A CLINICO-BACTERIOLOGICAL STUDY OF NEONATAL SEPTICEMIA IN GOVT. STANLEY HOSPITAL, CHENNAI.

Dissertation Submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

in partial fulfillment of the regulations

for the award of the degree of

M.D. (MICROBIOLOGY)
BRANCH – IV

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
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MARCH 2007

DECLARATION

I solemnly declare that this dissertation "**A CLINICO-BACTERIOLOGICAL STUDY OF NEONATAL SEPTICEMIA IN GOVT. STANLEY HOSPITAL, CHENNAI"** is the bonafide work done by me at the Department of Microbiology, Govt. Stanley Medical College and Hospital, Chennai, under the guidance and supervision of **Prof. Dr. P. R. THENMOZHI VALLI, M.D.,** Professor and Head, Department of Microbiology, Govt. Stanley Medical College, Chennai-600 001.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of degree of M.D. Branch IV Microbiology examinations to be held in March 2007.

Place : Chennai.

Date :

Dr. R. SECUNDA

CERTIFICATE

This is to certify that this dissertation entitled "**A CLINICO-BACTERIOLOGICAL STUDY OF NEONATAL SEPTICEMIA IN GOVT. STANLEY HOSPITAL, CHENNAI"** is the bonafide original work done by **Dr. R. SECUNDA,** Post graduate in Microbiology, under my overall supervision and guidance in the department of Microbiology, Stanley Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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CONTENTS

INTRODUCTION

"In every child who is born under no matter what circumstances, and of no matter what parents, the potentiality of the human race is born again, and in him too, once more, and of each of us , our terrific responsibility towards human life".

-James Agee

Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the first month of life. Neonatal sepsis encompasses systemic infections of the newborn including septicemia, meningitis and pneumonia[42].

Neonatal sepsis can be divided into two main classes depending on the onset of symptoms related to sepsis. Early onset sepsis usually presents within the first 72 hours of life. Late onset sepsis usually presents after 72 hours of life[42].

Neonatal mortality is very high in developing countries like India. The global burden of neonatal death is estimated to be 5 million of which 3.2 million occurs during the first week of life. Worldwide survey shows that one fourth of the burden of neonatal mortality worldwide is seen in India and about 1.2 million neonates die every year. In India, sepsis is one of the most important cause of neonatal death.

There are diverse modes of transmission of infectious agents from mother to foetus (or) new born infant. Transplacental haematogenous spread may occur at different times during gestation. Vertical transmission of infection may take place in utero, just prior to delivery (or) during the process.

Several factors contribute to the frequency and severity of the neonatal infection and emphasize the importance of early diagnosis and treatment.

a) With the increasing complexity of neonatal intensive care, gestationally younger and lower birth weight newborn are surviving and remaining for a longer time in an environment with a high risk of infection.

b) The clinical manifestations of newborn infections vary and include sub-clinical infection, mild to severe manifestations of focal or systemic infection and it may mimic the features of other disease so that the diagnosis of infection is missed (or) delayed until the process has become widespread.

c) The newborn infants are less capable of responding to infections because of one or more immunologic deficiencies.

d) A wide variety of etiologic agents infect the newborn, including bacteria, viruses, fungi and protozoa.

e) The important bacterial agents responsible for sepsis include gram negative bacteria like Klebsiella pneumoniae, Escherichia coli, Citrobacter freundii, Enterobacter aerogenes, Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter calcoaceticus, Salmonella typhi, Shigella dysentriae type 1, Serratia marcescens and gram positive organisms like Staphylococcus aureus, Coagulase negative staphylococcus, Streptococcus pyogenes, Enterococcal species, Streptococcus agalactiae, Streptococcus viridans and Streptococcus pneumoniae.

f) The laboratory tests available for diagnosis do not provide rapid result needed for early and quick recovery.

g) The emergence of antibiotic resistance among pathogens that infect newborns is of great concern.

h) Early treatment is of critical importance.

i) Failure to appreciate the symptoms, delay in starting the treatment (or) withholding antibiotics may make the difference between survival, death and permanent disability.

j) Hence in view of changing profile it has been decided to take up a study to identify the bacterial agents causing neonatal septicaemia along with their antibiotic sensitivity.

k) This will help to rationalize therapy and evaluate common programme of the management.

REVIEW OF LITERATURE

Definition:

When pathogenic bacteria gain access into the blood stream of neonates, they may cause an overwhelming infection without much localization (septicemia) or may get predominantly localized to the lung (pneumonia) or the meninges (meningitis). Systemic bacterial infections are known by the generic term neonatal sepsis which incorporates septicemia, pneumonia and meningitis of the newborn[15].

CLASSIFICATION OF NEONATAL SEPSIS

Early Vs. Late onset sepsis

Neonatal sepsis can be divided into two subtypes depending upon whether the onset of symptoms is before 72 hours of life (early onset) or later (late onset).

Early onset infections are caused by the organisms prevalent in the maternal genital tract or in the delivery area. The predisposing factors include low birth weight, prolonged rupture of membranes, foul smelling liquor, multiple per vaginal examinations, maternal fever, difficult or prolonged labour and aspiration of meconium. Early onset sepsis manifest frequently as pneumonia and less commonly as septicemia or meningitis.

Late onset septicemia is caused by the organisms thriving in the external environments of the home or the hospital. The infection is often transmitted through the hands of the care providers. The onset of symptoms is usually delayed beyond 72 hours after birth and the presentation is that of septicemia, pneumonia or meningitis. The predisposing causes of late onset sepsis include, low birthweight, lack of breast feeding, superficial infection (pyoderma, umbilical sepsis), aspiration of feeds, disruption of skin integrity with needle pricks and use of

intravenous fluids. These factors enhance the chances of entry of organisms into the body systems of the neonates who are much less immunocompetant as compared to older children and adults[15].

MODES OF TRANSMISSION AND PATHOGENESIS

Neonatal infections are unique for several reasons:

a) Infectious agents can be transmitted from the mother to the fetus or new born infants by diverse modes.

b) New born infants are less capable of responding to infection because of one or more immunological deficiencies.

c) Coexisting conditions often complicate the diagnosis and management of neonatal infections.

d) The clinical manifestations of newborn infections vary and include subclinical infection, mild to severe manifestation of focal or systemic infection and rarely congenital malformations resulting from infection in 1st trimester.

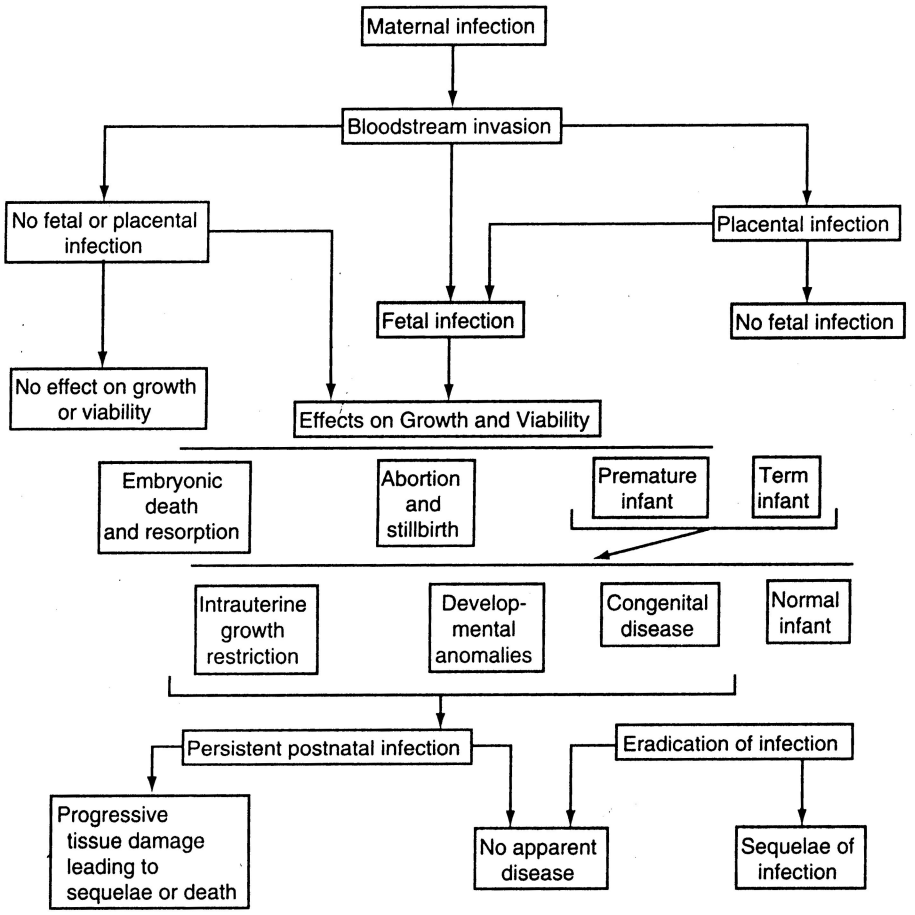
e) Maternal infection that is the source of transplacental fetal infection is often undiagnosed during pregnancy because the mother was either asymptomatic or had non specific symptoms at the time of acute infection.

f) Finally, with advance in neonatal intensive care, increasingly immature, very low birth weight newborns are surviving and remain in the hospital for a longer time, and environment that puts them at ongoing high risk for infection[37].

PATHOGENESIS OF INTRAUTERINE INFECTION

Intrauterine infection is a result of clinical or subclinical maternal infection with a variety of agents (e.g. Cytomegalovirus, Treponema pallidum, Toxoplasma gondii, Rubella virus, Varicella virus, Parvo virus B19) and hematogenous transplacental transmission to the fetus. Transplacental infection may occur anytime during gestation and signs and symptoms may be present at birth or be delayed for months or years.

The timing of infection during gestation affects the outcome. First trimester infection may alter embryogenesis with resulting congenital malformation (e.g. congenital rubella). Third trimester infection often results in active infection at the time of delivery (toxoplasmosis, syphilis). Infections that occur late may lead to delay in clinical manifestation until birth (syphilis)[37].



Pathogenesis of hematogenous transplacental infections

PATHOGENESIS OF ASCENDING OR INTRAPARTUM BACTERIAL INFECTION

The human birth canal is colonized with aerobic and anaerobic organisms that may result in ascending amniotic infection and / or colonization of neonate at birth.

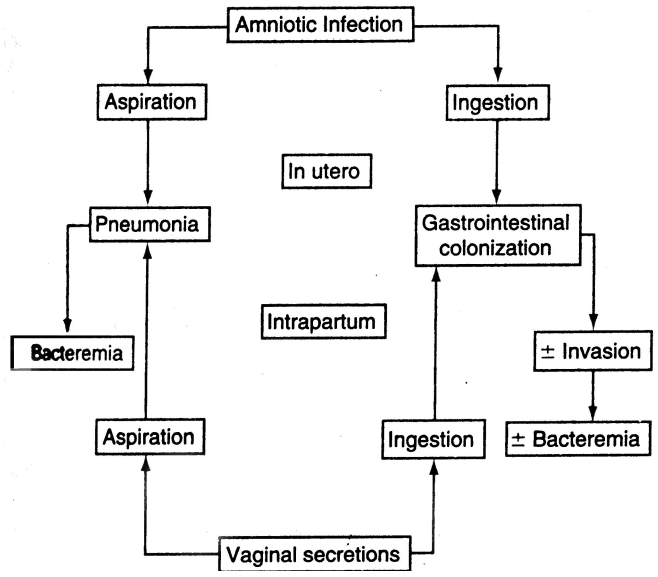
Chorioamnionitis results from microbial invasion of amniotic fluid, usually as a result of prolonged rupture of chorioamniotic membrane, on occasion, amniotic infection occurs with a relatively brief duration of membrane rupture. Amniotic fluid infection may be asymptomatic or may produce maternal fever, with or without local or systemic signs of chorioamnionitis.

A period longer than 18 hour is the current cut-off for increased risk of neonatal infection. Difficult or traumatic delivery and premature delivery are also associated with an increased frequency of neonatal infection.

Factors that influence that colonised infant to develop disease include prematurity, underlying illness, invasive procedures, inoculum size, virulence of the infecting organism and transplacental maternal anti bodies.

Aspiration or ingestion of bacteria in amniotic fluid may lead to congenital pneumonia or systematic infection with manifestations becoming apparent before delivery, at delivery or after a latent period of few hours.

Resuscitation at birth, particularly if it involves endotracheal intubation, insertion of an umbilical vessel catheter or both is an increased risk of bacterial infection[37].

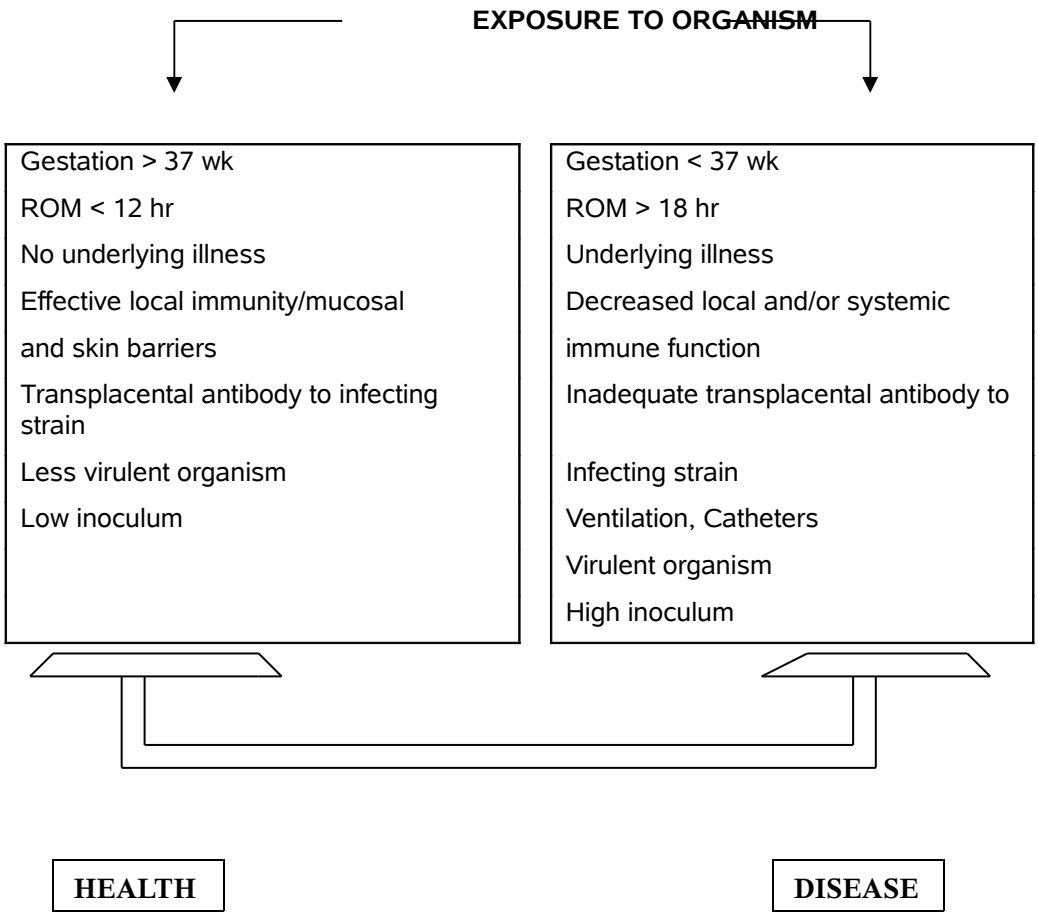


Pathways of Ascending or Intrapartum Infection

PATHOGENESIS OF LATE - ONSET POSTNATAL INFECTIONS

After birth, neonates are exposed to infectious agents in the nursery or community. Post natal infections may be transmitted by direct contact with hospital personnel, the mother or the family members, from breast milk (HIV, CMV), or from inanimate sources such as contaminated equipment. The most common source of post natal infections in hospitalized newborns is hand contamination of healthcare personnel.

Most cases of meningitis result from hematogenous dissemination . Less often, meningitis results from contiguous spread as a result of contamination of open neural tube defects, congenital sinus tracts, or penetrating wounds from fetal scalp sampling or internal fetal electrocardiographic monitors. Abscess formation, ventriculitis, septic infarcts, hydrocephalus, and subdural effusions are complications of meningitis that occur more often in new born infants than in older children[37].



Factors influencing the balance between health and disease in neonates exposed to a potential pathogen. ROM=rupture of membranes.

IMMUNITY

Diminished concentrations of immunoglobulins and other immunologic factors and decreased function of neutrophils and other cells involved in the response to infection have been demonstrated in both term and preterm infants.

IMMUNOGLOBULINS

IgG is actively transported across the placenta, with concentrations in a full term infant comparable to those in the mother.

The specificity of IgG anti body in the cord blood is dependent on the mother's previous antigenic exposure and immunologic response. Infants with birthweight less than 1500gm become significantly hypogammaglobulinemic, with mean plasma IgG concentrations in the range of 200 to 300 mg / dl in the first week of life.

The presence of passively transferred specific IgG antibody in adequate concentration provides neonates protection against infection to which protection is mediated by antibody. Specific bactericidal and opsonic antibodies against enteric gram - negative bacteria are predominantly in the IgM class. In general, a new born infant lacks antibody mediated protection against Escherichia coli and other enterobacteriacea[37].

COMPLEMENT

The complement system mediates bactericidal activity against certain organisms such as Escherichia coli and functions as an opsonin with antibody in the phagocytosed bacteria.

Essentially no transplacental passage of complement from the maternal circulation takes place. A fetus begins to synthesize complement components as early as 1st trimester. Premature infants have lower level of complement components and less complement activity than full term newborns do. These deficiencies contribute to diminish ability to opsonize certain organisms in the absence of antibody[37].

NEUTROPHILS

Quantitative and qualitative deficiencies of the phagocyte system are important factors contributing to newborns increased susceptibility to infection. Neutrophil migration (Chemotaxis) is abnormal at birth in both term and preterm infants. In addition, neonatal neutrophils have decreased adhesion, aggregation and deformability, all of which may delay the response to infection. Abnormal expression of cell membrane adhesion molecules (β_2 integrins and selectins) and abnormalities in the neonatal neutrophil cytoskeleton contribute to abnormal chemotaxis.

Neutropenia is frequently observed in pre-term infants and those with intrauterine growth restriction and it increases the risk of sepsis. The neutrophil storage pool in newborn infants is 20 to 30% of those in adults and is more likely to be depleted in the face of infection. Mortality is increased when sepsis is associated with severe sepsis - induced neutropenia and bone marrow depletion[37].

MONOCYTE - MACROPHAGE SYSTEM

The number of circulating monocytes in neonatal blood is normal, but the mass or function of macrophages in the reticuloendothelial system is diminished in newborns, particularly in pre-term infants. In both term and pre-term infants chemotaxis of monocytes is impaired, this impairment affects the inflammatory response in tissues and the results of delayed hypersensitivity skin tests. However, monocytes from neonates ingest and kill microorganisms as well as monocytes from adults do[37].

NATURAL KILLER CELLS

NK cells appear early in gestation and present in cord blood in numbers equivalent to those in adults, however, neonatal NK cells have decreased cytotoxic activity and ADCC in comparison to adult cells[37].

CYTOKINES / INFLAMMATORY MEDIATORS

The release of various inflammatory mediators in response to infection offers the potential opportunity to facilitate an early diagnosis of infection. Studies of potential surrogate markers for neonatal infection have focused on the association of bacterial sepsis, pneumonia and necrotizing enterocolitis with elevated cytokine levels. TNFX, IL-6 and IL-8 are elevated in neonates with sepsis[37].

ETIOLOGICAL AGENTS OF NEONATAL SEPSIS

Through the years, there has been a shift in the microorganisms responsible for neonatal septicemia and meningitis. This is clearly illustrated by the experience at Yale-New Haven Hospital (Freedman et al, 1981; Thompson et al, 1992; Unhanand et al, 1993; Yagupsky et al,

1991). During the 1930s, group A streptococci were the predominant organisms. In the 1950s, staphylococci (largely of phage group 1) became a major cause of nursery outbreaks throughout the world. Pseudomonas was prominent during the same decade, perhaps because of the introduction of respiratory support systems. Since the late 1950s, Escherichia.coli has been an important cause of neonatal sepsis. The dramatic increase in incidence of group B streptococci infections is notable and has been reflected in other centers as well. Both group D streptococci and Klebsiella are pathogens that have been found relatively recently, the latter accounting for a high proportion of antibiotic-resistant organisms that colonize and infect babies in neonatal intensive care units (Goldmann et al, 1978). During the 1980s and 1990s, S.epidermidis has been recovered from systemic cultures with increasing frequency (Battisti et al, 1981; Huebner et al, 1994; Kumar and Deliveria-Papadopoulos, 1985; Tan et al, 1994). This organism is most commonly seen in infants who are premature and who have required prolonged maintenance with central vascular catheters, peritoneal dialysis, or thoracostomy tubes. In most intensive care nurseries, this organism is the most common nosocomial systemic isolate. The prevalence rate for a specific bacterial pathogen vary from nursery to nursery and may change rather abruptly. Knowledge of the most commonly isolated bacteria in a nursery or intensive care unit, coupled with antimicrobial susceptibilities of these organisms, is valuable in treating suspected neonatal sepsis[5].

According to recent data from National Neonatal Perinatal Database, Klebsiella pneumoniae was the most frequently isolated pathogen followed by Staphylococcus aureus and Pseudomonas species[42].

BACTERIAL CAUSES OF SYSTEMIC NEONATAL INFECTIONS

- ***Gram Positive***

  Staphylococcus aureus

  Coagulase negative staphylococcus

  Enterococci species

  Group B streptococcus

  Streptococcus pneumoniae

  Viridans streptococcus

  Listeria monocytogenes

- ***Gram Negative***

  Klebsiella pneumoniae

  Escherichia coli

  Proteus mirabilis

  Pseudomonas aeruginosa

  Citrobacter freundii

  Enterobacter cloacae

  Salmonella typhi

  Serratia marcescens

  Haemophilus influenzae

- ***Others***

  Treponema pallidum

  Mycobacterium tuberculosis

NONBACTERIAL CAUSES OF SYSTEMIC NEONATAL INFECTIONS

- ***Viruses***

  Adenovirus

  Cytomegalovirus

  Enteroviruses

  Herpes simplex virus

  HIV

  Parvovirus

  Rubella virus

  Varicella-zoster virus

- ***Fungi***

  Candida species

- ***Protozoa***

  Plasmodia

  Toxoplasma gondii

  Trypanosoma cruzi[37]

STAPHYLOCOCCAL DISEASE

In the 1950s, phage group 1 Staphylococcus aureus was the most common bacterial agent that caused septicemia in neonatal units. Its unique invasive properties caused disseminated disease with widespread manifestations, including neonatal mastitis, furunculosis, septic arthritis, osteomyelitis and septicemia. Because infection of the bloodstream is usually secondary to local invasion, a careful search for the primary focus must

be made in all septic babies. Microbial surveillance, intensified infection control measures and increased local skin care have reduced colonization and disease rates caused by the group 1 organism.

In the 1970s, Coagulase-positive staphylococcal disease in nurseries was caused by organisms of the phage II group (Melish and Glasgow, 1971). These organisms produce an exotoxin (exfoliatin) that causes intraepidermal cleavage through the granular cell layer resulting from disruption of desmosomes (Melish et al, 1972). Clinical disease may take one of several forms, which include bullous impetigo, toxic epidermal necrolysis, Ritter disease and non-streptoccal scarlatina. The initial findings in Ritter disease are intense, painful erythema that is similar to a severe sun burn. Over the next few hours, the bulla may form, that when ruptured, leave a tender, weeping erythematous area. The characteristic desquamation of large epidermal sheets occur approximately 3 to 5 days after the onset of the illness. A fine desquamation is commonly seen in the perioral region. Bullous impetigo has been the most common disease associated with nursery outbreaks of phage group II staphylococcal infections.

In the 1980s, two additional kinds of staphylococcal infections have been recognized as major contributors to nursery infections, namely methicillin-resistant Staphylococcus aureus (MRSA) and S.epidermidis. Since the early 1980s, adult surgical and medical intensive care units in the United States and other countries have noted an increase in nosocomially acquired and community-acquired MRSA. Similarly, MRSA outbreaks reported with increasing frequency in neonatal intensive care units. Although standard infection control methods including contact isolation, hand-washing with chlorhexidine, and cohorting are frequently used to control outbreaks, eradication of MRSA may require hand-washing with hexachlorothene. The population at highest risk for colonization or infection include infants

under 1500g with long standing central vascular catheters, thoracostomy tubes, or central nervous system shunts, or those infants undergoing prolonged hospitalization. when colonization with MRSA is noted and clinical detoriation suggestive of systemic infection occurs, some authors have strongly suggested the inclusion of vancomycin in the initial antibiotic administration. Vancomycin has been shown to be effective therapy for systemic MRSA infection in both adults and children. However, regular use of vancomycin may cause the development of vancomycin-resistant organisms. Decisions concerning the use of antibiotics must be individualized and predicated on the clinical condition and history of the infant, the microbiologic history of the nursery and the contribution of indwelling catheters. Routine surveillance for MRSA in individual nurseries may be necessary if outbreaks or endemic colonization and infection are observed[5]. Rapid screening and identification of MRSA can be done directly from specimens using real time PCR[1,21].

GRAM NEGATIVE INFECTIONS

Gram negative organisms continue to be a menace to the ill, fragile and debilitated newborns in neonatal intensive care unit. Among these Klebsiella species is emerging as an important cause and are resistant to multiple antibiotics[40].

Escherichia coli is another common gram negative bacteria that causes septicemia during neonatal period. Approximately 40% of Escherichia coli strains that cause septicemia possess K1 capsular antigen[5].

In the newborns who receive broad spectrum antibiotics while in an environment that is potentially contaminated by bacteria from respirators or moist oxygen, the disease is most likely to be caused by Pseudomonas species[5].

Acinetobacter species is an important emerging nosocomial pathogen in neonatal

speticemia[23]. Other gram negative bacteria include Proteus species, Enterobacter species and Citrobacter species.

Since the early 1970s, number of nursery outbreaks caused by specific gram negative bacteria have been described, and virtually all have occurred in long stay intensive care nurseries. Among the causative organisms were Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcescenes and Escherichia colli.

Infected fomites represent a common source of nursery outbreaks caused by gram negative bacteria. Contaminated faucet aerators, sink traps and drains, suction equipment, bottled distilled water, cleansing solutions, humidification of apparatus and incubators have been incriminated.

In addition, healthy colonised infants or nursery personnel may act as a source of infection because the organism is transmitted among infants by way of the hands or gowns of the personnel[5].

CLINICAL MANIFESTATIONS

The earliest signs of sepsis are often subtle and nonspecific and need a high index of suspicion for early diagnosis. Babies with sepsis may present with one or more of the following symptoms[42].

Non-specific features of sepsis

a) Hypothermia or fever
b) Lethargy, poor cry, refusal to suck
c) Poor perfusion, prolonged capillary refill time
d) Hypoglycemia, hyperglycemia
e) Metabolic acidosis[42].

Specific features related to various system

Gastrointestinal system

Feed intolerance, abdominal distention, vomiting, diarrhea, hepatomegaly

Respiratory System

Apnea, dyspnea, tachypnea, retractions, flaring, grunting, cyanosis.

Renal System

Oliguria

Cardiovascular System

Pallor, mottling, cold, clammy skin, tachycardia, hypotension, bradycardia

Central nervous System

Irritability, lethargy, tremors, seizures, hyporeflexia, hypotonia, abnormal moro reflex, irregular respirations, full fontanel, high-pitched cry.

Hematological System

Jaundice, Splenomegaly, pallor, petechiae, purpura, bleeding[37].

DIAGNOSIS

The clinical findings are non-specific and poorly expressed. It is only foresight and high sense of suspicion that will form an early diagnosis and subsequently to be confirmed by laboratory help.

The diagnosis of systemic bacterial infection must start with a careful evaluation of the newborn's symptoms and signs, physical examination, information and longitudinal changes in vital signs and laboratory indicators and history including maternal and relevant recent nursery history.

LABORATORY INVESTIGATION

SEPTIC SCREEN

All newborns suspected to have sepsis should have a septic screen to corroborate the diagnosis of sepsis. The septic screen includes certain simple laboratory investigations which are practical and feasible even in the peripheral setup and are reliable early indicators of neonatal sepsis. The various components of septic screen are haematological changes in the peripheral blood, estimation of acute phase reactants, micro ESR and gastric aspirate[42].

HAEMATOLOGICAL CHANGES

1. Total WBC count

-useful and rapid test, but non-specific.

Total count <5000

Band cell/total ratio>0.2or 0.3

The normal range of WBC counts in newborn infants is wide and this wide range should be taken into account in interpreting the values (Manroe et al). Thrombocytopenia, as well as elevated Prothrombin time, partial thromboplastin time and international normalized ratio

(INR) and decrease fibrinogen will be found in severely ill infants[32].

ACUTE PHASE REACTANTS

These proteins are produced by the liver under the influence of interleukin - 1 when inflammation from any cause is present. The most important and widely used is C-reactive protein (CRP).

- CRP
- IL-6
- IL-8
- G-CSF
- TNF alpha
- IL-beta
- Haptoglobin

C-REACTIVE PROTEIN

C-reactive protein is a non-specific marker of inflammation or tissue necrosis It was the first protein to be discovered which behaves as an acute phase reactant. It was so named because of its ability to bind and precipitate the somatic C-polysaccharide of pneumococci. This binding reaction, which is calcium dependant, results from the specific capacity of CRP to recognize phosphoryl choline residues which are present in C-polysaccharide. CRP can also bind to some but not all other substances which contain phosphoryl choline, for e.g some phospholipids, plasma lipoproteins and the plasma membranes of damaged but not intact cells. In addition CRP binds even more avidly to nuclear chromatin, the DNA-histone complex, when this is exposed in dead or damaged cells or is isolated from preparation of nuclei[29].

Having bound to its various ligands, CRP then activates the classical complement pathway via Cl, and does so as efficiently as IgG antibodies. This means that CRP can induce

all the known inflammatory opsonizing and complex – solubilizing activities of the complement system. In particular complement activation by CRP – Chromatin complexes promotes their dissolution and the dissociation of the chromatin. A significant biological function of CRP may thus be to recognize and "scavenge" cell and tissue debris promoting its clearance from the circulation and the tissues[29].

CRP is synthesized in the liver in response to inflammatory cytokines. It is a trace protein in normal healthy individuals, the median value being 0.8 mg/l with an inter quartile range of 0.3-1.7 mg/l – 90% of healthy individuals have levels of less than 3 mg/l and 99% of them less than 10 mg/l. With the onset of an acute phase response the serum CRP concentration rises rapidly and extensively, reaching, for (eg) levels of over 300 mg/l by 48 hours after an acute severe stimulus such as a myocardial infarction or major trauma or surgery.With uncomplicated resolution of injury or effective treatment of infection the circulating CRP concentration generally falls equally rapidly[29].

The scope of practical, clinical application of serum CRP measurements is exceptionally broad, comprising (i) Screening for organic disease (ii) objective monitoring of the extent and activity of known infective, inflammatory, necrotic and neoplastic diseases and (iii) sensitive testing for the presence of inter current infection in patients immunocompromised by another primary disease and / or therapy. As a consequence of the increased awareness of a number of these applications a range of commercial assay methods have recently become available, including homogenous enzyme immunoassay, fluorescence polarization immunoassay, and various new light scattering methods, in addition to the already established nephalometric, gel precipitation and latex agglutination methods[29].

Elevations in CRP are found in bacterial sepsis and meningitis. A single determination of

CRP at birth lacks both sensitivity and specificity for infection, but serial CRP determination at birth and at 12 hours and beyond have been used to manage infants at risk for sepsis[54].

CRP levels fall quickly after efficient elimination of microbial stimulus, due to its short half life of 19 hours. Thus CRP may be used as a parameter to identify the time period when antibiotic therapy can safely be discontinued in case of suspected neonatal septicemia[41]. A good response to antibiotics is indicated by a rapid return to normal of CRP, whereas persistent elevations of serum CRP suggest that the treatment is inadequate or indicate some complication.

MICRO-ESR

Barrett (1980) described a micro-ESR method using 0.2 ml blood to fill a plastic disposable tube 230 mm long with a 1 mm internal bore. Capillary blood values correlated well with venous blood micro-ESR and westegren-ESR value[53]. It is simple, but not very reliable. A value >15mm in 1st hour is suggestive of infection[39].

GASTRIC ASPIRATE

Microscopic examination and culture of material obtained from gastric aspiration for leukocytes and bacteria are useful in identifying infants who are at risk of sepsis[27].

BACTERIOLOGICAL STUDY

Blood Culture

It is the gold standard for the diagnosis of septicemia and should be done in all cases of suspected sepsis prior to starting antibiotics. A positive blood culture and sensitivity of the isolate is the best guide to antimicrobial therapy[42].

Sample Collection

PREPARATION OF THE SITE

Because blood culture media have been developed as enrichment broths to encourage the multiplications of even one bacterium, it follows that these media will enhance the growth of any stray contaminating bacterium such as a normal inhabitant of human skin. Therefore careful skin preparation before collecting the blood sample is of paramount importance to reduce the risk of introducing contamination into blood culture medium. Universal precautions require that the phlebotomists wear gloves for this procedure[6].

SPECIMEN VOLUME

For infants and small children, only 1 to 5 ml of blood can usually be drawn for bacterial culture. Smaller volumes of blood is sufficient since high levels of bacteremia (more than 1000 cfu per ml of blood) are detected in some infants[6].

NUMBER OF BLOOD CULTURE

A single blood culture is satisfactory for confirming the diagnosis of neonatal septicemia. Repeated cultures of blood do not improve detection rate[43,47].

SAMPLE PROCESSING

The inoculated bottles are to be incubated at 37°C in carbon dioxide atmosphere and examined after 18 to 24 hours for any turbidity, discoloration or clotting. The first subculture done onto blood agar and macconkey agar within 24 hours of receipt along with gram film. These bolttles are reincubated and checked for turbidity twice daily. A final sub culture is made on the seventh day in the same media as before. The blood culture is considered negative if there is no growth on the final subculture. Any growth which occur through the seven day period of incubation is followed and the organism identified based on the colony morphology and biochemical parameters. Antibiotics susceptibility testing is to be done.

Self-contained subculture system

A recent modification of the biphasic blood culture medium is the BD septi-chek system consisting of a conventional blood culture broth bottle with an attached chamber containing a slide coated with agar or several types of agars. To subculture, the entire broth contents are allowed to contact with the agar surface by inverting the bottle, a simple procedure that does not require opening the bottle or using needles. The large volume of broth that is subcultured and the ease of subculture allow faster detection time for many organisms than is possible with conventional systems[6].

Lysis Centrifugation

This system consists of a stoppered tube containing saponin to lyse blood cells, polypropylene glycol to decrease foaming, SPS as an anticoagulant, EDTA to chelate calcium

ions and thus inhibit the complement cascade and coagulation and a small amount of an inert fluorochemical to cushion and concentrate the micro organisms during 30 minute centrifugation at 3000 x g. After centrifugation, the supernatant is discarded, the sediment containing the pathogens is vigorously vortexed and the entire sediment is plated to solid agar[6].

Instrument based systems

Conventional blood culture techniques are labour intensive and time consuming. Instruments can rapidly and accurately detect organisms in blood specimens[6].

Bactec systems

Measures the production of carbon dioxide by metabolizing organisms. Blood or sterile body fluid for routine culture is inoculated into bottles that contain the ^{14}C-labelled substrates (glucose, amino acids and alcohols). The bottles were incubated and often agitated on a rotatory shaker. At predetermined time intervals, the bottles are placed into the monitoring module, where they were automatically moved to a detector. The detector consists of two needles through a rubber septum seal at top of each bottle and withdrew the gas that had accumulated above the liquid medium. Any amount of radio labeled CO_2, the final end product of metabolism of the ^{14}C-labelled substrates was considered to be suspicious for microbial growth[6].

BacT / Alert microbial detection system

This measures CO_2 derived pH changes by a calorimetric sensor in the bottom of each bottle. The sensor is separated from the broth medium by a membrane that is only permeable to CO_2. As organisms grew they release CO_2 which diffuses across membrane and is dissolved in water present in the matrix of the sensor, free hydrogen ions are generated which cause a color change in sensor which is read by the instrument[6].

ESP system

Here microbial growth is detected by the consumption or production of gases as organisms metabolize nutrients in the culture medium. This is detected by monitoring changes in headspace pressure by a sensitive detector that is attached to the blood culture bottles[6].

Vital

A fluorescent molecule that decreases its fluorescence output in the presents of CO2, changes in pH, or modification of oxidation reduction is incorporated in the broth solution and serves as an indicator, detect any organism present in the culture[6].

CSF Culture

Since clinical features of sepsis and meningitis are non-specific in neonates, it is likely that meningitis may be present without specific symptomatology along with sepsis.

In early onset sepsis, a lumbar puncture is indicated in the presence of either a positive blood culture or presence of clinical picture of septicemia.

In situations of late-onset sepsis, a lumbar puncture should be done in all infants with signs and symptoms prior to starting antibiotics. The procedure should be postponed in a critically sick and hemodynamically unstable baby[42].

Urine Culture

In early onset sepsis, urine cultures have a low yield and are not indicated. Although a supra pubic bladder puncture sample or bladder catheterization is recommended in all cases of late onset sepsis, the procedure is painful and the yield is very poor. Urinary tract infection may be diagnosed in presence of one of the following:

>10 WBC / mm3 in a 10 ml centrifuged sample

>100000 organisms / ml in urine obtained by catheterization

Any organism in urine obtained by supra pubic aspiration[42].

Buffy coat smear

This is easy, cheap, rapid and reproducible technique of demonstrating micro-organisms in blood. Buffy coat smear is done by the technique of Faden et al[13]. One ml blood to be withdrawn with aseptic precaution and put into a vial containing 10 mg EDTA – using long sterile aspiration needle and syringe. Blood is transferred into sterile wintrobe tube and centrifuged at 2500 rpm for 15 minutes. The plasma to be removed and buffy coat is taken with sterile pipette. Two smears prepared, one stained by Grams stain and examined under oil immersion[3] and the other stained with acridine orange and examined under oil immersion magnification using fluorescent microscope. Smears are considered positive if two or more organisms are observed within cells.

TREATMENT

Institution of prompt treatment is essential for ensuring optimum outcome of neonates with sepsis who often reach the healthcare facilities late and in a critical condition.

Components of treatment

a) Supportive care

b) Antibiotics

Supportive Care:

The purpose of supportive care is to normalize the temperature, stabilize the cardiopulmonary status, correct hypoglycemia and prevent bleeding tendency. This can be done as follows:

- Provide warmth, ensure consistently normal temperature
- Start intravenous line

- Infuse normal saline 10 ml per kg over 5 to 10 minutes, if perfusion is poor as evidenced by capillary refill time of more than 3 seconds. Repeat the same dose 1 to 2 times over the next 30 to 45 minutes, if perfusion continues to be poor.
- Infuse glucose (10 percent) 2 ml per kg stat.
- Inject vitamin K 1 mg intramuscularly.
- Start oxygen by hood or mask, if cyanosed or grunting.
- Provide gentle physical stimulation if apneic.
- Provide bag and mask ventilation with oxygen if breathing is inadequate.
- Avoid enteral feed if very sick, give maintenance fluids intravenously.
- Consider exchange transfusion if there is sclerema[15].

Antimicrobial Therapy

The choice of antibiotics depend on the prevailing flora responsible for sepsis and their antimicrobial susceptibility.

Empiric antibiotic therapy includes broad coverage, usually a beta-lactam antibiotic and an aminoglycoside[32].

Suggested Antibiotic Regimens for Sepsis[32]

Organism	Antibiotic	Bacteremia	Meningitis
Escherichia coli	Cefotaxime or ampicillin and gentamycin	14 days	21 days
Klebsiella, enterobacter spp	Cefotaxime or cefipime or meropenem and gentamycin	14 days	21 days
Staphylococcus aureus MRSA	Nafcillin Ciprofloxacin or Vancomycin and Amikacin	10 to 14 days	21 days
Coagulase negative staphylococci	Vancomycin	7 days	-
Pseudomonas	Ceftazidime or Piperacilllin / Tazobactam and Gentamycin or Tobramycin	14 days	21 days
Enterococcus	Ampicillin or Vancomycin and Gentamycin	10 days	21 days
Listeria	Ampicillin and Gentamycin	10 to 14 days	21 days
Group B Streptococci	Ampicillin or Penicillin G	10 to 14 days	21 days

Adjunctive Immunotherapy

A variety of adjunctive immunotherapies for sepsis have been trialed since 1980s to address deficits in immunoglobulin and neutrophil number and function. They include:

- Double-volume exchange transfusion.
- Granulocyte infusions.
- Administration of intravenous immunoglobulin.

- ❖ Treatment with G-CSF and GM-CSF.

All these have been investigated with variable results[32].

PREVENTION

Observe recommendations of universal precautions with all patients.

- o Contact
- o Gloves
- o Gowns, mask and isolation as indicated

Nursery design engineering

- ➢ Appropriate nursing : patient ratio
- ➢ Avoid overcrowding and excessive workload
- ➢ Readily accessible sinks, antiseptic solutions, soap and paper towels

Handwashing

- ➢ Improve handwashing compliance
- ➢ Wash hands before and after each patient encounter
- ➢ Appropriate use of soap, alcohol-based preparations or antiseptic solutions
- ➢ Alcohol-based antiseptic solution at each patient bedside
- ➢ Provide emollients for nursery staff
- ➢ Education and feedback for nursery staff

Minimizing risk of CVC contamination

- Maximal sterile barrier precautions during CVC insertion
- Local antisepsis with chlorhexidine gluconate
- Aseptic technique when entering the line
- Minimize CVC days
- Sterile preparation of all fluids to be administered via a CVC

Meticulous skin care

- Encourage early and appropriate advancement of enteral feeding
- Education and feedback for nursery personnel

Continuous monitoring and surveillance of nosocomial infection rates in the NICU[37].

AIM AND OBJECTIVES OF THE STUDY

- To identify the organisms causing neonatal sepsis.
- To find out the rate of isolation of bacteria by blood culture.
- To know the susceptibility pattern of these organisms to institute early and effective antibiotic therapy.
- To detect emerging pattern of resistance in these organisms (MRSA / ESBL) by conventional and molecular methods.
- To estimate the CRP value in these cases.
- To correlate the results of bacterial isolation with CRP.

MATERIALS & METHODS

STUDY DESIGN : Prospective Cohort study.

The present study was carried out in the department of microbiology in collaboration with Institute of Pediatrics, Govt. Stanley Hospital and New born ward of Govt. RSRM Lying- in hospital.

STUDY PERIOD:

Over a period of one year from June 2005 to June 2006.

SAMPLE SPECIFICATION:

Blood samples were taken from 100 clinically suspected cases of neonatal septicemia with the following criteria.

Inclusion criteria

- Neonates suspected to have sepsis.
- Temperature > 99 degree Fahrenheit or < 95 degree Fahrenheit.
- Respiratory rate more than 60 per minute.
- Change in behavior.
- Abnormal cry.
- Not accepting feed.
- Drowsy or unconscious.
- Septic focus on skin or umbilicus.
- Diarrhoea.
- Seizures.

Exclusion Criteria

- Clinically not suspected septic neonates.
- No characteristics indicating probable sepsis.
- Prior antibiotic administration.

METHODOLOGY:

Blood Collection Procedure:

Under strict aseptic precaution the skin was cleansed over the site in a circle approximately 5cm, in diameter with 70% isopropyl alcohol rubbing vigorously. Then 2% iodine or povidone iodine was applied and left on the skin for atleast one minute. One to two ml of venous blood was drawn by venipuncture. The needle was withdrawn and the area of puncture was cleansed with 70% alcohol. About 1ml of blood was inoculated immediately into brain heart infusion broth aseptically with utmost care. Another 1ml of blood was allowed to clot in a dry test tube, serum separated and used for CRP estimation.

Sample Processing:

The inoculated bottles were incubated at 37 degree celcius in humid atmosphere and examined after 24 hrs for any turbidity, discolouration or clotting. The first subculture was done on to Blood agar and MacConkey Agar along with the gram film. These bottles were reincubated and checked for turbidity twice daily. A final subculture was made on the seventh day on the same media as before along with a gram film. The blood culture was considered negative if no growth occured on the final subculture. Any growth which occured through the seven day period of incubation was followed and the organism was identified based on the colony morphology and biochemical reactions.

Reading of Gram Film:

The gram film was examined as soon as subcultures had been setup and the positive films were reported at once to the clinician.

Examination of Subcultures:

The plates were examined after incubating for 18 to 24 Hrs. When any growth was seen, the colony morphology was noted .The colonies were identified by

1. Gram stain - to identify gram positive and gram negative organisms.
2. Hanging drop – to find out motile and non motile organism.
3. Preliminary tests like Oxidase, Catalase were performed.
4. Members of the species were identified based on biochemical parameters using IMViC reaction and sugar fermentation medium.

COLONIAL MORPHOLOGY:

Staphylococcus aureus :

Nutrient agar :-

Showed 1 to 3 mm diameter , circular , smooth, low convex, glistening , densely opaque colonies with golden yellow pigmentation.

Blood agar:-

Colonies were surrounded by a narrow zone of beta haemolysis.

MacConkey agar:-

Colonies were pink and small in size.

Organisms were confirmed with biochemical reactions.

Slide Coagulase Test :-

A staphylococcal colony was emulsified in a drop of saline to form a smooth milky

suspension. Similar suspensions were made with positive and negative control strains. To the suspension, a loopful of plasma was added. Coarse clumping of cocci visible to the naked eye within 10 seconds was considered positive and absence of clumping as negative[30].

Tube Coagulase Test : -

Staphylococcus to be tested was grown in brain heart infusion broth overnight at 37°C. To 0.1ml of this culture, 0.5ml of undiluted plasma was added and incubated at 37°C in a water bath for upto 4 hrs. Tubes were examined at1,2, and 4 hrs for clot formation by tilting the tube through 90°. Any degree of clot formation was considered positive. If the plasma remains wholly liquid it was considered negative.

Biochemical Reactions

Indole	Negative
MR	Positive
VP	Positive
Mannitol	Fermented
Urease	Positive

Slide coagulase and tube coagulase test were performed and those positive were identified as Staphylococcus aureus.

Klebsiella pneumoniae:

Nutrient agar:-

Large,dome shaped , mucoid colonies.

Blood agar:-

Large, greyish white, mucoid colonies.

MacConkey agar:-

Lactose fermenting mucoid colonies.

Species were identified with biochemical reactions

Biochemical Reactions

Indole	Negative
MR	Negative
VP	Positive
Citrate	Utilised
Glucose	Acid with Gas
Lactose	Acid with Gas
Sucrose	Acid with Gas
Maltose	Acid with Gas
Mannitol	Acid with Gas
TSI	Acid / Acid with gas no H_2S
Urease	Positive

Escherichia coli:

Nutrient agar:-

Colonies were 1-3mm diameter, circular, low convex, smooth colonies with no pigmentation and odour.

Blood agar:-

Greyish white colonies with haemolysis.

MacConkey agar:-

Flat, lactose fermenting colonies.

Species were identified with biochemical reaction.

Indole	Positive
MR	Positive
VP	Negative
Citrate	Not utilised
Glucose	Acid with Gas
Lactose	Acid with Gas
Sucrose	Not fermented
Maltose	Acid with Gas
Mannitol	Acid with Gas
TSI	Acid / Acid with gas no H_2S
Urease	Negative

Pseudomonas aeruginosa:

Nutrient agar:-

Colonies were large, low convex with serrated margins, with bluish green pigmentation and earthy odour.

Blood agar:-

Diffuse haemolysis present.

MacConkey agar:-

Non-lactose fermenting colonies with pigmentation.

Organisms were identified with biochemical reactions.

Indole	Negative
MR	Negative
VP	Negative
Citrate	Utilised
Glucose	Oxidatively utilised
Lactose	Oxidatively utilised
Mannitol	Non fermented
Xylose	Oxidatively utilised
TSI	Alkali / no change in the butt
O-F Test	Oxidatively utilized
Urease	Positive

Proteus mirabilis:

Nutrient agar:-

Swarming growth with fishy odour.

Blood agar:-

Swarming growth with fishy odour.

MacConkey agar:-

Non-lactose fermenting colonies.

Species were identified with biochemical reactions.

Indole	Negative
MR	Positive
VP	Negative
Citrate	Not utilized
Glucose	Acid only
Lactose	Not fermented
Sucrose	Not fermented
Maltose	Not Fermented
Mannitol	Not Fermented
TSI	Alkali / Acid with H2S
Urease	Positive

Acinetobacter species:
Blood agar:-

Smooth, opaque, raised, creamy colonies.

MacConkey agar:-

Non-lactose fermenting colonies with a purplish hue.

Indole	Negative
Citrate	Utilised
Glucose	Not fermented
TSI	Alkali / no change in the butt
O-F Dextrose	Positive
Urease	Negative

ANTIMICROBIAL SUSCEPTIBILITY TESTING:

After isolating and identifying the organism their antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion technique.

The test was performed on Muller Hinton agar using commercially available antibiotic discs. Turbidity of the actively growing broth culture was adjusted so that it is optically comparable to that of the 0.5 MacFarland standard.

A sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level.

The dried surface of a Muller Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times rotating the plate approximately 60 degree each time to ensure an even distribution of inoculum. As a final step the rim of the agar was swabbed.

The predetermined battery of antimicrobial discs which included Ampicillin, Gentamycin, Co-trimoxazole, Ciprofloxacin, Cefotaxime, Amikacin and Oxacillin was dispensed onto the surface of the inoculated agar plate. The discs were evenly distributed so that they were not closer than 24mm from center to center. Ordinarily, not more than six discs was placed on a 100mm plate.

The plates were incubated aerobically at 37 degree celcius for 16 to 18 hours. After 16 to 18 hours of incubation, each plate was examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformally circular and there will be a confluent lawn of growth. The diameter of the zones of complete inhibition was

measured, including the diameter of the disc. The sizes of the zones of inhibition were interpreted by referring to the NCCLS standards and reported as either susceptible, intermediate or resistant to the agents that were tested.

Controls used with each batch:-

1. Escherichia coli ATCC 25922

2. Pseudomonas aeruginosa ATCC 27853

3. Staphylococcus aureus ATCC 25923

Detection of Oxacillin/Methicillin Resistant Staphylococcus aureus (MRSA)

Here one microgram oxacillin disc was used[26]. Muller Hinton agar supplemented with an additional 5% NaCl was used and incubated at 35 degree celcius and the result was read as follows

Oxacillin	Susceptible	Intermediate	Resistant
Zone Size	>13mm	11-12mm	<10mm

Molecular Methods

Amplification test like Polymerase Chain Reaction (PCR) to detect the mec*A* gene was performed.

PCR assay for the detection of MecA gene

Extraction of DNA

PROTOCOL

- Cells were pelleted from overnight culture by centrifuging at 12000 rpm, 4°c for 5 minutes.
- Pellets were washed in 0.1 x SSC 5 ml by spinning at 10000 rpm 4°c for 10 minutes.
- The pellet was resuspended in 1 ml of sodium phosphate buffer and lysozyme solution.
- **The samples were then incubated for 1 hour at 37°c.**
- 9 ml of lysis buffer was added to the samples and it was incubated for 45 minutes at 37°c.
- Equal volume of phenol: chloroform was added and the samples were mixed well.
- The samples were then centrifuged at 10000 rpm for 10 minutes.
- The supernatant was transferred to new tube and to it equal volume of chloroform was added. The tubes were again centrifuged for 10 minutes at 10000 rpm.
- The aqueous phase was transferred to new tube and to it 0.1 volume of sodium acetate and 2 volumes of 95% of ethanol was added which led to precipitation of nucleic acid.
- The tubes were then kept for overnight incubation at -20°c.
- The pellets were then washed with 70% ethanol twice and then air dried so that the ethanol evaporates completely.

- The pellets were then suspended in TE buffer and was analysed using agarose gel electrophoresis.

<u>Agarose Gel Electrophoresis</u> (Joseph Sam brook David russel, 2001)[35]

Around 50 ml of 0.8% agarose was prepared in 1 x TAE buffer.

The agarose was properly stirred with a glass rod.

The beaker was heated in microwave oven till the agarose was completely dissolved.

When the agarose solution is 50c, Ethidium Bromide (SIGMA-ALDRICH) was added to a final concentration of 0.5µg / ml. The gel was swirled slowly and it was cast.

While the solution was cooling , the gel - casting tray was assembled. The comb was cleaned with soaked tissue in hot distilled water. A small space (0.5 - 1.0 mm) was allowed between the bottom of the comb and the casting tray.

The agarose solution was poured in the tray.

It was allowed to solidify in room temperature for 30 mins.

The gel casting tray was placed into the electrophoretic chamber which was filled with electrophoretic buffer till it reaches 3-5 mm over the surface of the gel.

The comb was slowly removed.

5µl of the sample was added with 2µl of 6 x loading buffer (xylene caynol - 0.25 w / v) (SIGMA - ALDRICH), Bromophenolblue (HIMEDIA) - 0.25% w / v sucrose (HIMEDIA) - 40% w / v.

The samples were run for 45 mins at 100 v.

The gel was observed under UV transilluminator for presence of DNA.

DNA QUANTIFICATION -

The DNA quantification was done approximately using Ethidium bromide spot test.

PCR

All pcr reaction were carried out in a thermal minicycler manufactured by MJ RESEARCH GERMANY.

FOR MECA

The following sets of primers were used.

Forward 5' CAT TTT GAG TTC TGC ACT ACC 3'

Reverse 5' GCA ATA CAA TCG CAC ATA CAT TAA TAG 3'

The following were the condition adopted for PCR (for mecA gene)

Initial denaturation at 94°C for 5 min.

Cycle denaturation at 94° C for 30 secs.

Primer annealing at 55°C for 1 min and 20 secs

The PCR was carried out for 30 cycles

A final extention at 72°C for 10 mins

The following was the set up for a single 25µl reaction

dNTP mix 20 mM	2.5µl
Taq buffer	2.5µl
Taq enzyme	2.0µl
Template DNA	1.0µl
Forward primer	2.5µl
Reverse primer	2.5µl

Molecular grade water 12.0µl

A negative control was employed which consisted of the PCR set up except the template DNA. All PCR products were stored at - 20°C until further analysis, all PCR products were analysed in 1.5% agarose gel, stained with ethidium bromide and observed under UV transilluminator[14].

C-REACTIVE PROTEIN ESTIMATION

CRP estimation was done using CRP check, latex agglutination slide test kit (Axis diagnostics and biotech).

Principle:

The test is based on the principle of latex agglutination. When latex reagent (polystyrene latex particles quoted with anti-CRP) was mixed with test serum containing CRP at level greater than 0.6 mg / dl it forms visible agglutination. If the concentration of CRP is lesser than 0.6 mg / dl then no agglutination.

Materials required:

Reagents

i) Anti-CRP latex reagent

ii) Positive control

iii) Negative control

iv) Accessories- slides, stirrer rods, droppers.

Sample: Serum

Test Procedure:

i) One drop (50 micro litre) of test sample was pipetted in the ring of the test slide using a disposable dropper.

ii) After shaking gently one drop of CRP latex reagent was added to the sample on the circle of the test slide.

iii) After mixing the sample thoroughly the slide was rocked for 2 minutes and was observed for agglutination under a high intensity light.

Interpretation of results:

Marked agglutination - CRP positive (>0.6 mg / dl)

No agglutination - CRP negative (<0.6 mg / dl)

Semi-quantitative determination:

Sera found positive was retested by preparing serial dilution of the test sample (1:2, 1:4, 1:8 and so on) using 0.9 % sodium chloride solution in clean labeled test tubes as below:

Tube No.	1.	2.	3.	4.	Saline Control
Serum Dilution	1:2	1:4	1:8	1:16	-
Saline	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Patient serum	0.5 ml	-	-	-	-
Transfer diluted Serum from pre-vious tube	-	0.5 ml	0.5 ml	0.5 ml	-

Each dilution was tested according to the qualitative procedure until no further agglutination was observed. The CRP concentration was estimated for the last dilution with visible agglutination.

CRP (mg / dl) = highest dilution with positive reaction x reagent sensitivity (0.6 mg / dl)

The titer for the various dilution is as follows:

Dilution	Titer
1 : 2	1.2 mg / dl
1 : 4	2.4 mg / dl
1 : 8	4.8 mg / dl

The results were observed, recorded and analysed.

RESULTS

The study was performed during June 2005 to June 2006 at Department of Microbiology, Govt. Stanley medical college & Hospital. The study group included 100 cases of suspected neonatal septicemia. The blood samples were taken prior to antibiotic administration.

TABLE -1

Sex distribution of cases (n=100)

Total	**100**	**Percentage**
Male	61	61%
Female	39	39%

TABLE -2

Age distribution of cases (n=100)

Total	**100**	**Percentage**
Term	59	59%
Pre-term	41	41%

TABLE -3

Occurrence of septicemia vs age of cases (n=100)

Age	Number of cases	Percentage
0-7 days	54	54%
8-14 days	29	29%
15-28 days	17	17%

TABLE - 1,2&3

Among the selected cases 61 (61%) were male infants and 39 (39%) were female infants. 59 (59%) were term neonates and 41 (41%) were preterm. 54 (54%) belong to early onset sepsis and 46 (46%) belong to late onset sepsis.

TABLE -4

Weight distribution of cases

Weight	1000-1500gms	1500-2000gms	2000-2500gms	>2500gms
No of cases	9	23	46	22
Percentage	9%	23%	46%	22%

TABLE -5

Place of delivery - Hospital vs Home

Place of delivery	Number	Percentage
Hospital	79	79%
Home	21	21%

TABLE -6

Clinical profile of neonates with sepsis (n=100)

S.No	Clinical Features	Percentage
1	Refusal of feeds	86%
2	Lethargy	53%
3	Diarrhea	12%
4	Vomiting	11%
5	Poor cry	68%
6	Abdominal distension	34%
7	Fever	27%
8	Respiratory distress	58%
9	Convulsion	4%
10	Jaundice	12%

TABLE - 4 &6

In the present study 78 (78%) of the neonates belong to low birth weight category(Birth weight < 2500g). The presenting symptoms in most of the neonates in our study were refusal of feeds, poor cry, lethargy, respiratory distress, abdominal distension, diarrhea and vomitting.

TABLE -7

Results of blood culture (n=100)

Result of Blood Culture	Number of cases	Percentage
Positive	47	47%
Negative	53	53%

TABLE -8

Organism isolated by Blood culture in neonates with sepsis (n=47)

Name of the Organism isolated in blood culture	Number	Percentage
Staphylococcus aureus	20	43%
Klebsiella pneumoniae	11	23%
Escherichia Coli	7	15%
Coagulase negative staphylococci	5	11%
Pseudomonas aeruginosa	2	4%
Acinetobacter species	1	2%
Proteus mirabilis	1	2%

TABLE- 9

Sensitivity pattern of various organisms isolated from neonates with sepsis (n=47)

TABLE - 7&8

47 (47%) were blood culture positive. The commonest organism isolated was Staphylococcus aureus and was present in 20 cases (43%), Klebsiella pneumoniae was isolated in 11 cases (23%) , Escherichia coli in 7 cases (15%), Coagulase negative staphyloccocus in 5 cases (11%), Pseudomonas aeruginosa in 2 cases (4%), Acinetobacter species in 1 case (2%) and Proteus mirabilis in 1 case (2%)

TABLE -10

Methicillin resistant Staphylococcus aureus by disc diffusion method vs PCR (n=20)

MRSA detection methods	Disc diffusion using 1microgram oxacillin	PCR for Mec A gene
Positive	9	9
Negative	11	11
Total	20	20

Out of the 20 Staphylococcus aureus isolated 9 were found to be MRSA which was detected by disc diffusion using 1microgram Oxacillin and by PCR for mecA gene with similar results.

TABLE -11

Correlation of CRP positivity with Culture positivity (n=100)

CRP test	Control	Bacteriologically	
		Positive	Negative
Positive	0	45 (95.74%)	7 (13.21%)
Negative	25	2 (4.26%)	46 (86.79%)
Total	25	47	53

CRP estimation was found to have a sensitivity of 96%, specificity of 87%, positive predictive value of 87% and negative predictive value of 96%

TABLE -12

Analysis of Clinical outcome/ Mortality in neonates with sepsis (n=100)

	CULTURE	
	Positive	Negative
Total No of Cases	47	53
Death	5	0
Baby well at time of discharge	42	53

TABLE - 13

Correlation of Mortality with type of organism (n=5)

Name of the Organism	Number	%
Klebsiella Pneumoniae	2	40%
Escherichia coli	1	20%
Staphylococcus aureus	1	20%
Pseudomonas aeruginosa	1	20%

TABLE - 12 &13

There were a total of 5 deaths in the study, out of which 4 deaths were due to gram negative septicemia.

DISCUSSION

In the present study, 100 neonates suspected of having septicemia were investigated.

A culture positivity rate of 47% was observed. The study of Arora et al obtained a culture positive rate of 46.8% and Roy et al observed a culture positive rate of 47.5%. Anitha sharma et al reported the rate of 20% and S.P.Khatua et al reported 59.8%. Other workers obtained the culture positive rate varied between 40% to 75%. Squire et al reported 7 cases with negative culture, who had fatal outcome and postmortem evidence of infection and hence negative blood cultures do not exclude sepsis[2,4,12,22,48,52].

Out of hundred cases in our sutdy, 61% were males and 39% were females. S.P.Khatua et al reported that culture positivity is more common in males ranging from 59-82%. The male infants in his study constituted 70.7%. Piyush Gupta et al and Anitha Sharma et al reported a male predominance of 64.7% and 74% respectively[2,40,46,49].

The factors regulating the synthesis of gamma globulins are probably situated on the X chromosome.Presence of one X chromosome in the male infant thus confers less immunological protection compared to the female counterpart[46].

In our study, neonatal septicemia was common in low birth weight neonates.

S.P.Khatua et al reported a higher incidence of septicemia in low birth weight infants. Higher incidence and mortality of low birth weight infants were also observed by other workers[7,18,20,46,51].

Low birth weight infants, both premature and term small for date infants have low IgG and they are more susceptible to infections. While placental transport of IgG from maternal to fetal circulation increases with maturity, this transport is hampered in small for date infants who are often the products of placental insufficiency[46].

In the present study, 86% of neonates had refusal of feeds as the main presenting symptom. Lethargy 53%, diarrhea 12%, vomiting 11%, poor cry 68%, abdominal distension 34% , fever 27%, convulsion 4%, jaundice 12% were the other observed finding.

N.Sinha et al observed that in gram negative infection, diarrhea, dehydration, abdominal distension, refusal to take feed, shock/circulatory failure were important presenting features[36].

S.P.khatua et al observed that refusal of feeds, lethargy, diarrhea, hypothermia, abdominal distension, jaundice and vomiting were the most common presenting feature[46].

Anitha Sharma et al reported the common symptoms to be refusal of feeds(76%) lethargy (60%) and temperature changes (52%)[2].

R.S. Jaswal et al observed that 66% of neonates with sepsis presented with refusal of feeds, followed by lethargy and jaundice which is similar to the observations of Guha et al[18,41].

Thus symptomatology given by various authors are generalized and does not pertain to a particular system. The frequently observed symptoms are refusal of feeds, poor cry, lethargy and respiratory distress. In essence symptomatology of septicemia is non-specific and multi systemic and hence high index of suspicion in appropriate situations is the only means of early

diagnosis.

54% of neonates were affected by gram positive organisms and 46% by gram negative organisms. In the present study Staphylococcus aureus and Klebsiella pneumoniae were the predominant organisms causing septicemia.

Karthikeyan et al reported that Staphylococcus aureus was the predominant pathogen followed by Klebsiella pneumoniae which correlates well with our present study[24].

Renuka Mohanty et al reported that staphylococcus aureus is a major cause of neonatal septicemia[44.]

Roy et al observed that the most frequent offender in neonatal sepsis were Klebsiella species followed by Enterobacter species, Coagulase negative staphylococci, Staphylococcus aureus and Escherichia coli[22].

Mandira Banerjee et al reporded an out break of neonatal septicemia with multi drug resistant Klebsiella pneumoniae[31].

According to Ohlsson et al the most commonly isolated bacteria were Escherichia coli, Klebsiella and Staphylococcus aureus[38].

Chaudhury et al reported that the ratio of gram positive to gram negative bacteremia was 1:1[10,45].

Monga et al reported that though Klebsiella showed a fall in incidence it still remained the commonest gram negative organism isolated. The incidence of Escherichia coli, Pseudomonas and Staphylococcal infections also doubled when compared to previous years[25].

The frequency of infection by various organisms varies from one institution to another and even from year to year in the same institution and also depending upon whether it is a early onset or late onset sepsis.

So in the present study 54% were early onset sepsis and 46% were late onset sepsis. In early onset sepsis most of the organism isolated was gram negative bacteria like Klebsiella and Escherichia coli. However in some cases Staphylococcus aureus was also isolated. In case of late onset sepsis it was predominantly due to gram positive organisms like Staphylococcus aureus and Coagulase negative staphylococci.

There were a total of 5 Coagulase negative staphylococci isolated in our study. Due to technical difficulties only one blood sample was taken. Since all babies had clinical features suggestive of sepsis and neonates also have one or more immunological deficiencies, hence Coagulase negative staphylococci was considered to be a pathogen in these cases.

In our study there was a total of 20(43%) Staphylococcus aureus isolated. Out of which 9(45%)were methicillin resistant.

The drug resistance patterns of Staphylococcus aureus to other drugs were as follows- Ampicillin-14(70%),Gentamycin-12(60%),
Co-trimoxazole-18(90%), Cefotaxime-10(50%), Amikacin 10(50%) and Ciprofloxacin-11(55%).

According to the latest report from the national nosocomial infection surveillance system approximately 60% of all Staphylococcus aureus nosocomial infections in intensive care units were methicillin resistant[11].

Karthikeyan et al reported that 66% of the staphylococcus aureus isolated from cases of neonatal septicemia was methicillin resistant[24].

The degree of resistance or sensitivity of Staphylococcus aureus towards commonly used antibiotics is diverse from region to region it is inevitable to look for MRSA in every Staphylococcus aureus isolated[33].

MRSA detection was done by 2 methods such as disk diffusion using one micro gram oxacillin disk and PCR for mecA gene. Similar results were obtained by the two methods in our study.

Gunter kampe et al reported that all mec A positive isolates were correctly identified as oxacillin resistant by using one micro gram oxacillin disk[19].

According to Flayhart et al detection of MRSA by Oxacillin screen agar and mec A PCR was 94.3% and 95.1% respectively[11].

In the present study the antibiotic sensitivity pattern of the gram negative organisms especially Klebsiella shows multiple drug resistance (Ampicillin, Gentamycin and co-trimoxazole). However majority of strains were sensitive to third generation cephalosporins. ESBL's were not detected in our study.

According to Mandira Banerjee et al the isolates of Klebsiella pneumoniae were resistant to all drugs except third generation cephalosporins[13].

The sensitivity pattern has been found to be different in different studies in different parts of the same country as well as at different times in the same hospital and this is due to the frequent emergence of resistant bacteria, a difficult problem in controlling neonatal septicemia[46].

CRP estimation was done and compared with the results of blood culture and the following results were obtained.

Sensitivity	-	96%,
Specificity	-	87%
Positive predictive value	-	87%
Negative predictive value	-	96%.

The p value was 0.001 which means that there is very good agreement between the two tests in our study.

According to Anitha Sharma et al the sensitivity and specificity of CRP was 80% and 93.8% respectively[2].

Anuradha et al reported a sensitivity of 100%, Specificity of 87.3%, Positive predictive value of 88.3% and Negative predictive value of 100% which correlates well with our present study[3].

The diagnostic value of CRP has been evaluated by many authors. some studies showed that CRP increases very early after the onset of infection. In addition these proteins are also helpful in following the course and prognosis of the disease. The early evaluations of CRP provide indications of the response to the treatment. A good response to antibiotics is indicated by a rapid return to normal of CRP, whereas persistent elevations of serum CRP suggest that the treatment is inadequate[28].

In the present study, the mortality due to gram negative septicemia was more. Out of 5 deaths, 4(80%) were due to gram negative organisms.

N.Sinha et al reported that the fatality rate was high in neonates and in infections with gram negative bacilli about 83%. This was in conformity with Gotoff et al, Bhakoo et al and Mishra et al[8,9,17,34,36].

Hence the mortality rate in neonatal septicemia is age and etiological agent dependant[16].

SUMMARY

1. The study was done at department of microbiology during June 2005 to June 2006. The study group included 100 suspected cases of neonatal septicemia. There were 61(61%)males and 39(39%) females in the clinically diagnosed cases of septicemia. 54(54%) cases were less than one week of age and 46(46%)cases belong to the age group of 8-28 days
2. 41(41%) cases were preterm and the rest 59(59%) cases were term neonates.78(78%) of the cases belonged to the low birth weight group(weight ranged from 1000gm - 2500gm)
3. The presenting symptoms in the present study were refusal of feeds, poor cry, lethargy, respiratory distress, abdominal distension, fever, diarrhea, vomiting and jaundice.
4. Blood culture was found to be positive in 47(47%) of clinically diagnosed cases of septicemia.The organisms isolated were Staphylococcus aureus 20(43%), Klebsiella pneumoniae 11(23%), Escherichia coli 7(15%), Coagulase negative staphylococci 5(11%), Pseudomonas aeruginosa 2(4%),Acinetobacter species 1 (2%) and Proteus species 1 (2%).
5. Antibiotic sensitivity pattern was observed for these organisms. Most of the organisms were suceptible to cefotaxime and amikacin. No ESBL's were detected.
6. Among the gram positive organism Staphylococcus aureus, 9(45%) of the strains were resistant to methicillin. This was detected by two methods - disk diffusion using one microgram oxacillin and PCR for mec A gene
7. Rapid test such as CRP assay was evaluated. A value greater than 0.6 mg/dl was considered positive. The test had a sensitivity of 96%, specificity of 87%, positive predictive value of 87% and negative predictive value of 96%.

8. The clinical outcome of the neonates included in the study was determined. There were 5 deaths out of which 4 deaths were due to gram negative septicemia.

CONCLUSION

1. Neonatal septicemia is a leading cause of morality and morbidity in neonates in our country.
2. Male infants are more commonly affected than female infants.
3. Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Coagulase negative staphylococci, Pseudomonas aeruginosa, Acinetobacter species and Proteus species are the common organisms isolated by blood culture.
4. Change in antibiotic sensitivity pattern was observed.Cefotaxime and amikacin are highly sensitive than other drugs.
5. Increased incidence of MRSA has been found.
6. Mortality rate high among low birth weight and preterm and also high in gram negative septicemia.
7. CRP estimation is a rapid , highly sensitive and specific test for early diagnosis and management of neonatal sepsis.
8. Early diagnosis will help the clinician to institute the antibiotics promptly which will help in reducing the morbidity and mortality

BIBLIOGRAPHY

1. A. Huletsky et al: New Real – time PCR assay for Rapid detection of methicillin – resistant staphylococcus aureus directly from specimens containing a mixture of staphylococci. Journal of clinical microbiology. Vol.42: pages 1875 – 1884, May 2004.

2. Anita Sharma, C .V. Krishnakutty, Uma Sabharwal, Sushila Rathee and Harash Mohan: Evaluation of Sepsis screen for diagnosis of neonatal septicemia. Indian Journal of Pediatrics. Vol. 60: pages 559 – 563, 1993.

3. Anuradha De, Kari Saraswathi, Alka Gogate and Kalyani raghavan: C-Reactive protein and Buffy coat smears in early diagnosis of childhood septicemia. Indian Journal of Pathology – Microbiology. Vol.41(1): pages 23 – 26, 1998.

4. Arora, J. Jaitwani: Acinetabacter spp – An emerging Pathogen in Neonatal Septicemia in Amritsar. Indian Journal of Medical microbiology. Page 81, January 2006.

5. Avery's Disease of the Newborn – 7th Edition Pg 490-512.

6. Bailey and scott's Diagnostic microbiology, 11th Edition, Pg 871- 879.

7. Bavikatte K, Schreiner RL, Lemons JA, Gresham EL. Group D streptococcal septicemia in the neonate. Amer. J Dis. Childhood. 113 : 493 – 496. 1979.

8. Bhakoo ON, Agarwal KC, Narang A, Bhattacharya S: Prognosis and treatment of neonatal septicemia a clinico bacteriological study of 100 cases. Indian Pediat. 11 : 519, 1974.

9. Bhakoo ON, Agarwal KC, Mohoni mahajan C, Walia BNS. Septicemia in infants and children – a bacteriological study. Indian Pediat 5 : 518, 1968.

10. Chaudhury A, Rao TV. Bacteraemia in a tertiary care urban hospital in South Inda, Indian J Pathol Microbiol. 42 : 317 – 320, 1999.

11. Diane Flayhart, Janet F. Hindler, David A. Bruckner, Geraldine Hall,Rabin K. Shrestha, Sherilynn A. Vogel, Sandra S. Richter, Wanita Howard and Karen C. Carroll : Multicenter evaluation of BBC CHROM agar MRSA medium for surveillance cultures of the anterior nares. Journal of Clinical microbiology, Vol.43: pages 5536 – 5540, 2005.

12. Edward Squire, Blaise Favara and James Todd : Diagnosis of neonatal bacterial infection – Hematological and Pathologic findings in fatal and non-fatal cases. Pediatrics Vol.64: pages 60 -64, 1979.

13. Faden Hs: Early diagnosis of neonatal bacteremia by buffy – coat examination. Journal of Pediatrics Vol. 88: pages 1034 – 1034, 1976.

14. George E.Killgore : A 5' Nuclease PCR (Taq, Man) High – Throughput Assay for detection of the Mec A Gene in staphylococci. Journal of clinical Microbiology. Vol.38: pages 2516 – 2519.

15. Ghai Essential Paediatrics – 6th Edition Pg. 161-163.

16. Ghosal SP, Chaudhuri M, Dutta N, Sarkar Ak, Mukherjee AK, Sen Gupta PC . Noma neonatorum. Indian Pediatrics. 14 : 709, 1977.

17. Gotoff SP, Behrman RE . Neonatal septicemia, J Pediat 76 : 142, 1970.

18. Guha DK, Dalbir J, Krishna MS, Guha AR, Khatri RL, Srikumar R . Outcome of neonatal septicemia, clinical and bacteriological profile, Indian Pediatrics, 15: 423-427, 1978.

19. Gunter Kampe, Christopher lecke, Ann-Katrin cymbal, Klaus weist and Henning ruden : Evaluation of Mannitol salt agar for detection of Oxacillin resistance in staphylococcus aureus by Disc diffusion and Agar screening. Journal of clinical microbiology. Pages 2254 – 2257, August 1998.

20. Hemming VG, Overall JC, Britt MR . Nosocomial infection in a newborn intensive care unit. N. Eng. J. Med. 294; 1310-1316, 1976.

21. Hong Fang et al: Rapid screening and Identification of Methicillin Resistant

Staphylococcus aureus from clinical samples by Selective-Broth and Real time PCR assay. Journal of clinical microbiology. Vol.41: pages 2894 – 2899, July 2003.

22. I Roy, A. Jain, M. Kumar, SK Agarwal : Bacteriology of neonatal septicemia in a tertiary care hospital of northern India. Indian Journal of Medical Microbiology. Vol.20(3): pages 156 – 159, 2002.

23. Indu Shukla, Solmaz Siddiqui, Sed manazir Ali : Acinetobacter sepsis in neonates, Indian J. Pathol Microbiology Vol. 49, Pg 59, 2006.

24. Karthikeyan G., Premkumar K : Neonatal sepsis – Staphylococcus aureus as the predominant pathogen. The Indian Journal of Paediatrics, Vol. 68, Issue 8, Pg. 715 – 717, 2001.

25. K. Monga, A. Fernandez and L. Deodhar : Changing bacteriological patterns in Neonatal Septicemia. Indian Journal of Pediatrics Vol.53: pages 505 – 508, 1986.

26. K.Rajaduraipandi, KR. Mani. K. Paneerselvam, M. Mani, M. bhaskar, P. Manikandan : Prevalence and antimicrobial susceptibility pattern of methicillin resistant staphylococcus aureus : A multicentre study. Indian Journal of Medical Microbiology. Vol.24 (1): pages 34 – 38, 2006.

27. Leibovich M, Gale R, Slater PE. : Usefulness of gastric aspirate examination in the diagnosis of neonatal infection. Trop Geogr Med. Vol. 39: 1987.

28. Leon Sann: Acute phase proteins for diagnosis and follow-up of neonatal infections. Indian Journal of Pediatrics Vol.53: pages 8 – 9, 1986.

29. M.B.Pepys, R. G. Gaines Das: International collaborative study of preparation of human C-reactive protein positive human serum. WHO / BS / 86 - 1514, 1986.

30. Mackie and Mc Cartney Practical Medical Microbiology. 14th Edition, Pg 255-256.

31. Mandira Banerjee, Krishna Sahu, S. Bhattacharya, sati Adhya, P. Bhownick and P. Chakraborty : Outbreak of Neonatal septicemia with multi drug resistant klebsiella

pneumoniae. Indian Journal of Pediatrics. Vol.60: pages 25 – 27, 1993.

32. Manual of Neonatal Care – John P . Cloherty 5th Edition Pg. 288 – 293.

33. Mary V. Jesudason, W. Selwyn Anandaraj and P. Jegadeesa . Incidence of Methicillin resistant coagulase positive and coagulase negative staphylococci in blood cultures : Indian J. Med. Res. 105, pp 155-157, April 1997,

34. Mishra JN, Rai MG, chakraborty s, Prasad S. . Study of neonatal septicemia. Indian Pediatri 22 : 281, 1985.

35. Molecular Cloning, A Laboratory Manual, Sambrook and Russel, 3rd Edition. Vol.2: pages 8.20 – 8.22.

36. N.Sinha, A. Deb and A. K. Mukherjee : Septicemia in neonates and early infancy. Indian Journal of Pediatrics Vol.53: pages 249 – 256, 1986.

37. Nelson Text book of Paediatrics – 17th Edition Pg 623 – 640.

38. Ohlsson A, Bailey T, Takieddine F : Changing etiology and outcome Acta Pediatrics Scand Vol.75: pages 540 – 544, 1986.

39. Philip AG & Hewitt JR: Early diagnosis of neonatal sepsis. Pediatrics Vol. 65: pages 1036 – 1041, 1980.

40. Piyush Gupta, M. V. Murali, M.M.A. faridi, P.B. Kaul, V.G. Ramachandran and V. Jalwar : Clinical profile of klebsiella septicemia in neonates. Indian Journal of Pediatrics. Vol.60: pages 565 – 572, 1993.

41. R.S. Jaswal, R.k. Kaushal, Asha Goel and Kushla Pathania : Role of C-Reactive Protein in deciding duration of antibiotic therapy in neonatal septicemia. Indian Pediatrics Vol.40: pages 880 – 883, 2003.

42. Rajiv Aggarwal, Nupur sarkar, Ashok K, deorari and Vinod K Paul : Sepsis in the newborn. Indian Journal Pediatrics. Vol.68(2): pages 1143 – 1147, 2001.

43. Ralpha A.Franciosi and Blaise E.Favara: A single blood culture for confirmation of the diagnosis of neonatal septicemia. American Journal. Clinical Pathology Vol.57: pages 215 – 219, 1972.

44. Renuka Mohanty, Subhranshu Sekhar Kar : Rold of clinical signs in the diagnosis of late onset neonatal septicemia. Proceedings of the conference PEDICON 2004, Pg 186.

45. S.Bhattacharya: Blood culture in India: A proposal for a national programme for early detection of sepsis. Indian Journal of Medical Microbiology. Vol. 23(4): pages 220 – 226, 205.

46. S.P.Khatua, A.K. Das, B.D. Chaterjee, S. Khatua, B. Ghose and A. Saha : Neonatal Septicemia. Indian Journal of Pediatrics Vol. 53: pages 509 – 514

47. Sarkar S, Bhagat I, Decristotaro JD, Wiswell TE, Spitzer AR. : A study of the role of multiple site blood cultures in the evaluation of neonatal sepsis. Journal of Perinatology. Vol.10: page 1038, November 2005.

48. Saxena S, Shrivastava JR, Goswami P. . Bacterial infections of newborns. Pediatr Clin India. 6 : 63-67, 1971.

49. Schaffer AJ. Septicemia Diseases of the newborn. 4th ed. Philadelphia. W.B. Sunders Company, pp 776, 1977.

50. Sharma M : Bacteraemia in children. Indian Journal of Pediatrics. Vol.69: pages 1029 – 1032, 2002.

51. Siegel JD, McCracken GH. Jr . Group D streptococcal infection. J. Pediatr. 93 : 542-543. 1978.

52. Somu N, Vasanthkumar Shetty M, Moser CG . A critical analysis of septicemia in newborn. Indian Pediatrics. 13 : 443-447, 1976.

53. Text Book of Laboratory Diagnosis – Todd and Stanford (4th edn).

54. William E. Benitz, Michael Y. Han, Ashima Madan and Pramela Ramachandra : Serial Serum C- reactive protein levels in the diagnosis of neonatal infection. Pediatrics Vol. 102 : 1998.

PROFORMA

Name : Age : Sex : I.P. No. : S.No.

Informant : Income :

Main Complaint :

1)

2)

HISTORY

a) Prematurity

b) Prolonged rupture of membrane more than 24 hr.

c) Maternal fever more than $100^{O}F$

d) Chorioamnionitis

e) Hospitalisation more than 7 days

f) FB like ET tube, chest drain, IV cannula, Ryle's tube

g) History of DM, HT, Syphilis

h) Maternal immunization

i) Birth weight

j) Present weight

k) Preterm or term

l) Mode of delivery by natural forceps or LSCS

m) Place of Delivery Home / Hospital

n) Resuscitation

o) Meconium aspiration

p) H/o native medicine

q) H/o. Blood transfusion

r) H/o. breast feed / artificial feed

SYMPTOMS / SIGNS

a) Apgar score
b) Poor feeding / abnormal cry
c) Vomitting
d) Lethargy / irritability
e) Temperature instability
f) Jaundice
g) Cyanosis
h) Convulsions
i) Abdominal distension
j) Hepatospleenomegaly
k) Bleeding diathesis
l) Respiratory rate more than 60/min
m) Heart rate more than 160/min or less than 100/min
n) Oliguria
o) Hypotension

IMPRESSION

a) Nature of the specimen
b) Media for culture
c) Growth in the
    1. Nutrient agar plate
    2. Blood agar plate
    3. Macconkey agar plate
    4. Gram stain
    5. Motility
    6. Oxidase
    7. Catalase
    8. Coagulase test
    9. Indole

10. Methyl red test
11. Voges-proausker test
12. Citrate test
13. TSI
14. Urease
15. Fermentation of sugars – glucose, lactose, sucrose, maltose and mannitol.
16. Antibiotic sensitivity testing :

RESULT OF THE BLOOD CULTURE TEST

C.R.P. ESTIMATION:

Antibiotics administered :

Condition at the time of discharge :

KEY TO MASTER CHART

B/O	-	Baby of
Mch	-	Male child
Fch	-	Female child
T	-	Term
PT	-	Pre-term
Kleb pneu	-	Klebsiella pneumoniae
Esch-coli	-	Escherichia coli
S.aureus	-	Staphylococcus aureus
CONS	-	Coagulase negative staphylococci
Pseudo	-	Pseudomonas aeruginosa

APPENDIX

1. Brain Heart Infusion broth:-

	Gm/Litre
Calf brain infusion	200
Beef heart infusion	250
Proteose peptone	10
Dextrose	2
Sodium chloride	5
Disodium phosphate	2.50

Add the contents and dissolve by heating.

Adjust the pH to 7.4 $\pm$ 0.2

Autoclave at 121°C for 15 minutes.

2. Peptone Water:-

Peptone	10 g
Sodium chloride, NaCl	5 g
Water	1 litre

Dissolve the ingredients in warm water, adjust the pH to 7.4-7.5 and filter. Distribute as required and autoclave at 121°C for 15 min.

3. MacConkey agar:-

This is a useful medium for the cultivation of enterobacteriacea. It contains a bile salt to inhibit non-intestinal bacteria and lactose with neutral red to distinguish the lactose-fermenting coli forms from the non-lactose fermenting salmonella and shigella groups. The concentration of sodium taurocholate may be reduced to suit less tolerant organisms. The omission of sodium chloride from the medium prevents the spreading of Proteus colonies.

Peptone	20 g

Sodium taurocholate, commercial	5 g
Water	1 litre
Agar	20 g
Neutral red solution, 2% in 50% ethanol	3.5 ml
Lactose, 10% aqueous solution	100 ml

Dissolve the peptone and taurocholate (bile salt) in the water by heating. Add the agar and dissolve it in the steamer or autoclave. If necessary, clear by filtration. Adjust the pH to 7.5. Add the lactose and the neutral red, which should be well shaken before use, and mix. Heat in the autoclave with 'free steam' (c. 100^{o} C) for 1hr., then at 115^{o} C for 15 min. Pour plates.

4. Nutrient agar:-

	Gm/L
Peptic digest of animal tissue	5.00
Beef extract	1.50
Yeast extract	5.00
Agar	15.00

Dissolve the contents in water and mix by heating Autoclave at 121^{o} C for 15 minutes. Adjust pH to 7.4 $\pm$ 0.2. Pour 20-25 ml of 9 cm dia. Petridishes to give 4 mm thickness.

5. Blood agar:-

Sterile sheep blood	50 ml
Peptone	10 g
Beef extract	3g
Sodium chloride	5 g
Distilled water	1000 ml

Autoclave the nutrient agar base at 121° C for 15 minutes and blood with sterile precautions and distribute in Petri dishes.

6. Muller Hinton agar:-

Beef infusion	300	ml
Casein Hydrolysate	17	gm
Starch	1.5	gm
Agar	10	gm
Distilled water	1000	ml

Emulsify the starch in a small amount of cold water, pour into the beef infusion and add the casein-Hydrolysate and the agar. Make up the volume to 1000 ml (1 litre) with distilled water. Dissolve the constituents by heating gently at 100° C with agitation.

Adjust the pH to 7.4. Dispense in screw-capped bottles and sterilize by autoclaving at 121 ° C for 20 minutes. 20 to 25 ml of it is poured into petridishes of 9 cm diameter to give a thickness of 4mm.

7. McFarland's Turbidity Standard for inoculum preparation

A Barium sulphate 0.5 McFarland standards was prepared as follows

1. 1. A 0.5 ml of 0.048mol/L of Barium chloride was added to 99.5 ml of 0.18 mol/L of H_2SO_4

with constant stirring to maintain a suspension.

2. 2. Correct density of the turbidity standard was verified by using a spectrophotometer. The absorbance of 625nm should be 0.08 to0.10 for the 0.5 McFarland standards.
3. The Barium sulphate suspension was transferred in 4-6 ml to a screw capped tube of the same size as those used in growing or diluting the bacterial inoculam.
4. These tubes were tightly sealed and stored in the dark at room temperature.
5. The Barium sulphate turbidity standard was vigorously agitated before each use and inspected for a uniform turbid appearance.

DNA EXTRACTION

Reagents required:

a) TE buffer:

Chemicals	Stock conc.	Final conc.	pH
Tris HCL	10 mM	1 M	8
EDTA	1 Mm	0.5 M	8

Mix 1 ml Tris HCL pH 8 and 0.5 ml of 0.5 M EDTA pH 8 and make the volume to 100 ml.

b) TAE buffer - 10 X:

242 gm of Tris base
57.1 ml of glacial acetic acid
100 ml of 0.5 EDTA

c) 0.1 M Sodium phospate buffer:

CHEMICALS	Stock conc.	Final conc.
Disodium hydrogen phosphate	0.1 M	05.01 M
Sodium dihydro orthophosphate	0.1	0.01 M
Sucrose	20%	20%

d) Lysis buffer - 50 ml

10 mM Tris HCL
1 mM EDTA
500 mg proteinase k
1% SDS

e) Lysozyme - 3 mg / ml
f) 3 M Sodium acetate acid

g) Chloroform: phenol (1 : 1)
h) 70% and 95% Ethanol

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