

## Original Research Article

# Comparative study between automated blood culture and conventional blood culture in neonatal septicaemia cases isolated in a tertiary care hospital in Odisha

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**Received:** 16 October 2020

**Accepted:** 23 October 2020

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### ABSTRACT

**Background:** Neonatal sepsis is the third leading cause of neonatal mortality and a major public health problem, especially in developing countries. In developing countries sepsis being cause of neonatal mortality is responsible for 30-50% of the 5 million of total neonatal deaths each year. The detection of microorganisms in a patient's blood has a great diagnostic and prognostic significance. Blood cultures provide essential information for the evaluation of a variety of diseases like endocarditis, pneumonia, and pyrexia of unknown origin particularly in patients with suspected sepsis. In our study we have done blood cultures from patients on a neonatal intensive care unit by both automated and conventional system simultaneously and have done comparative analysis between the two systems.

**Methods:** The aim of this study was to compare the results of blood culture employing the conventional and BacT/Alert and VITEK-2 methods for detection of neonatal septicaemia cases. A prospective study was carried out in the Department of Microbiology in association with Department of Paediatrics and NICU, of Kalinga Institute of Medical Sciences, Bhubaneswar. 250 neonates with clinically suspected septicaemia were included in the study group. Three (3) ml of venous blood was collected aseptically of which 2ml was cultured by automated BacT/Alert and VITEK-2 method for rapid isolation and sensitivity test and rest 1 ml of blood for conventional culture.

**Results:** Isolation of bacterial pathogens by culture using the automated system showed greater positivity (32.8%) as compared to 18% by conventional blood culture system.

**Conclusions:** This study shows that automated blood culture system is superior to conventional blood culture system in terms of rapid and specific isolation of organism.

**Keywords:** Neonatal mortality, Automated culture, VITEK-2, Blood culture, Septicaemia

### INTRODUCTION

Neonatal septicaemia refers to a clinical syndrome characterised by systemic signs and symptoms due to generalised bacterial infections with a positive blood culture in the first four weeks of life. In developing countries, the most common cause of neonatal mortality is sepsis and it is responsible for 30-50% of the 5 million of total neonatal deaths each year.<sup>1</sup> Although recent

medical advances have improved neonatal care, many challenges remain in the diagnosis and management of neonatal infections. The diagnosis of neonatal sepsis is complicated by the frequent presence of noninfectious conditions that resemble sepsis, especially in preterm infants, and by the absence of optimal diagnostic tests. The detection of microorganisms in a patient's blood has a great diagnostic and prognostic significance. Blood cultures provide essential information for the evaluation

of a variety of diseases like endocarditis, pneumonia, and pyrexia of unknown origin particularly in patients with suspected sepsis as well helps the treating physician in early and appropriate antimicrobial therapy.<sup>2</sup> This may help in reducing mortality and morbidity. For study purpose we were able to collect blood from 250 neonates to process the sample both by automated and conventional systems simultaneously. The pathogens recovered by conventional system were also recovered by automated system and we have critically analysed and compared between the two systems.

### ***Aims and objectives***

The aims and objectives of the study were 1) to know the bacteriological profile of septicaemic cases amongst neonates born in our hospital 2) to compare the efficacy of both conventional blood culture and Bac-Talart for isolation of bacterial pathogens in septicaemic cases.

## **METHODS**

A prospective study was carried out in the Department of Microbiology in association with Department of Paediatrics and NICU, of Kalinga Institute of Medical Sciences, Bhubaneswar with study duration of three years extending from November 2013 to October 2016. 250 neonates with clinically suspected septicaemia were included in the study group.

### ***Inclusion criteria***

Inclusion criteria were 1) neonates showing nonspecific and specific signs and symptoms of septicaemia. 2) nonspecific features include one or more of the following symptoms like-lethargy, hypothermia or fever, poor perfusion, hypotonia, respiratory distress and brady or tachypnoea 3) specific features include- central nervous system features like bulging of anterior fontanelle, vacant stare, excess irritability; cardiac system findings like shock, perfusion; gastrointestinal system finding like abdominal distension, paralytic ileus, necrotizing enterocolitis, hepatic features like hepatomegaly, direct hyperbilirubinemia; and haematological features like bleeding, purpura.

### ***Exclusion criteria***

Neonates without any clinical signs and symptoms of sepsis.

Three ml of venous blood was collected aseptically of which 2 ml was cultured by automated BacT/Alert and VITEK-2 method for rapid isolation and sensitivity test and rest 1 ml of blood for conventional culture.<sup>3</sup>

### ***Blood Culture by BacT/Alert system***

One blood culture consists of a FAN (fastidious antibiotic neutralization) aerobic and a FAN anaerobic bottle. For

patients <13 kg, either one FAN aerobic bottle or one paediatric FAN bottle is used. In our study pediatric FAN bottles were used. Positive samples were processed for identification in BacT/Alert system and sensitivity by VITEK-2 system. After the positive blood culture bottles were taken out from BacT/Alert system, gram's stained smear examination was done. Subculture from the broth of the blood culture bottle was done into Blood agar, MacConkey agar and chocolate agar. For subcultures, broth in each bottle was mixed gently and drawn out aseptically with a sterile syringe and needle. From the growth of the solid media, 0.5 McFarland standard saline suspension was prepared and it was put to VITEK-2 system for identification of the organism and antibiotic sensitivity test.

### ***Blood culture by conventional methods***

Total 1 ml of blood was collected from the neonates aseptically. Immediately after collection the blood was inoculated into the conventional biphasic medium with change of needle. BHI broth with 0.025% of sodium polyanethol sulphate as an anticoagulant was used. All the bottles were then incubated at 37°C. The biphasic bottle was examined for visible growth after overnight incubation and if negative, the blood broth mixture was tipped over the slant daily and incubated further for ten days. Identification of the bacterial growth was done by gram's staining and performing all enzymatic and biochemical screening tests as per standard guideline (Mackie and McCartney).<sup>3</sup> Subculture from the biphasic medium was done into Mac Conkey agar and blood agar. For subculture, broth in each bottle was mixed gently and drawn out aseptically with a sterile syringe and needle. In the absence of growth on Mac Conkey agar and Blood agar, a preliminary no growth report of culture after 24 hours of incubation was dispatched and the broth was further incubated for 14 days and was checked daily for the evidence of growth. Before discarding the biphasic medium, subculture was done for the identification of bacterial growth.<sup>3</sup>

### ***Statistical analysis***

The collected data was coded and entered into SPSS Version 16. The data was summarized using tables and graphs. Univariate analysis was performed separately for each of the variables. P values were calculated using the chi square test or fisher's exact test for categorical variables and student's t-test for continuous variables. A p value <0.05 was considered significant.

## **RESULTS**

Blood collected from 250 neonates who were clinically suspected of septicaemia were subjected to Bac Talart and VITEK system for identification of the organism and antibiotic sensitivity. All the 250 blood samples were subjected to both conventional and automated blood culture system. Isolation of bacterial pathogens by culture

using the automated system showed 32.8% positivity as compared to 18% by conventional blood culture system (Table 1). P value regarding isolation of pathogens by automated systems was found to be significant. In conventional blood culture, *S. epidermidis* was the commonest isolate 15 (6%) followed by *E. Coli* 10 (4%), *S. aureus* 5 (2%), *Enterobacter cloacae* 5 (2%)

*Acinetobacter iwoffii* 5 (2%) and *Candida albicans* 5 (2%) (Table 2). The frequency distribution of various organisms isolated by automated method shows *S. haemolyticus* as the commonest isolate 28 (34.2%) followed by *S. epidermidis* 12 (14.6%), *E. coli* 8 (9.8%), *S. aureus* 6 (7.3%), *E. cloacae* 6 (7.3%), *B. cepacia* 4 (4.9%) and *C. albicans* 8 (9.8%) (Table 3).

**Table 1: Isolation of bacterial pathogens by automated system and conventional system.**

Blood culture	Automated system		Conventional system		Chi-square	P value
	Number isolated	%	Number isolated	%		
Growth negative	168	67.2	205	82	88.973	0.000
Growth positive	82	32.8	45	18		
Total	250	100	250	100		

**Table 2: Bacterial pathogens isolated by conventional method.**

Bacteria isolated in conventional method	Number isolated	%
<i>S. aureus</i>	5	11.1
<i>S. epidermidis</i>	15	33.3
<i>E. coli</i>	10	22.2
<i>E. cloacae</i>	5	11.1
<i>A loffi</i>	5	11.1
<i>C albicans</i>	5	11.1
Total (n=250)	45	18

**Table 3: Frequency distribution of organisms isolated by automated System.**

Culture	Number isolated	%	
Gram Positive	<i>S. haemolyticus</i> ,	28	34.2
	<i>S. epidermidis</i>	12	14.6
	<i>S. weneri</i>	3	3.7
	<i>S. hominis</i>	3	3.7
	<i>S.aureus</i>	6	7.3
Gram Negative	<i>Enterobacter cloacae</i>	6	7.3
	<i>Burkholderia cepacia</i>	4	4.9
	<i>Acinetobacter iwoffii</i>	2	2.4
	<i>S. paratyphi</i>	2	2.4
	<i>E. coli</i>	8	9.8
	Fungi	<i>C. albicans</i>	8
Total	82	100.0	
Chi-square C <sup>2</sup> = 33.294			
P value= 0.000			

In our study all the gram-positive pathogenic isolates were sensitive to linezolid, tigecycline and vancomycin. co-trimoxazole was sensitive in 78.8% isolates followed by ceftriaxone (77%), azithromycin (76.9%), cefepime

(60%) erythromycin (59.6%) and clindamycin (53.9%). For gram negative pathogens; maximum sensitivity was observed for amikacin (86.4%) followed by imipenem (77.3%), meropenem (77.3%), tobramycin (77.3%), ciprofloxacin (68.2%), piperacilin- tazobactam (68.2%), ceftriaxone (59.1%) and cefepime (40.9%).

## DISCUSSION

One of the major difficulties in the management of neonatal sepsis is getting an accurate diagnosis. Unlike older patients, newborns have very subtle presentations, and multiple conditions resemble neonatal sepsis<sup>4</sup>. Blood culture is the gold standard for the diagnosis of neonatal sepsis. However, its positivity rate is low and is affected by blood volume inoculated, prenatal antibiotic use, level of bacteremia and laboratory capabilities.<sup>5</sup> In developing countries, culture negative sepsis is responsible for the majority of episodes.<sup>6</sup> We have done comparative study for isolation of pathogens between the automated blood culture system and conventional blood culture system. Further, antibiotic sensitivity pattern of the pathogens has been thoroughly analysed.

Isolation of bacterial pathogens by culture using the automated system showed 32.8% positivity as compared to 18% by conventional blood culture system in the present study which is close to the study conducted by Karen. K. Krisher et al where isolation of pathogens by conventional system is 10% as compared to 29% by automated system.<sup>7</sup>

In two different studies done by Vergnano S et al and BJ Stoll et al; it was found that the most common cause of septicaemia is group B streptococcus (GBS), isolated in half of episodes, followed by *Escherichia coli*, isolated in one-fourth of episodes.<sup>8,9</sup> Our study showed that *S. haemolyticus* was the commonest isolate 28 (34.2%) followed by *S. epidermidis* 12 (14.6%) and then *E. coli* 8 (9.8%) by automated method.

In our study all the gram-positive pathogenic isolates were sensitive to linezolid, tigecycline and vancomycin. co-trimoxazole was sensitive in 78.8% isolates followed

by ceftriaxone (77%), azithromycin (76.9%), cefepime (60%) erythromycin (59.6%) and clindamycin (53.9%). This finding is closely associated with the study conducted by Shahsanam Gheibi et al where maximum sensitivity was found to Vancomycin (90%) and ciprofloxacin (78.5%).<sup>10</sup> Study conducted by Katiyar R et al showed 40.74% sensitivity to amikacin and 25.92% to gentamicin.<sup>11</sup> Sensitivity to cefaclor, cefotaxime and ceftazidime were 40%,33.3% and22.2% respectively. Lowest sensitivity was found to Penicillin (7.41%) and Ampicillin (18.52%) which is close to the findings of our study. maximum sensitivity to linezolid (100%), vancomycin (95%), cefotaxime (73%), ceftriaxone (68%) and amikacin (68%) was observed in the study conducted by Maimoona Mustafa et al.<sup>12</sup> resistance pattern was maximum against ampicillin (86.4%), erythromycin (64%) and gentamicin (50%).

Maximum sensitivity was observed for amikacin (86.4%) followed by Imipenem (77.3%), Meropenem (77.3%), Tobramycin (77.3%) and Ciprofloxacin (68.2%) for gram negative pathogenic isolates. These findings are at par with the study conducted by Maimoona Mustafa et al where maximum sensitivity was observed for meropenem (100%), ciprofloxacin (70%), amikacin (68%) and cefotaxime. In our study resistance was maximum for cefadroxil (86.4%), ampicillin (72.7%), cefuroxime (68.2%) and gentamicin (54.5%) which was also nearer to the said study where maximum resistance was observed for cefadroxyl (86.4), ampicillin (72.7%) and cefuroxime (68.25%).<sup>12</sup> Mane AK et al also found maximum sensitivity to imipenem (100%), ciprofloxacin (66.6%) and levofloxacin (66.6%).<sup>13</sup> Maximum resistance was found against ampicillin (81.5%) and gentamicin (85.2%).

## CONCLUSION

Isolation of bacterial pathogens by culture using the automated system showed 32.8% positivity as compared to 18% by conventional blood culture system. This study shows that automated blood culture system is superior to conventional blood culture system in terms of rapid and specific isolation of organism. Long term surveillance is also needed to describe the varied pathogens causing neonatal sepsis as well as their changing antibiotic susceptibility profile.

## ACKNOWLEDGEMENTS

The authors are very much thankful to the HOD, Paediatrics, staff of NICU, KIMS especially to Dr Manas Nayak and the technical staffs and the HOD and faculty members of Department of Microbiology, KIMS for their support in completing the research project.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Sarangi KK, Pattnaik D, Mishra SN, Nayak MK, Jena J. Comparative study between automated blood culture and conventional blood culture in neonatal septicaemia cases isolated in a tertiary care hospital in Odisha. *Int J Res Med Sci* 2020;8:4267-70.