International Journal of Multidisciplinary Education and Research
ISSN: 2455-4588; Impact Factor: RJIF 5.12
Received: 15-11-2018; Accepted: 17-12-2018
www.educationjournal.in
Volume 4; Issue 1; January 2019; Page No. 14-17

Bacteriological profile of neonatal sepsis by blood culture: A prospective study in a tertiary care center of north India

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Abstract
Objectives: To assess the bacteriological profile of neonatal sepsis and its antibiotic susceptibility.
Methods: A 4 ml paired blood sample was collected in two culture vials at the time of enrolment and 24 hours after enrolment in case of suspected sepsis and sent for bacteriological assessment and antibiotic susceptibility pattern at the Department of Pathology and microbiology. Patients were observed for the outcome in terms of morbidity status, mortality and duration of hospital stay.
Results: Out of 180 cases of suspected sepsis, 51 (28.3%) cases were culture positive,
Gram positive pathogens were (n=32) (17.8%) and Gram negative pathogens were (n=19)(10.6%).Common isolates were CONS (n=32), S.aureus (n=1), Enterococcus (n=5) & Streptococcus (n=1) in gram positive and K. pneumoniae (n=11), P. aeruginosa (n=3), Acinetobacter (n=1) &E. coli (n=4) in gram negative while contamination was ~1%. Gram positive isolates were highly sensitivity to Tigecycline (93.8%), Nitrofurantoin (69.1%), Teicoplanin (54.5%), Linezolid (53.3%) while Gram negative isolates to Tigecycline (100%), Colistin (81.3%), Meropenem (61.8%).
Conclusion: The bacteriological profile of the neonates with sepsis showed a dominance of Gram positive (CONS) as compared to Gram negative isolates (Klebsiella pneumonia). Tigecycline and Colistin had the maximum sensitivity for both Gram positive as well as Gram negative isolates.

Keywords: neonatal sepsis, bacteriological profile, blood culture

Introduction

Neonatal sepsis or sepsis neonatorum refers to systemic infection of the newborn. It is characterized by a constellation of a nonspecific symptomatology in association with bacteremia. Prompt recognition, appropriate antimicrobial therapy and judicious supportive care are the key determinants of positive outcome in this neonatal emergency. Pathogens causing neonatal infections and their antibiotic susceptibility patterns may change over time [1] and differ between countries [2] and thus should be regularly monitored in a hospital setting which is essential to upgrade the treatment policy in clinical practice. Considering this fact, this study of bacteriological profile and antibiotic susceptibility pattern was done in suspected cases of sepsis admitted in our NICU.

In India according to National Neonatal Perinatal Database (NNPD) 2002-03, the incidence of neonatal sepsis has been reported to be 30/1000 intramural live births in the tertiary care institutions [3]. The pathogens most often implicated in neonatal sepsis are Gram negative organisms including *Klebsiella, Escherichia coli, Pseudomonas, and Salmonella* [4, 5], Of the Gram positive organisms, *Staphylococcus aureus* [5] coagulase negative *Staphylococci* (CoNS) *Streptococcus pneumoniae* and *Streptococcus pyogenes*are most commonly isolated. Many of them were found to be resistant to several groups of drugs commonly used in NICU like third generation cephalosporins and aminoglycosides.

Methods

The present study was carried out prospectively in a tertiary care centre in North India over a period of One year from April 2014 to March 2015. A total of 180 neonates who had clinical features of sepsis or had presence of risk factors (one major – prolonged rupture of membranes, evidence of chorio-amnnionitis, intrapartum fever, foul smelling liquor or Two minor – febrile illness of mother two weeks prior to delivery, meconium stained liquor amnii, more than three vaginal examinations during labour, low birth weight or prematurity, prolonged labour, low Apgar score) were enrolled in the study. Those with Congenital and Chromosomal anomalies or who expired within 24 hr of admission were excluded.

Permission for the study was obtained from Institutional Ethical Committee and Informed consent was obtained from guardians of all the participants. Demographic information, detailed maternal medical and obstetric history including complications during pregnancy, gestational age at delivery, mode of delivery and birth weight were noted. A thorough clinical examination of new born was done. Patients were observed for the outcome in terms of morbidity status, mortality and duration of hospital stay.

A 4ml paired blood sample was collected in two culture vials at the time of enrolment and 24 hours after enrolment and sent for bacteriological assessment and antimicrobial susceptibility pattern by using BACTEC METHOD, thus two sets of blood culture were collected from each neonate. Case Definitions: Early onset sepsis (EOS): Those cases in whom clinical manifestation of sepsis was seen within 72 hours after birth. Late onset sepsis (LOS): Those cases in whom clinical manifestation of sepsis was seen after 72 hours of birth. Criteria for defining contamination [6]:

a. In case of multiple pathogen and at least one of the specimens was CoNS, and Clinical improvement was seen on the first line treatment or on the basis of line of treatment for second pathogen.

b. Specimen for CoNS showed resistance to all the antibiotics tested and patient is improving on given treatment.

Data Analysis

The data was analyzed using Statistical Package for Social Sciences. Chi-square test was used for collection of data. The confidence level of the study was kept at 95%, hence a "p" value less than 0.05 indicated a statistically significant association.

Results

Out of 180 cases a total of 132 (73.3%) were in the early onset group whereas in remaining 48 (26.7%) cases were in the late onset group.

Table 1: General and Clinical Profile of Neonates in two groups

Characteristic	Total (n=180)	Early onset (n=132)	Late onset (n=48)	Statistical significance
	No.(%)	No.(%)	No.(%)	P
Male	140(77.8)	103(78)	37(77.1)	0.893
Female	40(22.2)	29(22)	11(22.9)	
Inborn	54(30)	51(38.6)	3(6.3)	<0.001
Out born	126(70)	81(61.4)	45(93.8)	
Term	125(69.4)	85(54.4)	40(83.3)	0.015
Preterm	55(55.0)	47(35.6)	8(16.7)	

Table 2: Maternal Demographics, Obstetric History & Perinatal complications

Characteristic	Early onset (n=132)		Late onset (n=48)		Statistical significance
	No.	%	No.	%	P
Maternal age					
<20 Years	17	(12.9)	10	(20.8)	0.042
20-30 Years	111	(84.1)	33	(68.8)	
≥30 Years	4	(3.0)	5	(10.4)	
Antenatal risk factor present	53	(40.2)	11	(22.9)	0.033
Major					
Leaking PV (>18hrs)	26	(49.1)	3	(27.3)	0.030
Chorioamnionitis	3	(5.7)	1	(9.1)	0.939
Maternal fever	3	(5.7)	3	(27.3)	0.189
Foul smelling liquor	2	(3.8)	0	(0)	0.391
Minor					
UTI	2	(3.8)	1	(9.1)	0.792
Meconium stained liquor	2	(3.8)	2	(18.2)	0.286
Prolonged labour	4	(7.5)	1	(9.1)	0.732
>3 during vaginal examination	5	(9.4)	0	(0)	0.171
Multiple factors	6	(4.5)	0	(0)	0.133
Perinatal problems at birth					
Cry with distress	47	(35.6)	6	(12.5)	0.004
No cry	47	(35.6)	18	(37.5)	
Reason for NICU admission					
Respiratory distress	55	(41.7)	10	(20.8)	0.010
Intolerance to feed	13	(9.8)	4	(8.3)	0.759
Abdominal distension	1	(0.8)	2	(4.2)	0.114
Yellowish skin discoloration	1	(0.8)	0	(0.0)	0.545
Prematurity	5	(3.8)	1	(2.1)	0.573
Irritability	4	(3.0)	1	(2.1)	0.732
Shock	0	(0)	3	(6.3)	0.004
Others	3	(2.3)	1	(2.1)	0.939
Multiple factors	50	(37.9)	16	(50.0)	0.576

Prevalence of low TLC (<5000) was significantly higher in the late onset (10.4%) as compared to the early onset (2.3%) group (p=0.019).Abnormal ANC was seen in 55% and low platelet (<1.5 lakh/mm^3) in 41.7%. An equal number (n=75; 41.7%) had CRP>1. In the late onset group, proportion of neonates with CRP>1 was significantly higher (54.2%) as compared to the early onset group (29.5%) (p=0.002). Respiratory distress was significantly higher in early onset group (p=0.010) while shock was significantly higher in late onset group (p=0.004).

Table 3: bacteriological profile of neonatal sepsis

SN	Pathogen	Early onset (n=132)	Late Onset (n=48)	Total (n=180)
		No. (%)	No. (%)	No. (%)
A.	Gram positive	20(15.2)	12(25.0)	32(17.8)
1.	CONS	16(12.1)	9(18.8)	25(13.9)
2.	*S. aureus*	1(0.8)	0(0)	1(0.6)
3.	*Enterococcus*	3(2.3)	2(4.2)	5(2.8)
4.	*Group B Streptococcus*	0(0.0)	1(2.1)	1(0.6)
B.	Gram negative	15(11.4)	4(8.3)	19(10.6)
5.	*K. pneumonia*	8(6.1)	3(6.3)	11(6.1)
6.	*P. aeruginosa*	3(2.3)	0(0)	3(1.7)
7.	*Acinetobacter sp.*	1(0.8)	0(0)	1(0.6)
8.	*E. coli*	3(2.3)	1(2.1)	4(2.2)

Out of the 720 (180x4) culture specimen, seven were contaminated and hence were excluded from the assessment. so, this assessment was evaluated for 713 specimen only.

Table 4: Antibiotic Susceptibility of Pathogens

SN	Antibiotic	% Sensitivity for gram positive 78	% Sensitivity for gram negetive 46
1.	Ampicillin	0.0	4.8
2.	Amikacin	0.0	33.3
3.	Colistin	100.0	81.3
4.	Cefotaxime	6.3	0.0
5.	Cefepime	6.3	22.7
6.	Gentamicin	53.3	25.0
7.	Levofloxacin	27.1	30.8
8.	Linezolid	60.3	100.0
9.	Meropenem	23.1	61.8
10.	Teicoplanin	54.5	0.0
11.	Tigecycline	93.8	100.0
12.	Piperacillin/Tazobactam	8.7	13.8
13.	Vancomycin	47.3	Not tested
14.	Cefazolin	22.2	8.3

Among these gram positive, Tigecycline had the maximum sensitivity (93.8%) followed by Nitrofurantoin (69.1%), Teicoplanin (54.5%) and linezolid (53.3%) with sensitivity over 50%. Among these seven antibiotics Ampicillin had the least sensitivity (0%).

Regarding sensitivity of gram negative, we found that Tigecycline was most sensitive (100%) followed by Colistin (81.3%) and Meropenem (61.8%) while cefotaxime (0.0%) and Gentamicin (25%) were the least sensitive antibiotics.

Discussion

In the present study the proportion of early to late onset neonatal sepsis cases was 2.75:1 similar to those reported by Aletayab *et al.* [7, 4] This may be due to better infrastructural facilities and patient management in early neonatal life itself,

other reasons being well-equipped NICU facilities with proper surveillance, screening mechanism, proper identification of risk factors for neonatal sepsis and prophylactic management. In our study LOS had a higher proportion of outborn cases ($p<0.001$) and Overall male to female ratio was 3.5:1 [8],

In the present study, majority of neonates were born at term (69.4%), however the proportion of preterms was significantly higher among early onset (35.6%) as compared to late onset (16.7%) Reasons could be the inclusion criteria which included prematurity as one of the criteria which is a known cause of EOS[3].In the present study EOS was seen to be significantly influenced by younger (<20 years) as well as older (>30 years) age of mothers. Similarly presence of antenatal risk factor (leaking PV) ($p<0.03$) were also found to be significantly associated with EOS due to pathogens is laid during the fetal or obstetric stages itself.

Abnormal ANC (55%), pneumonitis (49.4%), low platelet count (41.7%), raised CRP levels (41.7%) and haemodynamic instability (32.2%) were the major clinical/laboratory indicators. A significant difference between EOS and LOS was observed for factors like low TLC, raised CRP, and CSF findings suggestive of meningitis ($p<0.05$) onlyin LOS. The results of present study showed that CRP and leukocyte indices has a limited sensitivity for both types, though more so for EOS as compared to LOS[9]. Meningitis is commonly associated with LOS and some researchers recommend CSF evaluation to be the standard of care in these cases.

In the present study, culture positivity rate was 28.3%, and did not show a significant difference between EOS and LOS groups. Prevalence of culture positivity among clinical sepsis cases has been reported to be varying from 10% to 50% in various studies from India. [10]The variability in prevalence rate is dependent on the regional location, season, infrastructural facilities and most importantly on inclusion criteria. In the present study, we had a well-equipped infrastructure, a well-defined inclusion criteria and hence the positivity rate was not as high as 40-50% as indicated in some studies.

In the present study, Gram positive and Gram negative isolates were n=32(17.8%) n=19(10.6%) respectively. No significant difference between early onset and late onset groups was observed with respect to prevalence of different pathogens which is contrary to the results of previous studies from Asia [4, 5]. but our findings were similar to that of Mugalu *et al.*(2006) [8], Sivanandan *et al.*(2011) [11]. Whether this is an incidental finding or a changing trend needs to be examined in further studies.

Among Gram positive pathogens, CONS was the most common isolate (n=25; 13.9%) followed by *Enterococcus* (n=5; 2.8%). *S. aureus* and *Streptococcus* were seen in 1 (0.6%) patient only. Of these CONS, *S. aureus* and *Streptococcus* are often reported in literature as one of the most common pathogens in neonatal sepsis cases[12], while among Gram negative pathogens, *Klebsiella pneumoniae* (6.1%), *E. coli* (2.2%), *P. aeruginosa* (1.7%) and *Acinetobacter* (0.6%) were isolated in that order. This profile of Gram negative pathogens is similar to that reported in literature [12].

We found no statistically significant association of different pathogens with clinical profile, obstetric factors, maternal demographics and outcome.

In our study, The mortality rates in culture positive and culture negative groups were 29.4% and 9.3% respectively and results are in consistence with findings reported in literature too [13]. Reason could be growing antibiotic resistance and changing pattern of pathogens responsible for the sepsis. We found a significant difference in mortality rate of Gram negative pathogen positive (42.1%) and Gram negative pathogen negative (15.0%) groups of neonates [14].

Interestingly in the present study, a major shift from Gram negative to Gram positive isolates emphasized the changing pattern of pathogens and establishes the need for a revision in the first line of antibiotics in accordance with the changing microbial profile.

Among Gram negative pathogens, *E. coli* though positive in only 4 cases, showed a very poor prognosis with 75% mortalities [15]. Association of *E. coli* positivity in early onset neonatal sepsis has been reported to be associated with more morbidity and higher mortality rates, and the findings in present study reaffirmed these observations.

Seven specimen positive for CONS were adjudged as contaminated using the criteria described by Weistein[6]. A high incidence of false positivity of CONS owing to contamination has often been reported and debated in literature and our findings are consistent with these observations [6, 16].

For Gram positive isolates, Tigecycline had the maximum sensitivity (93.8%) followed by Nitrofurantoin (69.1%), Teicoplanin (54.5%) and linezolid (53.3%) respectively. The sensitivity of ampicillin was nil. Among Gram negative isolates too, Tigecycline was most sensitive (100%) followed by Colistin (81.3%) and Meropenem (61.8%) while Ampicillin (4.8%) and Amoxicillin (9.5%) were the least sensitive antibiotics.

Thus in effect, Tigecycline seemed to be an empirical choice for both Gram positive as well as Gram negative strains. In the present study contrary to empirically used antibiotics such as Gentamicin, cefotaxime, amoxicillin, vancomycin and penicillin [17, 18], Tigecycline was found to be superior. Trends similar to present study have also been obtained in a recent study from India [19]. Tigecycline, an expanded broad-spectrum antibiotic; is a semi-synthetic glycylcycline derived from minocycline and has activity against Gram-negative pathogens that are refractory as a result of both efflux and ribosomal protection mechanisms. The clinical efficacy of tigecycline in neonatal sepsis has not yet been established. In vitro evaluation of its efficacy in ESBL and MBL producing isolates in neonatal septicemia have been reported by Roy *et al*., in two different studies [20, 21,22].

Thus, the findings in present study indicated both changed microbial spectrum as well as antibiotic susceptibility pattern with a higher prevalence of Gram positive and increased resistance against conventionally used antibiotics, a finding well supported by systematic reviews [14], however, the present study also showed emergence of newer antibiotics showing high susceptibility pattern.

Limitations of the study: first, most of the cases were outborn and secondly, we could not collect data regarding indwelling catheter in babies positive for CONS. The high prevalence of Gram positive pathogens in present study could be attributed to a person-to-person transmission from caregiver to the newborn during the time of shifting from primary/secondary health centre to our facility. However, both these factors were beyond our control and hence a variability in spectrum of pathogens from other studies could be justified.

Conclusion

As neonatal sepsis is leading cause of neonatal morbidity and mortality in developing countries including India, and various studies have shown changing pattern of microorganisms and their antibiotic susceptibility. The results of present study also suggest change in bacteriological profile from gram negative (10.6%) to Gram positive pathogens (17.8%). The most common gram positive isolate being CoNS group while Gram negative being *K. pneumoniae* in both EOS and LOS groups. For Gram positive isolates, Tigecycline Nitrofurantoin, Teicoplanin and linezolid are highly sensitive while commonly used antibiotics like ampicillin, 3rd generation cephalosporins showed poor sensitivity. Similarly Gram negative isolates found to be highly sensitive for Tigecycline, Colistin and Meropenem while poorly sensitive for aminoglycosides and 3rd generation cephalosporins. Thus periodic assessment of bacteriological profile and antibiotic sensitivity is essential for revision of antibiotic policy and to improve the outcome in neonatal sepsis.

Financial support and sponsorship: The study does not cost any additional financial burden on participants as 2 pair of blood culture are routinely sent in our NICU as asepsis policy.

Conflicts of interest: None.

References

1. May M, Daley AJ, Donath S. Early onset of meningitis in Australia and New Zealand 1992-2002. Arch. Dis. Child. Fetal Neonatal Ed. 2005; 90:F324-7.
2. Zaidi AK, Huskins WC, Thaver D. Hospital-acquired neonatal infections in developing countries. Lancet. 2005; 365:1175-88.
3. Report of the National Neonatal Perinatal Database. Report 2002-2003. NNPD Network, 2005.
4. van den Hoogen A, Gerards LJ, Verboon-Maciolek MA, Fleer A, Krediet TG. Long-Term Trends in the Epidemiology of Neonatal Sepsis and Antibiotic Susceptibility of Causative Agents. Neonatology. 2009; 97(1):22-28.
5. Lim NL, Wong YH, Boo NY, et al. Bacteraemic infections in a neonatal intensive care unit: a nine months survey. Med J Malaysia. 1995; 50:59-63.
6. Weinstein P. Blood Culture Contamination: Persisting Problems and Partial Progress. J Clin Microbiol. 2003; 41(6):2275-2278.
7. Aletayeb SMH, Khosarvi AD, Dehdashtian M, Kompani F, Mortazavi SM, Aramesh MR. Identification of bacterial agents and antimicrobial susceptibility of neonatal sepsis: A 54-month study in a ertiary hospital. Afr. J Microbiol. Research. 2011; 5(5):528-531.
8. Mugalu J, Nakakeeto MK, Kiguli S, Kaddu–Mulindwa DH. Aetiology, risk factors and immediate outcome of bacteriologically confirmed neonatal septicaemia in Mulago hospital, Uganda. African Health Sciences. 2006; 6(1):120-126.
9. Mahbobeh NNF, Ahmadshah T, Parasto S, Setareh S, Mehvar A. Study of the bacterial agents in nosocomial and acquired infections based on the blood culture in neonatal intensive care unit of a hospital, northeast of Iran. Saudi Med J. 2007; 28(5):723-726.
10. Desai KJ, Malek SS. Neonatal Septicemia: Bacterial Isolates & Their Antibiotics Susceptibility Patterns. NJIRM. 2010; 1(3):12-15.
11. Sivanandan S, Soraisham AS, Swarnam K. Choice and Duration of Antimicrobial Therapy for Neonatal Sepsis and Meningitis. International Journal of Pediatrics 2011; Article ID 712150, 1-9.
12. Lim CT, Thong MK, Parasakthi N, et al. Group B streptococcus: maternal carriage rate and early neonatal septicaemia. Ann Acad Med Singapore. 1997; 26:421-5.
13. Kayange N, Kamugisha E, Mwizaholya DL, Jeremiah S, Mshana SE. Research article Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza- Tanzania. BMC Pediatrics. 2010; 10:39.
14. Vergnano S, Sharland M, Kazembe P, Mwansamba C, Heath PT. Neonatal sepsis: an international perspective. Arch Dis Child Fetal Neonatal Ed. 2005; 90:F220-F224.
15. Isaacs D. A ten years multicentre study of coagulase negative staphylococcal infections in Australasian neonatal units. Arch Dis Child Fetal Neonatal Ed. 2003; 88:F89-93.
16. Stoll BJ. Infections of the neonatal infant. In: Nelson Textbook of Pediatrics, Behrman E.d., Kleigman R.E., RM, Jenson HB. Philadelphia: W.B.Saunders, 17th ed, 2004, 623-39.
17. Nash C, Chu A, Bhatti M, Alexander K, Shreiber M, Hageman JR. Coagulase Negative Staphylococci in the Neonatal Intensive Care Unit: Are We Any Smarter? NeoReviews 2013; 14(6): e284-e298.
18. Muller-Pebody B, Johnson AP, Heath PT, Gilbert RE, Henderson KL, Sharland M; iCAP Group (Improving Antibiotic Prescribing in Primary Care). Empirical treatment of neonatal sepsis: are the current guidelines adequate? Arch Dis Child Fetal Neonatal Ed. 2011 Jan;96(1):F4-8.
19. Baltimore RS. Neonatal Sepsis: epidemiology and Management. Pediatric Drugs, 2003; 5(11): 723–740.
20. Gajul SV, Mohite ST, Mangalgi SS, Wavare SM, Kakade SV. Klebsiella pneumoniae in septicemic neonates with special reference to extended spectrum β-lactamase, AmpC, metallo β-lactamase production and multiple drug resistance in tertiary care hospital. J Lab Physicians 2015; 7:32-7.
21. Roy S, Datta S, Viswanathan R, Singh AK, Basu S. Tigecycline susceptibility in *Klebsiella pneumoniae* and *Escherichia coli* causing neonatal septicaemia (2007-10) and role of an efflux pump in tigecycline non-susceptibility. J Antimicrob Chemother 2013;68:1036-42
22. Roy S, Viswanathan R, Singh AK, Das P, Basu S. Sepsis in neonates due to imipenem-resistant Klebsiella pneumoniae producing NDM-1 in India. J Antimicrob Chemother 2011; 66:1411-3.