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Bacteriological profile of neonatal Septicaemia in MIMS, Mandya, India

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A B S T R A C T

Neonatal septicemia (NS) is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the first month of life. The gold standard for the diagnosis of neonatal septicaemia is the isolation of the bacterial agent from blood culture. The organisms responsible for neonatal sepsis vary across geographical boundaries and with the time of onset of illness. In most developing countries, gram negative bacteria remain the major source of infection. However, in the developed countries, Gram positive organisms have been implicated as the most common causes. In spite of great advances in antimicrobial therapy, neonatal life support measures and the early detection of risk factors, septicaemia continues to be a major cause of mortality and morbidity among neonates around the world. We conducted this study to know the bacterial profile and their antibiogram from clinically suspected neonatal septicaemic cases. This retrospective observational study was conducted in the Dept of Microbiology, MIMS, Mandya after obtaining approval from the Institutional Ethical Committee. The bacterial isolates and their antibiogram from blood samples of clinically suspected neonatal septicaemic cases processed in the Microbiology laboratory during one year (Jan 2014- Dec 2014) were studied from the records of Microbiology laboratory. A total of 128 blood samples from clinically suspected cases of neonatal septicaemia were processed and reported. Among them, 28 (21.9%) samples showed bacterial growth. Among 28 isolates, 15(53.6%) were from Early onset septicaemia and 13(46.4%) were from late onset septicaemia. In Early onset septicaemia, *Klebsiella pneumoniae* (9, 60%) formed the majority followed by *Staphylococcus aureus* (3,20%), Citrobacter(2,13.3%) and Coagulase negative *Staphylococcus*(1, 6.7%). In late onset septicaemia, *Klebsiella pneumoniae* (8,61.5%) was again the predominant isolate followed by Escherichia coli (2, 15.3%), *Citrobacter* and *Staphylococcus aureus* and CONS 1(7.4%) each. We noticed from this study that Klebsiella was the leading cause of neonatal sepsis and showed high resistance to commonly used antibiotics like ampicillin and gentamicin. In view of the changing spectrum of the causative agents and their antimicrobial susceptibility patterns, a positive blood culture and the antimicrobial susceptibility testing of the isolates are the best guide in choosing the appropriate antimicrobial therapy in treating neonatal septicaemia.

Introduction

Neonatal septicemia (NS) is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the first month of life (Freij BJ etal, 2005).The risk factors for neonatal septicemia include premature rupture of membranes, prolonged rupture, prematurity, UTI, poor maternal nutrition, LBW, birth asphyxia and congenital anomalies (Prabhu K *et al.,* 2010). Depending on the onset of symptoms, it can be classified into early onset sepsis within 72 h of life and late onset sepsis usually after 72 h of age (Cloberty JP *et al.,* 1998).

The gold standard for the diagnosis of neonatal septicaemia is the isolation of the bacterial agent from blood culture. For effective management of neonatal septicaemia, study of their antibiotic sensitivity plays a significant role (Forbes BA etal, 2007). The organisms responsible for neonatal sepsis vary across geographical boundaries and with the time of onset of illness (Al-Zwaini EJK, 2002) .In most developing countries, gram negative bacteria remain the major source of infection (Klein JO etal, 2001). However, in the developed countries, Gram positive organisms have been implicated as the most common causes (Plazek M Metal, 1983).

In spite of great advances in antimicrobial therapy, neonatal life support measures and the early detection of risk factors, septicaemia continues to be a major cause of mortality and morbidity among neonates around the world (Guerina NG, 1998).

An area based knowledge of the bacteriological spectrum is necessary because the first antibiotic administered can't wait for the culture results. With the high mortality associated with neonatal septicemia, a right choice of empiric therapy is very important.

Thus we conducted this study to know the bacterial profile and their antibiogram from clinically suspected neonatal septicaemic cases in MIMS, Mandya.

Material and Methods

Institutional Ethical Committee approval was taken and study was done after ethical clearance.

Study design: This is a retrospective observational study of the reports of blood cultures of all suspected cases of neonatal septicemia in the Dept. of Microbiology of a tertiary care hospital.

Study period: 1 year, Jan- Dec 2014.

Data collection: The bacterial isolates and the antibiogram from blood samples of clinically suspected neonatal septicaemic cases were studied from the records of Microbiology laboratory. 2ml blood drawn under aseptic precautions and inoculated into 20 ml blood culture bottles were received in the Microbiology laboratory. These 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, the second on the third day and a final on the seventh day. Subcultures were done onto blood agar and MacConkey agar plates. The inoculated plates were incubated aerobically at 37° C for 24 hours, and the plates were observed for growth. The growth was identified by colonial characteristics, gram's stain and standard biochemical tests (Collee JG, 1996). Antimicrobial susceptibility testing of all bacterial isolates was performed by the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar according to the

recommendations of the CLSI (CLSI, 2010).

Statistical analysis: Descriptive statistics was used to analyse the data and data was presented as percentage .

Results and Discussion

A total of 128 blood samples from clinically suspected cases of neonatal septicaemia were processed and reported. Among them, 28 (21.9%) samples showed bacterial growth. Among 28 isolates, 15(53.6%) were from Early onset septicaemia and 13(46.4%) were from late onset septicaemia (Fig 1).In Early onset septicaemia (15), *Klebsiella pneumoniae*(9, 60%) formed the majority followed by *Staphylococcus aureus* (3,20%), *Citrobacter* (2,13.3%) and Coagulase negative Staphylococcus (1, 6.7 %). In late onset septicaemia (13), *Klebsiella pneumoniae* (8, 61.5 %) was again the predominant isolate followed by *Escherichia coli* (2, 15.3%), *Citrobacter* and *Staphylococcus aureus* and CONS (1,7.4%) each. The total bacteria isolated and their frequency of distribution is shown in Table 1.Antibiotic sensitivity patterns of Gram negative & Gram positive bacteria are shown in Table 2 and Table 3 respectively.

The culture positivity rate of bacteria in our study was 21.9%. This is comparable to studies of Kenneth *et al.,*. who reported 22%(Kenneth C Iregbu *et al.,* 2006) and Sanjay etal. reported 17.09% (Sanjay etal., 2012). However a high culture positivity rate of 56% and 32% has been reported by Sharma et a1 and Mondal *et al.*, respectively (Sharma etal, 1987; Mondal *et al.*, 1991) .

A low blood culture isolation rate in this study might be due to several reasons like administration of antibiotics before blood collection either to the mother or to the baby or the possibility of infection with anaerobes. Chow *et al.*, reported that 26% of all neonatal septicemia was caused by anaerobes (Chow *et al.*, 1974).

In our study, early onset septicemia (53.6%) was more than late onset septicemia (46.4%), which is consistent with other studies (Aletayab *et al.,* 2011; Waseem R *et al.,* 2005; Al-Shamahy *et al.,* 2012) . This could be due to prematurity, low birth weight and unhygienic conditions during labor.

We found that Gram negative bacteria (22,78.6%) were more than Gram positive bacteria (6,21.4%).The fetus is frequently exposed to enteric bacteria during the course of maternal peripartal infections. The newborn infant has been shown to have a lack of serum bactericidins against Gram-negative bacilli. These antibodies against somatic or "O" antigens in Gram- negative bacteria are in the gamma-M fraction which is not passed transplacentally from mother to fetus. Hence Gram-negative organisms are commonest cause of neonatal septicemia Overall James, 1970) . This predominance of Gram negative organisms as the etiological agent in neonatal septicemia has been corroborated by other workers (Monga K etal 1986; Muley V. A etal, 2002).

We found that in Early onset septicaemia (15), *Klebsiella pneumoniae* (9,60%) formed the major isolate followed by *Staphylococcus aureus* (3, 20%) and *Citrobacter* (2,7.1%) and CONS (1,3.6%) . In late onset septicaemia (13), *Klebsiella pneumoniae* (8, 28.6%) was again the predominant isolate followed by E. coli (2,7.1%), *Citrobacter*, *Staphylococcus aureus* & CONS 1 (3.6%)each. Various studies have reported predominant Gram-negative organism as Klebsiella species with an isolation rate of 24.6% to 42.2%. (Roy I et al, 2002; Kumhar GD etal, 2002; Kapoor L et al, 2005).

Table.1 Frequency of Various Bacteria in Eos and Los.Figure in Paranthesis Indicate Percentage

Bacteria	**EOS**	**LOS**	**TOTAL**
Klebsiella pneumoniae	9(32.1)	8 (28.6)	17(60.7)
Staphylococcus aureus	3(10.7)	1(3.6)	4 (14.3)
Citrobacter	2(7.1)	1(3.6)	3 (10.7)
Escherichia coli	nil	2(7.1)	2 (7.1)
CONS	1(3.6)	1(3.6)	2(7.1)
TOTAL	15 (53.6)	13(46.4)	28

CONS-Coagulase Negative Staphylococcus,EOS-Early Onset Septicaemia, LOS- Late Onset Septicaemia

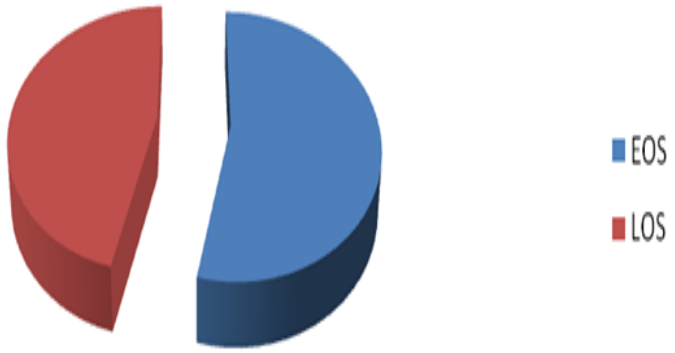
Table.2 Antibiotic Sensitivity Pattern of Gram Negative Bacteria. Figures in Paranthesis Indicate Percentage

	Klebsiella pneumoniae N=17	*Escherichia coli* N=2	*Citrobacter* N=3
Ampicillin	0	0	0
Cotrimoxazole	2(11.8)	0	0
Ciprofloxacin	6(35.3)	0	1 (33.3)
Ceftriaxone	10 (58.8)	1 (50)	2 (66.6)
Cefotaxime	9 (53)	1 (50)	2 (66.6)
Cefepime	15 (88.2)	1 (50)	3 (100)
Imipenem	16 (94.1)	2 (100)	3 (100)
Amikacin	14 (82.3)	2 (100)	3 (100)
Gentamicin	9 (53)	0	0
Tetracycline	2 (11.8)	0	0
Piperacillin-tazobactam	16 (94.1)	2 (100)	3 (100)

Table.3 Antibiotic Sensitivity Pattern of Gram Positive Bacteria. Figures in Paranthesis Indicate Percentage

	Staphylococcus aureus N=4	CONS N=2
Penicillin	1 (25)	0
Erythromycin	1(25)	0
Tetracycline	2 (50)	1 (50)
Oxacillin	1 (25)	1 (50)
Ciprofloxacin	2 (50)	2 (100)
Cotrimoxazole	2 (50)	2 (100)
Azithromycin	3 (75)	2 (100)
Linezolid	4 (100)	2 (100)

Fig.1 Prevalence of Bacteria in Early and Late Onset Septicaemia (Eos & Los) Respectively



Klebsiella pneumoniae is commonly found in the environment of the neonatal intensive care units and nursery. It can also be present as colonizers on the hands of the health care workers. There are also frequent reports of neonatal septicemia outbreaks due to *Klebsiella pneumoniae* in nursery and NICUs (Banerjee M *et al.,* 1993)

A comparatively low incidence (21.42%) of Gram positive organisms has been observed in our study, which correlates well with other studies (Sharma M. etal 2002; Mathur *et al.,* 1994).

The organisms causing neonatal septicaemia differ from area to area and also change with respect to time even in the same area, which may be due to different life conditions (Shrestha P etal, 2007)

The antibiotic sensitivity showed an alarming trend with most of the bacteria showing high resistance to the commonly used antibiotics like Ampicillin and Gentamicin usually employed as the first line of therapy. Similar findings have been reported by Guha *et al.,* and Monga *et al.,* (Guha *et al.,* 1978 and Monga *et al.,* 1986). However, most of the isolates were susceptible to Amikacin and third generation cephalosporins, comparable to the findings from New Delhi and Hubli (Anand NK *et al.,* 1966; Tallur S. S, 2000).

Gram positive bacteria showed 100% sensitivity to linezolid and Gram negative bacteria showed more than 90% sensitivity to imipenem. P. Jyothi *et al.,* reported maximum sensitivity of bacteria to imipenem and linezolid. (P Jyothi *et al.,* 2013).

Resistance to commonly used antibiotics is increasing. Establishment of appropriate rational antibiotic policy is essential to control this growing problem. There is an urgent need to do longitudinal surveillance of the microbial flora in every hospital (Deorari, A.K., 2006).

We noticed from this study that Klebsiella was the leading cause of neonatal sepsis, both in early and late onset septicaemia. Klebsiella showed high resistance to commonly used antibiotics like ampicillin and gentamicin. However, most of them were sensitive to amikacin and third generation cephalosporins. Neonatal septicemia is a life-threatening emergency and rapid treatment with appropriate antibiotics plays a important role for a good outcome. In view of the changing spectrum of the causative agents and their

antimicrobial susceptibility patterns, a positive blood culture and the antimicrobial susceptibility testing of the isolates are the best guide in choosing the appropriate antimicrobial therapy in treating neonatal septicaemia. A careful and regular monitoring of the use of antibiotics at regional and national level is required.

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