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THE BACTERIOLOGICAL AND DEMOGRAPHIC PATTERN OF TERM AND NEAR TERM NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Aims and Objective: of the study was to assess the bacteriological profile and demographic pattern of neonatal sepsis.

Material and Methods: Prospective analytic study was done from January 2015 to December 2015. Out of 1272 admissions, all term and near term neonates with weight appropriate for gestational age were 954, all were investigated on the basis of history, clinical findings, biochemical investigations, cultures for bacteriological profile and demographic pattern of neonatal sepsis.

Results: out of 954 who qualified inclusion criteria, sepsis was diagnosed in 396 (41.51%) neonates. Among 396 neonates 116 had at least one episode of positive culture, Out of 116 patients 110 were positive in blood cultures, four in CSF cultures and two in urine cultures. Gram negative sepsis occurred in 70 out of 116 (60.3%), gram positive sepsis occurred in 28 out of 116 (24.1%) and fungal sepsis was diagnosed in (15.5%). Acinetobacter was the most common organism in gram negative sepsis 26 out of 116 (22.4%) followed by klebsiella 15.5%, Escherichia Coli 12.9%, Pseudomonas 6.9% and Burkholderia Pseudomallei 2.6%. Among gram positive organisms the most common pathogen was Staphylococcus aureus 12.9% followed by Enterococcus 6.9% and Coagulase negative Staphylococcus 4.3% patients.

Conclusion: gram negative sepsis occurred more commonly than Gram positive in both early and late onset cases of sepsis. Incidence of acinetobacter and fungal sepsis was more in those patients which were referred from outside in more sick condition, required prolonged mechanical ventilation and more invasive procedures. All demographic parameters were insignificant across the study except age at time of admission.

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INTRODUCTION

Neonatal sepsis is a significant cause of morbidity and mortality among neonates worldwide. World Health Organization has estimated that 1.6 million deaths occur globally every year due to neonatal infections and 40% of all neonatal deaths occur in developing countries (WHO report 2006). In India, the incidence of blood culture proven sepsis was reported as 8.5 per 1,000 live births for the year 2002–2003 by the National Neonatal Perinatal Database (NNPD report 2002-03). Most of the neonatal sepsis related deaths are preventable if suspected early and treated with appropriate antibiotics. Neonatal sepsis is broadly categorized into early and late onset sepsis depending upon the postnatal day of presentation.

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Early-onset neonatal sepsis (EONS) occurs within first 72 h of life, while the late-onset neonatal sepsis (LONS) occurs between 72 h to 90 days of life (Sundaram *et al.,* 2009). The bacterial agents implicated in early-onset sepsis include group B Streptococcus (GBS), Escherichia coli, coagulase-negative Staphylococcus, Haemophilus influenzae and Listeria monocytogenes (Anderson-Belly *et al.,* 2010). The organisms commonly associated with late-onset sepsis include coagulase-negative Staphylococci (CONS), Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Enterobacter spp., Pseudomonas aeruginosa and Acinetobacter species (Kaistha *et al.,* 2009). The bacteriological profile for causative organisms of neonatal sepsis differs significantly between developed and developing countries (Sanghvi and Tudehope, 1996; Stoll *et al.,* 2002). Therefore, it is essential to establish the bacteriological profile of organism associated with septicemia. Prompt diagnosis and effective treatment is necessary to prevent deaths and complications due to

septicemia. Physical signs and symptoms are useful in identifying infants and children with septicemia. These clinical characteristics can be good predictors for positive blood culture but they have limited specificity and sensitivity (Tumbarello *et al.,* 2007; Weber *et al.,* 2003). Blood culture to isolate the offending pathogen remains the gold standard for definitive diagnosis of septicemia. But the results of blood culture takes hours to days, thus necessitating initial empirical treatment of suspected cases. However, the appropriateness of this empirical therapy is being challenged in the present era of changing bacteriological profile and increasing antimicrobial resistance (WHO report 1995). Therefore, the knowledge of common organisms causing neonatal sepsis in a particular area and their antibiotic sensitivity pattern should be borne in mind before setting guidelines for empirical therapy. Hence, the current study was designed to assess the bacteriological profile and demographic pattern of neonatal sepsis.

MATERIAL AND METHODS

The study was observational analytic study, conducted over a period of twelve months from january 2015 to december 2015 in neonatal intensive care unit of SGRRIM&HS Patel Nagar Dehradun Uttrakhand, a tertiary care hospital in North India.

Inclusion criteria

All babies who were admitted during this period were evaluated prospectively for evidence of sepsis. Near term and term babies with weight appropriate for gestation ages were included in this study.

Exclusion criteria

Babies with congenital malformations, and chromosomal anomalies were excluded. Sepsis was defined according to international sepsis definition conference (Levy *et al*., 2003) as "clinical syndrome characterized by presence of both infection and systemic inflammatory response syndrome".

Systemic inflammatory response syndrome in case of neonates is defined as two or more of the following:

- Tachypnea (respiratory rate more than 60 bpm + grunting or retractions.
- Temperature instability < 36°C or more than 37.9°C.
- Capillary refill time more than 3 seconds.
- White blood cell count < 5000/µl or more than 34000/µl.
- CRP more than 10 mg/dl IL-6 more than 70 pg/ml.
- Procalcitonin more than 8.1 mg/dl or more than 2 SD above normal values.

Sepsis is defined as one or more systemic inflammatory response syndrome criteria with signs of infection (MA Bhat *et al.,* 2009). Only first episode of sepsis in a patient was included. Sepsis evaluation was based on clinical signs and symptoms and rapid screening tests for sepsis, blood culture, urine microscopy and culture and CRP. Lumbar puncture for cerebrospinal fluid (CSF) analysis and culture, stool culture and chest radiographs, were obtained at the discretion of the attending pediatrician.

In case of CONS sepsis repeat blood culture was taken to rule out contamination. Blood for culture (1ml of blood) and complete blood counts was obtained by means of venipuncture. Blood cultures were monitored by an automated system (Bac T/ALERT 3D). The WBC count with differential and the platelet count were quantified using automated laboratory equipment (Sysmex KX-21). Urine was obtained by urethral catheterization using a sterile technique. A careful urinalysis, on a fresh urine sample, can identify children with a high likelihood of UTI to enable presumptive treatment while awaiting results of urine culture, the WBC in the urine were quantified by standard microscopic examination and expressed as WBC >5 leukocytes / high power field in a centrifuged sample or >10 leukocytes / mm^3 in an uncentrifuged sample, Urinary tract infection was defined as growth of single known

Table 1. Showing distribution of Organisms Causing Neonatal Sepsis

ORGANISM		Total number of patients (116)	Percent
Gram Negative (67.5%)	Acinetobacter	26	22.4%
	Klebiella	18	15.5%
	E.coli	15	12.9%
	Pseudomonas	8	6.9%
	Burkholderia Pseudomallei	3	2.6%
Gram Positive (26.25%)	Staph Aureus	15	12.9%
	Enterococcus	8	6.9%
	CONS	5	4.3%
Yeast (6.25%)	Candida aibicans	18	15.5%

Table 2. Demographic Details of Males and Females

Feature	Gestation (Weeks)	Birth Weight (gms)	Caesarean Section	Age at admission (hours)	Mortality
Male (64)	37.42±1.66	2480±288	15/116 (12.93%)	99.09±22.06	11/116 (9.48%)
Female (52)	*38±1.69*	2630±160	20/116 (17.24%)	94.20±20.60	5/116 (4.31%)
All (116)	37.92±1.48	2600±270	35 (30.17%)	98.88±22.22	16/116 (13.79%)

Table 3. Demographic Data of Septic Neonates

Feature		All Patient (116)	Gram Negative (70)	Gram Positive (28)	Fungal (18)	P.Value
1	Gestation (Weeks)	37.92±1.48	37.98±1.49	37.53±1.93	37.10±1.00	>0.05
2	Birth Weight (kgs)	2.60 ± 0.27	2.51±0.30	2.63±0.20	2.50 ±0.19	>0.05
3	Caesarean Section	35(30.17%)	21(18.1%)	9(7.75%)	5(4.31%)	>0.05
4	Age (in hours) at Admission	98.88±22.22	94.70±20.13	96.10±18.2	140.20±16.80	<0.05
5	Stay in hospital (days)	17.96±5.25	18.39±5.14	17.14±5.56	16.80±5.40	>0.05
6	Mortality	16(13.79%)	10(8.6%)	5(4.3%)	1(0.8%)	>0.05

pathogen on urine culture with ≥100,000 cfu/mL of urine obtained by urethral catheterization, (Consensus Statement on Management of Urinary Tract Infections 2001). Urosepsis was taken as UTI with signs of SIRS. The urine, CSF, and stool cultures were monitored using standard laboratory techniques. Normal CSF in neonates was defined as, cells up to 8(0-30)/mm^3, proteins 90(20-170)mg/dl, polymorphonuclear cells up to 60% of TLC, CSF glucose content 52(34-119)mg/dl and values beyond it were always taken abnormal in all patients (Sarff *et al*.,1976). Early-onset neonatal sepsis (EONS) occurs within first 72 hr of life, while the late-onset neonatal sepsis (LONS) was taken beyond 72hr of life (Sundaram *et al.,* 2009). Mortality was defined as death before discharge. Infants discharged to home were considered survivors. For statistical analysis data was expressed as mean ±SD. Analysis was done by using student t-test for parametric data. Proportions were compared using X2 test of significance. Values were considered significant if $p< 0.05$. The data was analysed using SPSS package.

RESULTS

During the study period, out of total only 954 patients fulfilled the inclusion criteria, sepsis was diagnosed in 396 (41.51%) neonates. Among 396 neonates 116 term and near term neonates with weight appropriate for gestational age had at least one episode of positive blood culture, urine culture and CSF culture for particular bacteria responsible for sepsis. Out of 116 patients 110 were positive in blood cultures, four in CSF cultures and two in urine cultures. Other 280 neonates had clinical features as well as biochemical evidences of sepsis but their cultures were persistently sterile. Out of 116 blood culture positive neonates 95 were term neonates and 21 were near term. Among these males were 64 (55.2%) and females were 52 (44.8%). Out of 116 neonates, gram negative sepsis occurred in 70 out of 116 (60.3%), Gram positive sepsis occurred in 28 out of 116 (24.1%) and fungal sepsis was diagnosed in (15.5%) i.e. 18 out of 116 shown in table 1. Acinetobacter was the most common organism in gram negative sepsis 26 out of 116 (22.4%) followed by klebsiella 18 out of 116 (15.5%), Escherichia Coli in 15 out of 116 (12.9%), Pseudomonas in 8 out of 116 (6.9%) and Burkholderia Pseudomallei 3 out of 116 (2.6%).

Among gram positive organisms the most common pathogen was Staphylococcus aureus in 15 out of 116 (12.9%) followed by Enterococcus was seen in 8 out of 116 (6.9%) and Coagulase negative Staphylococcus in 5 out of 116 (4.3%) patients. The most common fungal isolate was Candida albicans 18 out of 116 which is 15.5%. The demographic details of the male and female neonates infected with the three groups of organisms are shown in table 2 and 3. The average gestational age of all babies was 37.92 ± 1.48 weeks. Average Gestational age of babies with gram negative sepsis was 37.98 ± 1.49 weeks, with gram positive sepsis was 37.53 ± 1.93 and with fungal sepsis was 37.10±1 weeks ($p>0.05$). The average birth weight of all infected babies was 2.60±0.27 grams. Average weight of babies with gram negative sepsis was 2.51±0.30 gram, with gram positive sepsis was 2.63±0.20 gram and fungal sepsis was 2.50±0.19 gram. Although the average birth weight of babies having fungal sepsis was lower than babies with Gram positive or Gram negative sepsis, the difference was statistically insignificant. Out of 116 babies, 64 (55.17%) were males and 52 (44.83%) females. Incidence of caesarean deliveries was 30.17%. There was no difference in incidence of caesarean deliveries between groups ($p>0.05$). Average age at admission in all neonates was 98.88±22.22 hours. In gram negative sepsis it was 94.70±20.13 hours, in gram positive sepsis it was 96.10±18.20 hours. Average age at onset of fungal sepsis was 140.20±16.80. The difference was statistically significant ($p< 0.05$). Average duration of stay in hospital in all patients was 17.96±5.25 days. In gram negative sepsis it was 18.39±5.14 days, in gram positive sepsis it was 17.14±5.56days. In fungal sepsis average duration of hospital stay was 16.80±5.40days. The difference was statistically insignificant ($p>0.05$). The average mortality in all septic neonates was 16 out of 116 (13.79%). In gram positive sepsis mortality was 5/116(4.3%). In gram negative sepsis mortality was 10/116 (8.60%). In fungal sepsis it was 1/116(0.8%). The difference between the groups was statistically insignificant ($p>0.05$). Average age at admission in males was 99.09±22.06 hours and in females 94.20±20.60 hours. Average gestation of males and females was 37.42±1.66 weeks and 38±1.69 weeks respectively. Average birth weight of males and females was 2480±288 grams and 2630±160 grams respectively. Number of males with caesarean section was 15/116(12.93%) and females were 20/116(17.24%).

DISCUSSION

Infections such as pneumonia, septicemia, meningitis, and diarrhoea account for 30-50% of neonatal deaths in developing countries (Sarff *et al*.1976). This study from a tertiary care hospital in northern India reports on the incidence of neonatal sepsis in NICU, organism distribution, morbidity and mortality associated with neonatal sepsis. In our study the incidence of neonatal sepsis among term and near term was 41.51% (396 out of 954). Among 396, 116 patients were culture proven cases of sepsis. Neonatal blood culture positive rate have been found to range from 25–54% by English *et al.,* 2004; Klingenberg *et al.,* 2003; Mugalu *et al.,* 2006) in their studies. Pramila Verma *et al.* (2015) had noticed 36.8% (87/236) incidence of the neonatal sepsis in their study. High incidence of neonatal sepsis reported in our study has many reasons. As our hospital is a tertiary care hospital where sick patients are referred from the other hospitals and maternity centres. 64/116 (55.17%) babies in our study were born outside and were referred already in very sick condition. Also patients born outside had not received prior good follow up and many of them were not booked deliveries.

In our study we found gram negative organisms to be commonest cause of neonatal sepsis. Similar trend has been reported from developing as well as some developed countries by Issacs d *et al.* (1999). A sample of 11471 blood samples from all developing nations of the world revealed that gram negative rods were isolated in 60% of positive cultures with Klebsiella pneumoniae being the most common organism by Zaidi AK *et al.* (2005). In our study total incidence of Gram negative sepsis was 60.2% i.e. 70 out of total 116 patients with culture proven sepsis. But most common gram negative organism in our study was acinetobacter baumannii 26 out of 116(22.4%), followed by klebsiella 18 out of 116 (15.5%), E Coli 15 out of 116(12.9%), pseudomonas 8 out of 116(6.2%)

and Burkenholderia cepacia 3 out of 116(2.6%). Asit Mishra *et al.* (1998) had shown Acinetobacter to be responsible for 79 (31.5%) of the blood culture positive sepsis in their study. Reason for more incidence of acinetobacter sepsis in our study could be, the patients referred from outside were already in very sick condition, for which they required prolonged ventilatory support, recurrent suctioning of endotracheal tubes and more patient to nursing staff ratio in our NICU.

In our study the incidence of gram positive sepsis was 24.14% i.e. 28 patients out of 116, which was less than Gram negative sepsis. Commonest organism was Staphylococcus aureus (12.93%), followed by Enterococcus (6.9%) and finally CONS (4.3%). However Sanghvi KP *et al*.(1996) had shown gram positive organisms are the commonest cause of neonatal sepsis in developed countries with CONS being the commonest organism. In our study early onset sepsis occurred in only 9.48% i.e. 11 out of 116 patients, out of which five cultures were positive for klebsiella, four E Coli and two staph aureus, so Gram negative sepsis was more common than Gram positive. Gram negative organisms being the commonest cause of EOS has also been reported by Stoll JB *et al*. (2002). In developing countries Klebsiella is the commonest cause of early onset sepsis as shown by Zaidi AK *et al*. (2005). Vankatashan *et al*. (2009) had reported early onset sepsis in 62% of cases in his study with klebsiella pneumoniae as most common organism responsible during 1995-1998 but during the year 2001-2006 they noticed non fermenting gram negative bacilli (Acenitobacter and others) as the predominant organisms. In our study 90.51% (105/116) neonates had late onset sepsis. This is because of admission of out born babies, larger hospital stays and increased invasive procedures device usage. All these factors predisposed them to nosocomial/late onset sepsis. Gram negative organisms 58.09 % (61/105) were the major agents responsible for late onset sepsis. Data from both developed as well as developing countries have shown gram negative bacilli to be major pathogens of neonatal sepsis in one study done by Issacs d *et al*. (1999). Joshi *et al*. (2000) from India reported gram negative sepsis in 87% of their cases. But, in our study most common cause of late onset sepsis was acinetobacter with 24.76% i.e. 26 out of 105 followed by Klebsiella pneumoniae and staph aureus, each was responsible for 13 out of 105 i.e. 12.38% followed pseudomonas, E Coli and Burkenholdaria. Data from NNPD 2002-2003 from India has shown K. pneumoniae as the major pathogen in (27-32%) both intramural as well as extramural neonates.

In our study Staph.aureus was the second most common organism 13 out of 105 i.e. 12.38% responsible for late onset sepsis. E.Coli was found in 10.48% (11 out of 105) late onset sepsis. Vankateshen *et al*. (2009) had reported incidence of E.Coli in 11% cases of LOS. CONS was seen in 4.76% i.e. 5 out of 105 cases of late onset sepsis in our study. Venkatashan *et al*. (2009) had reported 5-6% incidence of CONS in late onset sepsis. In developed countries CONS is the major causative organism of late onset sepsis, which might indicate a high rate of invasive devise use as compared to our setup and setup in other developing countries. In our study yeast was grown in 17.14% i.e 18 out of 105 cases of LOS. Vankatashan *et al*. (2009) had reported that 11% of septic neonates were having fungal sepsis.The incidence of fungal sepsis was very high in comparison to other studies, especially in those patients which were referred from outside to this hospital. The incidence of meningitis on the basis of clinical features and abnormal CSF picture in our study was 15.51% i.e. 18 out of 116 patients, but there were only four cases of bacteriologically proven meningitis i.e. after isolation of bacteria on CSF Culture, out of four cases two were of EColi and one was of staph aureus and one acinetobacter, all of them presented as cases of late onset sepsis. The study by Sartaj Bhat *et al*. (2015) had shown the incidence of meningitis 17/80 (21.3%) in neonates. There were also two cases of E Coli urosepsis in our study. Demographic parameters like gestation age, birth weight, mode of delivery, stay in hospital, mortality were insignificant across the gram negative, gram positive and fungal infections except age at time of admission. Another study by Sartaj Bhat *et al.* (2015) had shown the similar pattern in their study.

Conclusion

In conclusion the incidence of neonatal sepsis was very high in our study. Gram negative sepsis occurred more commonly than Gram positive in both early and late onset cases of sepsis. Acinetobacter was the most common organism in gram negative sepsis 26 out of 116 (22.4%) followed by klebsiella 15.5%, Escherichia Coli 12.9%, Pseudomonas 6.9% and Burkholderia Pseudomallei 2.6%. Among gram positive organisms the most common pathogen was Staphylococcus aureus 12.9% followed by Enterococcus 6.9% and Coagulase negative Staphylococcus 4.3% patients. Incidence of acinetobacter and fungal sepsis was more in those patients which were referred fom outside in more sick condition and required prolonged mechanical ventilation and invasive procedures. All demographic parameters were were insignificant across the study except age at time of admission.

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