A STUDY ON NEONATAL SEPTICEMIA AND EVALUATION OF A RAPID TEST FOR EARLY DIAGNOSIS OF NEONATAL SEPSIS

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled "**A STUDY ON NEONATAL SEPTICEMIA AND EVALUATION OF A RAPID TEST FOR EARLY DIAGNOSIS OF NEONATAL SEPSIS"** submitted by Dr. D. THERESE MARY to the Tamilnadu Dr.M.G.R.Medical University, Chennai in partial fulfillment of the requirement for the award of M.D. degree Branch – IV (Microbiology) is a bonafide research work carried out by her under direct supervision & guidance.

Director,

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DECLARATION

I, **Dr. D. THERESE MARY** declare that, I carried out this work on "**A STUDY ON NEONATAL SEPTICEMIA AND EVALUATION OF A RAPID TEST FOR EARLY DIAGNOSIS OF NEONATAL SEPSIS"** at the Institute of Microbiology, Madurai Medical College. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, Degree or Diploma to any other University, Board, either in India or abroad.

This is submitted to the TamilNadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D. Degree examination in Microbiology.

Place: Madurai

Date: Dr. D.THERESE MARY.

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CONTENTS

INTRODUCTION

Sepsis is the commonest cause of neonatal mortality: it is responsible for about 30 – 50 % of the total neonatal deaths in developing countries. It is estimated that up to 20% of neonates develop sepsis and approximately 1 % die of sepsis related causes. Sepsis related mortality is largely preventable with rational antimicrobial therapy and aggressive supportive care.

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first 30 days of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections. Superficial infections like conjunctivitis and oral thrush are not usually included under neonatal sepsis.

The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live birth. Among intramural births, *Klebsiella pneumoniae* was the most frequently isolated pathogen (32.5%) followed by *Staphylococcus aureus* (13.6%). Among extramural neonates (referred from community/other hospitals) *Klebsiella pneumoniae* was again the commonest organism (27%) followed by *Staphylococcus aureus* (15%) and *Pseudomonas* (13%).

Through the years, there has been a shift in the microorganisms responsible for neonatal Septicemia. During the 1930's Group A *Streptococcus* were the predominant organisms. In the 1950's *Staphylococci*

(largely of phage group I) became a major cause of nursery outbreaks throughout the world. *Pseudomonas* was predominant during the same decade, perhaps because of the introduction of respiratory support systems. From the late 1950's onwards *E. coli* has been an important cause of neonatal sepsis. Finally both Group D *Streptococci* and *Klebsiella* have been relatively recent pathogens, the latter accounting for a high proportion of antibiotic- resistant organisms that colonize and infect babies in neonatal intensive care units **(Goldman et al, 1978)**. The prevalence rates for a specific bacterial pathogen vary from nursery to nursery and may change abruptly in any one unit.

The Group B *Streptococcus* is the most common Gram positive organism causing septicemia during the first month of life. Vertical transmissions from mother to infant are the most common route of infection. The incidence of Group B *streptococcal* disease has varied widely from place to place and from year to year. Group A *Streptococcal* disease is not as common now as in previous decades. Group D *Streptococci* include the *Enterococci* and several other species particularly *S.bovis*, which have been found in neonatal infection.

In the 1950s phage group I *Staphylococcus* aureus was the most common bacterial agent causing septicemia in neonatal units. More recently, coagulase positive *Staphylococcus* disease in nurseries has been caused by organisms of the phage II group.

Coagulase Negative *Staphylococci* may be identified in blood cultures of babies and are frequently dismissed as contaminants. Repeated isolation of the organism from blood associated with clinical signs of septicemia should alert the physician to its pathogenic role Coagulase negative *Staphylococci* are frequently, but not always associated with arterial or venous catheters or ventriculoperitoneal shunts. Eradication of infection requires removal of the catheter or prosthesis as well as appropriate antibiotic therapy.

E. coli are the most common gram negative bacteria causing septicemia during the neonatal period. Approximately 40% of *E.coli* strains causing septicemia possess K 1 capsular antigen and strains identical with that in blood can usually be identified in the patient's nasopharynx or rectal cultures.

The neonate who receives broad spectrum antibiotics while in an environment potentially contaminated by "water-bugs" (respirators, moist oxygen) is likely to develop disease caused by *Pseudomonas* species.

Klebsiella is the most common bacterial pathogen causing neonatal sepsis. A high proportion of antibiotic – resistant organisms that colonize and infect babies in Neonatal Intensive Care Units are *Klebsiella* species. Among the *Enterobacter* species *Enterobacter aerogenes*, *E. cloacae*, *E. sakazakii* and *E.bormaechei* have caused sepsis and a severe form of necrotizing meningitis in neonates. Contaminated infant formula has been

identified as a source of infection. Parenteral nutrition and bladder catheterization are identified as risk factors. *Citrobacter* is an occasional inhabitant of GIT and are responsible for disease in neonates and immunocompromised patients. It causes sporadic and epidemic neonatal sepsis and meningitis.

Neisseria meningitidis, Haemophilus influenzae and *Streptococcus pneumoniae* are relatively uncommon in neonates. *S.pneumoniae* cause early onset sepsis that mimic neonatal disease caused by group B. *Streptococci*. Most people exposed to *Listeria monocytogenes* do not develop illness, but the pregnant women may suffer pregnancy loss and the neonate may develop sepsis and meningitis.

Inhalation of infected amniotic fluid may produce pneumonia and sepsis in utero, manifested by fetal distress or neonatal asphyxia. Exposure to pathogens at delivery and in the nursery or community is the mechanism of infection after birth. The physiologic manifestations of the inflammatory response are mediated by a variety of proinflammatory cytokines, principally Tumor necrosis factor (TNF), Inter leukin – 1 (IL-1) and IL-6 and byproducts of activation of the complement and coagulation systems. Elevated levels of IL-6, TNF and Platelet activating factor have been reported in new born infants with neonatal sepsis. IL-6 is the cytokine most often elevated in neonatal sepsis.

Neonatal sepsis can be classified into two major categories depending up on the onset of symptoms.

1. Early onset of sepsis (EOS) presents within the first 72 hours of life. In severe cases, the neonate may be symptomatic at birth. Infants with EOS usually present with respiratory distress and pneumonia. The source of infection is generally the maternal genital tract. Some maternal / perinatal conditions have been associated with an increased risk of EOS. Knowledge about these potential risk factors would help in early diagnosis of sepsis. Based on the studies from India, the following risk factors seem to be associated with an increased risk of early onset sepsis; Low birth weight and prematurity, Intrapartum sepsis, rupture of membranes > 24 hrs, prolonged labor, perinatal asphyxia and Foul smelling and/or Meconium stained liquor. Presence of foul smelling liquor or three of the mentioned risk factors warrant initiation of antibiotic treatment. Infants with two risk factors should be investigated and then treated accordingly.

2. Late Onset Sepsis (LOS) presents after 72 hours of age. The source of infection in LOS is either nosocomial (hospital acquired) or community acquired and neonates usually present with septicemia, pneumonia or meningitis. Various factors that predispose to an increased risk of nosocomial sepsis include low birth weight, prematurity, admission in intensive care unit, mechanical ventilation, invasive procedures, administration of parenteral fluids, and use of stock solutions. Factors that

might increase the risk of community –acquired LOS include poor hygiene, poor cord care, bottle feeding, and prelacteal feeds. In contrast, breastfeeding helps in prevention of infections.

The earliest signs of sepsis are often subtle and nonspecific; indeed a high index of suspicion is needed for early diagnosis. Neonates with sepsis may present with one or more of the following symptoms and signs such as Hypothermia or fever, Lethargy, poorcry, refusal to suck, Poor perfusion, Hypotonia, absent neonatal reflexes, Brady / tachycardia, Respiratory distress, apnea and gasping respiration, Hypo/hyperglycemia and Metabolic acidosis.

A predominance of male infants is apparent in almost all studies of sepsis in the new born but not among infants infected in utero. The usual male predominance in neonatal sepsis has suggested the possibility of a sex linked factor in host susceptibility. A gene located on the X chromosome and involved with function of the thymus or with synthesis of immunoglobulins has been postulated. The female has double the number of genes affecting these factors and thus might posses a greater resistance to infection.

Since treatment should be initiated in a neonate suspected to have sepsis without any delay, only minimal and rapid investigations should be undertaken.

Blood culture is the gold standard for diagnosis of septicemia and should be performed in all cases of suspected sepsis prior to starting antibiotics. A positive blood culture with sensitivity of the isolated organism is the best guide to antimicrobial therapy. Therefore it is very important to follow the proper procedure for collecting a blood culture. The resident doctor/ staff should wear sterile gloves prior to the procedure and prepare a patch of skin approx. 5-cm in diameter over the proposed veni-puncture site. This area should be cleansed thoroughly with alcohol, followed by povidone- iodine, and followed again by alcohol. Povidone-iodine should be applied in concentric circles moving outward from the centre. The skin should be allowed to dry for at least 1 minute before the sample is collected. One-mL sample of blood should be adequate for a blood culture bottle containing 5-10mL of culture media. Since samples collected from indwelling lines and catheters are likely to be contaminated, cultures should be collected only from a fresh veni-puncture site.

Many laboratories use their BACTEC system which measures the production of carbon-dioxide by metabolizing organisms. Semi-automated and fully automated blood culture systems are available. These advanced techniques can detect bacteria at a concentration of 1-2 CFU/ml within 12 – 24 hours.

Lysis direct plating consists of lysis of 0.5 to 1.5 mL of blood in a tube containing a cell membrane active lysis agent followed by direct

inoculation of 0.25 mL amounts to one or more agar plates. Positive cultures are recognized by growth on agar and provide a rapid means to obtain quantitative blood culture results from pediatric patients. Sucrose 10% to 30% is used as an osmotic stabilizer. Sucrose is especially helpful in dealing with bacteria that have undergone cell wall damage to some extent and the resultant hypertonicity counteracts the normal bactericidal effect of blood. Growth of cell wall deficient bacteria (e.g., antibiotic damaged organisms, Mycoplasma) may be enhanced by adding osmotic stabilizers like sucrose, mannitol or sorbose to create a hyperosmotic medium. Rapid diagnosis of Bacteremia in infants can be done by examining a Gram stained smear of the plasma buffy coat layer, obtained by centrifuging anticoagulated capillary blood.

All neonates suspected to have sepsis should have a septic screen to corroborate the diagnosis. The various components of the septic screen include total leukocyte count, absolute neutrophil count, immature to total neutrophil ratio, micro-erythrocyte sedimentation rate and C reactive protein. The absolute neutrophil count varies considerably in the immediate neonatal period. The lower limit for normal total neutrophil counts in the newborn begins at 1800/cmm, rises to 7200/cmm at 12 hours of age and then declines and persists at 1800/cmm after 72 hours of age. The ratio of immature to total neutrophils (I/T ratio) is lesser than or equal to 0.16 at birth and declines to a peak value of 0.12 after 72 hours of age. Presence of

two abnormal parameters in a screen is associated with a sensitivity of 93-100%, specificity of 83%, positive and negative predictive values of 27% and 100% respectively in detecting sepsis. Hence, if two (or more) parameters are abnormal, it should be considered as a positive screen and the neonate should be started on antibiotics.

C- reactive protein is a nonspecific acute phase protein that rises in response to infectious and noninfectious inflammatory processes. It is synthesized by hepatocytes. Good evidence exists to support the use of CRP measurement in conjunction with other established diagnostic tests (WBC count, Differential count and Blood culture) to establish or exclude the diagnosis of sepsis in full term or near term infants.

The three clinical laboratory methods namely 1. Qualitative test, 2. Semi-qualitative test and 3. Quantitative test are carried out to measure serum CRP levels.

The qualitative latex agglutination test can be performed rapidly at the bedside within 10 – 15 minutes. A positive qualitative CRP test should always be followed by a semi-quantitative test which is a more sensitive measuring method to quantify the concentration of CRP. The semi-quantitative latex agglutination assay involves the use of serial dilutions of serum with saline. Each dilution of serum is mixed with a latex reagent and observed for agglutination. The highest dilution in which agglutination is visualized corresponds to an approximate titre or concentration of CRP –

ligand complexes. The quantitative immuno assay is the most rapid sophisticated sensitive method of detecting and measuring CRP. The ELISA and Immunofluorescence test are used in quantitative immuno assay.

Increased CRP levels can occur in infants for upto three days of life from non-infectious causes. Maternal fever, PROM, Fetal distress, Pneumothorax and Meconium aspiration pneumonitis could cause elevated CRP levels.

The CRP levels do not always rise above 10 mg / lit in preterm infants or those infants with overwhelming sepsis, resulting in a false negative test result.

Procalcitonin is a 116 aminoacid peptide that has an approximate MW of 14.5 KDa and belongs to the calcitonin super family of peptides. Its level increases significantly in severe systemic infections, as compared to other parameters of microbial infection.

The short half- life (25-30 hours in plasma) of PCT, coupled with its virtual absence in health and specificity for bacterial infections, gives it a clear advantage over the other markers of bacterial infection. Studies have also shown that an increase in PCT levels is minimal in viral infections while levels increase rapidly after a single injection with endotoxin. Increased PCT levels comparable to what is observed in severe sepsis were also seen in Addisonian crisis, transplant patients receiving T-cells antibody therapy.

The incidence of meningitis in neonatal sepsis has varied from 0.3-3% in various studies. Lumbar puncture is indicated in the presence of a positive blood culture or if the clinical picture is consistent with septicemia.

Chest x-ray should be considered in the presence of respiratory distress or apnea. An abdominal x-ray is indicated in the presence of abdominal signs suggestive of necrotizing enterocolitis (NEC). Neurosonogram and computed tomography (CT scan) should be performed in all patients diagnosed to have meningitis.

In early onset sepsis, urine cultures have a low yield and are not indicated. Urine cultures obtained by suprapubic puncture or bladder catheterization have been recommended in all cases of LOS.

Adequate and proper supportive care is crucial in a sick neonate with sepsis. He/she should be nursed in a thermo-neutral environment taking care to avoid hypo/hyperthermia. Oxygen saturation should be maintained in the normal range; mechanical ventilation may have to be initiated if necessary. If the infant is hemodynamically unstable, intravenous fluids should be administered and the infant is to be monitored for hypo/hyperthermia. There cannot be a single recommendation for the antibiotic regimen of neonatal sepsis for all settings.

The choice of antibiotics depends on the prevailing flora in the given unit and their antimicrobial sensitivity. Decision to start antibiotics is based upon the clinical features and / or a positive septic screen. However duration

of antibiotic therapy is dependent upon the presence of a positive blood culture and meningitis.

Sadana et al., (1997), have evaluated the role of double volume exchange transfusion in septic neonates with sclerema and demonstrated a 50% reduction in sepsis related mortality in the treated group. Non-specific pooled Intravenous Immuno globulin has not been found to be useful. Treatment with Granulocyte - Macrophage colony stimulating factor (GM – CSF) is still on experimental stage.

Aggressive management of suspected maternal chorioamnionitis with antibiotic therapy during labor along with rapid delivery of the infant reduces the risk of early – onset neonatal sepsis. Vertical transmission of Group B *Streptococcal* sepsis is significantly reduced by selective intrapartum chemoprophylaxis.

Principles for the prevention of nosocomial infection include adherence to universal precautions with all patient contact, avoiding nursery crowding, strict compliance with hand washing, meticulous neonatal skin care, minimizing the risk of catheter contamination, decreasing the number of veni-punctures and mechanical ventilation days and providing education and feedback to nursery personnel.

This study is aimed at finding a technically easy, rapid and sensitive test that would help reduce turn around time and enable early initiation of appropriate management.

AIM AND OBJECTIVES

THE STUDY ON NEONATAL SEPTICEMIA AND EVALUATION OF A RAPID TEST FOR EARLY DIAGNOSIS OF NEONATAL SEPSIS was done at Government Rajaji Hospital (GRH) for a period of one year from June 2009 to May 2010.

The study was aimed

1. To know the prevalence of Neonatal Septicemic cases at Government Rajaji Hospital, Madurai according to the
   a) Age
   b) Sex
   c) Place of birth.
2. To know the risk factors involved in Neonatal Sepsis.
3. To know the common clinical features of Neonatal Sepsis.
4. To evaluate a rapid, easily available and non laborious test for the early diagnosis of Neonatal Septicemia

REVIEW OF LITERATURE

Stoll BJ (1997) has documented in his article 'The Global Impact of Neonatal Infection' sepsis is the commonest cause of neonatal mortality and it is responsible for 30-50% of the total neonatal deaths in developing countries. This was also reported by **Agarwal et al., (2001)**. **Haque K H (1988)** has defined 'Neonatal Septicemia' is a clinical syndrome of bacteremia characterized by systemic signs and symptoms in the first month of life. It encompasses systemic infections of newborn including meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections of the new born. As per the report of **the National Neonatal Perinatal Database (National neonatology forum 2002-2003)** incidence of neonatal sepsis is 30 per 1000 live births.

Group D *Streptococci* and *Klebsiella* have been relatively recent pathogens the latter accounting for a high proportion of antibiotic resistant organisms that colonize and infect babies in neonatal intensive care units. **(Goldman et al., 1978)**. **Freedman et al, (1981)** have documented the shift in the microorganisms responsible for neonatal septicemia. According to him during the 1930s Group A S*treptococci* were the predominant organisms. In the 1950s *staphylococci* became a major cause of outbreaks throughout the world. *Pseudomonas* was prominent during the same decade. From the late 1950s onwards *E.coli* has been an important cause of neonatal sepsis.

The changing pattern of organisms responsible for neonatal sepsis has been reported by **Karpuch et al., (1983),** in their study at the Yale-New Haven Hospital covering the period of 1928 to 1988. According to their report *Klebsiella-Enterobacter* species are the most common bacterial pathogens in developing countries. **Roy et al., (2002)** have documented *Klebsiella* species is the most common isolate from neonatal sepsis. Septicemia due to gram negative bacilli was 59.4% according to the study done by **Kurien Anil Kuruvilla et al** (**1998).** The study on neonatal sepsis by **Trotman H and Bell Y.(2006)** reported 63% of the isolates were gram negative. According to **Malik et al., (2001)** and **Ahmed et al., (2002)** in a Bangladesh study *E.coli* was shown as the leading organism (30%): however *Klebsiella* was the dominant organism (55%) in Indian study.

Dillon et al., (1966) reported that Group A *streptococcal* disease is not as common now as in previous decades. **Geil et al., (1970)** have documented Group A *streptococcal* Infection in the neonate is now reported infrequently but may occur in epidemic form in nurseries. Disease caused by these organisms varies from a low grade, chronic omphalitis to fulminant septicemia and meningitis. **Howard and McCraken, (1974)** documented the common clinical manifestations of Group B *Streptococcal* infections as septicemia, pneumonia and meningitis, but other more localized syndromes also occur including oesteomyelitis, septic arthritis, otitis media, cellulitis, and conjunctivitis. According to **Siegel and McCracken(1978)** incidence of

Group D streptococcal infection appears to have increased during the past few years in many centers, so that they are recognized as often as, or more than those caused by *E.Coli*. Group D *Streptococci* include *Enterococci* and Nonenterococcal strains. **Alexander and Giacoia (1978)** reported that the clinical pattern of the disease is remarkably similar to that seen with Group B *Streptococci* and is frequently associated with complicated deliveries.

Melish and Glasgow, (1971) documented Coagulase positive *staphylococcal* disease in nurseries has been caused by organisms of the phage II group. **Melish et al., (1972)** reported these organisms produce an exotoxin (exfoliatin) that causes intraepidermal cleavage through the granular cell layer due to disruption of desmosomes. Most episodes of sepsis due to *S.aureus* are hospital acquired and mortality remains high with low birth weight as documented by **Esperson et al., (1989)**.

According to **Baumgart et al., (1983)** most hospital acquired strains of *S.epidermidis* produce B lactamase and many are resistant to B lactamase resistant penicillin. Many cases of sepsis caused by *S.epidermidis* are associated with the use of vascular catheters according to **Hall et al., (1987)**. Coliform organisms are prevalent in the maternal birth canal, and most infants are colonized during or just before delivery. **McCracken and co-workers (1974)** found K1 strains in the blood or cerebrospinal fluid of most infants with meningitis related to E.coli. These strains were also

cultured from the blood of some infants and adults with sepsis but without meningitis.

Pseudomonas aeruginosa is usually a cause of late onset disease in infants who are presumably infected from their gut flora or from equipment, aqueous solutions, or on occasion, the hands of health care personnel. **Ghosal et al., (1978)** observed Noma (gangrenous lesions of nose lip and mouth) has been associated with bacteremia caused by *P.aeruginosa*. **Stevens and colleagues (1982)** reported nine cases of *Pseudomonas* sepsis, four of which presented in the first 72 hrs of life. In three of these infants, the initial signs were those of respiratory distress, chest radiographs were consistent with Hyaline Membrane Disease. **Harris et al., (1994)** have documented that the inflammatory responses are mediated by elevated levels of cytokines.

Franciosi et al., (1973) observed generalized disease takes two clinically and epidemiologically distinct forms, early and late onset. **Siegel et al., (1980)** reported the annual incidence of Group B *streptococcal* disease ranged from 0.6 to 3.7 per 1000 live births. **Cloberty J P and Stark R (1998)** classified neonatal sepsis into two major categories depending upon the onset of symptoms. Early onset sepsis presents within 72 hrs of age and late onset sepsis presents after 72 hrs of age. According to **Daoud et al., (1995) and Sanghvi et al., (1996)** early onset sepsis contributed to 38% to

85% of neonatal sepsis. According to the study done by **Choudury et al., (2007)** early onset infection was 70.7%.

Takkar et al., (1974) and Singh et al., (1994) identified the risk factors associated with early onset sepsis based on studies from India. 63% of neonatal septicemia was due to premature birth according to the study done by **Khatua et al (1986)**. The increased incidence of septicemia among premature neonates was attributed to their poor immune response like low level of IgG, impaired cellular immunity and poor mucosal defense according to **Schreiber et al (1992**). Low birth weight infants are at the highest risk for both early and late-onset sepsis according to **Stoll et al., (1996)**, **Belady et al., (1997) and Kaftan et al., (1998).** The factor associated most significantly with bacterial sepsis and meningitis is low birth weight according to **Fanaroff et al., (1998)**. Reports from India show 50 – 60% of septic babies were premature babies and very low birth weight babies. This was documented by **Bang et al., (2001).** According to **Roy et al., (2002)** the most frequent neonatal risk factor was low birth weight affecting 63.8% of the neonates. Study of maternal risk factors revealed 32.08% of mothers have preterm labor, 28.9% had PROM and 5.2% had intrapartum fever. **Shah et al (2006)** have reported premature rupture of membranes contributed to 46% of cases of neonatal sepsis in their study. Late-onset–sepsis is either nosocomial (hospital-acquired) or community

acquired and neonates usually present with septicemia, pneumonia or meningitis. (**Wolach 1977).**

According to **Islam MN (2001)** in the developing countries most (75-90%) of the deliveries occur at home with the help of traditional birth attendant. Sepsis is 12 times more common in extramural admissions (39.7%). In extramural admissions *Klebsiella* is the commonest bacteria responsible (27.5%), the next common being *S. aureus* (14.9%). Sepsis is responsible for death in 38% of these extramural babies. This has been documented by **Sankar et al., (2008) and National Neonatal Perinatal Database report (2002 – 2003).**

Powell K R (1990) described the earliest signs of sepsis as subtle and nonspecific; poor feeding, diminished responsiveness or just 'not looking well' are the earliest symptoms. More prominent findings are respiratory distress, apnea, lethargy, fever or hypothermia, jaundice, vomiting diarrhea and skin manifestations including petechiae, abscesses and sclerema. According to the data given by **Nyhan et al., (1958), Moorman et al.,(1961), Buetow et al (1965) and Gluck et al., (1966).,** hyperthermia was 51%, hypothermia was 15%, respiratory distress was 33%, Jaundice was 35%, Hepatomegaly was 33%, lethargy was 25%, abdominal distension was 17%, vomiting was 25% and Diarrhoea was 11%. The clinical symptoms and signs described by **Das et al., (1980) and Shashikala et al., (2000)** are refusal of feeds (61%), respiratory distress (40%), convulsions (29%) and

abdominal distension (23%). 50% of infected newborn infants have a temperature higher than 37.8˚ C **(Nelson 1996).** Feeding intolerance and abdominal distension was 46%; lethargy and hypotonia was 37% as per the report of **Fanaroff et al., (1998).**

Neonatal sepsis is predominant among male infants. Term male infants have an approximately twofold higher incidence of sepsis than term females as stated by **Washburn et al., (1969).** A gene located on the X chromosome is involved with a function of the thymus or with the synthesis of immunoglobulins. The female has double X chromosomes and possess a greater resistance to infection as explained by **Schlegel R J, and Bellanli A J (1969). Neeraj kumar et al (2010)** reported that 72% male neonates were affected by neonatal sepsis in their study.

The Data of **Dietzman and co-workers (1974)** suggested that 0.2ml of blood is sufficient to detect bacteremia caused by *E.coli* in neonates. **Kennaugh et al.,(1984)** documented that common pediatric pathogens can be reliably recovered from 0.5 ml of blood even when cultured at blood to broth ratio 1:100. **Gerder J S and Polin R, (1998)** stated since treatment should be initiated in a neonate, suspected to have sepsis without any delay, only minimal and rapid investigations should be undertaken. Blood culture in the gold standard for diagnosis of septicemia and should be performed in all cases of suspected sepsis prior to starting antibiotics.

Lysis direct plating provides a rapid means to obtain quantitative blood culture results from pediatric patients. Lysis direct plating consists of lysis of 0.5 to 1.5 ml of blood in a tube containing cell membrane - active lysis reagent followed by direct inoculation of 0.25ml amounts to one or more agar plate as reported by **Campos J M and Spainhour J R, (1985)**. Sucrose 10% to 30% is sometime used as an osmotic stabilizer. Sucrose is especially helpful in dealing with bacteria that have undergone cell wall damage to some extent and the resultant hypertonicity counteracts the normal effect of blood **(Connie R Mahon, 2007).** Growth of cell wall deficient bacteria (e.g. antibiotic damaged organisms or Mycoplasma) may be enhanced by adding osmotic stabilizer like sucrose, mannitol or sorbose to create a hyperosmotic medium **(Bailey and Scott 2007).** Hypertonic sucrose (10%) has been advocated to improve the recovery of certain bacteria from patients receiving penicillin or cephalosporin analogs **(Koneman 2006).** Improved hypertonic culture media employing mannitol, erythritol or sorbitol in place of sucrose to provide high osmolarity were described by **Guze LB, et al (1969)** for the detection and growth of microorganisms in body fluids particularly of bacteria in blood specimens. **McGee et al (1971**) have documented that the incorporation of 10% sucrose and mannitol into one of the media recommended for recovery of aerobic and facultative anaerobic bacteria was suitable for culture of cell wall deficient forms.

According to **Michael et al., (1983)** lysis centrifugation system could detect 59% of clinically significant isolates while broth culture could detect 43% of isolates. Isolated colonies of clinically important organisms were detected 30 hrs earlier with lysis centrifugation technique. These results suggest that the lysis centrifugation technique may provide a substantial improvement over conventional methods for culture. Lysis centrifugation culture detected 91% of S. aureus while conventional broth system detected only 87%. Lysis centrifugation system was positive earlier than the broth system by one or more days. The study conducted on neonatal septicemia by **Roy et al (2002)** showed 47.5% were culture positive in neonatal septicemia. **Sharma et al (1987) and Tallur et al., (2000)** have reported culture positivity in the range of 26-50% in their study. It is often possible to make a rapid diagnosis of bacteremia in infants by examination of a Gram stained smear of the plasma buffy coat layer obtained by centrifuging anticoagulated capillary or venous blood **(Monica Cheesbrough, 2000).** A routine direct smear examination of all bottles by gram staining or acridine orange staining provides the most useful microscopic examination as per the text book on 'Diagnostic Microbiology' by **Connie R. Mahan (2007).** According to the text book of "Diagnostic microbiology" by **Bailey and Scott (2007)** when macroscopic evidence of growth is apparent a gram stained smear of an air dried drop of medium with methanol fixation

preserves bacterial and cellular morphology which may be especially valuable for detecting Gram negative bacteria among red cell debris.

Philip and Hewitt (1980) documented that measuring the peripheral blood cell count and differential count is probably the most useful non-specific and rapid test available. If the total count is under 5000 or if the band to neutrophil ratio exceeds or is equal to 0.2 bacterial sepsis should be strongly considered. According to **Meharbansingh(1999)**, micro-ESR is a simple inexpensive though not very reliable marker of neonatal infection. A value of more than 15 mm is considered as suggestive of infection. A number of acute phase proteins serve as useful indicators of infection in the neonates. The best studied among them is the C-reactive protein (CRP). A CRP level measured at the onset of signs of infection has an overall sensitivity between 35% to 94% and specificity between 60% and 96% in diagnosis of sepsis as documented by**, Russell et al., (1993), Pourcyrous et al., (1993) and Benitz et al., (1998)**. A single CRP level drawn early in the course of disease has a low sensitivity between 35% and 96% in detecting the presence or absence of infection because the sampling time may precede a measurable rise in CRP level; this rise may lag 12 to 24 hrs after the onset of symptoms. Serial measurements of CRP level every 24-48 hrs after the onset of signs of infection has an increased sensitivity between 78.9% and 98% in detecting sepsis. **(Benitz et al., 1998).** In a study done by **Khassawneh et al., (2007),** it was concluded that CRP, IL – 6 and IgM are

useful in the diagnosis of gram negative neonatal sepsis and CRP continues to be the best single test. According to them a CRP value 5 mg / L was the best among the three parameters with 95% sensitivity.

According to **Harmoinen et al., (1981)** quantitative immuno assay is the most rapid, sophisticated and sensitive method of detecting and measuring CRP. The ELISA and Immunofluorescence tests are used in quantitative immuno assay. A semi quantitative bedside latex agglutination technique gives results within 15 mts. According to **Philip A and Andrews P, (1986)** the semi quantitative latex agglutination assay involves the use of serial dilution of serum with saline. As per **Murex (1995)** diagnostics, Murex CRP the qualitative latex agglutination test can be performed rapidly at the bedside within 10 to 15 minutes. CRP is a useful serum marker to assess and monitor the presence, severity and course of the inflammatory response in infectious and non-infectious disorders including acute myocardial infarction, angina, malignancies, burns and trauma as reported by **Jaye D and Waites K (1997)**.

Increased CRP levels can occur in infants for up to 3 days of life from noninfectious causes like maternal fever during delivery, fetal distress, perinatal asphyxia and Pneumothorax as stated by **Shine et al., (1985)**. According to **Wagle et al., (1994)** CRP level do not always rise above 10 mg / l in preterm infants or those infants with overwhelming sepsis, resulting in a false negative test result.

Procalcitonin is a biomarker that exhibits greater specificity than other pro inflammatory marker (e.g. Cytokines) in identifying patients with sepsis and can be used in the diagnosis of bacterial infection. **Chiesa et al (1998)** reported that the sensitivity of diagnosing sepsis in neonates by PCT during the first 48 hrs of life as 85.7%; the sensitivity of detecting late onset sepsis was 100%. Infants receiving antibiotic therapy within the previous 72 hrs of PCT tests could also show decreasing levels of PCT. From the study by **Ugarte and colleagues (1998)** at a cut off value of 0.6 ngm / ml, PCT had a sensitivity of 67.6%, a specificity of 61.3%. **Resch et al (2003)** have documented that the reliability of Procalcitonin determination in the diagnosis of early onset neonatal sepsis as compared to CRP and IL-6 revealed sensitivity of PCT as 77% compared to 54% and 69% for IL-6 and CRP. **Carrigan et al., (2004)** reported that the broth culture method is the gold standard for the diagnosis of bacterial infection but a definitive result can take 24 hrs or more before a conclusive diagnosis. Procalcitonin owing its specificity to bacterial infection has been proposed as a pertinent marker in the rapid diagnosis of bacterial infections. Multivariate logistic regression identified that a PCT=2.6 ng / ml was independently associated with the development of septic shock.

Sabat et al., (2001) have documented that Procalcitonin levels comparable to what is observed in severe sepsis were also seen in Addisonian crisis, Transplant patients receiving pan T cell antibody therapy.

Simon et al., (2004) have stated that Procalcitonin level increases significantly in severe systemic infections as compared to other parameters of microbial infection.

Supportive therapy includes adequate oxygenation of tissues, ventillatory support for respiratory failure caused by sepsis. Refractory hypoxia and shock may require extracorporeal membrane oxygenation. Shock and metabolic acidosis should be managed with fluids and ionotropic agents. **(Nelson)**

Gladstone et al., (1990) documented the introduction of aminoglycoside antibiotics - Kanamycin in the early 1960s and Gentamycin later in the decade provide vastly improved therapy for bacteremia due to Gram negative organisms, the leading cause of sepsis at that time. Benzyl Penicillin is most suitable for the treatment of infections due to Group B *Streptococci*. Septicemia due to *Pseudomonas aeruginosa* is best managed by ceftazidime. Betalactamase resistant penicillins and vancomycin are indicated in the treatment of infection caused by *Staphylococcus aureus*. Ampicillin is the drug of choice for Listeriosis. A combination of amikacin and cefotaxime is ideal for treatment of neonatal meningitis**. (Meharbansingh 1999).**

Human immunoglobulin preparation for intravenous administration (IGIV) has been assessed for adjunctive therapy for early onset sepsis. **Sadana et al., (1997)** have evaluated the role of double volume exchange

transfusion in septic neonates with sclerema and demonstrated a 50% reduction in sepsis related mortality in the treated group.

Any improvements in the health of the pregnant woman and increased use of prenatal care facilities will lead to a lowering of the rate of prematurity and more appropriate management of prolonged interval after rupture of membranes, maternal post partum infections and fetal distress will reduce the incidence of neonatal sepsis. **Mathus et al., (1990)** documented breast fed infants have a lower incidence of gastroenteritis, respiratory illness, and otiitis media.

MATERIALS AND METHODS

This study was conducted at Government Rajaji Hospital, attached to the Madurai Medical College, Madurai. Blood samples were collected from neonates admitted at NICU with clinical evidence of Septicemia. The study period was one year from June 2009 to May 2010. A total of 250 suspected cases of Neonatal Septicemia were included in the study. Ethical committee clearance was obtained prior to the starting of the study and informed consent was obtained from the parent / guardian of each patient.

Selection of cases:

Single blood sample was taken from 250 clinically suspected cases of neonatal septicemia for culture. A detailed prenatal and postnatal history with special emphasis on any predisposing factors for infections of each baby was elicited. Histories related to maternal risk factors like preterm delivery, Intrapartum sepsis, and prolonged interval after rupture of membranes were elicited. Risk factors related to infants like low birth weight, prematurity and birth asphyxia were also included in the study. Intramural births and Extramural births were included in the study. Babies suffering from septicemia selected for this study had one or the other following symptoms such as a) Hypothermia or fever, b) Lethargy, poor cry, refusal to suck, c) Respiratory distress, apnea, gasping respiration d) Diarrhoea, vomiting, abdominal distension, e) Convulsions f) Jaundice,

Hepatomegaly g) Sclerema, Boils h) Umbilical Sepsis, i) Hypotonia or absent neonatal reflexes, j) Bradycardia / Tachycardia, k) Hypo / hyperglycemia, l) Metabolic acidosis and m) Poor perfusion, prolonged capillary refill time.

Sample collection:

Blood samples were collected by adopting strict aseptic precautions. The skin over the proposed veni-puncture site approximately 5cms diameter was cleansed thoroughly with alcohol, followed by povidone iodine, and followed again by alcohol. The skin was allowed to dry for atleast 1 minute before the sample was collected. 3-5 ml of blood was collected. 1 ml of blood was added to 10 ml of Brain Heart Infusion broth for conventional culture. 1 – 1.5 ml of blood was added to 10 ml Brain Heart Infusion broth containing lytic agents sucrose 10 % / mannitol 10% or both for lysis centrifugation technique. 2.5 ml of blood was centrifuged and serum was separated for doing CRP test and Procalcitonin measurement.

METHODS

1. CONVENTIONAL CULTURE METHOD:

The Blood culture was done with all samples and the samples with positive cultures were taken as the septicemic sample. The blood sample collected in the Brain Heart Infusion broth was labeled with name, date and time of collection and the culture bottle was brought to the laboratory. The

culture bottle was incubated at 37º C for 18 – 24 hrs, after which sub culturing on to the following plates were done using sterile technique.

a) Nutrient Agar Plate.
b) Blood Agar Plate.
c) MacConkey Agar Plate.

Plates were incubated at 37º C for 18 – 24 hrs aerobically and the growths in the culture plates were observed. The colony size, shape, edge, margin and consistency were noted. Broth was examined for any turbidity, pellicle formation and deposit. The organisms were identified by Colonial Morphology, Gram stain, Hanging Drop and Biochemical tests.

2. LYSIS CENTRIFUGATION TECHNIQUE:

1 to 1.5 ml of blood sample collected in 10 ml Brain Heart Infusion broth with lytic agent sucrose 10% / Mannitol 10% or mixture of equal volumes of 10% sucrose and mannitol was centrifuged at 3000 RPM for 30 mts. The supernatant was discarded. With a sterile loop the deposit was taken and a smear was prepared on a clean glass slide. The smear was methanol fixed and Gram staining was done. The smear was examined microscopically, using an oil immersion lens and Gram positive and Gram negative organisms were differentiated.

Colony Identification:

The colonies in conventional blood culture were identified as below:-

Small (1-3 mm in diameter), circular, low convex opaque colonies with golden yellow pigmentation on NAP and β haemolysis on BAP and pink colored colonies on MacConkey Agar were identified as *Staphylococcus* aureus. They were confirmed by slide and tube Coagulase test, Mannitol fermentation and DNase test.

Small (1-3mm in diameter), circular, convex, opaque, white colored colonies were identified as Coagulase Negative *Staphylococci* and Coagulase test (slide and tube) was negative.

Tiny, smooth, opaque white colored colonies on NAP, showing no haemolysis on Blood Agar Plate and magenta colored colonies on MacConkey Agar plate were identified as *Enterococcus* species. Confirmatory tests done were Catalase test, Heat test, Mannitol fermentation, Bile aesculin hydrolysis and inoculation on 6.5% NaCl Agar.

Large, convex, translucent, mucoid, white colored colonies on NAP with Lactose Fermenting (Pink) mucoid colonies on MacConkey Agar were identified as *Klebsiella* species. Biochemical tests Indole, MR, VP, Citrate, Urease, and TSI confirmed the identification of the organism as *Klebsiella* species.

Large, Flat, nonmucoid, translucent colonies on NAP with LF nonmucoid colonies on MacConkey Agar were identified as *Escherichia coli*. IMVic test, TSI and Urease test were done to confirm the organism as *Escherichia coli*.

Large, low convex, translucent colonies with swarming on NAP and Nonlactose fermenting colonies (colorless) on MacConkey Agar were identified as *Proteus* species. TSI showing A / K reaction with profuse H_2S production confirmed the identification. Other Biochemical tests (Indole, Urease, Citrate, PDA-phenylalanine deaminase) were also done to confirm the organism as *Proteus* species.

Large, convex, translucent colonies on NAP and LLF colonies (Light Park) on MacConkey Agar were identified as *Citrobacter* species. Further it was confirmed with Biochemical tests (Indole, TSI, Citrate, and Urease)

Large, flat and spreading, translucent with serrated edge and green pigmentation with a distinct odor on NAP and NLF colonies on MacConkey Agar were identified as *Pseudomonas* species. Positive Catalase and Oxidase test and TSI with Alkaline Butt and Alkaline slant (K/K) confirmed the identification. This is summarized in Table – 1.

Table -1

Growth on		Gram stain	Identification
NAP	**Mac**		
+	LF	Gram positive cocci in groups	*Staphylococcus* species
+	Magenta	Gram positive diplococci and short chains	*Enterococcus* species
+	LF Mucoid	Gram Negative Bacilli	*Klebsiella* species
+	LF Nonmucoid	Gram Negative Bacilli	*Escherichia coli*
+ Swarming +	NLF	Gram Negative pleomorphic bacilli	*Proteus* species
+ Green pigmentation	NLF	Gram Negative Bacilli, Discrete	*Pseudomonas aeruginosa*
+	LLF	Gram Negative Bacilli, Discrete	*Citrobacter* species.

The Gram Negative Bacilli were identified by the biochemical tests as follows.

Table -2 : Tests of Identification of GNB

Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	TSI	OF Test	Organism
+	-	+	+	-	-	-	A / A	Fermentative	*E.coli*
+	-	+	-	+	+	+	A / A Gas +	Fermentative	*K.oxytoca*
+	-	-	-	+	+	+	A / A Gas +	Fermentative	*K.Pneumoniae*
+	-	+			+	+	A / K H_2S +	Fermentative	*Proteus vulgaris*
+	-	-			+	+	A / K H_2S +	Fermentative	*Proteus mirabilis*
+	-	+			+	+	A / K	Fermentative	*Citrobacter koseri*
+	-	-			+	+	A / A H_2S	Fermentative	*Citrobacter freundii*
+	+	-	-	-	-	-	K / K	Oxidative	*Pseudomonas* species.

3. CRP TEST:

C-reactive protein is a nonspecific, acute phase protein that rises in response to infection and noninfectious inflammatory processes. Serum was used for estimating C - reactive protein (CRP). Estimation of CRP was done both qualitatively and semi quantitatively by the kit marketed by EURO Diagnostics Pvt. Ltd., Chennai which consisted of the following items.

1. CRP reagent. A uniform suspension of polystyrene latex particles coated with anti-CRP antibodies (monoclonal IgG).
2. Positive control, reactive with CRP reagent.
3. Negative control, non-reactive with CRP reagent.

The kit was stored at 2º to 8ºC in refrigerator.

In Qualitative method:

A clean slide was taken and divided into three portions. One drop of serum was placed on the first portion using a disposable pipette provided with the kit. One drop of positive control serum was placed on the second portion and one drop of negative control serum was placed on the third portion. One drop of CRP latex reagent was added to the test sample, positive control and negative control. Using a stick provided in the kit, test serum, positive control, and negative control and CRP reagent was mixed uniformly. Separate stick was used for the three specimens. The slide was

rocked gently back and forth, observing for agglutination macroscopically after 2 minutes.

In Semi quantitative method:

1. Using isotonic saline, doubling dilutions of the serum sample was prepared; 1:2, 1:4, 1:8, 1:16, 1:32, 1:64.
2. The same procedure as qualitative method was done for each dilution.

The results were interpreted as follows:

In Qualitative Method:

The presence of distinct coarse agglutination within 2 mts was considered as a positive test result and indicated the presence of C - reactive protein in the test specimen. Absence of agglutination was considered as negative result and indicated the absence of C-reactive protein in the test specimen.

In Semi quantitative method:

Agglutination in the highest serum dilution corresponded to the approximate amount of C-reactive protein in mg / dl present in the specimen. Concentration of C-reactive protein was calculated as follows.

CRP mg / dl = 0.6 x D where 'D' indicated the highest dilution of serum showing agglutination. CRP concentration above 0.6 mg / dl was taken as positive and CRP concentration below 0.6 mg / dl was taken as negative.

4. PROCALCITONIN MEASUREMENT:

Procalcitonin is a 116 amino acid peptide with a sequence identical to that of the pro-hormone of calcitonin. It is a biomarker that exhibits greater specificity than other inflammatory markers in identifying patients with sepsis and can be used in the diagnosis of bacterial infection.

The measurement was done by the BRAHMS PCT-Q test which is a Immunochromatographic test used for the semi quantitative detection of PCT. Serum was used for the determination of PCT concentration. Just before the measurement the package was opened and six drops of serum was pipetted into the round cavity of the BRAHMS PCT-Q. It was incubated for 30 minutes at room temperature. The time was documented when the test was begun on the reference card.

Documentation:

To document the test result, the concentration range which corresponded to the color intensity of the test band, was marked with the cross on the reference card.

Recording and assessment

After 30 minutes (max. 45 minutes), the PCT concentration range of the sample was determined. Initially, the validity of the test was checked with the help of the clearly visible control band.

A. No band or only test band visible**:** Tests, which showed no control band, were not valid and not evaluated.

B. Only control band visible**:** Tests, which showed only a control band, were negatively valid. The PCT concentrations are < 0.5 ng/ml.

C. Control and test band visible**:** Tests, which showed both a control band and a test band, were positively valid

D. The PCT concentration range was determined by comparing the color intensity of the test band with the color blocks of the reference card**.**

IDENTIFICATION TESTS:

I. CATALASE TEST: This test was done to detect the organisms which produced the enzyme catalase. It was done as follows.

Procedure:

1. 2-3 ml of 3 % H_2O_2 was taken in a test tube.
2. Using a sterile wooden stick or a glass rod several colonies of the test organism was removed and immersed in the hydrogen peroxide solution.

Interpretation:

The organisms which possessed the enzyme catalase split hydrogen peroxide into water and oxygen and produced brisk effervescence within 10 seconds due to the release of oxygen bubbles.

II. OXIDASE TEST:

Oxidase test is done to detect the presence of cytochrome oxidase produced by some organisms. This test helps to rule out Enterobacteriacaeae.

Procedure:

Oxidase disc was moistened with a drop of saline. Using a disposable plastic loop a portion of the colony was picked and rubbed onto an Oxidase disc.

Interpretation:

The organisms which produced cytochrome oxidase turned the reagent tetramethyl P- phenylene diamine hydrochloride into a purple compound within 10 secs. The appearance of purple color within 10 seconds was taken as positive. Pseudomonas was oxidase positive.

III. COAGULASE TEST:

This test is used to identify S.aureus which produces the enzyme Coagulase. Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of S.aureus.

a) Free coagulase which converts fibrinogen to fibrin by activating a coagulase – reacting factor present in plasma. Free coagulase is detected by clotting in the tube test.
b) Bound coagulase (clumping factor) which converts fibrinogen to fibrin without requiring coagulase reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test.

Procedure:

Slide test method

1. A drop of saline was placed on each end of a clean glass slide.
2. A colony of the test organism was emulsified in each of the drops to make two thick suspensions.
3. A loopful of plasma was added to one of the suspensions and mixed gently. Plasma was not added to the second suspension which was used as control.

Interpretation:

Clumping of organisms within 10 seconds was interpreted as positive slide Coagulase test.

Tube Test Method:

Three small test tubes were taken. One tube was used for testing the isolate from primary culture plate. The other two tubes were for positive and negative control. 0.8 ml of 18 – 24 hrs broth culture of *S.aureus* was added to one tube and kept as positive control. To another tube 0.8 ml of 18 – 24 hr broth culture of CONS was added as negative control. 0.2 ml of plasma was added to all the three tubes and incubated at 35 – 37˚ C. After four hours the tubes were examined for clotting. The tubes were left at room temperature overnight and examined again if no clotting occurred.

Interpretation:

Clotting of plasma in the tube with organisms from primary culture plate was interpreted as positive Coagulase test.

IV. DNase Test:

This test was done to demonstrate the ability of S.aureus to hydrolyse DNA.

Procedure:

1. The DNase agar plate was divided into 6 to 12 sections by drawing lines on its bottom and numbered the section to denote the strain to be applied to them.
2. A colony was picked from primary culture plate and spot – inoculated onto a small area of medium.
3. Known DNase positive and DNase negative cultures were spot – inoculated on other sections as control.
4. The plate was incubated aerobically at 37˚C for 18 – 24 hrs.
5. Then the plate was flooded with few mL of 1 mol / L (3.6%) hydrochloric acid to precipitate unhydrolysed DNA.
6. The plate was examined after few minutes.

Interpretation:

The organisms which were DNase positive produced clear uncloudy zones around the spot cultures.

V. INDOLE TEST:

Testing for indole production is important in the identification of Enterobacteriaceae. Most strains of *E.coli, P.vulgaris, M.morgagni* and *Providencia* species break down the amino acid tryptophan with the release of Indole.

Procedure:

1. The test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water and incubated at 35-37ºC for upto 48 hrs.
2. Indole production was tested by adding 0.5ml of KOVAC'S reagent.

Interpretation:

The organisms which possessed the enzyme tryptophanase hydrolysed trytoptophan into indole, pyruvic acid and ammonia; indole reacted with KOVAC's reagent and produced a bright fuchsia red color at the interface of the reagent and the broth within seconds. *E.coli, K.oxytoca, P.vulgaris*, *Citrobacter koseri* were indole positive.

VI. METHYL RED TEST: (MR test)

MR test is a quantitative test for acid production, requiring positive organisms to produce strong acids (lactic, acetic, formic acid) from glucose through the mixed acid fermentation pathway.

Procedure:

1. The test organism was inoculated in 5ml of Glucose phosphate peptone water and incubated at 37ºC for minimum 48 hrs.

2. 5 drops of Methyl red reagent was added to the broth.

Interpretation:

The organisms which produced large quantities of strong acids from carbohydrate substrate developed a stable red color immediately in the surface of the medium indicating pH < 4.4 and was taken as positive MR test. *E. coli and S.aureus* was MR positive

VII. VOGUES – PROSKAUER TEST:

Procedure:

1. The organism isolated from primary culture plate was inoculated in Glucose phosphate peptone water and incubated for 48hrs.

2. 1 ml of the broth was taken in a separate test tube, added 0.6ml of 5 % **α** napthal and 0.2ml of 40 % KOH and shaken well.

Interpretation:

A positive test was represented by the development of a red color within 15 min. to 30 min. after addition of the reagent indicating the presence of diacetyl, the oxidation product of acetoin.

Klebsiella species were VP positive.

VIII. CITRATE UTILIZATION TEST:

This test is used in the identification of Enterobacteriacaeae.

Procedure:

1. A well isolated colony was picked from the surface of a primary isolation medium and inoculated as a single streak on the slant surface of the citrate agar tube.
2. The tube was incubated at 35ºC for 24 – 48hrs.

Interpretation:

A positive test was represented by the development of a deep blue color within 24 – 48 hrs, indicating that the test organism was able to utilize the citrate in the medium as the sole source of carbon with the production of alkaline products. Visible colony growth along the inoculation streak line without change in color of Citrate agar was also taken as positive test. Citrate utilization test was positive in *Klebsiella* species and *Citrobacter* species.

IX. UREASE TEST:

Procedure:

The organism isolated from primary culture plate was heavily inoculated in Christensen's Urea Agar and incubated at 37ºC overnight.

Interpretation

The organisms which possessed the enzyme Urease hydrolyzed urea into ammonia and carbon dioxide and increased the pH of the medium producing pink color.

Proteus species were strongly urease positive.

Klebsiella, Citrobacter species were urease positive.

X. TRIPLE SUGAR IRON AGAR MEDIUM :

Triple sugar iron agar medium is useful in the presumptive identification of gram negative Enterobacteriaceae. It is useful in detecting the ability of the micro organisms to ferment carbohydrates, glucose, lactose or sucrose in TSI; to produce gas from the fermentation of sugars and to detect the production of H_2S.

Procedure:

A well isolated colony from primary culture media was picked with an inoculating needle; stabbed the butt and streaked the slope in a zig – zag pattern.

The cap was replaced loosely to allow oxygen to enter the tube and the medium was incubated at 35˚C for 18 – 24 hrs.

Interpretation:

1. The organisms which were nonfermentors produced alkaline butt / alkaline slant (Deep red color).

2. The organisms which fermented only glucose produced acid butt / alkaline slant (Yellow / Red).
3. The organisms which fermented lactose (and / or sucrose) produced acid butt / acid slant (Yellow / Yellow)
4. The organisms which produced H_2S developed acid butt / alkaline slant or acid butt / acid slant, H2S in butt.
5. The organisms which produced gas resulted in the formation of bubbles or splitting of the medium in the butt or complete displacement of the medium from bottom of the tube.

XI. HEAT TEST

Heat test was performed to demonstrate the ability of Enterococcus species to grow at 60ºC.

Procedure:

1. Few colonies were picked from primary culture plate and inoculated in peptone water and incubated at 37 ºC for 20 mts.

2. A nutrient agar plate and a MacConkey agar plate was divided into two Quadrants and labeled as BH (Before Heat) and AH (After Heat).

3. A loopful of inoculum was taken and streaked on the BH Quadrant of both NAP and MacConkey plate and incubated at 37 ºC overnight.

4. Then the peptone water with inoculum was placed in water bath at 60ºC for 30 mts.

5. A loopful of inoculum was taken and streaked on the AH Quadrant of both NAP and MacConkey plate and incubated at 37 ºC overnight

Interpretation:

Growth in both Quadrant (Before Heat and After Heat) confirmed the Organism as Enterococcus species.

XIII. OXIDATION – FERMENTATION TEST:

Two tubes of O-F medium were inoculated with the organism isolated from primary culture plate by stabbing 3 to 4 times halfway to the bottom of the tube. One tube was promptly covered with a layer of sterile melted petroleum jelly to a depth of 5 – 10 mm leaving the other tube open to the air. Both tubes were incubated at 35˚ C and examined daily for several days.

Results of O-F test were interpreted as below.

Table - 3

Open tube	Covered tube	Metabolism
Acid (Yellow)	Alkaline (Green)	Oxidative
Acid (Yellow)	Acid (Yellow)	Fermentative
Alkaline(Green)	Alkaline (Green)	Asaccharolytic

The septicemic cases were identified by the Gold standard conventional blood culture method.

The following rapid methods for identifying these cases were compared and results evaluated.

1. CRP test
2. Procalcitonin measurement
3. Lysis Centrifugation technique using, a) Mannitol, b) Sucrose and c) Both Mannitol and Sucrose, followed by Gram staining of the deposit.

Simple descriptive statistics Chi-square analysis, was used to findout the superiority of one test over the other to identify the organisms by Lysis Centrifugation followed by gram stain, CRP test and PCT estimation.

RESULTS

Blood samples from 250 cases of suspected neonatal septicemia admitted in Neonatal Intensive Care Unit at Government Rajaji Hospital, Madurai were analyzed in this study. The study involved the neonates from 0 to 30 days of age. Blood samples collected from all the cases were subjected to Conventional Culture, Lysis Centrifugation followed by Gram stain, CRP test, and Procalcitonin estimation. Among the 250 cases, results of rapid tests like Lysis Centrifugation Technique, CRP and Procalcitonin which detects septicemia rapidly were analyzed.

On analyzing the 250 cases of suspected neonatal septicemia the sex distribution was found to be 144 (57.6%) male neonates and 106 (42.4%) female neonates. Distribution of cases in relation to sex is given in Table – 1.

Table -1: Sex wise distribution of septicemia (n =250)

Gender	Number of cases	Percentage
Male	144	57.6
Female	106	42.4
Total	250	100

From Table – 1 it could be inferred that percentage of ***neonatal septicemia was high among male neonates than female neonates.***

The cases were further analyzed age wise and it was found that Early Onset Sepsis was more (160) than Late Onset Sepsis (90). Early Onset

Sepsis involved neonates of 0 - 72 hrs of age and Late Onset Sepsis involved neonates of more than 72 hrs to 30 days of age. This is depicted in Table 2.

Table – 2: Age wise distribution of Neonatal Septicemia (n =250)

Onset	Number	Percentage
Early onset (0 – 3 days)	160	64
Late onset (>3 – 30 days)	90	36
Total	250	100

It could be inferred from Table 2 that almost all age groups upto 30 days were invariably affected and admitted due to septicemia. ***The incidence of Early Onset Sepsis was higher (64 %) than Late Onset Sepsis (36 %).***

Analysis of the 250 neonates with suspected septicemia proved that low birth weight (< 2500 grams) neonates were more commonly affected than normal birth weight (>2500 grams) neonates. Distribution of cases in relation to birth weight is depicted in Table 3.

Table – 3: Birth weight Vs Neonatal Septicemia (n =250)

Birth weight	Number	Percentage
< 2500 grams	172	68.8
≥ 2500 grams	78	31.2
Total	250	100

From Table 3 it could be inferred that out of 250 cases studied for septicemia, low birth weight accounted for 172 (68.8%) cases of septicemia. Birth weight above 2500 Gms accounted for 31.2% of neonatal septicemia.

From the above table it was found that ***low birth weight babies were more prone for neonatal sepsis***.

Among the 250 cases admitted with clinical signs of septicemia 93 were intramural births and 157 were extramural births. Distribution of cases of septicemia among intramural and extramural birth is shown in Table - 4.

Table – 4: Distribution of cases in relation to place of birth (n =250)

Place of birth	Number	Percentage
Extramural	157	62.8
Intramural	93	37.2
Total	250	100

It could be inferred from Table – 4 among the 250 neonates admitted at GRH Madurai with clinical signs of septicemia ***incidence were more among Extramural births 157 (62.8%).***

The risk factors associated with an increased risk of early onset sepsis were analyzed among the 250 cases and this is tabulated in Table – 5.

Table – 5: Risk factors associated with early onset sepsis (n =250)

Risk factors	Number of cases	%
*PROM > 24 hrs.	75	30.0
IP Sepsis	32	12.8
Meconium stained liquor	26	10.4
Perinatal Asphyxia	31	12.4
Prematurity and Low birth weight (< 2500)	172	68.8

**Pre mature rupture of membranes (PROM)*

On analysis of the above table it was found that among the risk factors associated with early onset septicemia the commonest risk factor was ***prematurity with low birth weight and contributed to more number of cases of septicemia*** [172 (68.8 %)] out of 250 cases screened for septicemia. Other risk factors were also associated with neonatal septicemia. Some of the neonates had multiple risk factors.

Clinical symptoms and signs associated with neonatal sepsis were analyzed and presented in Table – 6.

Table – 6: Clinical features of neonates with septicemia (n =250)

Clinical features	Number	Percentage
Gastro intestinal symptoms		
• Refusal of feeds	155	62.0
• Diarrhoea	28	11.2
• Jaundice	31	12.4
• Abdominal distension	58	23.2
Respiratory tract symptoms		
• Respiratory distress	82	32.8
Central nervous system		
• Lethargy	86	34.4
• Poor cry	92	36.8
• Convulsion	52	20.8
Other Manifestations		
• Fever	73	29.2
• Hypothermia	42	16.8

From Table 6 it could be inferred that ***refusal of feeds was one of the important symptoms of neonatal septicemia.*** 62% of neonates admitted as

suspected cases of septicemia had refusal of feeds. Many neonates had more than one symptom.

Further analysis of the study showed that bacteria were isolated from 142 (56.8%) Blood samples out of the 250 blood samples processed. The samples were further analyzed organism wise and it was found that out of 142 samples processed, Gram Negative Bacilli were isolated from 86 (60.56%) blood samples. Among the Gram Negative Bacilli 48 (33.8%) were *Klebsiella* spp, 22 (15.49%) were *E.coli*, 13 (9.16%) were *Pseudomonas* spp and 3 (2.11%) were *Citrobacter* spp. Gram Positive Cocci isolated were 56 (39.44%). Out of the 56 Gram Positive isolates 29 (20.42%) were *S.aureus*, 21 (14.79%) were CONS and 6 (4.23%) were *Enterococcus* spp. This is given in Table – 7

Table –7: Organisms wise distribution (n =142)

Organism		Number	Percentage
Gram Negative	*Klebsiella spp*	48	33.80
	Escherichia Coli	22	15.49
	Pseudomonas spp	13	9.16
	Citrobacter spp	3	2.11
Gram Positive	*Staphylococcus aureus*	29	20.42
	CoNS	21	14.79
	Enterococcus spp	6	4.23
Total		142	100.00

From the above table it could be inferred that ***Gram negative organisms were common in neonatal septicemia***. Among ***Gram Negatives***

Klebsiella spp and among the Gram Positives S.aureus were the common organisms isolated.

The samples were further analyzed according to the rapidity of detection of septicemia by the three techniques Lysis centrifugation followed by Gram staining, CRP and Procalcitonin estimation.

Lysis of samples was done by 10% Sucrose, 10 % Mannitol and equal volumes of 10% Sucrose and 10% Mannitol. Out of 250 samples 126 (50.4%) were smear positive. The Gram reaction of the lysed samples by the three lytic agents is given below in Table – 8.

Table – 8: Gram reaction of Lytic agent (n =250)

Lytic Agent	Gram Positive		Gram Negative		Total %
	Number	Percentage	Number	Percentage	
10% Sucrose	43	17.2	83	33.2	50.4
10% Mannitol	41	16.4	82	32.8	49.2
10% Sucrose &10% Mannitol	43	17.2	83	33.2	50.4

From the above table it could be inferred that 50.4% samples were identified by Lysis centrifugation technique and the identification of organisms by lysis of samples followed by Gram reaction showed 17.2% Gram positive and 33.2% Gram Negative organisms. The Lysis centrifugation technique ***identifies the organisms equally irrespective of the***

Lytic agent used and also helps to differentiate the organisms as Gram positive and Gram negative.

Among the 250 samples analyzed, 90 (36%) showed rise in serum CRP. The CRP levels varied from 0.6 mg /dl to a maximum of 20 mg / dl. This is given in Table 9.

Table – 9: C- reactive protein level among CRP positive cases (n =90)

CRP level mgm / dl	Number	Percentage
0.6 – 4.9	27	30.00
5.0 – 9.9	24	26.67
10.0 -14.5	22	24.44
15 – 20	17	18.89
Total	90	100.00

From the above table it could be inferred that ***36% of neonatal septicemia were detected by CRP test***. As it is a rapid test and results could be interpreted within 15 minutes it could be ***used as a parameter in the early diagnosis of neonatal septicemia.***

Further analysis of the study showed that serum Procalcitonin was raised in 108 (43.2%) samples out of the 250 samples. The value of serum Procalcitonin among the 108 samples is depicted in Table 10.

Table- 10: Serum Procalcitonin level in Septicemia (n =108)

Procalcitonin ng / ml	Number	Percentage
≥ 0.5 to < 2.0	42	38.89
≥2 and < 10	35	32.41
≥ 10	31	28.70
Total	108	100.00

On analysis of the above table, it could be inferred that ***Procalcitonin estimation detected 43.2% of neonatal septicemia***. Among the 108 Procalcitonin positive cases, 33.89% showed a serum PCT level of ≥ 0.5 to < 2.0 ng /ml. 32.410% cases showed a serum PCT level of ≥2 and < 10 ng / ml; 28.70% of cases showed a serum PCT level of ≥ 10 ng / ml.

On comparison of serum CRP and Procalcitonin estimation which are indicators of septicemia it was observed that estimation of ***Procalcitonin detected more cases of septicemia than CRP estimation*** (43.2% and 36%) respectively. Table 11 depicts the comparison of CRP and Procalcitonin estimation in neonatal septicemia.

Table- 11: Procalcitonin Vs CRP estimation in neonatal septicemia (n =250)

Acute Phase Reactants			Positive	
			Number	Percentage
Procalcitonin			108	43.2
C-Reactive Protein			90	36
Chi Square value	df	p value	**Significant**	
2.561	2	0.031		

From Table 11 it could be inferred that Procalcitonin detected neonatal septicemia in 43.2% of cases and CRP detected septicemia in 36% of cases.

On comparing serum CRP and Procalcitonin with lysis centrifugation followed by Gram reaction which is also a rapid procedure, it was found that 50.4% samples were detected by Lysis centrifugation followed by Gram reaction whereas CRP detected 36% samples and serum Procalcitonin was detected in 43.2% proving that Lysis centrifugation detected more cases of neonatal septicemia. It was also found that by Lysis centrifugation technique the organism also can be differentiated into Gram Positive or Gram Negative. Table 12 depicts the comparison of Lysis centrifugation technique, serum Procalcitonin and serum CRP levels.

Table – 12.Lysis Centrifugation Technique Vs Acute Phase Reactants (n =250)

Investigation			**Positive**	
			Number	**Percentage**
Lysis centrifugation technique			126	50.4
Serum Procalcitonin			108	43.2
Serum CRP			90	36
Chi Square value	df	p value	**Significant**	
3.107	3	0.045		

Despite the fact that Serum Procalcitonin estimation and CRP test could be used as diagnostic markers in the early diagnosis of neonatal sepsis, the identification of organism is not possible by these methods. The

information thus obtained does not translate to patient care, since the specific etiology is unknown.

On the contrary ***Lysis centrifugation followed by Gram staining detects organism within four hours and the type of organism is detected, hence initiation of immediate treatment is made possible***.

Chi-square analysis of three techniques showed that Lysis centrifugation technique followed by gramstain was superior to CRP test and PCT estimation.

DISCUSSION

The study on neonatal septicemia and the evaluation of rapid diagnostic methods was carried out on 250 neonates admitted at Neonatal Intensive Care unit at Govt. Rajaji Hospital, Madurai. Various factors like sex distribution, age of onset, risk factors, etiology and rapid diagnostic methods were analyzed among the 250 cases of suspected neonatal septicemia.

In this study male neonates were more commonly affected (57.6%) than female neonates (42.4%). This is in accordance with the studies done by **Neeraj Kumar et al (2010)** at Department of Pediatrics, Himalayan Institute Hospital Trust, Rishikesh, India. In their study also there was a male preponderance (72%). This is similar to the previous studies done by various authors who hypothesized that incidence of septicemia was higher in males due to the presence of factors regulating the synthesis of gamma globulin on X chromosomes. **Schlegel and Bellanli (1969)** have explained in text book on "Infections Diseases of the fetus and newborn infant" (5^{th} edition) that a gene located on the X chromosomes is involved with the function of the thymus or with the synthesis of immunoglobulin. The female has double X chromosomes and possess a greater resistance to infection. Text book on Pediatrics by **Nelson (1996)** says that term male infants have an approximately two fold higher incidence of sepsis than term females.

In this study the incidence of early onset sepsis was 64% and late onset sepsis was 36%. It showed that incidence of early onset septicemia is more common than late onset septicemia. **Daud et al, (1995), Sanghvi et al (1996)** have reported in their study that early onset septicemia contributed to 38% to 85% of neonatal sepsis. The study on neonatal sepsis by **Choudary et al., (2007)** showed that 70.7% of these cases were early onset infections. The above studies support the present study. The source of infection in early onset sepsis is the maternal genital tract. Some maternal complications like febrile illness, prolonged rupture of membrane, prolonged labour etc leads to early onset sepsis.

In this study incidence of septicemia was analyzed based on the risk factors. Low birth weight (<2500gms) accounted for 68.8% of septicemia. All the low birth weight babies were preterm (<37 weeks of gestation). Hence preterm birth was the important factor which led to the birth of low birth weight babies in this study. **Roy et al (2002)** have reported in their prospective study on neonatal septicemia 63.8% of neonatal septicemia was due to low birth weight. Reports from India, based on the study by **Bang et al** (**2001**) showed 50-60% of septic babies were premature babies and low birth weight babies. **Khatua et al (1986)** have reported in their study septicemia in preterm neonates was 63%. The present study was in accordance with these studies. The increased incidence of septicemia among preterm neonates may be attributed to low level of IgG, impaired cellular

immunity, compromised granulocyte functions, poor mucosal defense and inability of B lymphocytes to produce antibodies as reported by **Schrieber and Berger (1992).**

Distribution of cases of neonatal septicemia in relation to place of birth was analyzed in this study and it was found 62.8% of septicemia cases were among extramural births. In the developing countries most (75-90%) of the deliveries occur at home with the help of traditional birth attendants. According to the report of the National Neonatal Perinatal Database and **Sankar et al (2008)** sepsis is 12 times more common in extramural admission. It is 3% among intramural births and 39.7% among extramural births. The present study was conducted at the tertiary care hospital and most of the cases admitted were referred from feeding rural areas where home deliveries were more prevalent. This could be the probable reason for the higher incidence (62.8%) of neonatal sepsis among extramural births in the present study.

Refusal of feeds was one of the important symptoms (62%) observed in this study. This study was in accordance with the study by **Neeraj kumar Jain** et al (2010) who have reported refusal of feeds in 61% of neonates with septicemia. **Das et al, (2010) and Shasikala et al (2000)** have also documented the same in their study. **Fanaroff et al (1998)** have observed feeding intolerance in 46% of the neonates. This may be attributed to the depressed neonatal reflexes like sucking reflex and rooting reflex.

The analysis of the etiology in this study showed Gram negative organisms were common in neonatal septicemia (60.56%). The study on neonatal sepsis by **Trotman and Bell (2006)** at the University Hospital of the West Indies reported 63% of the isolates were gram negative organism. **Kurien Anil Kuruvilla et al (1998)** have reported 59.4% of isolates from sepsis were gram negative bacilli. Hence the present study is in accordance with these two studies. Gram negative bacteria contain a substance in their cell walls called endotoxin, (Lipopolysaccharide) which has a strong effect on several physiologic functions. Endotoxins mediate numerous systemic reactions, including a febrile response, activation of complement system and certain blood clotting factor.

Among the Gram Negative organism, *Klebsiella* spp (33.8%) and among the Gram Positive organisms, *S.aureus* (20.42%) was the common organism isolated in this study. **Ahmed et al(2002), Malik et al(2001)** have reported *Klebsiella* was the dominant organism (55%) in Indian study. Based on the **National Neonatal Perinatal Network database (1995) Meharbansingh** has reported 29.7% of isolates were *Klebsiella* and 14.7% were *S.aureus*. These studies support the present study. The polysaccharide capsule and multiple adhesins present in *Klebsiella* protect it from phagocytosis and help the microorganism to adhere to host cells. Lipopolysaccharide activate complement causing selective deposition of C3b on LPS molecules which inhibit the formation of membrane

attack complex thereby preventing membrane damage and bacterial cell death.. *S.aureus* activates the alternate complement pathway by teichoic acid present in the cell wall.

Lysis centrifugation technique with lytic agents 10% sucrose, 10% mannitol and equal volumes of both was done in this study to identify the organisms rapidly. Gram stain was done after lysis centrifugation of the blood sample and detected 50.4% of the organisms. This procedure was done as per the references given in "Diagnostic microbiology" by **Bailey & Scott (2007), Koneman (2006) and Connie R. Mahan (2007).** Improved hypertonic culture media employing mannitol, erythritol or sorbitol in place of sucrose are described for the detection and growth of microorganism in blood specimen by **Guze, William and Wilkin (1969). Mc Gee et al (1971)** have reported that the incorporation of 10% sucrose or mannitol into one of the media was recommended for recovery of cell wall deficient forms. The recovery of antibiotic stressed organism might be related to the action of sucrose on leucocytes and erythrocytes. 59% of the clinically significant organisms were detected by lysis centrifugation culture and Gram stain by **Michael et al(1983)** in their study. But the lytic agent used in his study was saponin which is costly and not available everywhere.

In the present study 10% sucrose and mannitol were used as lytic agents and it was found that identification of organism was little lower than (50.4%) saponin. As the lytic agents lyse the cells and release the pathogens,

quantitatively organism yield is good. The procedure also release antibiotic stressed organism which is very useful in this study, since most of the neonates admitted at NICU are given prophylactic antibiotic. The organisms were identified equally irrespective of the lytic agent used.

Serum C-reactive protein estimation showed raised levels in 36% of neonatal sepsis in the present study. Serum CRP level of $\geq$ 0.6 mg / dl was found to be a good parameter in detection of sepsis in India according to **Stoll (1997).** A CRP level measured at the onset of infection has an overall sensitivity between 35% and 94% in diagnosing sepsis. Serial measurements of CRP levels every 24 to 48 hrs after the onset of signs of infection have an increased sensitivity between 78.9% and 98% as per the study report of **Benitz et al(198), Pourcyrous et al (1993)and Russell et al(1995).** The low detection rate found in this study might be due to single sample analysis at the onset of infection. As in most centers sophisticated tests are not available, it is advisable to restrict to hematological tests along with CRP level which is a simple method to diagnose neonatal sepsis.

A number of the inflammatory markers such as Leukocyte cell count, C- reactive protein, cytokines (TNFα, IL-6) have been applied in the diagnosis of inflammation and infection but their lack of specificity has generated a continued interest to develop more specific clinical laboratory tests. One promising marker has been Procalcitonin (PCT), whose concentration has been found to be elevated in sepsis. PCT has been

proposed as a pertinent marker in the rapid diagnosis of bacterial infection, especially for use in hospital emergency departments and intensive care units.

In this study a single blood sample was collected at the onset of the disease from all the 250 cases of suspected septicemia. Serum Procalcitonin was raised above 0.5 ngm / ml in 43% cases of septicemia. PCT had a sensitivity of 67.6% at a cut off value of 0.6 ngm / ml in the study of **Ugarte and Colleagues (1998). Chiesa et al (1998)** reported that the sensitivity of diagnosing sepsis in neonates during the first 48 hrs of life as 85.7% and 100% after 48 hrs of life. The present study is in accordance with the study by **Ugarte and Colleagues (1998)** and lesser than the study by **Chiesa et al(1998)**. Infants receiving antibiotic therapy within the previous 72 hrs were excluded from the **Chiesa et al(1998)** study as partially treated sepsis could be associated with decreasing levels of PCT. However in the present study neonates who had received antibiotics were also included. Hence sensitivity could be less compared to **Chiesa et al (1998)** study.

The results of acute phase reactants in the diagnosis of neonatal sepsis were compared and it was found serum PCT estimation (43.2%) was a better marker than CRP estimation (36%). **Resch et al (2003)** have reported in their study that Procalcitonin and CRP determination in the diagnosis of early onset sepsis revealed a sensitivity of 77% and 69% respectively. Thus PCT was more sensitive than CRP in the diagnosis of neonatal sepsis

according to the above study. However, considering the prohibitory cost of PCT as compared to that of a CRP test and the fact that lysis centrifugation followed by gram stain which proved more sensitive in the present study, the role of PCT in the diagnosis of neonatal septicemia is to be further evaluated. Hence CRP can be used as a diagnostic marker along with hematological tests.

The broth culture method is the gold standard for the diagnosis of bacterial infection but a definitive result can take 48 hrs or more before a conclusive diagnosis. **Singh et al (1994), Sharma et al (1987), Tallur et al (2000)** have reported positivity in the range of 26-50%. **Roy et al (2002)** reported in their study 47.5% were culture positive. These studies support the present study which detected 56.8% of sepsis by conventional blood culture.

The rapid methods employed in this study were lysis centrifugation followed by Gram stain, CRP and Procalcitonin estimation which could detect septicemia within 4 hours. CRP and Procalcitonin estimation are done in serum. The mere presence of these two markers cannot be taken as evidence of sepsis and only when they reach a cut off value sepsis is detected. The kits used must be highly sensitive. Another drawback of these two markers is they cannot identify the organisms. In this study Lysis centrifugation followed by Gram stain was done with 10% sucrose and mannitol as lytic agents. Sucrose and mannitol are cheap, easily available

and the method is simple and nonlaborious. The advantage of this method is its ability to differentiate Gram positive and gram negative organisms so that a preliminary report can be given within 4 hours to the clinician to initiate early appropriate management.

In a tertiary care hospital like Govt. Rajaji Hospital where the turnover of cases is very high and resources are limited, Lysis centrifugation followed by gram stain would be the method of choice in suspected septicemia cases, since it is apparently a simple and cost effective method.

SUMMARY

The study on Neonatal septicemia and evaluation of rapid diagnostic methods in a tertiary care hospital revealed a male preponderance (57.6%), prematurity and low birth weight (68.8%) as the important risk factor, higher incidence (62.8%) of sepsis among extramural births and refusal of feeds in 62% of neonates. Gram negative septicemia accounted for 56.8% and among the gram negative organisms *Klebsiella* spp were the common isolates (33.80%). Among gram positive isolates *S.aureus* was the common isolate (20.42%).

Conventional blood culture was done to identify the causative organism and 56.8% were culture positive. Rapid diagnostic methods used in this study were lysis centrifugation followed by gram stain, CRP test and Procalcitonin estimation. CRP test was positive in 36% of neonates with sepsis. As CRP test is a non specific test, it could be used as a diagnostic marker along with other hematological tests in the diagnosis of septicemia. Procalcitonin estimation was found to be a better marker (43.2%) than CRP in the early diagnosis of septicemia. However it could not be used at a tertiary care hospital where the turnover of cases is too high and resources are limited. In lysis centrifugation technique the lytic agents used were 10% sucrose and mannitol which are cheap, easily available in all the laboratories. The procedure is a simple one in which brain heart infusion broth with the lytic agent and the sample is centrifuged and the deposit is

used for doing a gram stain. By this method organism identification as gram positive and gram negative could be done and a preliminary report could be given to the clinician so as to initiate early appropriate management. Hence this method could be adopted as a rapid technique to diagnose neonatal sepsis in all the laboratories where the turnover of cases is high and resources are less.

CONCLUSION

The study on Neonatal septicemia and evaluation of a rapid test for early diagnosis of neonatal sepsis in a tertiary care hospital revealed the following findings.

- Among the 250 cases of suspected septicemia 56.8% blood samples were identified as septicemic by conventional culture.
- The incidence of early onset sepsis was higher (54%) than late onset sepsis (36%).
- Among the 250 cases of suspected septicemia 57.6% were male neonates showing a male predominance.
- Prematurity and low birth weights were the risk factors contributing to 68.8% of neonatal sepsis.
- Incidence of sepsis in extramural birth was 62.8% which is higher than in intramural births (37.2%).
- Refusal of feeds (62%) was seen as the commonest clinical features in these cases.
- Gram negative septicemia (60.56%) was the commonest cause of neonatal sepsis rather than Gram positive septicemia(39.44%). Among the Gram negative isolates *Klebsiella* spp were the predominant organism (33.80%) and among the Gram positive *S.aureus* was the predominant organism (20.42%).

- Among the rapid tests evaluated for the diagnosis of neonatal septicemia, Lysis centrifugation followed by Gram stain was found to be the best method rather than the estimation of CRP and PCT, the acute phase reactants in the diagnosis of neonatal septicemia.

Figure 1: Sex Wise Distribution of Septicemia

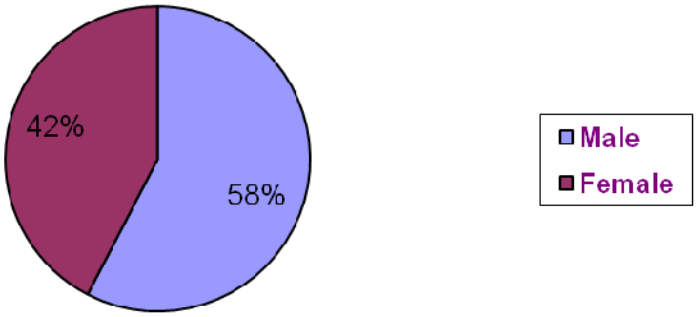


Figure 2: Birth Weight Vs. Neonatal Septicemia

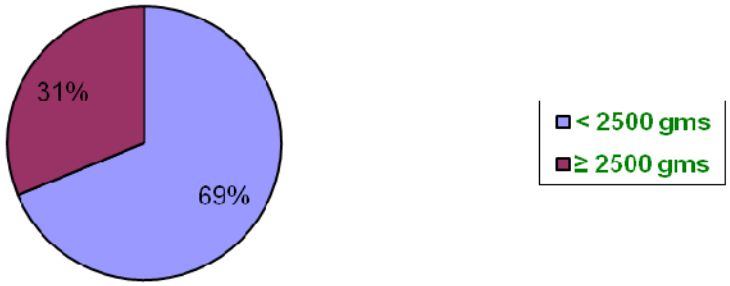


Figure 3: Distribution of cases in relation to place of birth

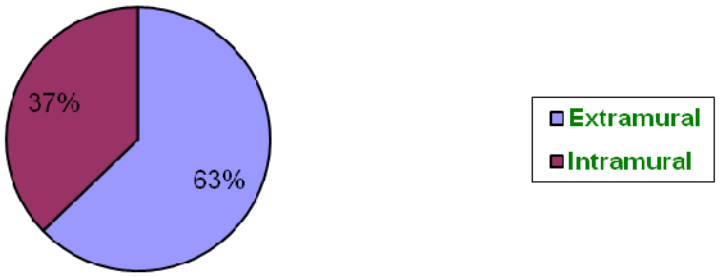
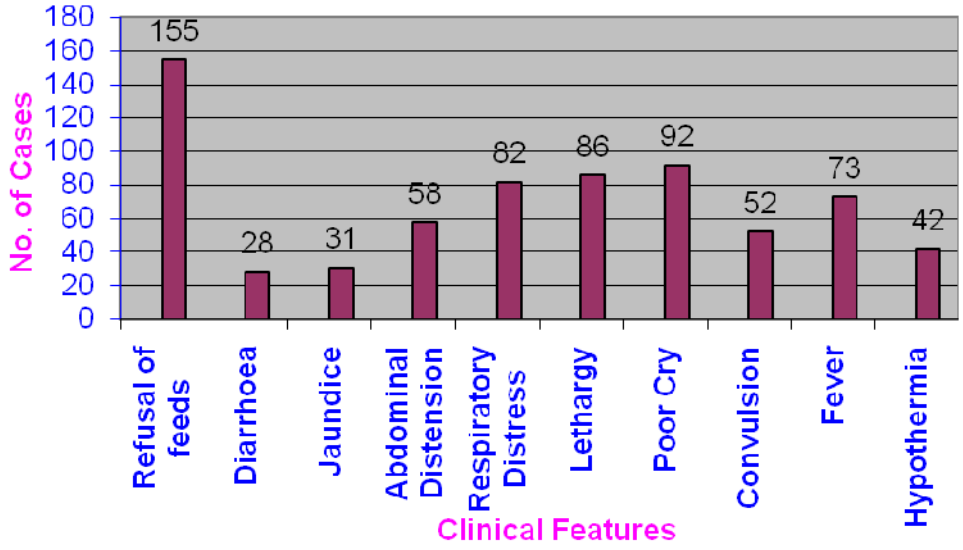


Figure 4: Clinical Features of Neonates with Septicemia



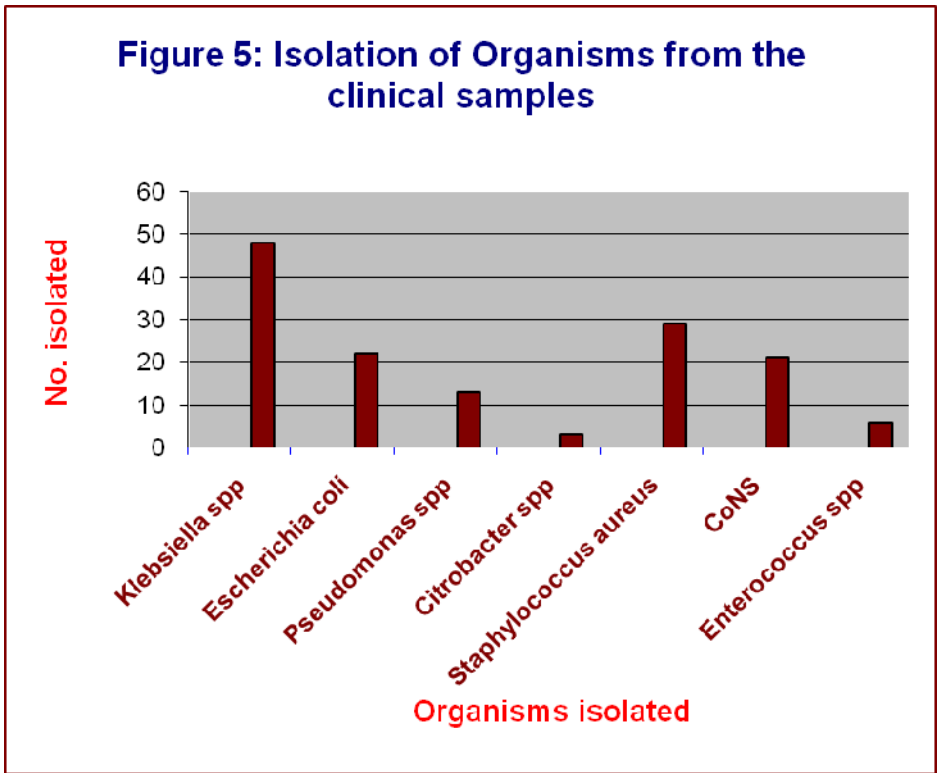
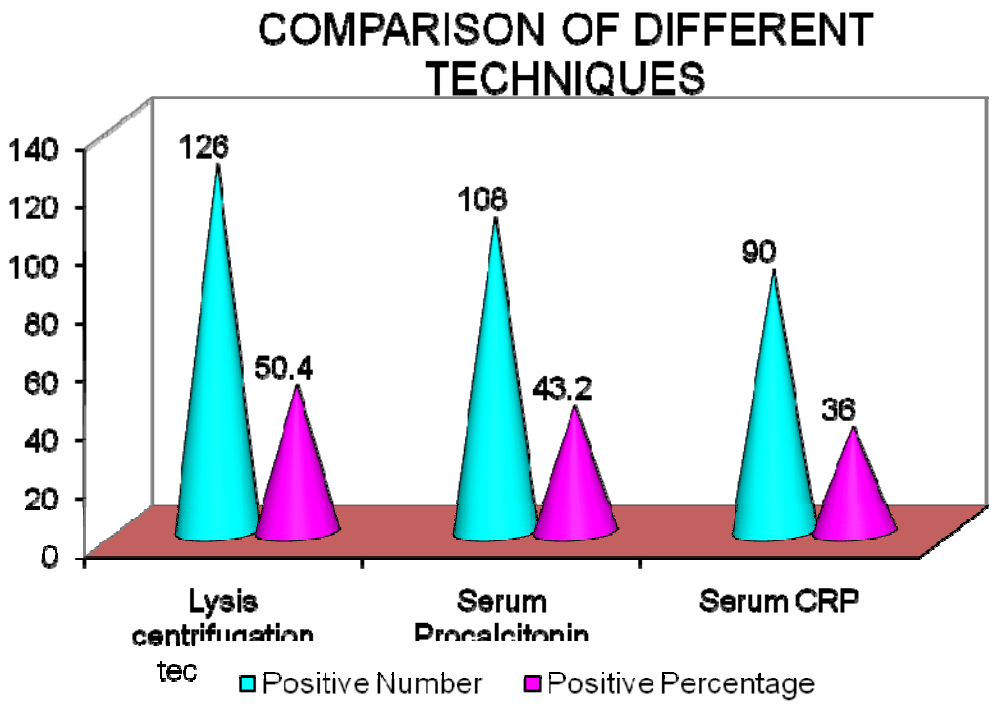


Figure 5: Isolation of Organisms from the clinical samples
No. isolated
60
50
40
30
20
10
0
Klebsiella spp
Escherichia coli
Pseudomonas spp
Citrobacter spp
Staphylococcus aureus
CoNS
Enterococcus spp
Organisms isolated

FIGURE - 6


COMPARISON OF DIFFERENT TECHNIQUES
140
120
100
80
60
40
20
0
126
50.4
108
43.2
90
36
Lysis centrifugation tec
Serum Procalcitonin
Serum CRP
Positive Number
Positive Percentage

ANNEXURE – I

Gram staining

Requirements:

Slides

Spirit lamp or Burner

Nichrome loop

Glass marker

Normal Saline

Distilled Water

Cedar-wood oil

Light microscope

Reagents:

- Crystal violet (2% or methyl violet (0.5%) or Gentian violet (1%)
- Grams Iodine

    Iodine crystal 1 gm

    Potassium iodide 2 gm

    Distilled water 100 ml

Dissolve 2 gm potassium iodide in 25 ml water and then add 1 gm iodine. When iodine is dissolved, make up to 100 ml with water.

- Acetone or Alcohol
- Dilute carbol fuchsin (1:10) or safranin (0.1%)

Procedure

A) Smear Preparation:

1. Grease – free clean slide was taken and made an oval shaped mark at the centre by using a glass marker.
2. The BHI broth with the lytic agent and blood sample was centrifuged and the supernatant was discarded.
3. With a sterile nichrome loop, a loopful of the sediment was taken and made a smear in the premarked area on the slide.
4. The smear was allowed to air dry.
5. The dry smear was fixed with Methanol.

B) Staining:

1. The slide was placed on the staining glass rods.
2. The smear was flooded with methyl violet (0.5%) and left for 1 minute.
3. The smear was washed with clean tap water.
4. The smear was then flooded with Gram's iodine and was allowed to stand for 1 minute.
5. It was again rinsed with clean tap water.
6. Few drops of acetone was added to decolorize the smear and washed immediately with clean tap water.
7. The smear was counterstained with dilute carbol – fuchsin and left for 1 minute.
8. Again it was washed with clean tap water and air dried.
9. The stained smear was examined microscopically using a bright – field condenser and an oil- immersion lens.

Hanging Drop:

Requirements:

- Glass slide
- Cavity slide
- Cover – slip
- Nichrome loop
- Burner
- Light Microscope
- Bacterial culture (suspension)
- Petroleum jelly

Procedure:

1. A cover slip was taken and petroleum jelly was applied on the four corners of the cover-slip.
2. With a sterile nichrome loop a drop of bacterial suspension was placed on the centre of the cover slip.
3. A clean cavity slide was inverted over the cover –slip, allowing cover-slip to adhere to the side.
4. The slide was turned around so that cover-slip is on the top and drop of bacterial suspension hangs in the centre of the cavity slide from the under surface of cover-slip.
5. The drop was examined under the microscope with condenser lying low. The iris diaphragm of the condenser was sufficiently closed to give good contrast. The preparation was brought into focus by focusing the edge of the drop under low power (10X) objective. Then focused under high power (40X) objective to observe motility of the organism.

ANNEXURE II

PREPARATION OF BLOOD AGAR PLATE: TSA + 5%Sheep Blood

To prepare 500ml of molten agar in 1 litre flask,

1. Add 20g of agar into 500ml of water. Heat to dissolve.
2. Autoclave at 121˚C for 20 mins, Cool to 50˚C.
3. Add 5% sterile, defibrinated sheep blood (25 ml sheep blood is added to 500 ml of molten agar).
4. Dispense 15 – 20 ml into Petri dishes. To remove the bubbles media can be flamed.

Allow to solidify, dry out. Place the plates in a plastic bag and store at 4˚C.

PREPARATION OF DNase AGAR:

This medium is prepared from ready to use dehydrated powder.

Ingredients:

Tryptose	20 gm/l
Deoxyribonucleic acid	2 gm/l
Sodium chloride	5 gm/l
Agar	12 gm/l
Distilled water	1 L

1. Mix 3.9 gms of dehydrated powder in 100 ml distilled water.
2. Autoclave at 121˚C for 15 min.
3. When the medium has cooled to 50 – 55 ˚C mix well and dispense in sterile Petri dishes.
4. Store the plates at 228 ˚C in sealed plastic bags.

PREPARATION OF BILE AESCULIN AGAR:

Ingredients:

Meat extract	3 gm
Peptone	5 gm
Oxbile	10 gm
Aesculin	1 gm
Ferric ammonium citrate	0.5 gm
Sodium Chloride	5 gm
Agar	15 gm
Distilled water	1 L

1. With heating dissolve the meat extract, peptone, Sodium Chloride and Agar in 400 ml distilled water, the Oxbile in 400 ml water, the Ferric Ammonium Citrate in 100 ml distilled water.
2. Mix the solutions and the adjust to pH 7.0

3. Autoclave at 121˚C for 15 minutes and cool to 50˚C
4. Dissolve the aesculin in 100 ml water, sterilize by filtration, and add to the melted medium at 50˚C
5. Dispense in screw – capped tubes as slants; store at 4˚C.

PREPARATION OF PHENYLALANINE DEAMINASE AGAR:

Ingredients:

Yeast extracts	3 gm
DL – Phenylalanine	2 gm
Disodium Hydrogen Phosphate	1 gm
Sodium Chloride	5 gm
Agar	12 gm
Distilled water	1 L

1. Mix ingredients in distilled water and adjust the pH to 7.4
2. Autoclave t 121˚C for 15 mts.
3. Dispense in tubes and allow to solidify as long slopes.

ANNEXURE – III

NEONATAL SEPSIS – CASE INVESTIGATION PROFORMA

Name of Patient :

Age / Sex : /

OP No / IP No : /

Occupation / Income : /

Birth weight : LBW / Normal

Apgar score :

Maturity : Preterm / Term / Post Term

Place of delivery : Tertiary care Hospital / Others

Blood Grouping / Rh typing :

Duration of labour :

PROM : Less than 24 hrs / More than 24 hrs

Associated illness specify. : Congenital anomalies if any

Immunization of Mother with TT : Yes / No

Antenatal period in Mother : Normal / Abnormal

H / O maternal fever during last Trimester / Labour: Yes / No

Previous Obstetrical History :

H / O Abortions :

Nature of Specimen :

Investigation required :

Date of Collection of specimen :

Results declared : Blood culture / CRP / PCT

Organisms Isolated :

LABORATORY FORM

Date:

Patients Name: Patient's I.D. No:

Age / sex :

Laboratory Reference No:

Specimen details:

Type of Specimen	Date of Collection	Date of Receipt in lab	Type of test	Remarks (if any)	Result

Interpretation:

Details of Investigator:

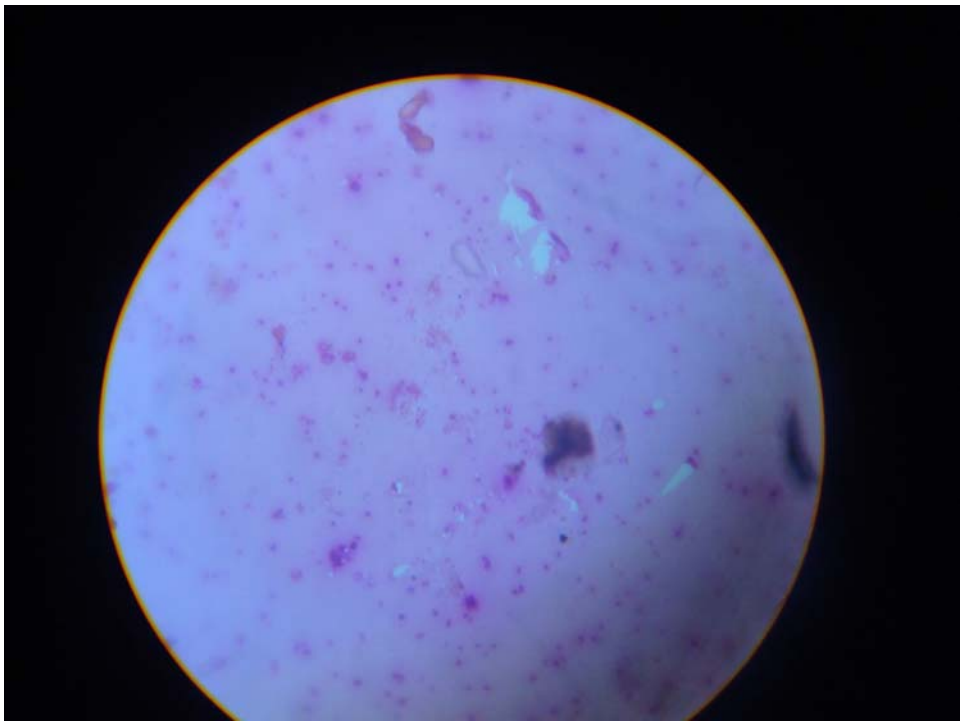
Name:

Address:

Signature

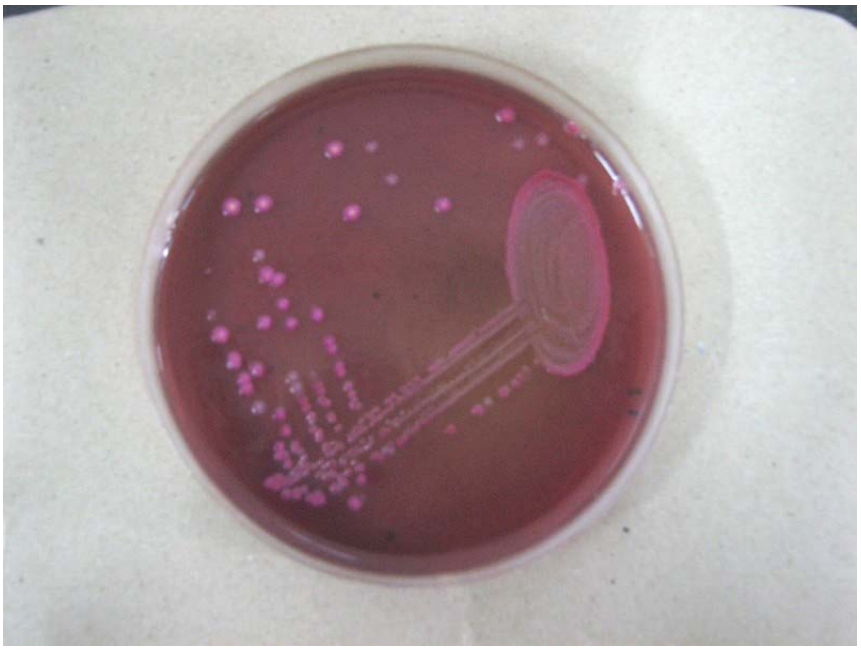
LYSIS CENTRIFUGATION TECHNIQUE

GRAM STAIN – GRAM POSITIVE COCCI



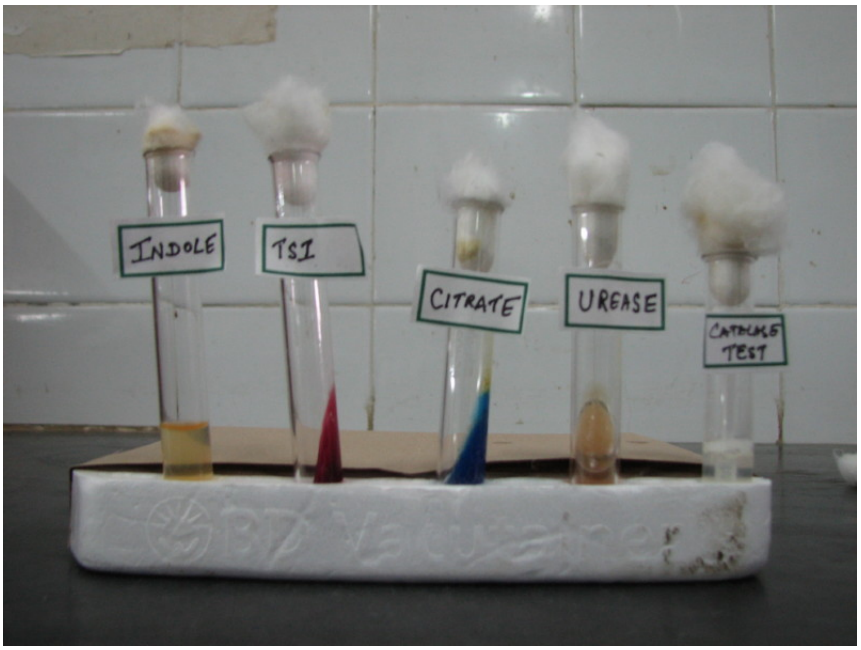
LARGE MUCOID COLONIES - KLEBSIELLA

LF COLONIES OF KLEBSIELLA



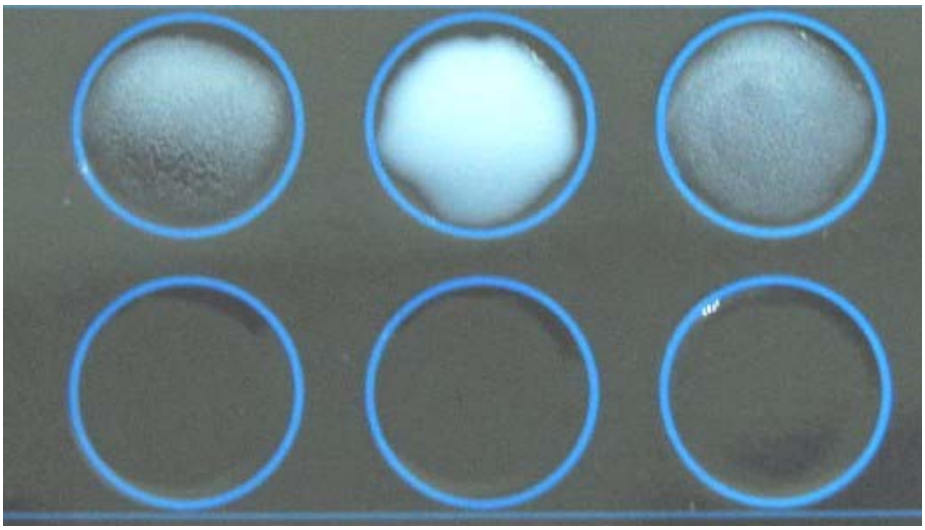
PSEUDOMONAS WITH GREEN PIGMENTATION

BIOCHEMICAL REACTIONS OF PSEUDOMONAS AERUGINOSA

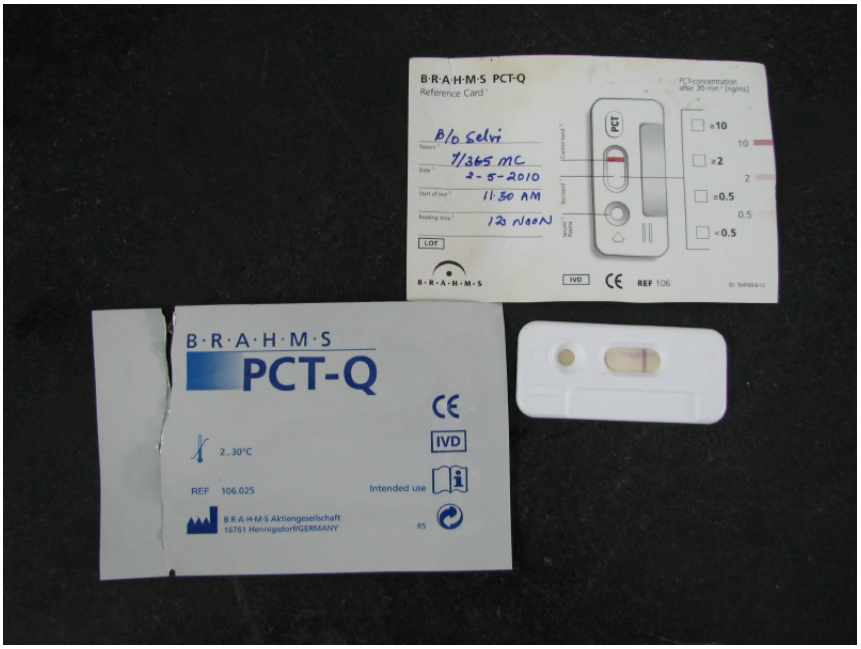


CRP KIT

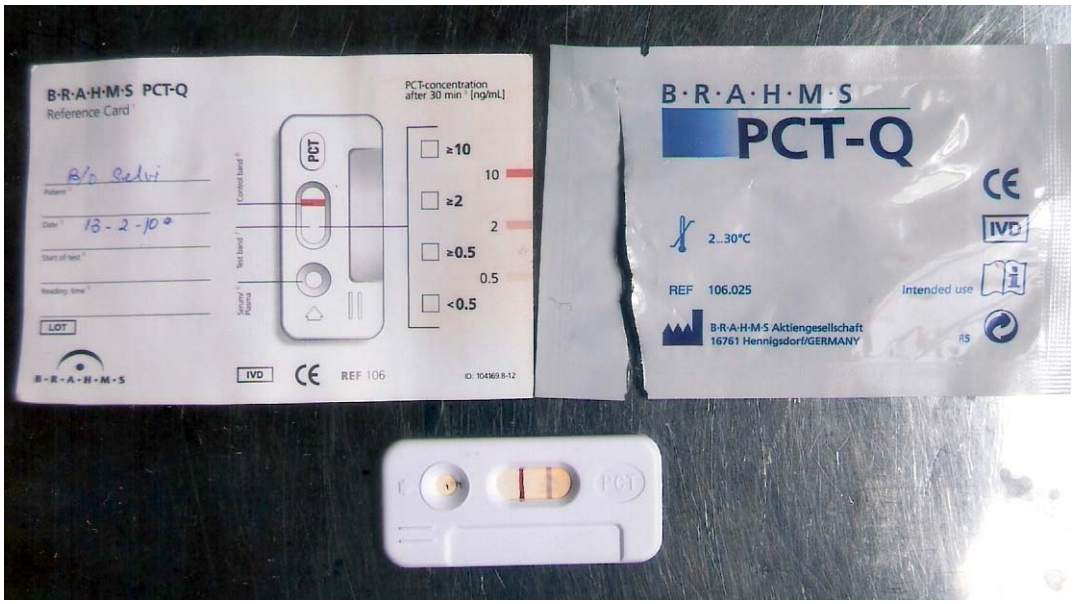
CRP TEST



PCT TEST



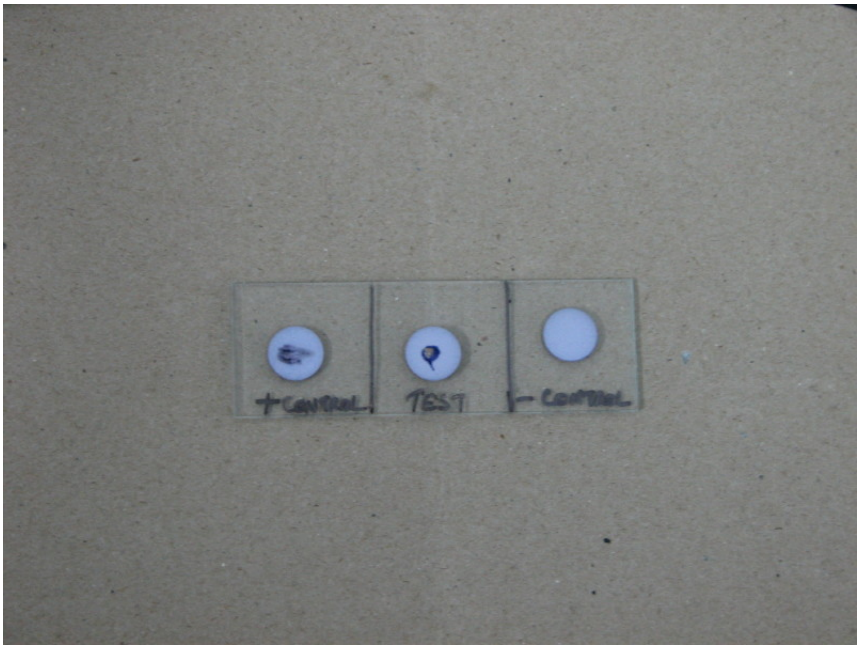
PCT NEGATIVE



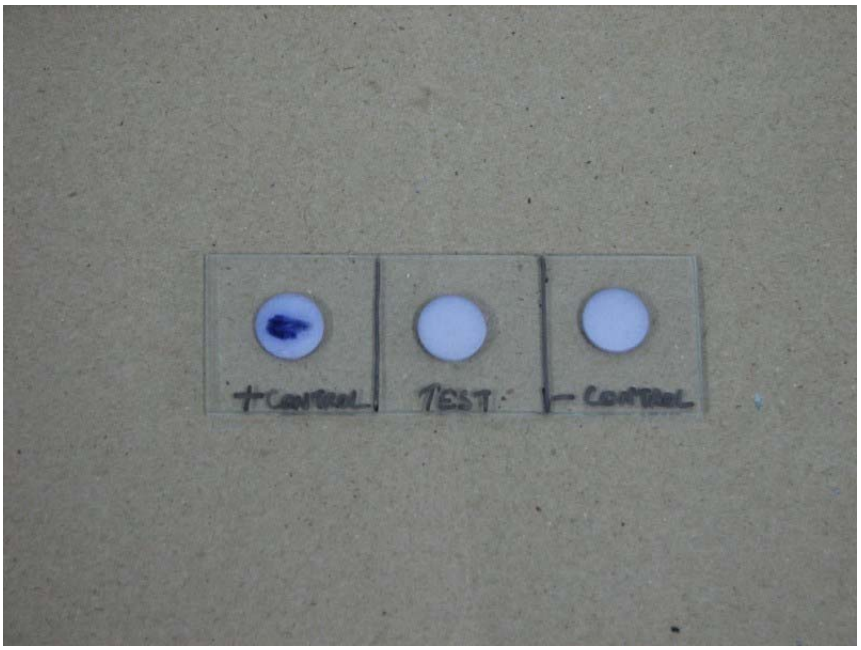
PCT POSITIVE

CATALASE TEST

OXIDASE TEST



OXIDASE POSITIVE



OXIDASE NEGATIVE

BIBILIOGRAPHY

1. Agarwal R, Sarkar N, Deorary A K and Paul V K. Sepsis in new born Indi. J. Pediatrics Dec 2001; 68
2. Ahmed A S M U, Chowdury M A K A, Hoque M, Damstadt GL. Clinical and Bacteriological profile of neonatal septicemia in tertiary level pediatric hospital in Bangladesh. Ind. Peadiatr.2002; 39:1034 - 9
3. Alexander and Giacoia. Early onset nonenterococcal Group D streptococcal infection in the newborn infant. J. Pediatr 1978; 93: 489.
4. Bailey and Scott, “Diagnostic Microbiology” Mosby Elsevier 2007 XII edition Page No. 787-792.
5. Bang AJ, Bang RA, Baitale B. Burden of Morbidities and unmet need for health care in rural Indian neonate. Ind Paediatr 2001; 38: 952-965.
6. Baumgart S, Hall S E, Campos J M, et.al. Sepsis with coagulase negative staphylococci in critically ill in newborns. AmJ Dischild 1983; 137; 461.

7. Belady PH, Farkouh LJ, Gibbs RS, Intra amniotic infections and premature rupture of membranes Clin Perinatol 1997; Mar, 24 (1) : 1143-1147.
8. Benitz W, Han M, Madan A, Ramachandra P. Serial serum C-Reactive Protein levels in the diagnosis of neonatal infection. Pediatrics 1998;
9. Buetow KC, Klien SW, Lane RB. Septicemia in premature infants. Am J Dis Child, 1965; 110 : 29.
10. Campos J M, Spainhour J R, Rapid detection of Bacteremia in children with modified lysis direct plating method. J.clin Microbial 1985; 22:674.
11. Carrigans S D, Scolt G, Tabrizian M, Toward resolving the challenges of sepsis diagnosis. Clin. Chem. 2004; 50: 1301 – 1314.
12. Chiesa C, Panero A, Rossi N et al. Reliability of Procalcitonin concentration for the diagnosis of sepsis in critically ill neonates. Clin Infect Dis 1998 ; 26 L 664 – 672.
13. Choudury Habibur Rasul, Abul Hassan M, Habilbullah M. Pak J Med Sci. 2007; Vol 23 No.1, 78-81.
14. Cloberty J P and Stark R(Ed) Manual of neonatal case 1998.

15. Connie R Mahon, ‘Diagnostic Microbiology’ III edition, 2007, Page No. 1003, 1006.
16. Das PK, Basu K, Chakraborty P, Bhowmilk PK. Clinical and bacteriological infection in metropolitan city based medical college nursery, J. Indian Med Ass, 2010.
17. Dauod et al. The changing face of neonatal septicemia. Ann Trop Paediatr 1995; 15: 93-96.
18. Dietzman DE Fisher GW, Schoenknecht FD Neonatal Escherichia coli septicemia, bacterial counts in blood J Pediatr 1974; 85: 128.
19. Dillon et al Group A Streptococcal infection in a newborn nursery AmJ. Dis. Child, 1966; 135-140.
20. Esperson F, Frimodt Moller N, Rosdah VT, Jesson O. Staphylococcus aureus bacteremia in children below the age of one year. Acta Pediatr 1989; 78: 56
21. Fanaroff A A, Korones S B, Wright L L et al., Incidence, presenting features, risk factors and significance of late onset septicemia in very low birth weight infants. Pediatr Infect Dis 1998; 17: 593 – 598.

22. Franciosi et al., Schaffers Diseases of the Newborn, Fifth edition, 1973; Page No:733.
23. Freedman et al., a half century of neonatal sepsis at Yale. AmJ Dis. Child 1981; 135 – 140.
24. Geil CC, Castle WK, and Mortimen EA Group A Streptococcal infections in newborn nurseries. Pediatrics 1970; 46, 849- 854.
25. Gerder JS, Polin R, Early diagnosis and treatment of neonatal sepsis. Indian J. Pediatr 1998; 65: 63 – 78.
26. Ghosal SP, Sengupta PC, Mukherjee AK, Noma neonatorum;its etiopathogenesis. Lancet 1978; 2: 289.
27. Gladstone IM, Ehrenkranz RA, Edberg SC et al., A ten year review of neonatal sepsis and comparison with the previous fifty years experience. Pediatr Infect Dis 1990; J 9: 819.
28. Gluck L, Wood HF, Fousek MD., Septicemia of the New born Pediatr Clin North Am, 1966; 13 : 1131.
29. Goldman et al., Bacterial colonization of neonates admitted to an intensive care unit. J. Pediatr 1978; 93. 288.

30. Guze LB, Williams and Wilkins. The extensive application of hyperosmolar media to the detection of bacteremia is more recent. Am J. Med. Tech, 1969; 35 : 702-705.

31. Hall RT, Hall SL, Barnes WG et al., characteristics of coagulase negative staphylococci from infants with bacteremia. Pediatr Infect Dis J 1987; 6: 377.

32. Haque KH, Infection and Immunity in the Newborn. In Forfar and Arneil's textbook of Pediatrics 5 th edition, 1988; p 273 - 289.

33. Harmoinen A, Perko M, Groroos P. Rapid Quantitative determination of CRP using LKB 8600 reaction rate analyzer. Clin Chim Acta 1981; 111: 117-120.

34. Harris MC, Costarino AT, Sullivan JS et al., Cytokine elevation in critically ill infants with sepsis and necrotizing enterocolitis. Journal of Pediatrics 1994; 124: 105.

35. Howard and McCracken: The spectrum of Group B streptococcal infections in infancy AmJ Dis child 1974; 128:815.

36. Islam MN, Neonatal infections in developing countries. Workshop on improving neonatal survival in developing countries. 2001;November, Dhaka 2-3

37. Jaye D, Waites K. Clinical applications of C-reactive protein in Pediatrics. Pediatr Infect Dis J 1997; 16: 735 – 746.

38. Kaftan H, Kinney JS, Early onset neonatal bacterial infections Semin perinatal 1998; Feb : 22(1) : 15-24.

39. Karpuch J, Goldberg M, Koheler et al., Neonatal bacteremia; a 4 year prospective study I sr J. Med Science.1983

40. Kennaugh JK, Gregory GW, Powell KR, Hendley JO. The effect of dilution during culture on detection of low concentrations of bacteria in Blood. Pediatr iInfect Dis 1984; J 3: 317.

41. Khassawneh M, Hayajnehwa, Kofahi H et al : Diagnostic markers for Neonatal sepis; Comparing C-Reactive Protein, Interleukin – 6 and Immunoglobulin M. Scand J Immunol. 2007; Feb; 65 (2) ;171 – 5

42. Khatua SP, AK, Chatterjee BD, Ghose B, Saha A, Neonatal septicemia, Indian J. Pediatr 1986; 53 : 504 – 509.

43. Koneman Text book of Colour Atlas and Diagnostic microbiology, fifth edition 2006; page 157.

44. Kurien Anil Kuruvilla, Swati Pillai, Mary Jesudasan,Atanu Kumar. Bacterial profile of sepsis in a Neonatal Unit in South India. Indian Paediatrics, 1998; 35: 851-858.
45. Malik A, Hassani S E, Khan H M, Ahmed A J, Nosocomial infection in new born. Ind. Peadiatr. 2001; 38: 68 – 71
46. Mathus NB,Sharma VK,et al., Anti infective factors in Preterm human colostrum. Acta Pediatr Scan 1990; 79: 1039.
47. McCracken et al; relationship between E.coli K-1 capsular polysaccharide antigen and clinical outcome neonatal meningitis. Lancet 1974; 2: 246.
48. McGee ZA, Wittler RG, Gooder H and Charache P, Wall Defective variants, terminology and experimental design, J Infect Dis. 1971; 123 : 433 – 438.
49. Meharbansingh ‘Care of the Newborn’ Sagar publication 5 th edition 1999; Page No: 215, 217, 218.
50. Melish and Glasgow. Staphylococcus scalded skin syndrome, the expanded clinical syndrome. J. Pediatr 1971; 78: 958.

51. Melish et al., The staphylococcal scalded skin syndrome isolation and partial characterization of the exfoliation toxin. J. Infect Dis 1972; 125: 129.

52. Michael et al., Clinical evaluation of a Lysis centrifugation technique for the detection of septicemia. JAMA 1983; 250: 2185 – 2188.

53. Monica Cheesbrough, District laboratory practice in tropical countries, Cambridge University press reprint 2007; Page 127.

54. Moorman RS Jr, Sell SH. Neonatal septicemia, South Med J 1961; 54: 137.

55. Murex Diagnostics, Murex CRP, Norcross GA: Inc: 1995.

56. Neeraj Kumar Jain, Deepak Seth, Vibha Mangal. A clinicomicrobial association in Neonatal septicemia. Pediatric Infectious Disease conference, 2010 page 1 - 5.

57. Nelson 'Text book of Peidiatrics' page No. 529. 15th Edition,1996; page no 798. 18th edition

58. Nyhan WL, Fousek MD, Septicemia of the New born Paediatrics, 1958; 22 : 368.

59. Philip A, Andrews P, Rapid determination of CRP levels: Semiquantitative versus quantitative. Clin Lab Observ. 1986; 110: 263 -265.

60. Philip A.G.S and Hewitt JR. Early diagnosis of neonatal sepsis. Pediatrics 1980; 65: 1036.

61. Pourcyrous M, Bada H, Korones S, Baselski V. Wong S. Significance of serial C- Reactive Protein responses in neonatal infection and other disorders. Pediatrics, 1993; 92: 431 – 435.

62. Powell KR. Evaluation and Management of febrile infants younger than 60 days of age. Pediatr Infect Dis 1990; J 9: 153.

63. Randall C, Walker M D, Nancy K, Hentry. Arch. Intern Med. 1986; 146 (12): 2341 – 2343.

64. Report on the National Neonatal Perinatal Database (National Neonatology Forum).2002-2003

65. Resch B, Gusenleitner W, Huller WD, Procalcitonin and Interleukin-6 in the diagnosis of early onset sepsis of the neonate. Acta pediatrica, 2003; 92: 243 – 245.

66. Roy I Jain A Kumar M, Agrawal SK. Bacteriology of neonatal septicemia in a tertiary care hospital of Northern India. Indian J. Med. Microbiol 2002; 20-156-9.

67. Russell G, Smyth A, Cooke R. Receiver operating characteristic curves for comparison of serial neutrophil band forms and C-Reactive Protein in neonates at risk for Septicemia. Eur J. Pediat. 1995; 154:138 – 144.

68. Sabat R, Holflich C, Docke WD,et al., Massive elevation of Procalcitonin plasma levels in the absence of infection in kidney transplant patients treated with Pan T cell antibodies. Intensive care Med. 2001; 27:987-991.

69. Sadana S, Mathur NB, Thakur A . Exchange transfusion in septic neonates with sclerema: effect on immunoglobulin and complement levels. Indian Pediatr 1997; 34 20 – 5.

70. Sanghvi KP, Tudehope DI. Neonatal bacterial sepsis in a neonatal intensive care unit : A 5 year analysis. J. Pediatr child health, 1996; 32: 333 – 338.

71. Sankar MJ, Agarwal A, Deorari AA, et al. Sepsis in the newborn. Indian J Pediatr, 2008; Mar ; 75(3) : 261 – 266.

72. Schelgel RJ, Bellanli AJ, Increased susceptibility of males to infection. Lancet 1967; 2:826.

73. Schreiber JR, Berger M. Intravenous immunoglobulin therapy for sepsis in premature babies. J. Pediatr 1992; 121: 401 – 404.

74. Shah GS, Budhathoki S, Das BK, Mandal RN. Risk factors in early onset neonatal sepsis, Kathmandu Medical Journal 2006; vol 4: No. 2, Issue 14, 187-191.

75. Sharma et al. Bac. Neonatal septicemia, Indian Pediatr 1987; 24: 1011 – 1017.

76. Shasikala ST, Kasturi AV, Shoba D, Krishna BVS. Clinical bacteriological specimen in Hubli, Indian J Paediatr 2000; 169 -174.

77. Shine B, Gould J, Campbell C, Serum C-Reactive protein in normal and infected neonates. Clin Chim Acta 1985; 148:97-103.

78. Siegel and McCracken Jr: Sepsis Neonatorum N Engl.J Pediatri 1978; 93: 542.

79. Siegel et al., Single dose penicillin prophylaxis against neonatal Group B Streptococcal infections a controlled trial in 18, 738 newborn infants.

80. Simon L, Gauvin F, Amre DK,et al.,Serum Procalcitonin and C-reactive protein levels as markers of bacterial infections A systematic review and metaanalysis. Clin Infect Dis 2004; 39: 206- 217.

81. Singh M, Narang, Bhakoo on Predictive perinatal score in the diagnosis of neonatal sepsis J Trop Pediatr 1994; Dec 40 (6): 365 – 368.

82. Stevens and colleagues. Early onset Pseudomonas sepsis of the neonate. Perinatal, Neonatal 1982; 6:75.

83. Stoll B, Gordon T, Tyson J et al., early onset sepsis, Late onset sepsis in the very low birth weight neonates: a report from the National Institute of Child Health and Human development. Neonatal research network. J. Pediatr 1996; 129: 63- 80.

84. Stoll BJ. The global impact of neonatal infections. Clin. Perinatal 1997; 24: 1-27.

85. Takkar VP, Bhakoo ON, Narang A. Scoring system for the prediction of early neonatal infection. Indian Pediatr 1974; 11: 507-600.

86. Tallur SS, Kasturi AV, Nadgir, Krishna BV. Clinico bacteriological study of neonatal sepsis in Hubli. Indian J. Pediatr 2000; 67: 169-174.

87. Trotman H and Bell Y. Neonatal sepsis in very low birth weight infants at the University Hospital of the West Indies. West Indies Med J 2006; 55 (3) : 165.

88. Ugarte H, Silva E, Mercan D, DeMendonca A, Vincent J L: Procalcitonin used as a marker of infection in the intensive care unit. Crit Care Med 1998; 24: 1052 – 1056.

89. Wagle S, Granaug A, Kohan R, Evans S, C-Reactive protein as a diagnostic tool of sepsis in very immature babies. J. Pediatr child Health 1994; 30:40-44.

90. Washburn TC, Medearis DN Jr. Child B. Sex differences in susceptibility to infections. Pediatrics 1965; 35: 57.

91. Wolach B, Neonatal sepsis: Pathogenesis and supportive therapy. Semin Perinatal 1997; 21 28 – 38.