BACTERIOLOGICAL DIAGNOSIS OF NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL: A LABORATORY CHALLENGE

By

DR PRARTHANA M S

Dissertation Submitted to the
Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore

In Partial fulfillment
of the requirements for the degree of

DOCTOR OF MEDICINE
In
MICROBIOLOGY

Under the Guidance of
DR. M.R. SANDHYA BELWADI

Department of Microbiology
Vydehi Institute of Medical Sciences and Research Centre
Bangalore
2011

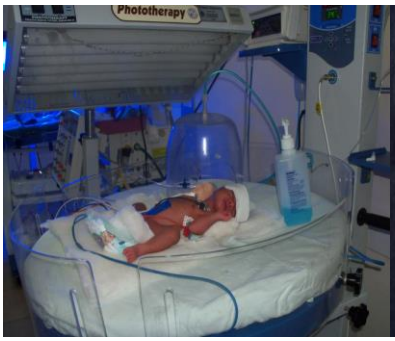
DEDICATED

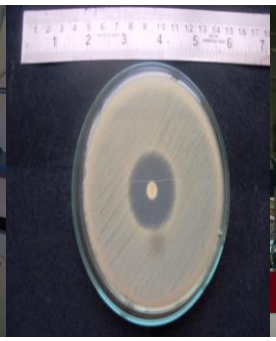
TO MY HUBBY SUNIL

My Children

ADITYA AND ANISH

& BELOVED PARENTS





Rajiv Gandhi University of Health Sciences, Karnataka

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation/thesis entitled

"BACTERIOLOGICAL DIAGNOSIS OF NEONATAL SEPSIS IN A **TERTIARY CARE HOSPITAL: A LABORATORY CHALLENGE" is** a bonafide and genuine research work carried out by me under the guidance of **Dr. SANDHYA BELWADI** Professor and Principal.

Date :

Place: Bangalore

Dr Prarthana M S

CERTIFICATION BY THE GUIDE

This is to certify that the dissertation entitled

"**BACTERIOLOGICAL DI**AGNOSISOF NEONATAL SEPSIS IN A **TERTIARY CARE HOSPITAL: A LABORATORY CHALLENGE** " is a bonafide research work done by **Dr.Prarthana M S** in partial fulfillment of the requirement for the degree of M.D (Microbiology).

Date :
Place: Bangalore

Dr. M.R.Sandhya Belwadi
Professor&Principal
Vydehi Institute of Medical Sciences

CERTIFICATION BY THE CO-GUIDE

This is to certify that the dissertation entitled

"**BACTERIOLOGICAL DI**AGNOSISOF NEONATAL SEPSIS IN A **TERTIARY CARE HOSPITAL: A LABORATORY CHALLENGE** " is a bonafide research work done by **Dr. Prarthana M S** in partial fulfillment of the requirement for the degree of M.D (Microbiology).

Date :
Place: Bangalore

Dr. Rajendran
Professor & HOD
Dept of Microbiology
Vydehi Institute of Medical Sciences

ENDORSEMENT BY THE HOD,

PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled "**BACTERIOLOGICAL DIAGNOSIS OF NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL: A LABORATORY CHALLENGE** " is a bonafide research work done by **Dr. PRARTHANA M S** in partial fulfillment of the requirements for the degree of M.D (Microbiology).

Dr. Rajendran
Professor&HOD
Dept of Microbiology
Vydehi Institute of Medical Sciences

Dr M.R.Sandhya Belwadi
Professor&Principal
Vydehi Institute of Medical Sciences

Date:
Place: Bangalore

Date:
Place: Bangalore

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the Rajiv Gandhi University of Health Sciences, Karnataka shall have the rights to preserve, use and disseminate this dissertation / thesis in print or electronic format for academic / research purpose.

Date:
Place: Bangalore

Dr Prarthana M S

ACKNOWLEDGMENT

I thank **Dr. S. Kantha** , Advisor, Vydehi Institute of Medical Sciences & Research centre for having permitted to counduct the study and helped in its completion.

I thank Mrs Kalpaja , Director , Vydehi Institute of Medical Sciences & Research centre for having permitted to counduct the study and helped in its completion.

With a deep sense of heartfelt gratitude, I owe my sincere thanks to my teacher, mentor and guide **Dr. Sandhya Belwadi.**, Professor & Principal, Department of Microbiology, Vydehi Institute of Medical Sciences & Research centre, Whitefield, Bangalore for her able guidance, patience, critical evaluation, constant encouragement and support at every single step, which aided in the timely completion of this dissertation. I am extremely thankful for her guidance and support rendered. I would like to thank my co-guide **Dr. Rajendran** Professor & HOD, Department of Microbiology, Vydehi Institute of Medical Sciences & Research centre, Whitefield, Bangalore for his academic help and guidance.

I wish to thank **Dr. Hemavathi**, Associate Professor, Department of Microbiology, VIMS, Bangalore for her cooperation, motivation and encouragement towards the completion of this dissertation.

I would also like to thank **Dr. Jayashree**, Assistant Professor for her support & valuable insight. Also I wish to thank **Dr. Ranjini & Dr. Leela Rani** for their valuable guidance.

I take this opportunity to express thanks to the technical staff for their cooperation in this venture.

This work would not have been possible without the constant encouragement and support from my family, specially my husband, parents and my child.

(Dr Prarthana M S)

LIST OF ABBREVIATIONS

- World Health Organization(WHO)
- National Neonatal Perinatal Database (NNPD
- Prolonged rupture of membrane (PROM)
- Very low birth weight (VLBW)
- Multiple Organs Dysfunction Syndrome (MODS)
- Disseminated Intravascular Coagulation (DIC),
- Acute Respiratory Distress Syndrome (ARDS)
- Multiple Organ Dysfunction Syndrome (MODS)
- Systemic Inflammatory Response Syndrome (SIRS)
- necrotizing enterocolitis (NEC).
- Granulocyte colony-stimulating factor (G-CSF)
- Procalcitonin (PCT)
- C Reative protein (CRP)
- Interferon γ (IFNγ)
- Erythrocyte sedimentation rate (ESR)
- Fluorescent in situ hybridization (FISH)
- extended spectrum beta lacta mases (ESBL)
- Matrix – assisted laser desorption ionization time-of-flight mass spectrometry
- (MALDI – TOF MS)
- Vancomycin resistant enteroccoci (VRE)
- Coagulase Negative Staphylococci- CONS
- Tumour necrosis factor (TNF)
- Polymerase chain reaction (PCR)
- Nucleic acid and Sequence-Based Amplification (NASBA)
- Low Birth Weight (LBW)
- Thrombin-antithrombin III complex (TAT)
- Plasminogen activator inhibitor-1 (PAI-1)
- Plasminogen tissue activator (tPA)
- methicillin resistant staphylococcus aureus (MRSA)
- methicillin resistant staphylococcus epidermidis (MRSE).
- Absolute Neutrophil count-ANC
- Early onset sepsis-EOS
- Late onset sepsis-LOS
- Intravenous immunoglobulin (IVIG)

ABSTRACT

Background and Objectives: Neonatal septicemia is a major cause of morbidity and mortality in new born infants. Early diagnosis and prompt treatment are the key words in the management of neonatal sepsis. This study was under taken to know the predisposing factors, outcome, and early indicators of sepsis and antibiotic sensitivity pattern of the organisms isolated from neonatal septicemia cases.

Materials and Methods: The 130 neonates with clinical suspicion of septicemia were included in this study. The blood were subjected to following investigations- Hb, TC, DC with absolute neutrophil count, Micro ESR, CRP , Blood Culture, Gastric aspirate (when indicated), CSF (when indicated) Urine, tracheal aspirate were subjected to culture and sensitivity when indicated.

Results : Culture was bacteriologically positive in 25.4% cases. Gram negative bacilli such as Klebsiella(3.8%) and E.coli(2.3%) constituted 36.3% of isolates, gram positive organisms isolated were S.epidermidis (6.9%), S.aureus(4.6%) and Enterococcus faecalis(4.6%). Ciprofloxacin had maximum sensitivity of 20 (15.4%). Leucopenia $\leq$ 5000/cmm had sensitivity of 47% specificity of 36.36 % and PPV of 46.15 %, Absolute neutrophil count had 48.48 % sensitivity, 90.72% specificity and 64 % PPV . m-ESR had sensitivity of 66.67 %, specificity of 85.57%, PPV of 61.11%, C-reactive protein had 78.79% sensitivity and 89.69% specificity and 72.22% PPV. Case fatality rate was 3.8%.

Conclusion: Clinical features of neonatal septicemia are non specific and vague. Sepsis screen had good sensitivity, specificity, and PPV. Combination of tests increases the specificity and PPV. As an individual test C-reactive protein has highest sensitivity.

Keywords: Neonatal Sepsis; Early Onset Sepsis; C-reactive protein.

CONTENTS

LIST OF TABLES

INTRODUCTION

Of the One hundred and thirty million babies born every year, about four million die in the first four weeks of life- the neonatal period.[1] The World Health Organization(WHO) estimates that, worldwide, approximately five million neonates die each year and that 98% of these deaths occur in developing countries.[2]

Neonatal mortality rate per 1000 live births varies from 5 in developed countries to 53 in the least developed countries.[3] Neonatal infections are estimated to cost 1.6 million annual deaths or 40% of all neonatal deaths in developing countries. The incidence of Neonatal sepsis according to data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live births. [4]

Bacterial sepsis in the neonate is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the first month of life. It encompasses systemic infections of the new born including septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections of the new born.[4, 5]

Neonatal sepsis can be divided into two main classes depending on the onset of symptoms related to sepsis. Early onset sepsis usually presents within 72 hrs of life. Source of infection is generally the maternal genital tract. It often manifests as pneumonia causing acute respiratory distress. The predisposing factor for early onset sepsis include:

- Prolonged rupture of membrane (PROM) (>12hrs)
- Foul smelling and/or meconium stained liquor amnii
- Repeated per vaginal examinations during labour

- Low birth weight (<2500gms) or pre term baby
- Maternal fever
- Difficult or prolonged labour with instrumentation

The commonest organism responsible for Neonatal sepsis and pneumonia included Escherichia coli, Staphylococcus epidermidis, S.aureus. and Klebsiella pneumonia.

Late onset sepsis usually presents after seventy two hours of birth. The source of infection is either nosocomial or community acquired. Neonate usually presents with septicemia, pneumonia or meningitis. Various factors that predispose to an increased risk of nosocomial sepsis include:

- NICU admission
- Invasive procedures
- Parenteral fluid therapy
- Low birth weight and prematurity
- Ventilation and use of stock solution

The clinical features are nonspecific and may present with one or more of the following symptoms and signs

1. hypothermia or fever
2. lethargy , poor cry , refusal to suck
3. poor perfusion , prolonged capillary refill time
4. hypotonia , absent neonatal reflexes
5. bradycardia or tachycardia
6. respiratory distress, apnea and gasping respiration
7. hypoglycemia , hyperglycemia , metabolic acidosis

The early and efficient diagnosis of neonatal bacterial sepsis remains a difficult task.

Blood culture is the gold standard for the diagnosis of septicemia [6] and should be done in all cases of suspected sepsis prior to starting of antibiotics .The other investigations include:

- Gastric aspirate culture
- Urine culture
- CSF culture
- Septic screen

  - total leucocyte count <5000/mm^3
  - absolute neutrophil count <1750/mm^3
  - micro ESR > 15 mm in 1 st hour
  - C- reactive protein > 5 mg/dl

Sepsis related mortality is largely preventable with rational antimicrobial therapy and aggressive supportive care. [4] Blood culture the gold standard for diagnosis of sepsis is time consuming and culture positivity rate is very low.

Early diagnosis and prompt treatment are the key words in the management of neonatal sepsis. Anticipation is forerunner of early diagnosis. Hence the need for minimum and rapid investigations apart from blood culture for the early diagnosis of neonatal sepsis and therefore the need for the study.

OBJECTIVES OF THE STUDY

The study was undertaken with the following objectives:

1. To assist the clinician in formulating the parameters of neonatal sepsis.
2. Early laboratory diagnosis by using Blood culture (the gold standard for diagnosis).
3. Recommending appropriate antibiotic regimen in treatment of neonatal sepsis.

REVIEW OF LITERATURE

The first scientific epidemiological studies, carried out by Ignaz Semmelweis and John Snow, were instrumental in suggesting how diseases were transmitted and how simple measures could interrupt transmission.

Ignaz Semmelweis was a Hungarian obstetrician who was shocked by the number of pregnant women in his hospital dying of puerperal fever (a type of blood poisoning also called childbed fever) during labour.

He determined the disease was more prevalent in the ward handled by medical students (29% deaths) than in the ward run by midwifery students (3% deaths) . this comparative study suggested to Semmelweis that the mode of transmission must involve his medical students. He decided that the source of contagion must be cadavers on which the medical students had previously had been performing autopsies because midwifery students did not work on cadavers

So in 1847 Semmelweis directed his staff to wash their hands with chlorine water before entering the maternity ward. Deaths from childbed fever droped , showing thet disease spread could be interrupted. Unfortunately, few physicians initially heeded Semmelweis recommendations.[7,8]

Systemic infection in the newborn is the commonest cause of neonatal mortality . Data from National Neonatal And Perinatal Database 2000 suggest that Klebsiella pneumoniae , Staphylococcus aureus and E.coli are the commonest cause of neonatal sepsis in India. Pseudomonas was isolated in 5.6% and 12.98% of neonates in intramural and extramural cohorts of neonates respectively. Group B strerptococcus was not an important agents of neonatal sepsis in India.6

Hospital – born babies in developing countries are at increased risk of neonatal infection because of poor intrapartum and postnatal infection control practices. Klebsiella pneumoniae , E.coli, Pseudomonas spp, Acinetobacter spp and Staphylococcus aureus .

Gram negative rods are major pathogens of neonatal sepsis in developing countries .A review of 11471 blood stream samples showed that gram negative rods were isolated from at least 60% of positive blood cultures in all developing regions of the world . .

Klebsiella pneumoniae is the major pathogen responsible for 16-28% of blood culture confirmed sepsis in different regions of the world.

Africa and south asia also have high rates of S. aureus infections , where as Latin America, Southeast Asia , and the Middle East have high reported rates of Coagulase negative staphylococcal infection. The prepondarence of Coagulase negative staphylococcal infection might indicate the latter regions adoptions of sophisticated tertiary neonatal care with a high rate of invasive devices use , although many of these isolates might in infact be contaminants. In developed Countries Group B Streptococci , E. coli and Coagulase negative staphylococci are the predominant pathogens.[8]

A study conducted by Chaturvedi P et all. Staphylococcal growth were maximum constituting approximately 35% of isolates , of which two third were Coagulase negative. Klebsiella and Coagulase negative staphylococci each , constituted approximately 25% of isolates , followed by E.coli (15.9%) , Pseudomonas(13.4%) , Staphylococci (3.3%) , other organisms were infrequently grown.a significant feature of this study was that a majour feature of Staphylococci isolated were Coagulase negative, either considered as non-pathogenic and discarded as contaminants, Coagulase negative staphylococci are now being rapidly recognized for their pathogenicity.in a neonate having clinical features of sepsis with Coagulase negative staphylococci as the only growth even in a single blood culture , this organiem should be considered as pathogenic. Recently various studies from India and abroad have reported a high incidence of Coagulase negative staphylococci septicemia in newborn. [9]

Barbara J Stoll et al, suggested that late onset sepsis is an important problem in very low birth weight (VLBW) infants. They found that vast majority of infants (73%) were caused by gram positive organisms with Coagulase negative staphylococci accounting for 55% of all infections. Rate of infection inversely related to birth weight and gestational age . Complications of prematurity associated with an increased rate of infection included intubations, respiratory distress syndrome, prolonged ventilation , broncopulmonary dysplasia, patent ductus arteriosis, severe intraventricular hemorrhage and necrotizing enterocolitis. [10]

Indian studies have reported incidence of Coagulase negative staphylococci in late onset neonatal septicemia varies from 2.8 to 24%. They found that the rise in serious

neonatal infections due to Coagulase negative staphylococci coincides with change in skin disinfectant usage and general increase in use of third generation cephalosporines to which Coagulase negative staphylococci were resistant. [11]

Neonatal sepsis is defined as a clinical syndrome with systemic signs and symptoms of infection and a positive culture from centralbody fluid. The national neonatology forum's definition for hospitals is as follows:[12]

Probable sepsis

Infants with clinical picture suggestive of sepsis with one or more of the following criteria:

Existence of predisposing factor(any one of the following):

- maternal fever
- foul smelling liquor
- prolonged rupture of the membranes(>12 hrs)
- presence of gastric polymorphs

Positive septic screen (two of the four parameters)

- TLC<5000/mm^3
- Immature to total polymorphs ratio>0.2
- CRP>6mg/ml
- ESR>10mm 1 st hr

Radiological evidence of pneumonia

Proven sepsis

Infants with clinic picture of either :

- Isolation of pathogen of blood, CSF, urine
- Autopsy evidence.

Risk factors

Three major routes of acquisition appears to be important- through mother prior to delivery, from organism present in the vaginal canal , or in the hospital environment.

Maternal factors

- Fever

- Symptomatic bacteriuria
- Prolonged rupture of membranes
- Chorioamnionitis
- Excessive bleeding
- Maternal genitourinary colonization
- Socioeconomic factor- poor nutrition and hygiene.

Neonatal factors

- LBW
- Preterm
- Male newborn
- First born twin
- Congenital malformations with interruption of skin or mucosa, eg , Meningo-myelocoele
- Exposure to certain drugs like steroids.

Environmental Factors

- Flora in the nurserAspiration of contaminated amniotic fluid
- Foetal monitoring, vigorous resuscitation, invasive procedures and intravenous lines.

Pathophysiology

Through out pregnancy and until the membranes rupture, the foetus is relatively protected from the microbial flora of the mother by the chorioamniotic membrane, the placenta & poorly understood antibacterial factors in amniotic fluid. Initial colonization of the neonate usually takes place after rupture of the maternal membranes. If the rupture of membranes lasts longer than 24hr, Vaginal bacteria may ascend & in some cases produce inflammation of the fetal membranes, umbilical cord and placenta. Infection may also result from aspiration of infected amniotic fluid. Finally, bacteria can be introduced after birth from the environment surrounding the baby. [13]

Neonates are considered immuno – compromised hosts due to their relatively immature immune defense mechanisms. Passively transferred specific IgG antibody in adequate concentration provides neonate protection against infection. Cord IgG levels are directly proportional to gestational age, levels in full term infant comparable to that in mother. Preterm infant experience hypogammaglobulinemia as significant placental transfer begins only after 32-34 wks of gestation. The average concentration of IgG in a preterm infant in 400mg/dl & term infant 1000 mg/dl. Even term babies, do not produce significant amounts of pathogen specific antibody & also their in deficiency in immunoglobulins, as other classes of immunoglobulins are not transferred across the placenta although a foetus can synthesis IgA & IgM in response to intrauterine infection. The absence IgM explains the increased susceptibility of the newborns to infections with gram negative organisms.

- Also deficiency in phagocyte system also contributes to increased susceptibility to infection.
- Immune compromise also includes- white cells are not fully functional due to lack of opsonins & complement in human neonate.
- Leucopenia occurs rapidly and neutrophil storage pool in newborn infant in 20-30% of that in adults.
- Impaired phagocytosis, chemotaxis, decreased adhesion, aggregation & deformability all of which may delay the response to infection. These are further compromised in preterm neonate.

- Decrease in T cell production, cytokine production by macrophages.
- Monocyte macrophages, natural killer cell function are also decreased.

No transplancental passage of complement from maternal circulation takes place. An important functions complement is to facilitate the uptake and destruction of pathogen by phagocytic cells. The classical complement pathway results in bacteriolysis & cytolysis. (Gram negative bacteria are generally sensitive to lysis, while gram positive cells are killed without lysis).[14]

- Fibronectin, a serum protein that assists with neutrophil adherence & has opsonic properties, is found in lower concertration in neonate.
- Certain specific enzymatic pathways are non functional in neonates eg antihyuronidase which antagonises the action of hyuronidase. These enzymes helps to prevent spread of infection. This explains that staphylococcal disease in neonate in often systemic than localized.

Once a newborn in infected, there is rapid multiplication & spread of infection to different organs through blood stream & produce varied systemic manifestation. Sepsis syndrome consists of septicaemia along with altered organ perfusion (hypoxia, increased blood lactate, oliguria and alteration in mental state).

Early Septic Shock- occur when systolic blood pressure decreases, the neonate responds to fluids & inotropes

. Refractory septic shock is said when shock that lasts for more than 1 hour despite vigorous therapeutic measures & necessitates vasopressor support.

Multiple Organs Dysfunction Syndrome (MODS)- is said when impaired organ perfusion is present along with either Disseminated Intravascular Coagulation (DIC), Acute Respiratory Distress Syndrome (ARDS), and acute renal, hepatic, neurologic dysfunction is called Multiple Organ Dysfunction Syndrome (MODS).

The term Systemic Inflammatory Response Syndrome (SIRS) describes the non specific inflammatory process consisting of several stages of infection.[15]

Clinical features:-

The signs and symptom of sepsis often are wage and nonspecific and therefore demand a high index of suspicion for early diagnose and prompt treatment. [16]

Non-specific features Neonates with sepsis may present with one or more of the following

symptoms and signs (a) Hypothermia or fever (former is more common in preterm low birth weight infants) (b) Lethargy, poor cry, refusal to suck (c) Poor perfusion, prolonged capillary refill time (d) Hypotonia, absent neonatal reflexes (e) Brady/tachycardia (f) Respiratory distress, apnea and gasping respiration (g) Hypo/hyperglycemia (h) Metabolic acidosis.

Specific features related to various systems:

Central nervous system (CNS): Bulging anterior fontanelle, vacant stare, high-pitched cry, excess irritability, stupor/coma, seizures, neck retraction. Presence of these features should raise a clinical suspicion of meningitis.

Cardiac: Hypotension, poor perfusion, shock.

Gastrointestinal: Feed intolerance, vomiting, diarrhea, abdominal distension, paralytic ileus, necrotizing enterocolitis (NEC).

Hepatic: Hepatomegaly, direct hyperbilirubinemia (especially with urinary tract infections).

Renal: Acute renal failure.

Hematological: Bleeding, petechiae, purpura.

Skin changes: Multiple pustules, abscess, sclerema, mottling, umbilical redness and discharge. Non specific finding like alteration in feeding behaviours, poor cry and activity, hypothermia, apnoea, may be early manifestation.

Blood culture:-

Blood culture in gold standard for diagnosis of sepsis. The yield in higher prior to initiation of antibiotic therapy. At least one set of blood culture is indicated in the diagnosis. It is time consuming and has got a success rate of about 40%.[17]

All blood cultures should be observed for at least 72 hours before they are reported as sterile. It is now possible to detect bacterial growth within 12-24 hours by using improved bacteriological techniques such as BACTEC and BACT/ALERT blood culture systems. These advanced techniques can detect bacteria at a concentration of 1-2 colony-forming unit (cfu) per mL.[4]

Gastric Aspriate:-

This can be viewed as sample of amniotic fluid plus or minus some swallowed secretion from the birth canal. Presence of >5 polymorphs / hpf or >75% neutrophil to epithelial cell ratio or presence of bacteria on gram staining suggests exposure to infection in utero but not necessarily an infected foetus. Its utility is limited to first 6 hours of life. It is of more value in preterms. [18]

Lumbar puncture:

Clinical suspicion of meningitis warrents lumbar puncture. The incidence of meningitis in neonatal sepsis has varied from 0.3-3% in various studies and 0.5% according to the NNPD 2000 data . The clinical features of septicemia and meningitis often overlap; it is quite possible to have meningitis along with septicemia without any specific symptomatology. In EOS, lumbar puncture is indicated in the presence of a positive blood culture or if the clinical picture is consistent with septicemia. In situations of late onset sepsis, LP should be done in all infants prior to starting antibiotics. The cerebrospinal fluid characteristics are unique in the newborn period and normal values are given in table.[4]

Normal Cerebrospinal Fluid Examination in neonates

CSF components	Normal range
Cell/ mm^3	8(0-30)
PMN (%)	60%
CSF proteins (mg/dl)	90(20-170)
Glucose (mg/dl)	52 (34-119)

CSF/ blood glucose (%)	51 (44-248)

Table1: **Normal Cerebrospinal Fluid Examination in neonates**

Urine culture:-

Sterilely acquired bladder tap or catheterised specimens may be obtained, but urine – output is low in newborn infant, as yield is low in first 72hrs of life. Therefore, a urine culture is not suggested as part of the work up for early onset disease. However, neonates at risk for fungal sepsis and very low birth weight infants with poor weight gain should have a urine examination done to exclude urinary tract infection (UTI). UTI may be diagnosed in the presence of one of the following: (a) >10 WBC/mm^3 in a 10 ml centrifuged sample (b) >10^4 organisms /ml in urine obtained by catheterization and (c) any organism in urine obtained by suprapubic aspiration.[18]

Tracheal aspirate culture:-

Tracheal aspirate culture have proven useful when obtained with in the first 12 hrs of life. The diagnostic accuracy is increased when obtained from neonates with suspected sepsis who require intubation and ventilation for presumed pneumonia or respiratory failure.

Table 2: Diagnostic markers of infection for preterm and newborn infants [19]

Hematological tests
Total white blood cell count
Total neutrophil count
Immature neutrophil count

Immature/total neutrophil ratio

Neutophil morphology, vacuolisaiton, toxic granulation, Dohle bodies, intracellular bacteria

Platelet count

Granulocyte colony-stimulating factor (G-CSF)

D-dimer

Fibrinogen

Thrombin-antithrombin III complex (TAT)

Plasminogen activator inhibitor-1 (PAI-1)

Plasminogen tissue activator (tPA)

Acute phase proteins and other proteins

∝ 1 Antitrypsin

C Reative protein (CRP)

Fibronectin

Haptoglobin

Lactoferrin

Neopterin

Orosomucoid

Procalcitonin (PCT)

Components of the complement system

C3a-desArg

C3bBbP

sC5b-9

Chemokines, cytokines and adhesion molecules

Interleukin (IL) 1β, IL 1 ra, IL2, sIL2, sIL2R, IL4, IL5, IL6, IL8, IL10

Tumour necrosis factor ∝ (TNF∝), 11sTNFR-p55, 12sTNFR-p75

Interferon γ (IFNγ)

E-selectin

L-selectin

Soluble intracellular adhesion molecule -1 (slCAM-1)

Vascular cell

Adhension molecule-1 (VCAM-1)

Cell surface markers

Neutrophil	**Lymphocyte**	**Monocyte**
CD11b	CD3	HLA-DR
CD11c	CD19	
CD13	CD25	
CD15	CD26	
CD33	CD45RO	
CD64	CD69	
CD66b	CD71	

Others

Lactate

Micro-erythrocyte sedimentation

Superoxide anion (respiratory burst)

1980's (Early & mid 1980s)

In the early and mid 1980's, neonatal clinician relied mainly on hematological indices as adjunct indicatory for early diagnosing of neonatal sepsis. The indices being mentioned in the table above were studied either singly or in combination.

Results of white cell counts and ratios varied widely across studies, with sensitivity and specificity ranging from 17% to 19% and 31% to 100% respectively.

1. WBC Count

WBC count varies with gestation and postnatal age. A low total leukocyte count <5000 to 7500/mm^3 has been correlated with diagnosis of neonatal sepsis. There in little guidance in the literature that an elevated total WBC in the first 3 days of life in useful in diagnosis of sepsis.

2. Neutrophil Count

Use of absolute peripheral blood neutrophil count has improved the sensitivity in screening for neonatal bacterial disease, but false positive and false negative results are frequent.

Neutropenia in the presence of respiratory distress in the first 72hrs had an 84% likelihood of signifying bacterial disease, where as presence of asphyxia had a 68% likelihood of signifying bacterial disease.[20]

3. MICRO ESR

ESR, a non-specific indicator of tissue damage in known to be elevated in infective states, and the rate of increase in dependent upon the severity of the morbid process. The sedimentation in low in normal newborn babies during first few days of life due to high hematocrit values. It is not affected significantly by sex, birth weight and correction for anemia is not essential during the newborn period, the rate of fall is not affected by injection of calcium, glucose or feeding.
The studies by Parida et al, found that 74.4% of definitely infected babies and 24% of probably infected babies had elevated values.[21]

"Micro" ESR when determined in heparinized capillary tubes when compared to ESR determined by wintrobe's method requires a small amount of blood which can be obtained heel stick and this test in easy to perform.[22]

More recently, granulocyte colony stimulating factor, a mediator produced by the bone narrow for facilitating the proliferation and differentiation of neutrophils, has been proposed to be a reliable infection markes for early diagnosis of neonatal sepsis. Based on a cut off of 200 pg/ml, it has a high sensitivity (95%) & NPV (99%) for predicating early neonatal bacterial & fungal injections.

4. CRP:

C-reactive protein (CRP) is a trace constituent of serum that was originally defined by its calcium dependent precipitation with the C-polysaccharide of pneumococcus. The protein was originally thought to be an antibody to C-polysaccharide and specific for patients with pneumococcus infection, but later studies dispelled this contention, and the relative non-specificity of CRP is now well recognized. The outstanding characteristic of the CRP is that it appears in the sera of individuals in response to a variety of inflammatory conditions and tissue necrosis and disappears when the inflammatory condition has subsided.

Human CRP is a homogeneous molecule (MW 120,000) with a sedimentation co-efficient of 6.5 & an electrophoretic mobility in the gamma region that consists of five probably identical, noncovalently bound subunits of approximattely 21,500 to 23,500 daltons each, linked in the form of a cyclic pentamer. It is made up of 100% peptide and has a amino acid composition similar to that of Immunoglobulin (IgG).

CRP shares with Immunoglobulin the ability to initiate certain functions of potential significance to host- defense, inflammation, precipitation, agglutination, opsonization, capsular swelling and complement activation. CRP also combines with T-lymphocyte and inhibits certain of their functions, and it inhibits the aggregation of platelets induced by aggregated human gamma globulin and thrombin.

CRP differs from immunoglobulin in antigenicity, tertiary structure, homogeneity, stimuli required for formation and release and binding specificities and it is produced

entirely by hepatocyte or liver parenchymal cells. In a physical sense, CRP is thermolabile, being destroyed by heating at 70^0C for 30 minutes and does not cross human placenta.

CRP a acute phase protein elevation in a patient above normal (i.e 5µg/ml or 0.5mg/dl) indicates tissue damage or inflammation or both, with great reliability. CRP levels increase with in 68 hrs after an acute tissue injury, whereas the serum levels of all the other acute phase reactants such as ∝ - 1 anti trypsin, haptoglobin, ceruloplasmin, ∝ - 1 acid glycoprotein increase from 12 to 24 hrs after injury. CRP therefore, is an earlier and more reliable indicator of clinical disease and its severity than the other reactants.[23]

CRP has both diagnostic & prognostic significance. It is more informative especially when serial measurements are done.

CRP rises 8-10 hrs following onset of neonatal infection culture proven sepsis is most unlikely if the CRP does not rise with in 24-48 hrs of onset of illness. The most accurate, rapid and reliable measurement of CRP is by laser nephalometry. (1.5 hrs assay time), rate immuno nephalometry or turbidometry (0.25-0.5hr) & enzyme immune assay (0.5hr) Other procedure involved are Laten agglutination test , Radioimmuno assay.[18, 24]

CRP using a latex agglutination, being a semiquantitative assay , a positive test with undiluted serum indicates CRP levels >or equal to 6 mg/L and with 1 in 4 dilution of serum a positive reaction indicates serum CRP concentration of >or equal to 24 mg/L.[25]

CRP is synthesised with in 6 to 8 hours of exposure to an infective process or tissue damage. It has a half life of 19 hours and may increase more than 1000 fold during an acute phase response. CRP as a diagnostic marker in neonates has higher sensitivity & specificity than total neutrophil count and I/T ratio.

However as the concentration of CRP increase rather slowly in the initial phase, the sensitivity at time to sepsis evaluation is only 60%.

Serial measurement at 24 and 48 hours after the onset of illness considerably improve the sensitivity (82% & 84% respectively). The specificity and positive predictive value of CRP range from 93% to 100% throughout the study period. Thus CRP is considered as a 'Specific' but late marker of neonatal infection.[19].

ACUTE PHASE PROTEIN & OTHER PROTEIN

Another acute phase marker that has attracted much attention recently in procalcitonin (PCT). The increase in circulating PCT concentration in independent of calcitonin and PCT has been shown to be associated with neurotransmission, immunomodulation, and vascular control during infection and in the systemic inflammatory response syndrome (SIRS).

The exact sites of production of PCT in sepsis is not known, monocytes and hepatic cells are believed to be potential sources. Serum concentrations of PCT begin to rise 4 hours after exposure to bacterial endotoxin, peak at 6 to 8 hrs and remain raised for at least 24 hours. Half life in 25-30 hrs and serum concentration is not affected by Gestational age.

PCT is useful in indicating the severity of infection, following progress of treatment, and predicting outcomes. Its diagnostic profile is superior to other acute phase proteins including CRP, with sensitivity and specificity ranging from 87% to 100%. However, false negative cases with height serum concentration have been detected in patients with respiratory distress syndrome, acute lung and inhalation injuries with out bacterial infections & it is relatively expensive.

In critically ill children PCT concentration is a better diagnostic marker of sepsis than CRP and serum amyloid. In critically ill neonates, however, PCT, CRP and SAA

are similar diagnostic marker of sepsis. A PCT concentration higher than 8.1ng/ml identified all children with bacterial sepsis.[26]

The Mid and Late 1990s

Chemokines, cytokines, adhension molecules and components of the immune pathway were extensively studied in the mid and late 1990s.

It is known that preterm as well as term newborns have immature inflammatory responses, a study has shown that these infants display a higher percentage of Interleukin (IL)6 & IL8 positive cells than do adults. The rational behind investigating this diverse group of intercellular messenger is that leucocytes indices and CRP are late marker and are not sensitive enough for early diagnosis of neonatal sepsis.

Of the many mediators studies (Table), much attention has been focused on IL6, IL8 and Tumour necrosis factor (TNF)∝. Umbilical cord blood IL6 has been consistently shown to be sensitive marker for diagnosing neonatal infection with in 72hrs of birth, the sensitivities and negative predictive values being 87-100% & 93-100%. However, it has a very short half life, and the concentrations fall precipitously with treatment and become undetectable in most infected patients with in 24hrs. Therefore is considered as an early and sensitive marker of neonatal infection.

Kustes.H, etal, showed that the use of IL 6 and IL-1 receptor antagonist (IL 1ra) together can predict neonatal sepsis two days before clinical manifestations and result in earlier initiation of antimicrobial treatment with better clinical outcome.[27]

The characteristics and kinetic properties of IL-8 and TNF ∝ are similar to those of IL-6. In both early and late onset sepsis IL8 concentration are substantially higher in infected than non-infected new borns. IL-8 is highly accurate marker with sensitivities ranging from 80% to 91% and specificity 76% to 100%. Accuracy in

further enhanced by simultaneous measurements of either CRP or neutrophil cell surface marker CD 11b.

LATE 1990s AND EARLY 2000s:

Advances in flow cytometric technology paved the way for easy detection of cell surface antigens on blood cells and have allowed simultaneous measurement of key markers using only minimal blood volume (0.05ml whole blood).

Neutrophil CD11b and CD 64 have been found to be promising markers for diagnosis of early and late infections respectively. CD11b is an $\propto$ subunit of the $\beta 2$ integrin adhesion molecule. It is normally expressed at a very low concentration on the surface of non-activated neutrophils. Its expression increases with in a few minutes after the inflammatory cells come into contact with bacteria and endotoxins.

In a study conducted by Ng PC showed that using two neutrophil (CD 11b, CD64) and two lymphocyte surface markers (CD 25, CD45 RO), CD64 has the highest sensitivity (97%), specificity (90%) and negative predictive value (99%).[28]

Diagnostic markers are useful indicators of neonatal sepsis. PCT, IL6, IL8, CD11b and CD 64 are “early sensitive” markers of infection, whereas CRP is a “late specific” diagnostic test. CD 64 is probably one of the most useful infection marker for diagnosis of late onset nosocomial sepsis.

Limitations of infection markers in clinical applications is due to cost, availability of specimens at the appropriate time, reliability of the tests and attitude of attending clinicians are all important factor in determining the suitability of a diagnostic marker for clinical application.

Mid and late 2000

Molecular diagnosis of neonatal sepsis

Among the current, commonly used diagnostic laboratory methods, growth in culture using an automated instrument is considered the gold standard.

Factors affecting the ability of blood culture to detect BSI include (1) the time of collection of the blood sample i.e. before initiation of antibiotics (2) volume of blood, minimum of 0.5 ml of whole blood to be added to blood culture bottle to optimize bacterial recovery. 3) Intermittent bacteremia 4) Low colony count per milliliter of blood 5) Collecting only a single bottle blood culture specimen 6) Inoculating only an aerobic blood culture bottle.

Molecular based approaches detecting BSI include,

Amplification methods for blood pathogen detection.

-Whole blood tested directly by target amplification

-Pre enrichment of whole blood before target amplification

-Fluids from positive blood culture bottles tested by polymerase chain reaction (PCR)

-Nucleic acid and Sequence-Based Amplification (NASBA)

-PCR in conjunction with sequencing or microarray analysis

Nonamplification methods for blood pathogen detection.

-DNA microarray .

-Fluorescent in situ hybridization (FISH)

-Matrix – assisted laser desorption ionization time-of-flight mass spectrometry (MALDI – TOF MS)

A number of studies in infants have been published in which whole blood was

used directly for screening for bacterial nucleic acid by a target amplification assay. Using a highly conserved universal 16s ribosomal DNA (rDNA) target prevent in all bacteria but not in human cells by conventional PCR and gel based detection methods. Sensitivity was found to be 66.7% & specificity was 87.5% compared with culture. By using real time PCR the sensitivity and specificity of real time PCR assay was 96.2% and 100% respectively. False positive blood cultures have been reported at a rate of 1% to 10% and generate uncertainty about whether these results represent growth of fastidious organism that cannot be subcultured or instrument false positive.

Newer amplification based technologies for blood pathogen detection have been developed to enhance sensitivity, speed, or case of pathogen detection, including NASBA and mass-tag PCR. NASBA in an isothermal nucleic acid amplification assay that targets single standard templates so it preferentially amplifies RNA targets and thus has an advantage over PCR in that ribosomal RNA (rRNA) genes are expressed at much higher levels at thousands of copies of per cells.

Bacterial detection techniques such as DNA microarray, FISH and mass spectrometry that do not require target amplification have been developed, thus shortening the time to obtain result, for eg. Cleven and colleagues developed an DNA microarray that directly identified 3 common blood stream pathogen (staphylococcus aureus, E.coli and pseudomonas aeruginosa) from positive blood culture bottles without prior nucleic acid target amplification. The array contained recombinant plasmid-based species specific probes, 200 to 800 base pairs in length, that targeted house keeping genes, virulence factors, and antibiotic resistance genes.

FISH has been used to rapidly identify micro organisms from positive blood

culture fluids. Jansen and colleagues developed FISH probes against variable regions of the 16s rRNA to identify streptococcus spp, E.faecalis, S.aureus, CONS, E.coli, P. aeruginosa , and the entire enterobacteriaceae family. These probes were used to analyze 182 positive blood culture bottles. The testing took only 25 to 45 minutes & with the exception of the S.aureus probe, demonstrated a sensitivity and specificity of 100%.

Among the most recent technologies being used to detect bacteria from positive blood culture, bottles in matrix–assisted laser desorption ionization time-of-flight mass spectrometry (MALDI – TOF MS). This technology seems to provide accurate results for BSI with single pathogens from positive blood culture bottles in about 1 hour time, making it potentially clinically useful.

Prior to the antibiotic era, the mortality from septicemia was 90% but it declined to 24-58% after antibiotics came into use. Neonatal septicemia in a life threatening emergency, and rapid treatment with antibiotics is essential for a favourable outcome. Current recommendation for treating early onset sepsis include broad spectrum antimicrobial therapy covering gram – positive and gram negative bacteria. Therefore, many Institutions prescribe 1 of 2 antibiotic regimens : ampicillin and gentamicin, or ampicillin and a third generation cephalosporin. [29]

Table 3:Empirical choice of antibiotics for treatment of neonatal sepsis

Clinical situation	Septicemia & pneumonia	Meningitis
First line Community-acquired or resistant strains unlikely	Ampicillin or penicillin and Gentamicin	Add Chloramphenicol

Second line Hospital–acquired or Some resistant strains likely	Ampicillin or Cloxacillin and Gentamicin or Amikacin	Add Cefotaxime
Third line Hospital – acquired sepsis Resistant strains are most likely	Cefotaxime and Amikacin	Same

With the advent of the third generation cephalonsporins, the empiric antimicrobial approach for neonatal sepsis has changed in many centres.

Agnihotri N et al, in their study found that S. aureus and gram negative isolates were frequently found to be resistant to amoxycillin / ampicillin, thus indicating that the use of these drugs above may be ineffective. Netilmicin was found to be the most effective drug against gram negative isolates, but resistance to it increased over 5 years study period. Ciprofloxacin was found to be the most effective drug against non – fermenters. Quinolones was found to be effective in treatment of multidrug resistant gram negative infections. Thus concluding that aminoglycosiades, third generation cephalosporins and quinolones are the most suitable drugs for the treatment of neonatal septicemia as per in vitro susceptibility results . [30]

India has an enormous and growing problems of antibiotic use and abuse in new born care. This is resulting in the increasingly resistant gram negative and gram positive bacteria. Gram negative bacteria like Klebsiella can produce extended spectrum beta lacta mases (ESBL) which render the Klebsiella resistant to almost all antibiotics. Gram positive bacteria can carry genes conferring vancomycin resistance, such as vancomycin resistant enteroccoci (VRE) & gene coding for methicillin resistance, such as methicillin resistant staphylococcus aureus (MRSA) and methicillin resistant staphylococcus epidermidis (MRSE). Prolonged use of broad

spectrum antibiotic is also causing a rising incidence of severe fungal sepsis in India. Neonatal fungal infection has almost exclusively been described in the very low birth weight (VLBW) baby weighing <1500g at birth.

Unless neonatologists stop using broad spectrum antibiotics for prolonged periods, resistance to antibiotics will rise. Resistance to the carbapenems, imipenem and meropenem is already appearing in Indian neonates. The long term result will be that neonatologists will have no antibiotics left to treat sepsis caused by some organisms. All doctors must make a combined and concerned effort to improve prescribing practices.

The choice of penicillin will depend on the organism causing sepsis. If it is necessary to cover for staphylococci, then oxacillin, cloxacillin or flucloxacillin may be most appropriate. Vancomycin is not necessary unless MRSA in common. In india, where gram negative bacilli predominate, but almost 100% are ampicillin resistant, piperacillin – tazobactam or ticarcillin – clavulanic acid might be appropriate. The choice of aminoglycoside will also depend on local data. Using antibiotics in rotation has been effective in some settings in reducing resistance. Prevention of infection by improved hand washing has consistently shown to reduce the incidence of nosocomial sepsis.[31]

Newer antibiotics like aztreonam, meropenem and imipenem are also now available in the market. Aztreonam has excellent activity against gram-negative organisms while meropenem is effective against most bacterial pathogens except methicillin resistant staphylococcus aureus (MRSA) and enterococcus. Imipenem is generally avoided in neonates because of the reported increase in the incidence of

seizures following its use. Empirical use of these antibiotics should be avoided; they should be reserved for situations where sensitivity of the isolated organism warrants its use.

Adjunctive therapy [4]

-Exchange transfusion

- Intravenous immunoglobulins(IVIG): Non-specific pooled IVIG has not been found to be useful.

-Granulocyte-Macrophage colony stimulating factor: This mode of treatment is still experimental.

MATERIALS AND METHODS

SOURCE OF DATA

All the neonates (Age 02 – 28 days) with clinical suspicious of septicemia admitted to NICU of Vydehi Institute of Medical Sciences & Research Centre, Whitefield, Bangalore – 66. Were prospectively enrolled over a period of one year from January – 2009 to December 2009.

COLLECTION OF SAMPLES:

1. Samples were collected with all aseptic precaution,

2. Blood sample – 2 different samples were collected from two different sites, to reduce the chance of introducing contaminating organisms from the skin, the venipuncture site was prepared as follows:

The staff involved should wear sterile gloves prior to the procedure. Prepare a patch of skin approximately 5 cm in diameter, over the proposed veni-puncture site. This area should be cleansed thoroughly with alcohol followed by povidone – iodine, followed again by alcolhol.

Application of povidone – iodine should be done in concentric circle moving to outwards from the centre.

The skin should be allowed to dry for at least 1 minute before the sample is collected.

1 ml sample of blood was added to a blood culture bottle containing 5 to 10 ml of culture media.

Blood cultures were collected from a fresh veni-puncture site because sample collected for involving lines and catheter are likely to be contaminated.

This are performed in special cases when ask by the pediatrician.

Urine colleted either through suprapubic aspirate are bladder catheterization sample.

Gastric aspirate

CSF sample collected aseptically through lumbar puncture.

Tracheal aspirate

C – Reactive protein – Rapid latex agglutination test on undiluted sample

WBC count

Absolute Neutrophil count

Micro ESR: is obtained by collecting capillary blood in a standard preheparinised micro haemoatocrit tube (75 mm length, internal diameter of 1.1 mm and outer diameter 1.5 mm) and reading the fall of erythrocyte column after one hour or move than days of life + 3 is considered to be significant all test were performed with in 2 hours of obtaining the blood.

RESULTS AND ANALYSIS

Table 4: Age, gender, Gestation age and Birth weight of neonates studied

Neonates characteristics	No of neonates	%
Age in days		
• 0-3 days	112	86.2
• 4-28 days	18	13.8
Gender		
• Male	70	53.8
• Female	60	46.2
Gestational age		
• Preterm	71	54.6
• Term	59	45.4
Birth weight (gms)		
• <1500	21	16.2
• 1500-2500	67	51.5
• >2500	42	32.3
Total	**130**	**100.0**

Out of 130 neonates with clinical suspision of septicemia studied

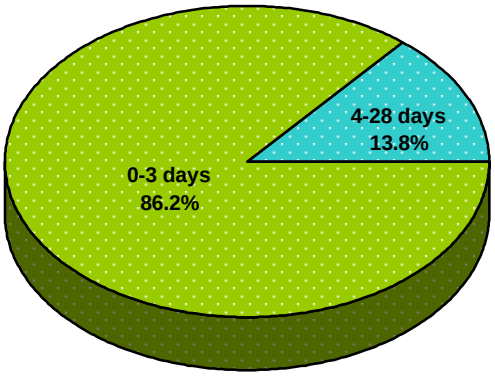
86.2% and 13.8% belonged to EOS (0-3 days) and LOS (4-28 days) respectively

53.8% were males and 46.2% were females

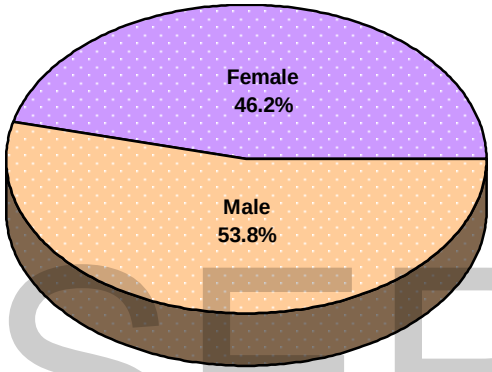
54.6% and 45.4% were preterm and term) respectively

16.2%, 51.5% and 32.3% belonged to VLBW(<1500 gms), LBW(1500-2500 gms) and >2500 gms

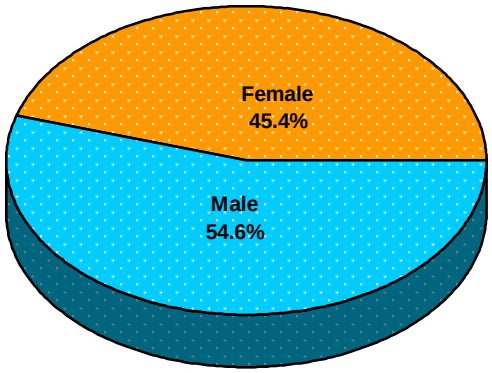
Respectively.



Age in days



Gender



Gestational age

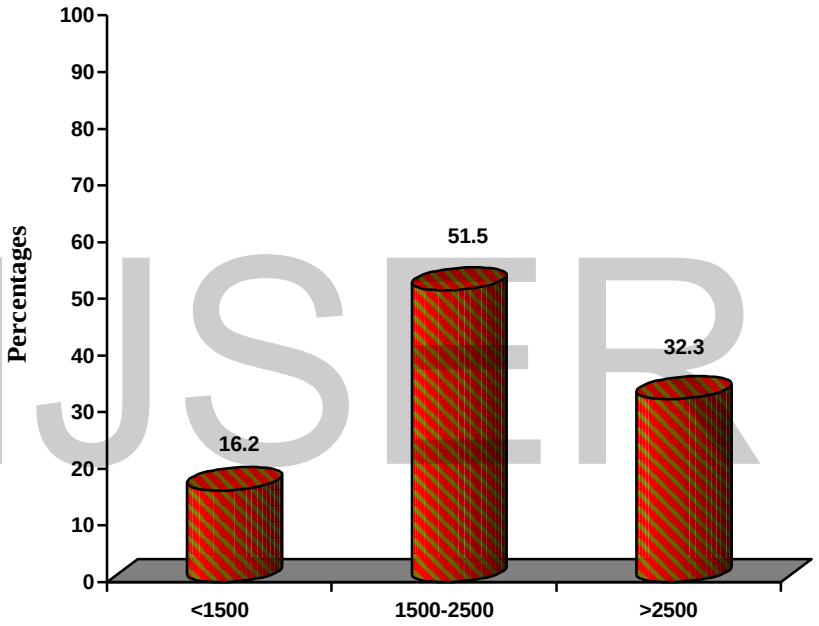

100
90
80
70
60
50
40
30
20
10
0
Percentages
16.2
51.5
32.3
<1500
1500-2500
>2500
Birth weight (gms)

Table 5: Predisposing factor

Predisposing factor	**No.of cases (%)**
Low birth-weight	88(67.7%)
Prematurity	71(54.6%)
Prolonged rupture of membranes > 12 hrs.	36(27.69%)
Maternal complications	9(6.9%)
Meconium stained liquor	19(14.6%)
Outside delivery	24(18.46%)
No obvious factor detected	26(20%)

The above table shows

Neonatal risk factors like low birth-weight was prevalent in 67.7% of cases, prematurity in 54.6% of cases.

Maternal risk factors observed were prolonged rupture of membranes > 12 hrs in 27.69%, Maternal complications (like abruption placentae, maternal fever) in 6.9%, Meconium stained liquor in 14.6%, outside delivery in 18.46 % and no obvious factor detected was detected in 20% of cases.

In 80% of cases there was one or more predisposing factor present.

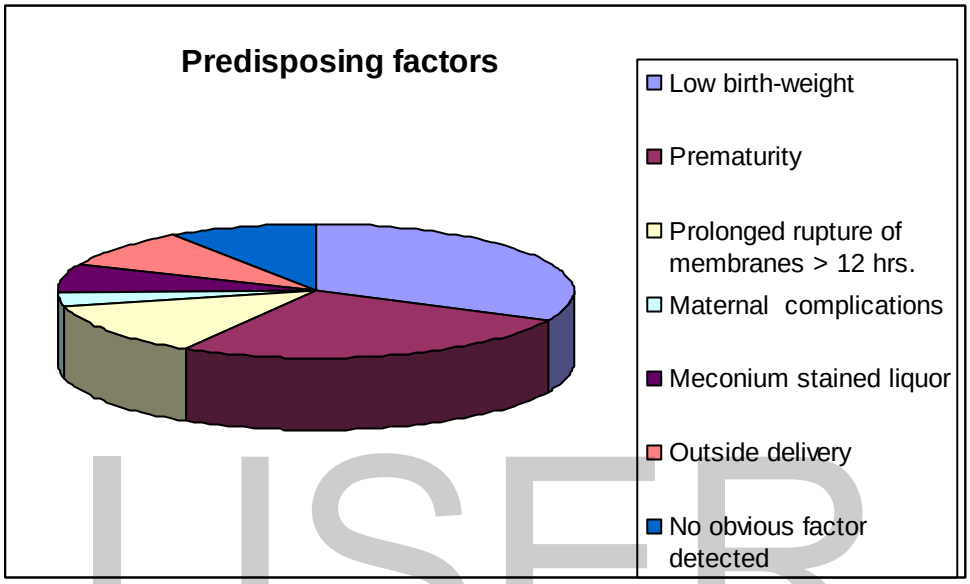

Predisposing factors
Low birth-weight
Prematurity
Prolonged rupture of membranes > 12 hrs.
Maternal complications
Meconium stained liquor
Outside delivery
No obvious factor detected

Table 6: Blood Investigations of neonates studied

Blood Investigations	No of neonates	%	95%CI
Micro ESR			
• Positive	36	27.7	20.72-35.94
• Negative	94	72.3	64.06-79.26
ANC			
• <1750	25	19.2	13.38-26.85
• >1750	105	80.8	73.15-86.62
WBC count			
• <5000	26	20.0	14.03-27.69
• >5000	104	80.0	72.31-85.97
CRP			
• Positive	36	27.7	20.72-35.94
• Negative	94	72.3	64.06-79.28
Total	**130**	**100.0**	**-**

Micro ESR >15mm/hr was taken as positive, the given table shows that 27.7% and 72.3% were

positive and negative for Micro ESR respectively.

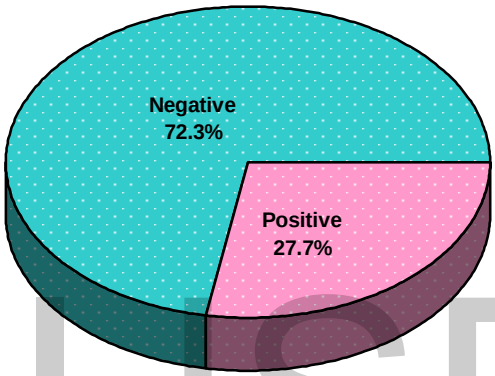
Absolute neutrophil count (ANC) <1750/cmm was present in 19.2% of cases and >1750/cmm was

seen in 80.8%.

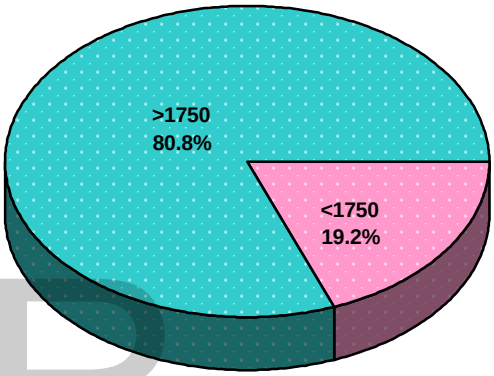
WBC count <5000/cmm was present in 20.0% of cases and >5000 /cmm was seen in 80.0%.

CRP >5mg/dl was taken as positive, the given table shows that 27.7% and 72.3% were positive and

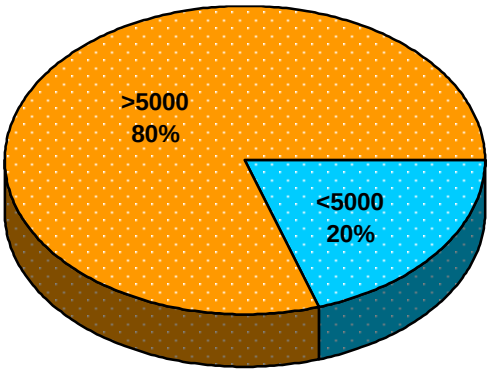
negative for CRP respectively.



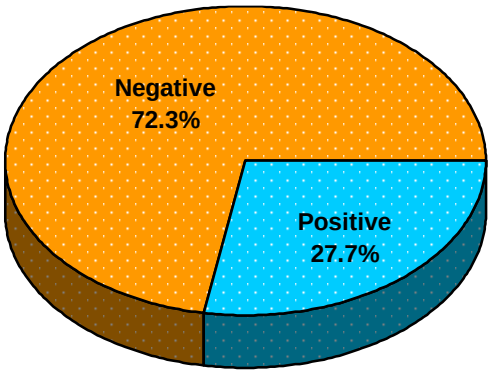
Micro ESR



ANC



WBC Count



CRP

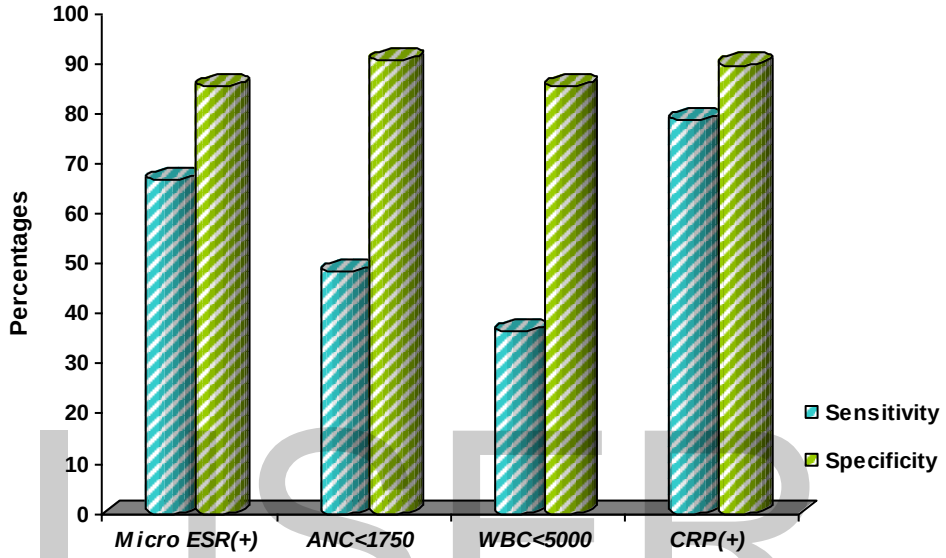
Table 7: Correlation of Micro ESR, Neutrophil count, WBC and CRP in relation to Culture findings (Growth/Nogrowth)-An observation

	True positive	False Positive	False Negative	True Negative	Total
Micro ESR (+)	22	14	11	83	130
ANC <1750	16	9	17	88	130
WBC <5000	12	14	21	83	130
CRP (+)	26	10	7	87	130
Combination					
CRP+ Micro ESR	29	20	4	77	130
CRP+ANC	28	16	5	81	130
CRP+WBC	28	21	5	75	130

Table 8: Correlation of Micro ESR, Neutrophil count, WBC and CRP in relation to Culture findings (Growth/Nogrowth)-An Evaluation

	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
Micro ESR (+)	66.67	85.57	61.11	88.30	80.77	<0.001**
ANC <1750	48.48	90.72	64.00	83.81	80.00	<0.001**
WBC <5000	36.36	85.57	46.15	79.81	73.08	0.011*
CRP (+)	78.79	89.69	72.22	92.55	86.92	<0.001**
Combination						
CRP+ Micro ESR	87.88	79.38	59.18	95.06	81.54	<0.001**
CRP+ANC	84.85	83.51	63.64	94.19	83.85	<0.001**
CRP+WBC	84.85	78.13	57.14	93.75	79.84	<0.001**

Statistically strongly significant(**), moderately significant (*).



From the above table and graph it can be observed that :

Sensitivity, specificity, PPV, NPV of Micro ESR were 66.67%, 85.57, 61.11, 88.30 respectively

Sensitivity, specificity, PPV, NPV of ANC were 48.48, 90.72, 64.00, 83.81 respectively

Sensitivity, specificity, PPV, NPV of WBC were 36.36, 85.57, 46.15, 79.81 respectively

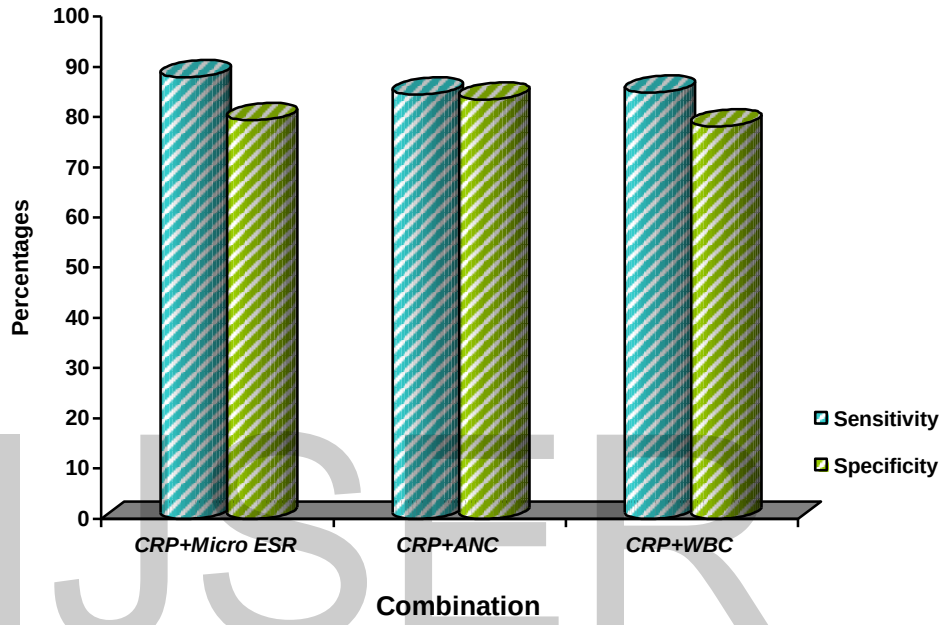
Sensitivity, specificity, PPV, NPV of CRP were 78.79, 89.69, 72.22, 92.55 respectively

Also observation shows that :

CRP has highest sensitivity , specificity, PPV, NPV.

All the test results were statistically strongly significant(**) with P value <0.001 except for WBC

count which was moderately significant (*) .



From the above table and graph it can be observed that :

among the Combination studied

CRP+ Micro ESR showed Sensitivity, Specificity, PPV, NPV of 87.88, 79.38, 59.18, 95.06 respectively

CRP+ANC showed sensitivity, specificity, PPV, NPV of 84.85, 83.51, 63.64, 94.19 respectively

CRP+WBC showed sensitivity, specificity, PPV, NPV of 84.85, 78.13, 57.14, 93.75 respectively

Also observation shows that :

The combination studied have increased the sensitivity of the tests and positive predictive values almost remained the same.

All the test results were statistically strongly significant(**) with P value <0.001 .

Table 9: Blood culture

Culture findings	No of neonates (n=130)	%
No Growth	**97**	**74.6**
Growth	**33**	**25.4**
1.S.epidermidis	9	6.9
2.S.aureus	6	4.6
3.Enterococcus faecalis	6	4.6
4.Klebsiella pneumoniae(4)+ Klebsiella oxytoca(1)	5	3.8
5.E.coli	3	2.3
6.Pseudomonas aeruginosa	2	1.5
7.Citrobacter freundii	1	0.8
8.Enterobacter aerogenes	1	0.8

From the above table it can be observed that 25.4% were Blood culture positive and rest 74.6% were Blood culture negative .

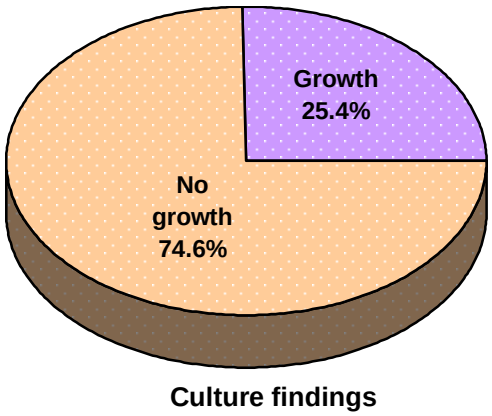


Table 10:Organisms isolated in culture positive cases.

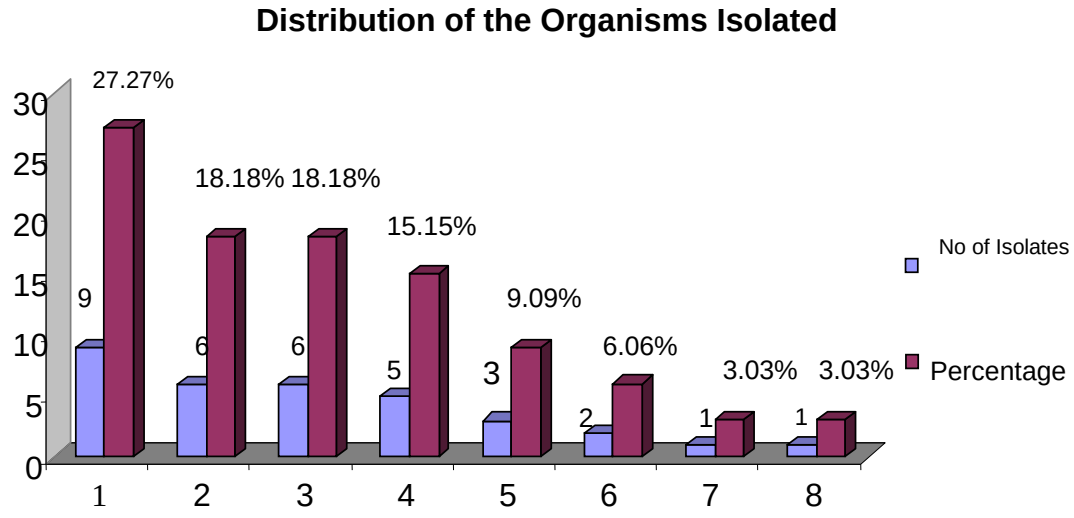
Sl no	Organisms Isolated	No	Percentage
1	S.epidermidis	9	27.27
2	S.aureus	6	18.18
3	Enterococcus faecalis	6	18.18
4	Klebsiella Sp	5	15.15
5	E.coli	3	9.09
6	Pseudomonas aeruginosa	2	6.06
7	Citrobacter freundii	1	3.03
8	Enterobacter cloacae	1	3.03

Out of 33 isolates the distribution of organisms was S.epidermidis 9(27.27%), S.aureus 6(18.18%),

Enterococcus faecalis 6(18.18%), Klebsiella pneumoniae(4)+ Klebsiella oxytoca(1) (15.15%),

E.coli 3(9.09%), Pseudomonas aeruginosa 2(6.06%), Citrobacter freundii 1(3.03%) and Enterobacter

aerogenes 1(3.03%).



1.S.epidermidis, 2. S.aureus, 3.Enterococcus faecalis, 4.Klebsiella Sp, 5.E.coli , 6. Pseudomonas aeruginosa , 7. Citrobacter freundii , 8. Enterobacter aerogenes .

Table 11: Organisms isolated in EOS and LOS

Sl no	Organisms Isolated	EOS(no of isolates)	LOS(no of isolates)
1	S.epidermidis	9	0
2	S.aureus	4	2
3	Enterococcus faecalis	3	3
4	Klebsiella Sp	3	2
5	E.coli	1	2
6	Pseudomonas aeruginosa	2	0
7	Citrobacter freundii	1	0
8	Enterobacter aerogenes	1	0

The table shows that S.aureus, Enterococcus faecalis, Klebsiella Sp and E.coli were isolated in both

Early onset sepsis (EOS) and Late onset sepsis(LOS).

Predominant organisms isolated in EOS were S.epidermidis, S.aureus along with Pseudomonas aeruginosa , Citrobacter freundii and Enterobacter aerogenes.

Organisms isolated in EOS and LOS

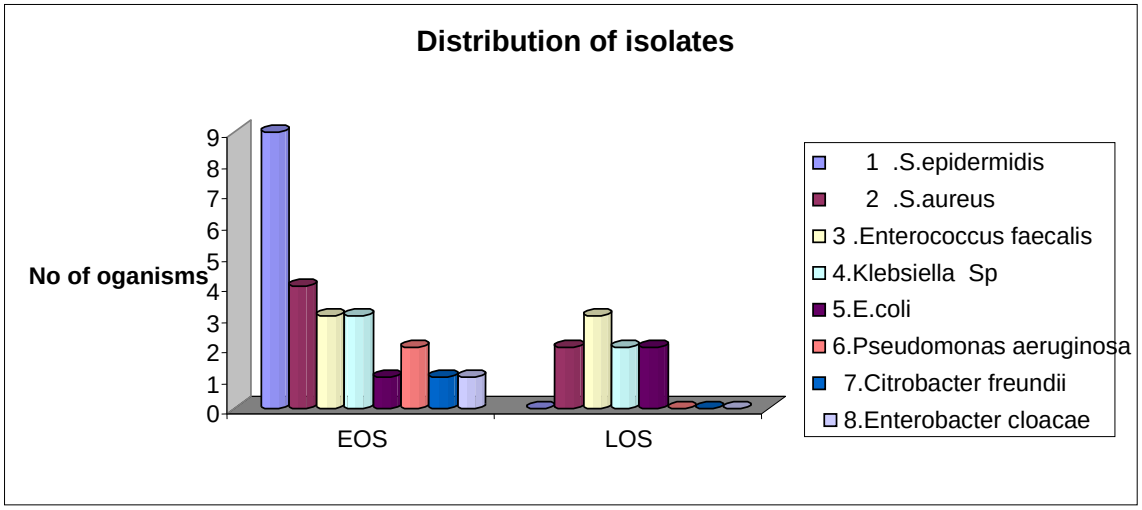
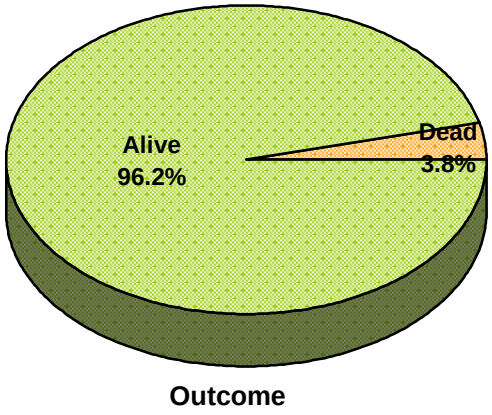


Table 12: Outcome

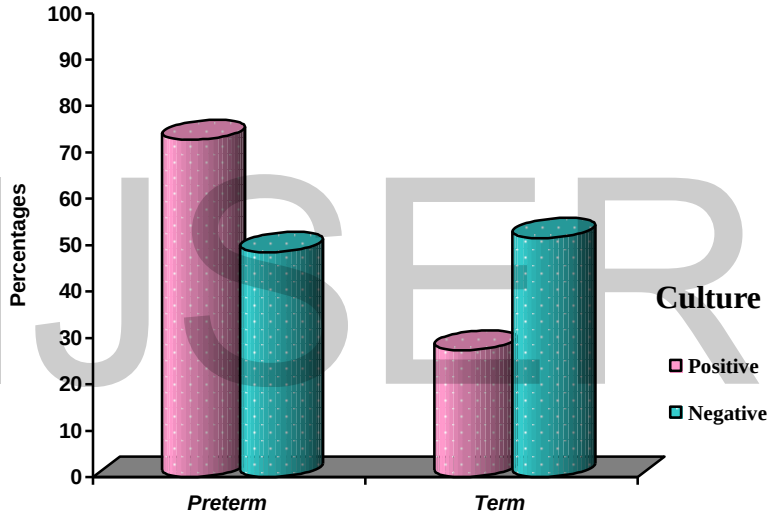
Outcome	No of neonates	%
Alive	125	96.2
Dead	5	3.8
Total	130	100.0



From the above table it can be observed that case fatality rate was found to be 3.8%.

Table 13: Correlation of gestation with culture positive

Gestational age	Number of neonates	Culture report			
		Positive		Negative	
		No	%	No	%
Preterm	71 (54.6%)	24	72.7	47	48.5
Term	59(45.4%)	9	27.3	50	51.5
Total	130(100.0%)	33	100.0	97	100.0
Inference	-	Preterm babies are significantly associated with culture positive with P=0.016*			



Gestational age

Table 14: Correlation of gestation with Birth weight

Gestational age	Number of neonates	Birth weight (gm)					
		<1500 gm		1500-2500 gm		>2500 gm	
		No	%	No	%	No	%
Preterm	71 (54.6%)	19	90.5	41	61.2	11	26.2
Term	59(45.4%)	2	9.5	26	38.8	31	73.8
Total	130(100.0%)	21	100.0	67	100.0	42	100.0
Inference		Preterm is significantly associated low birth weight with P<0.001*					

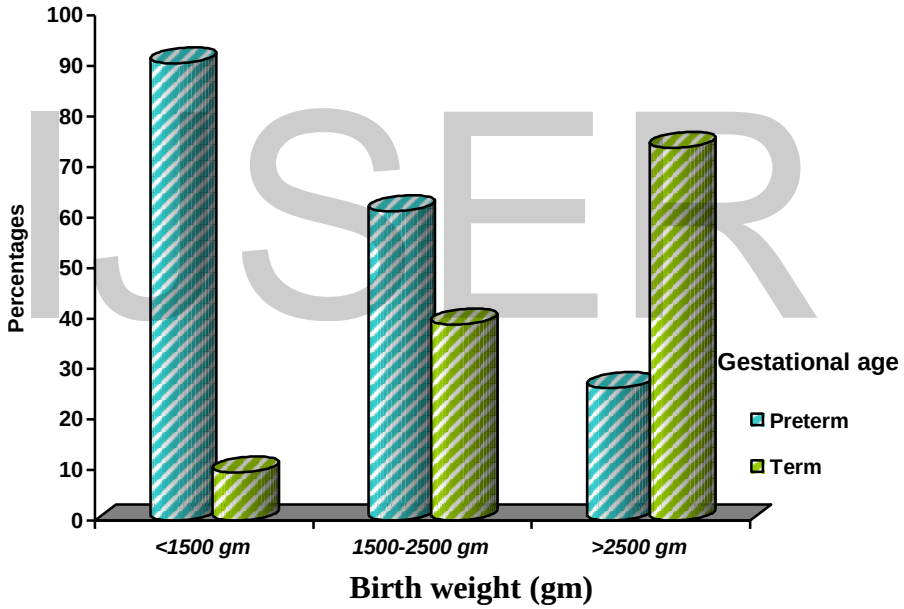
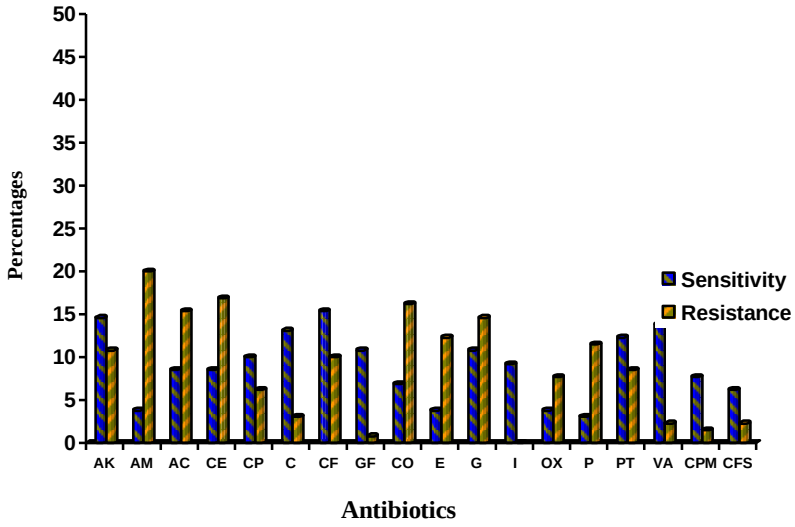


Table 15: Sensitivity resistance pattern of Antibiotics according to bacterial growth

Antibiotics	Sensitivity/Resistance pattern (n=130)	
	Sensitivity	Resistance
Amikacin (Ak)	19 (14.6%)	14 (10.8%)
Amoxycillin (Am)	5 (3.8%)	26 (20%)
Augmentin (Ac)	11 (8.5%)	20 (15.4%)
Cefotaxime (Ce)	11 (8.5%)	22 (16.9%)
Cephalexin (Cp)	13 (10%)	8 (6.2%)
Chloramphenicol (C)	17 (13.1%)	4 (3.1%)
Ciprofloxacin (Cf)	20 (15.4%)	13 (10%)
Gatifloxacin (Gf)	14 (10.8%)	1 (0.8%)
Cotrimoxazole (Co)	9 (6.9%)	21 (16.2%)
Erythromycin(E)	5 (3.8%)	16 (12.3%)
Gentamicin (G)	14 (10.8%)	19 (14.6%)
Imipenem (I)	12 (9.2%)	0 (0%)
Oxacillin (Ox)	5 (3.8%)	10 (7.7%)
Pencillin(P)	4 (3.1%)	15 (11.5%)
Piperacillin/ Tazobactam (Pt)	16 (12.3%)	11 (8.5%)
Vancomycin (Va)	18 (13.8%)	3 (2.3%)
Cefipime (CPM)	10 (7.7%)	2 (1.5%)
Cefperazone salbactum(Cfs)	8 (6.2%)	3 (2.3%)



The above table shows the antibiotic susceptibility pattern of aerobic organisms isolated from neonatal septicemia cases. Out of 33 isolates , 20 (15.4%) were sensitive to Ciprofloxacin (Cf) followed by 19 (14.6%) were sensitive to Amikacin (Ak), 18 (13.8%) to Vancomycin (Va), 17 (13.1%) to Chloramphenicol (C), 16 (12.3%) to Piperacillin/ Tazobactam (Pt), 14 (10.8%) to Gatifloxacin (Gf) , 13 (10%) to Cephalexin (Cp) , 14 (10.8%) to Gentamicin (G), 12 (9.2%) to Imipenem (I), 11 (8.5%) to Augmentin (Ac) , 11 (8.5%) to Cefotaxime (Ce), 10 (7.7%) to Cefipime (CPM), 9 (6.9%) to Cotrimoxazole (Co), 8 (6.2%) to Cefperazone salbactum(Cfs), 5 (3.8%) to Amoxycillin (Am), 5 (3.8%) to Erythromycin(E) , 5 (3.8%) to Oxacillin (Ox) and 4 (3.1%) to Pencillin(P) .

0% resistance was seen with Imipenem (I).

Table 16: Sensitivity pattern of antibiotics according to organisms

Antibiotics	Sensitivity	Organisms							
		1	2	3	4	5	6	7	8
Amikacin (Ak)	19	6	0	3	6	1	1	1	1
Amoxycillin (Am)	5	3	0	0	2	0	0	0	0
Augmentin (Ac)	11	4	1	1	3	2	0	0	0
Cefotaxime (Ce)	11	5	0	1	3	1	1	0	0
Cephalexin (Cp)	13	5	3	0	5	0	0	0	0
Chloramphenicol (C)	17	7	5	0	5	0	0	0	0
Ciprofloxacin (Cf)	20	7	2	3	4	2	1	1	0
Gatifloxacin (Gf)	14	8	0	0	6	0	0	0	0
Cotrimoxazole (Co)	9	3	0	2	2	2	0	0	0
Erythromycin(E)	5	3	1	0	1	0	0	0	0
Gentamicin (G)	14	5	1	2	4	1	0	1	0
Imipenem (I)	12	0	0	5	0	3	2	1	1
Oxacillin (Ox)	5	2	0	0	3	0	0	0	0
Pencillin(P)	4	2	0	0	2	0	0	0	0
Piperacillin/ Tazobactam (Pt)	16	5	0	2	3	2	2	1	1
Vancomycin (Va)	18	7	6	0	5	0	0	0	0
Cefipime (CPM)	10	0	0	4	0	3	1	1	1
Cefperazone salbactum(Cfs)	8	0	0	3	0	2	1	1	1

Organisms code
1. S.epidermidis
2. Enterococcus faecalis
3. Klebsiella pneumoniae(4)+ Klebsiella oxytoca(1)
4. S.aureus
5. E.coli
6. Pseudomonas aeruginosa
7. Citrobacter
8. Enterobacter Sp

Statistical Methods: Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. 95% Confidence Interval has been computed to find the significant features. Confidence Interval with lower limit more than 50% is associated with statistical significance. Diagnostic statistics viz. Sensitivity, Specificity, PPV, NPV and Accuracy have been computed to find the correlation of blood investigations with culture report

1.Chi-Square Test

$\chi^2 = \frac{\sum (Oi - Ei)^2}{Ei}$, Where Oi is Observed frequency and Ei is Expected frequency

2.Fisher Exact Test

	Class1	Class2	Total
Sample1	A	b	a+b
Sample2	C	d	c+d
Total	a+c	b+d	n

2x2 .Fisher Exact Test statistic= $\sum p = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!} \frac{1}{\sum a!b!c!d!}$

3. Diagnostic statistics

	Disease						
Test	**Present**	**N**		**Absent**	**n**		**Total**
Positive	True Positive	*A*		False Positive	*c*		*a + c*
Negative	False Negative	*B*		True Negative	*d*		*b + d*
Total		*A + b*			*c + d*		

The following statistics can be defined:

- Sensitivity: probability that a test result will be positive when the disease is present (true positive rate, expressed as a percentage).
  = a / (a+b)
- Specificity: probability that a test result will be negative when the disease is not present (true negative rate, expressed as a percentage).
  = d / (c+d)
- Positive predictive value: probability that the disease is present when the test is positive (expressed as a percentage).
  = a / (a+c)
- Negative predictive value: probability that the disease is not present when the test is negative (expressed as a percentage).
  = d / (b+d)
- Accuracy is the sum of true positive and True negative divided by number of cases

4. Diagnostic values based on accuracy

0.9-1.0 Excellent test

0.8-0.9 Good test

0.7-0.8 Fair test

0.6-0.7 Poor test

0.5-0.6 Fail

5.Significant figures

+ Suggestive significance (P value: $0.05<P<0.10$)

* Moderately significant (P value:$0.01<P \leq 0.05$)

** Strongly significant (P value : P≤0.01)

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

DISCUSSION

This study was conducted in Vydehi Institute of Medical Sciences and Research Centre, Whitefield, Bangalore during study period of one year. 130 neonates below the age of 28 days with clinical suspicion of neonatal septicemia were included in this study.
In this study clinical profile , bacterial isolates , their antibiotic susceptibility pattern , sepsis screen and out come was studied.

Clinical profile

In the present study out of 130 neonates with clinical suspision of septicemia studied Table 1 shows distribution of cases according to age , sex, gestational age and birth weight. It was absorbed that 86.2% belonged to EOS (0-3 days) and 13.8% belonged to LOS (4-28 days), 53.8% were males and 46.2% were females, 54.6% were preterm and and 45.4% were term ,16.2% belonged to VLBW(<1500 gms) group, 51.5% were LBW(1500-2500 gms) and 32.3% were appropriate for gestational age(>2500 gms **).**

Table 2 shows various predisposing factors detected in neonatal septicemia. In 80% of cases predisposing factors were present. Common neonatal factors observed were low birth weight (67.7%) and prematurity (54.6%). Gerdes J S et al[37] ,Tallur SS et al[33], Anand NK et. Al[38], Gupta P et. Al[39], observed low birth weight as important factor and found increase in incidence of neonatal septicemia in preterm babies. Nelson[40] and Cloherty[41] stated that the prematurity and low birth weights are the most important predisposing factors in neonatal septicemia.

Common maternal factors observed were prolonged rupture of membranes (27.69%), Outside delivery (18.46%) Maternal complications (like abruption placentae, maternal

fever) in 6.9%, Meconium stained liquor in 14.6%. Schuchat A . et al[42]. found neonatal sepsis was associated with intrapartum fever and frequent vaginal examinations . An obstetric risk factor like preterm delivery, intrapartum fever, or membrane rupture ≥18 hours was found in 49% of GBS cases and 79% of other sepsis. Anand NK et. Al[38]. observed prolonged rupture of membranes in 29.3% of cases . Gerdes J S et al[37] noted seven fold increase in the incidence of sepsis after prolonged rupture of membranes and with maternal fever risk increased four fold . Zardi KM et al[8]. observed that unsterile delivery provides an obvious source of inoculation of the new born with potentially pathogenic organisms. Berman[43] emphasized the important role of maternal infection in pathogenesis of neonatal infection.

Sepsis screen

Batteries of indirect markers of infection when collectively studied provide an extremely reliable index of neonatal sepsis much earlier and serve as an useful guide for initiating antibiotic therapy.

In this study sepsis screen was studied in bacterilogically positive and bacterilogically negative cases. Bacterial culture positivity gave definitive diagnosis of septicemia. In the present study out of 130 cases of suspected sepsis 33 cases were proved by positive culture. - Table 3, 4 and 5 depicts sepsis screen of cases studied

CRP

CRP >5mg/dl was taken as positive test for neonatal septicemia present , the given table shows that in 26 bacteriologically positive cases CRP >5mg/dl. while in 7 bacteriologically positive cases CRP <5mg/dl. where as in 10 bacteriologically negative cases CRP >5mg/dl. while in 87 bacteriologically negative cases CRP <5mg/dl.Thus in

our study this test had sensitivity of 78.79 %, specificity of 89.69 % and positive predictive accuracy of 72.22%.

WBC

WBC count <5000/cmm was taken as positive test for neonatal septicemia present

Table shows that in 12 bacteriologically positive cases WBC count <5000/cmm. while in 21 bacteriologically positive cases WBC count >5000/cmm. where as in 14 bacteriologically negative cases WBC count <5000/cmm. while in 83 bacteriologically negative cases WBC count >5000/cmm Thus in our study this test had sensitivity of 36.36 %, specificity of 85.57 % and positive predictive accuracy of 46.15%.

All the test results were statistically strongly significant(**) with P value <0.001 except for WBC count which was moderately significant (*) .

Micro-ESR

Micro-ESR $\geq$ 15 mm at the end of 1st hr was taken as positive test for neonatal septicemia

Table shows that in 22 bacteriologically positive cases m-ESR was $\geq$ 15 mm at the end of 1st hr. while in 11 bacteriologically positive cases m-ESR was < 15 mm at the end of 1st hr. where as in 14 bacteriologically negative cases m-ESR $\geq$ 15 mm at the end of 1st hr. while in 83 bacteriologically negative cases m-ESR was < 15 mm at the end of 1st hr.

Thus in our study this test had sensitivity of 66.67 %, specificity of 85.57 % and positive predictive accuracy of 61.11%.

ANC

Absolute neutrophil count (ANC) <1750/cmm was considered significant for sepsis.

Table shows that in 16 bacteriologically positive cases ANC was <1750/cmm . while in 17 bacteriologically positive cases ANC was >1750/cmm . where as in 9 bacteriologically

negative cases ANC was <1750/cmm . while in 88 bacteriologically negative cases ANC was >1750/cmm. Thus in our study this test had sensitivity of 48.48%, specificity of 90.72 % and positive predictive accuracy of 64%. Also observation shows that : CRP has highest sensitivity , specificity, PPV, NPV. Followed by micro ESR ,absolute neutrophil count and WEC count.

Combination studied

CRP+ Micro ESR showed Sensitivity of 87.88 %, Specificity of 79.38 %, PPV of 59.18 % and NPV of 95.06% .

CRP+ANC showed sensitivity of 84.85%, specificity of 83.51% , PPV of 63.64 and NPV of 94.19%.

CRP+WBC showed sensitivity of 84.85% , specificity of 78.13% , PPV of 57.14% and NPV of 93.75% .

Also observation shows that : All the test results were statistically strongly significant(**) with P value <0.001 .

Reference Table 18: Authors

Author	Year	CRP(%)			WBC(%)			Micro ESR(%)		
		SE	SP	PPV	SE	SP	PPV	SE	SP	PPV
Alistair G.S.et al.[44]	1980	47	86	22	50	94	40	30	97	43
Alistair G.S.et al. [45]	1982	75	71	41	33	90	57	50	83	43
GerdesJ.S et al. [37]	1998	47-100	83-94	6-83	29	91	27	-	-	-
Thakre R. et al. [18]	2006	70-93	78-94	7-43	100	83	27	-	-	-
Ramesh bhat . et al. [35]	2009	20	69	44	10	100	100	10	94	67
Present study	2010	78.79	89.69	72.22	36.36	85.57	46.15	66.67	85.57	61.11

Reference Table 19: Authors

Author	Year	ANC(%)			Combination(%)		
		SE	SP	PPV	SE	SP	PPV
Alistair G.S.et al.[44]	1980	-	-	-	93	88	39
GerdesJ.S et al. [37]	1998	38-96	61-92	20-77	100	83	27
Thakre R. et al. [18]	2006	38-96	61-92	20-77	47	64	36
Ramesh bhat . et al. [35]	2009				40	75	67
Present study	2010	48.48	90.72	64	**CRP+ESR-** 87.88 **CRP+ANC-** 84.85 **CRP+WBC-** 84.85	79.38 83.51 78.13	59.18 63.64 57.14

Various studies shows wide range of sensitivity and specificity for the above tests mentioned which is almost comparable with the present study.

The combination studied have increased the sensitivity of the tests and positive predictive values has slightly decreased which is comparable with the other studies by Alistair G.S.et al and GerdesJ.S et al.

Reference Table 20: Culture results of cases studied:

Author	Year	Total no cases	Positive culture No(%)
Rao P.S. et al.[46]	1993	640	255(40%)
Tallur S.S.et al. [33]	2000	242	156(64.87%)
Kumhar G.D. et al. [47]	2002	1828	770(42%)
Jain N.K. et al. [34]	2003	106	30(28.3%)
Shrestha P. et al. [48]	2007	513	103(20%)
Ramesh bhat . et al. [35]	2009	36	2(5.6%)

Khinchi Y.R. et al. [36]	2010	411	215(52.3%)
Present study	2010	130	33(25.4%)

Table 6 shows 33(25.4%) blood cultures were positive and 97(74.6%) were negative for the culture. The culture results are variable with the other workers also.

Negative cultures can be attributed to

– Non-bacterial growth

- Administration of antibiotic before blood collection either to mother or to the baby

- Possibility of infection with anaerobes.[49]

Different aerobic organisms isolated Refernce table 21

Table 7 shows the organisms isolated in culture positive cases

Staphylococcus epidermidis was the predominant organism 9(27.27%), followed by S.aureus 6(18.18%), Enterococcus faecalis 6(18.18%). Klebsiella pneumoniae 4(12.12%). E.coli 3(9.09%), Pseudomonas aeruginosa 2(6.06%) . One each of Citrobacter freundii 1(3.03%), Enterobacter cloacae 1(3.03%) and Klebsiella oxytoca 1(3.03%) Staphylococcus epidermidis was the most predominant organism (27.27%) in the present study. This finding is correlated with other workers shown in the table. However workers like Kumhar G.D. et al [47], Jain N.K. et al[34], Agnihotri N. et al[30], Jain N.K. et al. [55], found that Gram negative bacilli such as Klebsiella Sp and E.coli were most common organisms isolated followed by Staphylococcus aureus as the second most common cause of neonatal sepsis unlike in developed countries were Group B Streptococci predominates.

Bang A.T. et al[51], Stoll B.J. et al[52], Kumhar G.D. et al[47] and Agnihotri N. et al[30] had isolated 3.6%, 3.3%, 4.25%, 5.1% of Enterococci respectively.

Chaturvedi P. et al[50], Jain N.K. et al[34], Agnihotri N. et al[30], MovahedianA.H. et al[53] and Jain N.K. et al[55] had isolated Pseudomonas in the range of 13.4 - 36%.

Table 8 shows the organisms causing EOS and LOS

In the present study the predominant organisms isolated in EOS were S.epidermidis, S.aureus which is comparable with the study done by Rao P.S et al who found that, S.aureus and S.epidermidis were predominantly responsible for EOS and Pseudomonas and Salmonella typhimurium were responsible for LOS

Reference Table 22: Out come in different cases studied

Author	Year	Place	Total no cases	Expired no (%)
Kuruvilla K.A et al.[56]	1998	CMC Vellore	125	18(14.4%)
Tallur S.S.et al. [33]	2000	Hubli	242	114(47.52%)
Jain N.K. et al. [34]	2003	Nepal	106	12(11.32%)
Ramesh bhat . et al. [35]	2009	KMC Manipal	36	5(13.9%)
Khinchi Y.R. et al. [36]	2010	Nepal	215	22(10.2%)
Present study	2010	Bangalore	130	5(3.8%)

Table 10 shows the outcome in the present study and the case fatality rate was found to be 3.8% . Out of 5 neonatal deaths 2 were due to sepsis with Klebsiella Sp and all of them were preterm. 1 neonate had VLBW (800 gms), 3 neonates had low birth weight. The case fatality rates are variable with the other workers also.

Table 9 shows sensitivity and resistance pattern of antibiotics according to bacterial growth.

In the present study Out of 33 isolates , 20 (15.4%) were sensitive to Ciprofloxacin (Cf) followed by 19 (14.6%) were sensitive to Amikacin (Ak), 18 (13.8%) to Vancomycin (Va), 17 (13.1%) to Chloramphenicol (C), 16 (12.3%) to Piperacillin/ Tazobactam (Pt), 14 (10.8%) to Gatifloxacin (Gf) , 13 (10%) to Cephalexin (Cp) , 14 (10.8%) to Gentamicin (G), 12 (9.2%) to Imipenem (I), 11 (8.5%) to Augmentin (Ac) , 11 (8.5%) to Cefotaxime (Ce), 10 (7.7%) to Cefipime (CPM), 9 (6.9%) to Cotrimoxazole (Co), 8 (6.2%) to Cefperazone salbactum(Cfs), 5 (3.8%) to Amoxycillin (Am), 5 (3.8%) to Erythromycin(E) , 5 (3.8%) to Oxacillin (Ox) and 4 (3.1%) to Pencillin(P) . 0% resistance was seen with Imipenem (I).

Bang A T et al[51] showed that penicillin to be 28.2% sensitive, Erythromycin53.2%, Amoxycillin 87.5%, Chloramphenicol 85.7%, Cotrimoxazole 93.3% and Gentamicin 95% sensitive.

Shaw C.K. et al[57] , Wasseem R. et al[58] and Movahedian AH et al[53] found that gram positive organisms displayed a high degree of resistance to most penicillins and cephalosporins but Imipenem had 100% sensitivity. There was a high incidence of resistance noted with most third generation cephalosporins and aminoglycosides among most gram negative organisms . where-in cefepime and imepenem were effective in most cases. Kumhar G.D et al[47] found 80% gram positive isolates were sensitive to Vancomycin and 50-75% gram negative isolates were sensitive to Ciprofloxacin and Amikacin. our present study almost correlates with the data available from other studies.

There is an emerging resistance to cephalosposrins probably attributable to extended spectrum betalactamases. Further large-scale multicentre studies are required to generalise the data for the whole country.

SUMMARY

Neonatal septicemia is a major cause of morbidity and mortality in newborn infants. The clinical manifestations are non-specific and vague and therefore demand a high index of suspicion for early diagnose and prompt treatment.

So it is important to make the diagnosis early and to start the treatment as early as possible to prevent serious morbidity and mortality caused by untreated or lately treated septicemia.

The study was conducted in Vydehi Institute of medical sciences and research centre, Whitefield, over a study period of 1 year.

130 neonates below the age of 28 days, with clinical suspicious of neonatal septicemia were included in this study to find out predisposing factors , clinical profile, bacteriological antibiotic sensitivity pattern, sepsis screen and outcome of neonatal septicemias.

Clinical profile and predisposing factors :

53.8 % male babies were affected by neonatal septicemia.

Early onset septicemia i.e., $\leq$ 3 days was present in 86.2 % cases.

In 67.7% cases low birth weight is $\leq$ 2500 gms was present.

54.6 % preterm babies were affected by neonatal septicemia.

Predisposing factors were detected in 80% of cases. Common neonatal factors were prematurity (54.6 %) and low birth weight (67.7%). Common maternal factors observed were prolonged rupture of membranes (27.69%). Maternal complications in 6.9%, meconium stained liquor in 14.6%, outside delivery in 18.46% and no obvious factor detected was detected in 20% of cases.

Culture was bacteriologically positive in 25.4% cases. Organisms isolated were S.epidermidis 9(27.27%), S.aureus 6(18.18%), Enterococcus faecalis 6(18.18%), Klebsiella pneumoniae(4)+ Klebsiella oxytoca(1) (15.15%), E.coli 3(9.09%), Pseudomonas aeruginosa 2(6.06%), Citrobacter freundii 1(3.03%) and Enterobacter cloacae 1(3.03%).

S.aureus, Enterococcus faecalis, Klebsiella Sp and E.coli were isolated in both Early onset sepsis (EOS) and Late onset sepsis(LOS).

Predominant organisms isolated in EOS were S.epidermidis, S.aureus along with Pseudomonas aerogenosa , Citrobacter freundii and Enterobacter cloacae.

Sensitivity pattern of organisms against various antibiotics shows that, out of 33 isolates , 20 (15.4%) were sensitive to Ciprofloxacin (Cf) followed by 19 (14.6%) were sensitive to Amikacin (Ak), 18 (13.8%) to Vancomycin (Va), 17 (13.1%) to Chloramphenicol (C), 16 (12.3%) to Piperacillin/ Tazobactam (Pt), 14 (10.8%) to Gatifloxacin (Gf) , 13 (10%) to Cephalexin (Cp) , 14 (10.8%) to Gentamicin (G), 12 (9.2%) to Imipenem (I), 11 (8.5%) to Augmentin (Ac) , 11 (8.5%) to Cefotaxime (Ce), 10 (7.7%) to Cefipime (CPM), 9 (6.9%) to Cotrimoxazole (Co), 8 (6.2%) to Cefperazone salbactum(Cfs), 5 (3.8%) to Amoxycillin (Am), 5 (3.8%) to Erythromycin(E) , 5 (3.8%) to Oxacillin (Ox) and 4 (3.1%) to Pencillin(P) .

0% resistance was seen with Imipenem (I).

Sepsis screen :

Sepsis screen was studied in bacteriologically positive and negative cases. Bacterial culture positivity was criteria for definite diagnosis of septicemia. In this study out of 130 cases of neonatal sepsis, 33 were proved by positive culture.

Early indicators of neonatal sepsis being leucopenia ≤ 5000 / cmm, m-ESR ≥15 at the end of 1st hr, absolute neutrophil count of <1750/cmm and C-reactive protein >5mg/dl were considered to study sepsis screen.

Leucopenia ≤ 5000/cmm had sensitivity of 36.36%, specificity of 85.57% and positive predictive value 79.81%.

Micro ESR ≥ 15 mm at the end of 1st hr. had sensitivity of 66.67% specificity of 85.57% and positive predictive value of 88.3%.

C-reactive protein had 78.79% sensitivity and 89.69% specificity and 72.22% positive predictive value.

Combination studied showes that

CRP+ Micro ESR showed Sensitivity of 87.88 %, Specificity of 79.38 %, PPV of 59.18 % and NPV of 95.06% .

CRP+ANC showed sensitivity of 84.85%, specificity of 83.51% , PPV of 63.64 and NPV of 94.19%.

CRP+WBC showed sensitivity of 84.85% , specificity of 78.13% , PPV of 57.14% and NPV of 93.75% .

The combination studied have increased the sensitivity of the tests and positive predictive values almost remained the same

All the test results were statistically strongly significant (**) with P value <0.001.

Out come **:**

Case fatality rate was 3.8%. Out of 5 neonatal deaths 2 were due to sepsis due to Klebsiella Sp and all of them were preterm. 1 neonate had VLBW (800 gms), 3 neonates had low birth weight.

CONCLUSIONS

1. Clinical features of neonatal septicemia are nonspecific and vague and may be clinically indistinguishable from those occurring in noninfectious condition during neonatal period.
2. Male, preterm and low birth-weight neonates are more prone for septicemia.
3. Early-onset septicemia is more common than late-onset septicemia.
4. Prematurity, low birth weight, prolonged rupture of membranes, outside delivery predispose neonate to infections.
5. Gram-positive organisms are common cause of early-onset septicemia .
6. Sepsis screen has good sensitivity, specificity and positive predictive accuracy and is a valuable aid in early diagnosis of neonatal septicemia.
7. Sepsis screen is simple, cheap, less time consuming and easy to perform even at bedside.
8. As an individual test C-reactive protein has highest sensitivity, specificity and positive predictive accuracy and is a sensitive and responsive indicator of neonatal sepsis.
9. Combination of tests increases the specificity and positive predictive accuracy.
10. Mortality is higher in preterm and low birth-weight babies.
11. Mortality is higher in early-onset septicemia and Gram-negative septicemia.

BIBLIOGRAPHY

1) World health report 2005: Make every mother and child count. Geneva: WHO; 2005.
2) Bang AT, Bang RA, Bactule SB, Reddy HM, Deshmukh MD. Effect of home-based neonatal care and management of sepsis on neonatal mortality: field trial in rural India. Lancet 1999; 354:1955-61.
3) Neonatal and perinatal mortality: country, regional and global estimates. Geneva: WHO; 2006.
4)Report of the National Neonatal Perinatal Database (National Neonatology Forum) 2002-03.
5) Gupte S. Neonatal septicemia: Issues and controversies (Guest Lecture), Third Workshop of Asian Oceanian Perinatal Societies, Katamandu, Nepal 1997. Recent advances in Pediatrics: Neonatology.l999; 4(sp.vol):151-6.
6)Qazi SA, Stoll BJ. Neonatal sepsis: a major global public health challenge. Pediatr Infect Dis J. 2009;28:S1–S2. doi: 10.1097/INF.0b013e31819587a9.
7) Al-Taiar A, Hammoud MS, Cuiqing L, Lee JK, Lui KM, Nakwan N, Isaacs D. Neonatal infections in China, Malaysia, Hong Kong and Thailand. Arch Dis Child Fetal Neonatal Ed. 2013;98:F249–F255. doi: 10.1136/archdischild-2012-301767.
8) Loudon I. Semmelweis and his thesis. Journal of the Royal Society of Medicine. 2005 Dec;98(12):555-.
9) Tallur S S, Kasturi AV, Nadgir SD, Krishna B V. Clinico Bacteriological study of neonatal septicemia in Hubli. Indian J Paediatr. 2000; 67: 169-74.
10) Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, Lemons JA, Donovan EF, Stark AR, Tyson JE, Oh W. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002 Aug 1;110(2):285-91.
11) Gerdes J S , Polin R. Early diagnosis and treatment of neonatal sepsis. Indian Journal Pediatr 1998; 65: 63-78.
12) Anand NK, Gupta AK, Man Mohan, Lamba IMS, Gupta R, Shrivastava L. Coagulase negative staphylococcal septicemia in newborns. Indian Pediatrics 1991 ; 28 : 1241 – 1248.
13) Gupta P, Murali MV, Faridi MMA, Caul PB, Ramchandran V G, V Talwar.Clinical profile of Klebsiella septicemia in neonates. Indian Journal of Pediatrics 1993 ; 60 : 565 – 572.
14) Barbara J Stoll. Infections of neonatal infant. In : Richard EB, Robert MK, Hal BJ. Editors. Nelson text book of pediatrics. 17th edition. Philadelphia : Saunders ; 2004p 630-639.
15) Karen MP. Bacterial and Fungal infections. In: John P Cloherty, Eric C Elchenwald, Ann RS. Manual of Neonatal Care. 5th edition. Philadelphia : Lippincott ; 2004 p. 287-312..
16) Behrman RE. Children at special risk. In : Richard EB, Robert MK, Hal BJ editors. Nelson text book of pediatrics. 17th edition. Philadelphia : Saunders; 2004 p.148 – 151.
17) Kumhar G D, Ramachandran V G, Gupta P, Bacteriological Analysis of Blood culture Isolates from neonates in a Tertiary care hospital in India. J Health Popul Nutr 2002; 20(4): 343-47.
18) Edwards MS. Postnatal infections. In: Fanaoff, Martins editors. Neonatal-perinatal Medicine, 8th ed. Philadelphia: Mosby Elsevier, 2006. p. 791-804.
19) Vergnano S, Sharland M, Kazembe P, Wansambo CM, Heath PT. Neonatal sepsis. An international perspective. Arch Dis Child Fetal Neonatal Ed 2005;90: F220-F224.
20) National neonatal health strategy. Family health division, Department of health services, Ministry of health, Government of Nepal, 2004;1-4.
21) Stoll BJ. Infections of the neonatal infant. In: Behrman RE, Kliegman RM, editors. Nelson textbook of pediatrics. 18t ed. Philadelphia: WB Saunders Company; 2008, p.794-8.

22) Klein JO. Bacterial sepsis and meningitis. In: Remington JS, Klein JO, editors. Infectious Diseases of the Fetus,Newborn, and Infants. 5th ed. Philadelphia: WB Saunders Company; 2001:943–84.
23) Stoll BJ. Infections of the Neonatal Infant: Pathogenesis and Epidemiology. In: Behrman RE, Kliegman RM, editors. Nelson Textbook of Pediatrics. 17th ed. Philadelphia: Saunders; 2004: 623–40.
24) Colle JG, Duguid JP, Fraser AG, Marmion BP editors. Mackie & McCartney Practical Medical Microbiology. 13 th ed. New York: Churchil Livingstone; 1989.
25) Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 12th ed. St. Louis: Mosby Elsevier;
26) 2007. Chapter 13, Overview of Bacterial Identification Methods and Strategies; p.216-247.
27) Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests. 9th ed. Approved standard. Wayne, PA: Clinical and Laboratory Standards Institute, 2006. (CLSI document no. M2-A9).
28) Rahman S, Hameed A, Roghani MT, Ullah Z. Multidrug resistant neonatal sepsis in Peshhawar, Pakistan. Arch Dis Child Fetal Neonatal Ed 2002;87(1): F52-F54.
29) Karki BMS, Parija SC. Analysis of blood culture isolates from hospitalized neonates in Nepal. Southeast Asian J Trop Med Pub Health 1999;30(3):546-8.
30) Agnihotri N, Kaistha N, Gupta V. Antimicrobial susceptibility of isolates from neonatal septicemia. JPN J Infect Dis 2004;57(6):273-5
31) Bansal S, Jain A, Agarwal J, Malik GK. Significance of coagulase negative staphylococci in neonates with late onset
septicemia. Indian J Pathol Microbiol 2004; 47(4): 586-8
32) Kumar V C S, Neelagaud Y F. Incubation period for culture positivity to detect septicemia in neonates. Indian J Med Microbiol 2005; 23(4): 270-5.
33) Tallur S S, Kasturi AV, Nadgir SD, Krishna B V. Clinico Bacteriological study of neonatal septicemia in Hubli. Indian J Paediatr. 2000; 67: 169-74.
34) Jain N K, Jain V M, Maheshwari S. Kathmandu University Medical Journal.2003; 1(2): 117-20.
35) Ramesh Bhat Y, Kumar N. Outcome of sepsis Evaluation in Very-Low birth-weight premature neonates. Journal of clinical and Diagnostic Research 2009; 3: 1847-52.
36) Khinchi Y.R, Kumar A, Yadav S, Profile of Neonatal Sepsis. Journal of college of Medical Sciences Nepal. 2010; 6(2) : 1-6.
37) Gerdes J S , Polin R. Early diagnosis and treatment of neonatal sepsis. Indian Journal Pediatr 1998; 65: 63-78.
38) Anand NK, Gupta AK, Man Mohan, Lamba IMS, Gupta R, Shrivastava L. Coagulase negative staphylococcal septicemia in newborns. Indian Pediatrics 1991 ; 28 : 1241 – 1248.
39) Gupta P, Murali MV, Faridi MMA, Caul PB, Ramchandran V G, V Talwar.Clinical profile of Klebsiella septicemia in neonates. Indian Journal of Pediatrics 1993 ; 60 : 565 – 572.
40) Barbara J Stoll. Infections of neonatal infant. In : Richard EB, Robert MK, Hal BJ. Editors. Nelson text book of pediatrics. 17th edition. Philadelphia : Saunders ; 2004p 630-639.
41) Karen MP. Bacterial and Fungal infections. In: John P Cloherty, Eric C Elchenwald, Ann RS. Manual of Neonatal Care. 5th edition. Philadelphia : Lippincott ; 2004 p. 287-312.
42) Schuchat A et al. Risk Factors and Opportunities for Prevention of Early-onset Neonatal Sepsis: A Multicenter Case-Control Study **.** Pediatrics 2000; 105 (1) :21-26 .
43) Behrman RE. Children at special risk. In : Richard EB, Robert MK, Hal BJ editors. Nelson text book of pediatrics. 17th edition. Philadelphia : Saunders; 2004 p.148 – 151.
44) Alistair G S, Philip MB, Jean R. Hewitt B S. Early diagnosis of neonatal sepsis. Pediatrics 1980; 65(5): 1036-41.

45) Alistair G S, Philip MB. Detection of Neonatal Sepsis of Late onset. JAMA 1982; 247(4): 489-92.
46) Rao P.S, Baliga M, Shivananda P G. Bacteriology of neonatal septicemia in a rural referral hospital in South India. J Trop Pediatr 1993; 39: 230-33.
47) Kumhar G D, Ramachandran V G, Gupta P, Bacteriological Analysis of Blood culture Isolates from neonates in a Tertiary care hospital in India. J Health Popul Nutr 2002; 20(4): 343-47.
48) Shrestha P. Clinical and Bacteriological profiles of blood culture positive sepsis in new borns. J Nepal Paediatr Soc 2007; 27(2): 64-7.
49) Mathur M, Shah H, Dixit K, Khambadkone S, Chakrapani A, Irani S. Bacteriological profile of neonatal septicemia cases 1994;40(1):18-20.
50) Chaturvedi P, Agrawal M, Narang P. Analysis of Blood culture isolates from Neonates of a rural hospital 1989; 26: 460-65.
51) Bang A.T, Bang R A, Morankar V P, Sontakke PG, Solanki J M. Pnemonia in Neonates: Can it be managed in the community?. Archives of Disease in childhood 1993; 68: 550-56.
52) Stoll B.J. et al . Late onset sepsis in VLBW neonates: The experience of the NICHD Neonatal Research Network. Pediatrics 2002; 110(2): 285-91.
53) Movahedian A H, Moniri R, Mosayebi Z. Bacterial culture neonatal sepsis. Iranian J Publ Health 2006 ; 35(4) : 84-9.
54) Abed El Hakeem Noman El Jadba, Mansour Sobhi El Yazji. Neonatal Septicemia in Gaza city hospitals. 2009; 25(2) : 226-231.
55) Jain N K, Seth D, Mangal V. A clinicomicrobial association in neonatal septicemia. Pediatric oncall. 2010 ; 7; Art # 58.
56) Kuruvilla KA, Pillai S, Jesudason M, Jana A K. Bacterial profile of sepsis in a Neonatal Unit in South India. Indian Pediatrics 1998; 35: 851-58.
57) Shaw C K, Shaw P, Thapalial A. Neonatal sepsis bacterial isolates and antibiotic susceptibility patterns at a NICU in a tertiary care hospital in Western Nepal: A retrospective analysis. Kathmandu University Medical Journal 2007; 5(2): 153-60.
58) Wasseem R, Khan M, Izhar T S, Qureshi A W. Neonatal Sepsis. Professional Med J Dec 2005; 12(4):451-56.

ANNEXURE 1

Birth weight groups

Low birth weight (LBW) babies:

Babies with a birth weight of less than2500grams (up to and including 2499 grams)irrespective of the period of gestation.

Very Low birth weight (VLBW) babies:

Babies with a birth weight of less than1500grams (up to and including 1499 grams)

Extremely Low Birth Weight Babies (ELBW)

Babies with a birth weight of less than1000grams (up to and including 999 grams)

Gestational age

Gestational age is calculated fron the first day of last normal menstrual period till the date of birth and is expressed in completed weeks . eg 34 weeks + 6 days are considered 34 weeks only.

Gestatinal age group

Preterm (immature , born early, premature)

Preterm is defined as a baby with a gestation of less than 37 completed weeks (upto 36 weeks or less than 259 days)

Term

Babies with a gestational age between 37 to 41 weeks are called as term babies(259-293 days)

Post term (postmature)

Babies with a gestational age of 42 weeks or more are classified as post term babies.

Perinatal period

Perinatal period extends from