Evaluation of risk factors and bacteriological profile of Neonatal Sepsis among patients admitted at Navodaya Medical College, Hospital and Research Center, Raichur

Sneha S. Hegadi[1,*], Ashish Jitendranath[2]

[1]Assistant Professor, Shridevi Institute of Medical Sciences, Tumkur, Karnataka, [2]Associate Professor, Dept. of Microbiology, Sree Gokulam Medical College & Research Foundation, Venjaramoodis

***Corresponding Author:**
Email: sneha.hegadi@yahoo.com

Abstract

Introduction: Neonatal sepsis accounts for 69% of neonatal deaths in the developing world. Bacterial isolates and their antibiotic susceptibility has constantly been changing, which depends on several factors like gestational age, birth weight, maternal risk factors, place of delivery, mode of delivery.

Materials and Method: Blood samples from 200 clinically suspected neonatal septicemia cases were subjected to aerobic culture. Detailed history including risk factors and clinical findings were recorded. The culture isolates obtained were tested for antibiotic susceptibility pattern.

Results: Of the 200 cases studied, 92[46%} were blood culture positive which included 49[53.26%] males, 62[67.39%] were preterm and 53[57.60%] were very low birth weight, 68[73.91%] were spontaneous vaginal delivery and 59 [64.13%] were hospital out born neonates. Early onset septicemia was more common, seen in 66.33% of cases than late onset septicemia in 33.69% cases. Gram positive organisms were predominant in 59.78% of cases than Gram negative organisms in 42.39%. Majority of Gram negative isolates were susceptible to Ertapenem, Cefepime followed by Aztreonam and Gram positive isolates were 100% susceptible to Vancomycin, Linezolid and 100% resistance to Penicillin, Tetracycline.

Conclusion: In a setting with high fatality rates, high risk of mortality in neonatal sepsis has to be identified and targeted for intensive intervention.

Keywords: Neonatal septicemia, Blood culture, Susceptibility pattern, Risk factors

Introduction

Every year nearly 98% of neonatal deaths occur in developing countries. Neonatal sepsis causes an estimated 1.1 million deaths per year. Most neonates never reach the hospital; moreover paediatricians or facilities for bacterial culture are not available at most peripheral health facilities in developing countries. Simple methods to identify neonatal sepsis in the community for antibiotic treatment or referral are needed.Neonatal septicemia acquired either from mother (vertical transmission) or nosocomial (horizontal) is a formidable problem encountered in neonatal nurseries. It is the commonest cause of morbidity and mortality among neonates.[(1)] Mortality rate in neonatal sepsis differs according to the organism involved.

The risk factors for neonatal septicemia are poor maternal nutrition, birth asphyxia, LBW, premature rupture of membranes, prolonged rupture, UTI, prematurity, and congenital anomalies.[(3)]

Neonatal sepsis is divided into 2 groups: early onset or late depending on the onset of infection. Early onset septicemia usually caused by organisms prevalent in maternal genital tract. It presents as perinatal hypoxia and respiratory distress. Late onset is nosocomial usually or community acquired.[(1)]

The manifestations of neonatal sepsis are most often vague and hence for early diagnosis one needs to be vigilant. A WHO study [2003] identified 9 clinical features which predicted severe bacterial illness in young infants-

1. No spontaneous movement
2. Feeding ability reduced
3. Capillary refill time prolonged
4. Temperature > 38^{o}C
5. Respiratory rate > 60/ min
6. Cyanosis
7. Lower chest wall in drawing,
8. Grunting
9. H/o convulsions.[(4)]

Agnihotri N et al[2004][(5)] evaluated 3064 blood samples, out of which 588 were positive for bacterial isolates. Most of the cases detected by blood culture, occurred in the first week of life [64.4%].

Bacterial isolates and their antibiotic susceptibility has been changing constantly,[(6)] which depends on several factors like maternal risk factors, place of delivery gestational age, mode of delivery, birth weight. The antibiotic abuse has resulted in further confusion in diagnosis and the emergence of drug resistant bacterial strains in the nurseries with grave sequel. The successful treatment with an outcome favorable top the neonate depends on an ongoing review of the causative organisms and their antibiotic susceptibility pattern.[(7,8)]

Materials and Method

Blood samples from 200 clinically suspected cases of neonatal septicemia admitted in NICU, Navodaya

Medical College, Hospital and Research Centre for a period of 1 year from Nov. 2011 to Oct. 2012 were processed. Only Neonates with age less than 28 days were included. Exclusion criteria included, age is greater than 28days, neonates who received antibiotic dose before the septic workup and neonates with congenital malformation.

Risk factors for neonatal septicemia was evaluated which included history of:

- History of Birth asphyxia / Prematurity
- Unclean per vaginal examination before delivery
- Foul smelling liquor amnii
- Prolonged rupture of membranes >24 hrs
- Prolonged labour >24 hrs

❖ Clinical diagnosis: Early onset septicemia

1. **Late onset septicemia:** Blood samples were drawn into blood culture bottle before administration of antibiotic therapy by trained staff with all aseptic precautions. 1 ml of the blood sample was inoculated into a blood culture bottle aseptically. The blood culture bottles were incubated at 37° C under aerobic conditions in the incubator for 7 days. The first subculture was done after 24 hours of incubation, third day and on seventh day. Subcultures were then put up onto 5% sheep blood agar and MacConkey agar plates. The inoculated plates were incubated aerobically in the incubator at 37° C for 24 hours, and then observed for growth. The growth was identified by colonial characteristics, Gram's stain and standard biochemical tests described in Mackie and McCartney, Practical Medical Microbiology,[9] and Bailey and Scott's Diagnostic Microbiology[10] Cultures which did not show any growth following three subcultures were reported as negative at the end of 7 days. The culture isolates obtained were tested for antibiotic susceptibility pattern.
2. **Antibiotic Susceptibility Testing:** Antibiotic susceptibility testing was done for all the isolates on Mueller Hinton agar using commercially available discs (Hi media), by Kirby Bauer disc diffusion technique. As per the CLSI guidelines [2010]

Results

Of the total 200 clinically suspected neonates,92(46%) blood culture positive and 108 (54%)blood culture negative.126[63%] were males and 74[37%] females among which,49[53.26%] and 43[46.73%] were blood culture positive respectively.

Table 1: Showing the distribution of cases according to sex

Sex	Clinically suspected cases no	Culture positive cases no
Males	126	49
Females	74	43
Total	200	92

p> 0.05- statistically not significant

Early onset of septicemia was more common .Majority i.e. 118[59%] neonates were less than one week old, of which 61 cases yielded a positive growth in blood culture, indicating septicemia was more common in the first week of life. Neonates affected in 8-14 days were 47 and >14 days were 38 among which, 23 and 8 were culture positive respectively.

Table 2: Showing the distribution of cases according to the age

Age in days	Clinically suspected cases no	Culture positive cases no
1-7 days	118	61
8-14 days	47	23
>14 days	38	8
Total	200 (100)	92(100)

In the present study, culture positive septicemia cases were higher among the Very low birth weight neonates (57.6%) as compared to the Low (33.69%) and Normal birth weight (8.69%) neonates.

Table 3: Showing the distribution of cases according to birth weight

Birth Weight	Clinically suspected cases no	Culture positive cases no
Very low birth weight(<1.5kg)	68	53
Low birth weight (<2.5 kg)	89	31
Normal birth weight (>2.5 kg)	43	8
Total	200	92

p<0.05 statistically significant

72(36%) were preterm babies, 120 (60%) were term neonates and 8[4%) were post term neonates. Among culture positive cases, 68[73.91%] were preterm and 24[26.08%] were term. Maximum culture positive cases were seen among the preterm neonates.

Table 4: Showing the distribution of cases according to gestational age

Gestational Age (Weeks)	Clinically suspected cases no (%)	Culture positive cases no (%)
Pre term (<37 wks)	72	68
Term (37-41 wks)	120	24
Post term (>41 wks)	8	0
Total	200	92

P < 0.001 statistically significant

In the present study maximum culture positive (69.6%) cases were seen in neonates delivered by spontaneous vaginal delivery. The result of our study was comparable with the observations made by Kuruvilla et al,[21] Tallur et al[14] and Neema Kayange et al.[18]

Table 5: Showing the distribution of cases according to mode of delivery

Mode of Delivery	Clinically suspected cases no	Culture positive cases no
Spontaneous Vaginal	148	68
Caesarean Section	32	24
Total	200	92

p < 0.05 statistically not significant

Of the total 200 neonates, 116[58%] were hospital inborn (i.e.; delivered in our hospital/institution) babies, of which 28[30.43%] were culture positive whereas 79[39.5%] were hospital out born (i.e.; delivered in other hospital/institution) babies with 59[64.13%] being culture proven cases and 5[2.5%] were home born among which 5[5.43%] were culture positive. Maximum culture positive cases were from the hospital out born neonates.

Table 6: Showing the distribution of cases according to place of delivery

Place of Delivery	Clinically suspected cases no	Culture positive cases no
Hospital inborn	116	28[30.43]
Hospital out born	79	59[64.13]
Home	5	5[5.43]
Total	200	92

P<0.05 statistically significant

Table 7: Shows the distribution of perinatal risk factors among cases

Sl No		Clinically suspected cases		Culture positive cases		P Value
		EOS No N=146	LOS No N=54	EOS No N=61	LOS No N=31	
1	Foul Smelling Liquor AMNI[FSLA]	41.09%	40.74%	62.29%	35.48%	<0.0001
2	Unclean Vaginal Examination before Delivery[UPV]	65.75%	79.62%	91.80%	74.19%	0.110
3	Prolonged Labour> 24 Hrs	29.45%	27.77%	47.54%	11.47%	0.884
4	One Minute APGAR Score < 6	58.90%	22.22%	90.16%	13.11%	0.073
5	Prolonged Rupture of Membranes>24 Hrs	21.91%	35.18%	39.34%	29.05%	0.215
6	Gestational Age <37Wks and/ or Birth WT ≤ 2Kgs	91.09%	53.7%	68.85%	70.96%	<0.0001

Among the clinically suspected cases, 96 of EOS and 43 LOS cases had the major risk factor of unclean per vaginal (PV) examination prior to delivery. In the culture positive cases, 56 of EOS had an unclean PV examination; while 23 LOS cases had a history of unclean PV examination. The difference observed was statistically not significant in either the clinically suspected or the culture positive cases. Gestational age <37 weeks and / or a Birth weight ≤ 2 kgs was the second major risk factor seen among 133 of early onset and 29 of the late onset clinically suspected cases. Among the culture positive cases, 68.85% Of EOS cases and 70.96% of LOS cases had this risk factor. The difference observed was statistically significant in both the groups. The third major risk factor seen among the clinically suspected cases was a One minute Apgar score <6 among 86 cases of EOS cases and 12 of LOS cases had this risk factor. Among the culture positive cases, 90.16% Of EOS cases and 8% of LOS cases had this risk factor.

Culture Positive Cases

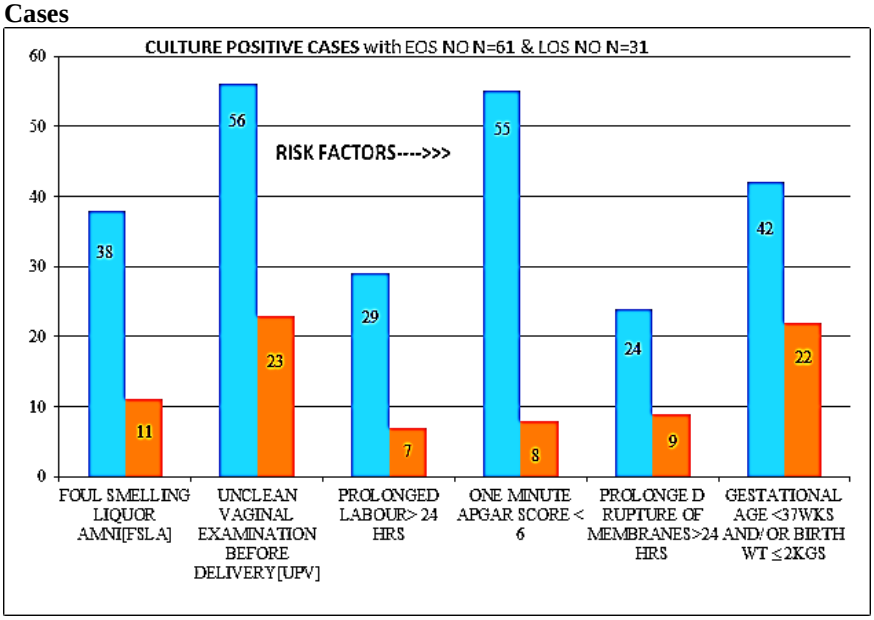

Table 8: Showing the total spectrum of isolates with EOS and LOS Cases

Bacterial isolates	Culture positive EOS (%)	Culture positive LOS (%)	Total No (%)
S.aureus	28[45.9]	12[38.7]	40[43.47]
CONS	8[13.11]	5[16.12]	13[14.13]
K.pneumoniae	13[21.31]	9[29.03]	22[23.91]
E.coli	8[13.11]	3[9.67]	11[11.95]
P.aeruginosa	4[6.55]	2[6.45]	6[6.52]
Total	61[100]	31[100]	92[100]

Of the total 92 positive blood culture, 40 were Staphylococcus aureus, the most common isolate followed by Klebsiella pneumonia -13. Other gram negative organisms isolated were Escherichia coli 11, and P.aeruginosa 6. Majority i.e.; 61 [66.30%] of the culture positive case were seen among the early onset septicemia followed by 31 (33.69%) among the late onset septicemia.

Table 9: Showing antibiotic sensitivity pattern of the isolates

Antibiotics	K.pne N=22		E.coli N=11		P.aer N=6		S.aur N=40		CONS N=13	
	S %	R %	S%	R%	S%	R%	S%	R%	S%	R%
Ampicillin (AMP)	-	-	27.27	72.27	-	-	-	-	-	-
Gentamicin(G)	63.63	36.36	72.27	27.27	50	50	57.5	42.5	76.92	23.07
Amikacin (AK)	77.27	22.72	36.36	63.63	50	50	-	-	-	-
Ciprofloxacin (CIF)	40.9	59.09	27.27	72.27	50	50	65	35	92.30	1[7.69]
Aztreonam (AZ)	27.27	72.27	54.54	45.45	50	50	-	-	-	-
Cefotaxime (CE)	22.72	77.27	27.27	72.27	0	100	-	-	-	-
Cefotaxime/ Clavulanate (CE/CL)	27.27	72.27	36.36	63.63	0	100	-	-	-	-
Ceftriaxone (CTR)	59.09	40.90	63.63	36.36	100	0	-	-	-	-
Cefuroxime (CU)	13.63	86.36	18.18	81.81	50	50	-	-	76.92	23.07
Ceftazidime (CA)	31.81	68.18	27.27	72.27	50	50	-	-	92.30	7.69
Cefoxitin (CX)	27.27	72.72	45.45	54.54	50	50	52.5	47.5	100	0
Cefepime (CEF)	77.27	22.72	100	0	50	50	-	-	-	-
Ertapenem (ETP)	86.36	13.63	100	0	50	50	-	-	-	-
Penicillin (P)	-	-	-	-	-	-	0	100	92.30	7.69
Tetracycline (T)	-	-	-	-	-	-	0	100	13	0

Chloramphenicol (C)	-	-	-	-	-	-	7.5	92.5	84.61	15.38
Erythromycin (E)	-	-	-	-	-	-	80	20	92.30	7.69
Clindamycin (CD)	-	-	-	-	-	-	100	0	100	0
Cotrimaxizole (COT)	-	-	-	-	-	-	55	45	92.30	7.69
Vancomycin (VA)	-	-	-	-	-	-	100	0	100	0
Linezolid	-	-	-	-	-	-	100	0	100	0

In the present study, majority isolates were Gram positive, accounting for 57.60% of the total cases, commonest was, S.aureus [43.47%] in which MSSA(methicillin sensitive S. aureus was seen 19 (47.5%), MRSA (Methicillin resistant S.aureus) was seen in 21(52.5%), 100% sensitive to Vancomycin and Linezolid and 100% resistant to Penicillin and Tetracycline. Other antibiotics like Erythromycin, Ciprofloxacin, Cotrimoxazole, Cefoxitn and Chloramphenicol showed 66.6% sensitivity. No inducible Clindamycin resistance was noted in these isolates.

Accounting to 42.39%, Gram negative organisms namely Klebsiella pneumoniae(23.91%) was commonest, susceptible to Ertapenem,(86.36%), Cepifime(77.27%), Amikacin(72.27%) and Ceftazidime(31.81%). Most of the isolates were found to be resistant to Ceftriaxone, Gentamycin, and Ciprofloxacin.

E.coli isolates [11.95%] were 100% susceptible to Ertapenem and Cepifime followed by Gentamicin, Ceftriaxone and Aztreonam. Majority were resistant to Ciprofloxacin, Amikacin, Cefotaxime and Cefoxitin.

Discussion

Despite the considerable progress in early diagnosis and treatment measures Neonatal septicemia is still a major cause of morbidity and mortality particularly, in the developing countries. This continues to pose a challenge to the pediatricians in making a definitive clinical diagnosis due to the subtle and non-specific signs and symptoms; hence laboratory diagnosis plays a major role. In the present study an attempt has been made to know the various risk factors responsible for neonatal septicemia, the common causative bacterias including their antibiotic profile.

In the present study culture positive septicemia cases were higher among males than the females. This co-related with studies of Varsha et al,[11] Dhumal P,[12] Y R Khinchi.[13] In neonatal septicemia male preponderance is probably linked to the X- linked immunoregulatory gene factor that results in the increase of susceptibility to infections in males. But Betty Chacko et al,[32] observed that both males and females were equally affected. In our study maximum culture positive cases were early onset septicemia as compared to late onset septicemia. This correlated to studies done by A.H. Movahedian, Shaw C K,[33] Vinod Kumar, Zardad Muhammad et al, Arpita Shah[18] reporting maximum cases of early onset septicemia. The ascending infections following rupture of membranes or the passage of the baby through the infected birth canal or resuscitation in the labour room could be one of the many reasons for early onset sepsis. According to Barbara J. Stoll[22] the higher number of EOS cases is due to the immature immunological responses of the neonates in the first week of life, and hence they are more susceptible to infections in this period. Our results differs from the study by G. Karthikeyan et al[10] who reported an equal number of EOS and LOS cases in their study, while the study by Zardad Muhammad et al[78] shows a higher percentage of LOS cases than EOS cases. These findings could be because of the nosocomial (hospital acquired) or community acquired source of infection.

In the present study, culture positive septicemia cases were higher among the Very low birth weight neonates (57.6%) as compared to the Low (33.69%) and Normal birth weight (8.69%) neonates. Our results correlate with studies done by I Roy.[8] According to Barbara J. Stoll et al,[22] the rate of infection is inversely proportional to the birth weight. In the very low birth weight neonates cellular immunity is impaired due to low IgG levels which contributes to the increased susceptibility to infections.

In the present study culture positive septicemia cases were higher among the preterm neonates. The results of our study were comparable to the studies conducted by Tallur et al[29] Roy I,[8] Betty Chacko et al,[32] Dhumal P,[11] Neema Kayange et al[35] and Arpita Shah et al.[18] Study conducted by Tallur et al,[29] showed a higher number of cases among the term. (60.30%) neonates compared to the preterm (39.70%) neonates, these variations probably due to predisposing factors among term neonates. Due to deficiencies of humoral and cellular defense mechanisms, pre terms are more susceptible to infections. According to Barbara J. Stoll et al,[22] the incidence of septicemia increased with the decreased gestational age of the neonates.

In the present study maximum culture positive (69.6%) cases were seen in neonates delivered by spontaneous vaginal delivery. The result of our study was comparable with the observations made by Kuruvilla et al,[28] Tallur et al[29] and Neem Kayange et al.[35] Neonatal septicemia was more prevalent in neonates delivered vaginally due to the surface colonization of the neonate with the microbial flora of the birth canal during vaginal delivery.

The present study showed, higher culture positive cases among hospital out born (64.13%) neonates compared to hospital inborn (30.43%) and home delivery (5.43%). Our results were comparable with the observations made by G.P. Mondal et al[24] in their study.

The results of the present study differs from the study by G.G. Christo et al,[57] who observed an equal number of culture positive cases among both the hospital inborn and out born neonates, while the study conducted by Uddin Ahmed et al,[52] showed a higher culture positivity among the home delivered neonates. The authors refer that majority of their study population were poor and delivered at home in the hands of untrained birth attendants under poor standards of asepsis, and were brought to the hospital for treatment. The higher percentage of septicemia among hospital out born neonates in the present study points to a probable community acquired source of infection in these neonates

In the present study, S.aureus 43.47% was the predominant isolate followed by Klebsiella pneumonia 23.91%. Gram positive organisms were more compared to Gram negative organisms 57.6%, 42.39% respectively causing septicemia in the present study.

In the present study, commonest organism isolated amongst Gram negative organisms was Klebsiella pneumoniae which was 86.36% susceptible to Ertapenem and 77.27% to Cepifime, 72.27% to Amikacin and 31.8% Ceftazidime. Most of other antibiotics were resistant. Our study was in co-relation with study done by A. Mohmood et al,[62] AH. Movhedian et al,[60] Shaw CK et al[75] and Neema Kayange et al.[77] In the present study, E.coli isolates were 100% susceptible to Ertapenem and Cepifime followed by Aztreonam[54.54%], Ceftriaxone[63.63%] and Gentamicin [72.27%]. Majority were resistant to Amikacin, Ciprofloxacin, Cefotaxime and Cefoxitin. Our study was in co-relation with study done by AH. Movhedian et al,[60] Shaw CK et al[75] and Neema Kayange et al.[77] In the present study, P.aeuriginosa was 100% sensitive to Ceftriaxone, Ceftazidime, 50% to Ertapenem, Cepifime, Aztreonam, Cifrofloxacin, Gentamycin and Cefoxitin and 100% resistance to Cefotaxime and Cefuroxime. Our study differed from study done by AH. Movhedian et al,[60] Shaw CK et al[75] and A. Mahmood et al[62] in showing increased percentage of Ertapenem resistance, Aztreonam and Cefotaxime. MSSA (methicillin sensitive S. aureus) were seen 47.5%, MRSA (Methicillin resistant S.aureus) were seen in 52.5% of the total isolates. MRSA strains were 100% sensitive to Vancomycin and Linezolid and 100% resistant to Penicillin and Tetracycline. Other antibiotics like Erythromycin, Ciprofloxacin, Cotrimaxazole, Cefoxitn and Chloramphenicol showed 50% sensitivity. No inducible Clindamycin resistance was noted in these isolates. Our study was in co-relation with the study conducted by A. Mahmood et al[62] and Shaw CK et al[75] as they also showed majority of the isolate were resistant Penicillin, Tetracycline and sensitive to Vancomycin, Linezolid. But study conducted by Neem Kayange et al[77] observed 86% of Vancomycin sensitive strains.

Conclusion

Neonatal septicemia is still a leading cause of mortality and morbidity in developing countries like India. It is more common among males, very low birth weight and preterm neonates. It was also found to be more common among the hospital out born neonates with spontaneous vaginal delivery. Majority of the cases were seen in early onset septicemia (60.9%) compared to late onset septicemia (39.1%). Gram positive organisms are the predominant causative agents in neonatal septicemia compared to Gram negative .Hospital acquired infections are a major threat to the premature and low birth weight neonates with multidrug resistant microorganisms emerging as a major cause of septicemia.

The present study showed most of the isolates resistant to Ampicillin, Amikacin and Cefotaxime, Penicillin and Tetracycline which are used as the first line therapy. Overall, majority isolates were susceptible to Gentamicin, Ciprofloxacin and the third generation cephalosporin like Ceftriaxone, and Ceftazidime which are the best alternatives for the first line therapy in neonatal septicemia. Other antibiotics like Aztreonam, Cefepime and Ertapenem were sensitive in majority of the cases which can be used as reserve drugs in case of multidrug resistant organisms. MRSA were 100% sensitive to Vancomycin and Linezolid.

In view of the changing spectrum of the causative agents of neonatal septicemia and their antibiotic susceptibility patterns from time to time and from one hospital to another, a positive blood culture and the antibiotic susceptibility testing of the isolates are the best guide to the antimicrobial therapy.

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