Clinico-Microbiological Analysis of CoNS Isolated from Blood Cultures of Neonatal Septicemia Cases in a Tertiary Care Hospital in Odisha

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ABSTRACT

Introduction: Neonatal septicemia forms the major cause of neonatal mortality; has been related directly or indirectly to the various customs and practices in the perinatal and neonatal period and geographical area and Coagulase Negative *Staphylococcus aureus* (CoNS) forms the major pathogen associated with these cases. Study aimed to know the bacteriological profile of septicaemic cases amongst neonates born in the hospital and to find out the prevalence of CoNS in neonatal septicaemic cases.

Material and Methods: A prospective study was conducted in the Department of Microbiology to analyze 250 blood samples obtained from the neonates admitted to Dept. of Paediatrics and NICU, of Pradyumna Bal Memorial Hospital (KIMS), Bhubaneswar, during the period from Nov. 2012 – April 2014. Two ml of venous blood was collected and cultured by automated BacT/Alert and VITEK2 method. Various parameters like age, gender, birth weight, gestational age, mode of delivery and age of onset of illness was considered for analysis.

Results: All the samples were subjected to automated blood culture system (BacT/Alert). From them 82(32.8%) became culture positive among which gram positive bacteria were the most predominant (65.8%). Out of all the gram positive pathogens, CoNS were the most common isolates (88.5%). Among the total culture positive pathogenic isolates (n=82), *S. haemolyticus* was the predominant bacteria 28 (34.1%) followed by *S. epidermidis* 12 (14.6%) and *Esch coli*. 8(9.8%). *Staph aureus* was isolated from 6 (7.3%) cases whereas candida spp. were isolated from only 8 cases(9.6%).

Conclusion: The study revealed that both gram positive and gram negative bacteriae were associated with neonatal septicaemia. Most common bacterial pathogens were coagulase negative Staphylococcus (CoNS) (88.5%) followed by Escherichia coli 8(9.8%) and Staphylococcus aureus (7.3%).

Keywords: CoNS, neonatal septicaemia, automated culture.

INTRODUCTION

The Neonatal septicaemia can be defined as a clinical syndrome characterised by systemic signs and symptoms due to generalised bacterial infections with a positive blood culture in the first four weeks of life.[1] The commonest cause of morbidity and mortality during the neonatal period is Bacterial infection. In developing countries sepsis is the commonest cause of mortality responsible for 30 - 50% of the 5 million of total neonatal deaths each year. The reported incidence of neonatal sepsis varies from 7.1 - 38 per 1000 live births in Asia. National Neonatal Perinatal Database (NNPD), (2002 - 2003) from India has reported an incidence varying from 0.1% - 4.5%. Fatal course of infection may result from complications such as shock, disseminated intravascular coagulation (DIC) and multisystem organ failure. Neonatal sepsis may be classified according to the time of onset of the disease, early onset sepsis (EOS) and late onset sepsis (LOS). This definition has clinical relevance, as EOS disease is mainly due to bacteria acquired before and during delivery and LOS disease due to bacteria acquired after delivery (Nosocomial and community sources). Early diagnosis of this life threatening condition leads to treatment and a favourable outcome.[2]

Study aimed to determine the bacteriological profile and antibiotic sensitivity of blood culture isolates from neonatal cases done by automated culture and sensitivity method in a neonatal intensive care unit (NICU) of Pradyumna Bal Memorial Hospital (PBMH and KIMS), Bhubaneswar, Odisha and to find out the prevalence of CoNS in neonatal septicaemic cases.

MATERIAL AND METHODS

A prospective study was carried out covering 250 suspected neonatal septicaemia cases reported in the Department of Microbiology in association with Department of Paediatrics and NICU, of Kalinga Institute of Medical Sciences, Bhubaneswar, during the period from Nov. 2012 – April 2014. The inclusion criteria were neonates showing non specific and specific signs and symptoms of septicaemia. The non specific features included one or more of the following symptoms like – lethargy, hypothermia or fever, poor perfusion, hypotonia, respiratory distress, brady or tachypnoea. The specific features included were (a) Central nervous system: Bulging anterior fontanelle, vacant stare, excess irritability, (b) Cardiac system: Shock, perfusion, (c) Gastrointestinal system: Abdominal distension, paralytic ileus, necrotizing enterocolitis, (d)Hepatic: Hepatomegaly, direct hyperbilirubinemia, (e) Hematological:Bleeding, Purpura. The neonates without any clinical signs and symptoms of sepsis were taken under exclusion criteria.

Two ml of venous blood samples for culture were collected with standard aseptic precautions. If antibiotics were already

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How to cite this article: Kausik Kumar Sarangi, Dipti Pattnaik, Surya Narayan Mishra, Manas Kumar Nayak, Jagadananda Jena. Clinico-microbiological analysis of cons isolated from blood cultures of neonatal septicemia cases in a tertiary care hospital in Odisha. International Journal of Contemporary Medical Research 2016;3(9):2617-2621.

started empirically, the collection of blood was done before the next dose of antibiotic was due or about half an hour before the predicted peak of temperature. After collection,these samples were transferred immediately to the Microbiology section of Central Laboratory, KIMS and were cultured by automated BacT/Alert and VITEK2 method for rapid isolation and sensitivity test.[2] The results were then analysed to find out the significance of various parameters.

STATISTICAL ANALYSIS

The collected data was coded and entered into SPSS (Statistical Package for Social Sciences), Version 16. The data was summerised using tables and graphs. Univariate analysis was performed separately for each of the variables. P values were calculated using the chi square test or Fisher's exact test for categorical variables and Student's t-test for continuous variables. A p value <0.05 was considered significant.

RESULTS

Table-1 shows the association between physical parameters and sepsis. Out of 250 clinically suspected septicaemic cases of neonates, 154 (61.6%) were males and 96 (38.4%) were females. Among 154 males, 45 (29.2%) were culture positive and 109 (70.8%) were culture negative. Out of 96 females, only 37 (38.5 %) were culture positive. Maximum culture positivity was seen in the neonates of 8-28 days of age (40.9%) followed by 4-7 days (28.6%) and 0-3 days (27.5%) of age. Total culture positivity in the study in all the age group was 82 (32.8%).

Table-2 shows the association of clinically suspected cases with various risk factors. Out of all the culture positive septicaemic cases, maximum percentage of positivity were seen in VLBW group (60%) followed by LBW neonates 47.6% (Table-2). Maximum bacterial growth was seen in pre term babies as compared to the term babies. PROM was also found to be the important risk factor for culture positivity (47.8%) than the absence of PROM (31.2%). Home delivery was seen as an important risk factors for sepsis (46.6%) than the hospital delivery both at KIMS or in other institutions (29.5% and 33.8% respectively). History of caesarean section was seen in 34.9% of culture positive septicaemic cases as compared to the normal delivery (28.2%). Significant p value was observed in relation to birth weight, gestational age and PROM.

Table-3 shows distribution pattern of various isolates. Out of total 82 pathogenic isolates recorded by automated system, *S. hemolyticus* was the predominant bacteria 28 (34.1%) followed by *S. epidermidis* 12 (14.6%) and *Esch. coli*. 8(9.8%). *S. aureus*

		Sepsis						Chi-square 'p' value
		Culture +Ve		Culture -Ve		Total		
		No.	%	No.	%	No.	%	
Sex	Male	45	54.9	109	64.9	154	61.6	$\chi2 = 2.331$ p = 0.127
	Female	37	45.1	59	35.1	96	38.4	
	Total	82	100.0	168	100.0	250	100.0	
Age	0-3 day	22	26.8	58	34.5	80	32.0	$\chi2 = 20.041$ p = 0.000
	4-7 day	22	26.8	55	32.7	77	30.8	
	8-28 day	38	46.3	55	32.8	93	37.2	
	Total	82	100.0	168	100.0	250	100.0	
Age (Mean ± S.E.)		6.62 ± 0.408		7.36 ± 0.432				

Table-1: Association between physical parameters and sepsis

		Sepsis						Chi-square 'p' value
		Culture +Ve		Culture -Ve		Total		
		No.	%	No.	%	No.	%	
Birth Weight	Very LBW	3	60.00	2	40.00	5	2.00	$\chi2 = 16.811$ p = 0.000
	LBW	41	48.20	44	51.80	85	34.00	
	Normal	38	23.80	122	76.20	160	64.00	
	Total	82	32.80	168	67.20	250	100.00	
Gestation	Term	31	19.50	128	80.50	159	63.60	$\chi2 = 35.072$ p = 0.000
	Preterm	51	56.00	40	44.00	91	36.40	
	Total	82	32.80	168	67.20	250	100.00	
PROM	Absent	71	31.30	156	68.70	227	90.80	$\chi2 = 2.595$ p = 0.107
	Present	11	47.80	12	52.20	23	9.20	
	Total	82	32.80	168	67.20	250	100.00	
Place of Delivery	Other Institution	44	33.80	86	66.20	130	52.00	$\chi2 = 1.884$ p = 0.039
	KIMS	31	29.50	74	70.50	105	42.00	
	Home Delivery	7	46.70	8	53.30	15	6.00	
	Total	82	32.80	168	67.20	250	100.00	
Mode of Delivery	C.S	60	34.90	112	65.10	172	68.80	$\chi2 = 1.086$ p = 0.297
	ND	22	28.20	56	71.80	78	31.20	
	Total	82	32.80	168	67.20	250	100.00	

Table-2: Association of Clinically suspected cases by Risk Factor

was isolated from 6 (7.3%) cases where as Candida was also isolated from 8 (9.8%) cases.

Table-4 shows the relationship of various organisms with respect to the onset of septicemia. Isolation of gram positive bacteria (75%) and fungi (10%) were more from late onset septicaemic cases as compared to the early onset septicaemia (31.8% and 9% respectively) but gram negative bacterial pathogens (59 %) were predominant from early onset septicaemic cases.

CONS were the predominant gram positive isolates 46 (88.5%) as compared to the other pathogens 6 (11.5%) in this study (figure-1).

CONS were the predominant gram positive isolates 46 (88.5%) followed by Staphylococcus aureus 6 (11.5%) in the study (table-5).

All the gram positive pathogenic isolates were sensitive to Linezolid, Tigecycline and Vancomycin. Co-trimoxazole was sensitive in 78.8% isolates followed by ceftriaxone (76.9%), Azithromycin (76.9%), Cefepime (65.4%) Erythromycin (55.8%) Clindamycin (51.9%) Levofloxacin (42.3%), Amikacin (42.3%), Cefadroxil (30.8%) and Gentamicin (25%). Maximum resistance was seen against Benzylpenicilin (86.6%) and Ampicilin (71.2%) (table-6).

Bacteria AS	No.	%
S.haemolyticus	28	34.10
S.epidermidis	12	14.60
S.werneri	3	3.70
S.hominis	3	3.70
S.aureus	6	7.30
Enterobacter cloacae	6	7.30
Burkholderia cepacia	4	4.90
Acinetobacter lwoffi	2	2.40
S.paratyphiA	2	2.40
Esch.coli	8	9.80
C.albicans	8	9.80
Total	82	100.00

Table-3: Distribution of pathogenic isolates by Automated System (n= 82)

Organism Type	Onset of Septicaemia		
	EOS	LOS	Total
	No.	No.	No.
Gram Positive	7(31.8)	45(75%)	52(63.1%)
Gram Negative	13(59%)	9(15%)	2226.3%)
Fungi	2(9%)	6(10%)	8(9.6%)
Total	22(n=22)	60(n=60)	82(n=82)
Chi-square 'p' value	$\chi 2 = 16.411$; p = 0.000		

Table-4: Organism Type Vs. onset of Septicaemia

Gram positive	No.	%
CONS	46	88.50
Staph. aureus	6	11.50
Total	52	100.00

Table-5: Prevalence of CONS among gram positive isolates (CONS- S.haemolyticus, S.epidermidis, S.werneri, S.hominis)

DISCUSSION

The present study was carried out by taking 250 neonatal

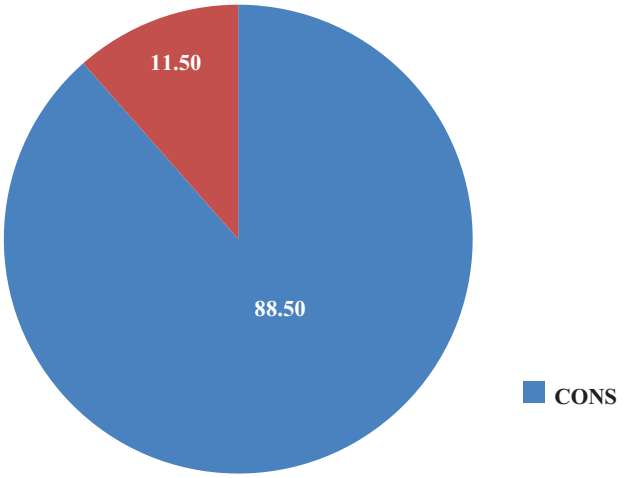

Figure-1: CONS among gram positive cases

Name of the antibiotic	Bacteria isolated in Automated System											
	S.haemolyticus (n=28)		S.epidermidis (n=12)		S.werneri (n=3)		S.hominis (n=3)		S.aureus (n=6)		Total (n=52)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Linezolid	28	100	12	100	3	100	3	100	6	100	52	100
Tigecycline	28	100	12	100	3	100	3	100	6	100	52	100
Cefadroxil	7	25	5	41.7	1	33.3	1	33.3	2	33.3	16	30.8
Benzyl Penicillin	4	14.3	2	16.7	0	0	0	0	1	16.7	7	13.5
Clindamycin	14	50	7	58.3	1	33.3	2	66.7	3	50	27	51.9
Ciprofloxacin	9	32.1	5	41.7	2	66.7	1	33.3	2	33.3	19	36.5
Oxacillin	14	50	5	41.7	2	66.7	2	66.7	4	66.7	27	51.9
Gentamicin	5	17.9	5	41.7	1	33.3	1	33.3	1	16.7	13	25
Ampicillin	6	21.4	3	25	0	0	0	0	3	50	12	23.1
Co-Trimoxazole	22	78.6	9	75	3	100	2	66.7	5	83.3	41	78.8
Cefuroxime	10	35.7	2	16.7	1	33.3	2	66.7	3	50	18	34.6
Ceftriaxone	20	71.4	9	75	3	100	3	100	5	83.3	40	76.9
Cefepime	18	64.3	8	66.7	2	66.7	3	100	3	50	34	65.4
Amikacin	10	35.7	5	41.7	2	66.7	2	66.7	3	50	22	42.3
Azithromicin	21	75	9	75	3	100	2	66.7	5	83.3	40	76.9
Erythromycin	16	57.1	7	58.3	2	66.7	0	0	4	66.7	29	55.8
Vancomycin	28	100	12	100	3	100	3	100	6	100	52	100

Table-6: Antibiotic sensitivity patterns among gram positive isolates (No and % showing sensitivity)

clinically suspected to be suffering from sepsis and admitted to the NICU of Paediatrics Department of KIMS. Distribution of cases organismwise, onset of septicaemia, types of organism have been analysed. Further, antibiotic sensitivity pattern of the pathogens have been thoroughly analysed.

In the present study, the total number of LBW babies (Table-2) were 55 out of 250 clinically suspected neonates (21.6%), amongst which culture positivity is associated with 47 cases (72.7.4%). This finding is nearer to the outcome of the study conducted by Manjushree H Bhalchandra[3] where majority (60%) of the LBW babies are associated with sepsis. Another study conducted by M.N.Shah et al[4], showed, 50% of LBW neonates associated with sepsis.

The number of VLBW neonates (Table-2) in our study were 5 (2%) out of which culture positivity was 60%. This finding is closely related to the study conducted by Jia-Horng Jiang et al[5] where culture positivity of VLBW is 54.4%.

In our study 51 (56%) neonates (Table-2) showed blood culture positivity in association with pre-term delivery. This finding is at par with the study conducted by M.N.Shah et al[4] where 51.04% bacteriologically positive cases were associated with pre-term delivery.

In the present study out of 250 clinically suspected neonates, 82 were bacteriologically confirmed, among which Staphylococcus haemolyticus was the predominant isolate 28 (34.1%) (table-3) followed by Staphylococcus epidermidis 12 (14.6%), S.werneri 3 (3.7%), S.hominis 3 (3.7%), and S.aureus 6 (7.3%). Other pathogens isolated were Enterobacter cloacae 6 (7.3%), Burkholderia cepacia 4 (4.9%), *Acinetobacter lwoffi* 2 (2.4%), *Salmonella paratyhi* A 2 (2.4%), *Esch.coli* 8 (9.8%) and *Candida albicans* 8 (9.8%). It shows that gram positive bacterial pathogens were the commonest isolates 52 (63.4%) followed by gram negative bacterial pathogens 26.8% and fungi 9.8%. This finding is similar to the outcome of the study conducted by Hilal Ozkan et al[6] where it was shown that gram positive isolates were 68.2% followed by gram negative isolates (16.5%) and fungi (15.8%).

Regarding onset of sepsis it has been shown in our study that gram positive bacterial pathogens are more commonly associated with late onset sepsis (table-4), which accounts to (72.5%) and gram negative pathogens are commonly associated with early onset sepsis (59%) which is close to the findings of the study conducted by F.Motara et al[7] where late onset sepsis was most common and CONS was the predominant organism causing late onset sepsis (67.6%) and also gram negative isolates were predominant in early onset sepsis. In another study conducted by Jia-Horng Jiang et al[8] CONS were also associated commonly with late onset sepsis.

The no. of gram positive isolates in our study were 52 (63.4%) amongst which Coagulase negative *staphylococcus aureus* were the most predominant 46 (88.4%) (table-5, figure-1). This finding is close to the outcome of the study conducted by Hilal Ozkan et al[6] where it was shown that gram positive isolates particularly CONS were the most predominant (74.7%). Another study conducted by Reza Ghotaslou et al[9] revealed gram positive organisms as predominant isolates among all (68.57%) and CONS among all gram positive isolates (91.6%). Study conducted by Shahsanam Gheibi et al[9] also revealed CONS as the predominant pathogen (54%) isolated in NICU.

In our study all the gram positive pathogenic isolates were sensitive to Linezolid, Tigecycline and Vancomycin. Co-trimoxazole was sensitive in 78.8% isolates (table-6) followed by ceftriaxone (77%), Azithromycin (76.9%), Cefepime (60%) Erythromycin (59.6%) and Clindamycin (53.9%). This finding is very closely associated with the study conducted by Shahsanam Gheibi et al[10] where maximum sensitivity was found to Vancomycin (90%) and Ciprofloxacin (78.5%). Study conducted by Katiyar R et al[11] showed 40.74% sensitivity to Amikacin and 25.92% to Gentamicin. Sensitivity to Cefaclor, Cefotaxime and Ceftazidime were 40%, 33.3% and 22.2% respectively. Lowest sensitivity was found to Penicillin (7.41%) and Ampicillin (18.52%) which is close to the findings of our study. Maximum sensitivity to Linezolid (100%), Vancomycin (95%), Cefotaxime (73%), Ceftriaxone (68%) and Amikacin (68%) was observed in the study conducted by Maimoona Mustafa et al.[11] Resistance pattern were maximum against Ampicillin (86.4%), Erythromycin (64%) and Gentamicin (50%).

CONCLUSION

During the new-born period, infection remains an important cause of morbidity and mortality, despite increasing sophistication in infant intensive care and the use of broad spectrum antimicrobial agents. There is an urgent need for such studies looking at simple and sustainable interventions to reduce the burden of neonatal infection. Long term surveillance is also needed to describe the varied pathogens causing neonatal sepsis as well as their changing antibiotic susceptibility profile.

ACKNOWLWDGEMENT

The authors are very much thankful to the HOD, Paediatrics, staffs of NICU, KIMS especially to Dr Manas.K. Nayak and the technical staffs and faculty members of Department of Microbiology, KIMS for their support in completing the research project.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 06-07-2016; **Published online**: 30-08-2016