aerobic gram negative rods found universally in the human intestinal tract and commonly in human vagina and urinary tract. They are motile by peritrichate flagella, non-sporing and some of the strains possess capsule and fimbriae. They also possess somatic (O), flagellar (H) and capsular (K) antigens. Kl antigen, surface fimbria or pili are associated with adherence to vaginal and uroepithelial surface and may function as a virulence factor in pathogenesis of early onset sepsis .Capsular antigens of Escherichia coli K1- types are associated with neonatal septicemia and meningitis. Strains that possess both a complete

Lipopolysaccharide and KI capsule have been shown to specifically evade both complement mediated bacteriolysis and neutrophil mediated killing. The Kl capsule is a poor immunogen and is usually little protective maternal antibody available to the neonate[26].

Klebsiella pneumoniae

The genus Klebsielia includes the species Klebsiella pneumoniae (Friedlander's bacillus), Klebsiella ozaenae, Klebsiella oxytoca and Klebsiella rhinoscleromatis. These are gram-negative, nonsporing, nonmotile bacilli. They are capsulated, the capsule enables the bacilli to resist phagocytosis from the action of many antibiotics. They possess fimbriae of one or more types. Colonies on nutrient agar and blood agar are large, raised, moist and mucoid, grayish white in colour and they form large, mucoid, dome shaped pink lactose fermenting colonies on MacConkey agar. Klebsiella pneumoniae cause septicemia, urinary tract infection, pyogenic infections such as abscesses, wound infections, pneumonia and meningitis[28]

Citrobacter species

These are normal intestinal inhabitants. Three species are recognized, Citrobacter freundii, Citrobacter koseri and Citrobacter amalonaticus. Citrobacter freundii strains are reported to be more pathogenic among these species.

These are gram negative, motile, non-sporing bacilli which utilize citrate, produce H_2S (C.freundii) and ferment lactose late or not at all. Citrobacter species cause neonatal septicemia, meningitis and formation of cerebral abscess[26].

Proteus species

These are widely distributed in nature as saprophytes. The two species are Proteus vulgaris and Proteus mirabilis. They are normal intestinal commensals and opportunistic pathogens. They are gram negative, actively motile, pleomorphic bacilli. They possess O, M, and K antigens. Cultures emits characteristics putrefactive (fishy) odour. Swarming growth occurs on solid culture media such as nutrient and blood agar. They utilize citrate, urease and produce H_2S. Proteus species cause urinary tract infections, pyogenic lesions of various types such as abscesses and septicemia[26].

Enterobacter cloacae

These are gram negative, non-sporing, motile capsulated short, thick bacilli, which exhibits slight mucoid growth on solid media. Found in the human and animal faeces and widely distributed in nature in the sewage, soil, and water, it is a common cause of hospital acquired infections. This organism is known to cause neonatal septicemia and urinary tract infections. Enterobacter species contain chromosomally encoded inducible beta-lactamases and treatment with cephalosporin can result in the emergence of cephalosporin resistant organism. Infection control measures and restriction of cephalosporin use are effective in controlling outbreaks[(26)].

Pseudomonas aeruginosa

Mostly a saprophyte; found in water, soil and decomposing organic matter. They are slender gram negative bacilli, non-sporing, non-capsulated, actively motile bacilli with a polar flagellum. They are known to cause nosocomial infections.

A number of bacterial factors including lipopolysaccharide (LPS), mucoid capsule, adhesions, invasions and toxins (exotoxin A) contribute to its extreme virulence. Lipopolysaccharide and mucoid capsule help avoid opsonization and proteases inactivate complement and Ig. Lipopolysaccharide also acts as typical Endotoxin resulting in septicemic shock and DIC. Exotoxin A inhibits protein synthesis and cell death. Selection of bacteria, likely due to the resistance of Pseudomonas to most common antibiotics used empirically, plays a role in colonization. So, prolong exposure to intravenous antibiotics is an identified risk factor for LOS with Pseudomonas in premature and low birth weight babies. Pseudomonas can be found in environment reservoirs in ICUs i.e. in sinks, respiratory equipment, humidifiers, incubators etc and outbreaks of nosocomial have been linked to both environment sources and spread by the hands of health care providers[(26)].

Organism Implicated In Early Onset Sepsis

The microorganisms most commonly reported from developed world to be associated with early onset infection include group B Streptococcus (40.7%) and Escherichia coli (17.2%) are predominant organism others being Streptococcus viridans, enterococcus and Staphylococcus aureus.[(8),(11)]

In developing countries gram negative bacilli are the predominant causative organisms for early onset sepsis mainly represented by Klebsiella, Eschrichia coli, and Pseudomonas. Of the gram positive Staphylococcus aureus, CONS, Streptococcus pneumoniae and Streptococcus pyogens are common isolate. Group B Streptococcus is generally rare or not seen at all.[8],[11]

Organism implicated in Late Onset Sepsis

Organisms that have been implicated in causing late onset sepsis syndrome in developed world were gram positive isolates constituting 70% of isolate , gram negative constituting 18% and others being fungi. Coagulase negative Staphylococci (48%) being the predominant isolate while GBS, Staphylococcus aureus, Eschrichia coli, Klebsiella, pseudomonas, Enterobacter, Serratia, Acinetobacter and anaerobes are other isolates.[8]

In developing world pathogens isolated are similar to those associated early onset sepsis, mainly gram negative organism such as Klebsiella, Escherichia coli and among gram positive Streptococcus pneumonia, Staphylococcus aureus and CONS.[8]

Blood culture isolates and antibiotic sensitivity pattern

Ashok K Deorari [6], in 2006, from AIIMS New Delhi, while analyzing the changing pattern of bacteriological profile in neonatal sepsis among intramural babies using National neonatal perinatal data for the year 1995, 2000 and 2002-03 around 18 centers from various institution throughout India concluded that the Incidence of culture positive sepsis also declined from 21 per 1000 live births in 1995 to 8.5 cases per 1000 live births in 2002-03.

National Neonatal Perinatal Database 2002-2003[3]

Organisms	Incidence
Klebsiella pneumoniae	32.5%
Staphylococcus aureus	13.6%
Escherichia coli	10.6%
Pseudomonas	5.6%
CONS	5.9%
Enterobacter species	3.8%
Acinetobacter	2.7%
Staphylococcus viridans	2.7%
Citrobacter .	0.6%
GBS	1%

Overall, gram negative organism predominated as the cause of neonatal sepsis. 66% of all cases of sepsis in 1995 and 56% of all cases in 2002-03. Klebsiella species remained the leading cause of neonatal sepsis, 30% in 1995 and 32.5% in 2002-03. This was followed by Staphylococcus aureus and Escherichia coli as second and third most common isolate.[3]

The trend in antibiotic sensitivity pattern with Klebsiella, Staphylococcus aureus and Escherichia coli was alarming. Presently nearly 80% of all Klebsiella isolates and 66% of Staphylococcus aureus are resistant to gentamycin ; 70% of all Klebsiella isolates and 60% of staphylococcus aureus are resistant to amikacin. Escherichia coli has also developed significant resistance to gentamicin but still 76% are sensitive to amikacin. Resistance to cefotaxime and ceftazidime is very common among all the three isolates. Ceftazidime is no more be useful antibiotic for near future except for Pseudomonas. Ciprofloxacin resistance is also menace, 75% of Klebsiella and 68% of staphylococcus aureus are resistant. Vancomycin sensitive staphylococcus.aureus isolates is also gradually reducing [3].

Joshi SG et al [13] in 2000, in their study on 1326 clinically suspected neonatal sepsis cases, reported blood culture positivity rate 25%. Gram negative sepsis accounted for 67.2%. Pseudomonas (38.3%) followed by Klebsiella (30.4%) and E.coli (15.6%) were the

predominant isolates. The isolates were predominantly resistant to extended spectrum cephalosporins (25-75%), piperacillin (68- 78%) and gentamycin (23-69%). They concluded that ampicillin + sulbactum with amikacin or ciprofloxacin is most effective regime.

I Roy et al[16], in 2002 ,in their study on 728 neonates, reported blood culture positivity rate 47.5% there was an overall predominance of gram negative organisms. Most frequent offenders were Klebsiella (24.6%), Enterobacter (22.9%), Coagulase negative staphylococcus aureus (16.6%), staphylococcus aureus (14%) and Escherichia coli(14%). More than 95% of enterobacteria were resistant to ampicillin and more than 40% were resistant to extended spectrum cephalosporins. Ciprofloxacin and amikacin resistance was infrequent. So ciprofloxacin and amikacin was recommended as first line of therapy for gram negative sepsis.

Madhu Sharma et al [28] in 2002, from Rohtak, in their study on 1014 neonates reported 33.94% of neonates were blood culture positive. Klebsiella (67.9%) was the commonest isolate followed by staphylococcus aureus, pseudomonas and Acinetobacter. The gram negative isolates were most sensitive to a cefaperazone-sulbactum combination (97.4%) followed by ceftizoxime (66.47%), amikacin (65.6%) and ciprofloxacin (46.4%). Ampicillin was only 13% sensitive. Gram positive isolates were mostly sensitive to cefoperazone-sulbactum combination (99%), followed by amikacin (76.3%).

Clinical Manifestations Of Neonatal Septicemia[11]

Neonatal septicemia is difficult to diagnose as it manifests with nonspecific signs and symptoms and need a high index of suspicion for early diagnosis. Some of the important clinical features suggestive of neonatal septicemia are:-

Clinical signs and symptoms:

General: Alteration in behavior and change in established feeding pattern is early sign. Lethargy, Refusal of feed, feed intolerance, failure to gain weight, Temperature instability (Hypothermia/ Fever).

Circulatory System: Pallor, cyanosis, cold clammy skin, bradycardia, Tachycardia, poor capillary filling and hypotension.

Respiratory System: Apnea, dyspnoea, tachypnoea with chest retraction, cyanosis, grunting and flaring

Central nervous system: Lethargy, irritability, high pitched cry, blank look, hypotonia, abnormal reflexes, seizures, tremors, bulging anterior fontanelle.

Gastrointestinal Tract: Vomiting, diarrhoea, abdominal distension & Hepatosplenomegaly

Renal System: Oliguria

Hematological system: Jaundice, Pallor, splenomegaly, Petechiae, purpura and mucosal bleeding.

Skin Changes: Multiple pustules, abscess, sclerema, mottling, umbilical redness and discharge.

Clinical syndromes associated with neonatal septicemia:

Meningitis, Pneumonia, Urinary tract infection (UTJ), Necrotising enterocolitis (NEC), Conjunctivitis, Otitis media and Osteomyelitis, etc

Pneumonia is more in early onset sepsis, whereas meningitis and / or bacteremia are more common in late onset sepsis [(11)]

LABORATORY DIAGNOSIS OF NEONATAL SEPTICEMIA

1.Specific Investigation

- Blood, cerebrospinal fluid and urine culture,
- Antigen detection test : Latex agglutination test, Counter immuno-electrophorosis (CIEP)
- Polymerase chain reaction (PCR)

2.Adjunctive Tests for Diagnosing Neonatal Sepsis

a) Hematological parameters

- White blood count ,
- Differential count,
- Band count, and immature to total neutrophil ratio(l/T) and
- Micro-ESR
- Nitro blue tetrazolium test (NBT)

b) Acute phase reactants

- C-reactive protein, orosomucoid (α-1 acid glycoprotein), haptoglobulin, α-l antitrypsin, α-1 antichymotrypsin and fibronectin assay.

c) Cytokines, Growth factors and its receptors

- IL-6, IL-8, IL-1, IL-10, Soluble interleukin receptors like, IL-l ra, IL-2rs.
- TNF-a, IFN-a, MIP l-a, Neutrophil CD1 Ib, sCD14, sICAM-1 G-CSF, Elastase alpha 1 proteinase inhibitor complex

d) Procalcitonin

e) Granulocyte elastase concentration in amniotic fluid.

f) Radiological investigation, Chest radiogram, cranial ultrasound, Computed tomography scan

A. Blood Culture

- Conventional method
- BACTEC system
- Lysis centrifugation

a) CONVENTIONAL / TRADITIONAL METHODS

Diagnosis of neonatal septicemia is ultimately based on the positive blood culture which is a gold standard. A positive blood culture is the best guide to antimicrobial therapy. However culture positive rates in neonates range from 30% to 75% in Indian literature and needs at least

48-72 hrs to show the growth. The success of isolating bacterial pathogens from blood depends upon the quantum of blood cultured, frequency of culture and duration of incubation[29],[30].

The volume of blood required for the isolation of the pathogen depends on the magnitude of septicemia, which is directly related to the age of the patient. The most widely accepted sample size in neonates is 0.5 - 1 ml collected before starting antibiotics. D.V Eitzman et al[31] (1957), Ralph et al[32](1972) have reported the reliability of a single pretreatment blood culture in newborns.

The ratio of volume of blood cultured to the volume of the medium should be one in 10 dilutions to reduce the concentration of any therapeutically administered antibiotic and also to reduce the natural bactericidal and bacteriostatic constituents to sub-effective levels.[29],[30]

Blood cultures media commonly used are:

Brain heart infusion (BHI) broth, Trypticase soya broth, Tryptone soya broth including agar slope (Castaneda), Thioglycolate broth, Glucose broth & Bile broth.

Culture media commonly used for subculture

Blood agar, MacConkey agar, Chocolate agar

Turn around time for blood culture:

Conventional methods of blood cultures normally need to be incubated for a minimum period of 7 days, which is substantially long enough to generate the growth of any significant bacteria.[29]

b. NON CONVENTIONAL METHODS

BACTEC system (Becton and Dikinson Microbiology system sparks Med)

These are automated blood culture systems, radiometric and non-radiometric types which measures the production of carbon dioxide by the metabolizing organisms. These systems aid in early detection of septicemia.

Advantages of BACTEC System include more rapid detection of pathogens, ability to monitor growth without visual inspection and automated handling of samples[29].

Lysis centrifugation

This is a commercially available Isolator where blood cells are lysed and centrifuged. The sediment is then plated on a solid agar medium, incubated and observed for growth. Advantages of Lysis Centrifugation include greater recovery of intracellular organisms due to lysis of host cells, ability to quantify the colony forming units (CPU) in blood and rapid detection of growth.[29]

B.Cerebrospinal fluid Analysis

CSF is collected aseptically by lumbar puncture and analyzed. Deposit of centrifuged sample is used for culture and gram staining. Samples should be plated promptly to avoid loss of viability of the organisms.[29]

The CSF cytology and biochemistry of neonates is different from that of older infants. Also there is overlap of values in normal neonates and those with meningitis.Normal CSF values was put forward by Larrie D. Sarff et al[33]1976 in her review study and also by Otila et al[34] (preterm babies) 1948.

Test	Term Mean (range)	Preterm Mean (range)
White blood cells	8 (0-32)	9 (0-29)
Polymorphonuclear cells	60% of WBC	57.2%
Protein (mg/dl)	90(20-170)	115(65-150)
Glucose (mg/dl)	52(34-119)	50(24-63)
CSF to Blood glucose ratio	81(44-248)	74(55-105)

Following parameters are highly suggestive of meningitis when infant has reported to you

very early or has received prior antibiotics that may render the culture negative.

- WBCs >32/cu.mm, Protein >150mg/dl and glucose < 75% of blood glucose.
- Gram stain during initial CSF analysis showed organism in 78% of culture proven gram negative meningitis

C.Urine Culture:

Urinary tract is a common site for infection in late onset septicemia. Urine cultures have low yield during the first 72 hours of life Urine sample is best collected in neonates by suprapubic aspiration method. Microscopic examination is done on centrifuged urine by wet film preparation to look for WBC. Urinary tract infections may be diagnosed in presence of one of the following:

a) > 10 WBC /cu.mm in a 10ml centrifuged sample,

b) $>10^4$ organism /ml in urine obtained by catheterization and

c) Any organism in urine obtained suprapubic aspiration [(1)].

D. Sepsis Screen

Sepsis screen tests involving WBC indices and CRP form simple, cheap, rapid and easily available parameters with reasonable diagnostic accuracy especially when they are used in combination. On this basis early and rational antibiotics therapy can be started in critical septicemic infants.[(5),(6),(7)]

All newborns clinically suspected of septicemia in the presence of any two risk factors, should have a sepsis screen to support diagnosis and initiate timely treatment. The various components of the sepsis screen include:

- C-Reactive protein
- Total leucocyte count
- Absolute neutrophil count
- Immature to total neutrophil count ratio (I/T ratio)
- Micro-Erythrocyte sedimentation rate.

D.1.C- Reactive protein

Philipson[35] in 1957 first described the presence of CRP in bacterial infection of neonates. Later on between 1962-1966 Fellix[36] and Hanson et al[37] observed that C- reactive protein increased invariably in neonatal infection and more consistently with septicemia and bacterial meningitis.

CRP is a rapidly responsive acute phase reactant, an abnormal β-globulin synthesized by the liver within 4-6 hrs of an inflammatory stimulus, peaks at 24-48hrs and then diminishes over time as the inflammation resolves [4]. Since the infection is most likely cause of inflammation in neonates, elevation of CRP has been a useful marker for sepsis in many studies, although sensitivity and negative predictive value are not high enough for CRP alone to be a definitive diagnostic test. Normal value of CRP is <1.6 mg/dl on day 1 and 2 and <1.0 mg/dl thereafter. The most rapid & quantitative method for determining CRP concentration is by nephelometry. Alternatively a positive CRP latex agglutination test on an undiluted sample corresponds to a plasma C-reactive protein concentration of 0.6 to 1.0 mg/dl. Normalization of CRP elevation appears to be a helpful tool in determining response to antimicrobial therapy & duration of treatment [38].

Elizabeth Mathai et al [52] in their study analyzed elevated CRP levels in both cord blood and at 24hrs sample. They reported elevated cord blood CRP levels significantly associated with PROM >24hrs, labor >12hrs and maternal fever. At 24 hrs, elevated CRP levels were associated with primiparity, more than 3 vaginal examinations after rupture of membranes, meconium staining of amniotic fluid and amnioinfusion. Negative predictive value for elevated CRP levels at 24hrs was 99%.

CRP sensitivity, specificity, positive predictive value and negative predictive value quoted by various studies:

Author	Year	Sensitivity	Specificity	PPV	NPV
Mathai et al[39]	2004	80.00%	60%	7.7%	98.6%
Gerdes et al[5]	2004	70%-93%	41%-98%	6%-83%	97- 99 %

Like WBC count, CRP may not be positive early in the course of infection. Mathers and Pohlandt[40] found that diagnostic sensitivity of CRP >1.0 mg/dl was 22% on admission for sepsis evaluation but 61% after 24 hours of admission. One point determination of CRP level was not considered to be a sensitive diagnostic index. Single determination is inadequate because rise may be due to stressful deliveries or a normal value due to estimation during incubation period or small inoculum.

Serial determination of CRP as diagnostic and therapeutic tool.

Pourcyrous et al[41] in their study assessed the CRP response of 491 infants on 691 occasions of suspected infection. CRP levels were measured initially and twice again at 12 hrs intervals. CRP responses were correlated with four designated clinical groups:

1) Positive blood culture or CSF culture
2) Negative blood culture - definite infection like NEC stage 2-3, pneumonia.
3) Negative blood culture - possible infection like antenatal risk factors.
4) Negative blood culture - no infection like RDS, PDA etc.

Results showed Gram-negative rods, Group B Streptococcus, Staphylococcus aureus were more often associated with raised CRP levels. In the negative blood culture - definite infection group, CRP was elevated in 88%; in the possible infection group, CRP was elevated in 33%; and in no infection group, CRP was elevated in 9%. So serial determination of CRP resulted in enhanced the sensitivity in culture proven and clinically suspected groups. So, author concluded that, if three serial CRP measurements are normal with no clinical signs suggestive of infection, antibiotics can safely be discontinued.

Ehl Stephen et. al.[42] published a study in 1997, which was conducted in Germany amongst 176 new born with birth weight more than 1500 grams. First dose of antibiotics was administered to newborns with suspected septicemia on admission, and after 24-48 hours, CRP estimation was done. If CRP less than 10 mg/L, infants were considered unlikely to be infected and the antibiotics were stopped (group 1). If CRP was more than 10 mg/L these infants were divided into two subgroups. In the first sub group (group2a) CRP was estimated daily and antibiotics were discontinued when CRP level returned below 10mg/L. In second subgroup (group 2b) CRP was estimated after 5 days of treatment and relapse rate of bacterial

infections were compared between two. These infants were followed for four weeks. So, CRP levels of less than 10mg/L determined later than 24hrs after beginning the antibiotics treatment had a negative predictive value with respect to further treatment of 99%. The mean treatment duration was 3.7 days in CRP guided group and 5.5 days in the other group. Relapse rate was nil in CRP guided group as compare to 5-day therapy group. They concluded that CRP could be a key parameter for individually guiding the duration of antibiotic treatment in newborns with suspected sepsis. This approach would allow considerably shorter courses of antibiotic therapy.

Phillip AG and Mills PC[38] (2000) published a study in which role of CRP was studied in minimizing antibiotics exposure. Infants born with a variety of perinatal risk factors or clinically manifestation suggesting possible infection were evaluated with white blood cell count, differential count and CRP soon after birth and 12hrs later. Discontinuation of antibiotic was primarily based on return to normal of the CRP. In 1894 newborns with suspected septicemia (25% of live births), out of which 425 were transferred to neonatal intensive care unit. There were 216 infants transferred because of risk factor and 209 because of clinical finding. In 162, antibiotics were discontinued within 48 hours, majority of the rest (244) were treated for 3-5 days, and 19 treated for 6 days or more. Peak CRP primarily determined the duration of antibiotic treatment. The mean duration of treatment was 3.1 days. No infant initially treated with antibiotics and discharged when the CRP returned to normal was readmitted within the next month. Therefore it was concluded that using a clinical pathway for neonatal sepsis which is based primarily or CRP determinations, can minimize antibiotic exposure and shorten hospital stay.

William E. Benitz et al[43] in their study on serial serum C-reactive protein levels in the diagnosis of neonatal infection categorized all as having proven sepsis (bacteria isolated from blood/CSF/urine), probable sepsis (clinical and laboratory findings consistent sepsis with negative culture), or no sepsis without consideration of CRP levels. CRP levels were determined at the initial evaluation and on each of the next two mornings. Sensitivity, specificity, predictive values and likelihood ratios were calculated for the first (CRP#1) ,second (CRP#2), higher of the second and third (CRP#2 and #3), or highest of all three CRP levels (CRPx3).

Sensitivity	Proven sepsis	Probable sepsis	EOS	LOS
CRP#1	39.4%	64.6%	35%	61.5%
CRP#2	92.9%	85%	78.9%	84.4%
3 serial CRP	97.8%	98.1%	88.9%	97.5%
CRP#2 and CRP#3	97,6%	94.4%	88.9%	96.4%

The negative predictive values for 3 serial CRP was 99.7% and 98.7% for both proven and probable sepsis and for early onset and late onset episodes respectively. And negative predictive value excluding initial CRP considering only CRP#2 and CRP#3 were 99.7% for both proven and probable early onset sepsis and around 97.6% for late onset group. So author concludes that two CRP levels <1mg/dl obtained 24 hours apart, 8hours and 48hours after presentation, indicate that bacterial infection is unlikely.

D.2.Total Leukocyte Count:

Total leukocyte count has a low predictive value in diagnosis of infection,because of wide range of normal count from 8,000 to 20,000/mm^3 and leucopenia(<5000/mm^3) is more specific for neonatal sepsis[44] .The most common widely used blood counts is neutrophil count and platelet count.Absolute neutropenia (<1000/ mm^3) is more predictive of neonatal sepsis than neutrophilia[45]. Gerdes JS and Polin R (1998)[5] in their study reported the sensitivity and specificity of leucopenia to be 26% and 91% respectively in diagnosing septicemia.

D.3.Absolute Neutrophil Count:

Reference values for absolute neutrophil count have been established by various researchers. All are of opinion that, shortly after birth there is an increase in number of neutrophil that reaches peak at 12hours and starts decreasing till 72hours and then remain constant. The reference values are; 1750 to 5400 /mm^3 after 3 days till 28 days as reported by Manroe et al[46]

Manroe et al[46] found perinatal factors other than bacterial disease like maternal hypertension, maternal fever prior to delivery, hemolytic disease and periventricular hemorrhage significantly altered the neutrophil dynamics. So, predictive value of reference range of neutrophil factor, in identifying bacterial disease in 1^{st} week of life depends on clinical setting

and neutrophil factor being evaluated. Elevation of either immature or total neutrophils were less specific.

D.4.Band Cell Count and I/T Ratio

The band neutrophil (also known as stab or non filamented neutrophil) measures 10-15um in diameter and the ratio of the nucleus to cytoplasm is 1:2. The band neutrophil has the width of the narrowest segment of it's nucleus indented to more than one half of the width of the hypothetic round nucleus, yielding a nucleus with S,C, or U shapes and the isthmus between the lobes being wide enough to reveal two distinct margins with nuclear material in between[47].The absolute value for band forms is not of much use because it tends to rise late in infection and in the most severely affected infants, band cell production is limited as the marrow becomes exhausted. A slightly more useful indicator of infection is the ratio of immature to total neutrophil ratio (I/T ratio). The maximum normal value is 0.16 during the first 24 hrs, 0.14 by 48 hrs, 0.13 by 60 hrs, where it remains until 5 days of age. Thereafter, the maximum normal I/T ratio is 0.12 until the end of the first month. I/T ratio >0.2 is a useful marker of infection and the ratio <0.2 makes infection unlikely. An abnormal I/T ratio in the presence of a low absolute neutrophil count is more strongly suggestive of infection[10],[46].

I/T ratio predictive values

Author	Year	Sensitivity	Specificity	PPV	NPV
Gerdes et al[5]	2004	90-100%	30-78%	11-51%	99-100%

5. Micro ESR

It is indirect evidence of sepsis that the rate of increase depends upon the severity of the morbid process. The micro ESR is done by standard haematocrit tubes of 75mm length by filling them completely and one end of the tube closed completely with plasticin. They were fixed vertically by means of sticking plaster and fall in the erythrocytes in one hour is measured accurately to the nearest mm. The ESR is low in normal newborn during the first few days of life due to high haematocrit values. It is not affected significantly be sex, birth weight, and infusions of calcium ,glucose or feeding. A value of more than 8 mm per hour during the immediate neonatal period and maximum 15 mm per hour(age in days + 3 is normal) is indicator of sepsis.

Intial higher values of micro ESR predict poor outcome and found to be highly sensitive(100%) in picking up maximum number of infected cases and useful in yearly diagnosis of neonatal infection. In the Indian study by Verma, Singh MB et al 74.4% of definitely infected babies and 24% probably infected babies had elevated values. Micro ESR has a sensitivity of 55% and specificity of 81%[48].It is less sensitive than CRP or I/T ratio. False positive elevated micro ESR values are found in DIC and Coomb's positive hemolytic anaemia.

In Walliullah SM et al 2006 study, Dhaka, I/T ratio more than 0.2 was considered positive for sepsis. Sensitivity and specificity of micro-ESR was 63.3% and 60% respectively. Sensitivity and specificity of I/T ratio was 70% and 56% respectively .Combination of micro-ESR and I/T ratio showed high sensitivity(80%) and specificity(70%).[49]

D.5.Platelet Count

A depression in the platelet count in the peripheral blood smear of < 1,50,000 per cu.mm (thrombocytopenia) may occur during septicemia and support the diagnosis. Thrombocytopenia accompanying bacterial infections is thought to be caused by a direct effect of bacteria or bacterial products on platelets and vascular endothelium leading to increased aggregation and adhesion or by increased platelet destruction caused by immune mechanisms. Thrombocytopenia noted in infected newborn has a poor Sensitivity (22%-38%) but the specificity and negative predictive accuracy are both more than 90% [5]

Sepsis Screen

Jeffrey S. Gerdes et al[5] in 2004 from Children's Hospital of Philadelphia in their study on diagnosis and management of bacterial infection in the neonate established sepsis screen using only three parameters leukocytes <5000/cu.mm, I/T ratio >/= 0.2 , and CRP> 1mg/dl and screen was considered positive if two of three test was positive. This sepsis screen has sensitivity of 100%, specificity of 83% and negative predictive value of 100%.

Screening parameter	Sensitivity	Specificity	PPV	NPV
ANC<1750/cu.mm	38- 96%	61-92%	20-77%	96-99%
I/T ratio >/=0.2	90-100%	30-78%	11-51%	99-100%
CRP >l mg/dl	70-93%	78-94%	7-43%	97-99.5%
WBC <5000/cu.mm, I/T>/=0.2 & CRP >l mg/dl (screen positive if 2/3 test are abnormal)	100%	83%	27%	100%

Sharmila Ghosh et al[50] in her study on early diagnosis of neonatal sepsis using a hematological scoring system (HSS) on 105 neonates used total leucocyte count (TLC)<5000/cu.mm or >25000/cu.mm, absolute neutrophil count (ANC) <7500 or 14500/cu.mm, I/T ratio >0.16, I/M ratio of 0.3, platelet count <1.5 lakhs and degenerative changes in neutrophils as hematological parameters and assigned score one for each. She analyzed the sensitivity, specificity and predictive value of each of parameters and all together. Author found that abnormal I/T ratio followed by I/M ratio were most sensitive (>90%) indicators in identifying neonates with sepsis. These two along thrombocytopenia (<1.5 lakh) had a high negative predictive value over 94%. The higher score on Hematological scoring system greater the certainty of sepsis being present.

According to shailesh Vartak et al[51] study conducted on 250 neonates admitted with a clinical diagnosis of neonatal sepsis.The best three test combination was absolute neutrophil count + ESR + I/T ratio having the best specificity and positive predictive value

Test	Sensitivity	Specificity	PPV	NPV
TC(<5000/cu.mm)	43%	91%	68%	79%
ANC(<1800/cu.mm)	47%	91%	69%	80%
I/T Ratio(>0.2)	79%	59%	45%	87%
Micro ESR(>15mm)	56%	95%	84%	84%
CRP Positive	89%	73%	59%	94%

TREATMENT

Neonatal septicemia is a serious life-threatening condition. Early initiation of effective and appropriate antibiotics with optimal supportive management is mandatory to reduce the morbidity and mortality due to neonatal sepsis.

ANTIBIOTIC THERAPY

Indications

The indications for starting antibiotics in neonates with early onset septicemia include[(1)].

- Presence of two or more adverse perinatal risk factors for early onset septicemia.
- A positive sepsis screen.
- A strong clinical evidence of septicemia.

The indications for starting antibiotics in neonates with late onset septicemia include[(1)] :

- A strong clinical evidence of septicemia.
- A positive sepsis screen

The Initial Choice of Antibiotics

In most cases, treatment of a neonate with a presumptive diagnosis of septicemia is initiated before the etiologic agent is identified and its susceptibility to antimicrobial agents is known. Thus, the initial choice of antimicrobial agents must be based on the following factors:

- The knowledge of the most probable pathogens depending on age of the neonate and the current bacteriological flora of the particular nursery.
- The antibiotic susceptibility patterns of these isolates.
- The hierarchy and the combinations of the drugs used.
- The cost and availability of the antibiotics.

Empirical Therapy

The Empirical choice of antibiotics should be unit specific that is determined by the probable source of origin of infection, the prevalent spectrum of etiological agents and their antibiotic susceptibility pattern. The common pathogens encountered in most neonatal units in our country are Klebsiella, Escherichia coli, Enterobacter, Staphylococcus aureus and Coagulase negative staphylococcus aureus. So, the initial antibiotic regimen must cover these pathogens.

Hence a combination of two or more antibiotics covering both the gram positive and gram negative organisms are recommended for initial empirical therapy while awaiting culture reports, and should be modified once definitive culture and sensitivity reports become

The guidelines for initial combination therapy are as follows

Clinical situation	First line therapy	Second line therapy
Community acquired infection and where Resistant strains are unlikely	Ampicillin and Gentamicin /Amikacin	Cefotaxime and Gentamicin/ Amikacin
Hospital acquired infection where Resistant strains are likely	Cefotaxime and Amikacin	Ciprofloxacin and Gentamicin / Amikacin

Combination therapy is used to exploit the synergistic action of the drugs and to prevent the emergence of antibiotic resistance. It is also necessary to periodically rotate the combination therapy in the NICU to overcome the problem of resistance to this drugs

Reserve Drugs

Third generation cephalosporins (e.g. Cefotaxime, Ceftriaxone etc) have excellent antimicrobial activity against gram negative organisms. In case of Pseudomonas septicemia, a combination of piperacillin or ceftazidime with amikacin is preferred and they also an excellent choice in the treatment of nosocomial infections. Newer antibiotics like Aztreonam/Imipenem should be reserved for multi-drug resistant organisms. Methicillin sensitive Staphylococcus aureus (MSSA) should be treated with cloxacillin or nafcillin. Methicillin resistant Staphylococcus aureus (MRSA) needs treatment with a combination of vancomycin/ teicoplanin / ciprofloxacin along with an aminoglycoside like amikacin. Vancomycin is also the drug of choice in case of Coagulase negative staphylococcus aureus, resistant to penicillin, cloxacillin, gentamicin and methicillin.

PREVENTION OF SEPTICEMIA IN NEONATAL NURSERIES

Strategies to be taken in the neonatal nursery to prevent infection among babies and reduce the chances of neonatal septicemia are as follows:

- Controlled admissions in the nursery should be undertaken to reduce the hazard of over crowding and chances of cross infections.

- Adequate and well trained staff, the nursing staff as well as the physicians are essential to prevent cross infections in the nurseries.
- Screening high risk patients during outbreaks and their cohorting helps to identify the infection and prevents the spread of infection.
- Correct method of hand washing by the hospital staff, mother and visiting relatives is the most important to prevent nosocomial spread of infections. Wash 2min at entry and 15-30 sec before and after every patient. There are 6 steps with 5 strokes each time.
- Use of masks, gowns and proper disinfection of the nursery environment, strict asepsis followed whenever any minor / invasive procedures are carried out.
- The nursery should be well equipped with incubators and resuscitative equipments and other emergency facilities. Sterilization / disinfection of these instruments after each use and periodic disinfection of the nursery are mandatory.
- Regular environment cultures, record of positive cultures, written down antibiotic policies and disinfection policies are required.

PATIENTS AND METHODS

SOURCE OF DATA:

This prospective study was conducted in the Department of Pediatrics, Santhiram Medical College and Hospital,Nandyal from November 2015 to October 2017. The blood sample was collected from 80 clinically suspected cases of neonatal sepsis admitted in Santhiram Medical College and Hospital, Nandyal constituted material for the study. Detailed history and clinical findings were recorded in the Proforma [Annexure-I].

INCLUSION CRITERIA

Neonates presenting with following:

1. Perinatal risk factors :
    - Low birth weight
    - Prematurity
    - Birth asphyxia
    - Home delivery
    - PROM more than 24 hours
    - Maternal fever
    - Instrumentation
2. Clinical risk factors:
    - Poor feeding, lethargy.
    - Sclerema
    - Hypothermia/fever
    - Jaundice
    - Apnoea, tachypnoea
    - Abdominal distension and vomiting.
    - Diarrhoea
    - Skin mottling
    - Bleeding tendencies
    - Seizures

EXCLUSION CRITERIA

- Neonates who received antibiotics before admission
- Neonates with major congenital malformations.

METHODS:

All neonates were categorized into early onset (0-72 hours) or late onset (>72 hours) sepsis based on age of presentation. Detailed history and clinical findings were recorded in the Proforma [Annexure-I].Among early onset sepsis, perinatal risk factors were noted and each baby was given a score based on Septic score as below.

PERINATAL INFECTION RISK SCORE[19]

SL.NO.	Perinatal factor	Risk Score
1	Foul smelling liquor	2
2	Unclean vaginal examination done before delivery	2
3	Duration of labor exceeding 24 hours	2
4	One minute APGAR score of 0 - 6	2
5	Duration of rupture of membrane before delivery > 24 hours	1
6	Birth weight 2 kg or less and / or gestation less than 37 wks	1
	Total Score	10

Action based on score: Score 0-3: observe clinically

Score>4 / =: Investigate.

All neonates who are clinically symptomatic or having septic score > 4 were screened using CRP and various hematological parameters with predetermined cut off value and at the same time blood culture was sent. Relevant to clinical situation CSF, Urine analysis and swabs of infective focus were taken.

The cut off values of the positive rapid screening tests in this study are as follows[5]

1. C-Reactive protein (CRP) : > 6ug/ml.
2. Total leukocyte count (Leukopenia) : < 5,000 cells/cu.mm.
3. Absolute neutrophil count (Neutropenia) : < 1,750 cells/cu.mm.
4. Band cell count to total neutrophil count ratio (I/T) : > 0.2.
5. Platelet count (thrombocytopenia) : < 1.5 lakh/cu.mm

The empirical antibiotic therapy was started according to antibiotic guidelines in the NICU, if CRP was positive, awaiting the culture reports.The duration of treatment and duration of hospital stay was noted in all neonates.The discharged neonates were followed up at NICU-OPD.

Sample collection:

An area of approximately 5 cm over the venipuncture site was disinfected with 70% alcohol, rubbing vigorously and allowed to dry. This was followed by application of Povidone Iodine in concentric circles over the site and allowed to dry for at least 1 minute. About 3-4 ml of blood was drawn using a sterile syringe, out of which 1 ml of the blood sample was inoculated aseptically into a culture bottle, 1 ml of the blood, was allowed to clot in a sterile bottle to collect serum for estimation of C-reactive protein and the remaining 2 ml was collected in a sterile bottle containing the anticoagulant EDTA for estimation of the Total WBC count, Absolute neutrophil count, Band cell count and I/T ratio**.**

1.Blood Culture

About 1 ml of blood was drawn aseptically and inoculated into a blood culture bottle containing 10 ml of Brain Heart Infusion broth, thus making a dilution of 1 in 10 to nullify the natural bacteriostatic/bacteriocidal activity of blood. Brain Heart Infusion broth was prepared using the commercially available ready to use powder. The broth was distributed into 10 ml quantity in McCartney bottles and sterilized by autoclaving at 121^{o}C for 15 minutes. After inoculation, the blood culture bottles were incubated at 37^{o} C under aerobic conditions in the incubator for 7 days. The first subculture was done after 24 hours of incubation, the second on third day and a final on the seventh day.

Subcultures were done onto Chocolate agar, 5% sheep blood agar and McConkey agar plates.

The inoculated plates were incubated aerobically in the incubator at 37^{O} C for 24 hours, and the plates were observed for growth. The growth was identified by colony characteristics, gram's stain and standard biochemical tests described in Mackie and McCartney, Practical Medical Microbiology[30] and Bailey and Scott's Diagnostic Microbiology.[29]Cultures which did not yield any growth following three subcultures were reported negative at the end of 7 days.

2. Antibiotic Susceptibility Testing:

Antibiotic susceptibility testing was done for all the isolates on Miller Hinton agar using commercially available discs, by Kirby Bauer disc diffusion technique as per the NCCLS guidelines (2002). The following antibiotics were tested for susceptibility.

List of Antibiotics Used:

GRAM POSITIVE ORGANISMS			**GRAM NEGATIVE ORGANISMS**		
Antibiotics	**Concentration (ug/disc)**	**Abbr**	**Antibiotics**	**Concentration (ug/disc)**	**Abbr**
Ampicillin	10	AMP	Ampicillin	10	AMP
Amikacin	30	AMK	Amikacin	30	AMK
Gentamicin	10	GM	Gentamicin	10	GM
Penicillin	10 units	P	Amoxyclav	30	AMC
Erythromycin	15	E	Ofloxacin	5	OFX
Amoxyclav	30	AMC	Cefotaxime	30	CTX
Ciprofloxacin	5	CIP	Ceftazidime	30	CAZ
Cefotaxime	30	CTX	Ceftriaxone	30	CTR
Ofloxacin	5	OFX	Cefuroxime	30	CFX
Oxacillin	1	OX	Ceftizoxime	30	CZX
Cephalexine	30	CN	Cephalexine	30	CN
Cefadroxil	30	CFR	Carbenicillin	100	CB
Cefazoline	30	CFZ			
Methicillin	5	MET			
Vancomycin	30	VA			

3.C-Reactive Protein Assay

This test is done by using diagnostic kit for in-vitro detection of CRP in human serum by the ***rapid slide latex agglutination qualitative*** method supplied commercially by Span Diagnostics Ltd.

A drop of undiluted patient's serum was mixed with a drop of latex reagent on a slide provided in the kit with the help of a disposable applicator stick spreading the fluid over the entire area of the circle. The slide was gently rocked to and fro for 2 minutes and observed for microscopic agglutination of latex particles under a direct light source. Agglutination of the latex particles within 2 minutes was taken as positive reaction. Controls were run along with the test.

The sensitivity of the antigen in the kit for visible agglutination is 6 ug/ml. so in our study, a ***CRP value of >6 ug/ml*** was taken as a ***positive test***.

4. Other Hematological Tests

A drop of EDTA blood was taken on a clean dry slide and a thin toungue shaped smear was made, air dried and stained with Leishman's stain. The Total leukocyte count, differential count, Absolute Neutrophil Count, Band cell count, I/T ratio and platelet count were calculated as per standard hematological method.

5.Statistical Analysis

The statistical analysis was done using the results of present study. Sepsis score and sepsis screen test results were compared with the blood culture results as the gold standard. The results for each parameter (numbers and percentages) are presented in Tables and charts.

The following methods of statistical analysis have been used in this study.

a). Proportions were compared using Chi-square test of significance (χ^2) for (r x c tables)

Rows	**Columns**		**Total**
1	1 2……	c	
1	a1 a2	ac	t1
2	b1 b2	bc	t2
.	. …….	.	.
.	. …….	.	
r	hr hr	hc	tr
Total	n1 n2	nc	N

a,b…….h are observed values. N is the grand total.

DF=(r-1) * (c-1), where r= rows and c= columns.

DF= Degree of Freedom (Number of observation that are free to vary after certain restriction have been placed on the data).

b). The number of True positives (TP), false positives (FP), True negatives (TN) and False negatives (FN) results were determined and Sensitivity, Specificity, Positive predictive accuracy and Negative predictive accuracy were calculated**.**

In the above test the "p" value of less than 0.05 was accepted as indicating statistical significance. Data analysis was carried out using statistical package for Social Science (SPSS, V 22.0) package.

OBSERVATIONS & RESULTS

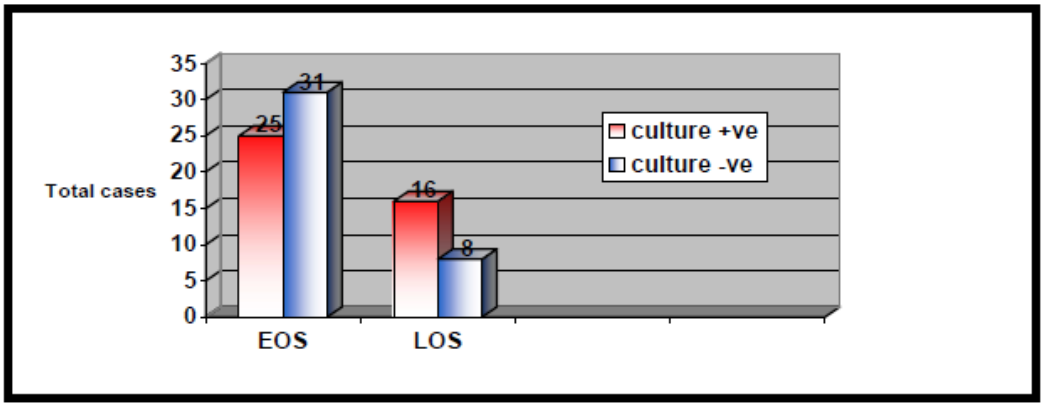
This prospective study was conducted in the Department of Pediatrics, Santhiram Medical College and Hospital, Nandyal from November 2015 to October 2017. The blood sample was collected from 80 clinically suspected cases of neonatal sepsis admitted in Santhiram Medical College and Hospital, Nandyal.

Out of 80 cases studied, 41 cases yielded a positive blood culture giving a success rate of 55.55%.

Table 2. SHOWS DISTRIBUTION OF CULTURE POSITIVITY IN SEPSIS

	EOS (%)	LOS (%)	Total (%)
Culture Positive	25 (44.64%)	16 (66.66%)	41 (51.25%)
Culture Negative	31 (55.35%)	8 (33.33%)	39 (48.75%)
Total	56	24	80

- Higher proportion of late onset sepsis (66.66%) were culture positive compared to early onset sepsis (44.64%).



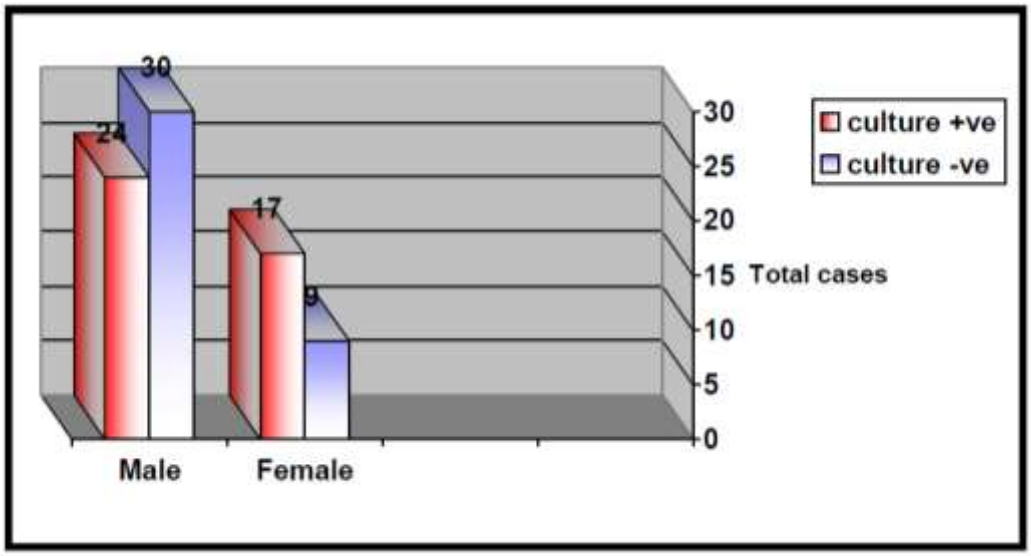
Graph 1. Distribution Of Culture Positivity in Sepsis

Table 3. SHOWS DISTRIBUTION OF CASES ACCORDING TO SEX.

	Male	Female	Total
Culture Positive	24 (58.5%)	17 (41.5%)	41
Culture Negative	30 (76.92%)	9 (23.08%)	39
Total	54 (67.5%)	26 (32.5%)	80

p-value=0.20

- Out of 80 cases studied, 54 (67.5%) were males and 26 (32.5%) were females.
- Males were more affected compared to females with 1.6:1.
- This is not significant with respect to culture positivity.



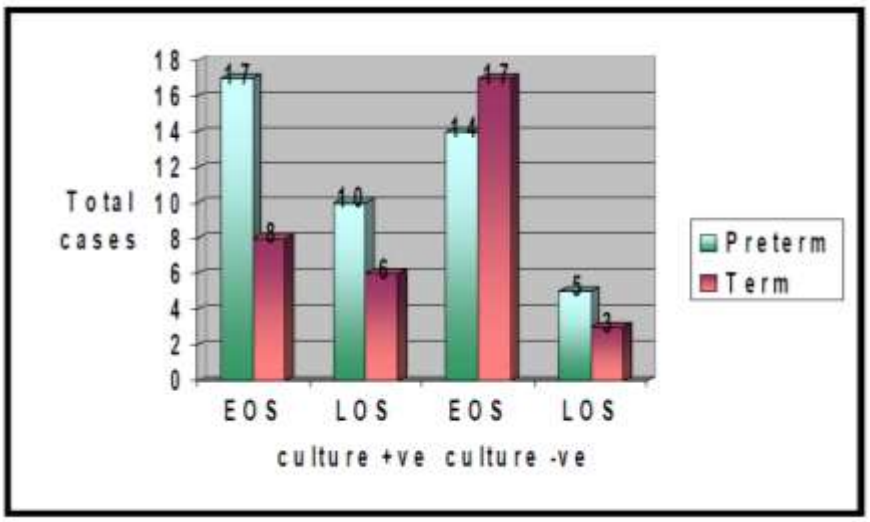
Graph 2. Distribution Of Cases According To Sex.

Table4. SHOWS DISTRIBUTION OF CASES ACCORDING TO THE GESTATIONAL AGE.

Gestational age	Culture positive		Culture negative		Total
	EOS	LOS	EOS	LOS	
Preterm	17 (68%)	10 (62.5%)	14	5	46 (57.5%)
Term	8 (32%)	6 (37.5%)	17	3	34 (42.5%)
Total	25	16	31	8	80

p-value = 0.062

- Out of 80 suspected sepsis, 46 cases (57.5%) were preterm.
- Among 41 culture positive, 27 were preterm.
- 68% of early onset and 62.5% of late onset culture proven sepsis babies were preterm.



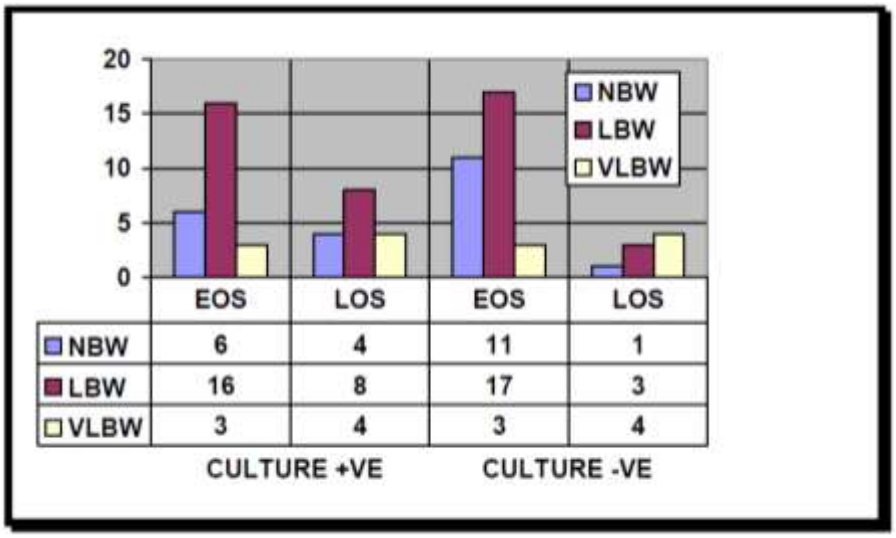
Graph 3.Distribution Of Gestational Age

Table 5. SHOWS DISTRIBUTION OF CASES ACCORDING TO THE BIRTH WEIGHT

Birth weight (kg)	Culture positive		Culture negative		Total
	EOS	LOS	EOS	LOS	
NBW (>2.5)	6 (24%)	4 (25%)	11	1	22 (27.5%)
LBW (1.5-2.5)	16 (64%)	8 (50%)	17	3	44 (55%)
VLBW (1-1.5)	3 (12%)	4 (25%)	3	4	14 (17.5%)

p-value = 0.002 (>2.5 kg Vs < 2.5 kg with respect to culture positivity)

- Out of 80 cases of sepsis 58 (72.5%) cases were below 2.5 kg; 17.5 % of them were very low birth weight (1-1.5kg).
- Among 41 culture positive, 31 (75%) were below 2.5 kg and it was significant.
- 19 of 25 (76%), of the early onset culture proven sepsis had their birth weight below 2.5 kg and among late onset sepsis 12 of 16 (75%) of them were below 2.5 kg.



Graph 4. Distribution Of Birth Weight

Table 6. SHOWS DISTRIBUTION OF CASES ACCORDING TO PLACE OF DELIVERY.

	Culture positive		Total Culture positive	Total cases
	EOS	LOS		
Inborn	18(72%)	10(62.5%)	28	46
Out born	7 (28%)	6 (37.5%)	13	34
Total	25	16	41	80

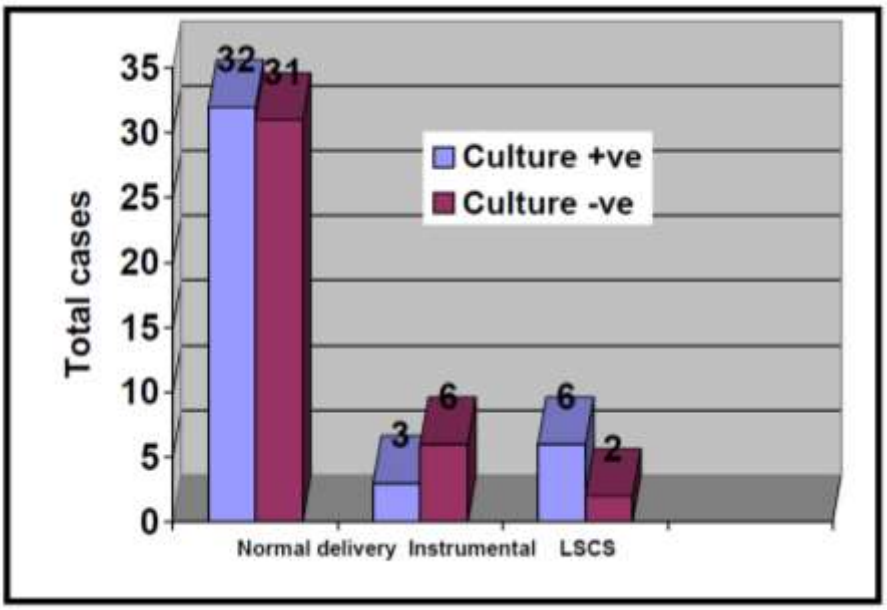
- Out of 80 cases, 46 were inborn babies. Inborn admissions with culture proven sepsis were more in EOS (72%) and LOS (62.5%) group.

Table 7. SHOWS DISTRIBUTION OF CASES ACCORDING TO THE MODE OF DELIVERY

Mode of delivery	Culture positive	Culture negative	Total
Normal Delivery	32 (78%)	31	63(78.75%)
Instrumental	3 (7.3%)	6	9 (11.25%)
LSCS	6 (14.6%)	2	8 (10%)
Total	41	39	80

- 32 of 41 (78%) of culture positive case were delivered spontaneously without assistance. Other 9 (22%) were assisted delivery.

- 17 of 80 (22%) of total septicemic newborns were born of assisted delivery.



Graph 5. Distribution Of Mode of Delivery

Table 8. SHOWS DISTRIBUTION OF PERINATAL RISK FACTORS AMONG NEONATES WITH EARLY ONSET SEPSIS.

Risk factor	**Culture positive n = 25 (%)**	**Culture negative n = 31 (%)**	**Total n = 56**	**p-value**
Birth wt < 2.5 kg or GA < 37 wks	19 (76%)	20 (64.5%)	39 (69.6%)	0.277
Birth asphyxia(APGAR<6@ 1 min)	18 (72%)	19 (61.2%)	37 (66%)	0.3125
PROM >24 hrs	16 (64%)	11 (35.4%)	27 (48.2%)	<0.005*
Duration of labor >24 hrs	16 (64%)	11 (35.4%)	27 (48.2%)	<0.005*
Unclean vaginal examination	23 (92%)	21 (67.7%)	44 (78.5%)	<0.005*
Foul smelling liquor	10 (40%)	7 (28%)	17 (30%)	<0.005*
Maternal fever	3 (12%)	4 (12.9%)	7 (12.5%)	1.00

Out of 56 early onset septic babies:

- Higher proportion of babies had preterm and birth asphyxia as risk factor, both in culture proven and culture negative cases. So no significance was found between two groups.
- Higher proportion of culture proven sepsis had intrapartum risk factor like duration of labor >24hrs, unclean vaginal examination and foul smelling liquor and they were significantly associated with culture proven sepsis.
- PROM > 24 hrs and maternal fever had no significant association.
- Higher proportion of babies with culture proven sepsis had more than two factors.

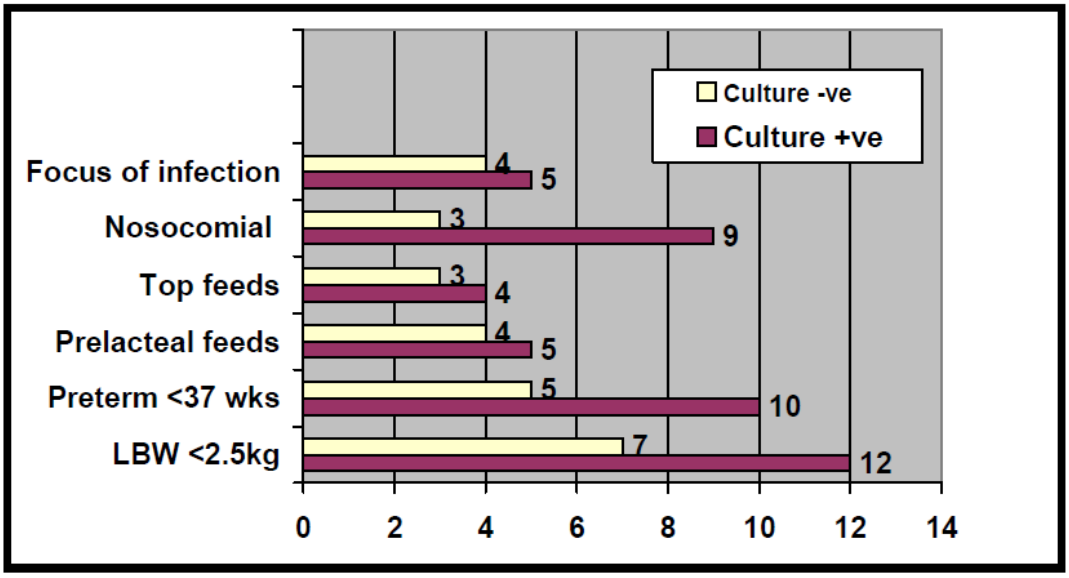
Table 9. SHOWS DISTRIBUTION OF PERINATAL RISK FACTORS AMONG NEONATES WITH LATE ONSET SEPSIS.

Risk factors	**Culture positive n = 16**	**Culture negative n = 8**	**Total n = 24**
Low birth weight <2.5 kg	12	7	19
Preterm <37 wks	10	5	15
Prelacteal feeds	5	4	9
Top feeds	4	3	7
Nosocomial	9	3	12
Focus of infection	5	4	9
• Diarrhorea	2	3	5
• Omphalitis	1	1	2
• IV site	1	0	1
• NEC	1	0	1

Out of 24 cases of late onset sepsis;

- ✓ Higher proportion of late onset sepsis babies were preterm and low birth weight and had previous admission to hospital in prior 1 wk as a risk factor.

- ✓ More often they had top feeding, prelacteal feeds and some focus of infection as risk factors for sepsis.

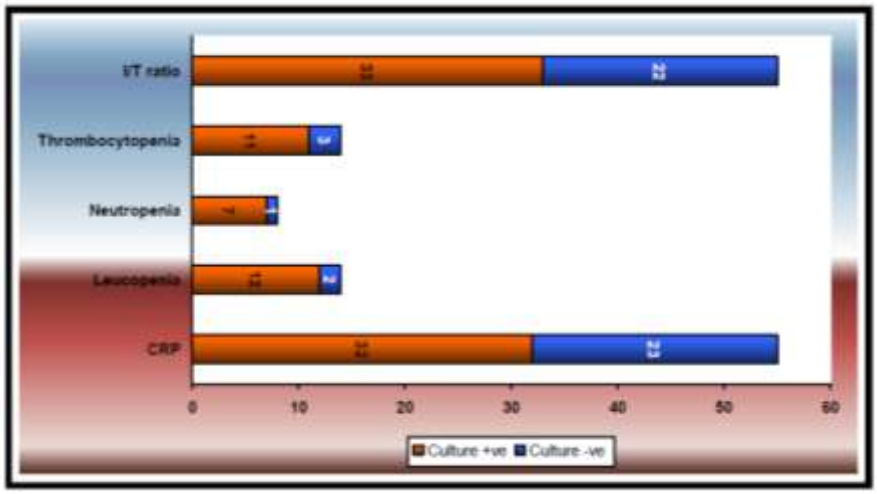


Graph 6. Distribution Of Risk Factors

Table10. SHOWS THE CORRELATION OF SEPSIS SCREEN PARAMETERS WITH THE BLOOD CULTURE STATUS.

Sl. No	Screening parameters	Culture positive n = 41 (%)	Culture negativen =39 (%)	Total cases n = 80 (%)	p- value
1.	CRP positivity	32 (78%)	23	55 (68.75%)	<0.005*
2.	Leucopenia (<5000/ cu.mm)	12(29.2%)	2	14 (17.5%)	<0.005*
3.	Neutropenia (<1750/cu.mm)	7 (17%)	1	8 (10%)	<0.005*
4.	Thrombocytopenia (<1.5 lakhs/cu.mm)	11(26.8%)	3	14(17.5%)	<0.005*
5.	I/T ratio > 0.2	33(80.4%)	22	55 (68.75%)	<0.005*
6.	Two or more tests	40(97.5%)	15	55 (68.75%)	<0.005*

- I/T ratio and CRP were positive in higher proportion of culture positive cases.
- Neutropenia, Leucopenia and Thrombocytopenia are positive in higher proportion in culture proven cases compared to culture negative cases.
- All screening parameters are significant with respect to culture proven sepsis.



Graph 7. Correlation of Sepsis Screen Parameters

Table 11 SHOWS THE SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE ACCURACY AND NEGATIVE PREDICTIVE ACCURACY OF SEPSIS SCREEN PARAMETERS.

Sl.no	Screening parameters	Sensitivity (%)	Specificity (%)	PPV	NPV
1.	CRP	78%	41%	58.18%	64%
2.	Leucopenia (<5000/ cu.mm)	31.70%	94.87%	85.7%	56%
3.	Neutropenia (<1750/cu.mm)	17%	97.4%	87.5%	57.5%
4.	Thrombocytopenia (<1.5 lakh/cu.mm)	26.8%	92.3%	78.5%	54.54%
5.	I/T ratio > 0.2	80.48%	43.58%	60%	68%
6.	Two or more tests positive	97.5%	61.5%	72.5%	96%

- ✓ I/T ratio and CRP have good sensitivity and negative predictive value.
- ✓ Neutropenia and Leucopenia have highest specificity and positive predictive value.
- ✓ If two or more of the above tests are positive, sensitivity and negative predictive value of the screening tool increased above 90%.

Table.12 SHOWS SPECTRUM OF CLINICAL DIAGNOSIS AMONG EARLY ONSET AND LATE ONSET SEPSIS.

CLINICAL DIAGNOSIS	Culture positive		Culture negative		Total n=80
	EOS n=25	LOS n=16	EOS n=31	LOS n=8	
Septicemia	14(56%)	2(12.5%)	22(70.9%)	0	38(47.5%)
Pneumonia	9(36%)	6(37.5%)	7(22.5%)	2(25%)	24(30%)
Meningitis	1(4%)	2(12.5%)	1(3.2%)	2(25%)	6(7.5%)
Infective diarrhoea	0	2(12.5%)	0	3(37.5%)	5(6.25%)
Umbilical sepsis	0	2(12.5%)	0	1(12.5%)	3(3.75%)
NEC	0	1(6.25%)	0	0	1(1.25%)
UTI	1(4%)	1(6.25%)	1(3.2%)	0	3(3.75%)

- ➢ Septicemia was the commonest mode of clinical presentation, constituting about 47.5 % of cases, followed by pneumonia and then meningitis.
- ➢ Among early onset sepsis, septicemia (56%), followed by pneumonia were the commonest presentation.
- ➢ Among late onset sepsis pneumonia (37.5%), followed by meningitis (12.5%) and infective diarrhoea were the commonest mode of presentation.

Table 13. SHOWS DISTRIBUTION OF ORGANISMS WITH RESPECT TO EARLY ONSET AND LATE ONSET SEPSIS.

Bacterial isolates	**Culture positive cases**		**Total n=41 (%)**
	EOS n=25	**LOS n=16**	
Gram positive isolates	**8** (32%)	**6**(37.5%)	**14(**34.14%)
Staphylococcus aureus MSSA MRSA	5(20%) 2(8%)	4(25%) 1(6.25%)	9(21.6%) 3(7.2%)
Coagulase negative staphylococcus	1(4%)	1(6.25%)	2(4.8%)
Gram negative isolates	**17**(68%)	**10**(62.5%)	**27**(65.8%)
Klebsiella pneumoniae	11(44%)	8(50%)	19(45.6%)
Escherichia coli	2(8%)	2(12.5%)	4(9.6%)
Pseudomonas aeruginosa	1(4%)	0	1(2.4%)
Enterobacter cloacae	1(4%)	0	1(2.4%)
Proteus vulgaris	1(4%)	0	1(2.4%)
Klebsiella pneumoniae & citrobacter freundii	1(4%)	0	1(2.4%)

- Out of 41 culture positive cases, 14 (34.14%) were gram positive isolates and 27 (65.85%) were gram negative isolates.

- Klebsiella, followed by Staphylococcus aureus were commonest isolates.

Table 14 SHOWS THE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF GRAM POSITIVE BACTERIAL ISOLATES

Antibiotics	Staphylococcus aureus n=12		Coagulase Negative Staphylococcus n=2
	MSSA n=9	MRSA n=3	
Ampicillin (AMP)	1(11.1%)	0	0
Penicillin (P)	1(11.1%)	0	0
Amoxyclav(AMC)	6(66.6%)	1(33.3%)	1(50%)
Oxacillin (OX)	6(66.6%)	0	0
Methicillin (MET)	9(100%)	0	1(50%)
Cefotaxime (CTX)	7(77.7%)	0	1(50%)
Cefadroxil (CFR)	6(66.6%)	0	1(50%)
Cefazolin (CEZ)	7(77.7%)	0	1(50%)
Amikacin (AMK)	7(77.7%)	2(66.6%)	1(50%)
Gentamicin (GM)	5(55.5%)	1(33.3%)	0
Ciprofloxacin (CIP)	6(66.6%)	1(33.3%)	0
Ofloxacin(OFX)	9(100%)	2(66.6%)	2(100%)
Erythromycin (E)	2(22.2%)	1(33.3%)	0
Vancomycin (VA)	9(100%)	3(100%)	2(100%)

- Methicillin sensitive Staphylococcus aureus (MSSA) was the major gram positive isolate showing 100% susceptibility to Ofloxacin, Methicillin and Vancomycin, while 75-80%were susceptible to Amikacin, cefotaxime and Cefazolin.
- MRSA was 100% susceptible to vancomycin, followed by Ofloxacin.
- All coagulase negative Staphylococci were susceptible to Ofloxacin and Vancomycin, while 50% of them were susceptible to Amikacin, Amoxyclav, Cefotaxime, Cefazolin and Methicillin.

Table 15. SHOWS THE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACTERIAL ISOLATES.

	Klebsiella	**E.coli**	**Pseudomonas**	**Enterobacter**	**Proteus**	**Citrobacter**
Total	19	4	1	1	1	1
AMP	1(5.2%)	0	0	0	0	0
AMK	4(21%)	1(25%)	1(50%)	0	0	1(100%)
GM	2(10.4%)	0	1(50%)	0	0	1(100%)
OFX	15(78.9%)	3(75%)	2(100%)	1(100%)	0	1(100%)
CTX	4(21%)	1(25%)	1(50%)	0	0	0
CTR	4(21%)	2(50%)	1(50%)	0	0	0
CAZ	5(26.3%)	2(50%)	2(100%)	0	0	1(100%)
CZX	15(78.9%)	2(50%)	1(50%)	0	0	1(100%)
CB	1(5.2%)	0	2(100%)	0	0	1(100%)

AMP-Ampicillin, AMK-Amikacin, GM-Gentamicin, OFX-Ofloxacin, CTX-Cefotaxime, CAZ-Ceftazidime, CTR-Ceftriaxone, CZX- Ceftizoxime, CB-Carbenicillin, E.coli-Escherichia Coli

- The major Gram negative isolates were Klebsiella pneumoniae , of these nearly 75% were sensitive to Ofloxacin and Ceftizoxime. 75% of Escherichia coli isolates were sensitive to Ofloxacin.
- All Pseudomonas aeruginosa were sensitive to Ofloxacin, Ceftazidime and Carbenicillin, while 50% of them sensitive to Amikacin, Gentamicin, Cefotaxime, Ceftriaxone and Ceftizoxime.

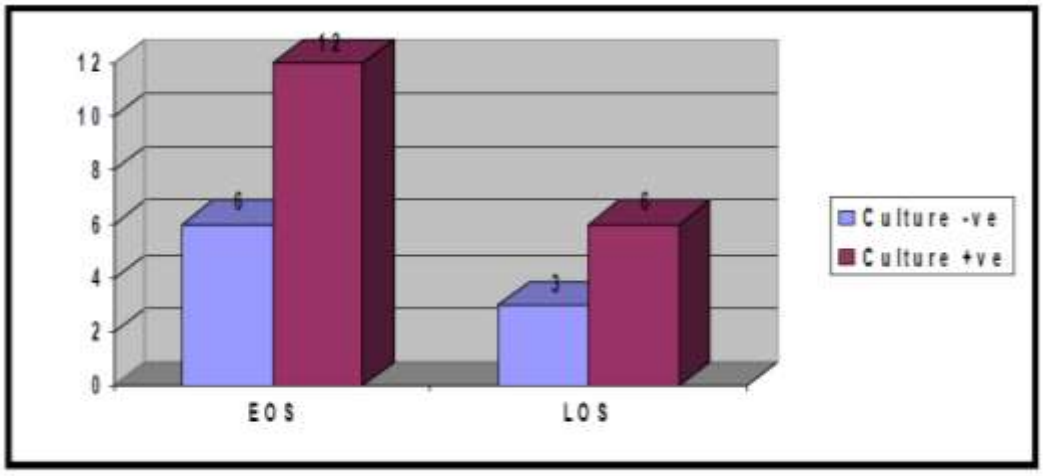
MORTALITY

Table: 16 CORRELATION OF CULTURE POSITIVITY WITH MORTALITY

	Total deaths (%)		Case fatality rate
	EOS n = 25	**LOS n= 16**	
Culture positive	12(48%)	6(37.5%)	43.9% (18/41)
	EOS n=31	**LOS n=8**	
Culture negative	6(19.35%)	3(37.5%)	23% (9/39)
Total	18	9	(27/80) 33.75%

p-value=0.005

- 27 of 80 cases died of sepsis, mortality rate being 33.75%.
- 43.9% of culture positive and 23% of the culture negative cases died of sepsis which was statistically significant. (p-value=0.005)



Graph 8.Correlation of Culture Positivity With Mortality

Table: 17 CORRELATION OF SEX AND PLACE OF DELIVERY WITH MORTALITY

	Culture positive n=41	**Total deaths n=18**	**Mortality rate**
Male	24	12	50%
Female	17	6	35.29%
Inborn	28	12	42.8%
Out born	13	6	46.15%

- Case fatality rate was 42.8% in inborn and 46.15% in outborns.
- Case fatality rate was 50% in male babies and 35.29% in female babies.

Table: 18 CORRELATION OF BIRTH WEIGHT WITH MORTALITY.

Birth weight	**Culture positive**		**Culture negative**		**Mortality rate n=27**
	Cases	**Deaths**	**Cases**	**Deaths**	
NBW	10	3	12	0	3 (11.11%)
LBW	24	8	20	6	14(51.85%)
VLBW	7	7	7	3	10(37.03%)

p-value<0.0005(>2.5 kg v/s <2.5 kg with respect to mortality)

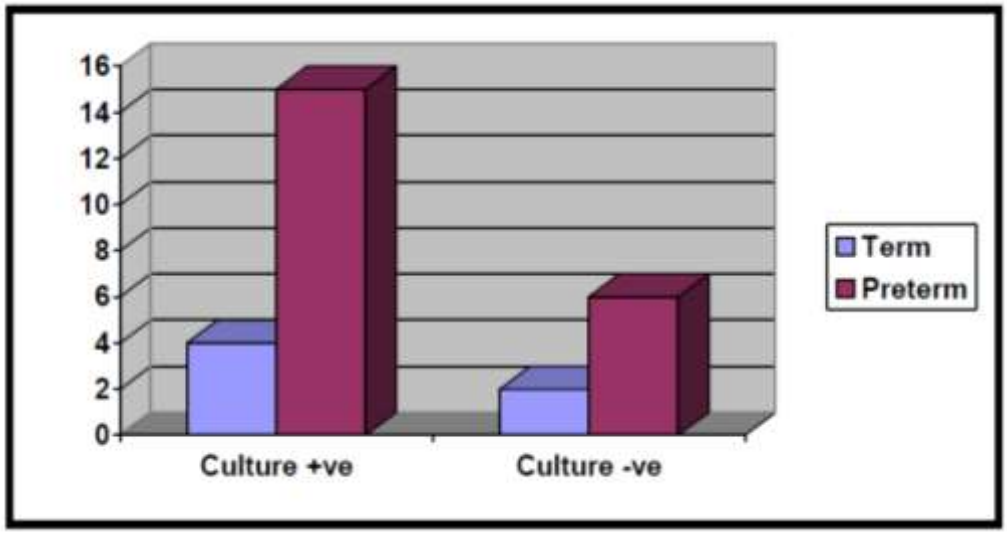
- Out of 58 low birth weight (<2500 gm) septic babies, 24 babies died with case fatality rate of 41.37% among low birth weight, which was statistically significant.
- Case fatality rate among more than 2500 gm was 11%.
- Out of 27 deaths, 24 (88.88%) cases were below 2500 gm birth weight.

Table:19 CORRELATION OF GESTATIONAL AGE WITH MORTALITY.

Gestational age	Culture positive n=41		Culture negative n=39		Mortality rate n=27
	Cases	Deaths	Cases	Deaths	
Preterm	27	15	20	6	21 (77.7%)
Term	14	4	19	2	6 (22.2%)

p-value=0.017

- Out of 27 deaths 21 (77.7%) were preterm babies.
- Case fatality rate among preterm babies was 44.68%, while it was 18.18% among term babies which was statistically significant.



Graph 9. Correlation Of Gestational Age With Mortality.

Table: 20 CORRELATION OF HEMATOLOGICAL PARAMETERS WITH MORTALITY.

	Total cases	Deaths	Case fatality rate
Neutropenia	8	7	87.5%
Leucopenia	14	8	57.14%
Thrombocytopenia	14	10	71.42%

- Neutropenia and thrombocytopenia are good predictors of mortality.

TABLE: 21 CORRELATION OF CLINICAL DIAGNOSIS WITH MORTALITY IN CULTURE POSITIVE CASES

Clinical diagnosis	**Culture positive EOS n=25**		**Culture positive LOS n=16**		**Mortality rate n=18**
	Cases	**Mortality**	**Cases**	**Mortality**	
Septicemia	7	1(14.2%)	1	0	1(5.5%)
Septicemia with MODS	6	6(100%)	1	1(100%)	7(38.8%)
Pneumonia	9	4(44.4%)	6	3(50%)	7(38.8%)
Meningitis	1	1(100%)	2	1(50%)	2(11.1%)
Infective diarrhoea	1	0	2	0	-
Umbilical sepsis	0	-	2	0	-
NEC	0	-	1	1(100%)	1(5.5%)
UTI	1	0	0	-	-
Septic arthritis	0	-	1	0	-

Among 41 culture proven sepsis:

- Septicemia with multiple organ dysfunction (shock, cardio-respiratory failure, DIC) was the commonest mode of death (38.8%) with 100% risk of mortality.
- Pneumonia accounted for 38.8% and meningitis accounted for 11.1% mortality.
- Septicemia with MODS and Necrotizing enterocolitis carries 100% risk of mortality.

Table:22 CORRELATION OF CLINICAL DIAGNOSIS WITH MORTALITY IN CULTURE NEGATIVE CASES

Clinical diagnosis	**Clinically suspected EOS n=31**		**Clinically suspected LOS n=8**		**Mortality rate n=9**
	Cases	**Mortality**	**Cases**	**Mortality**	
Clinically sepsis without focus	18	0	0	0	-
Septicemia with MODS	4	4(100%)	0	0	4(44.4%)
Pneumonia	7	2(28.5%)	3	1(33.3%)	3(33.3%)
Meningitis	1	-	2	1(50%)	1(11.1%)
Infective diarrhoea	0	-	3	1(33.3%)	1(11.1%)
Umbilical sepsis	0	-	-	-	-
NEC	0	-	-	-	-
UTI	1	0	0	-	-
Septic arthritis	0	-	0	0	-

Sepsis with MODS and Pneumonia were the commonest cause of death in clinically suspected group with 100% case fatality in MODS cases.

TABLE: 23 CORRELATION OF BACTERIAL ISOLATES WITH MORTALITY.

Bacterial isolates	**Total cases**		**Total deaths**		**Case fatality rate**	
	EOS n=25	**LOS n=16**	**EOS**	**LOS**	**EOS**	**LOS**
Gram positive isolates						
Staphylococcus aureus MSSA MRSA	5 2	4 1	1 1	1 1	20% 50%	25% 100%
Coagulase negative staphylococcus	1	1	1	0	100%	0
Total	8	6	3	2	37.5%	33.3%
Gram negative isolates						
Klebsiella pneumoniae	11	8	6	3	54.54%	37.5%
Escherichia coli	2	2	1	1	50%	50%
Pseudomonas aerusinosa	1	0	0	0	0	0
Enterobacter cloacae	1	0	1	0	100%	0
Proteus vulgaris	1	0	1	0	100%	0
Klebsiella pneumoniae & citrobacter freundii	1	0	0	0	0	0
Total	17	10	9	4	52.9%	40%

- Case fatality was highest among sepsis with gram negative organism and Klebsiella being commonest organism causing sepsis alone contributing to 54%of mortality.

DISCUSSION

In the present study an attempt has been made to know the various etiological agents responsible for neonatal septicemia and their antibiotic susceptibility patterns, and correlate the efficacy of the sepsis score and the sepsis screen parameters like C reactive protein with the blood culture results.

In this section we compare the results of our study with the studies done by different authors.

Table 24 COMPARATIVE STUDIES SHOWING THE DISTRIBUTION OF CULTURE POSITIVITY IN SEPSIS

SL No.	Author	Year	Total Culture positive	Early onset sepsis	Late onset sepsis
1.	**NNPD 2002[3]**	2002	1248	67%	33%
2.	**Present study**	2017	41	25 (60.98%)	16 (39.02%)

- Maximum culture positive cases were seen in neonates less than 3 days old (early onset septicemia) as compared to neonates aged more than 3 days (late onset septicemia) in the present study.
- Similar observations were seen in the studies done by National Neonatal and Perinatal Database who also reported a higher proportion of early onset septicemia cases.
- This could be due to ascending infection following rupture of membranes or during the passage of the baby through the infected birth canal or at the time of resuscitation in the labor room.
- The higher proportion of EOS cases may be due to the immature immunological responses of the neonates in the first week of life, making them more susceptible to infections in this period.[27]

Table.25 COMPARATIVE STUDIES SHOWING THE DISTRIBUTION OF CULTURE POSITIVITY CASES ACCORDING TO THE GESTATIONAL AGE

SI	Author	Year	Total culture positive	Preterm	Term
1	**Khatua et al**(12)	1986	92	63%	37%
2	**Dawodu et al**(17)	1997	1003	48%	52%
3	**Present study**	2017	41	65.85%	34.14%

- The proportion of culture positive septicemia cases was higher among the preterm neonates in the present study.
- The results of our study were comparable to the studies conducted by Khatua et al(12).
- The higher proportion of cases among the term neonates compared to the preterm neonates in other studies probably reflect differences in the population characteristics and the occurrence of the predisposing factors (preterm incidence) among them.
- Preterm neonates are more susceptible to infections duo to lack of inherent defensive mechanism, both humeral and cellular defense mechanisms. According to Barbara J(9), Stoll BJ el al(27) 1991, the incidence of septicemia inversely proportional to gestational age of the neonates.

Table.26 COMPARATIVE STUDIES SHOWING THE DISTRIBUTION OF CULTURE POSITIVITY CASES ACCORDING TO BIRTH WEIGHT

SI	Author	Year	Total culture positive	Low birth weight
1.	**Joshi et al**(13)	2000	230	50.4%
2.	**Tallur et al**(15)	2000	155	54.55%
3.	**I Roy et al**(16)	2002	346	63.8%
4.	**Nawshad et al**(14)	2002	30	60%
5.	**Present study**	2017	41	75.60%

- In the present study the higher proportion of culture positive cases were <2.5kg.
- The results of the present study are comparable with the other studies.
- Higher incidence of sepsis in low birth weight, both preterm and term small for gestational age is because, they have low maternally acquired IgG and they are inherent susceptibility to infection. While placental transport of IgG from maternal to fetal circulation increases with maturity, this transport is hampered in SGA neonates who are often the products of placental insufficiency.

Table.27 COMPARATIVE STUDIES SHOWING THE DISTRIBUTION OF CULTURE POSITIVITY CASES ACCORDING TO PLACE OF DELIVERY

SL.NO	Author	Year	Total Culture positive	Inborn	Out born
I	**Tallur et al**(15)	2000	155	59.5%	40.5%
2	**Joshi et al**(13)	2000	230	51%	49%
3	**Present study**	2017	41	68.29%	31.70%

Present study shows higher proportion of culture positive cases in inborn admission, which is comparable with other studies. This probably reflects that Hospital being a tertiary referral hospital for both Obstetrics and Pediatrics cases, has maximum late referral and intervened cases with higher proportion of babies born with adverse intrapartum and neonatal risk factors for neonatal sepsis.

Table.28 COMPARATIVE STUDIES SHOWING THE DISTRIBUTION OF SEX IN CULTURE POSITIVE CASES

SL.NO.	Author	Year	Total culture +ve	Male (%)	Female (%)
1	**Khatua et al**(12)	1986	92	70.7%	29.3%
2	**Tallur et al**(15)	2000	155	63.6%	36.4%
3	**Joshi et al**(13)	2000	230	55.5%	44.5%
4	**Present study**	2017	41	24(58.5%)	31 (41.4%)

- The culture positive neonatal septicemia cases were higher among males than the females in the present study, showing a ratio of 1.6: 1. These results are comparable with the observations made by other authors.
- The male preponderance in neonatal septicemia may be linked to the X- linked immuno-regulatory gene resulting in the host's susceptibility to infections in males.

Table.29 COMPARATIVE STUDIES SHOWING THE DISTRIBUTION OF PERINATAL RISK FACTORS AMONG NEONATES WITH EARLY ONSET SEPSIS

Perinatal risk factors	Yancey n=117	Dawod n=52	Tallur n-242	I Roy n-348	Present study n=25
Preterm	29.9%	48%	39.6%	32%	68%
Low birth weight	-	53.8%	54.5%	63.8%	76%
Birth asphyxia	-	36%	21.9%	37.5%	72%
PROM > 24hrs	36.75%	12%	14%	28.9%	64%
Prolonged labor>24hrs	29.9%	8%	19.1%	-	64%
Foul smelling liquor	49.57%	6%	-	-	40%
Maternal fever	-	6%	4.13%	5,2%	12%
Unclean vaginal examination	50.4%	-	-	-	92%

- The present study clearly shows a higher proportion of cases having unclean Pervaginal Examination before delivery, prolonged rupture of membranes for >24 hrs and prolonged labor for >24 hrs in developing definitive septicemia. This is comparable with other studies.

- It is also evident from present study that nearly an equal proportion of cases had birth weight ≤ 2.5 kg and Gestational age < 37 wks as risk factors for developing septicemia. This is comparable with study conducted by Dawodu et al[17], Tallur et al[15] and I Roy et al[16]

- APGAR score <6 suggestive of birth asphyxia was major risk factor associated with sepsis in present study compared to other studies. These variations probably reflect differences in the rates of occurrence of the predisposing risk factors in the various studies.

- Clinical chorioamnionitis diagnosed by presence of foul smelling liquor & maternal fever was present in 31.3% of the culture proven sepsis in present study which is comparable with other studies.The variations in the occurrence of perinatal risk factors probably reflect differences in the rates of occurrence of the predisposing risk factors in the various studies

COMPARATIVE STUDIES SHOWING THE SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE AND NEGATIVE PREDICTIVE ACCURACY OF VARIOUS SEPSIS SCREEN PARAMETERS

Table 30: COMPARATIVE STUDIES OF C-REACTIVE PROTEIN

SL.NO	Author	Year	Sensitivity	Specificity	Predictive Value	
					PPV	NPV
1.	Mathai et al [39]	2004	80.00%	60%	7.7%	99%
2.	Gerdes et al[5]	2004	70%-93%	78%-94%	7%-43%	97- 99 %
3.	Present study	2017	78%	41%	58.88%	64%

In the present study single CRP value has sensitivity of 78% & Negative predictive value of 64% which is comparable to observation made by other studies.

The difference in various studies is due to different cut-off value used in the qualitative test (kit). We had higher sensitivity because our cut-off was 6 ug/ml.

Table:31 COMPARATIVE STUDIES OF TOTAL WBC COUNT

SI	Author	Year	Sensitivity	Specificity	PPV	NPV
1.	Ghosh et al(50)	2001	22%	89%	51%	35%
2.	Gerdes et al(5)	2004	100%	83%	27%	100%
3.	Present study	2017	31.7%	94.87%	85.7%	57.5%

- In the present study, Leucopenia i.e. Total WBC counts <5000 cells/ cu.mm was taken as the diagnostic criteria for detecting neonatal septicemia. Leucopenia has high specificity and positive predictive value but low sensitivity and negative predictive value. This is comparable with results quoted by Ghosh et al(50).

- The differences in the results of this parameter shown by the different studies may be due to variations in the blood sampling time, the severity of infection, the age of the neonates, and the reduced sensitivity of this test in the first week of life.

Table: 32 COMPARATIVE STUDIES OF ABSOLUTE NEUTROPHIL COUNT

SI	Author	Year	Sensitivity	Specificity	PPV	NPV
1.	Ghosh et al(50)	2001	77%	86%	90%	69%
2.	Gerdes et al(5)	2004	38 to 96%	61 to 92%	20 -77%	96-99%
3.	Present study	2017	17%	97.4%	87.5%	52.7%

- Absolute Neutrophil counts <1,750 cells / cu.mm was taken as the diagnostic criteria for detecting neonatal septicemia in our study.

- Neutropenia had high specificity but low sensitivity in present study which is comparable with observation made by Ghosh et al(50), Gerdes et al(5)

- These variations in the results shown by the different authors may be due to differences in the blood sampling time, the severity of infection, the age of the neonates and the reduced sensitivity of this test in the first week of life.

Table: 33 COMPARATIVE STUDIES OF I/T RATIO

SI	Author	Year	Sensitivity	Specificity	PPV	NPV
1	Ghosh et al(50)	2001	93%	95%	92%	90%
2.	Gerdes et al(5)	2004	90-100%	30-78%	11-51%	99-100%
3.	Present study	2017	80.4%	43.58%	60%	68%

- In the present study with an I/T ratio > 0.2 as the diagnostic criteria for detecting neonatal septicemia.
- In the present study, I/T ratio has high sensitivity and high negative predictive value; low specificity and positive predictive value, which is comparable with observation made by Gerdes et al(5).
- The differences in the results of this parameter shown by the different studies may be due to the variations in the blood sampling time, the severity of infection, the age of the neonates, the diagnostic criteria followed and the reduced sensitivity of this test after the first week of life.

Table 34. COMPARATIVE STUDIES OF PLATELET COUNTS

Sl	Author	Year	Sensitivity	Specificity	PPV	NPV
1	Ghosh et al(50)	2001	70%	88%	88%	94%
2	Rodweli et al(54)	1988	22%	99%	60%	93%
3	Present study	2017	26.8%	92.3%	78.5%	54 %

- Thrombocytopenia i.e. Platelet counts < 1.5 lakh/cu.mm was taken as the diagnostic criteria for detecting neonatal septicemia in the present study.
- In present study, thrombocytopenia has high specificity and positive predictive value as sepsis screening tool which is comparable with observation made by Ghosh et al(5) .

Table 35: COMPARATIVE STUDIES OF TWO OR MORE POSITIVE SEPSIS SCREEN TESTS

SI	Author	Year	Sensitivity	Specificity	PPV	NPV
1.	Gerdes et al(5)	2004	100%	83%	27%	100%
2.	Present study	2017	97.5%	61.5%	72.5%	96%

- Considering any two combination test (hematological parameters and CRP) being positive as a screening tool for sepsis, the sensitivity and negative predictive value increased but specificity and positive predictive value decreased in the present study.
- This is comparable with the observation made by Gerdes et al.

Table 36: COMPARATIVE STUDIES SHOWING CULTURE POSITIVITY RATE

SI	Study Group	Year	Total culture +ve	Positivity rate
1	Joshi et al(13)	2000	1326/3427	25%
2	Tallur et al(15)	2000	155 / 242	64.87%
3	I Roy et al(16)	2002	346 / 728	47.50%
4	Madhu Sharma (28)	2002	521/l014	51.38%
5	NNPD 2002-03(3)	2002	1248/4360	28.6%
6	Present study	2017	41/80	51.25%

- In In the present study, 41 of 80 cases studied were culture positive, giving a positivity rate of 51.25%.
- Present study was comparable with the studies conducted by Tallur et al and madhu Sharma et al, while study conducted by Joshi et al, Roy et al and NNPD showed a very low culture positivity.
- The culture positivity depends on time of sampling, extent of bacteremia in neonate and prior antibiotic treatment in the neonate.

Table 37. COMPARATIVE STUDIES OF THE BLOOD CULTURE ISOLATES

Sl	Study Group	Year	Culture positive	Category of organism (gram stain)	Most common isolate
1.	Tallur et al[15]	2000	155/242	Gram negative 85%	Klebsiella (53.50%)
2.	I Roy et al[16]	2002	346 / 728	Gram negative 68%	Klebsiella(24.60%)
3.	Madhu Sharma[28]	2002	521/1014	Gram negative 88.8%	Klebsiella (6 1.4%)
4.	NNPD2002- 03[3]	2002	1248/4360	Gram negative 56%	Klebsiella (32. 5%)
5.	Present study	2017	41/80	Gram negative 65.8%	Klebsiella (46.3%)

- In the present study, Klebsiella pneumoniae 46.3% was the predominate isolate, followed by Staphylococcus aureus (29.2%) Gram negative organisms formed the majority of the isolates as compared to Gram positive organisms (65.8%Vs 34.1% respectively) in the present study

Gram negative organism like Escherechia coli & other enteric bacilli are killed by complement mediated bactericidal activity & the protective antibodies are of the Ig M class which is deficient in neonates who acquire only maternally derived Ig G class antibody.

MORTALITY

Table 38. COMPARATIVE STUDIES SHOWING THE CORRELATION OF BIRTH WEIGHT, GESTATIONAL AGE AND SEX WITH MORTALITY

	Khatua[12] n=92	Joshi n=230	Present study n=80
Mortality	53(57.6%)	74(32%)	27(33.7%)
Low birth weight	45(84.9%)	116(50.4%)	50(89.6%)
Preterm	38(71. 6%)	121(52.6%)	43(76.7%)
Sex(Male:Female)	41:12	--	36:20

Higher proportion mortality was associated with early onset culture proven sepsis and they were preterm and low birth weight. This was statistically significant in the present study which is comparable with other authors.

Table 39: COMPARATIVE STUDIES SHOWING THE CORRELATION OF MORTALITY WITH LABORATORY PARAMETERS

	Squire[52] n=44	**Mathur[53] n=171**	**Present study n=41**
Mortality rate	23(52%)	83(48.5%)	18(43.9%)
Leucopenia	9(39.1%)	--	8(57.1%)
Neutropenia	12(52.1%)	67(80.7%)	7(87.5%)
Thrombocytopenia	14(60.1%)	--	10(71.4%)

Neutropenia and thrombocytopenia are good predictors of mortality with 80-90% case fatality rate which is comparable with other studies.

Table 40: COMPARATIVE STUDIES SHOWING THE CORRELATION OF MORTALITY WITH ISOLATES

	Khatua[12]	**Dawodu[17]**	**Present study n-80**
Mortality rate	53(57.6%)	14(23%)	27(33.7%)
Culture positive	69.1%	23%	43.9%
Gram positive	38.5%	-	35.7%
Gram negative	78%	-	48.8%

- Mortality rate was high among culture positive cases. Gram negative sepsis is associated with high mortality in present study which is comparable with other studies. This is probably related to lack of specific IgM antibodies and complement deficiency in newborn which are required for protection of gram-negative organism.

CONCLUSIONS AND SUMMARY

1. Blood culture though considered gold standard, culture positive rate is low

2. Prematurity and low birth weights are significant risk factors for both early onset and late onset sepsis.

3. Early onset sepsis is significantly associated with intrapartum risk factors like PROM>24hrs, prolonged duration labor, unclean per vaginal examination and foul smelling liquor. Septic score >4 involving above parameters is a good predictor of risk for sepsis.

4. Late onset sepsis is more often due to faulty feeding practices & these neonates have some focus of infection like omphalitis, gastroenteritis etc, as risk factor for sepsis.

5. Signs and symptoms of sepsis are subtle and more difficult to recognize at early stages of infection and ominous signs like shock, grunting, increasing abdominal girth with bilious vomiting and bleeding manifestation are late manifestation and is uniformly fatal.

6. Septicemia was the commonest mode of presentation followed by pneumonia and meningitis

7. Sepsis screening parameters using CRP, haematological parameters are easily available, cost effective, rapid screening tests with good sensitivity and negative predictive value so that if any of the two screening test being negative, the infection is unlikely. Neutropenia and thrombocytopenia have high specificity and high positive predictive value and are predictors of mortality.

8. Overall gram negative organisms are the predominant causative agents for neonatal sepsis. Klebsiella followed by Staphylococcus aureus being the commonest isolate.

9. Antibiotic resistance to commonly used cephalosporin and aminoglycosides like Cefotaxime and Amikacin is increased among gram negative organism. Ofloxacin and Ceftizoxime are best alternatives to which organism are highly sensitive. MRSA and multidrug resistant organism is grave risk for epidemic among admitted neonates

10. Higher proportion mortality was associated with early onset culture proven sepsis and they were preterm and low birth weight. This was statistically significant.

11. Culture proven septic neonate who is born Preterm/low birth weight presenting with septicemia with shock or bleeding manifestation or respiratory failure or meningitis or MODS with associated neutropenia/ thrombocytopenia have highest risk of mortality.

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