International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

Original Research Article.

Correlation of Haematological Parameters with Blood Culture in Neonatal Sepsis: A Prospective Study

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ABSTRACT

Background: Sepsis is the commonest cause of neonatal mortality. It is responsible for about 30-50% of the total neonatal deaths in developing countries. The aim of present study is to find the correlation of haematological parameters with microbiological one (blood culture).

Methods: The prospective, cross sectional hospital based study conducted over a period of 2 years on 250 newborns. In the setting of present study blood culture is done by a conventional method & a gram smear is also made and examined at subculture stage. Data is subjected to appropriate statistical analysis.

Results: There is statistically significant association between cases of sepsis and mode of delivery. There is statistically significant association between prematurity and sepsis. Common clinical manifestations of neonatal septicaemia were feeding difficulty (75.12%), reduced movement (60%), temperature abnormality (56.1%), respiratory distress (19.02%), convulsions (18.05%), sclerema (8.78%), prolonged capillary refill time (18.54%) and redness around umbilicus (7.31%). Among the 45 culture proven babies, 22 (48.89%) babies had grown Klebsiella, of which 11:11 (M:F); 12 (26.67%) babies had grown Staph. aureus, of which 9:3 (M:F); 5 (11.11%) babies had grown Coagulase Negative Staphylococcus, of which 4:1 (M:F); 3 (6.67%) babies had grown E. coli, of which 2:1 (M:F); 2 (4.44%) babies had grown Pseudomonas, of which 1:1 (M:F); 1 (2.22%) baby had grown Enterococcus, which was a male child. Among the 45 culture proven cases, 27 (60%) were gram negative and 18 (40%) were gram positive.

Conclusion: Blood culture positivity and organism isolated on culture is helpful in instituting appropriate antibiotic , to which the organism is sensitive and help to develop an antibiotic policy for the neonatal intensive care unit of institution.

Keywords: Haematological Parameters, Blood Culture, Neonatal Sepsis.

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Article History:

Received: 29-01-2018, **Revised:** 22-02-2018, **Accepted:** 27-03-2018

Access this article online	
Website: www.ijmrp.com	Quick Response code
DOI: 10.21276/ijmrp.2018.4.2.014	

INTRODUCTION

Sepsis is the commonest cause of neonatal mortality. It is responsible for about 30-50% of the total neonatal deaths in developing countries.[1,2] Estimates show upto 20% of neonates develop sepsis while 1% die of sepsis related causes.[2] Sepsis related mortality is largely preventable if diagnosed early and treated aggressively with antibiotics and good supportive care.

The inability of any single laboratory test to provide rapid, reliable and early identification of neonates with bacterial sepsis has led to efforts to devise a panel of screening tests combining data from several different determinants, as a means of increasing predictive accuracy. In general, the results have shown little increase in positive predictive accuracy compared with most of the individual tests.[3]

Prerequisite of any such, kind of screening panels would be that the result should be available in a short period of time. Many authors have evaluated the efficacy of the "sepsis screen" using various parameters.

In 1950, researchers first documented the efficacy of blood culture in neonates using 10 ml of blood collected from jugular vein. They showed that a single blood culture was satisfactory for confirming the diagnosis of neonatal septicaemia. Singh M[4] in 2006 from AIIMS New Delhi, while analysing the changing pattern of bacteriological profile in neonatal sepsis among intramural babies using National Neonatal Perinatal Database for the year 1995, 2000 and 2002-03 from around 18 centres from various institution throughout India concluded that the incidence of sepsis decreased

from 38.7 per 1000 live births to 29.9 /1000 live births in 1995 to 8.5 cases per 1000 live births in 2002-03. Overall, gram negative organism predominate as the cause of neonatal sepsis , 66 % of all cases of sepsis in 1995 and 56 % of all cases in 2002-03 Klebsiella species remained the leading cause of neonatal sepsis, 30% in 1995 and 32.5% in 2002 -03.This was followed by staph aureus and and E.coli as second and third most common isolate.[5,6] The neonate's immune system is underdeveloped and infection is caused by a group of organisms that are unique to the perinatal period.[7-9] Any delay in the treatment raises the risk of mortality. So, an algorithmic approach utilizes sepsis screen for management of asymptomatic as well as symptomatic neonates with sepsis. Clinical symptomatology can be mild and nonspecific, while definite diagnosis based on culture is not available for at least 2 days. So there is a dilemma, to give antibiotic therapy or not. In this case indirect markers of sepsis provide practical help. Because none of these tests alone have sufficient accuracy and reliability, a combination of tests is used to diagnose probable sepsis. This combination is called Sepsis Screen. The aim of present study is to find the correlation of haematological parameters with microbiological one (blood culture).

MATERIALS AND METHODS

This is a prospective cross sectional hospital based study conducted in Pathology department and neonatology division of department of Pediatrics, in Rural Medical College Loni over a period of 2 years from August 2009 to July 2011. This study was done to find out the predisposing risk factors, utility of sepsis screen to diagnose sepsis, which is to evaluate the efficacy of various hematological parameters in diagnosing early onset sepsis and identification of causative organisms and its correlation with hematological parameters. The study was done on 250 neonates selected by stratified random sampling, out of which 45 had proven sepsis, 160 had probable sepsis based on clinical features and sepsis screen and 45 had no sepsis.

Exclusion Criteria

1. Extreme prematurity (<28wks gestation).
2. Very low birth weight (< l000gms).
3. Multiple gestations.
4. Neonates with congenital anomalies.
5. Late onset sepsis (> 72 hrs of age).
6. Babies delivered outside the hospital and admitted.

Methods

A printed questionnaire will be used to elicit detailed maternal history. A printed proforma for recording detailed history and thorough clinical examination for neonatal septicaemia was studied. Blood culture is the gold standard for diagnosis of septicaemia and should be performed in all cases of suspected sepsis prior to starting antibiotics. A positive blood culture with sensitivity of the isolated organism is the best guide to antimicrobial therapy. Therefore it is very important to follow the proper procedure for collecting a blood culture.

The resident doctor/staff should wear sterile gloves prior to the procedure and prepare a patch of skin approximately 5-cm in diameter over the proposed veni-puncture site. This area should be cleansed thoroughly with alcohol, followed by povidone iodine, and followed again by alcohol. Povidone-iodine should be applied in concentric circles moving outward from the centre. The skin should be allowed to dry for at least 1 minute before the sample is collected. One-mL sample of blood should be adequate for a blood culture bottle containing 5-10 mL of culture media. Since samples collected from indwelling lines and catheters are likely to be contaminated, cultures should be collected only from a fresh veni-puncture site. All blood cultures should be observed for at least 72 hours before they are reported as sterile.

In the setting of present study blood culture is done by a conventional method & a gram smear is also made and examined at subculture stage. Data is subjected to appropriate statistical analysis.

Table 1: Distribution of cases of sepsis according to mode of delivery.

MODE OF DELIVERY	MALE	FEMALE	TOTAL
Vaginal	80	64	144(70.24%)
LSCS	43	18	61(29.76%)
Total	**123 (60%)**	**82 (40%)**	**205**

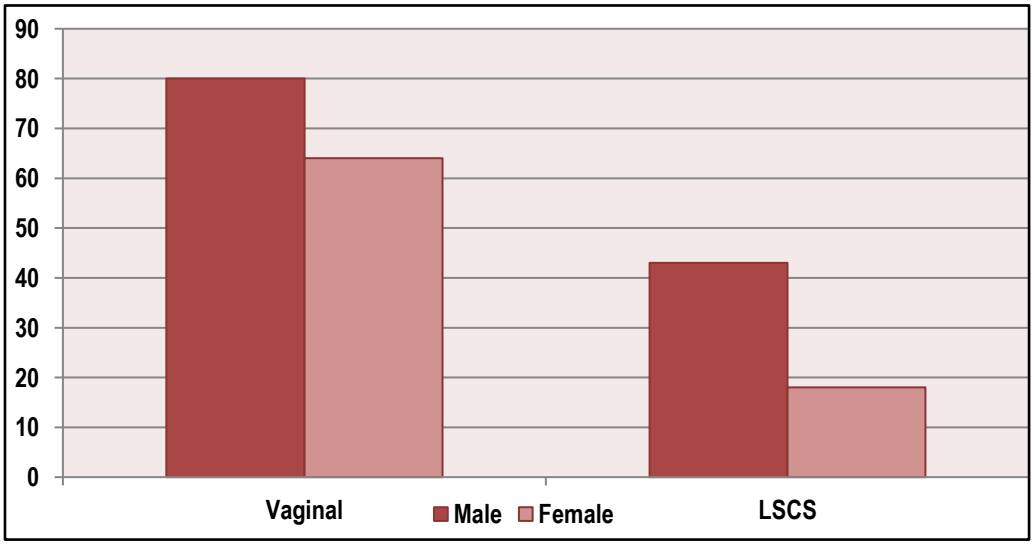

Figure 1: Mode of delivery.

Table 2: Distribution of cases of sepsis (n=205) according to gestational age.

Gestational Age (Wks)	Male	Female	Total
≥ 28-30	3	2	5(2.44%)
≥30-32	17	11	28(13.66%)
≥32-34	18	8	26(12.68%)
≥34-36	21	17	38(18.54%)
≥36-38	30	19	49(23.90%)
≥38	34	25	59(28.78%)
Total	**123 (60%)**	**82 (40%)**	**205**

Table 3: Distribution of cases of sepsis (n=205) according to age of onset.

Age of Onset (Hours)	Male	Female	Total
<24	93	67	160(78.05%)
24-48	22	13	35(17.07%)
48-72	8	2	10(4.88%)
Total	**123 (60%)**	**82 (40%)**	**205**

Table 4: Distribution of cases of sepsis (n=205) with predisposing perinatal risk factors.

Risk Factors	Male	Female	Total	Percentage
Low birth weight/ prematurity	91	53	144	70.24%
Single unclear or >3 sterile vaginal examinations	46	38	84	40.98%
Premature rupture of membranes > 18 hrs	49	19	68	33.17%
Instrumental delivery	43	18	61	29.76%
With 1 or none risk factors	23	19	52	25.37%
Foul smelling / meconium stained liquor	24	26	50	24.39%
Febrile illness in the mother	03	07	10	4.88%
Prolonged labour (1^{st} and 2^{nd} stage > 24 hrs)	06	02	08	3.9%

Table 5: Distribution of cases of sepsis (n=205) according to clinical presentation:

Clinical Parameters	Male	Female	Total	Percentage
Not able to feed	98	56	154	75.12%
Not attaching to breast	92	52	144	70.24%
Reduced movements	81	42	123	60.00%
Temperature >37.7ºC or <35.5ºC	74	41	115	56.1%
Not sucking at all	70	38	108	52.68%
Lethargic or unconscious	38	16	54	26.34%
Cyanosis	23	24	47	22.93%
Respiratory rate >60/min	23	16	39	19.02%
Severe chest indrawing	16	22	38	18.54%
Nasal flaring	16	22	38	18.54%
Grunting	16	22	38	18.54%
Crepitations	16	22	38	18.54%
Prolonged capillary refill time	28	10	38	18.54%
Convulsions	27	10	37	18.05%
Bulging fontanel	12	07	19	9.27%
Sclerema	10	08	18	8.78%
Redness around umbilicus	11	04	15	7.31%

Table 6: Distribution of cases of sepsis (n=205) according to organism isolated:

ORGANISMS	MALE	FEMALE	TOTAL
Klebsiella	11	11	22(48.89%)
Staph. aureus	9	3	12(26.67%)
Coagulase negative staphylococus	4	1	5(11.11%)
E.Coli	2	1	3(6.67%)
Pseudomonas	1	1	2(4.44%)
Enterococcus	1	0	1(2.22%)
Total	**28(62.22%)**	**17 (37.78%)**	**45**

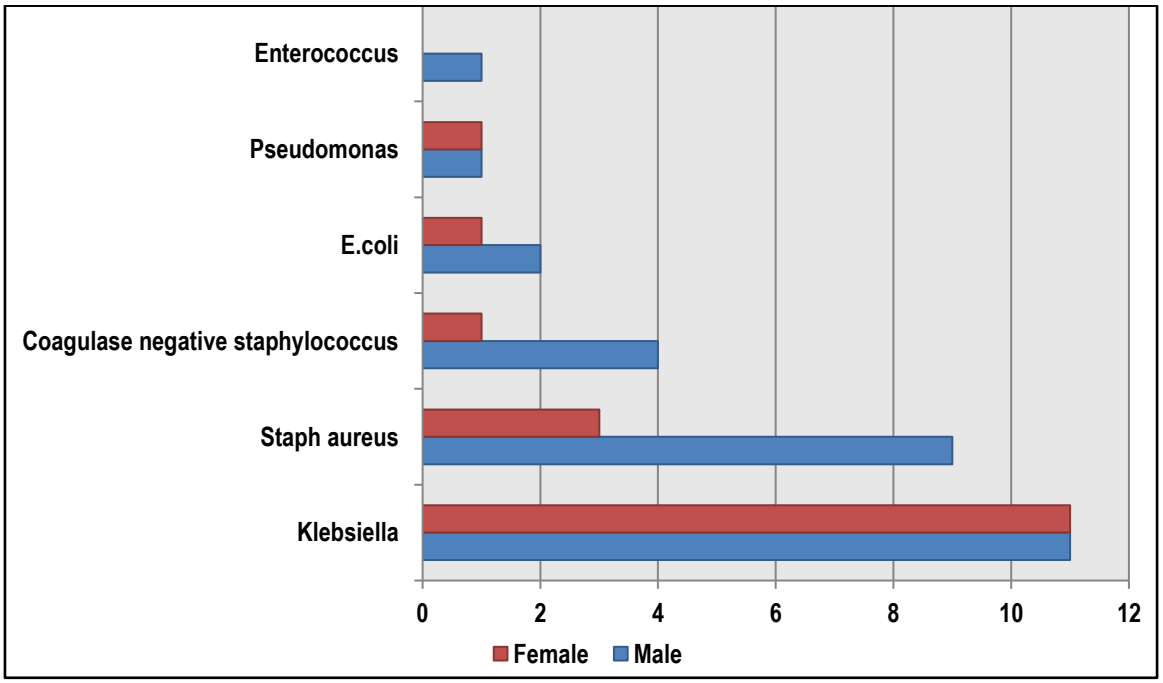

Figure 2: Organisms isolated.

Table 7: Distribution of organisms isolated by blood culture, according to their gram staining:

Gram Staining	Gram-Negative	Gram-Positive	Total
No. of cases	27(60%)	18(40%)	**45**

RESULTS

After applying Chi-square test there is a significant association between cases of sepsis and mode of delivery. (i.e. p<0.05) [Value of Chi Square test = 3.98, d. f. = 1, significant, p<0.05] Out of the 205 babies studied, 144(70.24%) were born through vaginal delivery and 61(29.76%) were delivered through LSCS. Among these 144 babies born through vaginal delivery, 80 were male and 64 were female. Among the 61 LSCS born babies, 43 were male and 18 were female [Table 1].

Mean gestational age (wks) for total patients = 35.10. For male: 35.05, for female: 35.19. After applying Chi-square test there is a significant association between gestational age and cases of sepsis (i.e. p<0.05) [Value of Chi Square test = 25.48, d. f. = 4, significant, p<0.05] Among the 205 cases of sepsis studied, 5 (2.44%) babies were of ≥28-30wks of gestation, out of which 3 babies were male and 2 were female; 28(13.66%) babies were of ≥30-32wks of gestation, out of which 17 were male and 11 were female; 26 (12.68%) babies were of ≥32-34wks of gestation, out of which 18 babies were males and 8 were females; 38(18.54%) babies were of ≥34-36 wks of gestation, out of which 21 babies were male and 17 were female; 49(23.90%)babies were of ≥36-38 wks of gestation, out of which 30 babies were male and 19 were female; 59(28.78%)babies were of ≥38 wks of gestation, out of which 34 babies were male and 25 were female. There is statistically significant association between prematurity and sepsis. [Table 2] Mean age of onset (hours) for total patients = 18.44. For male: 19.41, for female: 16.97. After applying Chi-square test there is a significant association between age of onset (hours) and cases of sepsis (i.e. p<0.05) [Value of Chi Square test = 12.44, d. f. = 2, significant, p<0.05] Among the 205 cases of sepsis , 160(78.05%) babies had onset of <24 hrs, out of which 93 babies were male and 67 were female; 35(17.07%) babies had onset between 24-48 hrs, out of which 22 were male and 13 were female; 10(4.88%) babies had onset between 48-72 hrs, out of which 8 were male and 2 were female [Table 3]. Risk factors like low birth weight & prematurity were present in 70.24%, Single unclear or >3 sterile vaginal examinations in 40.98%, Premature rupture of membranes > 18 hrs (33.17%), instrumental delivery in 29.76%, foul smelling & meconium stained liquor in 24.39%, febrile illness in mother in 4.88% and prolonged labour in 3.9%. In 74.63% of cases there was two or more predisposing factor present. Positive predictive value of 2 or more risk factors is 84.32% [Table 4]. Common clinical manifestations of neonatal septicaemia were feeding difficulty (75.12%), reduced movement (60%), temperature abnormality (56.1%), respiratory distress (19.02%), and convulsions (18.05%), prolonged capillary refill time (18.54%), sclerema (8.78%) and redness around umbilicus (7.31%) [Table 5]. Value of Chi Square test = 2.01, d. f. = 5, Not significant, p>0.05. After applying Chi-square test there is a no significant association between organism and cases of sepsis (i.e. p>0.05). Among the 45 culture proven babies studied, 22(48.89%) babies had grown Klebsiella, out of which 11 babies were male and 11 were female; 12(26.67%) babies had grown Staph. aureus, out of which 9 babies were male and 3 were female; 5(11.11%) babies had grown Coagulase negative staphylococcus aureus, out of which 4 babies were male and 1 was female; 3(6.67%) babies had grown E. coli, out of which 2 babies were male and 1 were female; 2(4.44%) babies had grown Pseudomonas, out of which 1 baby was male and 1 was female; 1(2.22%) baby had grown Enterococcus, which was a male child [Table 6]. Among the 45 culture proven cases 27(60%) were gram negative and 18(40%) were gram positive [Table 7].

DISCUSSION

In present study, 144 (70.24%) were born through vaginal delivery and 61(29.76%) were delivered through LSCS. In a study conducted by, Kuruvilla et al[10] 73.3% were born through vaginal delivery and 26.7% were born through LSCS, Sunaina et al[11] 85.3% were born through vaginal delivery and 14.7% through LSCS, and in Shrestha et al[12] 73.53% were born through vaginal delivery and 26.47% through LSCS.

Among the 205 early onset sepsis babies studied, 5(2.44%) babies were of ≥28-30wks of gestation, out of which 3 babies were male and 2 were female; 28(13.66%) babies were of ≥30-32wks of gestation, out of which 17 were male and 11 were female; 26 (12.68%) babies were of ≥32-34wks of gestation, out of which 18 babies were male and 8 were female; 38(18.54%) babies were of ≥34-36 wks of gestation, out of which 21 babies were male and 17 were female; 49 (23.90%) babies were of ≥36-38 wks of gestation, out of which 30 babies were male and 19 were female; 59(28.78%)babies were of ≥38 wks of gestation, out of which 34 babies were male and 25 were female. So, 71.21% of babies were preterm and 28.79% were term. There is statistically significant association between prematurity and sepsis which is comparable with other studies showing a higher incidence among preterm.

In a study conducted by Khatua et al[13] the incidence of early onset sepsis in preterm was 63% and 37% in term, Ahmed et al[14] showed incidence of 50% in preterm, 47% in term and 3% in post term. Chacko et al[15] showed incidence of 80.55% in preterms.

Table 8: Comparative studies showing the distribution of predisposing perinatal risk factors among neonates with early onset sepsis

Perinatal risk factors	St. Geme et al[16] n=33 (1984)	Yancey et al[17] n=117 (1996)	Dawodu et al[18] n=61 (1997)	Tallur et al[19] n=203 (2000)	Roy et al[20] n-346 (2002)	Present study n=205 (2011)
LBW/Preterm	78%	29.9%	53.8%	54.5%	63.8%	70.24%
Maternal fever	18.2%	-	6%	4.13%	5.2%	4.89%
Foul smelling liquor/MSL	36.4%	49.57%	6%	-	-	24.39%
PROM > 18hrs	100%	36.75%	12%	14%	28.9%	33.17%
Unclean vaginal exam.	-	50.4%	-	-	-	40.98%
Pro longed labour >24hrs	24.2%	29.9%	8%	19.1%	-	3.9%

The present study clearly shows a higher proportion of cases having unclean per vaginal examination before delivery, PROM (premature rupture of membranes) >18 hrs and prolonged labour for >24 hrs in developing definitive septicemia. This is comparable with study conducted by St Geme et al[16] and Yancey et al[21] 1996. It is also evident from present study that nearly an equal proportion of cases had birth weight < 2.5kgs and Gestational age < 37 wks as risk factors for developing septicemia. This is comparable with study conducted by Dawodu et al[18], Tallur et al[19] and Roy et al.[20]

Clinical chorioamnionitis and Meconium stained liquer (MSL) was present in 24.39% in present study which is comparable with studies conducted by St. Geme et al[16] & Yancey et al.[17] The variations in the occurrence of perinatal risk factors probably reflect differences in the rates of occurrence of the predisposing risk factors in the various studies.

Table 9: Comparative studies showing the distribution of perinatal risk factors among neonates with early onset sepsis

CLINICAL PARAMETERS	Khatua et al[13] n=92 (1986)	Ahmed et al[14] n=12 (2002)	Sunaina et al[11] n=225 (2004)	Present study n=205 (2011)
Feeding difficulty	92.3%	33.3%	79.5%	75.12%
Respiratory distress	24%	75%	71.5%	19.02%
Temperature abnormality	71.6%	25%	54.2%	56.1%
Convulsion	10.8%	8.3%	32%	18.05%
Lethargy	74%	33.3%	41.8%	26.34%
Cyanosis	13%	16.7%	74.2%	22.93%
Redness around umbilicus	60.1%	16.7%	4.4%	7.31%
Prolonged Capillary refill time	-	-	47.5%	18.54%
Sclerema	17.4%	-	-	8.78%

In present study, common clinical manifestations of neonatal septicemia were feeding difficulty (75.12%), reduced movement (60%), temperature abnormality (56.1%), respiratory distress (19.02%), convulsions (18.05%), prolonged capillary refill time (18.54%) and redness around umbilicus (7.31%). In present study feeding difficulty was the most common (75.12%) clinical feature which is comparable with Sunaina et al[11] and Khatua et al[13] Temperature abnormality was a common (56.1%) clinical feature, which is comparable with Sunaina et al[11] and Khatua et al.[13] In present study respiratory distress was present in 19.02% which is comparable with Khatua et al[13] but not with Sunaina et al[11] and Ahmed et al[14] because of less incidence of pneumonia in our study. In present study, incidence of convulsion (22.93%) and cyanosis (18.05%) is comparable with Khatua et al[13] and Ahmed et al.[14] In present study redness around umbilicus was present in 7.31% which is comparable with Sunaina et al[11] and Ahmed et al.[14] In present study Prolonged Capillary refill time was present in 18.54% which is comparable with Sunaina et al.[11] In present study Sclerema was present in 8.78% which is comparable with Khatua et al.[13]

Table 10: Comparative studies showing culture positivity rate

SL. No	Study Group	Year	Total culture +ve	Positivity rate
1	Joshi et al[20]	2000	332 /1326	25%
2	Tallur et al[19]	2000	157 / 242	64.87%
3	Roy et al[22]	2002	346 / 728	47.50%
5	NNPD (National Neonatal Perinatal Database)2002-2003[23]	2003	1248/4360	28.6%
6	KennethC.Iregbu et al[24]	2006	86/390	22%
7	Neeraj kumar et al[25]	2010	52/167	31%
8	Present study	2011	45/205	21.95%

In the present study, 45 of 205 cases studied were culture positive, giving a positivity rate of 21.95%. Present study was comparable with the studies conducted by Joshi et al[20], NNPD[23] and Kenneth C.Iregbu et al.[24]

While study conducted by Tallur et al[19] showed a high culture positivity because multidrug resistance was frequent. The culture positivity depends on lab setup, incidence of sepsis in the study area, time of sampling, extent of bacteremia in neonate and prior antibiotic treatment in the neonate. In the present study, Klebsiella pneumoniae 48.89% was the predominate isolate, followed by S. aureus (26.67%). Gram negative organisms formed the majority of the isolates as compared to Gram positive organisms in the present study. This is comparable with studies conducted by others. Predominate organisms isolated in different studies vary, but in present study it is compared only on Gram positivity or negativity.

Table 11: Comparative studies showing the distribution according to organism isolated

ORGANISMS	I Roy et al[22] n=728 (2002)	NNPD 2002 2003[23] n=4360	Neeraj kumar et al[25] n=167 (2010)	Present study n=205 (2011)
Klebsiella	24.6%	32.5%	37%	48.89%
Staph. aureus	14%	13.6%	6%	26.67%
E. coli	14%	10.6%	23%	6.67%
Pseudomonas	3.33%	5.6%	15%	4.44%
Coagulase Negative Staphylococus Aureus	16.6%	5.9%	10%	11.11%
Enterococcus	22.9%	3.8%	2%	2.22%

CONCLUSION

There is statistically significant association between cases of sepsis and mode of delivery. There is statistically significant association between prematurity and sepsis.

Common clinical manifestations of neonatal septicaemia were feeding difficulty (75.12%), reduced movement (60%), temperature abnormality (56.1%), respiratory distress (19.02%), convulsions (18.05%), sclerema (8.78%), prolonged capillary refill time (18.54%) and redness around umbilicus (7.31%).

Among the 45 culture proven babies, 22 (48.89%) babies had grown Klebsiella, of which 11:11 (M:F); 12 (26.67%) babies had grown Staph. aureus, of which 9:3 (M:F); 5 (11.11%) babies had grown Coagulase Negative Staphylococcus, of which 4:1 (M:F); 3 (6.67%) babies had grown E. coli, of which 2:1 (M:F); 2 (4.44%) babies had grown Pseudomonas, of which 1:1 (M:F); 1 (2.22%) baby had grown Enterococcus, which was a male child.

Among the 45 culture proven cases, 27 (60%) were gram negative and 18 (40%) were gram positive.

Blood culture positivity and organism isolated on culture is helpful in instituting appropriate antibiotic , to which the organism is sensitive and help to develop an antibiotic policy for the neonatal intensive care unit of institution.

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Source of Support: Nil. **Conflict of Interest:** None Declared.

Cite this article as: Lakshika Chauhan, Swati Chouhan, N N Agarkar, Gurmit Singh. Correlation of Haematological Parameters with Blood Culture in Neonatal Sepsis: A Prospective Study. Int J Med Res Prof. 2018 Mar; 4(2):66-72.
DOI:10.21276/ijmrp.2018.4.2.014