RESEARCH PAPER

# Simultaneous Two-site Blood Culture for Diagnosis of Neonatal Sepsis

**PRIYA TOMAR, *AMIT GARG, RASHEE GUPTA, ABHISHEK SINGH, NAVRATAN KUMAR GUPTA AND AMIT UPADHYAY**

*From Departments of Pediatrics and *Microbiology, LLRM Medical College, Meerut, Uttar Pradesh, India.*

*Correspondence to: Dr Amit Upadhyay, Head, Department of Pediatrics, LLRM Medical college, Meerut, Uttar Pradesh. (India). au.llrm@gmail.com*

*Received: May 17, 2016; Initial review: May 31, 2016; Accepted: December 27, 2016.*

**Objective:** To evaluate efficacy of two blood cultures taken simultaneously from two different sites as compared to standard practice of single blood culture in diagnosis of neonatal sepsis.

**Study Design:** Prospective cohort study.

**Setting:** A tertiary-care center at a public hospital.

**Participants:** 475 neonates admitted to intensive care unit with suspected sepsis, from August 2014-July 2015.

**Intervention:** Two blood cultures drawn from two different peripheral veins in patients with suspected neonatal sepsis.

**Main outcome measures:** Increase in culture-positivity rate with use of two blood cultures.

**Results:** 475 babies with suspected sepsis were enrolled. 185 patients had only first culture positive (38.9%). When we added second culture positivity, yield increased to 221 (46.5%). Adding on second culture increased the culture yield by 36 (7.6%; 95% CI 2.41 to 12.79; *P*=0.018). The most common organisms isolated were *E. coli*, *S. aureus and Candida* spp. Major morbidities and mortality were more common in blood culture positive patients Contamination was ruled out in 25 babies who grew Coagulase negative Staphylococcus (CONS) *(n*=10) and Candida spp. *(n*=15) in either of the two cultures.

**Conclusion:** Two blood cultures taken simultaneously from two different sites improve rate of pathogen detection as compared to routine practice of single blood culture.

**Keywords:** *Bacteremia, Identification, Organisms, Septicemia.*

**Published online: February 02, 2017. PII:S097475591600038**

Early diagnosis and targeted therapy are essential for reducing the burden of neonatal sepsis in terms of mortality and emerging antibiotic resistance [1]. Blood culture remains the gold standard for diagnosis of sepsis [2,3]. Non-availability of blood cultures or false-negative results hamper in provision of organism-specific antibiotics. Blood culture in neonatal period faces challenges of diagnostic accuracy owing to use of intrapartum antibiotics, use of prior antibiotics in referring hospitals, and growth of organisms with low colony counts [3].

Various methods have been tried to enhance the yield of blood cultures. These include inoculation of high volume of blood into culture bottles, automated continuous blood culture monitoring systems and use of 2 or more blood cultures, maintaining blood-broth ratio of 1:5 to 1:10 and avoiding samples from indwelling catheters for risk of contamination [4-6]. In developing countries, the lack of good antenatal care, sub-standard hospital care and irrational antibiotic usage might affect the culture positivity rates. We could not find any published data from India or any other developing country on the efficacy of two blood cultures in enhancing the isolation rates of organisms in suspected neonatal sepsis.

We designed this prospective cohort study to evaluate the increase in culture positivity rate by taking two blood cultures drawn during the same time frame.

## METHODS

This study was a prospective cohort study conducted at the Neonatal division of Department of Pediatrics of a tertiary-care institute from August 2014 to July 2015. The study was approved by the institutional ethics committee.

All neonates >30 weeks of gestation admitted to the unit till 28 days of life were evaluated for sepsis. Criteria for inclusion were presence of at least one clinical feature with two or more risk-factors for sepsis. Risk factors considered were prolonged rupture of membranes (>24 hours prior to delivery), foul smelling liqor, maternal fever (temperature >100.4ºF) within 2 weeks of delivery or during labor, prolonged labor (>24hr), multiple vaginal examinations (>3 sterile or single unclean per vaginal examination), *dai* handling, delayed cry (>5min in extramural births or APGAR score <4 at 1 min in intramural births) and prematurity (<37 completed weeks), previous hospital stay and history of faulty feeding in the form of bottle feeding or use of diluted

animal milk. The clinical indicators of sepsis were respiratory distress, refusal to feed, abdominal distension, regurgitation, loose stools, hypothermia, fever, lethargy and bleeding, in absence of any other identifiable cause. Exclusion criteria were patients who had received antibiotics before blood culture sampling (***Fig.* 1**). Neonates included in the study underwent standard sepsis screen. Blood cultures were taken after cleansing the skin site with 70% isopropyl alcohol for 30 seconds followed by 1-2% tincture iodine and isopropyl alcohol again. The usual time between two cultures was about 5-8 minutes but never more than 15 minutes. 1 mL blood samples were drawn separately from two peripheral veins and inoculated into BactT/Alert (Paed Plus) bottles. The cultures were observed for 5 days before reporting as negative. Identification of bacteria and antibiotic sensitivity testing was done by standard bacterial methods as per the CLSI guidelines [7]. All Gram negative pathogens and *S. aureus* were considered true positive even if isolated in any one of the blood cultures. Isolation of CONS and *Candida* spp. in only one of the cultures was considered true culture positive only with positive sepsis screen. If two or more of the following parameters were positive, it was considered a positive sepsis screen: *(i)* Total leukocyte count <5000/cumm; *(ii)* Absolute neutrophil count: Low counts (as per Manroe chart for term and Mouzinho's chart for VLBW infants); *(iii)* Immature/total neutrophil >0.2; 4) Micro-ESR >15 mm in 1st hour; 5) C reactive protein >10 mg/l and clinical course consistent with sepsis. Candida spp. or CONS grown in both cultures were considered true culture positives. Only one of the cultures positive for CONS or Candida, with normal sepsis screen or clinical course not suggestive of sepsis, was defined as contaminant. The study participants were divided into 3 groups *i.e.* Group I (both cultures sterile), Group II (either culture positive) and Group III (both cultures positive) and their outcomes were compared (***Fig.* 1**).

A pre-designed proforma was used to gather data and patients were enrolled after taking informed written consent of the parents. The neonates were evaluated for the course of the disease and complications like pneumonia, meningitis, acute renal failure, bleeding, sclerema and death.

Primary objective of this study was diagnosis of extra cases of culture-proven sepsis with the use of two blood cultures. Our secondary objectives included comparison of clinical outcomes in the three groups, determination of common pathogenic organisms, and diagnosis of contaminants in blood culture.

In the year prior to start of this study, the culture positivity rate with use of one blood culture was 30%. In a pilot study run over one month in our unit, the overall culture positivity was 40% with double blood culture

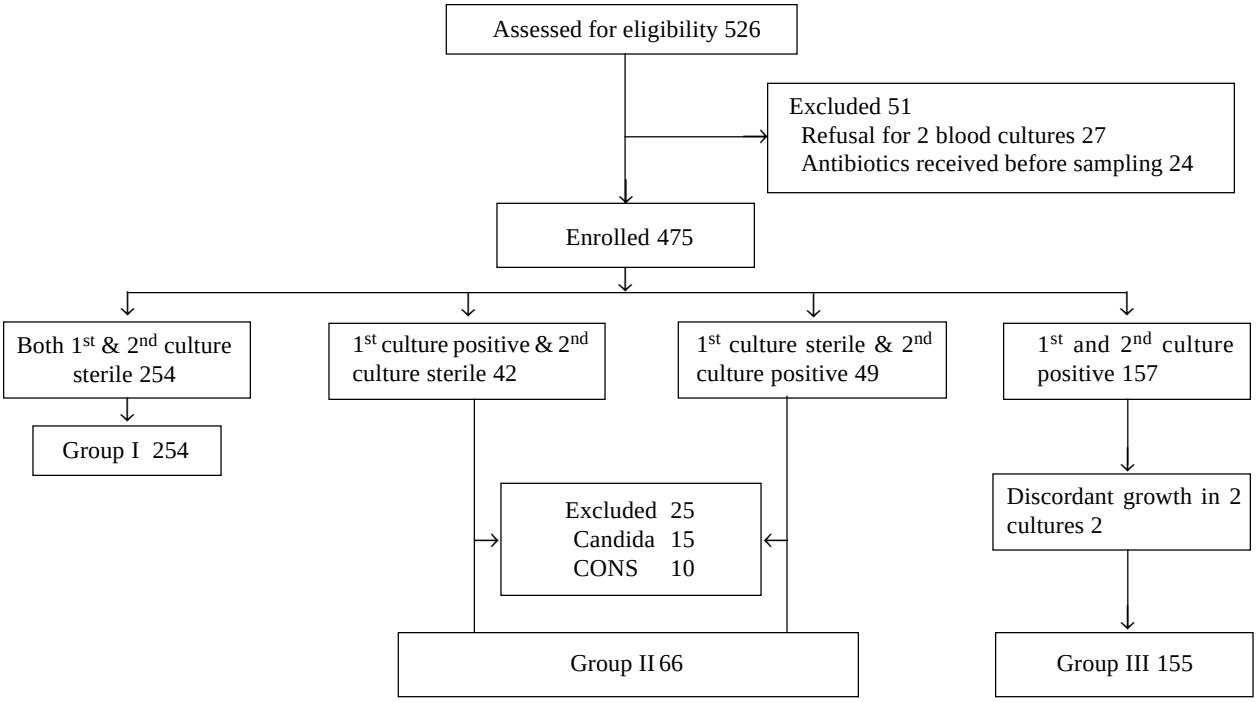


**FIG. 1** *Flowchart showing study participants.*

method. To increase the culture positivity from 30% to 40%, with risk difference of 0.1 and 2-tailed p value of 90, the estimated sample size was calculated to be 475, at an alpha error of 5%.

*Statistical analysis***:** Statistical analysis was done using Stata 12 software. Continuous variables were analyzed using t test, ANOVA/Kruskal Wallis test and multiple comparisons were done using Bonferonni adjustment. Categorical variables were analyzed using chi-square/ Fisher's exact test.

## RESULTS

There were 526 patients admitted with an initial diagnosis of suspected sepsis, of which 475 neonates were enrolled **(*Fig* 1)**. The mean (SD) gestational age was 35.1 (2.4) weeks and mean (SD) birth weight was 2.1 (0.58) kg. The median age of neonates at admission was 72 (range 1-696) hours. The most common presenting complaints were respiratory distress, poor feeding, lethargy and hypothermia.

First blood culture positive was seen in 185 cases (38.9% first culture positivity rate). When we added the second culture positivity, the yield increased to 221 (46.5% total culture positivity rate). The increase in culture yield was 36 (7.6%), with 95% CI of 2.41 to 12.79, (*P*= 0.018). Those with culture positivity had a significantly higher proportion of positive sepsis screen (45.2%) compared to those with both culture sterile (29.5%).

*E. coli*. was the most common organism isolated. *E. coli* and *Candida* spp.were the most common organisms responsible for early and late onset sepsis, respectively. None of the cultures showed polymicrobial growth. On initial evaluation, there were 91 patients in group II and 155 in group III. In group II (only one of the two cultures positive), there was growth of *E. coli* in 16, *K. pneumoniae* in 10, *S. aureus* in 38, *Candida* in 17 and CONS in 10 babies. Among 17 babies with *Candida* and 10 with CONS, only 2 with *Candida* growth were considered true culture positive. Other 25 babies (15 with candida and 10 with CONS) had negative sepsis screen and clinical course not consistent with sepsis, and were considered as contaminats.

There were two patients with discordant results: one with CONS and *Candida* spp. and the other with CONS and Klebsiella in first and second blood culture, respectively. The former was discarded as contaminant and the latter was counted as Klebsiella-positive under group II. The details of organisms isolated in the cultures is provided in ***Table* I**.

There were significantly more LBW babies (74%) and preterms (49%) in the culture-positive group than in Group I (59% and 24%, respectively). Major morbidities and mortality were more common in blood culture-positive patients (Group II or Group III) as compared to culture-negative babies in Group I. However, there was no significant difference between Group II and Group III (***Table* II**).

## DISCUSSION

It was observed in the present study that taking two blood cultures increased the culture yield by 7.6%. Major morbidities were comparable in babies with either one or both cultures positive but they were more than in babies with both cultures sterile.

Previous studies on culture positivity in neonates have reported an isolation rate ranging from 25-60% [8–10]. Although few studies have reported lower culture positivity rates but it could be due to low blood volume

**TABLE I** DISTRIBUTION OF ORGANISMS IN BLOOD CULTURE SAMPLES

| *Organism isolated* | *Isolated only in 1st culture* | *Discarded as contaminant* | *Included in Group II** | *Isolated only in 2nd culture* | *Discarded as contaminant* | *Included in Group II** | *Group III** | *Different organisms in two cultures* |
|---|---|---|---|---|---|---|---|---|
| *Escherichia coli (n*=63) | 8 | 0 | 8 | 8 | 0 | 8 | 47 | 0 |
| *Klebsiella pneumonia (n*=38) | 4 | 0 | 4 | 6 | 0 | 6 | 28 | 1 |
| *Pseudomonas aerugi- nosa (n*=2) | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *Acenitobacter baumannii (n*=2) | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *Staphylococcus aureus (n*=56) | 17 | 0 | 17 | 21 | 0 | 21 | 18 | 0 |
| CONS** *(n*=8) | 6† | 6 | 0 | 4† | 4 | 0 | 8 | 2 |
| Candida sps (*n=52*) | 7† | 7 | 0 | 10‡ | 8 | 2 | 50 | 1 |

***Coagulase negative Staphylococcus, †All discarded as contaminants, ‡8 discarded as contaminants; *Group I- Culture negative patients, Group II- Single blood culture positive, Group III- Both cultures positive.*

**TABLE II** OUTCOMES IN BLOOD CULTURE POSITIVE AND NEGATIVE GROUPS

| | *Group (%)* | | | *P values* | | |
|---|---|---|---|---|---|---|
| *Morbidities/outcome* | *I*(n=254)* | *II*(n=66)* | *III* (n=155)* | *I vs II* | *I vs III* | *II vs III* |
| Pneumonia *(n*= 54) | 10 (4) | 11 (16.7) | 33 (21) | <0.001 | <0.001 | 0.431 |
| Meningitis *(n*=32) | 0/77 (0) | 12/44 (27) | 20/82 (24.4) | <0.001 | <0.001 | 0.723 |
| Bleeding *(n*=86) | 24 (9.5) | 19 (29) | 43 (27.7) | <0.001 | <0.001 | 0.874 |
| Acute renal failure *(n*=12) | 3 (1.2) | 3 (4.5) | 6 (4) | 0.073 | 0.072 | 0.816 |
| Sclerema *(n*=7) | 0 (0) | 2 (3) | 5 (3) | 0.005 | 0.004 | 0.939 |
| NEC *(n*=12) | 0 (0) | 3 (4.5) | 9 (6) | 0.049 | 0.026 | 0.830 |
| Thrombocytopenia *(n*=166) | 54 (21) | 31 (47) | 81 (52) | <0.001 | <0.001 | 0.472 |
| Mortality *(n*=128) | 44 (17) | 20 (30) | 64 (41) | 0.019 | <0.001 | 0.124 |

**Group I- Culture negative patients, Group II- Single blood culture positive, Group III- Both cultures positive.*

taken for culture or administration of antibiotic before blood collection [11,12].

Wiswell, *et al*. [13] were among the first to document advantage of two site blood cultures in initial evaluation of neonatal sepsis. They had taken 2 sets of blood cultures (1 aerobic and 1 anerobic) from different sites in 460 inborn infants during 1st week of life. In 8 neonates, bacteremia was confirmed while in 10 cases, contamination from skin flora was documented. This was a retrospective study and included neonates upto first week of life only.

Sarkar, *et al*. [14], in a prospective study had taken blood cultures from two different peripheral sites 15- 30 minutes of each other in 216 neonates with suspected sepsis. In their study, 20 (9.2%) of 216 neonates had 22 episodes of culture-proven sepsis. All neonates with positive cultures grew the same organism with a similar sensitivity pattern from the two different peripheral sites. The remaining 196 neonates had negative blood cultures from both the sites. They did not document any advantage of double site cultures in detection of neonatal septicemia. The difference in results from the present study could be due to smaller sample size and inclusion of only inborn neonates.

CONS and *Candida* spp. are frequently isolated organisms in neonates admitted in neonatal intensive care units [15]. As these organisms form part of the skin flora, they can be mere contaminants in a blood culture growth if the skin is not prepared well before taking culture [16–18]. Struthers, *et al.* [19] conducted a prospective study with the objective of differentiating pathogenic from contaminant CONS and reducing antibiotic usage. One hundred pairs of cultures were drawn from two percutaneous sites from 69 babies with suspected sepsis after 48 hours of life. They also considered one positive culture of CONS as contaminant and both positive cultures as infection. They differentiated between contaminant CONS in 5 neonates who had growth in one of the two cultures only and pathogenic CONS in 16 neonates with both positive cultures. In contrast, the present study had a significant proportion of isolates which were reported as contaminants.

The strength of the study is its prospective design, large sample size, a representative sample including extramural babies with both early and late neonatal sepsis. Limitations of our study were that colony count and time to positivity could not be documented.

It is concluded that two simultaneous blood cultures significantly increased culture positivity in neonates with sepsis. This could be useful in referral neonatal units where the admission rates for babies with sepsis are high. As the overall incidence of CONS and fungal sepsis is on an upsurge, the policy of two blood cultures can be helpful to rule out contamination in units with high rates of these organisms.

Contributors: PT, AG, AU: have contributed to conception and design of the work, analysis and interpretation of data; PT, RG: have worked on data compilation together; AS, AG, NG: have revised the work critically. All the authors have approved for this version to be published. AU: will act as guarantor of the study.
*Funding*: None; *Competing interests*: None stated.

## REFERENCES

1. Bedi N, Gupta P. Antimicrobial stewardship in pediatrics: An Indian perspective. Indian Pediatr. 2016;53:293-8.
2. Baltimore RS. Neonatal nosocomial infections. Semin Perinatol. 1998;22:25-32.
3. Paolucci M, Landini MP, Sambri V. How can the microbiologist help in diagnosing neonatal sepsis? Int J Pediatr. 2012;2012:120139.
4. Li J, Plorde JJ, Carlson LG. Effects of volume and periodicity on blood cultures. J Clin Microbiol.

**WHAT IS ALREADY KNOWN?**

- Blood culture is the gold standard for diagnosis of neonatal sepsis.

**WHAT THIS STUDY ADDS?**

- Two simultaneous blood cultures are useful in detecting more cases of neonatal sepsis and ruling out contamination.

1994;32:2829-31.
5. Lee A, Mirrett S, Reller LB, Weinstein MP. Detection of bloodstream infections in adults: How many blood cultures are needed? J Clin Microbiol. 2007;45:3546-8.
6. Buttery JP. Blood cultures in newborns and children: Optimising an everyday test. Arch Dis Child - Fetal Neonatal Ed. 2002;87:F25-8.
7. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI document M100-S18. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
8. Mugalu J, Nakakeeto MK, Kiguli S, Kaddu-Mulindwa DH. Aetiology, risk factors and immediate outcome of bacteriologically confirmed neonatal septicaemia in Mulago hospital, Uganda. Afr Health Sci. 2006;6:120-6.
9. Jain NK, Jain VM, Maheshwari S. Clinical profile of neonatal sepsis. Kathmandu Univ Med J. 2003;1:117-20.
10. Kaistha N, Mehta M, Singla N, Garg R, Chander J. Neonatal septicemia isolates and resistance patterns in a tertiary care hospital of North India. J Infect Dev Ctries. 2010;4:55-7.
11. Agnihotri N, Kaistha N, Gupta V. Antimicrobial susceptibility of isolates from neonatal septicemia. Jpn J Infect Dis. 2004;57:273-5.
12. Galhotra S, Gupta V, Bains H, Chhina D. Clinico-bacteriological profile of neonatal septicemia in a tertiary care hospital. J Mahatma Gandhi Inst Med Sci. 2015;20:148-52.
13. Wiswell TE, Hachey WE. Multiple site blood cultures in the initial evaluation for neonatal sepsis during the first week of life. Pediatr Infect Dis J. 1991;10:365-9.
14. Sarkar S, Bhagat I, DeCristofaro JD, Wiswell TE, Spitzer AR. A study of the role of multiple site blood cultures in the evaluation of neonatal sepsis. J Perinatol. 2005;26:18-22.
15. Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev. 2006;19:788-802.
16. Agarwal J, Bansal S, Malik GK, Jain A. Trends in neonatal septicemia: emergence of non-albicans Candida. Indian Pediatr. 2004;41:712-5.
17. Mirrett S, Weinstein MP, Reimer LG, Wilson ML, Reller LB. Relevance of the number of positive bottles in determining clinical significance of coagulase-negative staphylococci in blood cultures. J Clin Microbiol. 2001;39:3279-81.
18. Thylefors JD, Harbarth S, Pittet D. Increasing bacteremia due to coagulase-negative staphylococci: fiction or reality? Infect Control Hosp Epidemiol. 1998;19:581-9.
19. Struthers S, Underhill H, Albersheim S, Greenberg D, Dobson S. A comparison of two versus one blood culture in the diagnosis and treatment of coagulase- negative staphylococcus in the neonatal intensive care unit. J Perinatol. 2002;22:547-9.