ORIGINAL RESEARCH

Utility of an Extra Blood Culture in the Diagnosis of Neonatal Sepsis

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ABSTRACT
Background: Sepsis is a major cause of preventable neonatal mortality and morbidity around the world. We evaluated increase in culture positivity rate of two blood cultures taken simultaneously from different sites as compared to single blood culture in diagnosing sepsis in neonates. **Material and methods:** This was a descriptive cross sectional study done in NICU of a tertiary-care centre from September 2017 to June 2018 on 200 neonates. Two blood cultures were drawn from two different peripheral veins in neonates with suspected sepsis. Primary outcome measure was increase in culture positivity rate with use of two blood cultures. **Results:** Thirty eight (19%) neonates had positive diagnostic blood cultures from both sites. Twenty five (12.5%) neonates grew organism from one site but not from the other site. 137 (68.5%) patients had sterile cultures from both sites. Overall, first culture positivity was seen in 50 cases (25% first culture positivity rate). On adding the result of second culture, the yield of positivity increased to 63 cases (31.5% total culture positivity rate). Thus adding on second culture, yield increased in 13 cases but it was statistically not significant (6.5%) (CI: -2.3%-15.3%) (p=0.148). **Conclusion:** There is no role of sending simultaneous two site blood cultures in the diagnosis of neonatal sepsis.
Keywords: Blood culture, India, NICU, Sepsis, Yield

What is already known?
Blood culture is the gold standard test for diagnosing sepsis in neonates.

What this study adds?
There is no role of sending simultaneous two site blood cultures in the diagnosis of neonatal sepsis.

INTRODUCTION
Neonatal sepsis leads to an estimated 1.6 million deaths per year globally and 40% of neonatal mortality in developing countries likes India [1]. Neonatal sepsis is a significant health issue in India (30-40 cases per 1000 live births) [2]. Better NICU care has been able to save more preterm babies but also added on to increased sepsis-related mortality due to use of invasive procedures like endotracheal intubation, ventilation and placement of central lines. Prompt diagnosis and antimicrobial therapy is vital for decreasing morbidity and mortality associated with neonatal sepsis.

Drug resistance due to indiscriminate and injudicious use of antibiotics is a rising cause of failure of medical treatment and contributes significantly to increasing mortality [3]. An important strategy to prevent drug resistance is judicious use of antibiotics. Blood culture is the gold standard test for diagnosis of neonatal sepsis [4]. Blood culture may have poor yield owing to mother getting intra-partum antibiotics, babies getting prior antibiotics from referring hospitals and growth with low colony counts.

To increase the yield of blood cultures, inoculation of greater volume of blood into culture bottles, automated blood culture monitoring system, getting multiple blood cultures, taking blood/broth ratio of

1:5-10 and avoiding samples from indwelling lines, has been used [5]. Multiple blood cultures taken from two different sites have found to improve the culture yield in adults. Few studies from developed countries have suggested the advantage of multiple blood cultures in diagnosing or ruling out neonatal sepsis [6]. We found only one published study from India on usefulness of double site blood cultures as compared to conventional single site blood culture in neonatal sepsis. The role of two site blood cultures in diagnosis of neonatal sepsis needs to be further explored.

With this background, a descriptive cross sectional study was planned to evaluate improvement in culture positivity rate by getting two blood cultures from different sites within a time period of 15 minutes.

MATERIAL AND METHODS

We conducted a descriptive cross sectional study in the NICU of paediatrics department, Sanjay Gandhi Memorial Hospital, New Delhi from September 2017 to June 2018. Prior to the commencement, the study protocol was approved by the Institutional Ethical and Research Committee (Approval no: SGMH/Sandeep Singh/Secondary DNB/ 11-09-2017).

Neonates with gestational age >30 weeks were evaluated for neonatal sepsis who presented within 28 days of life. Neonates presenting with at least one clinical feature suggestive of sepsis or at least two risk factors of sepsis or positive septic screen (at least 2 out of 5 positive parameters) were included in the study. Risk factors for neonatal sepsis considered were: rupture of membranes for >24 hours prior to delivery, foul smelling liquor, history of maternal fever (temperature >100.4ºF) within 2 weeks prior to delivery or during labour, prolonged labour (>24hours), multiple per vaginal examinations (>3 sterile or single unclean), handling of newborn by dai during birth, history of delayed cry (>5 min; extramural births) or APGAR score <4 at 1 min (intramural births) and prematurity (<37 completed weeks), history of prior hospital admission and history of bottle feeding or feeding of diluted animal milk.

The clinical features of sepsis included respiratory distress, decreased or refusal to feed, distension of abdomen, regurgitation, loose stools, hypothermia, fever, lethargy and bleeding from any site, without any other identifiable cause. Exclusion criteria included neonates receiving inadvertent antibiotics after admission before blood sample could be collected or refractory shock.

All the parents/ caregivers of the children who fulfilled the selection criterion were explained about the study and a written, valid and informed consent was obtained before enrolment of each patient. Detailed history and clinical examination were done as necessary in each patient at enrolment.

All babies underwent bed side sepsis screen. Sepsis screen consisted of the following tests: total white blood cell count, absolute neutrophil count, immature/total neutrophil (band cell) ratio, C-reactive protein and micro-erythrocyte sedimentation rate. A baby with at least two positive parameters was considered to have positive sepsis screen.

Two blood cultures were sent prior to start of antibiotics under aseptic precautions with samples taken from two different sites but within 15 minutes of duration. One ml of blood was taken from both sites after part preparation under complete asepsis and transferred to BACTEC paediatric blood culture bottles. Bottles were shifted to microbiology lab as soon as possible. If a delay was anticipated, bottles were kept at room temperature. It was observed for 5 days before reporting as negative. Standard methods were used for bacterial identification. KirbyBauer disc diffusion method was employed for sensitivity testingas per the Clinical and Laboratory Standards Institute guidelines [7]. The microbiologist promptly informed positive reports to concerned clinician. Lumbar puncture for CSF analysis and other relevant investigations were done as and when indicated.

Depending on blood culture results, enrolled neonates were destined to be divided into 3 groups, i.e. Group I (both culture sterile), Group II (either culture positive) and Group III (both culture positive).

Sample size calculation: From previous studyit was observed that yield increases by 7.6% after adding second culture positivity [8]. So, the minimum required sample size with 90% power of study and 5% level of significance is 158. To reduce margin of error, total sample size taken was 200. (By using formula $n \geq [P_C(1-P_C)+P_E(1-P_E)][(Z_\alpha + Z_\beta)^2]/\delta_0^2$ with P_C=Percentage of increase in yield, P_E= Null hypothesis value (0%), $\delta_0 = P_E - P_C$ and Z_α is value of Z at two sided alpha error of 5% and Z_β is value of Z at power of 90%.)

Statistical analysis: Categorical variables were presented in number and percentage (%). Continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non-parametric test were used. McNamer test was used to compare paired proportion i.e. yield of first culture positivity and yield of both culture positivity. Qualitative variables were compared using Chi-Square test /Fisher's exact test. Quantitative variables were compared using ANOVA/Kruskal Wallis test (when the data sets were not normally distributed) between three groups and unpaired ttest/Mann-Whitney Test (when the data sets were not normally distributed) between the two groups. A p value of <0.05 were considered statistically significant. The data were entered in MS Excel spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.

RESULTS

There were 458 admissions in the unit during the study period with 222 patients with a provisional

diagnosis of neonatal sepsis. Finally, 200 patients were enrolled for the study out of which 94 (47%) were institutional births and 106 (53%) were referred from elsewhere for further management.
Mean (SD) gestational age of enrolled babies was 35.6± 5.7 weeks and mean (SD) birth weight 2120±630 kg. There was 104 were males and 96 females (male: female ratio of 1.1:1). The median age of neonates at admission was 72 hours **(Table 1)**.

Primary outcome: 38(19%) neonates had positive diagnostic blood cultures from both sites. 25 (12.5%) neonates grew organism from one site but not from the other site. 137 (68.5%) patients had sterile cultures from both sites. Of the 200 cultures sent, first culture positivity was found in 50 cases (25% 1st culture positivity rate). When we added the result of second culture, the yield of positivity increased to 63(31.5% both culture positivity rate). Thus adding on second culture, yield increased in 13 cases but it was statistically not significant (6.5%) (CI: -2.3%-15.3%) (p=0.148).

Clinical presentation: Neonates most commonly presented with clinical features of respiratory distress, decreased feeding or refusal to feed, lethargy, hypothermia, abnormal movements, abdominal distension and loose stools. Meningitis, need for ventilation and mortality rate were statistically more common in culture positive than in culture negative patients. Meningitis was seen in 11 culture positive and 1 culture negative patients (Odds ratio 28.77, p value= 0.001). Need of ventilation was seen in 18 culture positive and 16 culture negative patients (Odds ratio 3.02, p value= 0.004). Similarly, mortality was seen in 23 culture positive and 23 culture negative patients (Odds ratio 2.72, p value= 0.004) (**Table 2**).
Lumbar puncture was indicated in 102 patients but could be carried out on 80 babies. Of these 12 (15%) had CSF findings suggestive of meningitis. 1 (1.2%), 4 (5%), 7(8.7%) patients belonged to Group I, II and III respectively.
Sepsis rates were comparable in institutional and referred babies from other hospitals (**Table 1 and 2**). Low birth weight and preterm babies had significantly higher chances of sepsis (**Table 3**). Antenatal factors like prolonged rupture of membranes, prolonged labour, maternal fever, foul smelling liquor, dai handling and multiple vaginal examinations prior to delivery were significantly associated with sepsis. Significant post natal factor for sepsis was faulty feeding (**Table 3**). The various components of the sepsis screen were more positive in culture positive than culture negative patients. (**Table 4**) However there was no difference in one or both culture positive patients.
The incidence of confirmed Gram negative, Gram positive and fungal septicaemia was 57%, 25% and 17% respectively.

Comparison of study groups (Group I vs. II vs. III): Enrolled neonates were divided into 3 groups, i.e. Group I (both culture sterile), Group II (either culture positive) and Group III (both culture positive). There were 137 patients in Group I, 25 patients in Group II and 38 patients in Group III. In Group II, there was *E. coli* in 6, *K. pneumoniae* in 4, *S. aureus* in 13 and Candida in 2 patients. Gram negative pathogens were considered true culture positive. Two babies with Candida growths had sepsis screen positive and clinical course consistent with sepsis and were taken as culture positive and managed accordingly. So, there were no contaminants found. In Group III, there was *E. coli* in 12, *K. pneumoniae* in 13, *S. aureus* in 2, Candida in 9, CoNS in 1 and *Acinetobacter* in 1 patient. Significant neonatal morbidity and mortality were found to be more common in blood culture positive patients but no difference in neonates with one or both blood cultures positive (**Table 5**).
Gram positive versus Gram negative sepsis: The incidence of gram negative bacteraemia i.e. 36 (57%) was more than gram positive bacteraemia i.e. 16 (25%). Complications like pneumonia, acute renal failure, thrombocytopenia, meningitis and requirement of ionotropic support were comparable in both groups. However, need for ventilation and mortality were more common with Gram negative bacteraemia but it was statistically not significant. (**Table 6**)
Early onset vs. late onset sepsis:Of the culture proven cases, 42 babies (66.6%) had early onset sepsis (<72 hours of life) and 21(33.3%) had late onset sepsis (>72 hours of life). Incidence of pneumonia, meningitis, acute renal failure and need for ventilation were comparable in both groups. Occurrence of thrombocytopenia, ionotropic requirement and development of NEC were higher in early onset septicaemia but statistically it was not significant. Mortality was seen to be higher in late onset sepsis . Antibiotic susceptibility pattern of gram positive and negative isolates are shown in **Tables 5 and 6**

Outcome in neonatal septicaemia: Of the 63 patients with confirmed sepsis, 23 died during the period of hospitalization (Case fatality rate: 36.5%) and 40 (63.5%) were discharged to home. Mortality with late onset sepsis was more than with early onset sepsis but it was found to be statistically not significant.

Table 1: Baseline variables in study participants

Baseline variables	Total Culture negative 137	Total Culture Positive 63	Odd's ratio n95% CI	P Value
Gender Male n=104	70(51.1)	34(53.9)	1.12(0.61-2.04)	0.70
Place of delivery Extramural n=106	71(51.8)	35(55.6)	1.16(0.63-2.11)	0.62
Place of delivery Intramural n=94	66(48.2)	28(44.4)	0.86(10.7-18.2)	0.5
Gestational age <37 weeks n=120	75(54.7)	45(71.4)	2.06(1.81-6.21)	0.02
Birth weight<2.5 kg n= 126	80(58.4)	46(73.0)	1.92(1.0-3.69)	**0.048**
Birth weight<1.5 kg n= 47	19(13.8)	28(44.4)	5.14(2.57-10.26)	**<0.0001**

*Values in brackets represent figures in percentage

Table 2: Clinical features in culture negative and culture positive patients

Clinical feature	Total Culture negative 137	Total Culture Positive 63	Odd's Ratio 95% CI	P Value
Respiratory distress n=160	109(79.6)	51(80.9)	1.09(0.512.31)	0.81
Need for ventilation n= 34	16(11.7)	18(28.6)	3.02(1.426.43)	**0.0041**
Poor feeding n=30	20(14.6)	10(15.9)	1.10(0.482.52)	0.8147
Regurgitation n=6	4(2.9)	2(3.2)	1.09(0.196.11)	0.92
Abdominal distension n=4	2(1.5)	2(3.2)	2.21(0.30-16.08)	0.432
Seizures n=8	6(4.4)	2(3.2)	0.71(0.143.64)	0.68
Lethargy n=14	9(6.6)	5(7.9)	1.22(0.393.81)	0.72
Jaundice n=11	9(6.6)	2(3.2)	0.46(0.092.22)	0.33
Hypothermia n=12	8(5.8)	4(6.4)	1.09(0.313.77)	0.88
Meningitisn=12	1(0.7)	11(17.5)	28.77(3.62228.43)	**.001**
Mortality n=46	23(16.8)	23(36.5)	2.72(1.37-5.39)	**0.004**

Table 3: Risk factors in culture negative and culture positive patients

Risk factors	Total Culture negative 137	Total Culture positive 63	Odd's Ratio 95% CI	Univariate p value
Prematurity n=120	75(54.7)	45(71.4)	2.98(1.816.21)	**0.02**
Low birth weight n=126	80(58.4)	46(73.0)	1.92(1.003.69)	**0.048**
Asphyxia n=60	41(29.9)	19(30.2)	1.01(0.521.93)	0.97
Prolonged PV Leaking n=29	12(8.8)	17(26.9)	3.84(1.708.67)	**0.001**
Prolonged labour n=31	2(1.5)	29(46.0)	57.57(13.08253.28)	**<0.0001**
Dai handling n=20	5(3.6)	15(23.8)	8.25(2.84-23.92)	**0.0001**
Foul smelling Liquor n=9	2(1.5)	7(11.1)	8.43(1.7-41.87)	**0.0091**
Maternal fever n=14	5(3.6)	9(14.3)	4.4(1.40-13.73)	**0.0107**
Multiple vaginal examinations n=24	11(8.0)	13(20.6)	2.97(1.257.08)	**0.0136**
Faulty feeding n=22	7(5.1)	15(23.8)	5.80(2.23-15.10)	**0.0003**

*Values in brackets represent figures in percentage

Web Table 1: Laboratory parameters in the three groups

S.No.	Lab parameter	Group I* 137	Group II* 25	Group III* 38	P 1 vs.2**	P 1 vs. 3**	P 2vs. 3**
1	Total leukocyte count<5000/cumm	7(5.1)	5(20.0)	8(21.1)	**.028**	**.005**	.082
2	Low absolute neutrophil count#	7(5.1)	6(24.0)	10(26.3)	**.005**	**.0003**	.92
3	Micro-ESR≥ 15mm in first hour	13(9.5)	8(32.0)	14(36.8)	**.006**	**.0001**	.91
4	Reactive CRP≥ 10mg/l	20(14.6)	10(40.0)	16(42.1)	**.006**	**.0005**	.92
5	Immature/total neutrophil≥ 0.2	8(5.8)	5(20.0)	9(23.6)	**.046**	**.003**	.97
6	Platelet count<1.5 lac/cumm	70(51.1)	20(80.0)	32(84.2)	**.014**	**.0005**	.93
7	Sepsis screen Positive## n=61	30(21.9)	12(48.0)	19(50.0)	**.013**	**.001**	.92

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

#as per Manroe chart for term and Mouzinho's chart for VLBW infants
two or more parameters are abnormal
^Values in brackets represent figures in percentage

Web Table 2: Secondary outcomes in three groups

Outcomes	Group I* 137	Group II* 25	Group III* 38	P 1 vs. 2**	P 1 vs. 3**	P 2 vs. 3**
Pneumonia n= 17	5(3.6)	4(16.0)	8(21.1)	**0.033**	**0.001**	0.51
Meningitis n=12	1(0.7)	4(16)	7((18.4)	**0.002**	**<0.0001**	1.00
Acute renal failure n=6	1(0.7)	3(12.0)	2(5.3)	**0.012**	**0.11**	0.37
Sclerema n=3	0(0)	1(4.0)	2(5.3)	0.15	**0.04**	1
Thrombocytopenia n=122	70(51.1)	20(80.0)	32(84.2)	**.014**	**.0005**	.93
NEC n=3	0(0)	1(4.0)	2(5.3)	0.15	0.46	1
requirement of Inotropes n=57	28(20.4)	11(44.0)	18(47.4)	**0.02**	**0.002**	0.99
Need for ventilation n=34	16(11.7)	6(24.0)	12(31.6)	0.18	**0.007**	0.71
Mortality n=46	23(16.8)	8(32.0)	15(39.5)	0.13	**0.005**	0.73

*Group I- Culture negative patients, Group II- Single blood culture positive patients, Group III-Both cultures positive patients, ** post hoc analysis,
^Values in brackets represent figures in percentage

Web Table 3: Outcomes in patients with Gram negative and Gram positive growths

Outcome	Gram Negative 36	Gram Positive 16	p value
Sepsis Screen Positive n=21	15(41.7)	6(37.5)	0.98
Pneumonia n= 11	7(19.4)	4(25.0)	0.71
Meningitis n=10	7(19.4)	3(18.8)	1.0
Acute renal failure n=4	3(8.3)	1(6.2)	1.0
Sclerema n=3	2(5.5)	1(6.2)	1.0
Thrombocytopenia n=35	24(66.7)	11(68.8)	0.86
NEC n=3	2(5.5)	1(6.2)	1.0
requirement of Inotropes n=21	17(47.2)	4(25.0)	0.22
Need for ventilation n=16	12(33.3)	4(25.0)	0.74
Mortality n=20	17(47.2)	3(18.8)	0.68

^Values in brackets represent figures in percentage

Web Table 4: Outcomes in Early onset and late onset sepsis

Outcome	EOS 42	LOS 21	P value
Sepsis Screen Positive n=31	20(47.6)	11(52.4)	0.72
Pneumonia n= 12	8(19.0)	4(19.0)	1.0
Meningitis n=12	7(16.7)	5(23.8)	0.50
Acute renal failure n=5	3(7.1)	2(9.5)	1.0
Sclerema n=2	1(2.4)	1(4.8)	1.0
Thrombocytopenia n=46	33(78.6)	13(61.9)	0.27
NEC n=2	2(4.8)	0(0)	0.54
Requirement of Inotropes n =29	22(52.4)	7(33.3)	0.24
Need for ventilation n=18	12(28.6)	6(28.6)	0.76
Mortality n=23	13(30.9)	10(47.6)	0.30

^Values in brackets represent figures in percentage

Web Table 5: Antibiotic resistance in Gram positive isolates

Antibiotics	*S. aureus* 15	CoNS 1
Ampicillin	9 (60.0)	1 (100)
Amoxycillin/ clavulanic acid	8 (53.3)	0(0)
Levofloxacin	6 (40.0)	0(0)

Ciprofloxacin	5 (33.3)	0(0)
Amikacin	9 (60.0)	0(0)
Netilmycin	10(66.7)	0(0)
Clindamycin	4 (26.7)	0(0)
Erythromycin	9 (60.0)	0(0)
Meropenem	1(6.7)	0(0)
Linezolid	0 (0)	0(0)
Teicoplanin	0 (0)	0 (0)
Vancomycin	0 (0)	0 (0)

^Values in brackets represent figures in percentage

Web Table 6: Antibiotic resistance in Gram negative isolates

Antibiotics	*E. coli* 18	*K. pneumoniae* 17	*A. baumannii* 1
Amoxycillin/ clavulanic Acid	7(38.9)	10(58.8)	1 (100)
Ciprofloxacin	7 (38.9)	7 (41.2)	1 (100)
Amikacin	6(33.3)	6(35.3)	1 (100)
Gentamycin	5 (27.8)	5 (29.4)	1 (100)
Clindamycin	7(38.9)	8(47.1)	1 (100)
Ceftriaxone	5(27.8)	6 (35.3)	1 (100)
Piperacillin/ Tazobactam	5(27.8)	6 (35.3)	1 (100)
Meropenem	2(11.1)	1(5.9)	1 (100)
Polymixin	0 (0)	0 (0)	0 (0)
Colistin	0 (0)	0 (0)	0 (0)

^Values in brackets represent figures in percentage

Figure 1: Flowchart showing study participants

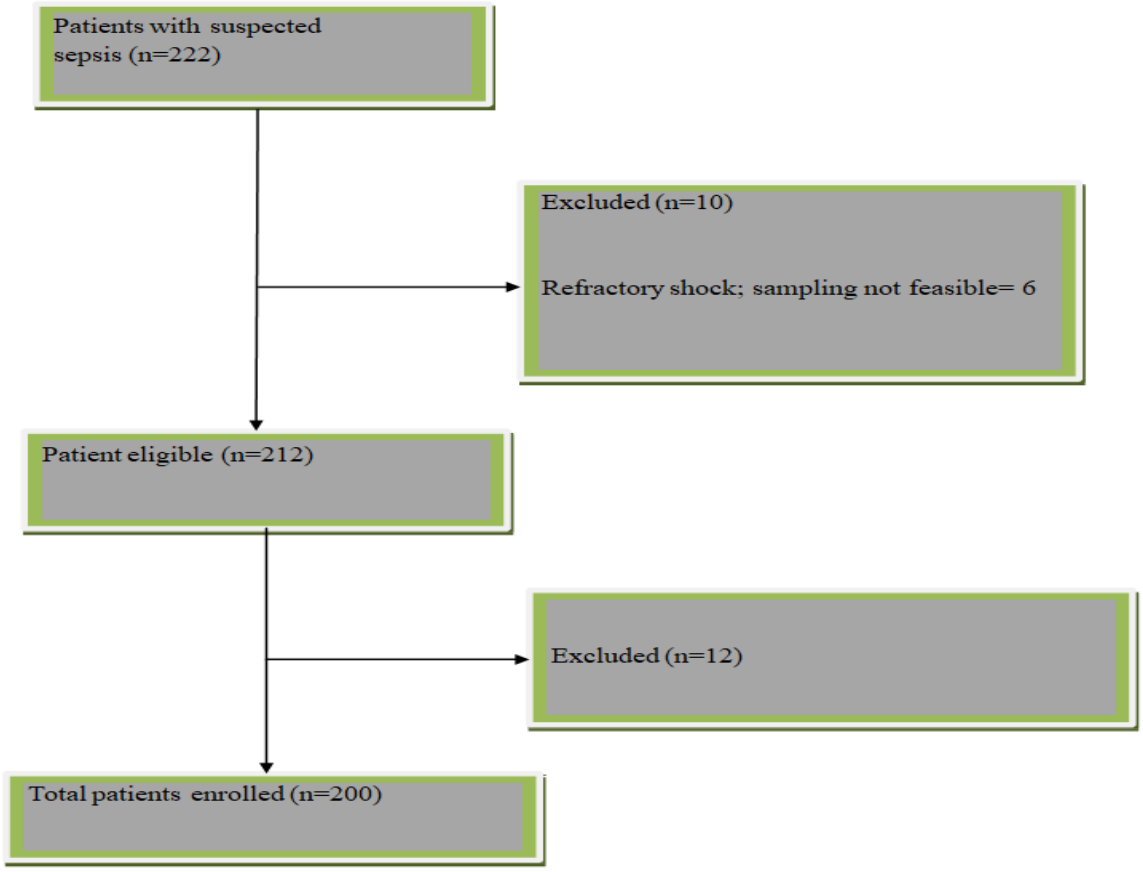


Figure 2 A: Distribution of study groups

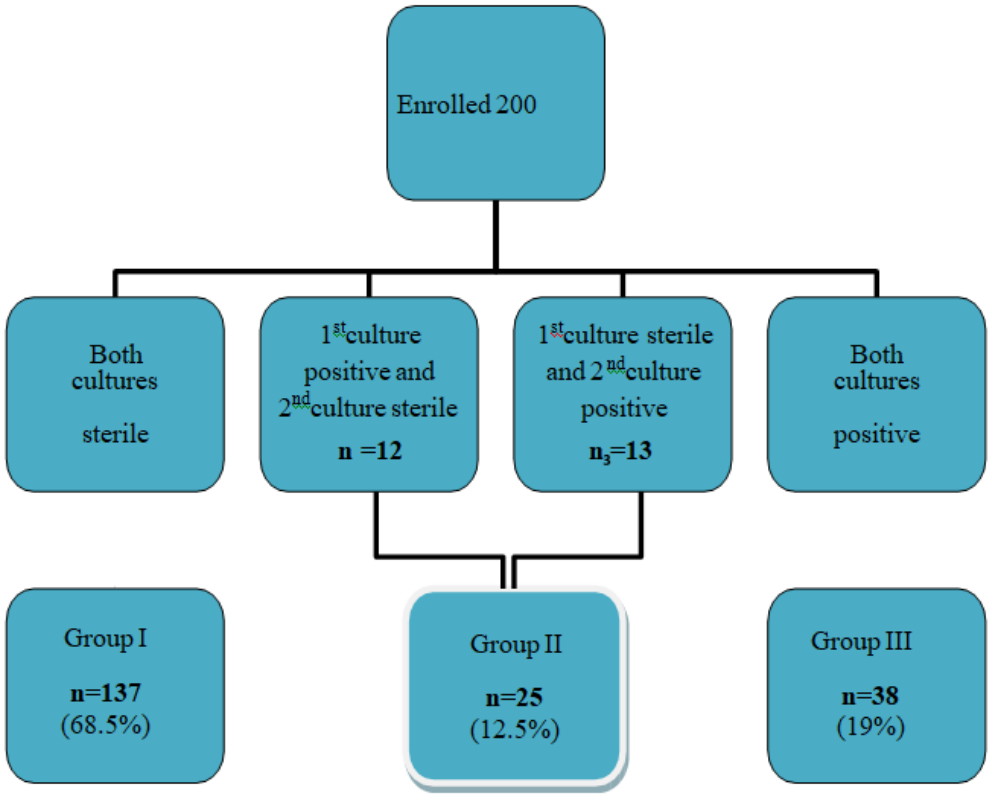
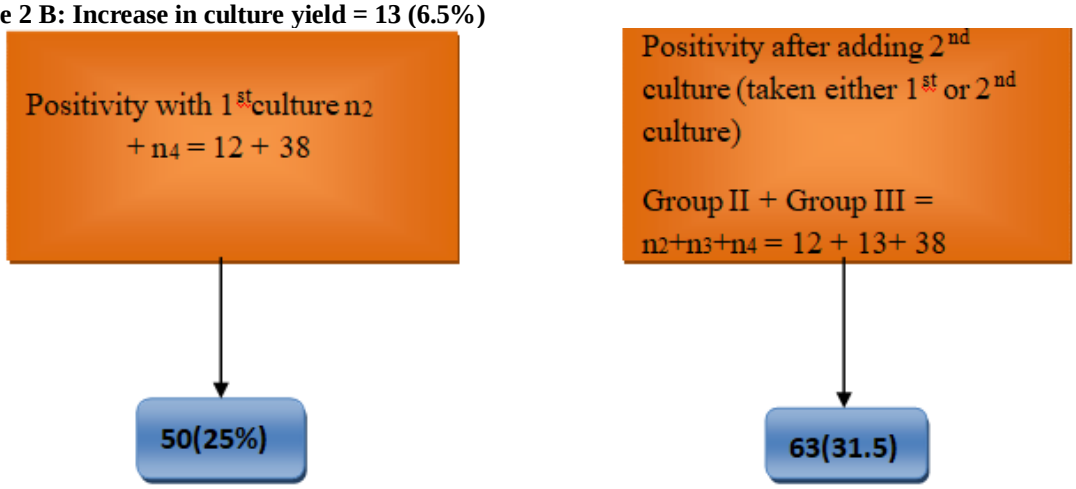


Figure 2 B: Increase in culture yield = 13 (6.5%)



DISCUSSION

Neonatal sepsis is one of the leading causes of mortality and responsible for 30 to 50% of neonatal deaths in developing countries like India [9]. Up to 20% of the neonates develop sepsis and approximately 1% die of complications of sepsiswhich is largely preventable with prevention of sepsis itself, prompt diagnosis, rational antimicrobial therapy and aggressive and timely supportive care. As per National Neonatal Perinatal Database (NNPD), the incidence of neonatal sepsis is 30 per 1000 live births [2]. The isolation of organisms remains the most accurate and valid method of diagnosing neonatal sepsis.

The present study was planned to evaluate improvement in culture positivity rate by getting two blood cultures from different sites versus standard practice of single blood culture in definitive diagnosis of neonatal sepsis.

Summary of outcomes: Our study showed that first blood culture positivity was seen in 50 cases (25% first culture positivity rate). With second culture, the yield increased to 63 (31.5% both culture positivity rate). Thus adding on second culture, yield increased in 13 cases but it was statistically not significant (6.5%) (CI: -2.3%-15.3%) (p=0.148.)

It was found that culture positivity increased with use of two blood cultures instead of routine one culture in

babies suspected of neonatal sepsis but it was not significant. Morbidity and mortality were more in blood culture positive patients. However, there was no difference in major morbidity or mortality in neonates with one or both blood cultures positive (Group II and III).

Comparison with other studies: Our study had culture positivity rate of 31.5%. As seen in various studies, culture positivity in neonates has been reported to range from 25-60% [10-12] Few studies have demonstrated lower culture positivity rates, reasons could be low blood volume taken or administration of antibiotic prior to sample collection or due to anaerobic, fungal or viral pathogens [13, 14]. Higher culture positivity rates like 48%, 63% , 64% have been reported from different studies [15-17]. In contrary to our study, **Tomar et al**found advantage of two different site blood cultures in initial evaluation of neonatal sepsis [8]. However, there was higher culture positivity rate. This difference from our result could be due to large sample size, different demographic profile, poor neonatal care and improper procedure for collecting blood culture.

Not getting sufficient blood sample is a not uncommon in children and neonates as the sensitivity of blood culture improves with adequate blood volume [18-20]. Many studies have already emphasized on blood volume for culture to be ≥1 ml in one culture bottle [21]. We used 1 ml volume for all cultures like previous studies.

Summary of secondary outcomes: At our centre, there is high prevalence of sepsis in preterm babies similar to previous studies from south East Asia [8, 22-24]. Presentation of sepsis varied depending on disease severity and immune status of newborns. Respiratory distress (80%) was the most common complaint in our study as in studies by **Galhotra, Jyothi, Jain and Tomar et al** [8, 13, 23, 25]. Majority of patients had early onset sepsis (66.6%) as compared to late onset sepsis (33.3%) in the culture proven group. Similar prevalence has been reported by **Galhotra et al** and **Jyothi et al** [13, 23].

Majority of complications and clinical outcomes of sepsis like pneumonia, meningitis, acute renal failure, NEC, need for ventilation, sclerema and mortality were more common in blood culture positive patient (group II or group III) as compared to culture negative patients (group I). However, it was statistically not significant between these two groups (II and III).

The incidence of Gram negative bacteraemia i.e. 36 (57%) was more than Gram positive bacteraemia i.e. 16 (25%). The commonest pathogen isolated was *E. coli*, more in early onset sepsis, similar to other studieswhile data from National Neonatal Perinatal Database (NNPD, 2003) showed *K. pneumoniae* to be the most common agent [2, 8, 13]. *S. aureus* was the commonest organism in late onset sepsis. In our study, patient with Gram negative bacterial sepsis shows higher mortality rate as compared to Gram positive bacterial sepsis. Similar incidence has been reported in studies by Jain et al and **Viswanathan et al** [11, 26]. The organisms commonly implicated in neonatal sepsis in developing countries differ from developed countries [1].

We found that 60% of *S. aureus* isolates showed resistance to commonly used antibiotic Ampicillin. **Kumhar, Galhotra and Jain** demonstrated high level of Penicillin resistance in various studies [13, 25, 27]. Universal sensitivity to vancomycin was seen for Staphylococcal isolates like many studies from India and South East Asia.

We found that out of 63 patients with confirmed sepsis, 23 died during the period of hospitalization (case fatality rate: 36.5%) and 40 (63.5%) were discharged to home. Mortality from late onset sepsis was more than with early onset sepsis but it was statistically not significant. High mortality rates of 37% and 48.5% have been observed by Indian authors [17, 28, 29].

CoNS and Candida spp. are commonly isolated species in neonates admitted in NICUs [30]. Being part of the skin flora, they may be contaminants in a blood culture growth if aseptic precautions are not taken well [22]. **Struthers et al** tried to differentiate pathogenic from contaminant CoNS and reducing antibiotic usage concluded one positive culture of CoNS as contaminant and both positive cultures as infection [22]..

In the present study there were 25 patients in group II (one out of two cultures positive) and 38 patients in group III (both cultures positive). In group II, there was growth of Candida in 2 patients and no CoNS growth was detected. These 2 babies with Candida growths had positive sepsis screen and clinical course was consistent with the sepsis and were taken as culture positive. So, in contrast to mentioned previous studies, the present study had no growth of contaminants [8].

STRENGTHS AND LIMITATIONS

Our study has one of the largest sample sizes among similar studies. Neonates in both early and late neonatal period up to 28 days of life were enrolled.

The limitation of the study was that this was done as a postgraduate thesis and single centre study to be completed in a definite time frame. A multi centre study would have added more value to the research question enrolling patients from across the country. Taking blood culture from two sites demands physician and nurse time, costs money, increases baby handling.

IMPLICATIONS FOR PRACTICE

Two site blood cultures does not seems to improve rate of pathogen detection in case of neonatal sepsis.

CONTRIBUTORS

SS: collected the data, reviewed the literature and drafted the first version of the manuscript; SAS, RKY: conceptualized the study and revised the manuscript; AT, NM, MS, SKS: reviewed the literature and statistical analysis; MVS, SAS, MM, AS: critically reviewed the final version of submitted manuscript All authors contributed to drafting of the manuscript and approved the final version of the manuscript;

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COMPETING INTRESTS: None

CONCLUSION

Our study concluded that taking two simultaneous blood cultures from different sites does not significantly increase culture positivity. Single blood culture remains gold standard in the diagnosis of neonatal sepsis. This might give to the clinician a better choice of targeted, narrowspectrum antibiotic instead of upgrading antibiotics on a clinical ground alone. This study gives us an insight into the prevailing NICU flora and antibiotic sensitivity pattern which can be used as a guide to review the antibiotic policy being currently followed in our unit.

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