www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i7.193

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Original Research Article

Microbiological Aspects and Conventional Methods for Diagnosis of Neonatal Sepsis

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Abstract
Background: *septicaemia is a major cause of morbidity and mortality among neonates. Its early diagnosis can save lives of many neonates.*
Objective: *neonatal septicaemia is very difficult to diagnose due to non specific signs and symptoms. Early diagnosis of septicaemia is very important for saving lives of neonates. Study design: the study is conducted in the department of Microbiology and the department of Pediatrics at Patna medical college, Patna. 120 neonates with signs and symptoms of septicaemia were included in this study. Diagnosis is done by conventional method of culture technique.*
Result: *result shows that among culture positive cases 68% are male, 33% belong to first week of their lives, and 53% are of low birth weight. Among clinically suspected cases 52% are blood culture positive and the most common organism isolated is Klebsiella pneumoniae.*
Discussion and Conclusion: *male predominance in neonatal septicaemia shows that there is sex linked factor in host susceptibility. Incidence of septicaemia is highest in first week of life, low birth weight is predisposing factor for septicaemia,. Gram negative bacteria Klebsiella is involved in most of the cases of septicaemia.*
Keywords: *Sepsis, NICU, SIRS, hypoglycemia, hypothermia, PROM, PPROM, unbooked, leukocytosis, leucopoenia, tachypnea, and tachycardia.*

Background

The term neonatal septicaemia refers to circulation and multiplication of infecting bacteria with their toxic products in the new born within 28 days (4 weeks) of birth.

Septicaemia is a common cause of morbidity and mortality among children in developing world. Definitive diagnosis is done by bacteriological culture of blood samples [1].

Changing bacterial flora and emergence of resistant strains make it imperative to the known

prevailing pattern of antibiotic susceptibility of etiological agent of septicaemia [2].

Neonatal septicaemia is difficult to diagnose clinically as it presents with non-specific signs and symptoms like birth asphyxia, intracranial haemorrhage, respiratory distress syndrome, hypoglycaemia, hypothermia etc[3]. Blood stream infections have been quoted as the most common infection in paediatric age group. A very wide spectrum of organisms have been described for cases of neonatal septicaemia and this spectrum is subject to geographical and time variations. The organisms isolated are often resistant to multiple antimicrobials which make the treatment difficult with grave prognosis. Thus the need to monitor bacteriology and its antimicrobial susceptibility pattern becomes a necessity [4].

Etiology & Microbiological aspects

SIRS (Systemic inflammatory response syndrome) - Fever or hypothermia, leukocytosis or leucopenia, tachypnea, and tachycardia are the cardinal signs of the systemic response often called SIRS.

Microorganisms involved commonly are a) gram-negative bacteria (non typhoidal Salmonella species, *Haemophilus influenza, Enterobacter-iaceae* and *Pseudomonas* etc. b) Gram-positive (*Staphylococcus aureus, coagulase-negative Staphylococci, Enterococci, Streptococcus pneumoniae*, other *Streptococci* and other gram-positive cocci) [5]. Now a day's gram –negative non-fermenters such as *Acinetobacter* spp. and *Pseudomonas* spp. are emerging as frequent causes of neonatal septicaemia [6].

Aims and Objectives

In spite of great advances in antimicrobial therapy, neonatal life support measures and the early detection of risk factors, neonatal septicaemia continues to be a major cause of morbidity and mortality around the world. Thus, the needs for microbial monitoring in neonatal wards cannot be overemphasized. The present study has been undertaken with the following objectives:

1. To study the clinico-etiological profile of neonatal sepsis among neonates admitted to NICU in Patna Medical College, Patna.
2. To isolate and identify the causative organisms of neonatal septicaemia
3. To study the role of various laboratory culture techniques for the identification of bacterial isolates from clinical samples from patients of septicaemia.

Materials and Methods

The present study was conducted in department of Microbiology, Patna Medical College & Hospital, Patna. Blood samples were collected from patients admitted in the NICU in Department of paediatrics, Patna Medical College & Hospital, Patna. One hundred twenty neonates up to 4 weeks of age, with clinically suspected septicaemia were studied prospectively.

Selection of cases

1. **Maternal risk factors**
   Prolonged rupture of membranes (PROM, rupture of membranes for >18 hours before delivery), preterm prolonged rupture of membranes (PPROM, rupture of membranes <37 weeks of gestation and >18 hours before delivery), unbooked mother (less than three antenatal check-ups of the mother during pregnancy), outside hospital delivery, delivery by untrained personnel, meconium stained amniotic fluid and vaginal delivery.
2. **Associated perinatal risk factors**
   Low birth weight (LBW, <2.5 kg), Preterm (<37 weeks), gestational age, birth asphyxia, presence of intravascular catheter, congenital abnormality, non-breast feeds.
3. **Signs and symptoms**
   Feeding intolerance, refusal of feed, lethargy, temperature instability, icterus, apnea, respiratory distress, poor perfusion, seizures, bleeding diathesis.

Blood culture

Materials

- Blood culture bottles containing 2.5 ml Brain –Heart Infusion Broth (BHI).
- 70% Alcohol for swab.
- 2% Tincture of iodine for swab.
- Tourniquet.
- Sterile disposable syringe and 21-23 G needle.
- Sterile gauze.

Methods

1) Two blood culture bottles were labelled with the name and patient identification and hospital detail.
2) A peripheral vein on the hand or foot of the neonate was selected. The skin over the venipuncture site was cleansed with 70% alcohol– soaked sterile gauze, starting in the centre of a circle, approximately 5 cm in diameter, rubbing vigorously. The alcohol was allowed to air dry.
3) Starting in the centre of the circle, 2% tincture of iodine, or, povidone -iodine was applied in ever widening circles until the entire circle had been saturated with iodine.
4) The screw top of the blood culture bottle was removed and the diaphragm top was swabbed with 70% alcohol. The alcohol was allowed to air dry.
5) With the help of a sterile disposable syringe and needle, 0.5 to 1.0 ml of blood was withdrawn from the selected venipuncture site. The blood culture bottle used in the process was Mc- Cartny blood culture bottle. The ratio of blood to B.H.I broth should be 1:5 to 1:10.
6) Using another sterile, disposable syringe and needle, a second sample of blood was collected at the same time, from the selected venipuncture site and inoculated as described above into the second BHI broth.
7) The venipuncture sites were cleansd with 70% alcohol again, after withdrawing blood.

Culture

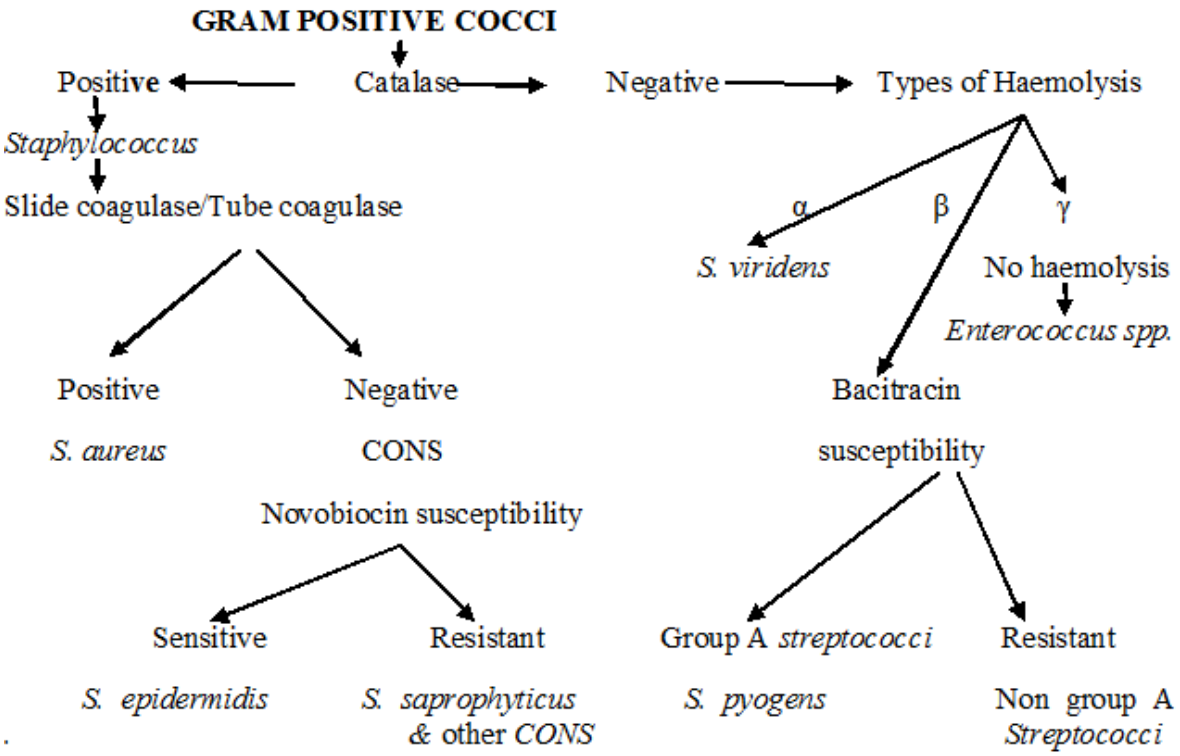
1) The inoculated Brain-Heart Infusion broth was incubated aerobically at 37°C for 18 hours. Growth was indicated by haemolysis of red blood cells (RBC'S), gas bubbles in the medium, or, turbidity.
2) Gram stained smear of an air dried drop of the medium, on a sterile glass slide, was performed when macroscopic evidence of growth was apparent.
3) In addition to daily visual examination, subcultures were performed after the first 6-12 hours of incubation by aseptically removing few drops of the well-mixed medium and spreading this inoculum onto a Blood agar, MacConkey agar and Nutrient agar plate.
4) The isolates on subcultured plates were identified using standard methods.
5) Culture-negative bottles were reincubated for 7 days.

Methods of Identification of Organism From Blood Culture

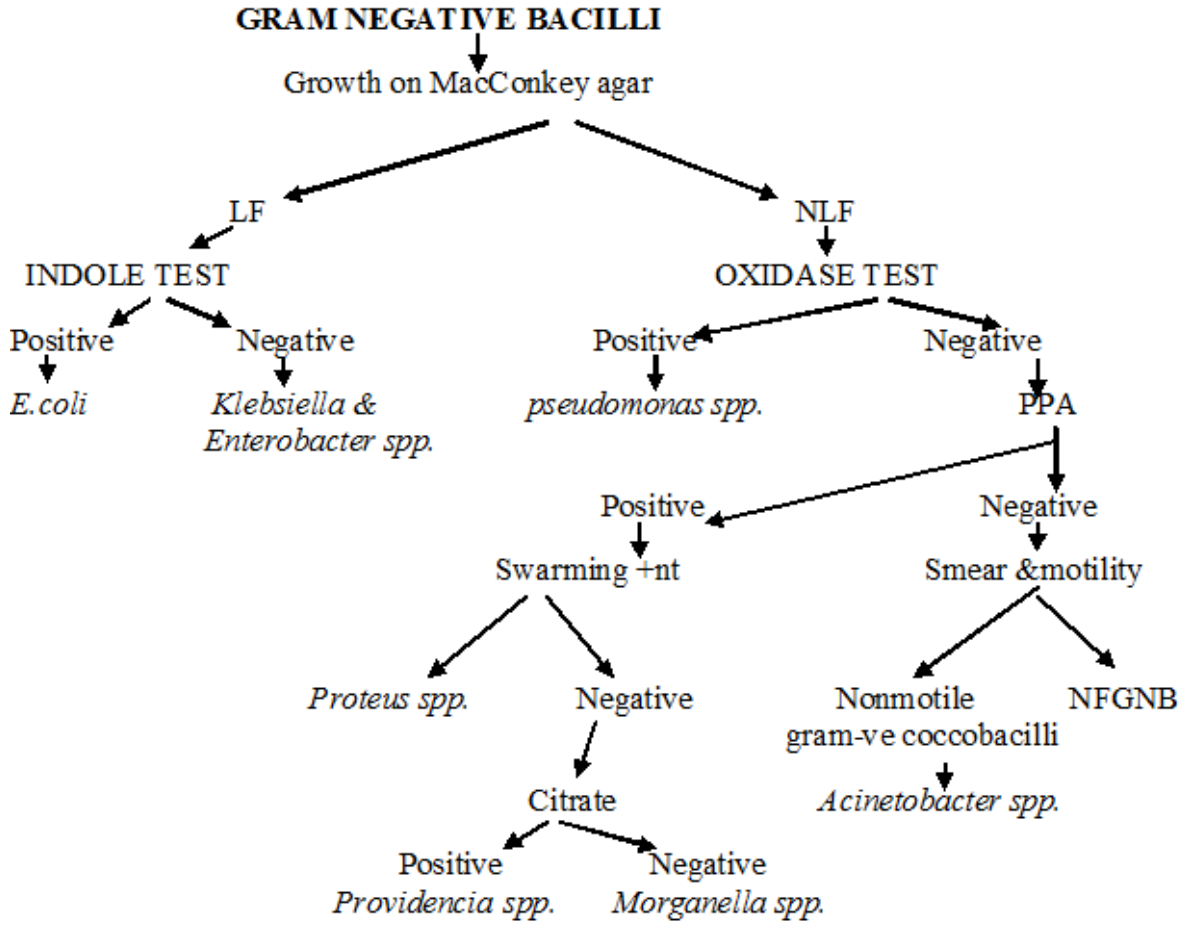
Identification of micro-organism: - Identification was done on the following grounds

a) **Colony characters:** Among colony characters size, shape, margin, surface, consistency, haemolysis, appearance, swarming of the colony were seen.
b) **Gram's staining:** Smear was prepared from isolated colony on culture plate and Gram's staining was done. Size, shape, arrangement and morphological characters are seen under high power of microscope.
c) **Motility test:** Hanging drop preparation was done for motility test.
d) **Biochemical tests:** Series of biochemical tests were required for identification of different bacterial isolates as per method described by Collee et al. (1996).

FLOW CHART, SHOWING IDENTIFICATION OF GRAM POSITIVE COCCI.



FLOW CHART SHOWING IDENTIFICATION OF GRAM NEGATIVE BACTERI.



Results and Observation

Table -1 Sex wise distribution of cases (total no. of cases-120)

Sex	Suspected cases		Culture Positive case	
	Number	Percentage	Number	Percentage
Males	82	68 %	46	57.09 %
Females	38	32 %	16	42.10 %
Total	120	100 %	62	

Table 1 showed out of 120 suspected cases 82 were males (68%) and 38 were females (32%). Among 62 culture positive cases 46 (57.09%) were males and 16 (42.10%) were females. So males were higher in number compared to females.

Table-2 Age wise distribution of cases (total no. of cases-120)

Age (days)	Suspected cases		Culture Positive cases	
	Number	Percentage	Number	Percentage
1-7 (1st week)	40	33 %	22	55.00 %
8-14 (2nd week)	28	23 %	15	53.57 %
15-21 (3rd week)	27	23 %	14	51.85 %
22-28 (4th week)	25	21 %	11	45.83 %
Total	120	100 %	62	

Table 3 showed out of 120 suspected cases 40 (33%) belonged to first week, 28 (23%) belonged to second week, 27 (23%) belonged to third week and 25 (21%) belonged to fourth week. Among 62 culture positive cases 22 (55.00%), 15 (53.57%), 14 (51.85%) and 11 (45.83%) belonged to first, second, third and fourth weeks respectively. So the incidence was highest in first week followed by second, third and fourth weeks.

Table-3 Distribution of cases according to the birth weight

Birth weight	Suspected cases		Culture Positive cases	
	Number	Percentage	Number	Percentage
Low birth weight (<2.5kg)	63	53 %	38	60.30 %
Normal birth weight (≥2.5kg)	57	47 %	24	42.10 %
Total	120	100 %	62	

Table 4 showed out of 120 suspected cases 63 (53%) were of low birth weight and 57 (47%) were of normal birth weight. Among 62 culture positive cases 38 (60.30%) were of low birth weight and 24 (42.10%) were of normal birth weight. So incidence of neonatal septicaemia was higher in low birth weight neonates compared to normal birth weight.

Table -4 Results of Blood Culture

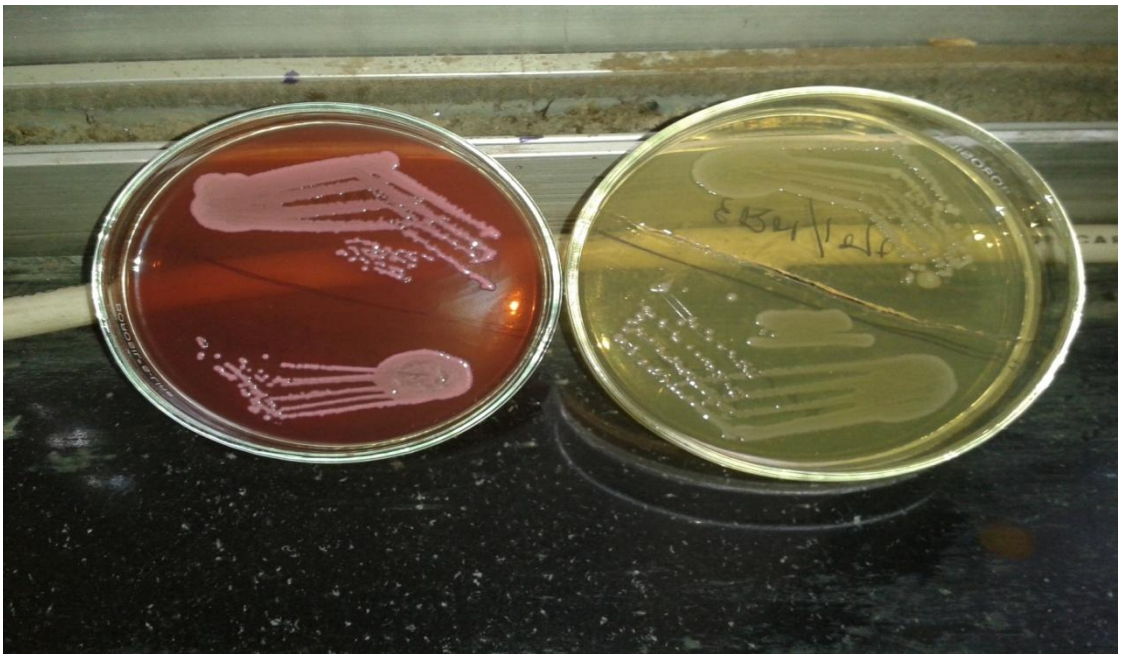
Blood culture	cases	
	Number	Percentage
Positive	62	52 %
Negative	58	48 %
Total	120	100 %

Table 6 showed out of 120 clinically suspected cases of neonatal septicaemia 62 (52%) were blood culture positive and 58 (48%) were blood culture negative.

Table -5 Organism isolated by blood culture

Sl.No.	Organism	Isolates	
		Number	Percentage
1	*Klebsiella pneumonia*	21	34.50 %
2	*Staphylococcus aureus*	16	25.80 %
3	*Escherichia coli*	10	18.00 %
4	*Coagulase negative Staphylococci*	11	15.41 %
5	*Pseudomonas aeruginosa*	03	04.83 %
6	*Proteus mirabilis*	01	01.61%
	Total	62	100 %

Table 7 showed commonest organisms isolated was *Klebsiella pneumoniae* (34.50%) followed by *Staphylococcus aureus* (25.80%), *Escherichia coli* (18.00%), Coagulase negative *Staphylococci* (15.41%), *Pseudomonas aeruginosa* (04.83%), *Proteus mirabilis* (1.61%).



MacConkey's agar & Nutrient Agar Showing *Klebsiella pneumoniae*

Biochemical Tests For *Klebsiella pneumoniae*
(INDOLE, METHYL-RED, CITRARE, UREASE, & T.S.I)

Figure-I, Pie Chart Showing Organism Isolated by Blood Culture

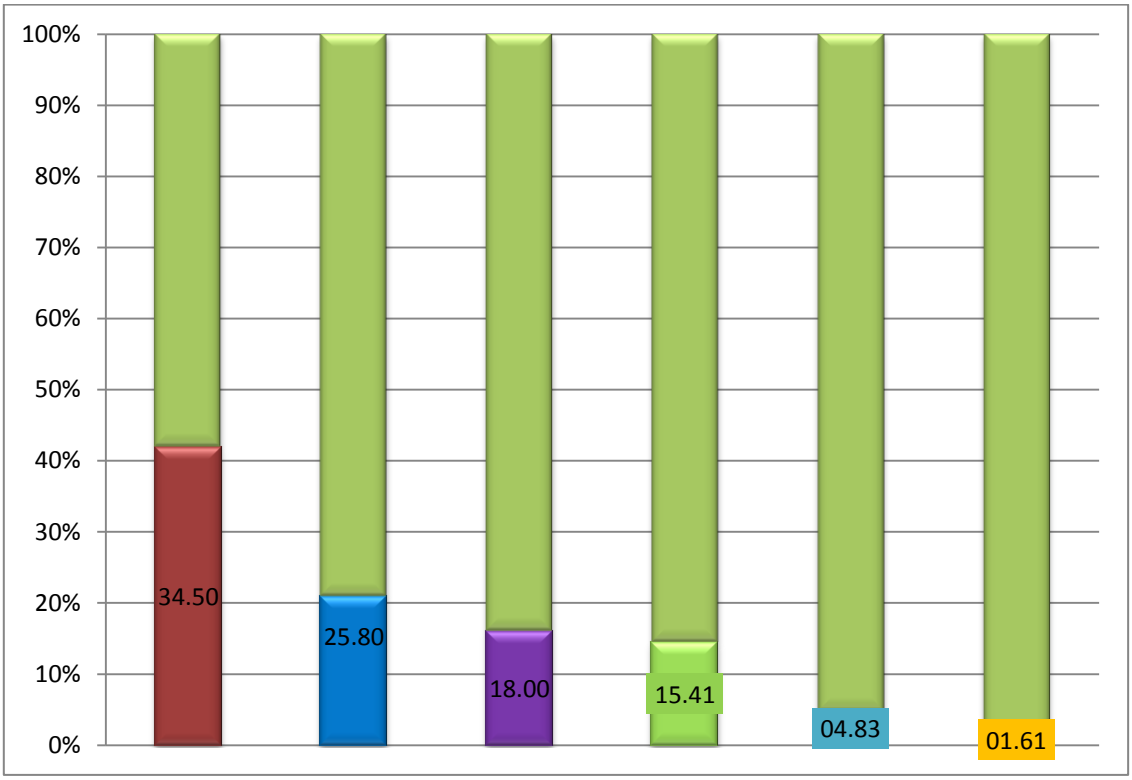


Figure II

Klebsiella pneumoniae (34.50%)
Staphylococcus aureus (25.80%)
Escherichia coli (18.00%)
Coagulase Negative Staphylococci (15.41%)
Pseudomonas aeruginosa (04.83%)
Proteus mirabilis (01.61%)

Discussion

Patna Medical College & Hospital is a 1700 bedded tertiary care centre in Patna, Bihar. It provides health care services to the vast population of whole Bihar, Eastern U.P, and Nepal. This study was conducted in the department of Microbiology and Paediatrics at Patna Medical College & Hospital, Patna. One hundred neonates with clinical suspicion of septicaemia on the basis of clinical features and associated perinatal risk factors and maternal risk factors were included in the study. Blood culture, was done by the conventional method.

In the present study an attempt was made to know the various bacterial flora responsible for neonatal septicaemia, through blood culture.

Sex

In our study neonatal septicaemia was more common in males (57.09%) as compared to females (42.10%). Similar reports are reported by other workers. Khatua et al., reported 70.7% cases of neonatal septicaemia to be males. In the study conducted by U. Vaidya et al., Male: Female ratio was 1.6:1.[10] Anitha Sharma et al., also reported male predominance, that is out of 50 cases 37 (74%) were males.[15] In a study conducted by Anuradha De et al., out of 200 suspected cases of neonatal septicaemia 114 (57%) were males and 86 (43%) were females.[11] The percentage of females in present study with neonatal septicaemia were 42.10%. The usual male predominance in neonatal septicaemia has suggested sex linked factor in host susceptibility.

Age

In our study, incidence of septicaemia was highest in first week of life (55.00%) followed by second week (53.57%), third week (51.85%), and fourth week (45.83%). According to Barbara J Stoll *et al*., 1975 neonates are more susceptible for infection in the first week of life. High incidence of Gram negative bacteraemia was noted between the age of 6 days to 17 days.[13] K.K. Anand *et al*., reported 58.6% of neonates were less than 10 days in the study conducted in Safdar Jang Hospital, New Delhi.[12]

Birth Weight

In our study septicaemia was more common in low birth weight neonates (60.30%) as compared to the normal weight neonates (42.10%). Sinha et al 1986 reported 64.9% incidence in low birth weight neonates. K.K. Anand reported 81.3% of neonatal septicemia cases were below 2200gms. According to study conducted by K.Chug et al., the mean birth weight of septicaemic neonates was 1.84 kgs. G.G. Christo et al., 1990 reported high rate of septicaemia among low birth weight neonates.[7] According to Barbara J. Stoll et al., 1975 rate of infection is inversely proportional to birth weight.[13]

Blood culture

Total 120 cases of clinically suspected neonatal septicaemia were selected for the present study, 62 cases were positive by blood culture and 58 cases were culture negative. So the blood culture positivity was 52%. Madhubala Parikh and Nandan Singh in 1995 reported out of 254 cases, 199 were blood culture positive (47%).[16] U. Vaidya et al., in 1991 reported out of 381 cases, blood culture was positive in 156 cases (41%).[14] In the study conducted by Khatua et al., 1986, culture was positive in 59.8%, Namdeo et al., 1987 showed 50%, P.P.Sharma et al., 1987 reported 56%, P.S. Rao's study showed 40.0%, Marina Thomas 1999 showed 40% and S.G. Joshi in 2000 reported 25% culture positivity respectively.[17]

Table – 6 Results of Blood Culture by other workers [8, 14, 16]

Sl. No.	Author	Percentage of positive blood culture
1.	Khatua et al. 1986	59.8 %
2.	Namdeo et al. 1987	50.0 %
3.	P.P Sharma 1987	56.0 %
4.	Madhubala Parikh et al. 1995	47.0 %
5.	Vaidya U et al. 1991	41.0 %
6.	Rao P.S et al. 1993	40.0 %
7.	Joshi S.G et al. 2000	25.0 %
8.	Sriparna Basu et al.2012	41.36%
9.	B. Patel, R. Prasad, et al.2013	47.5 %
10.	Present study	52.0 %

Organism isolated

Klebsiella pneumoniae 21 (34.50%), *Staphylococcus aureus* 16 (25.80%), were commonest organisms isolated in our study followed by *Escherichia coli* 10 (18.00%), CONS 11 (15.41%), *Pseudomonas aeruginosa* 3 (04.83%), and *Proteus mirabilis* 01 (1.61%). In 62 isolates gram negative organisms were 35 (56.45%) and gram positive organisms were 27 (43.54%).

So gram negative septicaemia was commonest. Anitha sharma et al., reported 85% of culture positive cases to be gram negative organism.[15] Where as in another study by Narang et al., 61.1% neonates had gram negative septicaemia. Anuradha De et al., 1995 reported 72.4% of gram negative septicaemia. [11] Sinha et al., 1986 reported *pseudomonas* as most common isolates (34.5%), then *Klebsiella* and *Escherichia coli* as 16.4% each, *Staphylococcus aureus* as 14.5% of isolates. Chug et al., 1988 reported *Staphylococcus aureus* as a most common isolates 20.1%, then *Escherichia coli* and *Klebsiella* as 14% each, Pseudomonas 06.2% CONS as 07.5% and Streptococcus faecalis as 06.7% of isolates. Marina Thomas et al,. 1999 reported Klebsiella 08.0%, E. coli as 04.0%, pseudomonas 12.0%, *Staph. aureus* 50.6%, Streptococcus faecalis 09.3%. S.G Joshi et al,. In 2000 reported *Klebsiella* 30.4%, E. coli 15.6%, *Pseudomonas* 38.3%, etc.

Table – 15 Organisms isolated by other workers [8, 9, 16, 17]

Organisms isolated	Sinha et al. 1986	Chug et al. 1988	Madhubala Parikh 1991	Madhu-bala parikh 1995	Marina Thomas et al. 1999	S.G Joshi et al. 2000	Sriparna Basu et al. 2012	Bheemasam udra. patel et al. 2013	Present study
Klebsiella	16.4%	14.2%	77.3%	14.2%	08.0%	30.4%	27.8%	16.84%	34.5%
E.coli	16.4%	14.1%	6.5%	41.1%	04.0%	15.6%	13.9%	09.47%	18.0%
Pseudomonas	34.5%	6.2%	6.5%	06.7%	12.0%	38.3%	19.4%	05.26%	04.8%
Staph. Aureus	14.5%	20.1%	9.7%	20.1%	50.6%	-	5.6%	11.58%	25.8%
CONS	-	07.5%	-	07.5%	-	-	2.8 %	10.53%	15.4%
Strepto. Feacalis	-	06.7%	-	06.7%	09.3%	-	-	3.16%	-
Others	-	03.3%	-	03.3%	15.9%	15.6%	36.1%	-	01.6%

This study on Microbial Etiology and Diagnosis of Neonatal Septicaemia was a prospective study conducted in the Departments of Microbiology and Pediatrics at the Patna Medical College & Hospital.

1) In the present study, out of 120 clinically suspected cases of neonatal septicaemia there were 82 males (68%) and 38 (32%) females. Among 62 culture positive cases 46 (57.09%) were males and 16 (42.10%) were females. So incidence was higher in males compared to females.
2) Out of 120 suspected cases 40 (33%) belonged to first week, 28 (23%) belonged to second week, 27 (23%) belonged to third week and 25 (21%) belonged to fourth week. Among 62 culture positive cases 22 (55.00%), 15 (53.57%), 14 (51.88%) and 11 (45.83%) belonged to first, second, third and fourth weeks respectively. So the incidence was highest in first week followed by second, third and fourth weeks respectively.
3) Out of 120 suspected cases 80 (67%) were term (mature delivery) neonates and 40 (33%) were preterm (premature delivery). Among culture positive cases 40 (50.00%) were term neonates and 22 (55%) were preterm neonates. So incidence was higher in preterm neonates than term neonates.
4) Out of 120 suspected cases 63 (53%) were of low birth weight and 57 (47%) were of normal birth weight. Among 62 culture positive cases 38 (60.30%) were of low birth weight and 24 (42.10%) were of normal birth weight. So incidence of neonatal septicaemia was higher in low birth weight neonates compared to normal birth weight.
5) The clinical presentations in our study were refusal of feeds (67%), lethargy (30%), fever (18%), abdominal distension (13%), jaundice (12%), convulsions (8%), vomiting (7%), excessive cry (5%), coldness of body (3%), cough (3%), irregular breathing (2%), diarrhoea (1.66%), cyanosis (1.66%) and pustules (1%). Refusal of feeds, lethargy and fever were commonest presenting combination.
6) The isolation and identification of microbial isolates was done according to standard methods.
7) Blood culture was positive in 62 (52%) cases of clinically suspected cases of neonatal septicaemia, of them 35 (56.45.%) showed gram negative septicaemia and 23 (43.54.%) showed gram positive septicaemia.
8) Among the culture positive cases *Klebsiella pneumoniae* an *Escherichia coli* were the commonest among gram negative isolates i.e, *Klebsiella pneumoniae* 21 (34.50%), *Escherichia coli* 10 (18.00%), Coagulase negative *Staphylococcus* 11 (15.41%), *Staphylococcus aureus* 16

(25.80%), *Pseudomonas aeruginosa* 3 (04.83%), and *Proteus mirabilis* 1(1.61%).

9) Out of 62 blood culture positive cases 15 (24.19%) expired. In our study mortality rate was 24.19% among culture positive cases.
10) Coagulase-negative *Staphylococcus* were the predominate isolates from neonates with intravenous catheter and prolonged rupture of membranes.
11) *Escherichia coli* were the predominant isolate in neonates with maternal preterm prolonged rupture of membranes.

Conclusion

Neonatal septicaemia is leading cause of mortality and morbidity in developing countries like India. Neonatal septicaemia presents with non specific signs and symptoms. It is more common in males, low birth weight, and preterm neonates. A positive blood culture is the only definitive method of confirming a case of septicaemia, which helps in prompt and timely administration of antibiotics which could be life saving.

References

1. Meremikwu Martin M, Nwachukwu Chukwuemeka, Asuquo Anne E, Okebe Joseph U, Utsalo Simon J, bacterial isolate from blood cultures of children with suspected septicaemia in Calabar, Nigria. *BMC Infectious Diseases* 2005;5;110.
2. Yardi D, Gaikwad S, and Deodhar L. Incidence, mortality and bacteriological profile of septicaemia in paediatric patients. *Indian journal of paediatrics*; 1984;51: 173-176.
3. Vishnu Bhat et al, "Neonatal sepsis in Tertiary Care Hospital in South India" Indian J of pediatrics2011; vol:78 (4):413-417.
4. Roy I, Jain A, Kumar M, Agrawal SK. Bacteriology of neonatal septicaemia in a tertiary care hospital of northern India. *IJMM*, 2002; 20(3): 107-116.
5. Robert S. Munford, Harrisons principal of internal medicine, 17th edn, 1695-1699.
6. U.K Mishra et al, "Newer approaches to the diagnosis of early onset neonatal sepsis" 2006; 91(3): F208-212.
7. Christo G.G. et al., "Neonatal sepsis clinical and Epidemiological Aspects" Indian J. Pediatr. 1990; 57: 781-784.
8. Joshi S.G. et al., "Neonatal Gram negative bacteraemia". Indian J. Pediatrics. 2000; 67: 27: 32.
9. Chugh, K et al., "Bacteriological profile of neonatal septicemia". Indian J. Pediatr. 1988; (55) 961-965.
10. Vaidya U. et al. "Neonatal Septicemia A reapprasial with special reference to use of Cefotaxime". Indian Paediatrics: 1991; 28: 1265-70.
11. De Anuradha et al., "Bacteraemia in Hospitalized children – A one Year Prospective study". IJMM: 1995; 13: 72-75.
12. Anand K.K. et al., "Coagulase negative staphylococcus septicemia in newborns". Indian paediatrics. 1991; 28: 1241-47.
13. Stoll Barbara J. et al., "Early onset sepsis in very low birth weight neonates. A report from the National Institute of child Health and Human Development neonatal research Network". Hammer Smith hospital. 1967-75; 29: 63-70.
14. Vaidya U. et al. "Neonatal Septicemia A reapprasial with special reference to use of Cefotaxime". Indian Paediatrics: 1991; 28: 1265-70.
15. Sharma Anitha et al., "Diagnostic and prognostic role of blood culture in neonatal septicaemia". Indian paediatrics. 1993; 30: 347-349.
16. Parikh Madhubala and Singh Nandan. "Rapid diagnosis of neonatal bacteraemia" IJMM. 1995; 13: 37-40.
17. Thomas Marina et al. Microbial profile of neonatal septicaemia in Coimbatore". Indian J. Paediatrics. 1999; 66: 11-14.