Gram-negative Late Onset Neonatal Sepsis in a Tertiary Care Center From Central India: A Retrospective Analysis

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ABSTRACT

BACKGROUND: Neonatal sepsis has been a major cause of neonatal mortality and morbidity globally. Late onset sepsis is on the rise mostly due to better health care services and improved survival of premature neonates. Gram-negative sepsis has emerged as a major public health problem constituting significant morbidity and mortality. There is limited data on gram-negative late onset sepsis from the central part of India, therefore this study was conducted at a tertiary care center from rural part of India.

OBJECTIVES: To determine the clinical profile and outcome among neonates with gram-negative late onset sepsis.

DESIGN: It is a retrospective analysis conducted among neonates with gram-negative late onset sepsis at a tertiary care center from central India.

METHODS: All neonates below 28 days of age suspected to have late onset sepsis were enrolled in the study. The data for the period of January 2019 to December 2021 was collected and analyzed using software SPSS version 29. The outcome variables studied were discharge (good outcome) and death (poor outcome).

RESULTS: In the present study, overall prevalence of gram-negative late onset sepsis was 4.8%. Respiratory distress (52.2%), seizure (18.9%), jaundice (15.6%), and lethargy (15.6%) were common clinical symptoms among neonates with sepsis. The most common organism isolated was *Klebsiella* spp. (36.7%) followed by *Acinetobacter* spp. (31.1%) and *E. coli* (17.8%). Low gestational age (n = 20 vs n = 7, *P* = .002) and low birth weight (n = 33 vs n = 4, *P* = .02) were associated with poor outcomes in neonates with gram negative LOS. The overall mortality rate was found to be 30% among neonates with gram negative sepsis.

CONCLUSION: The prevalence of gram-negative sepsis was found to be 4.8%. Factors associated with poor outcome in gram-negative sepsis were low birth weight, and prematurity. *Klebsiella* spp. was found to be a common cause of gram-negative LOS, therefore, the empiric antibiotic policy must provide coverage against these micro-organisms.

KEYWORDS: Gram-negative micro-organisms, late onset sepsis, low birth weight, neonatal sepsis, neonatal mortality

Author Summary

Why was this study done?

- Neonatal sepsis is a significant cause of neonatal mortality with marked disparities among various regions and neonatal intensive care units across the country.
- Gram-negative bacteria causing late onset neonatal sepsis is increasing at an alarming rate, particularly in low and middle income countries including India.
- Growing antimicrobial resistance among these gram-negative organisms leading to high case fatality rate is alarming.
- The exact data for incidence of gram-negative LOS and the bacteriological profile from neonatal intensive care units from various parts of India is a major hurdle for designing and implementing center specific empirical antibiotic policies, posing a risk of multidrug resistance.
- We analyzed the data on gram-negative LOS among neonates admitted in NICU of a tertiary care center from Central India to determine the clinical, bacteriological profile and outcome of gram-negative LOS.

What did the study find?

- We studied the data of neonates admitted with late onset sepsis and had gram-negative bacteria in their blood culture from previous years and summarized the clinical symptoms and the proportions of late onset sepsis caused by various gram-negative bacteria.
- Gram-negative LOS accounted for 4.8% of neonatal sepsis in our neonatal intensive care unit. Respiratory distress was the most common clinical symptom among neonates with gram-negative late onset sepsis.
- The common organisms isolated were *Klebsiella*, *Acinetobacter*, *E. coli*, and *Pseudomonas*. Low birth weight and lower gestational age were associated with higher risk of gram-negative LOS with poor outcome.

- The mortality rate of gram-negative sepsis in the present study was found to be 30%.

What do these findings mean?

- Gram-negative late onset sepsis is more prevalent than was previously thought. This is expected to increase in the future with the advancement in medical facilities and improved survival of premature neonates. Lack of center or region specific empirical antibiotic recommendations are likely to result in increased morbidity and mortality among neonates with gram-negative LOS.
- It is necessary to have data for the incidence of neonatal sepsis and the knowledge of common causative organisms for late onset sepsis for strengthening preventive strategies for better outcomes.

Introduction

Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection during the first month of life. Neonatal Sepsis has been a challenge for the health care system across the world as it contributes significantly to the neonatal morbidity and mortality. Late onset sepsis (LOS) presents after 72 hours of life and is caused by nosocomial or community acquired contamination after delivery.[1] With the improvement in survival of premature and low birth weight neonates, incidence of late onset sepsis has been on the rise lately. Improper hand washing and medical equipment are significant sources of infection in neonatal intensive care units.[2] LOS have been reported to affect 30% to 40% very low birth weight babies, and approximately 15% of late preterm babies.[3]

In recent times, LOS caused by gram-negative bacilli has been a major cause of concern not only because of the more severe infection and the higher mortality but also the increasing incidence. The gram-negative LOS has shown to prolong the hospital stay and has been associated with poor neurodevelopmental outcome.[4] Emergence of antimicrobial resistance poses a greater challenge in the management of neonates with gram-negative sepsis. Various studies have focused on very low birth weight and preterm neonates ignoring the term and late preterm neonates.

The epidemiological data for neonatal sepsis shows varied bacterial profiles in different regions from time to time. The data for the exact incidence of gram-negative LOS and microbiological profile is limited especially from the central part of India.[5-7] Region and center specific data on neonatal sepsis is needed for prevention and better management of gram-negative late onset sepsis. The knowledge of microbiological profile will help in designing appropriate empirical antimicrobial policies, therefore, ultimately reducing the risk of antimicrobial resistance. There is limited literature specifically on gram-negative LOS from India, hence, the present study was conducted to determine the prevalence, clinical, and microbiological profile of gram-negative late onset sepsis at a neonatal intensive care unit of a tertiary care center from central part of India.

Materials and Methods

Study design, period, and setting

This retrospective study was conducted at a level III neonatal intensive care unit of a tertiary care center from central India. In this study, data was collected for a period of 3 years from 01 January 2019 to 31 December 2021.

Study population

All neonates aged 3 to 28 days with clinical features of late onset sepsis at the time of admission or developed during their hospital stay were enrolled in the study. All neonates with blood culture showing growth of gram-negative organisms were included in the study. Whereas those with sterile blood cultures or cultures with growth of gram-positive or polymicrobial organisms were excluded from the study. All neonates with congenital anomalies or chromosomal anomalies were also excluded from the study. Total 90 neonates with late onset sepsis showing growth of gram-negative micro-organisms on blood culture were included in the study.

Data collection

Data was collected retrospectively from the Hospital Information System (HIS) for the study duration and entered in the data collection form. The demographic details, clinical history, laboratory investigations including blood culture, and outcome variables were collected in the data collection form. The good outcome means discharge of neonate while poor outcome means death of neonate.

Operational definitions

Late onset neonatal sepsis. It is defined as infection occurring after 72 hours of life.[8]

Gram negative sepsis. Neonates with a positive blood culture showing growth of gram-negative bacilli. In the study, gram-negative bacilli refer to the following species: *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas* spp., *Serratia* spp., *Citrobacter koseri, Proteus,* and *Stenotrophomonas* spp.[9]

Data analysis

Data collected in the data collection forms were entered in MS excel sheet and exported to SPSS version 29 for analysis. The categorical and nominal data were expressed as frequency and percentage. Chi-square test was used for analysis and *P*-value <.05 was considered significant.

Results

Total admissions in the neonatal intensive care unit during the study period were 1875. All these neonates were evaluated for sepsis with blood culture, and out of these, 90 neonates had gram-negative late onset sepsis. Out of 90 neonates, 56 (62.2%) were males and 34 (37.8%) were females. There were an equal number of term and preterm neonates in the study. There were 55 (61.1%) small for gestational age neonates, and 35 (38.9%) appropriate for gestational age.

Among 90 neonates, 39 (43.4%) had low birth weight, 30 (33.3%) had very low birth weight, and 6 (6.7%) had extremely low birth weight. In the current study, 48 (53.3%) were born vaginally and 42 (46.7%) were born by cesarean section. There were 60 (66.7%) inborn neonates and 30 (33.3%) outborn neonates. The vast majority (58.9%) of mothers were primiparous. The basic characteristics of study neonates are shown in Table 1.

In this study, the prevalence of gram-negative late onset neonatal sepsis among neonates admitted to the neonatal intensive care unit of a tertiary care center was found to be 4.8%. Regarding the clinical characteristics of neonates with late onset sepsis, 47 (52.2%) had respiratory distress, 17 (18.9%) had seizures, 14 (15.6%) had jaundice and lethargy each, 12 (13.3%) had apnea, and 11 (12.2%) had abdominal distension (Table 2).

Among 90 neonates with gram-negative LOS, the predominant organisms identified were *Klebsiella* (36.7%) and *Acinetobacter* (31.1%). The details of organisms isolated are shown in Table 3. In the present study, out of 90 neonates, 63 (70%) were discharged successfully, and 27 (30%) died (Figure 1).

In this study, the low gestational age was associated with higher mortality rate (*P* = .002). Similarly, high mortality was associated with low birth weight (*P* = .02). Furthermore, neonates showing growth of *Citrobacter koseri* (1/1) and *Klebsiella* spp. (12/21) were found to have a higher risk of mortality (Table 4).

Discussion

The overall prevalence of gram-negative late onset sepsis among neonates admitted in NICU was found to be 4.8% in the present study. This finding is in line with a previous study from Taiwan conducted by Tsai et al[10] (5.8%). A study done by Chaudhari et al[7] reported an incidence of 2.8% for gram-negative late onset sepsis. The difference could be attributed to the duration of the study and the selection criteria.

Gram-negative LOS was more among neonates with low gestational age. Previous studies by Chaudhari et al[7] from India and Jatsho et al[11] from Bhutan found similar results. The current study also found that the neonates with lower birth weight, especially low birth weight and very low birth weight were at higher risk of developing gram-negative LOS. This finding is in accordance with the previously conducted studies.[9-12] This could be because premature and low birth weight neonates require NICU

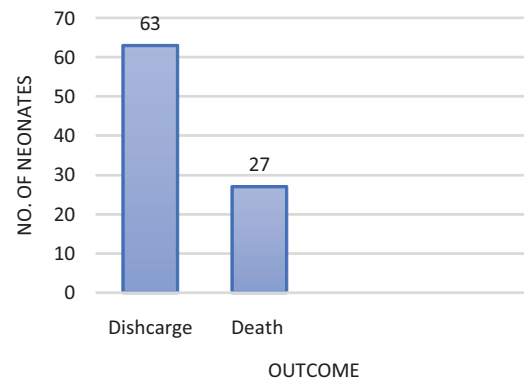**Table 1.** Characteristics of neonates with gram-negative late onset sepsis (n = 90).

CHARACTERISTICS	CATEGORIES	FREQUENCY (%)
Gender	Male	56 (62.2)
	Female	34 (37.8)
Gestational age	Term	45 (50)
	Preterm	45 (50)
Classification based on GA	Small for GA	55 (61.1)
	Appropriate for GA	35 (38.9)
Birth weight	Extremely Low birth weight	6 (6.6)
	Very Low birth weight	30 (33.4)
	Low birth weight	39 (43.4)
	Normal weight	15 (16.6)
Type of admission	Inborn	60 (66.7)
	Outborn	30 (33.3)
Mode of delivery	Vaginal	48 (53.3)
	Cesarean section	42 (46.7)
Parity	Primiparous	53 (58.9)
	Multiparous	35 (38.9)
	Grand-multiparous	2 (2.2)
History of abortion	Yes	15 (16.7)
	No	75 (83.3)

Table 2. Clinical characteristics of neonates with gram-negative LOS.

CLINICAL CHARACTERISTICS	FREQUENCY (N)	PROPORTION (%)
Respiratory distress	47	52.2
Seizures	17	18.9
Jaundice	14	15.6
Lethargy	14	15.6
Apnea	12	13.3
Abdominal distension	11	12.2
Refusal to feed	10	11.1
Vomiting	9	10
Pallor	9	5.6
Diarrhea	3	3.3
Sclerema	3	3.3

Table 3. Details of organisms isolated from neonates with gram-negative LOS.

ORGANISM ISOLATED	FREQUENCY	PERCENTAGE
Klebsiella spp.	33	36.7
Acinetobacter spp.	28	31.1
E. coli	16	17.8
Pseudomonas spp.	8	8.9
Enterobacter spp.	3	3.3
Sphingomonas spp.	1	1.1
Citrobacter koseri	1	1.1

Figure 1. Outcomes among neonates with gram-negative LOS.

Table 4. Analysis of various characteristics associated with the outcome in gram-negative LOS.

CHARACTERISTICS		OUTCOMES		*P*-VALUE
		DISCHARGE	DEATH	
Gender	Male	39	17	.92
	Female	24	10	
Gestational age	Preterm	25	20	.002
	Full term	38	7	
Birth weight	ELBW	2	5	.02
	VLBW	18	11	
	LBW	32	7	
	Normal	11	4	
Type of admission	Inborn	42	18	>.05
	Outborn	21	9	
Mode of delivery	Vaginal	31	17	.23
	Cesarean section	32	10	
Parity	Primiparous	38	15	.78
	Multiparous	24	11	
	Grand-multiparous	1	1	
Abortion	Yes	11	4	.75
	No	52	23	
Organism isolated	*Klebsiella* spp.	21	12	
	Acinetobacter spp.	20	8	
	E. coli	11	5	
	Pseudomonas spp.	7	1	
	Enterobacter spp.	3	0	
	Sphingomonas spp.	1	0	
	Citrobacter koseri	0	1	

care and invasive procedures like surfactant administration, vascular access, parenteral nutrition, and ventilation.

In contrast to the previous studies, this study reported that respiratory distress, seizures, jaundice, and lethargy were common clinical symptoms among neonates with gram-negative sepsis. This could be due to the fact that the study by Tsai et al[10] had most of the extremely low birth weight and very low birth weight neonates as the study population. Other previous studies included neonates with late onset sepsis caused by gram-positive and gram-negative organisms.[13-15]

Most common isolated organism in the study was the *Klebsiella* spp. This finding is in accordance with those of the previously published studies which reported *Klebsiella* spp. as the most common isolated organism from neonates with gram-negative LOS. Contrary to the mortality rate of 15% reported in previous studies, the mortality rate in the present study was observed to be 30%.[10,12] This could be because these studies were from upper-middle and high income countries. Data on mortality rate in neonates with gram-negative late onset sepsis from lower-and middle-income countries like India is unknown due to lack of dedicated studies on gram-negative LOS.[15-17] Data from various Indian studies reported an overall mortality rate of 30% to 40% for late onset sepsis including gram-positive and gram-negative organisms.

Limitations and Strengths

The study's strength was that it was conducted in a level III neonatal intensive care unit of a tertiary care center from central India. This study included only a gram-negative late onset sepsis cohort, which other studies lack. The study is limited by its retrospective nature. Since the study was based on hospital record review, some factors may have been ignored. The study did not consider the antibiotic sensitivity pattern among isolated organisms.

Conclusion and Recommendation

In this study, the prevalence of gram-negative sepsis was 4.8%. Factors associated with poor outcome in gram-negative sepsis were lower birth weight, and prematurity. *Klebsiella* sepsis was a common cause of gram-negative LOS, therefore, the empiric antibiotic policy must provide coverage against these organisms. This study also warrants the need for emphasizing importance of preventive strategies such as hand hygiene practices and targeted empiric antibiotic policies to reduce the morbidity and mortality due to gram-negative late onset sepsis.

Declarations

Ethics approval and consent to participate

The study was carried out after obtaining ethical clearance from the Institutional Ethics Committee (IEC MGIMS, Sevagram) vide letter no. PED/133/2022. Only clinical records were collected and confidentiality of information was ensured. Furthermore, no personally identifiable information about the study participants was obtained. The waiver of consent was obtained as per the ICMR-guidelines as it is a retrospective study.

Author contributions

Akangksha Pataskar: Formal analysis; Methodology; Resources; Writing – original draft. **Anuragsingh Chandel:** Conceptualization; Formal analysis; Methodology; Writing – original draft; Writing – review & editing. **Varsha Chauhan:** Methodology; Supervision; Writing – review & editing. **Manish Jain:** Conceptualization; Writing – review & editing.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

The data used for analysis are available from the corresponding author on reasonable request.

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