Research Article

Bacteriological profile and antibiogram of blood culture isolates done by automated culture and sensitivity method in a neonatal intensive care unit in a tertiary care hospital in Odisha, India

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ABSTRACT

Background: Sepsis is one of the commonest cause of neonatal mortality. The aetiology of neonatal sepsis has variations according to the various customs and practices in the perinatal and neonatal period and geographical area. This study was designed to analyse the magnitude and aetiological characteristics of neonatal sepsis & also the antibiotic sensitivity pattern of the isolates.

Methods: This prospective study was conducted in the Department of Microbiology including 250 neonates with suspected neonatal sepsis admitted to Dept. of Paediatrics & NICU, of Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar, during the period from Nov. 2012 - April 2014. Two ml of venous blood was taken from each neonate & cultured by automated BacT/Alert & VITEK2 method for rapid isolation & sensitivity test. A structured proforma was used to collect the information for the baseline characteristics like age, gender, birth weight, gestational age, mode of delivery of the neonate and age of onset of illness.

Results: Amongst the total samples processed, 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%).

Conclusions: Present study revealed that both gram positive and gram negative bacteria were responsible for blood stream infections and most of the strains were multidrug resistant. Among gram positive bacterial pathogens, most common were Coagulase Negative *Staphylococcus* (CoNS) (88.5%) followed by *Staphylococcus aureus* (7.3%).

Keywords: Automated culture, Neonatal sepsis, CoNS

INTRODUCTION

Neonatal septicaemia refers to 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] Bacterial infections are the commonest cause of morbidity and mortality during the neonatal period. Fulminate and fatal course of infection may result from complications such as shock, disseminated intravascular coagulation (DIC) and multisystem organ failure, mandating early diagnosis of

this life threatening condition for a timely treatment and a favourable outcome.[2]

Aims and objectives

To find out the bacteriological profile and antibiogram of blood culture isolates from neonatal septicaemic cases done by automated culture & sensitivity method in a Neonatal Intensive Care Unit (NICU) of Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar, Odisha, India.

METHODS

A prospective study covering 250 suspected neonatal septicaemic cases was carried out in the Department of Microbiology in association with Dept. of Paediatrics & NICU, of Kalinga Institute of Medical Sciences, Bhubaneswar, during the period from Nov. 2012 - April 2014.

Two ml of venous blood samples for culture were collected following standard aseptic precautions in specific automated aerobic blood culture bottle [FAN (Fastidious Antibiotic Neutralization bottle (yellow cap) - BioMerieux #259794]. If empirical antibiotics were already started, the collection was timed before the next dose of antibiotic was due or about half an hour before the predicted peak of temperature.

The samples thus collected were transferred immediately to the Microbiology section of Central Laboratory, KIMS and were cultured by automated BacT/Alert & VITEK2 method for rapid isolation & sensitivity test. The results thus obtained were analysed to find the significance of various parameters.

The blood culture bottles positive for bacterial growth changed its colour from blue green to yellow & positive bottles are determined by one of three software algorithms and are indicated as a yellow sensor and high reflectance units. Negative bottles result from darker sensors and low reflectance units.

RESULTS

Out of 250 clinically suspected septicaemic cases of neonates, 154 (61.6%) were males and 96 (38.4%) were females. Among the males culture positivity was 45 (54.9%) & for females it was 37 (45.1%). Neonates of the age group 8-28 days showed maximum culture positivity 38 (46.3%). Total culture positivity in the study in all the age group was 82 (32.8%) (Table 1).

Table 1: Association between physical parameters and sepsis.

		Sepsis						Chi-square 'p' value
		Culture +ve		Culture -ve		Total		
		No.	%	No.	%	No.	%	
Sex	Male	45	54.9	109	64.9	154	61.6	$X^2 = 2.331$ $p = 0.127$
	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	$X^2 = 20.041$ $p = 0.000$
	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 ± SE)		6.62 ± 0.408		7.36 ± 0.432				

Out of all the culture positive septicaemic cases, maximum percentage of positivity were seen in VLBW group. (60%) followed by LBW neonates (47.6%). Maximum bacterial growth was seen in pre term babies (56%) as compared to the term babies (19.5%). 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 factor for sepsis (46.6%) than the hospital delivery both at KIMS or in other institutions (29.5% & 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.

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* was isolated from 6 (7.3%) cases whereas *Candida albicans* was also isolated from 8 (9.8%) cases (Table 3).

Isolation of Gram positive bacteria (75.0%) and fungi (10%) were more from late onset septicaemic cases as compared to the early onset septicaemia (31.8% and 9.0%) but Gram negative bacterial pathogens (59.0%) were predominant from early onset septicaemic cases (Table 4).

Table 2: Association of clinically suspected cases by risk factor.

		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	X^2 = 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	X^2 = 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	X^2 = 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	X^2 = 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	CS	60	34.90	112	65.10	172	68.80	X^2 = 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 3: Distribution of pathogenic isolates by automated System (n= 82).

Bacterial pathogens	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. paratyphi A	2	2.40
Esch. coli	8	9.80
C. albicans	8	9.80
Total	82	100.00

Table 4: Organism type vs. onset of septicaemia.

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%)	222 (6.3%)
Fungi	2 (9%)	6 (10%)	8 (9.6%)
Total	22 (n=22)	60 (n=60)	82 (n=82)
Chi-square 'p' value	X^2 = 16.411; p = 0.000		

Among all the gram positive isolates, CoNS were the predominant pathogen 46 (88.5%) as compared to the other pathogens 6 (11.5%) in this study (Figure 1).
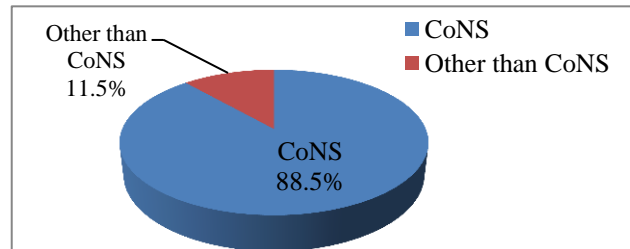

Figure 1: CoNS among Gram positive cases.

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 5).

Among the Gram negative isolates maximum antibiotic sensitivity was observed for tigecycline (90.9%), levofloxacin (86.4%) & amikacin (86.4%) followed by imipenem (77.3%), meropenem (77.3%), tobramycin (77.3%), ciprofloxacin (68.2%), piperacilin-tazobactum (68.2%), ceftriaxone (54.5%) and cefepime (40.9%). Resistance was maximum for cefadroxil (86.4%), ampicilin (72.7%) and cefuroxime (68.2%) (Table 6).

Table 5: Antibiotic sensitivity patterns among gram positive isolates (No & % showing sensitivity).

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 negative isolates (No & % showing sensitivity).

Name of the antibiotic	Bacteria isolated in automated system											
	Enterobacter cloacae (n=6)		*Burkholderia cepacia* (n=4)		*Acinetobacter lwoffi* (n=2)		*S. paratyphi A* (n=2)		*Esch. coli* (n=8)		Total (n=22)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	2	33.3	0	0	0	0	2	100	2	25	6	27.3
Gentamicin	2	33.3	2	50	1	50	1	50	4	50	10	45.5
Amikacin	5	83.3	3	75	2	100	2	100	7	87.5	19	86.4
Ciprofloxacin	5	83.3	2	50	1	50	1	50	6	75	15	68.2
Levofloxacin	5	83.3	3	75	2	100	2	100	7	87.5	19	86.4
Cefadroxil	1	16.7	0	0	0	0	1	50	1	12.5	3	13.6
Cefuroxime	2	33.3	1	25	0	0	2	100	2	25	7	31.8
Ceftriaxone	4	66.7	2	50	0	0	2	100	5	62.5	13	59.1
Cefepime	3	50	1	25	0	0	2	100	3	37.5	9	40.9
Tobramicin	4	66.7	3	75	2	100	2	100	6	75	17	77.3
Piperacillin-Tazobactum	5	83.3	2	50	0	0	2	100	6	75	15	68.2
Imipenem	6	100	1	25	0	0	2	100	8	100	17	77.3
Meropenem	6	100	1	25	0	0	2	100	8	100	17	77.3
Tigecycline	6	100	2	50	2	100	2	100	8	100	20	90.9
Co-trimoxazole	5	83.3	4	100	1	50	2	100	4	50	16	72.7

DISCUSSION

The present study included 250 clinically suspected neonates suffering from sepsis and admitted to the NICU of Paediatrics Department, KIMS. Distribution of cases based on onset of septicaemia & type of organism have been analysed. Further, antibiotic sensitivity pattern of the bacterial pathogens have been thoroughly analysed.

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

Shah et al.,[4] showed, the association of LBW neonates & sepsis as 50%.

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

In our study 51 (56%) neonates 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 cases among which *Staphylococcus haemolyticus* was the predominant isolate 28 (34.1%) followed by *Staphylococcus epidermidis* 12 (14.6%), *S. werneri* 3 (3.7%), *S. hominis* 3 (3.7%), & *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%) & *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% & 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%) & fungi (15.8%).

Regarding onset of sepsis it has been shown in our study that Gram positive bacterial pathogens were more commonly associated with late onset sepsis which accounts to (72.5%) & Gram negative pathogens were 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 & CoNS was the predominant gram positive pathogen (67.6%) & Gram negative isolates as predominant pathogens in early onset sepsis. In another study conducted by Jia-Horng Jiang et al.[8] CoNS were also associated commonly with late onset sepsis.

In our study all the Gram positive pathogenic isolates were sensitive to linezolid, tigecycline and vancomycin. Co-trimoxazole was sensitive in 78.8% isolates followed by ceftriaxone (77%), azithromycin (76.9%), cefepime (60%), erythromycin (59.6%) & clindamycin (53.9%). This finding is closely associated with the study conducted by Shahsanam Gheibi et al.[9] where maximum sensitivity was found to vancomycin (90%) & ciprofloxacin (78.5%). Study conducted by Katiyar R et al.[10] showed maximum resistance to penicillin (7.41%) & ampicillin (18.52%) which is close to the findings of our study. Similar to our study maximum sensitivity to linezolid (100%), vancomycin (95%), cefotaxime (73%), ceftriaxone (68%) & amikacin (68%) was also observed in the study conducted by Maimoona Mustafa et al.[11]

For Gram negative pathogenic isolates, our study showed maximum sensitivity to amikacin (86.4%) followed by imipenem (77.3%), meropenem (77.3%), tobramycin (77.3%), ciprofloxacin (68.2%), piperacilin-tazobactum (68.2%), ceftriaxone (59.1%) and cefepime (40.9%) & resistance to cefadroxil (86.4%), ampicilin (72.7%), cefuroxime (68.2%) & gentamicin (54.5%) which was at par with the study conducted by Maimoona Mustafa et al. In another study conducted by A. K. Mane et al.[12] maximum sensitivity was also found to imipenem (100%), ciprofloxacin (66.6%) & levofloxacin (66.6%). & maximum resistance was found against ampicillin (81.5%) & gentamicin (85.2%).

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