Indian Journal of Applied Microbiology
ISSN (Online): 2454-289X, ISSN (Print): 2249-8400

Volume 21 Number 2
July - September 2018, pp. 20-28

A Microbiological Profile of Early Onset of Neonatal Sepsis in A Tertiary Care Hospital in North India

Yogeeta Bala[1*], V.S. Randhawa[1], Arvind Saili[2], Ravinder Kaur[1], Shweta Chitkara[1] and Aditya Duggal[3]

[1] Department of Microbiology, Lady Hardinge Medical College & Associated Hospitals, New Delhi.

[2] Department of Neonatology, Lady Hardinge Medical College & Associated Hospitals, New Delhi.

[3] Department of Neurology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi.

Abstract:

Introduction: Sepsis is the commonest cause of neonatal mortality and is responsible for 30-50% of total neonatal deaths each year in developing countries

Aims &Objective: To identify the common bacterial pathogens, risk factors associated with early onset neonatal sepsis (EONS) and their antibiotic susceptibility pattern.

Methods: Neonates with suspected early onset sepsis were enrolled in the study. Blood culture was done and growth, if any was identified and standard antibiotic susceptibility testing was done according to CLSI 2017 guidelines.

Results: Out of 150 neonates, 44 were culture proven cases of neonatal sepsis. *Klebsiella spp*. was isolated from 36.6% cases followed by *Staphylococcus aureus* 22.7% and *Acinetobacter spp*.(20.4%). The proportion of resistance to first line antibiotics like penicillin, erythromycin, clindamycin, gentamicin, amikacin, amoxicillin-clavulanic acid was high. On multivariate analysis, premature rupture of membranes (p value=0.0001), maternal fever (p value=0.01) and birth asphyxia (p value=0.007) were significantly associated with EONS.

Conclusion: Characterisation of causative organisms of EONS can aid in instituting prompt and appropriate therapy, in order to minimize morbidity and mortality.

*Author for Correspondence. E-mail: yogeetabala90@gmail.com

Keywords: Antibiotic susceptibility testing; Blood culture; Early onset neonatal sepsis; Neonate; Risk factors.

Introduction:

Neonatal sepsis is defined as infection with evidence of systemic inflammatory process [1] Neonatal sepsis (NS) is a clinical syndrome characterized by systemic signs and symptoms of infection in the first month of life [2]. It is one of the major causes of morbidity and mortality in the newborn. Surviving infants can have significant neurologic sequelae; as a consequence of central nervous system involvement, septic shock or hypoxemia secondary to severe parenchymal disease [3]. Neonatal infections are estimated to cause 1.6 million deaths every year globally, and 40% of all neonatal deaths occur in developing countries [4]. The disease is classified into early-onset neonatal sepsis (EONS-<7 days of birth) and late-onset neonatal sepsis (LONS >7 days) [5]. The source of infection in EONS is primarily from maternal genital tract. Various factors that may affect it include low birth weight, prematurity & invasive procedures. The diagnosis of neonatal sepsis is a challenge. Early diagnosis of neonatal sepsis is important and requires awareness of risk factors and a high index of suspicion. Neonates with clinical signs of sepsis should have a complete blood count Differential Count with smear, blood culture, urine culture (not necessary for evaluation of early-onset sepsis), and lumbar puncture (LP), if clinically feasible, as soon as possible. Neonates with respiratory symptoms require chest x-ray. The gold standard for confirming neonatal sepsis is a positive culture from a sterile site, including blood, cerebrospinal fluid (CSF) or urine. However, culture results may only become available after 48 - 72 hours [6]. The organisms responsible for NS vary across geographical locations [3,7].In most developing countries, gram-negative bacteria remain the major source of infection. However, in developed countries, gram-positive bacteria are the most common causes of NS [8]. The bacterial agents implicated in early-onset sepsis include group B *Streptococcus* (GBS), *Escherichia coli*, *coagulase negative Staphylococcus*, *Haemophilus influenza* and *Listeria monocytogenes* [9]. The overall improvement in the neonatal survival due to newer drugs, better neonatal care and advanced life support facilities has led to change in the spectrum of agents causing neonatal sepsis. However data on the recent trends of organisms causing neonatal sepsis in developing countries are limited [10].

Microorganisms implicated in neonatal sepsis have developed increased resistance to commonly used antibiotics, thus making treatment extremely difficult. Against this background, it is important that the etiology and epidemiology of NS is constantly monitored locally to detect changes in the pattern of pathogens and their antibiotic susceptibilities. This study was therefore undertaken to determine the common microbial agents associated with neonatal sepsis, their antibiotic susceptibility pattern and associated risk factors.

Material and Methods:

This prospective study was conducted on a total of 159 neonates admitted in the neonatology ward of Kalawati Saran Children Hospital, New Delhi over a period between November 2016-March 2018 in collaboration with Department of Microbiology, Lady Hardinge Medical College,

New Delhi. This study was approved by Ethics Committee for Human Research of our institute. Written informed consents were obtained from the parents. The inclusion criteria were a case having clinical suspected neonatal sepsis with age <7 days. The exclusion criteria were neonates who were on antibiotics or had known congenital anomalies (9 neonates were excluded) [30].

Neonates were considered to be at risk of developing early onset neonatal sepsis, if any one of the following potential maternal risk factors are present- prolonged rupture of membranes (PROM) for >24 hours, foul smelling liquor, maternal urinary tract infection within 2 weeks prior to delivery or peri-partum fever. Sepsis is clinically suspected, if the neonate had symptoms and signs suggestive of sepsis; such as poor feeding, poor activity, respiratory distress, apnea, seizure, lethargy, bulging anterior fontanella, fever, hypothermia, jaundice, vomiting, loose stools, abdominal distension, cyanosis, bleeding, mottling, tachycardia, weak pulse, grunting, retractions, nasal flaring etc. A study specific case proforma was used to collect the demographic and clinical data of the cases. A detailed antenatal, natal and postnatal history was taken. The birth weight, sex and day of onset of sepsis were noted.

Blood culture was performed by BacT/Alert automated blood culture system in all the cases. Approximately 0.5 ml of blood was inoculated aseptically into BacT/Alert paediatric blood culture bottle. Bottle was immediately transported to the microbiology laboratory. BacT/Alert bottles were incubated in BacT/Alert 3D microbial detection system (3D, BioMeriux Inc. Durham, NC) till it signals positive for growth (maximum incubation period was 7 days). Once the bottle was taken out, Gram's stain & subculture on BHIA agar/ blood agar/ MacConkey agar were performed. The microbes isolated were identified by standard microbiological techniques. Identification of the organisms was based on cultural characteristics, and various standard biochemical tests like catalase, oxidase, urease, coagulase etc [11]. Samples from other sterile sites; as CSF, Urine & other body fluids were processed for diagnosing neonatal sepsis, wherever necessary.

Antimicrobial susceptibility was performed on Mueller-Hinton agar for rapidly growing organisms according to Clinical Laboratory Standards Institute guidelines (CLSI 2017) for interpretation; as resistant (R), intermediate susceptible (IS) and susceptible (S) [12]. All the antibiotic disks were obtained from Hi Media, Mumbai, India. Susceptibility to the following antibiotics was tested: For Gram negative organisms - Gentamicin (30µg), ciprofloxacin (5µg), ceftazidime (10µg), amikacin(30µg), piperacillin-tazobactam (30/6 µg), Colistin (10 µg), cefepime (30 µg), amoxicillin-clavulanic acid (30 µg), imipenem (10 µg), meropenem (10 µg). For Gram positive organisms: penicillin (10units), cefoxitin(30µg), ciprofloxacin(5µg), chloramphenicol (25µg), erythromycin (15 µg), clindamycin (2µg), vancomycin (30 µg), teicoplanin (30 µg), high level gentamicin (120 µg), linezolid(30µg) .

All the statistical data analysis was done with the help of the SPSS software (Statistical Package for the Social Sciences, version [16]. For qualitative and quantitative data, Mann Whitney U test, T test, Chi square test and Fisher exact test were done to analyse the data. Univariate analysis and multivariate analysis was performed on the various parameters P value less than or equal to 0.05 was considered to be statistically significant.

Results:

During the study period, there were a total of 150 clinically suspected cases of neonatal sepsis. Of 150 neonates, majority (84.6%) presented with respiratory distress followed by tachycardia (42%), fever (31%), hypothermia (22%) and lethargy (15%).

Out of 150 clinically suspected neonates, 44 (29.3%) were culture proven cases of neonatal sepsis. The males (70.4%) were predominantly affected. Among neonates with EONS, 93.1% were low birth weight and 86.3% were preterm. According to mode of delivery, 52.2 % of neonates with sepsis were delivered by caesarean section, compared to 47.7 % of neonates that were delivered normally. Study of maternal and neonatal risk factors showed significant association with premature rupture of membranes, (p value=0.0001), maternal fever (p value=0.01) and birth asphyxia (p value=0.007) with neonatal sepsis by using multivariate analysis. Bacteriological profile of early onset neonatal sepsis is depicted in Figure 1.

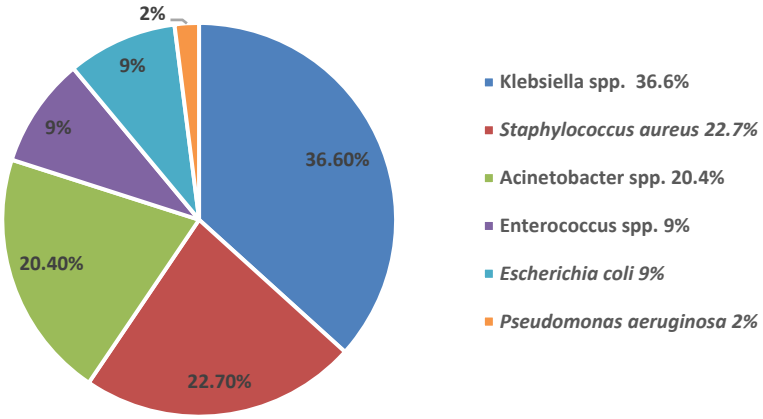
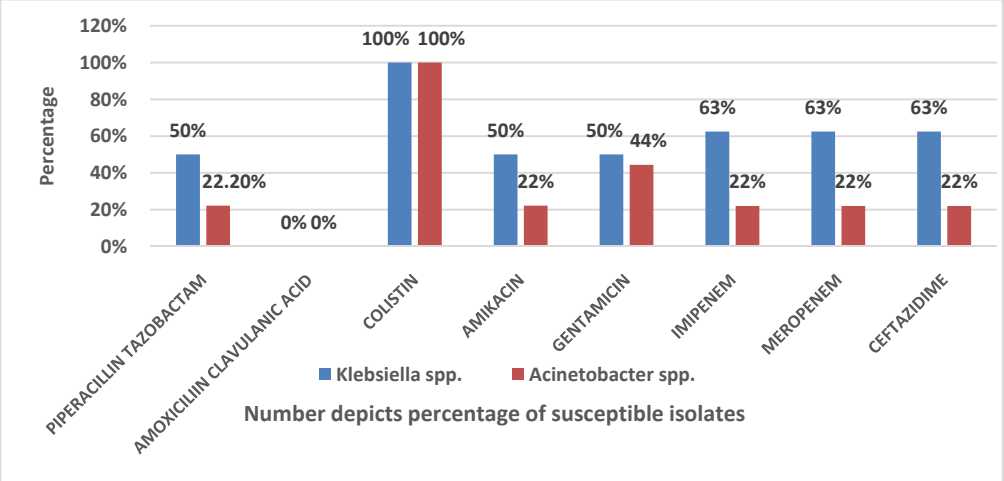


Figure 1: Bacteriological profile of early onset neonatal sepsis (n=44)

Klebsiella spp. (53.3%) was found to be major agent amongst gram negative isolates followed by *Acinetobacter* spp. (30%), *Escherichia coli* (13.3%) and *Pseudomonas aeruginosa* (3.3%). *Staphylococcus aureus* (71.4%) was predominant isolate amongst gram positive isolates followed by *Enterococcus* spp. (28.5%). Amongst gram negative organisms the antimicrobial susceptible percentage of *Klebsiella* spp. (n=16) as depicted in figure 2 showed 100% susceptibility to colistin followed by imipenem (62.5%), meropenem (62.5%), and ceftazidime (62.5%). Whereas 50% of the isolates were susceptible to piperacillin-tazobactam, amikacin and gentamicin. However, it was found to be 100% resistant to amoxicillin-clavulanic acid.

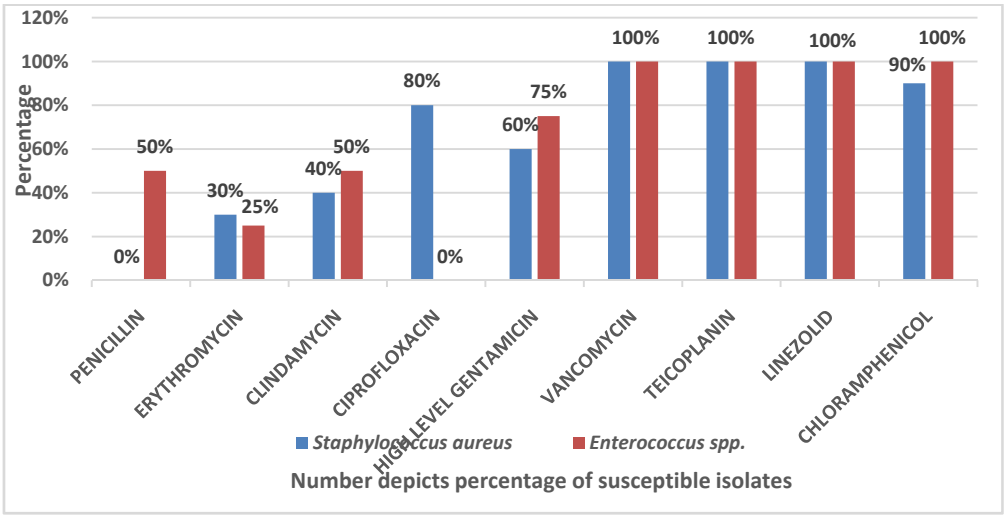
Acinetobacter spp.(n=9) which was second most common isolate showed 100% susceptibility to colistin followed by gentamicin(44.4%). However, only 2 isolates (22.2 %) were susceptible to piperacillin-tazobactam, amikacin, imipenem, meropenem, and ceftazidime as shown in figure 2.

Figure 2: Antimicrobial susceptibility profile of *Klebsiella spp.* and *Acinetobacter spp.*



Amongst gram positive organism all the strains of *Staphylococcus aureus* as shown in figure 3, were 100 % susceptible to vancomycin, linezolid and teicoplanin. This was followed by susceptibility to chloramphenicol (90%), ciprofloxacin (80%), high level gentamicin (60%). There was high resistance to penicillin (100%), erythromycin (70%) and clindamycin (60%). All the strains of *Enterococcus* spp. demonstrated 100% susceptibility to vancomycin, linezolid and teicoplanin followed by high level gentamicin (75%), ciprofloxacin (50%), penicillin (50%) and clindamycin (50%) as shown in figure 3.

Figure 3: Antimicrobial susceptibility profile of *Staphylococcus aureus* and Enterococcus spp.



Discussion:

The culture positivity rate of EONS in the current study was 29.3%. The rate reported in other studies varied from 21% by Naher *et al.* (13), 23% by Kuruvilla *et al.* [7] and 18.2% by Nwadioha *et al.* [14]. However, other studies have also reported a higher rate of culture positivity [9,15,16]. This difference could due to the differences in the blood volume used for culture, blood culture techniques applied, administration of antimicrobials to the mother or neonate before the collection of sample. Although bacteria are the most common agents implicated in neonatal sepsis, it is also reported to be caused by viruses and parasites. Viruses such as adenovirus, enterovirus, Coxsackie virus, rubella virus, protozoans like *Toxoplasma* species and fungi like *Candida* species [31]. Therefore, only a proportion of the blood cultures from cases with clinical sepsis were positive for pathogenic organisms.

The detection of microorganisms in the blood of a neonate with sepsis has a great therapeutic and prognostic significance. In view of this, timely detection of blood borne pathogens is one of the most important functions of a microbiology laboratory. In the current study, using BacT/ Alert system, majority (72%) of clinically significant pathogens were recovered within the first 12 hours of incubation and all pathogens were recovered within 24 hours. Similar findings were also reported in studies of Gopi *et al.* [17] and Pal *et al.* [18] which also used the BACTEC system.

In the present study gram negative organisms (68%) were more common than gram positive organisms (32%) as isolated pathogens. This is in accordance with many other studies [7,9, 14,15,19] *Klebsiella* spp. was the most common isolate amongst the gram negative organisms. This trend is similar to the studies of Viswanathan *et al.* [19], Tallur *et al.* (15), Zakariya *et al.*[9] Amongst the gram positive organism *Staphylococcus aureus* was the commonest isolate. Mustafa *et al.* [20] reported a similar trend.

Males (70%) were more affected than females (30%) in our study. This is consistent with the studies of Naher *et al.* [13], Mustafa *et al.*(20), Abdel *et al.* [21]. The reason for male preponderance is not exactly known, but this could be because of sex-dependent factors [20]. The synthesis of gamma globulins is probably regulated by X-linked immunoregulatory genes and males have one X chromosome, are more prone to neonatal sepsis than females. However, Chacko *et al.* reported an equal proportion of the males and females in his study of neonatal sepsis.[3] Premature rupture of membranes (PROM) (p value=0.0001) and maternal fever (p value=0.0006) were significant maternal risk factors associated with EONS. PROM is likely to facilitate ascending infection and hence neonatal sepsis. Similarly, maternal fever probably suggests chorioamnionitis in absence of any other source of infection. Similar trend was also reported by Bhutta *et al.*[22] who reported a significant association with maternal fever and maternal UTI in early onset of neonatal sepsis and Das *et al.*[23] reported maternal fever and meconium stained amniotic fluid to be significantly associated with EONS. Martius *et al.* [24] and Oddie *et al.* [25] reported premature rupture of membranes to be a significant maternal risk factor in premature neonates with sepsis.

Amongst the neonatal risk factors, birth asphyxia was significantly associated with neonatal sepsis (p value=0.01) which is in accordance with the studies by Chacko *B et al.* [(3], Mathur *et*

al. [26]. Birth asphyxia represents respiratory complications requiring mechanical ventilation, which conditions them for greater susceptibility for sepsis. In our study no statistical significant relation of low birth weight with neonatal sepsis was observed. However, Khinchi *et al.* [27] reported birth weight to be inversely related with neonatal sepsis. Low birth weight neonates are mostly premature and are predisposed to sepsis due to multiple reasons like reduced immunocompetance at various levels of defense, more subjected to invasive interventions etc.

The current trends of the antimicrobial susceptibility pattern of the etiological agents are helpful in making right choice for empirical therapy. The antimicrobial susceptibility of gram positive isolates of our study showed 100% susceptibility to teicoplanin, linezolid and vancomycin, which is in accordance with the study of Mustafa *et al*. [20] who reported 100% susceptibility to linezolid and 95% susceptibility to vancomycin. All the *Staphylococcus aureus* isolates in our study were Methicillin-resistant *Staphylococcus aureus*, however 100% susceptible to vancomycin and linezolid. Kuruvilla *et al*. [7] and Roy *et al*. [28] also reported 100% susceptibility to vancomycin. There was high degree of resistance to penicillin (100%), erythromycin (70%) and clindamycin (60%) in our study which is in accordance with the studies of Kuruvilla *et al*.[7] and Dipmala Das *et al*.[23].

Amongst gram negative isolates, all isolates were susceptibile to colistin, followed by meropenem (63%) and imipenem (63%). Similar findings were reported by Mustafa *et al*. [20] and Roy *et al* [28].

The case fatality rate in the present study for early onset neonatal sepsis (proven cases) was 18%. This is comparable to 16.7% reported by Kuruvilla *et al*. [7] and 19.4 % in Chacko B *et al.* [3] in their studies. However other studies have reported higher rates [15,29]. This may be attributed to increased clinical severity of the cases included in their study.

Conclusion:

Knowledge of the likely causative organisms and their antibiotic susceptibility patterns would contribute towards a more rational and appropriate use of antibiotics. The on time information obtained from automated blood culture system would assist the clinician in making important patient management decisions and duration of hospitalization and also help to curb the unnecessary use of antibiotics in non-infected babies which would ultimately reduce multidrug resistance in bacterial pathogens. In the present study, gram negative bacteria especially *Klebsiella* spp. and *Acinetobacter* spp. were found to be the commonest agent of neonatal sepsis. An alarming degree of antibiotic resistance was observed for commonly used antibiotics.

Conflict of interest

The authors declare no conflict of interest.

References

1. Ebrahim GJ., 2011,"Sepsis, septic shock and the systemic inflammatory response syndrome", *J Trop Pediatr.* 57(2), pp.77–9.
2. Aggarwal R, Sarkar N, Deorari AK and Paul VK,2001"Sepsis in the newborn", *Indian J Pediatr.* 68(12), pp.1143-47.
3. Chacko B and Sohi I, 2005,"Early onset neonatal sepsis", *Indian J Pediatr*. 72(1),pp.23.
4. Sundaram V, Kumar P, Dutta S, Mukhopadhyay K, Ray P, Gautam V, et al.,2009," Blood culture confirmed bacterial sepsis in neonates in a North Indian tertiary care center: changes over the last decade", *Jpn J Infect Dis*.,62(1)pp.46–50.
5. Nizet V and Klein JO,2011,"Bacterial Sepsis and Meningitis", *In Infectious Diseases of the Fetus and Newborn (7th Edition). Philadelphia: W.B. Saunders*. pp. 222–75.
6. Labib AZ, Mahmoud AB, Eissa N, El Gendy FM, Soliman MA and Aly AA,2013. "Early diagnosis of neonatal sepsis: a molecular approach and detection of diagnostic markers versus conventional blood culture", *Int J Microbiol Res*., pp.77–85.
7. Kuruvilla KA, Pillai S, Jesudason M and Jana AK,1998, "Bacterial profile of sepsis in a neonatal unit in south India", *Indian Pediatr,*35(9) pp.851–8.
8. Nasir I, Mele H, Babayo A and Yahaya F,2015,"Serum Procalcitonin Assay for Investigations and Clinical Management of Neonatal Sepsis: A Review", J *Pediatr Infect Dis*.,10(01)pp.003–11.
9. Zakariya BP, Bhat V, Harish BN, Babu TA and Joseph NM,2011,"Neonatal sepsis in a tertiary care hospital in South India: bacteriological profile and antibiotic sensitivity pattern, *Indian J Pediatr*,78(4) pp.413–417.
10. Zaidi AKM, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA,2005, "Hospital-acquired neonatal infections in developing countries", Lancet 365(9465) pp.1175–88.
11. Koneman E,Winn W, Allen S, Janda W, Procop G. Woods G, Schreckenberger P,2006. "The Enterobacteriaceae", *In Koneman's color atlas and textbook of diagnostic microbiology. 6th Edition, Lippincott Williams and Wilkins, London*, pp.211-264.
12. Wayne, PA: Clinical and Laboratory Standards Institute; 2017. *Performance standards for antimicrobial disk susceptibility tests. 27th edition, (M 100)* Vol.37 no.1.
13. Naher HS and Khamael AB,2013, "Neonatal sepsis; the bacterial causes and the risk factors", *Int Res J Med Sci*.,1(6)pp.19–22.
14. Nwadioha SI, Nwokedi EOP, Kashibu E, Odimayo MS and Okwori EE,2010,"A review of bacterial isolates in blood cultures of children with suspected septicemia in a Nigerian tertiary Hospital", *Afr J Microbiol Res*.,4(4)pp.222–225.
15. Tallur SS, Kasturi AV, Nadgir SD and Krishna BVS,2000,"Clinico-bacteriological study of neonatal septicemia in Hubli", *Indian J Pediatr*., 67(3)pp.169–174.
16. Mishra UK, Jacobs SE, Doyle LW andGarland SM,2006,"Newer approaches to the diagnosis of early onset neonatal sepsis", *Arch Dis Child-Fetal Neonatal* Ed. 91(3)pp.F208–F212.

17. Gopi A, Arun KR and Harindranath D,2014,"Evaluation of time to positivity in detection of microorganism using BACTEC [TM] 9050 in a tertiary care hospital over a period of one year", *J Evol Med Dent Sci*., 3(64)pp.13999–14006.

18. Pal N, Sharma R, Rishi S and Vyas L,2009,"Optimum time to detection of bacteria and yeast species with BACTEC 9120 culture system from blood and sterile body fluids", *J Lab Physicians.*, 1(2)pp.69.

19. Viswanathan R, Singh AK, Mukherjee S, Mukherjee R, Das P and Basu S,2011,"Aetiology and antimicrobial resistance of neonatal sepsis at a tertiary care centre in eastern India: a 3 year study", *Indian J Pediatr*.,78(4)pp.409–412.

20. Mustafa M and Ahmed SL,2014,"Bacteriological profile and antibiotic susceptibility patterns in neonatal septicemia in view of emerging drug resistance", *J Med Allied Sci*. 4(1)pp.2.

21. Abdel Mohsen AH and Ahmed Kamel B.,2015,"Predictive values for procalcitonin in the diagnosis of neonatal sepsis", *Electron Physician*. (4)pp.1190–1195.

22. Bhutta ZA and Yusuf K.,1997,"Early-onset neonatal sepsis in Pakistan: a case control study of risk factors in a birth cohort", *Am J Perinatol*., 14(09)pp.577–581.

23. Das D, Barua N and Sharma J.,2016,"Evaluation of blood culture and serum procalcitonin for diagnosis of septicaemia in paediatric patients", *Indian J Microbiol Res*., 3pp.250.

24. Martius JA, Roos T, Gora B, Oehler MK, Schrod L, Papadopoulos T, et al.,1999, "Risk factors associated with early-onset sepsis in premature infants", Eur J Obstet Gynecol Reprod Biol.,85(2)pp.151–8.

25. Oddie S.,2002,"Risk factors for early onset neonatal group B streptococcal sepsis: case-control study", *BMJ*,325(7359)pp.308–308.

26. Mathur NB, Marmu K and Kumar S.,2010, "Systemic Inflammatory Response Syndrome in Home Delivered Neonates: A Prospective Observational Study", *Indian J Pediatr*.,77(10)pp.1109–13.

27. Khinchi YR, Kumar A and Yadav S.,2010, "Profile of Neonatal sepsis", *J Coll Med Sci-Nepal*,6(2)pp.1–6.

28. Roy I, Jain A, Kumar M and Agarwal SK,2002,"Bacteriology of neonatal septicaemia in a tertiary care hospital of northern India", *Indian J Med Microbiol*., 20(3)pp.156.

29. Stoll BJ,1997,"The global impact of neonatal infection",*Clin Perinatol*., 24(1)pp.1–21.

30. Zea-Vera, Alonso, and Theresa J. Ochoa. 2015, “Challenges in the Diagnosis and Management of Neonatal Sepsis.” *Journal of Tropical Pediatrics*,61(1) pp.1–13. 31.

31. Kliegman, Robert., et al., 2016, *Nelson Textbook of Pediatrics. 20th Edition, Phialdelphia, PA: Elsevier*.