



Early Onset Neonatal Sepsis in a Tertiary Care Centre

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Abstract

Sepsis prevails to be a major threat for neonates. Neonatal sepsis is an important cause of mortality and morbidity in neonatal intensive care units. It is responsible for 30-50% of the total deaths in developing countries. One year study was conducted in the Dept of microbiology, Govt. medical college, Thrissur, Kerala from July 2012 to June 2013 to find out the different species of bacteria causing sepsis in neonates and the antibiotic susceptibility pattern of the isolates. A total no of 230 blood samples were collected from neonates with clinical signs and symptoms of sepsis admitted within 72 hours of life in the newborn nursery of New Medical College Hospital, Thrissur. Culture positivity was 30% and the predominant species of bacteria isolated are staphylococcus aureus [34.8%] and Klebsiella pneumoniae [27.33%], Acinetobacter baumannii [4.35%]. CRP was positive in 68% of cases. Initial empirical therapy with cefazolin and Amikacin was given followed by administration of appropriate antibiotics based on antibiotic susceptibility pattern. Case fatality rate was 14.3% in this study.

Keywords: *sepsis, antibiotic susceptibility testing.*

Introduction

Neonatal sepsis is a clinical syndrome of bacteremia characterised by systemic signs and symptoms of infection in the first month of life. The term early onset sepsis (EOS) and late onset sepsis (LOS) refer to the age of onset of infection in the neonatal period. Originally divided arbitrarily as infections occurring before and after 1 week of life, it is more useful to separate early and late onset infections according to peripartum pathogenesis. Early onset of infections are acquired before or during delivery. Late onset

infections are acquired after delivery in the normal new born nursery, Neonatal ICU or the community. The age of onset depends on the timing of vertical transmission and the virulence of the infecting organism.

Materials and Methods

Study Design: Cross Sectional Study

Duration of study: 1 year

Study population: All neonates with signs and symptoms of sepsis admitted during the study period

Methodology

A total of 230 blood samples were collected from neonates with clinical signs and symptoms of sepsis admitted within 72 hours of life in the inborn nursery of New Medical College Hospital of Govt. Medical College, Thrissur.

Collection of blood samples

The vein from which the blood was to be drawn was chosen before skin disinfection. A soft tubing tourniquet was applied to the upper arm to enable the veins to be seen and felt. Using 70% alcohol, the skin over the venipuncture site was cleansed in a circle approximately 5 cm in diameter. It was allowed to air dry. 2% tincture iodine was then applied and allowed to dry for 1 minute. A sterile disposable syringe was used and with the thumb of the left hand holding down the skin below the puncture site, the venipuncture was made with the bevel of the needle, directed upwards in the line of the vein. The tourniquet was released after collecting 2 ml of blood. The needle was removed and the puncture site was pressed with a piece of sterile dry cotton. The site was cleansed with 70% alcohol. The needle was removed from the syringe and 1 ml of blood was inoculated directly into blood culture bottle containing 10 ml of brain heart infusion broth. This was transported immediately to the laboratory and was incubated at 37 degree centigrade. The remaining 1 ml of blood was transferred to a sterile penicillin bottle and was used for detecting CRP. After overnight incubation, subcultures were performed by removing a few drops of the well mixed medium and spreading this inoculum on to a blood agar and Mac Conkey agar plate. The plates were examined at the end of 24 and 48 hours for growth and colony characteristics.

Identification of bacteria

Identification of bacteria was based on staining and cultural characteristics as well as biochemical reactions of the isolates using standard laboratory procedures. The Antibiotic sensitivity testing was

done using the disk diffusion method as per CLSI guidelines.

Identification of fungi

Ear discharge collected using sterile cotton swab was inoculated into Sabouraud's dextrose agar slope. One tube was incubated at room temperature and the other at 37 degree centigrade. The tubes are examined after 24 hours and 48 hours. When growth is observed, wet mount preparation with lactophenol cotton blue was done to identify the morphology of the fungus. When budding yeast cells were found, germ tube test was performed to differentiate *Candida albicans* and non *albicans*. When the isolate is positive for germ tube test then it is inoculated into corn meal agar to observe Chlamydospore formation.

Results

Blood samples were collected from 230 neonates with clinical suspicion of sepsis within the first 72 hours of life. Of these, 69 (30%) had culture positive sepsis and 138 (60%) were culture negative. Rest of the 10% samples yielded mixed bacterial growth and skin contaminants only.

Samples	Number & Percentage
Sterile	138 (60%)
Culture positive (pure growth)	69 (30%)
Mixed bacterial growth	17 (7.39%)
Skin flora	6 (2.6%)
Total	230 (100%)

Sex wise distribution of clinical sepsis

Among the neonates from whom the blood samples were collected, 148 were males and 82 were females. Male babies were found to be more prone to develop sepsis than females. 64.3% of clinical sepsis was seen in male babies as compares to 35.6% in female babies.

Maternal risk factors in suspected sepsis

Neonatal sepsis was found to be associated with one or more maternal risk factors. 41.3% had premature rupture of membrane and 23.9% had pregnancy induced hypertension and 11.3% of neonates were born to mothers with gestational diabetes mellitus.

Maternal risk factors	Number & Percentage
PROM	95 (41.3%)
Preterm labour	88 (38.2%)
PIH	55 (23.9%)
Gestational diabetes	26 (11.3%)
Maternal pyrexia	16 (6.95%)
Abruption placentae	15 (6.52%)
Oligamnios	13 (5.65%)

Neonatal risk factors in suspected sepsis

The most important neonatal risk factors associated with clinical sepsis is low birth weight which was presenting 78.2% of cases followed by prematurity which was noted in 63.4% of the total cases.

Neonatal risk factors	Number & Percentage
Low birth weight	180 (78.2%)
Prematurity	146 (63.4%)
IUGR	88 (38.2%)

Mode of delivery in culture positive sepsis

Of the 69 cases of culture positive sepsis, 42 (60.8%) were delivered by normal vaginal delivery and 23 (33.33%) were delivered by LSCS.

Mode of delivery	Number & Percentage
Normal vaginal delivery	42 (60.8%)
LSCS	23 (33.33%)
Assisted delivery – forceps/vacuum	4 (5.79%)
Total	69 (100%)

Preterm versus term babies in culture positive sepsis

Neonatal sepsis is more common among preterm babies. Of the 69 cases of culture positive sepsis 72.4% were delivered preterm and 27.5% were term babies. Among the preterm babies 32 (64%) were males and 18 (36%) were females. Among term babies, 11 (58%) were males and 8 (42%) were females.

Preterm babies		Term babies	
Male	32 (64%)	Male	11 (58%)
Female	18 (36%)	Female	8 (42%)
Total	50 (100%)	Total	19 (100%)

Birth weight and culture positive sepsis

Neonatal sepsis was found to be more in babies with birth weight less than 2.5kg. (63.8%). Culture positive sepsis was contributed by 20.2% of the Very Low Birth weight babies (VLBW).

Only 16% of culture positive sepsis was seen in term babies.

Birth weight	Number & Percentage
More than 2.5kg	11 (16%)
Less than 2.5kg	44 (63.8%)
Less than 1.5 kg	14 (20.2%)
Total	69 (100%)

Maternal risk factors in culture positives

Maternal risk factors most frequently present in culture positive cases were preterm labour (72.5%), pre mature rupture of membrane (PROM) 49.3% or both (45%).

Risk factors	Number & Percentage
Preterm labour	50 (72.46%)
PROM	34 (49.27%)
Maternal pyrexia	11 (15.94%)
Meconium stained liquor	9 (13.04%)
Prolonged duration of labour	4 (5.79%)
Multiple pregnancy	3 (4.34%)

Neonatal risk factors in culture positives

Most common neonatal risk factor present in culture positive cases was prematurity (74%) followed by Low Birth weight (63.8%) and birth asphyxia (56.5%). Two or more risk factors were present in 28 (40.6%) cases.

Normal Risk factors	Number & Percentage
Prematurity	51 (73.9%)
Low birth weight	44 (63.76%)
Birth asphyxia	39 (55.52%)
Respiratory distress syndrome	28 (40.57%)
Meconium aspiration	10 (14.49%)

Correlation between CRP and blood culture

CRP was positive in 68% of culture positive cases and negative in 32% of cases. Of the total 230 cases of suspected sepsis, CRP was positive in 23.5% cases.

CRP	BS positive	BC negative	Total
	No & Percentage	No & Percentage	No & Percentage
CRP positive	47 (68%)	7 (4.4%)	54 (23.5%)
CRP negative	22 (32%)	154 (95.6%)	176 (66.5%)
	69 (100%)	161 (100%)	230 (100%)

Clinical signs in culture positive sepsis

Most common clinical sign elicited from neonates was retractions and grunting (48%). This was followed by Jaundice (22%) and tachypnoea (20%). In 7% cases there were no findings.

Clinical signs	Number & Percentage
Retraction, Grunting	32 (48%)
Jaundice	15 (22%)
Tachypnoea	13 (20%)
Bulging Fontanel	5 (7%)
Tachycardia / Fever/ Cyanosis	1 (1%)

Symptoms in culture positive sepsis

Most common symptoms seen in babies with neonatal sepsis were dyspnoea (35%) and poor feeding (27%).

Presenting symptoms	Number & Percentage
Dyspnoea	24 (35%)
Poor feeding	19 (27%)
Reduced movements	13 (19%)
Lethargy	5 (7%)
GI symptoms	5 (7%)
Fever	3 (5%)

Organisms isolated from blood culture

The organism most frequently isolated from blood culture of neonates with suspected early onset sepsis (EOS) was staphylococcus aureus (34.7%) followed by Klebsiella pneumoniae (27.53%).

Organisms	Number & Percentage
Staphylococcus aureus	24 (34.8%)
MRSA	4 (5.8%)
Staph. epidermidis	5 (7.25%)
Staph. saprophyticus	4 (5.8%)
Klebsiella pneumoniae	19 (27.54%)
Acinetobacter baumannii	3 (4.35%)
Candida albicans	10 (14.49%)
Total	69 (100%)

Sensitivity pattern of staphylococcus aureus

Of the 24 isolates of Staphylococcus aureus 8.3% were sensitive to penicillin and 91.67% were resistant to penicillin 70.83% of Isolates were sensitive to Erythromycin and 29.17% were resistant 91.67% of the isolates were sensitive to cloxacillin and Gentamicin and 8.33% were resistant to both drugs..

Antibiotic	Sensitive	Resistant
Penicillin	2 (8.3%)	22 (91.67%)
Erythromycin	17 (70.83%)	7 (29.17%)
Gentamicin	22 (91.67%)	2 (8.33%)
Cloxacillin	22 (91.67%)	2 (8.33%)

MRSA isolated in the study of 4 nos. (5.8%) all the isolates were sensitive to Vancomycin and Amikacin and 75% were sensitive to Clindamycin. Of the 9 isolates of Coagulase Negative Staphylococci (CONS), 5 were staphylococcus epidermidis and 4 were staphylococcus saprophyticus differentiated by Novobiocin resistance.

Klebsiella isolated in Blood culture

Klebsiella pneumoniae	Number & Percentage
Sensitive	7 (36.8%)
Resistant	12 (63.2%)

Of the 19 Klebsiella pneumoniae isolated in culture, 12 were multi drug resistant and only 7 were sensitive.

Sensitivity pattern of Klebsiella isolate in culture

None of the 19 Klebsiella Pneumoniae isolates were sensitive to Ampicillin. Sensitivity to ciprofloxacin and Cotrimoxazole was 36.8%. The isolates were more sensitive to Amikacin (63%) compared to gentamicin (26.3%). 94.7% of isolates were sensitive to Imipenem.

Antibiotic	Sensitive	Resistant
Ampicillin	0 (0%)	19 (100%)
Gentamicin	5 (26.3%)	14 (73.7%)
Cephalexin	4 (21%)	15 (79%)
Ceftriaxone	6 (31.6%)	13 (68.4%)
Cotrimoxazole	7 (36.8%)	12 (63.2%)
Ciprofloxacin	7 (36.8%)	12 (63.2%)
Amikacin	12 (63%)	7 (37%)
Imipenem	18 (94.7%)	1 (5.3%)
Piperacillin – Tazobactam	9 (47.4%)	10 (52.6%)
Cefepime	9 (47.4%)	10 (52.6%)

Sensitivity pattern of Acinetobacter spp.

Antibiotic	% Resistant	% Sensitive
Gentamicin	66.67%	33.33%
Ceftriaxone	66.67%	33.33%
Cotrimoxazole	33.33%	66.67%
Ciprofloxacin	33.33%	66.67%
Amikacin	0	100
Imipenem	0	100
Piperacillin – Tazobactam	0	100
Cefepime	33.33%	66.67%

Isolates were highly sensitive to Amikacin, Imipenem and Piperacillin – Tazobactam (100%). Sensitivity to ciprofloxacin and gentamicin was 33.33%. Ceftriaxone and Cotrimoxazole showed 66.67% sensitivity.

Of the 69 cases of culture positive sepsis 44 babies survived and 25 expired. All of those who expired were preterm and Low Birth Weight babies. 21 (84%) of them were having IUGR as well.

Discussion

Septicemia is a major cause of morbidity and mortality in the new born. The bacteriological profile of neonatal sepsis is constantly under change with advances in the early diagnosis and treatment of sepsis and the increased survival of preterm babies.

In this study, 230 neonates with clinical signs and symptoms of sepsis and babies born to mothers with high risk factors were selected according to the universal sampling technique. In clinical sepsis as well as culture positive sepsis in the study, male babies were more prone to develop sepsis. Ratio of male to female in the study was 1.8:1. In a study conducted by SMH Aletayeb et al in a tertiary care centre in Iran in 2011, the male: female ratio was 2:1.

Maternal risk factors most frequently associated with clinical and culture positive sepsis in the present study were Preterm labour(72.5%), PROM (49.3), Maternal pyrexia(25%) and PIH (20%). A study by Shashi Gandhi et al in 2007 found that 77% of PROM was associated with culture positive sepsis. Betty et al found that PROM contributed to 20% of sepsis. In another study conducted by Roy et al in 2002 in Uttar Pradesh, the maternal factors revealed 32.8% of preterm labour, 28.9% PROM, and 5.2% intrapartum fever. 60.8% of babies with sepsis were delivered by normal vaginal delivery and 39.2% delivered by LSCS in the present study. In a study by Kuruvila et al, culture positive sepsis in normal vaginal delivery and LSCS were found to be 53.3 % and 26.7% respectively.

In the present study, the most common presenting symptoms were dyspnea and poor feeding (27%). The most common clinical signs elicited were retractions and grunting in 48% cases followed by Jaundice in 22% cases. The national Neonatal Perinatal Database (NNPD) reports of 2002-2003 identified respiratory distress as the commonest presentation of early onset sepsis.

CRP was positive in 23.5% of suspected clinical sepsis and 68% of culture positive sepsis. In a study conducted by Shanthi Anantha krishnan, Gunasekharan D in 2009 in Puducherry, CRP was positive in 32% of suspected clinical sepsis.

The organism most frequently isolated from blood culture of neonate with suspected EOS in the study was *Staphylococcus aureus* (34.8%) followed by *Klebsiella pneumoniae* (27.54%). In a study conducted by Karthikeyan G and Prem Kumar K in Chennai in 1997, *Staphylococcus aureus* (61.5%) and *Klebsiella pneumonia* (21.9%) were reported.

In this study, only 8.33% of the *Staphylococcus aureus* isolates were sensitive to Penicillin and 91.67% were resistant. These findings are echoed by Roy et al in their study where the Penicillin resistance of *Staphylococcus aureus* was found to be 95.9%.

Of the gram negative bacteria, *Klebsiella pneumonia* was the predominant isolate with maximum sensitivity to Imipenem (94.75) which was noted in studies by Giorgiana Brad et al (88%), Chandra Madhur Sharma et al(100%) and Kuruvilla et al (100%).

Candida albicans constituted 14.5% of the total isolates of this study. Bhattacharya S in 1994 in Calcutta reported 16.4% of *Candida* species. In another study Ghanshyam D Kumhar et al found only 2.43%.

Case fatality of culture positive sepsis was found to be 36.2 % which was consistent with the study by Tallur et al from Hubli, India in 2000 and Leibovitz E et al from Israel in 1997 where the case fatality rate range from 37-47.5%. Overall case fatality rate of EOS was 14.3%. This was

similar to the findings of Kuruvila et al in which the fatality rate was 14.4%.

Conclusion

Culture positivity was 30% of the clinical cases of sepsis. *Staphylococcus aureus* (40.6%) and *klebsiella pneumonia* (27.53%) were the predominant species obtained in culture. Other bacteria isolated are CONS(13.04%) and *acinetobacterbaumani* (4.35%). the only fungi isolated was *candida albicans* (14.49%). Among the *klebsiella pneumonia* isolates, 63.2% are multidrug resistant. There were clustering of cases and this leads to investigation of an outbreak. *Klebsiella pneumonia* was isolated from the neonatal ICU and labourroom. An extensive infection control programme was initiated by the Hospital Infection Control Committee under the guidance of HOD Microbiology. Proper sterilisation of all patient care equipments and articles were done. Repeat sterility testing showed that the organisms had been eliminated.

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