



A Bacteriological Study of Neonatal Sepsis and its Antibiotic Pattern among Blood Culture Isolates in Western India

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KEYWORDS

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ABSTRACT:

BACKGROUND: Neonatal sepsis remains a significant cause of morbidity and mortality in neonates, particularly in developing regions like Western India. Prompt identification of causative organisms and appropriate antibiotic therapy are critical, as the clinical manifestations are often nonspecific and may rapidly progress to life-threatening conditions.

MATERIALS AND METHODS: A Retrospective study was conducted at The Department of Microbiology, GGGH, and Jamnagar on 300 neonates that were admitted in the Neonatal Intensive Care Unit (NICU), Department of Paediatrics, GGGH, and Jamnagar for a period of one year. Based on the identification of suspected neonates using inclusion criteria, the data was gathered. Prior to starting antibiotics, blood samples were collected.

RESULTS: A total of 300 neonatal blood samples were studied, out of which 146 (48.6%) showed bacterial growth. The majority of these isolates were Gram-Negative bacteria (87.67%), while Gram-Positive bacteria accounted for 12.32%. *Klebsiella pneumoniae* was the most commonly isolated Gram-Negative organism, seen in 45.2% of cases. Among Gram-Positive isolates, Coagulase Positive Staphylococcus was the most frequent, present in 8.89% of cases. The most common clinical signs among culture-positive neonates included refusal to feed (39.7%) and lethargy (21.9%). Most affected neonates (59.5%) were full-term, with normal birth weight, delivered vaginally. A significant majority (82.1%) of infections occurred within the first 7 days of life, indicating early-onset sepsis. Breastfeeding was the most common feeding method (58.2%), followed by nasogastric tube feeding (32.1%). Only a small proportion of the infected neonates were preterm (1.3%) or had low birth weight (11.6%). Antibiotic susceptibility testing for each isolate was performed according to CLSI guidelines, with separate panels used for Gram-Positive and Gram-Negative organisms.

CONCLUSION:

Early diagnosis and prompt initiation of treatment were found to be crucial for achieving favorable outcomes in neonatal sepsis. The observed high levels of resistance to first- and second-line empirical antibiotics underscore the urgent need to revise current treatment protocols based on the sensitivity patterns identified.

INTRODUCTION:

Neonatal sepsis refers to an infection involving the bloodstream in newborn infants less than 28 days old. It remains a leading cause of morbidity and mortality among neonates, especially in middle and lower-income

countries.¹ Neonatal sepsis can be caused by bacteria such as *Escherichia coli* (E coli), *Klebsiella*, *Listeria*, and some strains of streptococcus. Group B Streptococcus (GBS) has been a major cause of neonatal sepsis.



Neonatal sepsis is divided into two groups based on the time of presentation after birth: Early-Onset Sepsis (EOS) and Late-Onset Sepsis (LOS). EOS refers to sepsis in neonates at or before 72 hours of life (some experts use seven days), and LOS is defined as sepsis occurring at or after 72 hours of life.²

Maternal factors that increase the risk of neonatal sepsis include chorioamnionitis, GBS (Group B Streptococci) colonization, delivery before 37 weeks, and prolonged rupture of membranes greater than 18 hours.³ The following factors increase an infant's risk of early-onset bacterial sepsis such as GBS colonization during pregnancy, Preterm delivery, Rupture of membranes longer than 18 hours before birth and Infection of the placenta tissues and amniotic fluid (chorioamnionitis).

Babies with late-onset neonatal sepsis are infected after delivery. An infant's risk for sepsis after delivery is increased in cases having a catheter in a blood vessel for a long time and staying in the hospital for an extended period of time. There are an estimated 1.3 to 3.9 million annual neonatal sepsis cases and 400 000 to 700 000 annual deaths worldwide, depending on a study. Group B Streptococcus and E. coli infections account for 70% of early-onset neonatal sepsis.

The first 28 days of life (the neonatal period) are the most vulnerable time for child survival. Every year, an estimated 2.5 million neonates die in their first month of life, accounting for nearly one-half of deaths in children under 5 years of age, according to estimates from the United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME).⁴

Mortality from neonatal sepsis may be as high as 50% for infants who are not treated. Infection is a major cause of mortality during the first month of life, contributing to 13%-15% of all neonatal deaths. Low birth weight and gram-negative infection are associated with worse outcomes. Hence studying a complete bacteriological profile of all the commonly occurring bacteria and their respective antibiotic patterns can help in reducing the morbidity and mortality rates to a great extent.⁵

Hence the objective of our study is to

- To evaluate the most common organisms that cause neonatal sepsis both early and late in our hospital.
- To describe the various sensitivity patterns of these organisms
- To describe the changing patterns of resistance in organisms that were isolated in the study
- To help in choosing the local empirical antibiotics to be used for treatment.

MATERIALS AND METHODS:

This Retrospective study was conducted at The Department of Microbiology, GGGH, and Jamnagar on 300 neonates that were admitted in the Neonatal Intensive Care Unit (NICU), Department of Paediatrics, GGGH, and Jamnagar for a period of one year.

Inclusion Criteria

- Patients aged between 0 to 28 days of life
- Includes both males and females
- All suspected cases of sepsis within 28 days of life

Exclusion Criteria

- Patients with age more than 28 days of life
- Immuno-compromised patients
- Patients with Congenital diseases

Data collection procedure:

The data was collected based on Identification of suspected neonates by Inclusion Criteria. The collection of blood samples was done before initiation of antibiotics. The blood samples were collected from peripheral site preferably using a sterile needle and transferred to Glucose broth under aseptic conditions and all the samples were transferred to the Department of Microbiology immediately and processed.

On Day 1, Direct microscopic examination like Gram stain was done for all specimens. Inoculations were done on MacConkey agar, Chocolate agar, Blood agar, Sabouraud Dextrose Agar (SDA) along with Brain Heart Infusion (BHI) and are incubated aerobically for 18 to 24 hours at 37°C.⁶



On Day 2, After incubation, all the bacterial isolates were confirmed by various tests like Colony Characteristics, Gram stain, Motility test, Lactose fermentation, Oxidase test, Triple Sugar Iron test, Citrate utilization. Urease test, Phenyl Pyruvic acid test, Indole test, Oxidative Fermentation test, Catalase test and Coagulase test. Antibiotic Susceptibility testing was done using Modified Kirby Bauer Disc Diffusion method and The Zone of Inhibition was measured as per Clinical Laboratory Institute (CLSI) guidelines,2020.⁷ Blood samples were inoculated in agar plates as mentioned before thrice over a period of 7 days and reported negative in case there is no growth at the end of 7th day.

Antibiotic Discs:

Table- 1: Antibiotic discs used for Gram Positive bacteria

Gentamycin	Amikacin
Cephalexin	Cloxacillin
Ciprofloxacin	Linezolid
Tetracycline	Lincomycin
Ampicillin and Sulbactam	Cefotaxime
Cefoxitin	Cotrimoxazole
Roxithromycin	Levofloxacin
Vancomycin	

Table- 2: Antibiotic discs used for Gram Negative bacteria

Gentamycin	Piperacillin
Amikacin	Piperacillin and Tazobactam
Ciprofloxacin	Imipenem
Tetracycline	Cefotaxime
Ampicillin and Sulbactam	Cotrimoxazole
Chloramphenicol	Ofloxacin
Ceftizoxime	Gatifloxacin

Individual organisms will be tested to specific groups of antibiotics according to Clinical Laboratory Institute (CLSI) guidelines,2020.⁸

Data Analysis:

Data will be entered into Microsoft Excel and Analysed by SPSS Software.

Antibiotic Susceptibility testing:

Antibiotic susceptibility testing was done using Disk Diffusion method. All the isolates were inoculated into appropriate media for antibiotic susceptibility and the results are interpreted according to the Clinical Laboratory Institute (CLSI) guidelines,2020.^{7,9}

Tests were done on Muller Hinton Agar (MHA), which has a Ph between 7.2 to 7.4. The surface of the agar is gently and uniformly inoculated using a sterile cotton stick dipped in a broth containing pure colony of the isolate in each sample that showed growth. The bacterial suspension contained turbidity with visual equivalence to 0.5 McFarland standards. The cotton swab stick is dipped in the suspension, taken out and then squeezed gently onto the wall of the test tube to take off extra suspension before inoculation. The plates were then incubated at 37°C for 24 hours after which measurements of the Zone of Inhibition were taken.

Zones were measured from the surface of the plates with their lids open using a ruler. Plates are kept on a black, non-reflecting surface, illuminated with a reflected light and then read. Results were taken and denoted as Sensitive(S), Intermediate (I)and Resistant (R). Standard control ATCC 25922, ATCC 25923 and ATCC 27853 are used according to CLSI,2020.

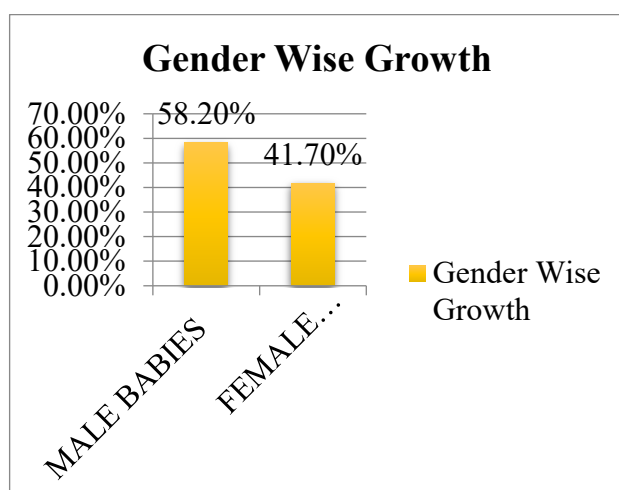
RESULTS:

A total of 300 samples were studied for a period of 12 months. Out of these,146 samples showed bacterial growth giving a positive rate of 48.6% and 154 samples showed no bacterial growth (51.3%) as shown in **Table- 3**.

**Table- 3: Bacterial Growth**

	Number of Samples	Percentage
Growth	146	48.6%
No Growth	154	51.3%
Total	300	100%

The Bacterial growth was seen in 85 Male babies (58.2%) and 61 Female babies (41.7%) out of total 146 positive samples in the study (Figure 1).

**Figure -1: Gender wise Growth**

Among the total 146 positive samples that were isolated, Gram-Positive isolates were 18 (12.32%) and the total Gram-Negative isolates were 128 (87.67%).

Among the total 146 positive cultures, the prominent signs and symptoms observed among the suspects were examined. Refusal to take feeds (39.7%), Lethargy (21.9%), Fever and Abdominal distension (9.5%), Poor cry, Irritability, etc are some of the alarming signs noticed among these newborns (Table- 4).

Table- 4: Clinical Features

S. No	Clinical Features	No of isolates	Percentage
1.	Refusal to Feeds	58	39.7%
2.	Lethargy	32	21.9%
3.	Fever	14	9.5%

4.	Respiratory distress	12	8.2%
5.	Abdominal distension	14	9.5%
6.	Convulsions	7	4.7%
7.	Poor Cry	2	1.3%
8.	Diarrhoea	5	3.4%
9.	Vomiting	1	0.6%
10.	Jaundice	1	0.6%
	Total	146	100%

The total number of Normal delivered babies with normal birth weight among the 146 positive isolates were 87(59.5%). Babies that were delivered by Caesarean section and had normal birth weights were 40(27.3%) and the ones with Low birth weight were 17 (11.6%). Total Preterm babies were 2 (1.3%) (Table- 5).

Table- 5: Birth wise Associations

S.No	Mode of delivery	Number of Isolates	Percentage
1.	NVD/NBW*	87	59.5%
2.	LSCS/NBW*	40	27.3%
3.	LSCS/LBW*	17	11.6%
4.	Preterm	2	1.3%
	Total	146	100%

- NVD/NBW*: Normal Vaginal Delivery / Normal Birth Weight
- LSCS/NBW*: Lower Segment Caesarean Section/Normal Birth Weight
- LSCS/LBW*: Lower Segment Caesarean Section/Low Birth Weight



Age of neonates was studied from 0 hours to 28 days of life and their associations were charted based on the onset of illness. Incidence of Positive culture was noted in each study interval. The first 7 days showed 82.1% of the total growth whereas the last 7 days showed the least 6.1%. Feeding of the neonates were based on various factors like the general health status, Birth weight, system involved (Respiratory, Gastrointestinal). Breast feeding was given to all newborns that took feeds well. Out of 146 positive samples 85 were breastfed, 47 were fed through NG tube and 14 were given Formula feeds.

Out of the total 146 isolates, 128 were Gram Negative and 18 were Gram Positive. Highest isolates were Coagulase Positive Staphylococcus were in 13(8.89%) among Gram Positive isolates and Klebsiella pneumonia were isolated in 66(45.2%) among Gram Negative isolates respectively (**Figure 2**).

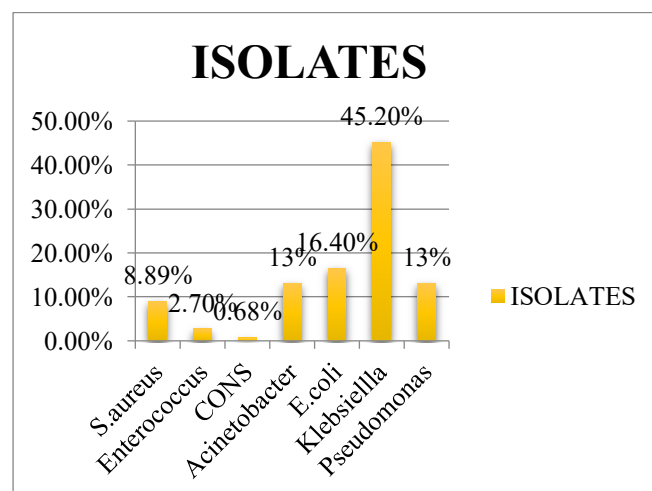


Figure -2: Isolates

Antibiotic susceptibility patterns were studied based on analysing the measurements of Zone of Inhibition of each isolate as per CLSI guidelines. Each isolate was noted for its own antibiotic susceptibility pattern based on its Gram nature. Gram-Positive and Gram-Negative isolates were kept different groups of Antibiotic discs and read accordingly. The overall Susceptibility pattern of the Antibiotics used in my department was analysed (**Table- 6 & 7**).

Table -6: Gram Positive Isolates (n=18)

S.No	Drug	Sensitive	%	Resistant	%
1.	AS	9	50%	9	50%
2.	BA	12	66.6%	6	33.3%
3.	PR	1	5.5%	17	94.4%
4.	TE	7	38.8%	11	61.1%
5.	CF	8	44.4%	10	55.5%
6.	RC	9	50%	9	50%
7.	QB	15	83.3%	3	16.6%
8.	LZ	14	77.7%	4	22.2%
9.	CX	11	61.1%	7	38.8%
10.	AT	14	77.7%	4	22.2%
11.	LM	17	94.4%	1	5.5%
12.	GM	12	66.6%	6	33.3%
13.	Cx	13	72.2%	5	27.7%



Table -7: Gram Negative Isolates (n=128)

S.No	Drug	Sensitive	%	Resistant	%
1.	AS	48	37.5%	80	62.5%
2.	BA	86	67.1%	42	32.8%
3.	CF	91	71%	37	28.9%
4.	PC	122	95.3%	6	4.6%
5.	CH	70	54.6%	58	45.4%
6.	RC	84	65.6%	44	34.3%
7.	CI	104	81.25%	24	18.25%
8.	TE	99	77.3%	29	22.7%
9.	ZN	102	79.6%	26	20.4%
10.	GM	97	75.7%	31	24.3%
11.	AK	90	70.3%	38	29.7%
12.	GF	110	85.9%	18	14.1%
13.	PT	128	100%	0	0%
14.	IPM	128	100%	0	0%

The sensitivity and resistance patterns of both Gram Positive and Gram-Negative isolates are tabulated and analysed for each organism separately.

Table -8: Sensitivity patterns of Gram-Positive Isolates:(n=18)

Drug	CONS (n=1)	Enterococcus (n=4)	S.aureus (n=13)
AS	100%	75%	81.25%
BA	100%	75%	57.5%
PR	R	100%	6.25%
TE	R	100%	18.75%
CF	100%	100%	18.75%
RC	100%	50%	41.25%
QB	100%	100%	70%
LZ	100%	75%	73.75%
CX	100%	50%	65%
AT	100%	100%	75%
LM	100%	100%	93.75%
GM	100%	100%	55%
Cx	100%	100%	50%



Table -9: Sensitivity patterns of Gram Negative isolates (n= 128)

Drug	E.coli (n=24)	Klebsiella (n=66)	Pseudomonas (n=19)	Acinetobacter (n=19)
AS	83.3%	19.6%	R	78.9%
BA	91.6%	78.7%	R	63.15%
CF	79.1%	80.3%	R	100%
PC	100%	95.4%	84.2%	100%
CH	75%	78.7%	R	R
RC	79.1%	60.6%	73.6%	57.89%
CI	100%	100%	26.3%	47.3%
TE	79.1%	92.4%	R	100%
ZN	100%	84.8%	57.89%	57.89%
GM	95.8%	77.2%	36.8%	84.2%
AK	79.1%	71.2%	63.15%	63.15%
GF	87.5%	77.2%	52.6%	94.7%
PT	100%	100%	100%	100%
IPM	100%	100%	100%	100%

DISCUSSION:

In the present study, we have described the Bacteriological profile of all the blood samples of all the suspects of sepsis that were received for culture and sensitivity from the NICU. Bacterial growth was detected and the specific organisms were identified. Antibiotic susceptibility testing was done the next day and the sensitivity pattern was noted. Various factors and associations that were associated with Neonatal sepsis were studied and graphs were plotted accordingly. Out of 300 samples that were received for blood culture in our laboratory, 146 (48.6%) showed bacterial growth and the remaining 154 (51.3%) had no bacterial growth.

The study of Roy et al¹⁰ shows a culture positivity rate of 47.5% and Arora et al¹¹ shows a culture positive rate of 46.8%. S.P. Khatua et al¹² reported 59.8% of samples were positively tagged. The present study showed 48.6% positive cultures which is consistent with these studies and hence holds good. Out of 146 positive

samples during the present study, Male babies were affected more 85 (58.2%), than the Female babies 61 (41.7%) which shows male predominance among the affected patients.

A Study by Aytenew Getabelew et al¹³ reported a Male predominance of 58.2% and Veerendra Mehal et al¹⁴ showed 62.4% where Male gender showed dominance. Piyush Gupta et al¹⁵ and Barbara J Stoll et al¹⁶ showed the same results as the previous studies. They showed a higher Male Gender Predominance of 64.7% and 54% respectively in their studies. The present study also showed Male dominance of 58.2% which is consistent with all the previously mentioned studies.

Associating the Onset of the illness, neonates that were aged less than 7 days showed more positive cultures, than the later age groups. Among the total of 146 babies that were aged between 0 and 7 days, 120 gave positive cultures (82.1%). This signifies that more Positive cultures were seen during the first 7 days of life



than the remaining neonatal period. Thida et al¹⁷ studied 72.4% of culture positive neonatal sepsis during first 7 days of birth of the newborn. In a study by Barbara J Stoll et al¹⁶ showed that 93.6% newborns were affected during the first 7 days of life which is consistent with the present study (82.1%).

In the present study, among the total 146 positive cultures, Gram Negative organisms were the predominating (82.1%) among the isolates as compared to Gram Positive (12.32%). When comparing with the study done by Appiah Korang Labiet al¹⁸ which revealed Gram Negative organisms were more when compared to Gram positive organisms (81.1%). The same was found in studies which were conducted by Macharashvili et al¹⁹ 78% of Gram Negative among the total isolates. Kamath et al²⁰ showed 71% and Xinlu et al²¹ showed 92.45% Gram Negative isolates. Our Present study has also showed the same result of Gram-Negative organisms' predominance and is concordant with the other studies.

In the present study, among the total 146 positive samples, the various signs and symptoms of the infected newborns were studied. The most common symptom was Refusal to Feeds (39.7%) followed by Lethargy (21.9%) Fever and Abdominal distension (9.5%). A study conducted by Kayange et al²² concluded that 39% of the culture positive newborns showed Refusal to take feeds by any form and 20.9% babies showed lethargy. The present study showed 39.7% of babies that refused feeds and 21.9% babies that were lethargic and holds good with all the above-mentioned studies.

In the present study, the most susceptible antibiotic for Gram positive isolates was Lincomycin (94.4%) followed by Levofloxacin (83.3%) and Linezolid and Roxithromycin (77.7%). Most resistant antibiotics were Cephalexin (94.4%), followed by Tetracycline (61.1%) and Cefotaxime (55.5%). According to the study conducted by Tessema et al²³ the sensitivity with Linezolid was higher when compared to that of Levofloxacin, but in our study, Levofloxacin has shown more efficacy than that of Linezolid. The sensitivity of Linezolid, which is commonly used in Neonatal Intensive units, which came out to be 87.8% by a study conducted by Tessema et al²³ and it is consistent

with the present study which showed 77.7% sensitivity. Comparing sensitivity of Levofloxacin, Tessema et al²³ showed 80% whereas the present study showed 83.3% and holds good. The resistance against Cefotaxime among Gram Positive isolates was 58.9% in a study conducted by Shaikh et al²⁴ Nikita et al²⁵ showed 63% resistance to Cefotaxime. The present study is consistent with these patterns showing a resistance of 55.5%.

In the present study, the most susceptible antibiotic for Gram negative isolates was Piperacillin + Tazobactam and Imipenem (Carbapenems) (100%) followed by Piperacillin monotherapy and Gatifloxacin (85.9%). Most resistant were Ampicillin +Sulbactam (62.5%) followed by Chloramphenicol (45.4%) and Ciprofloxacin (34.3%). A Study by Mohammed. Atif et al²⁶ showed 91% sensitivity to Piperacillin+ Tazobactam. Mutlu et al²⁷ showed 97.4% sensitive to Imipenem. Both of these studies are consistent with the present study. Shaikh et al (64) showed 52.5% resistance to Ampicillin and Sulbactam and 38.89% to Ciprofloxacin. Najeeb et al²⁸ showed 26% resistance to Ciprofloxacin whereas Thida et al¹⁷ showed 42.9% resistance to Ampicillin and Sulbactam. Vergnano et al²⁹ showed 50% resistance to Chloramphenicol. All these studies are consistent with the present study.

CONCLUSION:

In the present study, Male predominance is observed probably due to absence of one extra X Chromosome that carries the Immunoglobulin coding genes. Early Diagnosis and Treatment Initiation are very important in obtaining Positive Treatment Outcomes. The high levels of resistance to First and Second Line Empirical Antibiotic Regimens highlight the need for modifying the treatment regimens considering the most effective antibiotics observed in this study. Periodic Surveillance at hospital settings to monitor changes in pathogens causing neonatal sepsis, and their antibiotic resistance patterns in order to implement optimal prevention and treatment strategies is recommended. Moreover, antibiotic rotation through systematically rotating antibiotics or antibiotic classes for empirical treatment might also be helpful to reduce antibiotic resistance. Further studies should be done to compare



antibiotic resistance in neonatal sepsis patients to devise targeted and effective interventions.

CONFLICT OF INTEREST:

The authors declare that they have no conflicts of interest.

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