

# Comparative microbial analysis and assessment of paired umbilical cord blood culture and peripheral venous blood culture in neonates with high risk factors to detect early onset neonatal sepsis: A study from tertiary care hospital of central India



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## ABSTRACT

**Background:** Neonatal sepsis is a prime cause of neonatal morbidity and mortality. Peripheral venous blood (PVB) culture is a gold standard for the diagnosis of neonatal sepsis. Low sensitivity of blood culture in newborn is due to small volume of blood sample and antibiotics given before sampling. **Aims and Objectives:** The study aims to compare and analyze the microbial spectrum in the Umbilical cord blood (UCB) culture with PVB culture and evaluation of both for detection of early onset neonatal sepsis (EONS) in high risk neonates and to use appropriate microorganism specific antibiotic for treating the sepsis. **Materials and Methods:** About 100 inborn neonates with two or more risk factors for EONS, chosen by sequential sampling method were included in this prospective analytical study. Blood samples were collected from umbilical cord and peripheral vein for culture. Sepsis screen was done to substantiate the diagnosis of neonatal sepsis. **Results:** Out of 100 neonates, 21 belongs to sepsis; 14 to probable sepsis; 65 to no sepsis. Among these 27 had UCB culture & 24 had PVB culture + ve. Organisms grown on culture were *Klebsiella(m/c isolate)*, *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter*, *Coagulase-ve Staphylococcus* (blood culture contaminant), *Pseudomonas* and *Enterococcus*. **Conclusion:** UCB culture is simple and convenient method for the diagnosis of neonatal sepsis as compared to PVB culture with Sensitivity of 66% and specificity of 93%. Organisms grown are comparable to PVB culture sample (*Klebsiella* - most common isolate).

**Key words:** Neonatal sepsis; Umbilical cord blood; Blood culture; Early onset neonatal sepsis; Neonate

## INTRODUCTION

Neonatal sepsis is the most common cause of neonatal mortality. Incidence of neonatal sepsis in India is 30 per 1000 live births (National Neonatal-Perinatal database of India [NNPD]).<sup>1</sup> Early onset neonatal sepsis (EONS) can have a wide spectrum of clinical presentation. Aggressive approach to diagnosis and management is the essential

determinant of the prognosis. Early diagnosis of neonatal sepsis is important to reduce the case fatality rate.<sup>2</sup>

EONS usually occurs in the first 72 h of life, with 80 to 90% of cases presenting up to 48 h after birth. Early onset sepsis typically manifests as fulminant, multisystem illness usually acquired by vertical transmission from the mother with high case fatality rate.<sup>2</sup>

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Blood culture to identify the organism is the gold standard for the diagnosis of EONS. However, the yield is low due to - low inoculum in the sample, inability of the laboratory to identify all the organisms, prior antibiotic usage. Added to this is the delay in obtaining the results as it takes 48 h of incubation of blood samples. This period could be too late for the clinician to initiate any useful treatment.

Umbilical cord blood (UCB) can be collected for blood culture for diagnosing EONS, as it is-painless and ensures adequate volume of blood for culture.

### Aims and objectives

The study aims to compare & analyze the microbial spectrum in the UCB culture with peripheral venous blood (PVB) culture and evaluation of both for detection of EONS in high risk neonates and to use appropriate microorganism specific antibiotic for treating the sepsis.

## MATERIALS AND METHODS

This prospective analytical study was carried out in nursery of tertiary care center of Central India over a period of 1 year from May 2019 to May 2020 after getting clearance from Ethics & Scientific Review Committee in which 100 inborn neonates delivered with presence of 2 or more of the following risk factors (Inclusion Criteria<sup>2</sup>) were included in the study by sequential sampling method:

- a. Prematurity (<37 weeks)
- b. Preterm premature rupture of membranes
- c. More than 3 vaginal examinations after rupture membranes/1 unclean vaginal examination
- d. History of maternal fever
- e. Foul – smelling liquor
- f. Chorioamnionitis
- g. prolonged rupture of membrane (>18 h)
- h. prolonged labor >24 h
- i. Perinatal asphyxia (Apgar score of <4 in 1 min)
- j. Meconium stained liquor.

Neonates born with lethal congenital anomalies and outborns were excluded.

UCB is collected (4–6 ml) at birth under asepsis from the placental end of the umbilical artery or vein and sent for CBC, peripheral smear, sepsis screen and blood culture. Similarly, PVB samples are also taken within 6 h of birth for above investigations before starting antibiotics according to NICU protocols.

Demographic, birth and clinical details of all the subjects are recorded and tabulated. After admission, babies are assessed for the clinical signs of sepsis and the peripheral blood smear parameters and culture reports once arrived

were recorded. Diagnostic outcome of all subjects were divided into three categories.

- Neonates with no signs of sepsis in next 72 h with peripheral vein blood culture (PVBC) sterile will be considered normal or no sepsis group
- Neonates with clinical signs of sepsis with sepsis screen  $\pm$  with PVBC sterile, were diagnosed as probable/clinical sepsis
- Neonates with clinical signs of sepsis and PVBC showing growth were grouped as proven sepsis.<sup>3</sup>

In our study, true positive are those which are culture +ve with clinical signs of sepsis present (sepsis screen can be  $\pm$ ); false positive are those which are culture +ve with no clinical signs of sepsis (sepsis screen –ve; contaminants); false negative are those which are culture –ve with clinical signs of sepsis present (sepsis screen can be  $\pm$ ); true negative are those which are culture –ve with no clinical signs of sepsis (sepsis screen –ve). So,

- Sensitivity=true positive/(true positive+false negative)
- Specificity=true negative/(true negative+false positive)
- ppv=true positive/(true positive+false positive)
- npv=true negative/(true negative+false negative).

### Statistical analysis

Statistical analysis was carried out for all 100 inborn high risk neonates for EONS. The data were expressed in terms of rates, ratio and percentages. Chi-square test was used to compare or associate nominal data. A probability value (P-value) of less than 0.05 was considered statistically significant. Using the above data statistical parameters – sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of umbilical cord blood culture (UCBC) and PVBC is also calculated and compared.

## RESULTS

Baseline characteristics of subjects were studied. Mean gestational age was 34 weeks and mean birth weight was 2.2 kg (Table 1). The risk factor found to be statistically significant ( $P<0.05$ ) associated with sepsis are maternal fever, prematurity, prolong rupture of membrane, preterm premature of rupture of membrane, chorioamnionitis, foul smelling liquor (Table 1).

Out of 35 sepsis cases which presented with clinical features of EONS (Table 2), the most common clinical manifestation was respiratory distress (69%).

Distribution of diagnostic outcome of all 100 neonates (Figure 1):

- No sepsis – 65
- Probable sepsis – 14
- Proven sepsis – 21.

**Table 1: Association of neonatal profile with early onset neonatal sepsis**

Patient's profile	No sepsis (n=65) No. (%)	Probable sepsis (n=14) No. (%)	Proven sepsis (n=21) No. (%)	Total No.(%)	P-value
Gender					
Male	30 (46.1)	5 (35.7)	6 (28.6)	41 (41.0)	
Female	35 (53.8)	9 (64.3)	15 (71.4)	59 (59)	
Gestational age (week)					
Preterm (<37)	40 (61.5)	9 (64.2)	13 (61.9)	62 (62.0)	
Term (>37)	25 (38.5)	5 (35.8)	8 (38.1)	38 (38.0)	
Birth weight (kg)					
BW (<2.5)	38 (58.4)	9 (64.2)	15 (71.4)	62 (62.0)	
BW (> 2.5)	27 (41.5)	5 (35.7)	6 (28.6)	38 (38.0)	
Mode of delivery					
LSCS	28 (43)	5 (35.7)	7 (33.3)	40 (40.0)	
NVD	37 (57)	9 (64.3)	14 (66.7)	60 (60.0)	
Risk factors distribution					
Prematurity (<37 weeks)	40 (62.0)	6 (42.8)	13 (61.9)	59	0.0009
Maternal fever	19 (30.4)	8 (57.1)	14 (66.7)	41	0.002
Prolonged rupture of membrane (>18 h)	23 (35.4)	5 (35.7)	9 (42.8)	37	0.03
Preterm premature rupture of membrane	20 (31.6)	4 (28.5)	7 (33.3)	31	0.04
3 or more vaginal examination after rupture of membrane	20 (30.4)	4 (28.5)	6 (28.5)	30	0.34
Meconium stained liquor	18 (27.8)	1 (7.1)	3 (14.3)	22	0.20
Birth asphyxia (apgar score of<4 in 1 min)	12 (19.0)	4 (28.5)	2 (9.5)	18	0.30
Prolong labor (>24 h)	13 (19.0)	3 (21.4)	1 (4.7)	17	0.55
Foul smelling liquor	0 (0.0)	1 (7.1)	8 (38.1)	9	0.001
Chorioamnionitis	0 (0.0)	1 (7.1)	8 (38.1)	9	0.001

**Table 2: Distribution of clinical features of early onset neonatal sepsis**

Clinical features	Sepsis (proven+probable) (n=35)	
	No.	%
Respiratory distress (tachypnea, grunting, chest retraction)	24	68.57
Shock	20	57
Lethargy	16	45.7
Abdominal distension	13	37
Feeding difficulty/intolerance	11	31
Bleeding/altered orogastric aspirate	11	31
Seizure	10	28.6
Hypoglycemia	8	22.8
Jaundice	7	20
Fever	5	14
Others	14	40

In UCBC out of 100 neonates (Table 3), 27 were culture positive and 73 were sterile and in 27 positive 23 belong to sepsis (clinically significant with sepsis screen +ve) and 4 belong to no sepsis (probably contaminant, not clinically significant with sepsis screen -ve). And in 73 sterile UCBC, 12 belong to sepsis (clinically significant with sepsis screen can be ±) and 61 (not clinically significant with sepsis screen -ve) belong to no sepsis.

In PVBC out of 100 neonates (Table 3), 24 are culture positive in which 21 belong to sepsis (clinical significant with sepsis screen +ve) and 3 belong to no sepsis (probably contaminants, not clinically significant with

sepsis screen - ve). And in 76 sterile PVBC, 14 belong to sepsis (clinical significant with sepsis screen can be ±) and 62 belong to no sepsis (not clinically significant with sepsis screen -ve).

Out of 100 UCBC samples (Table 4), 27 were +ve in which *Klebsiella* was most commonly detected and in 4 samples Coagulase negative *Staphylococcus* (CONS) is detected which is the contaminant. Out of 100 PVBC samples (Table 4), 24 were +ve in which also *Klebsiella* was most commonly detected and in 3 samples CONS is detected which is the contaminant.

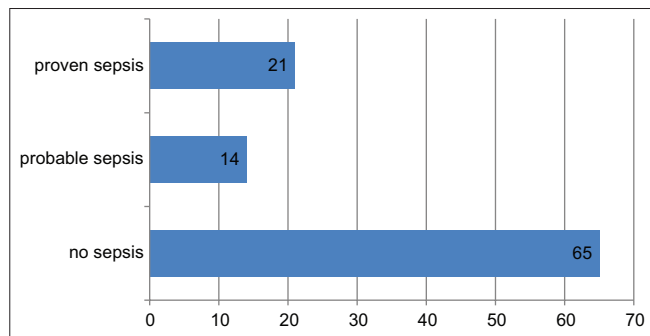
Statistical parameters values of UCBC are comparable to PVBC with higher sensitivity for UCBC to detect EONS (Table 5).

## DISCUSSION

Over the past two decades, neonatal morbidity associated with sepsis has increased due to changing microbial spectrum. To establish early diagnosis of EONS is a challenge because of varied clinical presentations. For definite diagnosis of EONS peripheral blood culture results are the gold standard which are time consuming and cumbersome. Therefore, there is always a need for test which helps in early detection of EONS that could be easily performed. In this study, we have evaluated the effectiveness and importance of UCB culture (UCBC) in the detection of the neonatal sepsis in high risk neonates and its comparison with PVBC and along with it we

compare & analyze the microbial spectrum in the UCBC with PVBC so to use appropriate microorganism specific antibiotic for treating the sepsis.

Males predominantly develop sepsis in our study (Table 1), which is consistent with other studies by Pramana *et al.*,



**Figure 1:** Diagnostic distribution for early onset neonatal sepsis

	Diagnosis		Total (n=100)
	Sepsis (proven+probable) (n=35)	No sepsis (n=65)	
Umbilical cord blood culture			
Positive	23	4	27
Negative	12	61	73
Peripheral vein blood culture			
Positive	21	3	24
Negative	14	62	76
Total	35	65	100

**Table 3: Umbilical cord and peripheral venous blood culture distribution**

Organisms in culture	Umbilical blood culture positive (n=27)	Peripheral blood culture positive (n=24)	Sensitivity
<i>Klebsiella</i>	6	5	Amikacin, gentamicin, imipenem, levofloxacin, colistin
<i>Staphylococcus aureus</i>	4	4	Gentamicin, vancomycin, erythromycin, linezolid, clindamycin, levofloxacin
<i>Acinetobacter</i>	4	2	Amikacin, gentamicin, imipenem, ciprofloxacin, ceftazidime
<i>Coagulase –ve staphylococcus</i>	4	3	Levofloxacin, linezolid, gentamycin, vancomycin, erythromycin, linezolid
<i>Escherichia coli</i>	3	4	Amikacin, gentamicin, imipenem, piperacillin/tazobactam, ceftriaxone, colistin, levofloxacin
<i>Pseudomonas</i>	3	3	meropenem, levofloxacin, amikacin, colistin, gentamicin, piperacillin/tazobactam, ceftriaxone
<i>Enterococcus</i>	3	3	gentamycin, ciprofloxacin, vancomycin, erythromycin, linezolid, vancomycin
Total	27	24	

**Table 4: Spectrum of organisms in umbilical cord blood culture and peripheral vein blood culture with sensitivity**

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Umbilical cord blood culture	66	93	85	83
Peripheral vein blood culture	60	95	87	81

Makkar *et al.*, Dutta *et al.*<sup>3-5</sup> Preterm neonates predominantly develop sepsis in our study (Table 1), which is consistent with other studies by Pramana *et al.*, Makkar *et al.*, Dutta *et al.*<sup>3-5</sup> LBW neonates predominantly develop sepsis in our study (Table 1), which is consistent with other studies by Dutta *et al.*, Pramana *et al.*, Makkar *et al.*<sup>3-5</sup> There was no significant correlation of sepsis status with NVD and LSCS delivered neonates (Table 1), it is consistent with other studies by Makkar *et al.*, Pramana *et al.*<sup>3,4</sup>

Risk factors distribution among 100 neonates, the following risk factors shown a statistically significant correlation with EONS with P<0.05, maternal fever, prematurity, prolonged rupture of membrane, premature rupture of membrane, foul smelling liquor, chorioamnionitis (Table 1). While other risk factors did not show any statistically significant correlation with EONS with P>0.05. Similar findings were reported in other studies by Mandot *et al.*, Dutta *et al.*, Pramana *et al.*<sup>4-6</sup>

Out of 35 sepsis (proven +probable sepsis) cases which presented with clinical features of EONS, the m/c clinical manifestation was respiratory distress (Table 2). Majority of neonates has shown >2–3 clinical features. Similar findings were present in other studies done by Pramana *et al.*, Dutta *et al.*<sup>4,5</sup>

In this study, PVBC is considered gold standard for the diagnosis of neonatal sepsis, and it requires a minimum of 48–72 h to yield a result. Out of 100 neonates, for the purpose of this study diagnostic accuracy and easy calculation 21 neonates (proven sepsis) and 14 neonates

(probable sepsis) are included in sepsis group (n=35) and remaining 65 neonates are included in No sepsis group (Figure 1).

In the 27 +ve UCBC (Table 4), the bacterial isolates are predominantly gram-ve - 16 isolates (m/c is *Klebsiella*, being sensitive to amikacin, gentamicin, imipenem, levofloxacin, colistin) and gram+ve -11 isolates (m/c is *Staphylococcus aureus*, being sensitive to gentamicin, vancomycin, erythromycin, linezolid, clindamycin, levofloxacin). Common Organisms detected in positive UCBC are *Klebsiella* f/b *S. aureus*, *Acinetobacter*, *Coagulase-ve Staphylococcus* (blood culture contaminant), *Escherichia coli*, *Enterococcus*, *Pseudomonas* (Table 4). Similarly in the 24 +ve PVBC (Table 4), the bacterial isolates are predominantly Gram-ve - 14 isolates (m/c is *Klebsiella*) and Gram+ve – 10 isolates (m/c is *S. aureus*). Common Organisms detected in positive PVBC are also *Klebsiella* f/b *S. aureus*, *E. coli*, *Pseudomonas*, *Enterococcus*, *Coagulase-ve Staphylococcus* (blood culture contaminant), *Acinetobacter* (Table 4). Out of 27 UCBC +ve neonates, 14 have shown similar bacterial isolates in both UCBC and PVBC in which *Klebsiella* was most common, then *S. aureus*, *E. coli*, *Acinetobacter*, *Enterococcus*, *Pseudomonas*. Out of the remaining 13 positive UCBC, 9 positive UCBC were found in probable sepsis cases with clinical signs of EONS present with sepsis screen can be  $\pm$  but PVBC sterile and the remaining 4 positive UCBC isolates had no clinical signs of EONS with PVBC being sterile and was considered insignificant (*Coagulase-ve Staphylococcus*-blood culture contaminant), most probably the result of contamination due to improper collection of blood from the umbilical cord.

These results are consistent with other studies by Mandot et al., Pramana et al., Dutta et al.<sup>4,6</sup> According to NNPD of India of 2002–2003, organism causing sepsis in intramural babies were *Klebsiella* (32.5%), *S. aureus* (13.6%), *E. coli* (10.6%), *Pseudomonas* (5.6%), and *Acinetobacter* (2.7%).<sup>1</sup> Our study also showed almost similar organism profile as in NNPD.

Statistical parameters of UCBC for detecting neonatal sepsis are – Sensitivity-66%, Specificity-93%, PPV-85%, NPV-83% and diagnostic parameters of PVBC are – Sensitivity-60%, Specificity-95%, PPV-87%, NPV-81% (Table 5). These results are consistent with the study by Meena et al.<sup>7</sup>

In our study, UCBC has a slightly higher sensitivity as compared to PVBC, it could be because of higher yield, due to more blood sample availability as UCB can be collected easily and effortlessly and is easily accessible without causing any pain to neonate. It is well-known that withdrawing large amounts of blood from a neonate can be challenging and

may also lead to iatrogenic anemia.<sup>8</sup> For neonates, blood sampling by venipuncture can be difficult, as in routine clinical practice a large proportion of CBC report were spurious because of the submission of an inadequate volume of blood<sup>9</sup> and repeated needle prick for adequate blood sample can cause more pain to the neonate and prior antibiotic exposure also leading to poor yield in peripheral blood in general. UCB sample is collected before instillation of antibiotics which also adds to its higher sensitivity in general. Other added benefit of UCB is that it prevents iatrogenic anemia and introduction of infection.

Despite the advantages of UCBC, it is also documented in the study conducted by Fos et al.,<sup>10</sup> in 2009 that UCBC results had excess of contamination lacking clinical correlation. The technique of collection of cord blood is critical to ensure meaningful results without contamination. Collection of blood from the umbilical cord on perineum before delivery of the placenta has been reported to have higher contamination.

#### Limitations of the study

The present study has some limitations. The sample size was small and UCB culture potential in replacing PVB culture in detection of EONS needs to be evaluated in multicentric trials or larger sample size.

## CONCLUSION

UCBC is a good method to have etiological diagnosis for EONS and can be good alternative to PVBC for enhanced detection of EONS in high risk neonates. Organisms grown in UCBC samples are comparable with PVBC with *Klebsiella* being most common and have certainly an additional value in detecting and treating neonatal sepsis with appropriate antibiotics.

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## REFERENCES

1. Deorari A. Investigators of national neonatal perinatal database (NNPD) changing pattern of bacteriologic profile in neonatal sepsis among intramural babies. *J Neonatol*. 2006;20:14. <https://doi.org/10.1177/0973217920060103>
2. Eichenwald E, Hansen A, Martin C and Stark A. Cloherty and Stark's Manual of Neonatal Care. 8<sup>th</sup> ed. Netherlands: Wolters Kluwer Publisher; 2017. p. 598-627.
3. Makkar M, Gupta C, Pathak R, Garg S and Mahajan NC.

- Performance evaluation of hematologic scoring system in early diagnosis of neonatal sepsis. *J Clin Neonatol.* 2013;2(1):25-29. <https://doi.org/10.4103/2249-4847.109243>
4. Pramana K, Kardana I and Nilawati G. Diagnosis accuracy of hematological scoring system in early identification of neonatal sepsis. *Bali Med J.* 2016;5(3):139-143. <https://doi.org/10.15562/bmj.v5i3.310>
  5. Dutta NR, Medhi P and Hazarika R. Haematological scoring system (HSS) in early diagnosis of neonatal sepsis: A study from tertiary care hospital from North East India. *J Evol Med Dent Sci.* 2016;5(52):3359-3366. <https://doi.org/10.14260/jemds/2016/777>
  6. Mandot S and Gandhi JS. Umbilical cord blood culture versus peripheral venous blood culture in early onset neonatal sepsis. *Int J Contemp Pediatr.* 2017;4(1):53-56. <http://dx.doi.org/10.18203/2349-3291.ijcp20164302>
  7. Meena R, Meena K, Athwani V and Gothani S. Umbilical cord blood culture in diagnosis of EONS. *Indian J Paediatr.* 2020;87(10):793-797. <https://doi.org/10.1007/s12098-020-03345-5>
  8. Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM and Ascher DP. Volume of blood required to detect common neonatal pathogens. *J Pediatr.* 1996;8(129):275-278. [https://doi.org/10.1016/S0022-3476\(96\)70254-8](https://doi.org/10.1016/S0022-3476(96)70254-8)
  9. Connell TG, Rele M, Cowley D, BATTERY JP and Curtis N. How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. *Pediatrics.* 2007;119:891-896. <https://doi.org/10.1542/peds.2006-0440>
  10. Fos NI, Gomis RV, Gomis CV, Rubio J, Justich P, Valera JC, et al. Blood culture from the umbilical vein in the diagnosis of neonatal sepsis. *Internet J Pediatr Neonatol.* 2010;12:1.

**Authors' Contributions:**

**GS-** Interpreted the results; reviewed the literature and manuscript preparation; **SA-** Concept and design of the study, prepared first draft of manuscript; **PG-** Preparation of manuscript and revision of the manuscript; **UC-** Concept, coordination, statistical analysis and interpretation.

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