



Clinico-microbiological study of neonatal sepsis

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Abstract:

Neonatal sepsis, a systemic infection manifesting in the first month of life is a leading cause of mortality in the newborn. Blood culture is the main stay in the diagnosis of neonatal sepsis. The present study focuses on the bacterial agents, the antibiogram and the clinical risk factors associated with neonatal sepsis. One hundred and ten neonates with clinical suspicion of neonatal sepsis were included in this study. Bacterial pathogens isolated in positive blood cultures were identified and the antibiotic susceptibility testing was performed. The risk factors were noted from the case records & statistical analysis was done using the Chi square test. Thirty six (32.72%) cultures were positive among 110 suspected cases of neonatal sepsis. 22(61.11%) cases presented with early onset sepsis and 14(38.89%) presented as late onset sepsis. The common bacteria isolated were *Klebsiella* spp., *Escherichia coli* & *Staphylococcus aureus*. Antibiotics effective against gram negative bacilli were cefoperazone/sulbactam and piperacillin/tazobactam. The rate of Methicillin resistant *Staphylococcus aureus* isolation was 57%. Gram negative bacilli predominate as agents of neonatal sepsis & antibiotic resistance among bacteria is on rise. Thus there is a need for continuous screening and surveillance for antibiotic resistance in NICU.

Key words: Neonatal sepsis, Risk factors, Antibiotic resistance, Early onset sepsis

Introduction:

Neonatal sepsis is an important cause of mortality and morbidity in the developing countries. Incidence in India is 30/1000 live births.¹ Risk factors significant for neonatal sepsis are preterm delivery, low birth weight, maternal fever, premature rupture of membrane (PROM) > 16hrs.² Early onset neonatal sepsis (EOS) presents within 72 hours of birth and late onset sepsis presents 4-90 days after birth. Source of infection in EOS is the colonized mother's genitourinary tract. The bacteria associated with EOS are Group B Streptococci, *E.coli*, Coagulase negative staphylococci, *Haemophilus influenzae* and *Listeria monocytogenes*. The bacteria implicated in LOS are from the environment: Coagulase negative staphylococci, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Candida* spp., *Acinetobacter* spp. and anaerobes.³

The clinical presentation and risk factors are different for EOS and LOS, though the bacteria associated with both early and late onset sepsis is the same in India.⁴ Early onset sepsis presents with pneumonia whereas in late onset sepsis, meningitis and bacteremia are the common presentations.⁴ The diagnosis of neonatal sepsis is based on clinical presentation and the laboratory investigations including C-reactive protein, procalcitonin, complete blood count, differential count, blood cultures and molecular methods: PCR. Blood culture remains the gold standard in diagnosis of neonatal sepsis with the identification of the pathogens and the antibiotic susceptibility pattern of the isolate.⁵

Timely diagnosis of sepsis in newborn, especially in those with risk factors is important in controlling the mortality and morbidity. The knowledge of bacterial spectrum and the antibiogram of the pathogens will help in adapting

appropriate control measures and antibiotic policy in the NICU in our hospital. The study was undertaken to study the spectrum of bacteria associated with neonatal sepsis. The study also assessed the various risk factors and the antibiotic susceptibility pattern of the pathogens.

Objectives

- To identify the common bacterial pathogens associated with neonatal sepsis
- To determine the antibiotic susceptibility pattern of the bacterial pathogens
- To evaluate the clinical risk factors associated with early onset sepsis

Materials & Methods:

Study period: 6 months from February 2014.

Study Design: This observational cross-sectional study was carried out in tertiary care hospital.

Study population: The study included 110 cases of clinically suspected septicemia in neonates.

The study has received clearance from the Institutional Ethics Committee.

The findings of TLC, DLC, ESR and CRP were noted. CRP values were measured by turbidometry. Blood culture was done using the BacT ALERT microbial detection system (Biomérieux, Inc. Durham, North California, USA).⁶

Positive samples were subcultured on blood agar, chocolate agar, and MacConkey agar and incubated overnight at 37°C. Growth of gram negative bacteria, gram positive bacteria, and yeasts were identified and the susceptibility pattern assessed by conventional methods and Vitek Compact 2 system. ESBL production in *Klebsiella* spp. and *E. coli* was confirmed using ceftazidime and Ceftazidime/clavulanic acid discs. Methicillin resistant *Staphylococcus aureus* was detected by using the cefoxitin

disc and interpreted according to the CLSI guidelines.⁷

Details regarding place of birth, age at onset of symptoms, symptoms & signs of presenting illness & other associated conditions (prematurity <37 weeks, low birth weight <2500 g, respiratory distress, and neonatal jaundice) were recorded.⁸ Any significant events during pregnancy and labour in mother were noted with the help of proforma. The clinical features and the risk factors were documented.

The association between the risk factors was found using the Chi Square Test. The collected data were entered and analyzed using SPSS (statistical package for the social sciences) version 11.

Results:

Of the 110 blood cultures received from cases of clinically suspected sepsis, 36(32.72%) were positive, 4(3.63%) grew skin contaminants, 70(63.3%) were negative. Out of 36 positive blood cultures, 22(61.11%) cases presented with early onset sepsis and the remaining 14(38.89%) presented as late onset sepsis. CRP was elevated in 61% of the cases with positive blood cultures.(n=36). The total leucocyte count was high in 58% of the cases with positive blood cultures.

The pathogens isolated from positive blood cultures is shown in **Table I**. Antibiogram of the gram positive and gram negative bacterial isolates are shown in **Tables II** and **III**. The risk factors studied are depicted in **Table IV**. The significant risk factors evaluated by Chi square test were low birth weight, premature rupture of membranes and maternal fever.

Candida krusei showed 100% sensitivity to amphotericin B, voriconazole. Resistance to fluconazole, flucytosine was seen in both the isolates of *Candida krusei*.

Discussion:

Neonatal sepsis is associated with high morbidity and mortality. Knowledge of

Table I: Spectrum of pathogens associated with neonatal sepsis

Pathogens	Number and percentage
<i>Klebsiella species</i>	9(25%)
<i>Escherichia coli</i>	8(22.22%)
<i>Staphylococcus aureus</i>	7(19.44%)
<i>Acinetobacter spp.</i>	5(13.89%)
<i>Coagulase negative staphylococcus</i>	3(8.33%)
<i>Candida krusei</i>	2(5.55%)
<i>Enterobacter cloacae</i>	1(2.77%)
Total	36

the bacterial profile and the antibiotic susceptibility pattern of the isolates play an important role in the management of sepsis. The blood culture positivity rate from the suspected cases of neonatal sepsis in this study was 32.3%. *Klebsiella spp.*, *Staphylococcus aureus*, *Escherichia coli* were the bacteria isolated from the suspected cases of neonatal sepsis. The antibiotics effective against *Staphylococcus aureus* were vancomycin, linezolid, clindamycin. The antibiotics effective against most of the gram negative bacterial isolates of neonatal sepsis were cefaperazone sulbactam, tigecycline and carbapenems. The important clinical risk factors were low birth weight and premature rupture of membranes and maternal fever in this setup.

The blood culture positivity rate in our study was 32.72%. Culture positivity rates in the previous studies on neonates with sepsis reported were as high as 56% by Sharma et al. to 40% by Tsering et al, which is similar to our study.⁹ The

comparatively lower rates of positivity in blood culture in our study could be because of prior antibiotic usage. Early onset sepsis was more in number (61.11%) compared to the cases with late onset sepsis which is consistent with the findings of Chung et al.¹⁰ The bacteria associated with EOS were *Klebsiella spp.*, *E.coli* and the common bacteria associated with late onset sepsis were *Staphylococcus aureus*, CONS. The other studies report *Klebsiella species* (25%) and *E. coli* (15%) as common agents of EOS and strains of *S. aureus* (14%), GBS (12%), *Streptococcus pneumoniae* (12%), and non-typhoidal *Salmonella species* (13%) as most frequently associated with LOS.¹⁰

C- reactive protein was positive (more than 6mg/dl) in 32% in previous studies. In our study CRP was elevated in 61% cases with positive blood cultures.¹⁰

The most common bacteria associated with neonatal sepsis in our study was *Klebsiella pneumoniae*, which is similar to the previous studies. Many of the previous published reports have *Klebsiella pneumoniae* as the commonest etiological agent of neonatal sepsis in developing countries. The isolation of *Escherichia coli* was also high in this study. Mondal et al has reported *E.coli* as the commonest pathogen associated with neonatal sepsis. The overall isolation of gram negative bacteria was more in our study compared to gram positive isolates.¹¹ Among the Gram positive bacteria, *Staphylococcus aureus* was the commonest followed by Coagulase negative staphylococci, comparable to other studies. We did not encounter *Group B streptococcus* in our study. Prenatal screening of pregnant women for vaginal colonization of *Group B Streptococcus* and antibiotic prophylaxis if vaginal cultures were positive could explain the above finding.

Antibiotic susceptibility pattern of the isolates varies from region to region. Prior knowledge of the antibiogram of the isolates in the region would help the clinician to choose on the right antibiotic.

In case of Gram negative bacterial isolates,

Table II: Antibiotic Resistance pattern of the Gram positive blood culture isolates of neonatal sepsis *

	P	Cd	E	Lz	Met	G	Cf	Ri	Va	Te
<i>S.aureus</i> (n= 7) %	7 100%	2 28.57%	4 57.14%	1 14.28%	4 57.14%	5 71.42%	5 71.42%	2 28.57%	0 0%	0 0%
<i>Coagulase negative Staphylococci</i> (n=3) %	3 100%	1 33.33%	0 0%	0 0%	1 33.33%	1 33.33%	1 33.33%	0 0%	0 0%	0 0%

*Resistance percentage.

P = Penicillin, Cd = Clindamycin, E = Erythromycin, Lz = Linezolid, Met = Methicillin, G = Gentamycin, Cf = Ciprofloxacin, Ri = Rifampicin, Va= Vancomycin, Te = Teicoplanin

the antibiotics effective were cefaperazone/sulbactam, carbapenems piperacillin/tazobactam. Penicillin plus an aminoglycoside is the preferred therapy. But majority of the gram negative bacteria were resistant to ampicillin and amoxiclav in our study and the level of aminoglycoside resistance of the gram negative bacteria exceeded 50%. The proportions of resistance in *E. coli* were ampicillin 75%, cotrimoxazole 75% and 62.5% to third generation cephalosporins. Among *Klebsiella species*, almost all were resistant to ampicillin, 44% to cotrimoxazole, and 62% to third generation cephalosporins. Resistance to aminoglycosides was lower in *E.coli* than *Klebsiella spp.* The rates of ESBL production in *Klebsiella spp.* and *E. coli*

were 61% and 56% respectively, thus the third generation cephalosporins, penicillins would prove ineffective in these cases. ESBL production rates in the previous studies reported are 58%, a finding consistent with this study. Carbapenems are believed to be the last resort for these ESBL producers. The worrisome fact is that carbapenem resistance was seen in 39% of gram negative bacterial isolates. *Acinetobacter spp.* was resistant to many of the antibiotics. Multi-drug resistant *Acinetobacter spp.* were sensitive to polymyxin B, colistin, tigecycline. The previous studies have also reported cefaperazone/sulbactam and piperacillin/tazobactam as the most effective drugs against the gram negative bacterial isolates associated with neonatal sepsis.^{12,13}

Table III: Antibiotic resistance pattern of the Gram negative bacterial pathogens from blood culture of neonatal sepsis cases*

Antibiotics	Gram Negative Bacilli			
	<i>Klebsiella spp.</i> (9)	<i>E.coli</i> (8)	<i>Acinetobacter spp.</i> (5)	<i>Enterobacter spp.</i> (1)
A	9(100)	6 (75)	4(80)	0
Ac	6(66.66)	3 (37.5)	4(80)	1(100)
Ak	2(22.22)	1(12.5)	2(40)	1(100)
Ci	5(55.55)	5(62.5)	4(80)	1(100)
Ce	4(44.44)	4(50)	3(60)	1(100)
Cu	4(44.44)	4(50)	3(60)	1(100)
Cfs	2(22.22)	1(12.5)	1(20)	0
Cf	3(33.33)	6(75)	2(40)	1(100)
Co	4(44.44)	5(62.5)	4(80)	1(100)
G	5(55.55)	3(37.5)	2(40)	0
I	3(33.33)	2(25)	2(40)	1(100)
Mr	2(22.22)	1(12.5)	2(40)	1(100)
Etp	2(22.22)	1(12.5)	NT	0
Pc	5(55.55)	3(37.5)	3(60)	0
Pt	4(44.44)	2(25)	2(40)	0
Tig	0	0	0	0

*Resistance percentage

A= Ampicillin, Ac= Amoxiclav, Ak= Amikacin, Ci= Ceftriaxone, Cu=Cefuroxime, Cfs= Cefaperazone/sulbactam, Co= Cotrimoxazole, G= Gentamicin, I= Imipenem, Mr= Meropenem, Etp= Ertapenem, Pc= Piperacillin, Pt= Piperacillin/tazobactam, Tig= Tigecycline, NT= not tested

Table IV : Risk factors associated with neonatal sepsis

Risk factors		Blood culture positive(n=36)	Blood culture negative(n=74)	p value
Mode of Delivery	Normal vaginal delivery	21	50	p=0.34
	LSCS	15	24	
Gestational Age	Preterm	23	32	p=0.064
	Term	10	38	
	Post-term	03	04	
Birth Weight	Low birth weight	22	16	p=0.001 SIGNIFICANT
	Normal	14	58	
Maternal Fever	Present	19	18	p=0.005 SIGNIFICANT
	Absent	17	52	
PROM	Present	21	18	p=0.001 SIGNIFICANT
	Absent	15	52	
Meconium Stained Liquour	Present	10	12	p=0.201
	Absent	26	58	

p value<0.05 is statistically significant

In case of *Staphylococcus aureus*, vancomycin remains as the sensitive drug, no resistance was found.¹⁴Clindamycin and Linezolid were the other two antibiotics quite effective against this organism. The rate of MRSA isolation in our study was 57.14%, which is higher than the other studies reported.

The two strains of *Candida krusei* showed 100% susceptibility to amphotericin B. *Candida krusei* is known for its innate resistance to fluconazole.¹⁵

Of the risk factors evaluated for neonatal sepsis, the factors which were statistically

significant are low birth weight, maternal fever and premature rupture of membranes. Similar studies carried out on neonatal sepsis have reported pre-term and low birth weight babies, gravida less than or equal to two, maternal fever and PROM for more than 16 hours as significant risk factors for developing neonatal sepsis.¹⁶

Prompt antibiotic therapy has a great influence on the outcome of neonatal sepsis. Thus, the knowledge of the antibiogram and the groups at risk for sepsis will help the clinician to choose the right antibiotic therapy.

Conclusions:

The spectrum of bacteria associated with neonatal sepsis is constantly changing. The rates of Antibiotic resistance among the bacterial pathogens is high. Hence there is a need to constantly monitor the same in NICU's.

The most common pathogens associated with neonatal sepsis were *Klebsiella pneumoniae*, *E.coli* and *Staphylococcus aureus*. The significant perinatal risk factors for sepsis were low birth weight, maternal fever and PROM. Antibiotic resistance is on rise especially among the gram negative bacterial pathogens.

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