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## Bacterial Culture Profile, Antibiotic Susceptibility And Resistance Pattern In Neonatal Sepsis: A Tertiary Care Experience.

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### ABSTRACT

Neonatal sepsis is the second leading cause of new born mortality in India. A knowledge of existing maternal and new born risk factors, bacteriological profile and their antibiogram in individual NICUs can guide the physician to take appropriate, prompt and rational decision in combating and preventing new born sepsis. A retrospective cross sectional study was conducted in a level three neonatal intensive care unit of a teaching hospital in south India from Jan 2021 to June 2022, to study the clinical and bacteriological profile of all new born admitted for evaluation of sepsis. During the study period 234 newborn were admitted for evaluation of new born sepsis, 63.6% were early onset sepsis (EOS). Blood culture was positive in 9.4% of the cases, predominant isolates were staphylococcus aureus, followed by coagulase negative staphylococcus (CONS). Most of the isolates were highly resistant to first line antibiotics like Ampicillin and Amoxycillin. Better collection technique and culture methodology may further improve bacterial yield, in new born suspected of sepsis in our set up. Judicious and rational use of antibiotics as per sensitivity profile may help in combating the development of resistant organisms.

**Keywords:** Bacterial Culture, Antibiotic Susceptibility, Resistance Pattern

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## INTRODUCTION

As per the UNICEF 25 million babies are born each year in India, accounting for 1/5<sup>th</sup> of world's annual child birth. However, one of these babies dies every minute [1]. Neonatal sepsis (33%) is the second most common cause of neonatal mortality, after prematurity (35%) [1]. Prematurity, per se, is a very high-risk factor for sepsis too. Neonatal mortality in India has reduced from 1/3<sup>rd</sup> of global new born deaths in 1990 to less than 1/4<sup>th</sup> today [1]. However, we are far from the ambitious goal of India New Born Action Plan (INAP) of single digit NMR by 2030 [1].

Rates of clinically diagnosed neonatal sepsis reported were as high as 170/1000 live births in rural India [2] and blood culture-confirmed sepsis was reported to be 5.5/1000 live births in resource poor developing countries [3]. The organisms causing new born sepsis differ from region to region and also changes with time, in the same set up [4,5].

The rampant use of antibiotics has led to the emergence of multi drug resistant organisms [6, 7]. Every NICU must be aware of local bacterial isolates and their antibiogram in order to guide the primary care physicians to institute rational, appropriate and empiric antibiotics at the earliest, in the time critical condition of new born sepsis, in order to decrease neonatal mortality and morbidity. Equally important is the identification of maternal and infant risk factors for sepsis like age, sex, birth weight, gestational age, PROM>18 hrs etc. This can give a high index of suspicion of sepsis in the individual NICU, prompt identification and institution of antibiotics. Therefore, we attempted to identify the risk profile of the babies admitted to our NICU, the bacteria associated with sepsis, and their antibiotic profiles, in order to improve new born sepsis outcome.

## MATERIALS AND METHODS

A retrospective study was carried out in a tertiary care teaching hospital neonatal unit in South India. The study was carried out after obtaining ethical clearance from institutional ethical committee. All newborn (0-28 days) delivered as inborn and out born admitted in neonatal intensive care during the period of January 2021 to June 2022 either with risk factors for sepsis or clinical features suggestive of sepsis were included in the study. Blood culture was obtained with strict aseptic precautions before starting antibiotics in all newborn with clinical features suggestive of sepsis such as hemodynamic and temperature instability, hypoglycemia, lethargy, poor feeding, hypotonia, respiratory distress, mottling, seizures, with maternal risk factors like maternal fever, premature membrane rupture >18 hours, uncleaned vaginal examination more than or equal to 3, foul smelling liquor and newborn risk factors like preterm babies born before 37 completed weeks, 5 min APGAR < 6. Newborn who had received antibiotics prior to sampling for blood culture were excluded from the study. About 2 ml of blood was inoculated into BacT/ALERT. Culture bottles were processed using the BacT/ALERT 3D 240 Microbial Detection System. Positively identified bottles were subjected to gram stain and subculture on 5% sheep blood agar, chocolate agar and MacConkey agar plates and put for overnight incubation at 37°C. Antimicrobial Sensitivity and resistance pattern was tested for both first-line and second-line antibiotics. After taking blood culture, the newborn was started on empirical first-line antibiotics according to the standard NICU protocol followed in the hospital. If there was no clinical response after 72 hr, antibiotics were upgraded to IV vancomycin or meropenem. When the blood culture reports were available we selected antibiotics accordingly.

The clinical profile, maternal and neonatal risk factors profile and laboratory profile like total WBC count, CRP and platelet count were obtained from case sheets.

Neonatal Sepsis which was the infection involving blood stream in newborn infants < 28 days. Early onset sepsis and late onset sepsis were defined based on the time of onset of sepsis within 72 hours of birth or after 72 hours of birth within 28 days of life respectively.

Data was analysed using SPSS 24v. Descriptive statistics like percentages, mean, median, standard deviation were calculated. The association between variables was calculated by chi-square test at 5% level of significance.

## RESULTS

### Clinical profile

During the study period from Jan 2021 to June 2022, there were 714 admissions to the NICU of which 234 were evaluated for suspected sepsis. 131 (56%) were boys, 219 (93.6%) were inborn babies, 165 (70.5%) were normal weight babies, 62 (26.5%) were low birth weight (LBW) and 5 (2.1%) were very low birth weight (VLBW).

52 (22.2%) were preterm. Maternal risk factors were present in 51 (21.8%), Premature rupture of membranes >18hr (PROM) in 45 (19.2%), antepartum fever in 5 (2.1%) and unclean vaginal examination in 1 (0.4%). Newborn risk factors were present in 73 (31.2%), LBW or preterm in 73 (31.2%) and Apgar at 5 minutes <6 in 2 (0.9%) (Table 1).

### Lab Profile

Elevated WBC count >20,000 in 14 (6%), Normal in 219 (93.6%), Decreased < 5000 in only one baby with suspected sepsis. CRP was > 10 in 25 (10.7%) and thrombocytopenia in 5 (2.1%).

Culture was positive in 22 (9.4%), of all cases of suspected sepsis, of which 14 (63.6%), was early onset sepsis (EOS), and 8 (36.4%) were late onset sepsis. Among the isolates 13 (59%) were gram positive cocci, of which staphylococcus aureus in 6 (27%) was the most common, followed by coagulase negative staphylococcus aureus (CONS) in 3(14%). Pseudomonas in 3(14%) was the most common gram negative isolate (Fig 1).

Of the organisms causing EOS, 56.9% were gram positive, predominantly staphylococcus aureus and CONS, While 43% were gram negative predominantly pseudomonas and other non fermenting gram negative bacteria (NFGNB).

However, in LOS 62.5% were gram positive cocci (Table 2).

Antibiogram of the organisms, revealed that staphylococcal species and pseudomonas were highly resistant to Ampicillin. Gram positive species showed moderate resistance to AmoxicillinClavulanic acid, while NFGNB were sensitive to it. For Ciprofloxacin gram positive species shows significant resistance, but gram-negative species showed good susceptibility. However, gram negative species showed uniformly significant resistance to Amikacin (Table 3). Staphylococcal species including CONS and Methicillin resistant staphylococcus aureus (MRSA) and Methicillin Resistant CONS were uniformly sensitive to Vancomycin, linezolid and Clindamycin.

Pseudomonas and NFGNB were highly sensitive to Piperacillin tazobactam and Carbapenams (Table 4). Only one species of enterococcus showed multi drug resistance (MDR) in our series.

### Risk Factors

In our NICU, maternal antepartum fever had high association with culture positive sepsis, whereas other maternal and new born risk factors were not significantly associated with culture positivity (Table 5).

Among lab parameters, high TLC (Total Leukocyte Count), CRP positive and thrombocytopenia were significantly positively associated with culture proven sepsis.

## DISCUSSION

Neonatal sepsis is a significant contributor to neonatal mortality in India. Blood culture is the gold standard in the diagnosis of new born sepsis. However, it takes hours to days and is positive in only 25 to 45 % of cases [8]. In the context of "time critical condition" like new born sepsis, empiric antibiotic treatment becomes vital. Hence a knowledge of the bacterial isolates and their susceptibility pattern in individual NICUs helps guide the physician to initiate appropriate and rational, empiric antibiotics before blood culture sensitivity results become available.

Tasneem reported 15.3% blood culture positivity in her study [9]. Chacko et al reported 43% culture positivity [10] and bhat 48.3 % [11]. Our study showed culture positivity of 9.4%. Better focus on stringent collection technique and improved culture methods might increase the yield.

Overall 59% of our isolates were gram positive and the rest gram negative. staphylococcus aureus was the commonest organism noted followed by CONS. MRSA accounted for 9% of the isolates. Karthikeyan et al also demonstrated staphylococcus aureus as the predominant pathogen in 51.5% [12]. Several studies have shown that staphylococcus aureus is evolving as the predominant pathogen in developing countries [4, 5].

Of the gram-negative species, Pseudomonas was the commonest single organism in our study. jyothi et.al reported gram negative organism as the predominant yield in their unit [13] and, Klebsiella was the most frequent in the study by Pokhrel et al [14]. These differences may be due to differences in study setting, population under study and adherence to hand hygiene policies. When comparing isolates of EOS vs LOS, we noted that gram positive and gram negative were equally prevalent. However, in LOS gram positive organisms were most prevalent, ie 62.5%. In the study by Pokhrel et al both EOS and LOS had similar distribution of bacteria. However Ingale showed klebsiella predominance in EOS and CONS as the predominant bacteria in LOS [15].

Our study showed that the common organism isolated were highly resistant to first line antibiotics like Ampicillin and Amoxycyclavulinic acid. Pokhrel et al also reported similar picture, as also studies from neighboring Nepal /Pakistan [16, 17]. For Ciprofloxacin gram positive organism showed significant resistance, but gram-negative organisms showed high susceptibility. Low susceptibility to Ciprofloxacin has also been reported by other studies [18, 19]. The susceptibility of gram-negative bacilli in our babies to Amikacin was also low, similar to that reported by Pokhrel et al.

In our series, all the gram-negative isolates were highly sensitive to Piperacillin tazobactam and Carbepenams, which is similar to Pokhrel et al. We had one isolate of enterococcus with multi drug resistance. Several studies including Pokhrel et al have reported emergence of highly resistant organisms [6, 7]. Group B streptococcus, is the most common cause of EOS in high income countries, but has low reported incidence in low- and middle-income countries [20]. In our series of new born sepsis also we did not identify GBS.

Maternal fever was the only risk factor statistically associated with culture positivity. All other maternal and new born risk factors did not yield statistical significance. Pokhrel also reported high association of maternal fever with culture positivity. However Shruti in a systematic review [21] showed the following as risk factors for new born sepsis : neonatal risk factors included male gender and out born admissions, and maternal risk factors gestational age <37 weeks and PROM.

Among lab parameters high TLC, CRP positivity and thrombocytopenia were highly statistically significant. Pokhrel et al also noted thrombocytopenia in 75% of the cases and increased CRP in 84%.

### CONCLUSION

Staphylococcus aureus followed by CONS and Pseudomonas was the predominant isolate in our NICU. Resistance to first line antibiotics like Ampicillin , Amoxycillin Clavulanic acid and Amikacin and the presence of MRSA is highly concerning.

Stringent hand hygiene measures in the labour ward, post-natal ward and NICU can reduce the occurrence of these strains.

Judicious and responsible use of initial empiric antibiotics based on the above antibiogram profile may help in antibiotic stewardship.

**Table 1: General characteristics of newborn enrolled in the study**

Variables	Frequency (n)	Percentage (%)
Gender		
Male	131	56.0
Female	103	44.0
Place of delivery		
Inborn	219	93.6
Out born	15	6.4
Mode of delivery		
Instrumental	24	10.3
LSCS	122	52.1
NVD	88	37.6
Birth weight		
< 1.5 Kg	5	2.1
1.5 Kg - < 2.5 Kg	62	26.5
2.5 Kg - < 4 Kg	165	70.5
≥ 4 kg	2	0.9
Gestational age		
Preterm	52	22.2
Term	182	77.8
Risk factors in mother		
PROM >18 hours	45	19.2
Antepartum fever	5	2.1
Uncleaned vaginal examination	1	0.4
Risk factors in newborn		
Low birth weight or preterm	73	31.2
Apgar score at 5 min <6	2	0.9

**Table 2: Bacterial Isolate in EOS & LOS**

Organism	EOS < 72 hours n (%)	LOS > 72 hours n (%)
Gram positive cocci		
Staphylococcus aureus	3 (21.4%)	3(37.5%)
CONS	2 (14.2%)	1(12.5%)
MRSA	1 (7.1%)	1(12.5%)
enterococcus	1 (7.1%)	
MRCONS	1 (7.1%)	
Gram negative bacilli		
Pseudomonas	3 (21.4%)	
NFGNB	3 (21.4%)	1(12.5%)
klebsiella		1(12.5%)
ESBL E-coli		1(12.5%)
Total	14(100%)	8(100%)

**Table 3- antibiotic sensitivity among common bacterial isolates**

Antibiotics Vs Specify the organism	Ampicillin		Amoxycillin clavavulinic acid		Ciprofloxacin		3rd Generation Cephalosporin		Aminoglycosides	
	S / (S+R)	S%	S / (S+R)	S%	S / (S+R)	S%	S / (S+R)	S%	S / (S+R)	S%
Staphylococcus aureus	2 / 6	33.3	3 / 6	50	4 / 6	67	4 / 5	80	NT	
CONS	1 / 3	33.3	2 / 3	66.7	2 / 3	66.7	1 / 3	33.3	NT	
MRSA	0 / 2	0	1 / 2	50	0 / 2	0	0 / 2	0	NT	
Pseudomonas	0/1	0	1 / 3	33.3	2 / 2	100	3/3	100	2/3	66.7
NFGNB except pseudomonas	NT*		2 / 2	100	4 / 4	100	3/4	75	1/2	50
Klebsiella	NT		1 / 1	100	1 / 1	100	1 / 1	100	1/1	100

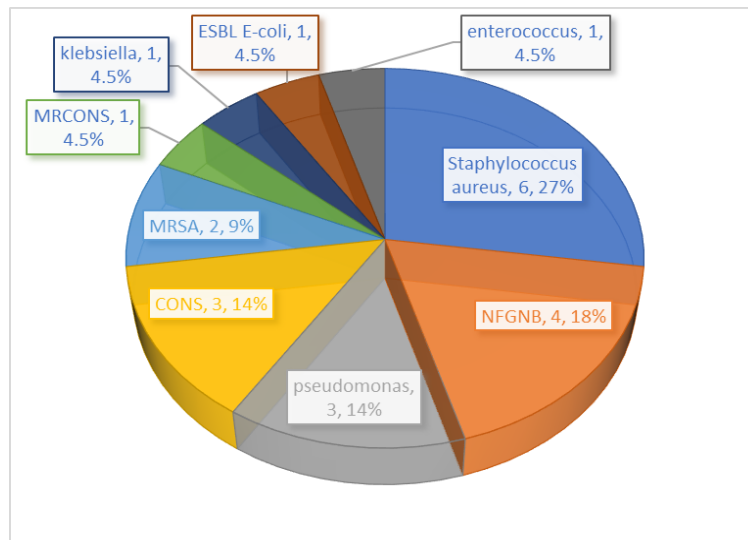
**Table 4: Antibiotic sensitivity among common bacterial isolates**

Antibiotics Vs Specify organism	Piperacillin tazobactam		Meropenem/ Imipenam		Vancomycin		Linezolid		Clindamycin	
	S / (S+R)	S%	S / (S+R)	S%	S / (S+R)	S%	S / (S+R)	S%	S / (S+R)	S%
Staphylococcus aureus	NT		NT		5 / 6	83.3	6 / 6	100	4 / 4	100
CONS	NT		NT		3 / 3	100	3 / 3	100	1/1	100
MRSA	NT		NT		2 / 2	100	2/2	100	1/1	100
Pseudomonas aeruginosa	2 / 2	100	3 / 3	100	NT		NT		NT	
NFGNB except pseudomonas	4/4	100	4/4	100	NT		NT		NT	
Klebsiella	NT		NT		NT		NT		NT	

\*NT – Not Tested

**Table 5: Association between Cultures Reports and risk factors**

Maternal Risk Factors		Cultures Report		P-Value
		Growth present	Growth absent	
PROM > 18 hours	Present	6 (27.3)	39 (18.4)	0.315
	absent	16 (72.7)	173 (81.6)	
Antepartum Fever	Present	2 (9.1)	3 (1.4)	0.018
	absent	20 (90.9)	209 (98.6)	
Uncleaned Vaginal Examination ≥ 3	Present	0 (0.00)	1 (0.5)	0.747
	absent	22 (100.0)	211 (99.5)	
Newborn risk factors				
LBW or Preterm	Present	3 (13.6)	70 (33.0)	0.062
	absent	19 (86.4)	142 (67.0)	
APGAR < 6 at 5min	Present	0 (0.00)	2 (0.9)	0.647
	absent	22(90.9)	210(99.1)	



**Figure 1: Bacterial isolates in culture positive sepsis (frequency, percentage)**

**Table 6: Association between Cultures Reports and lab profile**

Lab parameters		Cultures Report		P-Value
		Growth Present	Growth absent	
TWBC	Elevated	2 (9.1)	12 (5.7)	0.006
	Low	1 (4.5)	0 (0.00)	
	Normal	19 (86.4)	200 (94.3)	
CRP	Positive	6 (27.3)	19 (9.0)	0.029
	Negative	16 (72.7)	193 (91.0)	
Thrombocytopenia	Present	2 (9.1)	3 (1.4)	0.018
	Absent	20 (90.9)	209 (98.6)	

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