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Original Article

Paediatrics Section

Clinical and Microbiological Profile of Neonatal Sepsis at a Tertiary Care Hospital in Dehradun, India

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ABSTRACT

Introduction: Neonatal sepsis is the most common cause of neonatal mortality. It is responsible for 30-40% of neonatal deaths in developing countries. Due to the non-specific nature of presentation, neonatal sepsis is very difficult to diagnose, despite its high incidence. Blood culture is still considered as the gold standard for the diagnosis of neonatal septicaemia. However, sensitivity and specificity of blood culture varies considerably due to many factors, and the final diagnosis of neonatal sepsis is based on a combination of clinical, microbiological and haematological parameters.

Aim: To find sensitive clinical indicators for suspecting neonatal sepsis and to ascertain the microbiological profile of neonatal sepsis.

Materials and Methods: This six months (01st May 2017-31st October 2017) prospective observational study was conducted in the Department of Microbiology of Sri Guru Ram Rai Institute of Medical and Health Sciences, Dehradun, Uttarakhand, India, on 45 neonates admitted in the Neonatal Intensive Care Unit (NICU). Half ml to 2 mL of blood was drawn following strict aseptic precautions, before the start of antibiotics. Microbial detection and identification were by fully automated BACT/ALERT 3D and VITEK 2 systems, respectively. The data was expressed in terms of frequency and percentage, and statistical results were analysed with help of Microsoft Excel.

Results: In this study, culture yielded positive results in a relatively high proportion (60%) of suspected cases. Respiratory distress, reduced movements, and poor feeding were very frequently encountered in both suspected and confirmed cases of neonatal sepsis. Fever was seen only in around half of all neonates of suspected and confirmed neonatal sepsis. In this study, bacteria were 81.48% of the isolates, whereas fungi were 18.51%. Although, as a group, gram-negative bacteria formed the predominant group isolated in cases of neonatal sepsis, yeasts like *Candida* species were the predominant isolate (18.51%). In this study, isolation of gram-negative bacteria (74.07%) predominated over gram-positive bacteria (7.4%).

Conclusion: Respiratory distress, reduced movements, and poor feeding were sensitive indicators for suspecting neonatal sepsis. Fever was a relatively uncommon finding in this study. *Candida* species and *Acinetobacter baumanii* were more frequently isolated. Automation can significantly help in reducing mortality in neonatal sepsis.

Keywords: Early onset sepsis, Late onset sepsis, Septicaemia, Systemic inflammatory response syndrome

INTRODUCTION

Neonatal sepsis is defined as a Systemic Inflammatory Response Syndrome (SIRS) which is associated with suspected or proven infection, in the first 28 days of life [1]. Neonatal sepsis is the most common cause of neonatal mortality, being responsible for around 30-40% of neonatal deaths in developing countries like India [2,3]. In India, the incidence of blood culture-confirmed 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) [4]. Newborn babies develop sepsis due to numerous maternal and neonatal risk factors. Some common maternal risk factors include prolonged rupture of membranes and febrile illness of the mother during delivery or within two weeks of it. Common neonatal risk factors include prematurity, low birth weight, and asphyxia [5]. Infection may be 3-10 folds higher in preterm babies (less than 37 weeks gestational age). This may be explained by the facts that preterm infants have a weaker immune response as compared to term infants and often require invasive procedures that provide an opportunity for the pathogens to enter [6]. Microbial agents may enter and infect the foetus through the placental route, the ascending route (after rupture of membranes) or during passage through the birth canal. After delivery, microorganisms may also infect the neonate from the extrauterine environment (e.g., nursery) [7,8]. Lastly, the foetus may also be infected by improperly disinfected instruments used during pregnancy or delivery (an additional risk factor) [9]. Pathogenic bacteria can cause localised infection of organs like the lung (pneumonia) or meninges (meningitis), or they may cause generalised infection (septicaemia) without any localisation [10].

As the clinical presentation of neonatal sepsis is very non-specific, it is difficult to accurately diagnose neonatal sepsis. Blood culture is regarded as the gold standard for the diagnosis of neonatal sepsis [11]. However, due to limitations in sensitivity and specificity of blood cultures, diagnosis of neonatal sepsis is based on a combination of clinical, microbiological, and haematological parameters [12]. Neonatal sepsis is further divided into Early Onset Sepsis (EOS) if the neonate presents in the first 72 hours of birth or Late Onset Sepsis (LOS) if the neonate presents after 72 hours of birth [13]. The timing of onset can be helpful in suspecting the microbial agents causing sepsis, as EOS and LOS are usually caused by distinct groups of microorganisms [14]. Neonatal sepsis is caused by a variety of microorganisms such as gram-positive and gram-negative bacteria, and even yeasts [5]. Group B Streptococcus (GBS) and *Escherichia coli* (*E. coli*) have been implicated in more than 70% cases of neonatal sepsis in the Western countries. However, in developing countries, gram-negative organisms are the major group [15]. GBS and *E. coli* are the most frequently isolated pathogens in cases of EOS. However, a minority of cases, may be caused by other streptococci (most commonly viridans group streptococci, but also *Streptococcus pneumoniae*) [16], *Staphylococcus aureus*, *Enterococcus* spp., gram-negative enteric bacilli such as *Enterobacter* spp., *Haemophilus influenzae* (virtually all non-typeable *Haemophilus* spp. in the *H. Influenzae*

type B {Hib} vaccine era), and *Listeria monocytogenes* [17-19]. The organisms commonly associated with LOS include Coagulase-Negative Staphylococci (CoNS), *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* spp., *Pseudomonas aeruginosa* and *Acinetobacter* species [20].

Septicaemia due to fungi is secondary to long hospital stays, multiple invasive procedures, and injudicious use of antibiotics [21]. *Candida* spp. are the most common fungi, although a few cases may be due to *Malassezia* spp. [22]. Classically, *Candida albicans* has been reported as the causative agent in half of the cases of fungal neonatal sepsis [23]. However, non-*albicans Candida* infections have also gained importance in the last few years [24]. The organisms which cause neonatal sepsis changes over time and vary from region to region. This is because of difference in selection pressure due to different antibiotics used in different areas [25]. The aim of this study was to find sensitive clinical indicators for suspecting neonatal sepsis and to ascertain the microbiological profile of neonatal sepsis. Although many studies are available on the microbiological profile of neonatal sepsis from the Indian subcontinent [12,25-33], only one study [12] has used fully automated methods for detection of microorganisms and their subsequent identification. Two studies [25,32] have used automated methods for detection only. Furthermore, from the studies using conventional method for detection of microorganisms from blood cultures, very few have followed standard protocols [27,31]. Using automation for detection from blood cultures decreases the likelihood of contamination, and increases its sensitivity; and using automation for identification aids in a more accurate identification of isolates as compared to conventional methods. This study bridges this gap by using state of the art automation methods (BACT/ALERT 3D and VITEK 2) for detection and identification, respectively.

MATERIALS AND METHODS

This observational study was conducted in the Department of Microbiology of Sri Guru Ram Rai Institute of Medical and Health Sciences, Patel Nagar, Dehradun, Uttarakhand, India, on 45 neonates (aged 0 to 28 days) admitted to the NICU in six months period (01st May 2017- 31st October 2017). The study was conducted in accordance with the ethical standards of the Institutional Ethics Committee, and informed consent was obtained from the parents. Suspected neonates with one or more signs and symptoms suggestive of sepsis like fever, cough, respiratory distress, reduced movements, poor feeding, abdominal distention, and diarrhoea, with or without any risk factors for sepsis, in whom blood culture or any other culture was sent, were recruited for the study. All neonates who did not satisfy the inclusion criteria were excluded from the study.

Blood samples were collected by healthcare staff following strict aseptic precautions. Samples were collected before the start of antibiotics. Half ml to 2 mL of blood was drawn using a sterile syringe, which was immediately inoculated into a paediatric bottle for BACT/ALERT 3D automated microbial detection system. If no growth was flagged till five days, the culture was regarded as sterile. If the growth was flagged as positive, subculture was done on sheep blood agar, MacConkey agar, and chocolate agar. Identification of organisms was done using the VITEK 2 automated microbial identification system [12].

STATISTICAL ANALYSIS

The data was expressed in terms of frequency and percentage. Statistics results were analysed with help of Microsoft Excel. The p-values for Odds Ratios (OR) were calculated using the Fisher's-exact test. A p-value <0.05 was considered statistically significant, and a p-value <0.01 was considered highly significant.

RESULTS

Of the 45 cases of suspected neonatal sepsis, organisms were isolated in 27 (60%) of cases [Table/Fig-1]. Organisms were predominantly isolated from blood, followed by Cerebrospinal Fluid (CSF) and urine [Table/Fig-2]. Frequency of EOS and LOS was almost alike in confirmed cases of sepsis, whereas in suspected sepsis, early onset sepsis (40%) was less frequent than late onset sepsis (60%) [Table/Fig-3].

Gender	Suspected cases of sepsis (Total=45)	Confirmed cases of sepsis (n=27)	Unconfirmed by culture (n=18)
Male infants	26	14	12
Female infants	19	13	6

[Table/Fig-1]: Gender distribution of cases of neonatal sepsis.

Sample	n (%)
Blood	23 (85.18%)
CSF	03 (11.11%)
Urine	01 (3.70%)

[Table/Fig-2]: Sepsis confirmed by culture (n=27).

Onset	Suspected (n=45)	Confirmed (n=27)
≤3 days	18 (40%)	14 (51.85%)
>3 days-28 days	27 (60%)	13 (48.14%)

[Table/Fig-3]: Early onset and Late onset (EOS and LOS) Sepsis.

Respiratory distress was the most common feature (71.11%) of suspected neonatal sepsis, followed by reduced movements (60%) and poor feeding (53.33%) [Table/Fig-4]. Respiratory distress (66.66%) along with reduced movements (66.66%) were also the most common features in cases of confirmed sepsis, followed by poor feeding (62.96%) [Table/Fig-5]. None of the suspected and confirmed cases had abdominal pain and diarrhoea. Fever was seen only in around half of all neonates of suspected and confirmed neonatal sepsis, and other symptoms like cough were seen only in a minority of cases of suspected and confirmed neonatal sepsis.

In cases of confirmed neonatal sepsis, reduced movements were also seen more commonly in preterm (<37 weeks) and low birth weight (<2500 gm) neonates, than in term and normal birth weight neonates (p=0.01) [Table/Fig-6,7]. Fever was more common in late onset sepsis compared to early onset sepsis in cases of neonates with confirmed neonatal sepsis (p<0.01) [Table/Fig-8]. The p-value of other symptoms were not significant.

Age	Fever	Cough	Respiratory distress	Reduced movements	Poor feeding	Abdominal distension	Diarrhoea
Less than or equal to 72 hours	4	0	12	13	12	0	0
More than 72 hours, and till 28 days	18	7	20	14	12	0	0
Total	22 (48.88%)	7 (15.55%)	32 (71.11%)	27 (60%)	24 (53.33%)	0	0

[Table/Fig-4]: Clinical features in suspected cases of neonatal sepsis (n=45).

Age	Fever	Cough	Respiratory distress	Reduced movements	Poor feeding	Abdominal distension	Diarrhoea
Less than or equal to 72 hours	3	0	10	10	10	0	0
More than 72 hours, and till 28 days	11	2	8	8	7	0	0
Total	14 (51.85%)	2 (7.47%)	18 (66.66%)	18 (66.66%)	17 (62.96%)	0	0

[Table/Fig-5]: Clinical features in confirmed cases of neonatal sepsis (n=27).

Weeks	Reduced movements	Normal movements	p-value (using Fisher's-exact test)
<37 weeks	17	4	0.01
≥37 weeks	1	5	

[Table/Fig-6]: Movements in pre-term versus term neonates in confirmed cases of sepsis (n=27).
p<0.05 is statistically significant

Birth weight	Reduced movements	Normal movements	p-value (using Fisher's exact test)
<2500 gm	14	2	0.01
≥2500 gm	4	7	

[Table/Fig-7]: Movements in low birth weight versus normal birth weight neonates in confirmed cases of sepsis (n=27).
p<0.05 is statistically significant

Age	Fever	No fever	p-value (using Fisher's exact test)
Less than or equal to 72 hours	3	11	<0.01
More than 72 hours, and till 28 days	11	2	

[Table/Fig-8]: Fever in early and Late Onset Sepsis (EOS and LOS) in confirmed cases of neonatal sepsis.
p<0.05 is statistically significant

Bacteria were more commonly isolated than fungi in cases of neonatal sepsis [Table/Fig-9], of which gram-negative organisms constituted around three-fourths of the isolates (74.07%), whereas gram-positive organisms were only a minority (7.4%). *Candida* spp. was the most common isolate (18.51%). This was followed by *Klebsiella pneumoniae* (14.81%) and *Acinetobacter baumanii* (14.81%), which were followed by *E. coli* (11.11%) and *E. cloacae* (11.11%) [Table/Fig-10].

Organism	Frequency	Percentage (%)
Bacteria	22	81.48
Fungi	5	18.51

[Table/Fig-9]: Organisms isolated in neonatal sepsis (bacteria versus fungi).

Organism	Less than or equal to 72 hours	More than 72 hours, and till 28 days	Total (%)
Klebsiella pneumoniae	3	1	4 (14.81%)
Klebsiella aerogenes	1	0	1 (3.70%)
E. coli	0	3	3 (11.11%)
Enterobacter cloacae	2	1	3 (11.11%)
Pseudomonas aeruginosa	0	2	2 (7.40%)
Pseudomonas stutzeri	1	0	1 (3.70%)
Acinetobacter baumanii	1	3	4 (14.81%)
Burkholderia cepacia	1	1	2 (7.40%)
Enterococcus faecalis	1	1	2 (7.40%)
Candida species	4	1	5 (18.51%)
Total	14 (51.85%)	13 (48.14%)	27

[Table/Fig-10]: Organisms isolated in neonatal sepsis.

DISCUSSION

In this study, organisms were isolated in 27 (60%) of cases. Culture positivity varies across studies from as low as 10.30% (Pandita N et al., [25]) to as high as 66.67% (Garg A et al., [26]). In this study, the frequency of EOS and LOS was almost alike (51.85% versus 48.14%). However, in literature, most studies have a predominance of EOS, when only the confirmed cases are taken into account [27-29]. In this study, fever was seen only in around half (51.85%) of the neonates with confirmed sepsis. This finding was very similar to a study by Chhabra GS et al., where fever was seen in 54% of neonates with sepsis [30]. On the contrary, Satyamurthi B et al., reported hypothermia in around one-third neonates (35%) [29]. In this study, respiratory distress, reduced movements, and poor feeding were more commonly seen in suspected as well as confirmed cases of neonatal sepsis. In a study by Ghosh S et al., reduced movements, poor feeding, and poor cry were seen in almost all cases of sepsis [31]. In the study, by Chhabra GS et al., poor feeding (87%) and reduced movements (76%) were the 2nd and the 3rd most common features of neonatal sepsis, followed by respiratory distress (72%) [30]. In this study, bacteria and fungi accounted for 81.48% and 18.51% of isolates, respectively. *Candida* species were the predominant isolates (18.51%) in this study. Ghosh S et al., had a very incidence of isolation of *Candida* species in their study (47.05%) [31]. However, studies like Satyamurthi B et al., and Kamble R and Ovhal R, had a very low isolation rate (<3%) of *Candida* species [29,32].

In this study, isolation of gram-negative bacteria (74.07%) predominated over gram-positive bacteria (7.40%). *Klebsiella pneumoniae* was the predominant gram-negative bacteria along with *Acinetobacter baumanii* (14.81%). Isolation of *Klebsiella pneumoniae* varies widely across studies, with the range being from 13.04-49.64% [12,27]. In this study, isolation of *E. coli* and *Enterobacter* were 11.11%. Many studies reported *E. coli* as the second most common gram-negative bacteria, with a range of 7.84-29.10% [31,32]. *Citrobacter* and *Pseudomonas* species (including *aeruginosa*), and *Enterococcus faecalis* were also isolated occasionally in this study. In this study, there was a relatively high proportion of isolation of *Acinetobacter baumanii* (14.81%) compared to other studies [12,25,31,33]. However, Satyamurthi B et al., also had a similarly high proportion of isolation of *Acinetobacter* species (14.68%) [29].

Staphylococcus species were not isolated in this study. This was in contrast to many other studies [12,25-29,32,33] [Table/Fig-11]. There was also no isolation of *Streptococcus* species like the studies by Ghosh S et al., and Kamble R and Ovhal R, [31,32]. These anomalies were probably due to the low sample size. *Burkholderia cepacia* was isolated twice in this study. *Burkholderia cepacia* is a rare cause of neonatal sepsis [34], and is responsible for outbreaks in NICUs [35]. *Burkholderia cepacia* is an important nosocomial pathogen, and its isolation in this study may be an attributed to inadequate infection control practices in the hospital. The incidence

Studies	KS	ESC	ENT	CIT	PS	AS	BC	SA	CNS	SS	ES	CS
Shah M and and Desai P, 2013 [33]	16.47	23.57	4.54	1.98	7.67	5.68	NA	15.9	13.35	7.38	NA	3.4
Sharma M et al., 2015 [12]	13.04	8.69	NA	NA	6.52	4.34	NA	39.13	26.08	2.17	NA	NA
Pandita N et al., 2016 [25]	26.6	11.29	5.64	5.64	3.22	6.45	NA	8.06	25.8	0.8	2.41	NA
Singh HK et al., 2016 [27]	49.64	26.95	NA	NA	7.8	NA	NA	7.09	4.96	NA	3.55	NA
Sathyamurthi B et al., 2016 [29]	37.06	8.39	NA	NA	4.89	14.68	NA	18.18	13.98	NA	NA	2.79
Kamble R and and Ovhal R, 2016 [32]	23.13	29.10	1.49	0.74	11.9	1.49	NA	17.91	5.97	2.23	4.47	1.49
Garg A et al., 2017 [26]	17.5	27.5	NA	NA	12.5	NA	NA	21.25	7.5	NA	13.75	NA
Ghosh S and Basu G, 2018 [31]	25.49	7.84	NA	NA	NA	1.96	NA	1.96	13.72	1.96	NA	47.05
Present study	18.51	11.11	11.11	NA	11.11	14.81	7.4	NA	NA	NA	7.4	18.51

[Table/Fig-11]: Comparison of organisms (in %) isolated in cases of neonatal sepsis from various studies.
KS: *Klebsiella* species; ESC: *Escherichia coli*; ENT: *Enterobacter* species; CIT: *Citrobacter* species; PS: *Pseudomonas* species; AS: *Acinetobacter* species; BC: *Burkholderia cepacia*; SA: *Staphylococcus aureus*; CNS: Coagulase negative staphylococci; SS: *Streptococcus* species; ES: *Enterococcus* species; CS: *Candida* species; NA: Not applicable

of *Burkholderia cepacia* in neonatal sepsis may be grossly under-reported, as hospitals using conventional methods of identification of bacteria routinely do no perform additional testing for seeking this organism.

Limitation(s)

The sample size was relatively small, and the entire spectrum of signs and symptoms were not recorded in this study.

CONCLUSION(S)

In this study, blood culture was positive in a relatively high proportion of suspected cases. Respiratory distress, reduced movements, and poor feeding were very frequently encountered, and these can be sensitive indicators for suspecting neonatal sepsis. Fever was seen only in around half of all neonates of suspected and confirmed neonatal sepsis, and this fact should be kept in mind for suspecting neonatal sepsis. Authors recommended, that as far as possible, automation should be used for detection and identification of organisms causing neonatal sepsis. Automation has higher sensitivity and specificity, and can help in significantly reducing mortality in neonatal sepsis.

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