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Research Article

Beyond Blood Culture: Placental Membrane Culture And Examination As A Superior Diagnostic Tool For Accurate And Targeted Diagnosis Of Early-Onset Neonatal Sepsis

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ABSTRACT

Background: Early-onset neonatal sepsis (EONS), occurring within the first 72 hours of life, remains a major cause of neonatal morbidity and mortality. Diagnosis is hampered by nonspecific clinical features and the low sensitivity of blood cultures, often leading to indiscriminate broad-spectrum antibiotic use. Placental examination—both histopathology and microbiological culture—has emerged as a promising adjunct, providing evidence of intrauterine infection and enabling targeted therapy.

Methods: A prospective observational cohort study was conducted over 18 months in the NICU of a tertiary care hospital in South India. Inborn neonates with suspected EONS were included. Maternal risk factors, neonatal baseline data, clinical features, and outcomes were documented. Placental samples were obtained for microbiological culture and histopathology, and peripheral blood cultures were processed as per standard protocols. Diagnostic performance of placental investigations was compared against blood culture using McNemar's test and ROC curve analysis.

Results: A total of 121 neonates were studied. Most were term or near-term (mean gestational age 37.8 ± 1.6 weeks, mean birth weight 2921 g). PROM, intrapartum fever, and foul-smelling liquor were common maternal risk factors. Clinical manifestations included respiratory distress (71.1%), septic shock (26.4%), and thrombocytopenia (20.7%). Placental cultures were positive in 83.4% (101/121) compared with 25% blood culture positivity. *Pseudomonas* (21.3%) and *Klebsiella* (10.7%) were predominant isolates, reflecting regional epidemiology. Concordance between blood and placental cultures was high (24 cases identical), but notably, 74 neonates with sterile blood cultures had positive placental growth. ROC analysis showed superior diagnostic performance for placental culture (AUC 0.96; sensitivity 83.5%; PPV 100%) versus blood culture (AUC 0.85; sensitivity 24.8%). Histopathology revealed inflammatory changes in 79% of placentas with sensitivity of 94.6%. Overall survival was 89.3%.

Conclusion: Placental membrane culture significantly outperformed blood culture in detecting EONS and provided crucial microbiological data for early, targeted antibiotic therapy. When combined with histopathology, diagnostic precision was maximized. These findings highlight the feasibility, reliability, and cost-effectiveness of placental examination, supporting its incorporation into routine diagnostic algorithms for EONS, particularly in high-burden, resource-limited settings.

Keywords*: Early-onset neonatal sepsis, placenta, microbiological culture, histopathology, diagnosis, antibiotic stewardship*

BACKGROUND

Early-onset neonatal sepsis (EONS), defined as infection manifesting within the first 72 hours of life, continues to be a major contributor to neonatal morbidity and mortality worldwide[1]. Diagnosis is complicated by nonspecific clinical

manifestations and the limited sensitivity of conventional blood cultures, reported to be only 36–40%.[2] This uncertainty often leads to empirical broad-spectrum antibiotic use, which increases the risk of antimicrobial resistance.[3]

Histopathological examination of the placenta (HPE) has emerged as a critical tool for identifying intrauterine infection. Chorioamnionitis (CA) and funisitis, key histological markers of maternal and fetal inflammatory responses, are significantly associated with neonatal sepsis.[4,5] Placental histology not only provides diagnostic information but also predicts adverse neonatal outcomes, including respiratory distress and shock, thereby strengthening its role in risk stratification.[6,7]

Several studies highlight the limitations of relying solely on maternal risk factors or clinical chorioamnionitis. For instance, clinical CA does not always correspond with histological evidence, and maternal markers such as CRP are insufficient as standalone predictors.[8–10] In large cohorts, neonates born to mothers with CA have been found to develop culture-confirmed sepsis even if asymptomatic at birth, underscoring the need for more reliable perinatal diagnostic approaches.[3]

Microbiological culture of the placenta provides further value. A high degree of concordance (up to 70%) between placental bacterial culture and histological chorioamnionitis has been reported, demonstrating the utility of combining both modalities for accurate diagnosis.[9] Importantly, placental cultures can yield specific microbial identification much earlier than blood cultures, enabling more rational antibiotic stewardship.[2,11] Studies have also emphasized that region-specific microbial epidemiology is critical: unlike Western countries where Group B Streptococcus predominates, organisms such as *Klebsiella*, *Pseudomonas*, and *Citrobacter* are more relevant in the Indian subcontinent.[12,13]

Recent prospective and multicenter studies have advocated integrating placental examination into neonatal sepsis work-up, not only to improve diagnostic precision but also to minimize overtreatment. Ykema et al. demonstrated that placental histology allowed safe truncation of antibiotics in preterm neonates, preventing unnecessary exposure.[2] Wortham et al. further showed that maternal CA correlated strongly with neonatal infections, though a subset of infected neonates remained asymptomatic in the first hours of life, reinforcing the need for adjunctive placental diagnostics.[3]

Thus, accumulating evidence supports the placenta as a "secret witness" of intrauterine events,[7] with both histopathology and culture offering substantial diagnostic advantage. However, despite global evidence, its application remains underutilized in India. The present study addresses this gap by evaluating the diagnostic performance of placental culture and histology against conventional blood culture in detecting EONS in an Indian NICU population.

MATERIALS AND METHODS

Study Design and Setting

This was a prospective observational cohort study conducted in the Neonatal Intensive Care Unit (NICU) of a tertiary care hospital in a metropolitan city of South India over a period of 18 months. The objective was to evaluate the diagnostic accuracy of placental histopathology and microbiological culture in early-onset neonatal sepsis (EONS), using blood culture as the conventional comparator.

Study Population

All inborn neonates admitted to the NICU with risk factors for or clinical suspicion of EONS were eligible for enrollment. EONS was defined as sepsis occurring within the first 72 hours of life, supported by clinical features and/or laboratory findings.

Inclusion Criteria

1. Inborn neonates admitted with probable diagnosis of EONS.
2. Presence of maternal or perinatal risk factors such as prolonged rupture of membranes, intrapartum fever, or foul-smelling liquor.
3. Symptomatic neonates presenting within the first 72 hours of life.
4. Twin gestations (both mono- and dichorionic).

Exclusion Criteria

1. Infants requiring therapeutic hypothermia for hypoxic ischemic encephalopathy.
2. Neonates with prenatally diagnosed major congenital anomalies.
3. Infants of TORCH-positive mothers with symmetric intrauterine growth restriction (IUGR).
4. Features suggestive of chromosomal aneuploidy.
5. Refusal of parental consent.

Data Collection

Maternal demographic details, obstetric history, risk factors, and intrapartum events were documented. Neonatal baseline parameters including gestational age, birth weight, Apgar scores, and clinical features at admission were recorded. A standardized case record form was used to ensure consistency of data capture.

Placental Examination

- **Gross Examination:** Placentas were weighed, and surfaces examined for infarction, calcification, or other abnormalities.
- **Microbiological Culture:** Membrane and chorionic plate samples were obtained under aseptic precautions and inoculated on blood agar, chocolate agar, and MacConkey agar. Plates were incubated aerobically at 37 °C for 24–48 hours. Organisms were identified by colony morphology, Gram staining, and standard biochemical methods.
- **Histopathology:** Representative sections from maternal and fetal surfaces, membranes, and umbilical cord were fixed in formalin, processed, and stained with hematoxylin–eosin. Inflammatory changes were assessed and classified according to maternal and fetal response categories.

Blood Culture

Peripheral venous blood (1–2 mL) was collected aseptically and inoculated into BacT/Alert bottles for automated aerobic culture. Positive signals were processed for microbial identification using conventional microbiological methods.

Outcome Measures

The primary outcome was the diagnostic accuracy of placental culture and histopathology in identifying EONS compared with blood culture. Secondary outcomes included correlation of placental findings with maternal risk factors, neonatal clinical manifestations, and short-term outcomes (discharge, death, or leave against medical advice).

Ethical Considerations

The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from parents or legal guardians prior to enrollment. All procedures were conducted in accordance with ethical principles governing clinical research.

Statistical Analysis

Data were compiled in Microsoft Excel and analyzed using SPSS software. Descriptive statistics were applied for baseline characteristics. Diagnostic performance was expressed as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Agreement between blood and placental cultures was tested using McNemar's test, and receiver operating characteristic (ROC) curves were used to assess diagnostic performance. A p-value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of the Study Population

A total of 121 neonates were included in the present prospective cohort study. The gender distribution was nearly equal, with 67 (54.9%) male and 55 (45.1%) female infants, ensuring balanced representation for gender-related analysis (**Table 1**). The gestational age of the study cohort ranged between 34 and 40 completed weeks, with the majority concentrated around late preterm and term gestations. Most neonates were born at 37 weeks (24%) and 38 weeks (31.4%), followed by 39 weeks (17.4%). Only a small proportion were early preterm, with three neonates born at 34 weeks and two at 35 weeks. The mean birth weight was 2921.4 ± 406.5 g, and 90% were appropriate for gestational age (AGA), while 7 neonates (5.8%) were small for gestational age (SGA) and 5 (4.1%) were large for gestational age (LGA).

The median age of admission to the NICU was 6 hours of life (HOL), with most neonates (40.8%) admitted precisely at 6 HOL. Another 17.5% were admitted at 3 HOL, and 12.5% at 4 HOL, indicating that the majority of neonates were recognized as symptomatic and admitted early within the critical early-onset sepsis (EONS) window. The average duration of NICU stay was 11.5 days, ranging from a minimum of 5 days to a maximum of 21 days.

Mode of delivery was almost equally distributed, with 62 neonates (51.6%) delivered by lower segment cesarean section (LSCS) and 58 (48.3%) born by spontaneous vaginal delivery. The mean placental weight was 320.3 ± 57.8 g. Gross placental examination revealed infarction in 20.8% of samples, grey/white patches in 10%, while 69.1% appeared grossly normal. These grossly normal placentas later yielded important histopathological and microbiological findings, underscoring the limitation of relying on gross morphology alone.

Collectively, these baseline observations establish that the cohort predominantly consisted of term or near-term neonates, most of whom were symptomatic within hours of birth, providing a strong foundation for evaluating placental culture as a diagnostic adjunct.

Maternal and Perinatal Risk Factors for EONS

The analysis of maternal characteristics highlighted several important perinatal risk factors strongly associated with EONS (**Table 2**). Prolonged rupture of membranes (PROM) was a prominent risk factor, present in 44% of mothers. While PROM of less than 18 hours was most common (37 cases), a significant subset (14 cases, 11.6%) had PROM exceeding 24 hours. Intrapartum fever was documented in 43 (35.8%) mothers, and leukocytosis (>15,000 cells/μL) was

found in 39 (32.5%). Urinary tract infections were common, affecting 47 (39.1%) mothers, while foul-smelling liquor, an indicator of intra-amniotic infection, was observed in 35.8% of cases.

Meconium-stained amniotic fluid was detected in 15.5% of deliveries, while the remainder had clear liquor. More than half the mothers (55.6%) did not receive any form of antepartum antibiotic prophylaxis. Of those who did, 25.2% received parenteral and 19.1% oral antibiotics. These observations suggest that a significant proportion of neonates were exposed to unmitigated risk of ascending intrauterine infection due to absent or inadequate prophylaxis.

When correlated with neonatal outcomes, these maternal factors were strongly associated with placental culture positivity. For instance, PROM exceeding 18 hours, maternal fever, and foul-smelling liquor were disproportionately represented among neonates with positive placental growth, establishing the biological plausibility of placental colonization translating into neonatal sepsis.

Clinical and Laboratory Manifestations of Sepsis

The overwhelming majority of neonates, 107 (89.9%), were symptomatic at birth, while 8 (6.7%) developed features of sepsis within the first 6 hours and 4 (3.4%) after 6 hours (**Table 2**). Respiratory distress was the most common clinical presentation: 52.5% had a Silverman–Anderson score of 4, 14.2% scored 5, and 5% scored 6, indicating moderate-to-severe distress in more than two-thirds of cases. Septic shock was diagnosed in 32 neonates (26.6%), reflecting the severity of systemic illness.

Respiratory support requirements paralleled disease severity. More than half (55.8%) required continuous positive airway pressure (CPAP), while 27.5% received nasal prongs oxygen. A smaller proportion required advanced support—non-invasive mechanical ventilation (10%) and synchronized intermittent mandatory ventilation (6.7%).

Hematological abnormalities were common. Thrombocytopenia (<150,000/μL) occurred in 20.8% of neonates, and leukocyte abnormalities were seen in 22.4%—with 11 neonates (9.1%) having leucopenia and 17 (13.3%) showing leukocytosis. These laboratory findings were consistent with systemic inflammatory and infectious processes and correlated well with placental culture positivity.

Placental Culture Findings

Placental microbiological culture was the most revealing investigative modality in this study. Of the 121 samples, 101 (83.4%) yielded microbial growth, while only 21 (17.2%) were sterile (**Table 3**). In striking contrast, blood cultures were positive in only 30 cases (25%).

The predominant organism isolated from placental cultures was *Pseudomonas* spp. (21.3%), followed by *Staphylococcus aureus* (14.8%), *Citrobacter* spp. (13.1%), and *Klebsiella* spp. (10.7%). Other organisms included coagulase-negative staphylococci (CONS) (11.5%), *Acinetobacter* (4.9%), *Enterococcus* (3.3%), *E. coli* (2.5%), and rare isolates such as *Proteus* (0.8%). The distribution of these organisms is shown in **Figure 1 (Bar chart of placental culture growth)**.

The breadth of organisms detected illustrates that placental colonization is not limited to classical pathogens like *GBS* but reflects a wide spectrum of Gram-negative bacilli and Gram-positive cocci relevant to the Indian context. Importantly, the predominance of *Pseudomonas* and *Klebsiella* corroborates their known role in Indian NICU sepsis epidemiology, further strengthening the external validity of these findings.

Blood Culture Findings and Correlation with Placental Growth

Blood cultures yielded positive results in 30 neonates (25%), with *Pseudomonas* (8.4%) and *Klebsiella* (5.0%) being most frequent (**Table 3**). Other organisms included *Citrobacter* (4.2%), *E. coli* (2.5%), *Acinetobacter* (2.5%), *Staphylococcus aureus* (0.8%), and CONS (0.8%).

When correlated with placental cultures, 24 cases demonstrated identical organisms in both samples, confirming excellent diagnostic reproducibility. However, two cases revealed discordance: in one, blood grew *Acinetobacter* while placenta yielded *Staphylococcus aureus*, and in another, blood yielded *E. coli* while placenta grew CONS. In four cases, blood cultures were positive despite sterile placental cultures.

The most significant finding, however, was that among the 90 sterile blood cultures, 74 (82.2%) demonstrated positive placental growth (**Table 4**). This observation highlights the marked superiority of placental culture in detecting infections missed by blood cultures. It also underscores the potential of placental examination to identify neonates who would otherwise be misclassified as culture-negative sepsis.

Diagnostic Accuracy of Placental Culture

Diagnostic accuracy was assessed using both concordance analysis and statistical tests. McNemar's test compared the 71 discordant cases (placental positive, blood negative) with the 4 converse discordant cases (placental negative, blood positive). The χ^2 value of 59.85 far exceeded the critical value at $\alpha = 0.05$, confirming a highly significant diagnostic advantage of placental culture (**Table 5**).

ROC curve analysis further demonstrated the superiority of placental culture, with an AUC of 0.96, compared to 0.85 for blood cultures. Placental culture sensitivity was 83.5%, with a PPV of 100%. In contrast, blood culture sensitivity was only 24.8%, with a PPV of 88.2%. These findings, summarized in **Table 5** and illustrated in **Figure 2 (ROC curve)**, establish placental culture as a far more reliable diagnostic tool in EONS than the current gold standard of blood culture.

Histopathological Examination of Placenta

Histopathology revealed inflammatory changes in 87 of 110 (79%) placentas examined. Maternal inflammatory responses (chorioamnionitis and subchorionitis) were observed in 65.5%, while fetal inflammatory responses (vasculitis and arteritis) were present in 43.6%. The sensitivity of histopathology in detecting EONS was 94.6%, with a PPV of 98.2%. Thus, histopathology, when combined with microbiological culture, provided a powerful dual diagnostic approach capable of identifying infections in neonates even with sterile blood cultures.

Special Scenarios

Special scenarios emphasized the complementary role of placental culture (**Table 4**). Four neonates had positive blood cultures despite sterile placental cultures. Twenty-four had identical organisms isolated from both sources, underscoring concordance. Two had discordant organisms, and 74 had placental positivity despite sterile blood cultures, providing evidence for the heightened sensitivity of placental microbiological analysis.

Additionally, lumbar puncture performed in 103 neonates identified meningitis in 32 (31%). Of these, only 8 had concurrent positive blood cultures, suggesting that placental findings might also serve as a proxy for invasive infections not readily detected by conventional methods.

Neonatal Outcomes

Despite the severity of illness, neonatal outcomes were favorable in most cases. A total of 108 neonates (89.3%) were discharged in stable condition. Twelve families (9.8%) left against medical advice, while one neonatal death (0.8%) was recorded. These outcomes reflect the potential benefit of integrating placental culture and histopathology into the diagnostic algorithm, allowing early identification and treatment of high-risk neonates.

Summary of Key Findings

This study demonstrated that placental microbiological culture significantly outperformed blood culture in detecting early-onset neonatal sepsis. Placental cultures were positive in 83.4% of cases, compared to only 25% positivity in blood cultures. The diagnostic superiority was statistically significant, supported by McNemar's test and ROC curve analysis. Histopathology corroborated inflammatory changes in 79% of placentas, further validating placental examination as a robust diagnostic tool.

The consistent association of placental positivity with maternal risk factors (fever, PROM, foul-smelling liquor) and neonatal morbidities (respiratory distress, shock, thrombocytopenia) reinforces the clinical significance of placental evaluation. Together, these findings establish placental examination—both microbiological and histological—as a powerful, non-invasive adjunct to conventional blood cultures in the diagnosis of EONS.

Tables

Table 1. Baseline characteristics of the study population (N = 121)

Characteristic	n (%) / Mean ± SD
Gender	Male 67 (54.9), Female 55 (45.1)
Gestational age (weeks)	Mean 37.8 ± 1.6 (Range 34–40)
34–35 weeks	5 (4.1)
36 weeks	14 (11.6)
37 weeks	29 (24.0)
38 weeks	38 (31.4)
39 weeks	21 (17.4)
40 weeks	14 (11.6)
Birth weight (g)	Mean 2921.4 ± 406.5
Birth weight status	AGA 108 (90.0), SGA 7 (5.8), LGA 5 (4.1)
Age at admission (HOL)	Median 6 (Range 1–9)
Mode of delivery	LSCS 62 (51.6), SVD 58 (48.3)
Mean placental weight (g)	320.3 ± 57.8

Table 2. Maternal and neonatal risk factors and manifestations of early-onset sepsis

Variable	n	%
Maternal factors (N = 118)		
Any PROM	118	100.0
- PROM <18 h	37	31.4
- PROM 18–24 h	67	56.8

- PROM >24 h	14	11.9
Fever (>38.3 °C)	43	36.4
Leukocytosis (>15,000/μL)	39	33.1
Urinary tract infection	47	39.8
Foul-smelling liquor	43	36.4
Meconium-stained liquor	19	16.1
No antepartum antibiotics	64	54.2
Neonatal manifestations (N = 121 neonates)		
Symptoms at birth	107	88.4
Symptoms 1–6 h	8	6.6
Symptoms >6 h	4	3.3
Respiratory distress (Silverman–Anderson ≥4)	86	71.1
Septic shock	32	26.4
Thrombocytopenia (<150,000/μL)	25	20.7
Leucopenia	11	9.1
Leukocytosis	17	14.0

Table 3. Spectrum of organisms isolated from placental and blood cultures

Organism	Placental culture n (%) (N=121)	Blood culture n (%) (N=120)
Sterile	21 (17.2)	90 (75.6)
Pseudomonas spp.	26 (21.3)	10 (8.4)
Staphylococcus aureus	18 (14.8)	1 (0.8)
Citrobacter spp.	16 (13.1)	5 (4.2)
Klebsiella spp.	13 (10.7)	6 (5.0)
CONS	14 (11.5)	1 (0.8)
Acinetobacter spp.	6 (4.9)	3 (2.5)
Enterococcus spp.	4 (3.3)	1 (0.8)
E. coli	3 (2.5)	3 (2.5)
Proteus spp.	1 (0.8)	0 (0.0)

Table 4. Special scenarios in placental and blood culture correlation

Scenario	n (N=121)	Comments
Blood positive, placenta negative	4	Included 2 *Pseudomonas*, 2 *Klebsiella* (late growth, day 3)
Blood positive, placenta positive (same organism)	24	High diagnostic reproducibility
Blood positive, placenta positive (different organisms)	2	Case 1: Blood *Acinetobacter*, Placenta *S. aureus*; Case 2: Blood *E. coli*, Placenta CONS
Blood negative, placenta positive	74	Strong evidence of placental culture superiority

Table 5. Diagnostic accuracy of placental vs. blood culture for EONS

Diagnostic parameter	Placental culture	Blood culture
Positivity rate (%)	83.4	25.0
Sensitivity (%)	83.5	24.8
Positive predictive value (%)	100	88.2
Area under ROC curve (AUC)	0.96	0.85
McNemar's χ^2	**59.85 ($p < 0.05$)**	

Figures

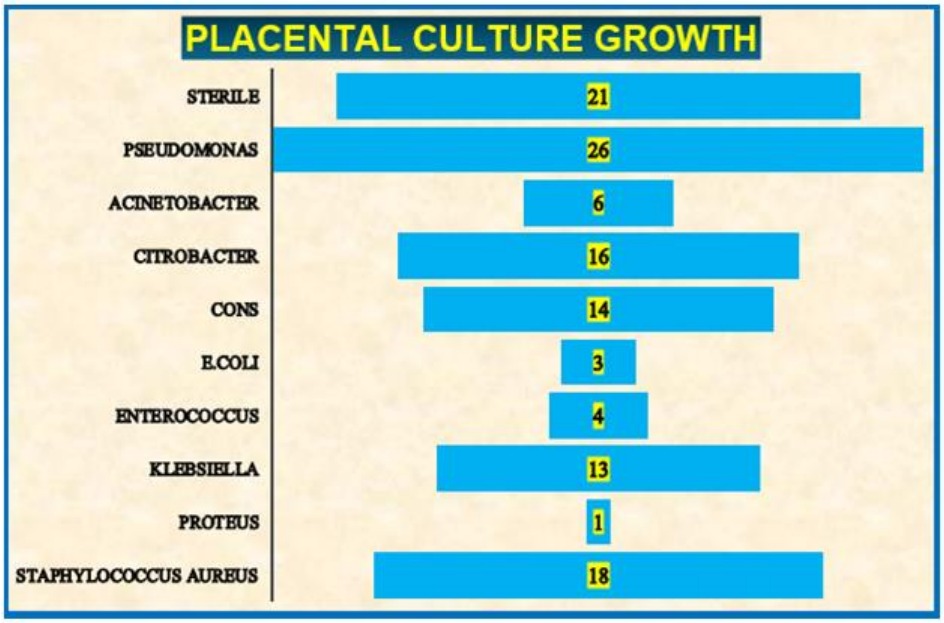

Figure 1. Distribution of organisms isolated from placental cultures.

Bar chart showing frequency (%) of different organisms isolated from placental samples (N=121). Pseudomonas was most frequent (21.3%), followed by Staphylococcus aureus (14.8%), Citrobacter (13.1%), and Klebsiella (10.7%).

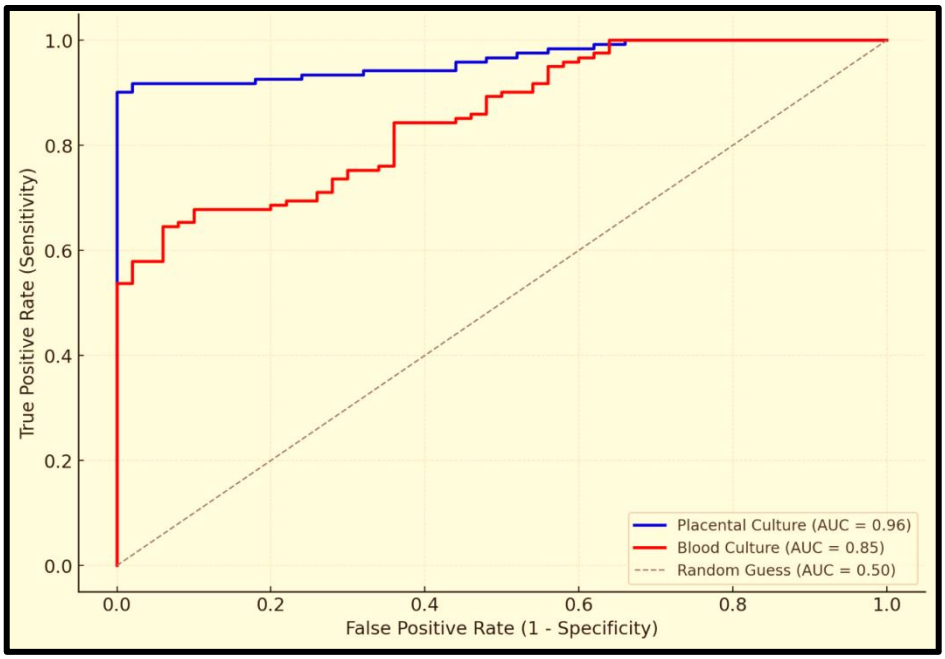

Figure 2. ROC curve comparing diagnostic accuracy of placental and blood cultures.

Receiver operating characteristic (ROC) curve analysis demonstrated superior diagnostic efficacy of placental culture (AUC 0.96, sensitivity 83.5%) compared to blood culture (AUC 0.85, sensitivity 24.8%).

DISCUSSION

Early-onset neonatal sepsis (EONS) remains a formidable challenge in neonatal care, particularly in low- and middle-income countries where it contributes significantly to morbidity and mortality.[1] Despite advances in neonatal intensive care, the diagnosis of EONS continues to rely heavily on clinical suspicion and laboratory markers that lack specificity. Blood culture, though considered the gold standard, demonstrates poor sensitivity, with reported yields as low as 36–40%.[2] This diagnostic uncertainty frequently results in the widespread empirical use of broad-spectrum antibiotics, which, although lifesaving in some cases, has far-reaching consequences such as disruption of neonatal microbiota, prolonged hospital stay, and the emergence of antimicrobial resistance.[3]

In this context, the placenta has been increasingly recognized as a "secret witness" of intrauterine events. Histopathological evidence of maternal and fetal inflammatory responses, such as chorioamnionitis, funisitis, and

vasculitis, has long been correlated with neonatal sepsis.[4,5] However, histology alone does not provide microbiological confirmation. Our study underscores that **placental microbiological culture offers a major diagnostic advantage**, complementing histopathology and outperforming blood culture in detecting causative organisms in EONS.

Placental Cultures vs. Blood Cultures

The present findings demonstrated that placental cultures were positive in 83.4% of neonates compared to only 25% positivity in blood cultures. This corroborates earlier observations that placental bacteriological cultures show a high concordance with histological chorioamnionitis[9] and provide a more accurate reflection of intrauterine microbial exposure. In fact, studies such as Wortham et al. have shown that maternal chorioamnionitis strongly predicts neonatal infection, even in infants who are asymptomatic at birth.[3] Our results align with this, as a large proportion of neonates with sterile blood cultures had positive placental growth, suggesting that blood culture alone may underestimate the true burden of infection.

The discordance between blood and placental results also highlights an important biological distinction. While a sterile blood culture may indicate either absence of bacteremia or insufficient inoculum volume, placental positivity reflects the neonate's exposure to pathogens during intrauterine life, thereby serving as an early marker of sepsis risk.[12,14]

Placental Culture and Targeted Antibiotic Therapy

A major implication of our findings is the potential role of placental cultures in guiding **early, targeted antimicrobial therapy**. Ykema et al. demonstrated that the use of placental histology in suspected EONS cases enabled early discontinuation of antibiotics in 74% of preterm neonates without compromising safety.[2] When combined with microbiological culture, this diagnostic yield is even higher, as it not only identifies inflammation but also specifies the causative organism.

This has profound clinical significance in antibiotic stewardship. Unnecessary empirical use of broad-spectrum antibiotics, such as third-generation cephalosporins or carbapenems, not only increases antimicrobial resistance but also adversely affects the developing neonatal gut microbiota.[10] Placental culture results, which are often available earlier than blood culture, can enable clinicians to narrow therapy to organism-specific regimens. For example, in our study, the predominance of *Pseudomonas* and *Klebsiella* mirrors the Indian NICU pathogen profile, distinct from Western countries where *Group B Streptococcus* dominates. Knowledge of such regional epidemiology from placental cultures allows centers to optimize empirical antibiotic policies and reduce reliance on unnecessary broad-spectrum coverage.

Correlation with Maternal Risk Factors

Placental cultures also demonstrated strong associations with maternal risk factors such as prolonged rupture of membranes, intrapartum fever, and foul-smelling liquor. These correlations support findings by Martius et al. [4]and Velemínský and Tosner [5] who emphasized the role of intrauterine infection pathways in precipitating EONS. The ability of placental cultures to capture these associations adds another dimension to their diagnostic value: they serve not only as a confirmatory test but also as a biological link between maternal events and neonatal outcomes.

Histopathology and Placental Cultures Together

Histopathology remains indispensable in identifying maternal and fetal inflammatory responses. Studies have shown that histological chorioamnionitis and funisitis are independent predictors of EONS.[4,7,15] However, histology does not identify the causative organism. Our study demonstrates that combining placental cultures with histology yields both inflammatory evidence and microbiological confirmation. This dual approach can provide clinicians with higher confidence in initiating or de-escalating therapy, improving both diagnostic precision and antibiotic stewardship.

Clinical Implications

The clinical impact of integrating placental examination into EONS evaluation is manifold:

1. **Early diagnosis:** Placental cultures, processed soon after delivery, can provide microbiological evidence even before blood culture results become available.
2. **Guided therapy:** Identification of specific organisms facilitates rational antibiotic choices, minimizing unnecessary broad-spectrum use.
3. **Stewardship:** By avoiding prolonged empirical regimens, placental cultures support global goals of antimicrobial stewardship.[2,11]
4. **Regional adaptability:** The absence of *Group B Streptococcus* in our cohort, compared to its predominance in Western cohorts, underscores the necessity of region-specific diagnostic and treatment strategies.[12]
5. **Outcome prediction:** Positive placental findings correlated with adverse neonatal outcomes such as respiratory distress, septic shock, and thrombocytopenia, demonstrating predictive clinical value beyond diagnostic confirmation.

Strengths and Limitations

The strength of this study lies in its prospective design, systematic collection of placental samples, and combined evaluation of histopathology and microbiology. The relatively high yield of placental cultures in neonates with sterile blood cultures highlights its incremental diagnostic value. Limitations include the single-center design and the potential

for contamination in placental sampling despite strict aseptic precautions. Additionally, long-term outcomes beyond hospital discharge were not assessed.

This study thus demonstrates that **placental cultures are significantly more sensitive than blood cultures in detecting EONS and provide crucial microbiological data for targeted antibiotic therapy**. When used alongside histopathology, placental examination enhances diagnostic accuracy, supports rational antibiotic use, and aligns with antimicrobial stewardship goals. Incorporation of placental cultures into diagnostic algorithms holds promise for improving neonatal outcomes, especially in LMIC settings where the burden of EONS remains high.

Future Directions

Future research should focus on multicenter validation of placental culture protocols, integration with molecular diagnostic techniques, and evaluation of cost-effectiveness in routine practice. Incorporating placental examination into neonatal sepsis diagnostic algorithms may redefine standards of care, particularly in regions with high neonatal sepsis burden and distinctive microbial epidemiology.

CONCLUSION

The present study establishes that reliance on blood culture alone for the diagnosis of early-onset neonatal sepsis (EONS) is no longer sufficient. In our cohort of 121 neonates, blood culture positivity was limited to only 25%, whereas placental membrane cultures demonstrated growth in 83.4% of cases, underscoring their superior diagnostic yield. Importantly, 74 neonates with sterile blood cultures had positive placental cultures, thereby identifying a substantial group of at-risk infants who would otherwise have been missed by conventional methods.

Placental cultures also revealed a spectrum of pathogens consistent with the regional epidemiology of Indian NICUs, where *Pseudomonas* (21.3%) and *Klebsiella* (10.7%) predominated, in contrast to the Group B Streptococcus profile observed in Western cohorts. Such region-specific diagnostic insights are crucial for tailoring empirical antibiotic regimens and curbing indiscriminate use of broad-spectrum antimicrobials. Diagnostic accuracy was further validated by ROC curve analysis, which showed an AUC of 0.96 for placental culture compared to 0.85 for blood culture, with a sensitivity of 83.5% and positive predictive value of 100%.

These findings clearly highlight the feasibility, cost-effectiveness, and reliability of placental membrane culture as a frontline diagnostic tool. When combined with histopathological evaluation—where 79% of placentas showed inflammatory changes and sensitivity reached 94.6%— the diagnostic precision for EONS is maximized. Together, these modalities provide both microbial identification and tissue-level confirmation, enabling early initiation of targeted therapy and supporting antimicrobial stewardship.

It is therefore high time to acknowledge the limitations of blood culture and move beyond its inadequacies. The authors strongly advocate for redefining the diagnostic paradigm of EONS by incorporating placental membrane culture as a **routine standard of care**, with histopathology as an adjunct wherever feasible. By doing so, neonatal care can shift from empiricism to precision, ensuring timely treatment, rational antibiotic use, and improved neonatal outcomes.

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