

Original Research Article

Incidence, bacteriological profile and risk factor analysis of neonatal sepsis in a peri urban set up of North India

Shalini Upadhyay*, Anita Chakravarti, Tanisha Bharara, Priti Agarwal

Department of Microbiology, SGT University, Gurugram, Haryana, India

Received: 29 March 2020

Accepted: 22 April 2020

*Correspondence:

Dr. Shalini Upadhyay,

E-mail: shalini.upd@gmail.com

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ABSTRACT

Background: The high incidence and antimicrobial resistance among the pathogens causing neonatal sepsis is alarming. In addition to substantial immediate mortality, survivors of infections in the neonatal period are at increased risk of long-term disability. The present study was conducted to know the bacteriological profile and risk factors associated with culture proven neonatal sepsis in a peri urban population.

Methods: This study was conducted over a period of 4 year (2015-2019). On clinical suspicion, blood culture specimens were sent to microbiology laboratory. The organisms isolated from blood cultures were identified and tested for antimicrobial susceptibility. As part of infection control practices, environmental samples from the neonatal intensive care units were tested.

Results: Of 907 blood cultures of neonates received in the microbiology laboratory, 20.7% were culture positive. Majority of the episodes occurred at or before 72 hours of life (81.4%). 54.3% were Gram positive cocci including Coagulase negative *Staphylococci*, *Staphylococcus aureus* and *Enterococcus spp.* The common Gram-negative pathogens included *Escherichia coli*, *Klebsiella spp.* and *Pseudomonas spp.* Common risk factors involved were preterm birth, low birth weight, premature rupture of membrane, prolonged labour and iatrogenic causes.

Conclusions: The early signs of sepsis are often subtle and nonspecific. Therefore, a high index of suspicion is needed for early diagnosis. Rapid, reliable detection and appropriate case management can save lives of many newborns.

Keywords: Antimicrobial resistance, Bacteraemia, Low birth weight, Neonatal septicaemia, Preterm

INTRODUCTION

The neonatal period is a vulnerable phase in the lives of both mothers and their new-born babies and still it is the most neglected period regarding the quality of care.¹ According to United Nations International Children's Emergency Fund data, children are at highest risk of dying in the first month of life at an average global rate of 18 deaths per 1,000 live births in 2018.² Most of the neonatal deaths are concentrated in the first day and week of life.¹

Globally, the common causes of neonatal deaths are preterm birth complications, intrapartum complications, neonatal sepsis and congenital malformation.^{3,4} Being the second most populated country India, claims a large proportion of neonatal sepsis burden.⁴ In India, Gram-negative bacteria have been reported to be the most common cause of neonatal sepsis till recently, *Klebsiella pneumoniae* (*K. pneumoniae*) being predominant. However, the proportion of cases due to Gram-positive bacteria, especially *Staphylococcus aureus* (*S. aureus*) and coagulase negative *Staphylococci* (CoNS), has

increased gradually over the last two decades.^{5,6} There is paucity of published data about neonatal sepsis from low- and middle-income countries.^{7,8} Such information is helpful in guiding the priorities of health-programme and making policies to end preventable child deaths. In this study we report the incidence, risk factors, profile of pathogens causing neonatal sepsis and their antimicrobial resistance in a peri urban population. We also did a comparative analysis of published literature from various states of India to estimate the overall incidence, pathogen profile and common risk factors involved in neonatal sepsis.

METHODS

This is a retrospective observational study conducted between October 2015 to October 2019 (4 years) by retrieving data from the case files of neonates admitted during the study period with the diagnosis of culture proven sepsis. Data abstracted from case sheets included demographics, perinatal risk factors for sepsis and laboratory data. All patient details were anonymized, coded by randomization and delinked from any identity of the patients.

There is a lot of heterogeneity among studies regarding the definition of neonatal sepsis. The presence of a positive blood culture constitutes the gold standard for the diagnosis of neonatal sepsis. This conclusion is based upon 2 assumptions; one that the infant would not have been evaluated in the absence of clinical suspicion of sepsis and second that the isolated bacteria did not represent contamination.⁹

Inclusion and exclusion criteria

All blood specimens received in the microbiology laboratory with clinical suspicion of neonatal sepsis were included in the study. Blood samples from patients who could not be followed up were excluded from the study.

Sample processing

On clinical suspicion, two millilitres of blood collected with aseptic precautions was sent to the microbiology laboratory. The specimen received were inoculated into bottles and incubated in BacT/ALERT 3 D system (bioMérieux). When the machine flashed positive for the bottle, Gram's stain was prepared. Subculture from the bottle was done on blood agar and macConkey agar. Antimicrobial susceptibility testing (AST) was performed by disc diffusion method on MHA (Mueller Hinton Agar). The antimicrobial susceptibility of the isolates was determined as per Clinical and Laboratory Standards Institute guidelines (CLSI).¹⁰ In case of non-identification by conventional methods, the isolates were processed in the automated system, Vitek 2 (bioMérieux). As part of our infection control protocol, regular environmental sampling from the NICU (Neonatal Intensive Care Unit) was done. In case of suspected outbreak, samples from

surroundings of the index case were collected and processed as above.

Quality control

S. aureus ATCC 25923, *Escherichia coli* (E. coli) ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603 were the control strains.

Definitions^{11,12}

Culture-positive sepsis - Isolation of a recognised pathogen from blood in neonates suspected to have sepsis on the basis of clinical features or maternal or perinatal risk factors. Cases of sepsis with positive culture for CoNS were labelled so only if the clinical course was suggestive of sepsis and appropriate antibiotic therapy was given. The date of sepsis onset was taken as the date on which first blood sample yielding a pathogenic strain was collected.

- Early-onset sepsis (EONS)- Occurrence of sepsis at or before 72 h of life.
- Late-onset sepsis (LONS) - Occurrence of sepsis after 72 h of life.

Multidrug resistance (MDR) was defined as resistance to any three of the antibiotic groups.

Statistical analysis

Data were analysed using SPSS software Version 20.0. A p-value <0.05 was considered statistically significant. Means and standard deviations (SD) were calculated as required for numerical variables.

RESULTS

During the study period there were 3734 livebirths, out of which 907 were clinically suspected of neonatal sepsis and their blood culture specimens were received in the microbiology laboratory. Of the 907 neonates who were worked up for sepsis, 47.2% (428) were females and 52.8% (479) were males. The overall incidence of neonatal sepsis was 5 per thousand live births (188/3734). The culture positive cases among suspected cases were 20.7% (188/907). There has been significant decrease in prevalence of neonatal sepsis from 24% (23/96) in the year 2015-2016 to 18.3% (73/398) in 2018-2019 (p value<0.05) (Figure 1).

Majority of the episodes, 81.4% (153/188) occurred at or before 72 hours of life i.e. EONS. Of the 188 bacterial isolates, 54.3% (102/188) were Gram positive, 41.5% (78/188) Gram negative and 4.3% (8/188) *Candida* spp. Among Gram positive microorganisms, CoNS and *S. aureus* were common. Common species of CoNS isolated were *S. epidermidis* followed by *S. hemolyticus*. *Klebsiella* spp, *E. coli* and *Pseudomonas* spp were common Gram-negative microorganisms isolated from

blood cultures (Table 1). The pathogen profile in EONS did not vary much from that of LONS.

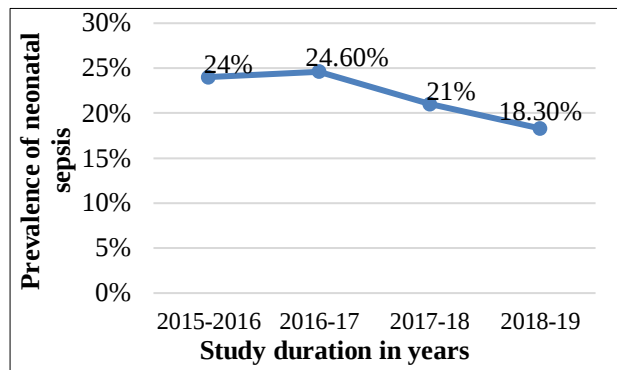


Figure 1: Year wise prevalence of culture proven neonatal sepsis during the study period.

Table 1: The microbiological profile of neonatal sepsis.

Microorganism isolated		Number
Gram positive	CoNS	54
	<i>S. aureus</i>	38
	<i>Enterococcus</i> spp.	10
Gram negative	<i>Klebsiella</i> spp.	32
	<i>E. coli</i>	12
	<i>Pseudomonas</i> spp.	13
	<i>B. cepacia</i>	11
	<i>Acinetobacter</i> spp.	5
	Others*	5
	Fungi	<i>Candida</i> spp.
Total		188

*Others include *Enterobacter* spp., *Citrobacter* spp. and *Sphingomonas paucimobilis*

Table 2: The antimicrobial susceptibility pattern of common pathogens causing neonatal sepsis.

Microorganisms	Antibiotics (Percentage sensitivity)								
	CX	E	CD	COT	VA	TE	LZ	MI	TGC
Gram positive									
CoNS	24	13	43	74	100	78	100	100	100
<i>S. aureus</i>	33	17	37	76	100	84	100	100	100
<i>Enterococcus</i> spp.		20	30	70	90	40	100	100	100
Gram negative	AMP	GEN	AK	LE	CTR	CPM	COT	ETP	COL
<i>Klebsiella</i> spp.	22	84	84	72	72	78	72	81	100
<i>E. coli</i>	16	83	83	75	75	83	75	83	100
<i>Pseudomonas</i> spp.	8	69	85	77	69	77	69	85	100

Note: CoNS-coagulase negative Staphylococci, CX-cefoxitin, E-erythromycin, CD-clindamycin, VA-vancomycin, TE-tetracycline, LZ-linezolid, MI-minocycline, TGC-tigecycline, AMP-ampicillin, GEN-gentamicin, AK-amikacin, LE-levofloxacin, CTR-ceftriaxone, CPM-cefepime, COT-cotrimoxazole, ETP-ertapenem, COL-colistin.

Among the Gram-positive cocci, methicillin resistance was detected in of 76% (41/54) CoNS and 66% (25/38) of *S. aureus*. All isolates of CoNS and *S. aureus* were susceptible to vancomycin and linezolid while 10% (1/10) of the *Enterococcus* spp were resistant to vancomycin. *K. pneumoniae* showed carbapenem resistance in 19% (6/32) and *E. coli* in 17% (2/12) of cases. Multidrug resistance was observed in 15.4% (2/13) of the *Pseudomonas* spp. isolates. Four out of five *Acinetobacter* spp. were multidrug resistant (Table 2).

During the study period in two incidences 4 clustered cases of *Burkholderia cepacia* (*B. cepacia*) were isolated from blood culture. The remaining three cases of *B. cepacia* were sporadic. The *B. cepacia* isolated were sensitive to meropenem and tigecycline while resistant to ceftriaxone and piperacillin-tazobactam. The sensitivity to gentamicin, amikacin and fluoroquinolones was 36.4%, cotrimoxazole 45.5% and cefepime 18.2%. The environmental sampling was done to know the source of the clustered cases.

Table 3: Risk factors associated with neonatal sepsis.

Factors associated with neonatal sepsis	Percent of culture positive (n=188)
Low birth weight (birth weight <2500 grams)	46
Preterm (<37 weeks of gestation)	39
Continuous positive airway pressure (CPAP)	16
Intravenous fluids	38
Neonatal hyperbilirubinemia (NNH)	25
Meconium stained liquor (MSL)	23
Premature rupture of membrane (PROM)	30
Maternal fever within 7 days before delivery	17
Prolonged labour (≥24hrs)	11

The same microorganism was isolated from the ventilator circuit in one of the incidences while no correlation to environmental samples could be traced in another instance. Low birth weight (46%) and preterm birth (39%) were the most common risk factors leading to neonatal sepsis. Invasive access for nutrition or drug delivery too had a role to play in increased risk of infection. Perinatal and neonatal factors associated with neonatal sepsis are enumerated in Table 3. In a number of cases, more than one factor was implicated.

DISCUSSION

The incidence of EONS is 0.9-1.5 per 1000 live births while for LONS it is 3-3.7 per 1000 live births in developed countries. In the developed countries common causes of EONS are Group B *Streptococci* (GBS) followed by *E. coli*, *S. aureus*, CoNS, *Listeria monocytogenes*. For LONS main pathogen is CoNS,

followed by *E. coli*, *Klebsiella* spp. and *Candida* spp.¹³⁻¹⁵ In developing countries, the common pathogens reported are *K. pneumoniae*, *E. coli* and *S. aureus*, whereas GBS is responsible for only 2-8% of cases.¹⁶ GBS infection may be underreported since it usually presents very early in life and lead to death of neonate before coming to medical attention.¹⁷ The decrease in the frequency of GBS may also be related to prenatal screening and treatment with intrapartum antibiotics.

In recent studies done around different states of India, variable findings have been reported.¹⁸⁻²⁰ This variability can be attributed to quality of healthcare systems and also prevalent socio-cultural practices which may influence infection rates.²⁰⁻²² Most of the studies show higher rates of EONS while a few have higher rates of LONS.^{4,9,22-25} The higher rate of EONS over LONS can be either due to very early transmission from delivery rooms and NICUs or vertical transmission from the mother.

Table 4: Review of neonatal sepsis studies from different parts of India.^{4,9,18-29}

Citation	Study period	Design	Study location	Incidence of neonatal sepsis*	EONS	LONS	Mortality*
Panigrahi et al ⁴	2002-2005	Prospective	Odisha	10%	6%	94%	7.20%
Zakariya et al ¹⁸	2004-2006	Prospective	Puducherry	41.60%	57.5	42.5	NA
Jajoo et al ¹⁹	2011-2015	Prospective	Delhi	13.10%	NA	NA	23.40%
DeNIS Collaboration ⁹	2011-2014	Prospective	Delhi	6.20%	83%	17%	24%
Thakur et al ²⁰	2012-2013	Cross sectional	Himachal Pradesh	42%	51%	49%	12%
Bandyopadhyay et al ²¹	2012-2014	Retrospective	Delhi	NA	59%	41%	29.50%
Bangi et al ²²	2013-2014	Prospective	Andhra Pradesh	6.03%	2.57	3.44	48%
Kumar et al ²³	2013-2015	Prospective	Tamil Nadu	26.20%	94.40%	5.60%	NA
Verma et al ²⁴	2014	Prospective	Rajasthan	7.60%	69%	31%	23.40%
Jajoo et al ²⁸	2015	Prospective	Delhi	17%	47%	NA	NA
Pathak et al ²⁹	2015-2016	Retrospective	Uttar Pradesh	37.40%	NA	NA	NA
Agrawal et al ³¹	2015-2016	Cross sectional	Madhya Pradesh	5.06%	NA	NA	NA
Sethi et al ³⁰	2016-2017	Prospective	Telangana	39%	54%	46%	NA
Rath et al ²⁵	2017-2018	Retrospective	Odisha	NA	52%	48%	17%

Note: *In culture proven cases, EONS-early onset neonatal sepsis, LONS-late onset neonatal sepsis, NA-Not applicable.

The maternal genital tract may be colonised with the pathogens responsible for sepsis due to unhygienic personal and obstetric practices.^{9,26,27} Very high rates of neonatal sepsis were seen in some of the studies while over the years most of the studies have shown a

decreasing rate for neonatal sepsis in India (Table 4).^{9,22,24,28-31} This trend is the result of improved intensive care facilities, better diagnostics and prompt treatment strategies to prevent mortality of the newborns. The changing trend of organisms from Gram negative towards

Gram positive has been attributed to longer stay in hospital, especially intensive care units and invasive procedures to save sick newborns.¹⁷ Sophisticated tertiary care neonatal units being implemented at various centres including our centre, may be the reason for increased prevalence of CoNS. Furthermore, the surge might be due to better care to very-low-birth-weight newborns without assessing the dangers of sources that cause common outbreaks. The similar situation happened in developed countries while they were introducing the high-end care facilities.^{13,27}

In our study, cefoxitin, erythromycin and clindamycin showed maximum resistance among Gram positive microorganisms. Furthermore, there has also been rise in MDR Gram negative pathogens. A study by Joseph Cantey et al showed high frequency of antibiotic use in NICU, inspite of the fact that only 5% have a positive blood culture.^{13,32} Patel et al observed that approximately 35% of neonates receive at least one course of antibiotics not backed up by diagnostic reports during their NICU stay.³³ The inappropriate use of antibiotics is associated with the development and spread of resistant pathogens in the neonates.³⁴

Neonatal factors associated with sepsis include prematurity, low birth weight, delivery at home and invasive procedures. The preterm neonates have immature immune system having low immunoglobulin levels which is related to decreased transplacental transfer of maternal IgG.³⁵ Furthermore, the barrier function of the skin and mucus membranes is compromised in these infants leading to increased risk of sepsis in preterm newborns. The improvement in medical facilities has led to better survival of low birth weight babies.

However, these neonates are exposed to NICU flora, they undergo multiple invasive procedures, including intravenous access and intubation. All the interventions and exposures lead to breach of the natural immunity of the neonate making them susceptible to infections. Premature rupture of membrane, prolonged labour, maternal fever within seven days before delivery were some of the factors significantly related to neonatal sepsis. Late and inadequate prenatal care, low socioeconomic status of the mother, poor maternal nutrition are additional factors associated with neonatal sepsis.³⁶

Our study has a few limitations. It was a single centre; retrospective study and we did not separately record community acquired and hospital acquired infections. Also, the incidence of LONS might have been underestimated as the neonates could not be followed up after discharge from the hospital due to practical difficulties. The strength of our study lies in the fact that it covers peri-urban population of a developing country which is less explored. Strict, protocol-driven practices for sample collection were followed reducing any chance of contamination.

The presentations of neonatal sepsis are ambiguous which may lead to delay in diagnosis. Surveillance studies and early identification of infectious agents are necessary to formulate guidelines for empirical and definitive treatment. The findings serve as a wakeup call for regional centres in developing countries to take necessary actions to contain neonatal infections and devise measures to prevent related morbidity and mortality. Proper implementation of antimicrobial stewardship program as a part of institutional infection control policy can curb the menace of antimicrobial resistance.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: Not required

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Cite this article as: Upadhyay S, Chakravarti A, Bharara T, Agarwal P. Incidence, bacteriological profile and risk factor analysis of neonatal sepsis in a peri urban set up of North India. *Int J Contemp Pediatr* 2020;7:1349-54.