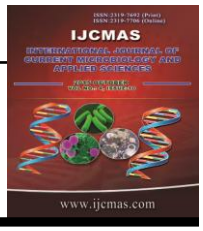
Int.J.Curr.Microbiol.App.Sci (2015) 4(10): 977-984

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 10 (2015) pp. 977-984**
http://www.ijcmas.com



Original Research Article

Bacteriological Profile and Antibiotic Susceptibility Pattern of Neonatal Septicaemia in a Tertiary Care Hospital

Natasha Sawhney*, Pottathil Shinu and Varsha A. Singh

Department of Microbiology, Maharishi Markendeshwar Institute of Medical Sciences and Research, Mullana, Ambala-133207, Haryana, India
**Corresponding author*

Keywords

Neonatal sepsis, Multidrug resistance, Septicemia

A B S T R A C T

Neonatal sepsis refers to systemic and generalized bacterial infection of the newborn documented by a positive blood culture in the first 4 weeks of life, and is one of the four leading causes of neonatal mortality in India. Prompt diagnosis and effective treatment is necessary to prevent deaths and complications due to septicemia. The objective of the study is to identify the common bacterial pathogens associated with neonatal sepsis and their antibiotic sensitivity pattern. During the study period, a total of 83 neonates admitted in neonatology ward were screened for sepsis by physical examination and clinical features. All infants satisfying the criteria for sepsis were subjected for blood culture. Growths, if any were noted and standard antibiotic sensitivity was performed by the Kirby-Bauer disc diffusion method as per the CLSI recommendations. Out of 84 clinically suspected and positive screening test cases of neonatal sepsis, 41.7% were culture proven cases of neonatal sepsis. *Klebsiella* spp has been found to be the predominant pathogen (45.75%) of the culture positive cases followed by staphylococcus aureus in 25.7% cases. Majority of the pathogens isolated were resistant to the commonly used antibiotics. Maximum sensitivity was seen by cefoperazone/ sulbactum and piperacillin/ tazobactum for gram negative organisms and vancomycin for gram positive organisms. To conclude, multidrug resistance organisms were isolated from septicemia in neonates. Therefore great caution is required in selection of antibiotic therapy.

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. 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; Edmond and Zaidi, 2010; Maayan-Metzger *et al.,* 2009)**.** 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) a pneumoniae is the most common bacterial agent causing neonatal sepsis in developing countries, while group B Streptococcus and coagulase-negative staphylococci (CONS) are the common agents in developed countries (Iregbu *et al.,* 2006). Even among developing countries, regional variation in prevalence of the bacterial agents causing neonatal sepsis exists (Kuruvilla *et al.,* 1998; Chacko and Sohi, 2005). 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). Rapid immunological techniques like C-Reactive Proteins (CRP) assays may help in the diagnosis of septicemia; however they lack the capacity to detect specific pathogens and are not available in many centers in developing countries (Singh *et al* 2003). Neonatal blood culture positive rate have been found to range from 25–54% (English *et al.,* 2004; Klingenberg *et al.,* 2003; Mugalu *et al.,* 2006). 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 determine the common bacterial agents associated with neonatal sepsis and their antibiotic susceptibility pattern in a tertiary care hospital in Northern India.

Materials and Methods

Study setting and duration

This prospective observational cohort study was conducted in the department of microbiology and Paediatrics of Maharishi Markendeshwar Institute Of Medical Sciences and Research, Mullana (Ambala) tertiary care hospital (750 bedded), over a period of one year from March 2012 through February 2013. This study was approved by the Research and Ethical committees of our institute and informed consent was obtained from each patient's next of kin.

Study population

During the study period, A total of 83 neonates admitted in neonatology ward were screened for sepsis by the examining physician and sepsis was 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 fontanel, fever, hypothermia, jaundice, vomiting, loose stools, abdominal distension cyanosis, bleeding, mottling, tachycardia, weak pulse, grunting, retractions, nasal flaring were noted. Septic screening tests like band cell count, C- reactive protein, and micro erythrocyte sedimentation rate were done in all these cases. All neonates in whom sepsis was suspected and had at least two positive screening tests were included in the study.

Specimen collection and processing

All blood cultures were collected from a peripheral vein with proper aseptic precautions before starting any antibiotic therapy. A minimum of 3 mL of blood was collected from each patient using proper aseptic precautions and inoculated immediately into 30 mL of brain heart infusion broth with 0.025% Sodium polyanethol sulfonate as anticoagulant (HiMedia Laboratories, Mumbai). A second similar sample was obtained on the same day from a different site after few hours to rule out contamination with skin flora. Both the bottles were incubated aerobically at 37°C for 7 days. Subcultures were made on sheep blood agar and MacConkey agar (HiMedia, Mumbai, India) and chocolate agar routinely after 48 h and 7 days. Subculture was also done in between if visible turbidity appeared. All the inoculated culture plates were incubated at 37°C for overnight.

Identification of bacterial isolates and Antibiotic sensitivity testing

After incubation, the growth was identified by colony characteristics and standard biochemical tests (Bailey and Scott., 1994; Chap. 13). Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method as per the CLSI recommendations (NCCLS Supplement 2000).

Quality control

Every new batch of culture media was incubated at 37^0C for overnight to check the sterility. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains for antimicrobial susceptibility testing. However, a non-ESBL producing organism *E. coli* ATCC 25922 and an ESBL-producing organism K. pneumoniae ATCC 700603 was used while testing ESBL screening and phenotypic confirmatory tests.

Results and Discussion

During the study period, there were a total of 84 clinically suspected cases of neonatal sepsis. The demographic profile and the predisposing factors of these 84 cases are summarized in (Table 1). Of these 84 neonates, 42(50%) were inborn, while the other 42(50%) were outborn. Amongst all the clinically suspected cases of neonatal sepsis, 48(57.2%) were early onset and 36 (42.8%) were late onset sepsis. Also, 27(56.2%) early onset cases were positive for the blood culture while only 8(22.2%) late onset sepsis cases were positive for blood culture (p value 0.003). Overall, of all the neonates screened, 41.7% (35 of 84) of the clinically suspected cases were culture proven cases of neonatal sepsis. Out of 35 culture proven cases, 23 (65.7%) sepsis

cases were attributable to gram negative species while only 11 (31.5%) cases were attributable to gram positive. *Candida* was also isolated from one (2.8%) case. *Klebsiella* species and *Staphylococcus aureus* were the most common gram negative and gram positive organisms, together accounting for 45.7% and 25.8% of the isolates respectively. Other common gram negative isolates were *Escherichia coli* (11.4%), *Pseudomonas* spp (5.8%), *Proteus* spp (2.8%) and other less frequent isolates (Table 2).

Table 3 and 4 shows the antibiotic sensitivity patterns of the common organisms isolated. In most number of cases, *Staphylococcus aureus* was resistant to the commonly used antibiotics, including penicillin, cloxacillin and cephalexin. None of the gram negative isolates were resistant to vancomycin. Of the two amino glycosides studied, amikacin scored over gentamicin in terms of sensitivity for *Staphylococcus aureus*. Amongst the gram negative isolates, most of them were resistant to commonly used antibiotics. Ciprofloxacin was sensitive in about 50–60% of cases isolated. Newer combinations of antibiotics like piperacillin/tazobactum and cefoperazone/sulbactum were sensitive in more than 95% 0f cases.

Neonatal sepsis refers to systemic and generalized bacterial infection of the newborn documented by a positive blood culture in the first four weeks of life and is one of the four leading causes of neonatal mortality in India (Mustafa and Laeeq Ahmed, 2014). The gold standard for diagnosis of septicemia is the isolation of bacterial agent from blood culture. The prevalence of bacterial profile of blood culture and their susceptibility patterns in an area, provide guidance to start empirical treatment which is the cornerstone in the management of sepsis (Mustafa and Laeeq Ahmed, 2014).

In this study, blood culture positivity rate in neonatal septicemia cases was 41.7%, similar results found in various studies (Kumhar *et al.,* 2002; Desai *et al.,* 2011). Also, the gram negative organisms constituted the major group of isolates (65.7%) from neonatal septicemia cases, which correlates with the findings (67.85%) of Desai *et al.* (2011) and 65% in another study (Mustafa and Laeeq Ahmed, 2014). Among this group, *Klebsiella* spp has been found to be the predominant pathogen (45.75%) which correlates with the findings (47.1%) of Madhu Sharma *et al.* (2002). A total of 31.5% of gram positive organisms has been observed in our study which correlates with the findings of Roy *et al* (2002) and Desai *et al.* (2011). *Staphylococcus aureus* was the predominant pathogen (25.7%)**.** The results of antibiotic sensitivity revealed that majority of gram negative organisms were resistant to commonly used antibiotic like amoxicillin. It has been also shown that piperacillin/ tazobactum and cefoperazone/sulbactum were the two most effective antibiotics against gram negative organisms. Majority of *Staphylococcus aureus* was resistant to amoxicillin. Vancomycin still remains the most sensitive drug for *Staphylococcus aureus*, concluding not a single case of resistance which correlates with Roy *et al.* (2002).

Conclusion

Neonatal septicemia is a life threatening emergency and rapid treatment with antibiotics is mandatory for a favourable outcome. Clinical recognition of neonatal sepsis is not always straight-forward. Appropriate intervention requires an early aetiological diagnosis. In the present study,

S. aureus and Gram negative isolates were frequently found organisms. Classical empirical treatment of neonatal sepsis consists of amoxicillin and an aminoglycoside. In present study, frequent found pathogens were resistant to these antibiotics thus concluding that the use of these drugs might be ineffective. The empirical regimen should be modified later based on the antibiogram of the isolates. Therefore, the present study reiterates the earlier findings and emphasizes the importance of antibiotic sensitivity testing of the pathogens encountered in particular neonatal settings to recognize the trend.

Table.1 Demographic details of 84 neonates with clinical sepsis

PARAMETER	VALUE (%)
Neonates' characteristics	
Number of preterm neonates (%)	30 (35.7%)
Birth weight (g) mean + SD (range)	2.25+0.66 (1.2-3.9)
Sex	
Number of males	50 (59.5%)
Number of females	34 (40.5%)
Maternal data	
Number of cases with PROM>24 hrs	9 (10.8%)
Number of cases with meconium stained liquor	12 (14.2%)
Type of delivery	
Spontaneous vaginal delivery	63 (75%)
Caesarean section	10 (11.9%)
Instrumental delivery	9 (10.7%)
Assisted breech delivery	1 (1.1%9)

Table.2 Number of microbial isolates from culture positive neonates (n=35)

ISOLATES	FREQUENCY OF ISOLATES (%)
Klebsiella	16 (45.7%)
Staphylococcus aureus	9 (25.7%)
Escherichia coli	3 (8.5%)
Pseudomonas spp	2 (5.8%)
Proteus	1 (2.8%)
Acinetobacter	1 (2.8%)
CONS	2 (5.8%)
Candida	1 (2.8%)

Table.3 Antibiotic sensitivity pattern of gram positive isolates

Antibiotic	Resistant percentage of isolates (n)	
	Staphylococcus aureus (n=25)	CONS (n=2)
Amoxicillin	84% (21)	100% (2)
Erythromycin	44% (11)	50% (1)
Cephalexin	36% (9)	50% (1)
Gentamicin	48% (12)	50% (1)
Vancomycin	0	0
Ciprofloxacin	40% (10)	50% (1)
Amikacin	12% (3)	0

Table.4 Antibiotic sensitivity pattern of gram negative isolates

Antibiotic	Resistant percentage of isolates (n)			
	Klebsiella (n=16)	*E. coli* (n=3)	*Proteus* (n=1)	*Pseudomonas* (n=2)
Amoxicillin	100% (16)	67% (2)	100 %(1)	100 (2)
Cotrimoxazole	81.2% (13)	67% (2)	100% (1)	100 (2)
Gentamicin	75% (12)	67% (2)	100% (1)	50 (1)
Piperacillin-tazobactum	6.25% (1)	33% (1)	0	0
Cefoperazone-sulbactum	6.25% (1)	33% (1)	0	0
Cefotaxime	37.5% (6)	33% (1)	100 (1)	50% (1)
Ciprofloxacin	68.7% (11)	33% (1)	0	50% (1)
Ceftriaxone	31.2% (5)	33% (1)	100% (1)	50% (10

Acknowledgement

I whole heartedly thank the laboratory technician staff Miss Suman and Mr. Gulab Singh for their help in media preparation and culture work.

Reference

Anderson-Berry, A.L., Bellig, L.L., Ohning, B.L. 2010. Neonatal sepsis.[Internet]. emedicine Pediatrics: Cardiac Disease and Critical Care Medicine 2010; 978352 [Updated 2010 Feb 23; Cited 2010 Sep 22]. Available from: http://emedicine.medscape.com/ article/978352-overview

Baron, E.J., Finegold, S.M. (Eds), 1994. Overview of conventional methods for bacterial identification, Chapt. 13. In: Bailey and Scott's diagnostic microbiology. Mosby Publishers, St. Louis. 167 Pp.

Chacko, B., Sohi, I. 2005. Early onset neonatal sepsis. *Indian J. Pediatr.*, 72: 23–26.

Desai, K.J., Malek, S.S., Parikh, A. 2011. Bacterial isolates and their antibiotics susceptibility patterns. *Gujarat Med. J.*, 66(1): 13–15.

Edmond, K., Zaidi, A. 2010. New approaches to preventing, diagnosing, and treating neonatal sepsis. *PLoS Med.*, 7: e1000213.

English, M., Ngama, M., Mwalekwa, L., Peshu, N. 2004. Sign and Symptoms of illness in Kenyan Infants aged less than 60 days. *Bull. WHO*, 82: 323–329.

Iregbu, K.C., Elegba, O.Y., Babaniyi, I.B. 2006. Bacteriological profile of neonatal septicaemia in a tertiary hospital in Nigeria. *Afr. Health Sci.*, 6: 151–154.

Kaistha, N., Mehta, M., Singla, N., Garg, R., Chander, J. 2009. Neonatal septicemia isolates and resistance patterns in a tertiary care hospital of North India. *J. Infect. Dev. Ctries*, 4: 55–57.

Klingenberg, C., Olomi, R., Oneko, M., Sam, N., Langeland, N. 2003. Neonatal morbidity and Mortality in Tanzanian tertiary care referral hospital. *Ann. Trop. Paediatr.*, 23: 293–299.

Kumhar, G.D., Ramchandran, V.G., Piyush Gupta, *et al.* 2002. Bacteriologiacal analysis of blood culture isolates from neonates in a tertiary care hospital in India. *J. Health Popul. Nutr.*, 20(3): 156–159.

Kumhar, G.D., Ramchandran, V.G., Piyush Gupta, *et al.* 2002. Bacteriologiacal analysis of blood culture isolates from neonates in a tertiary care hospital in India. *J. Health Popul. Nutr.*, 20(3): 156–159.

Kuruvilla, K.A., Pillai, S., Jesudason, M., Jana, A.K. 1998. Bacterial profile of sepsis in a neonatal unit in south India. *Indian Pediatr.*, 35: 851–858.

Maayan-Metzger, A., Barzilai, A., Keller, N., Kuint, J. 2009. Are the "good old" antibiotics still appropriate for early-onset neonatal sepsis? A 10 year survey. *Isr. Med. Assoc. J.*, 11: 138–142.

Madhu Sharma, Nidhi Goel, Uma Choudhary, Ritu Aggarwal, Arora *et al.*, 2002. Bacteraemia in children. *Indian J. Pediatr.*, 69(12): 1029–1032

Mugalu, J., Nakakeeto, M.K., Kiguli, S., Kaddu-Mullindwa, D.H. 2006. Aetiology, risk factors and immediate outcome of bacteriologically confirmed neonatal septicaemia in Mulago hospital, Uganda. *Afr. Health Sci.*, 6: 120–126.

Mustafa, M., Laeeq Ahmed, S. 2014. Bacteriological profile and antibiotic susceptibility patterns in neonatal septicemia in view of emerging drug resistance. *J. Med. Allied Sci.*, 4(1): 2–8.

National Neonatal Perinatal Database. [Internet]. NNPD report 2002-03 [cited 2010 Sep 22]. Available from: http:// www.newbornwhoccorg/pdf/nnpd_report_2002-03 PDF; 2005

Performance Standards for antimicrobial susceptibility testing. Eighth Information Supplement 2000. National Committee for Clinical Laboratory Standards (NCCLS). M2A7 Vol. 20, No. 1 and 2.

Roy, I., Jain, A., Kumar, M., Aggarwal, S.K., *et al.* 2002. Bacteriology of neonatal septicemia in a tertiary care hospital of northern India. *Indian J. Med. Micobiol.*, 20(3): 156–159.

Sanghvi, K.P., Tudehope, D.I. 1996. Neonatal bacterial sepsis in a neonatal intensive care unit: a 5 year analysis. *J. Paediatr. Child Health*, 32: 333–338.

Singh, S.A., Dutta, S., Narang, A. 2003. Predictive clinical scores for diagnosis of late onset neonatal septicemia. *J. Trop. Pediatr.*, 49(4): 235–239.

Stoll, B.J., Hansen, N., Fanaroff, A.A., *et al.* 2002. Changes in pathogens causing

early-onset sepsis in very-low-birth-weight infants. *N. Engl. J. Med.*, 347: 240–247.

Sundaram, V., Kumar, P., Dutta, S., *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: 46–50.

Tumbarello, M., Sanguinetti, M., Montuori, E., Trecarichi, M.E., Posteraro, B., Fiori, B., Citton, R., D'Inzeo, T., Fadda, G., Cauda, R., Spanu, T. 2007. Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-lactamase-producing enterobacteriaceae: Importance of inadequate initial antimicrobial treatment. *Antimicrob. Agents Chemother.*, 51: 1987–1994.

Weber, M.W., Carlin, J.B., Gatchalian, S., Lehmann, D., Muhe, L., Mulholland, E.K. 2003. WHO Young infants study group: predictors of neonatal sepsis in developing countries. *Pediatr. Infect. Dis. J.*, 22(8): 711.

World Health Organization (WHO), 1995. Essential newborn care. In a report of a technical working group. WHO, Geneva.

World Health Organization (WHO), 2006. Neonatal and perinatal mortality: country, regional and global estimates. WHO, Geneva.