BRIEF COMMUNICATION

The diagnostic utility of obtaining two blood cultures for the diagnosis of early onset sepsis in neonates

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INTRODUCTION

A positive blood culture is the gold standard for diagnosing neonatal sepsis [1, 2]. Several factors may affect blood culture reliability, including blood volume obtained, bacterial colony counts, sterile technique, antibiotic exposure, laboratory techniques and culture media, and number of sites cultured [1–4]. The practice of obtaining two blood cultures from different sites in neonates is common when evaluating for late-onset sepsis (LOS) as a means of potentially increasing yield and differentiating true infection from contamination or line colonization [5]. While this practice has been widely adapted for LOS, limited data exist on its utility for early-onset sepsis (EOS) [5, 6].

The 2018 American Academy of Pediatrics guidelines for EOS state that "use of two separate culture bottles may help determine if commensal species are true infections by comparing growth in two cultures [2]." At the Yale New Haven Children's Hospital Neonatal Intensive Care Unit (NICU), the practice of obtaining two separate site blood cultures when evaluating for suspected EOS was adopted for this purpose as well as to increase the likelihood of organism recovery. This study examines the utility of a two-blood culture approach in the evaluation for EOS over a 9-year period.

DESIGN/METHODS

This investigation is part of a Yale Institutional Review Board approved retrospective study of all cases of neonatal EOS and LOS. Clinical and microbiological data were collected retrospectively on all neonates hospitalized at the Yale New Haven Children's Hospital NICU who underwent an EOS evaluation from 2013–2021. During this period, our standard antepartum/intrapartum antibiotic regimens consisted of Penicillin or Ampicillin for Group B *Streptococcus* colonization, Ampicillin/Amoxicillin and Erythromycin or Azithromycin as latency antibiotics for premature rupture of membranes, and broad-spectrum treatment (e.g., Ampicillin and Gentamicin) for suspected intra-amniotic infection.

All EOS evaluations included either an "unpaired" culture (i.e., a single culture obtained via either a newly placed central line or a peripheral site) or "paired" cultures (i.e., two central cultures, two peripheral cultures, or one of each). Peripheral cultures were obtained after cutaneous antisepsis via arterial or venipuncture. Central cultures were all obtained at the time of umbilical catheter placement. A single positive blood culture, unpaired or paired, that yielded an organism(s) not commonly associated with EOS and where the clinical team opted not to treat for ≥5 days was categorized as a contaminant. EOS was defined as one or more positive blood cultures for a common neonatal pathogen (e.g., Escherichia coli) obtained from an infant at <72 h of age and treated for ≥5 days. Infection positivity rates were estimated (with surrounding Clopper-Pearson 95% Confidence Intervals, CI) and compared between EOS evaluations from unpaired versus paired cultures using Fisher's Exact test. Infection rates were also compared between cultures obtained peripherally and centrally using Fisher's Exact test. Agreement between two different site blood cultures was estimated using the kappa statistic and reported with 95% CIs.

RESULTS

Among 43,078 live births from 2013–2021, EOS evaluations were performed in 2259 infants (44.2% females) with median gestational age of 35 weeks (range 22–44) and birth weight of 2360 grams (range 350–5340). Paired blood cultures were obtained in 1476 (65.3%) infants and a single/unpaired culture in 783 (34.7%). Thirteen of 3836 blood cultures (overall contamination rate of 0.34%), all from paired cultures, were deemed contaminants with only one contaminant (7.7%) originating from a central specimen and twelve (92.3%) from a peripheral site. Of paired cultures, excluding contaminants, both were negative in 98.3% (1481/1507), both positive in 1.1% (16/1507), and discordant (one positive and one negative) in 0.7% (10/1517) of pairs. Among unpaired/single cultures and excluding contaminants, EOS positivity rate was higher when the culture was obtained centrally versus peripherally (Table 1). Similarly, among paired cultures, the EOS positivity rate was highest and agreement strongest when there was at least one central culture obtained, and EOS positivity rate and agreement lowest when both cultures were obtained peripherally (Table 1).

DISCUSSION

The two-blood culture approach for EOS and the comparison between centrally and peripherally obtained blood cultures as part of the evaluation for EOS has not been extensively studied. Our findings reveal that agreement and precision were lowest

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Table 1. EOS evaluations and EOS positivity by paired or unpaired and site of cultures, and agreement among paired cultures[a].

	All paired cultures ($n = 1507$ pairs)	1 Central, 1 peripheral ($n = 98$ pairs)	Both central ($n = 250$ pairs)	Both peripheral ($n = 1159$ pairs)	All unpaired cultures ($n = 796$)	Peripheral unpaired ($n = 713$)	Central unpaired ($n = 83$)
Gestational age (weeks)[b]	34.1 (30.1, 38.9)	30.1 (27.9, 33.3)	27.9 (25.4, 30.6)	34.9 (33.0, 39.4)	36.3 (33.3, 39.6)	37.0 (34.0, 39.7)	31.1 (27.2, 35.7)
Birth weight (grams)[b]	2180 (1370, 3140)	1360 (1040, 2170)	1070 (740, 1510)	2440 (1830, 3310)	2650 (1905, 3370)	2735 (2065, 3400)	1465 (875, 2815)
Female[c]	660 (44.0)	37 (38.1)	108 (43.7)	515 (44.5)	350 (40.2)	319 (44.8)	31 (38.8)
EOS positivity[d]	1.2 (0.7–1.9)	4.1 (1.1–10.1)	2.0 (0.7–4.6)	0.8 (0.4–1.5)	1.5 (0.8–2.6)	1.1 (0.5–2.2)	4.8 (1.3–11.9)
Agreement[d]	75.9 (61.4–90.3)	88.4 (65.8–100.0)	79.6 (52.0–100.0)	69.3 (47.7–90.9)	N/A	N/A	N/A

EOS early onset sepsis, *Q1* 25th percentile, *Q3* 75th percentile, *CI* confidence interval.
[a]Values exclude 13 contaminant cultures.
[b]Median (Q1, Q3).
[c]N(%).
[d]Percent (95% CI).

when two peripheral cultures were obtained, with most contaminants originating from a peripheral specimen.

Furthermore, EOS positivity rates were lowest when either paired peripheral cultures or an unpaired peripheral culture were obtained.

Thirty five percent of EOS evaluations involved a single blood culture, 90% of which were peripheral, likely reflecting the inherent challenges in obtaining even a single adequate peripheral specimen from a neonate. Multiple needlesticks are often required, increasing pain and potentially increasing rates of blood culture contamination. Alternatively, the inability to obtain an adequate blood volume increases the likelihood of false positive results.

Agreement amongst paired blood cultures in our study was strongest when at least one was obtained centrally, and the highest EOS positivity rate was observed when an unpaired central culture was obtained. While blood culture volume was not available, our findings suggest that an adequate blood volume, which can be obtained more readily and reliably from a free-flowing central catheter than from a peripheral site, is a more important factor in diagnosing EOS than the number of cultures obtained.

DATA AVAILABILITY

The data that support the findings of this study are not openly available. Deidentified data are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

Dr. Fleiss participated in the data collection and statistical analysis, drafted the initial manuscript, and reviewed and revised the final manuscript. Dr. Shabanova led the data analysis, created the table in the manuscript, and reviewed and revised the initial and final manuscript. Dr. Murray participated in the study design, contributed to the initial draft of the manuscript, and reviewed and revised the final manuscript. Dr. Gallagher participated in the study design, contributed to the initial draft of the manuscript, and reviewed and revised the final manuscript. Dr. Bizzarro designed the study from the earliest stages, managed the data and participated in the data analysis, drafted the initial manuscript, and reviewed and revised the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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