

## Original Research Article

# Optimizing neonatal care: streamlined positive blood culture reporting via laboratory automation with BacT/ALERT 3D

Premjith Lal U. A., Rupali S. Mantri\*, Nitin A. Ambhore, Sharmila S. Raut

Department of Microbiology, Government Medical College Akola, Akola, Maharashtra, India

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### \*Correspondence:

Dr. Rupali S. Mantri,

E-mail: [r.mantri@rediffmail.com](mailto:r.mantri@rediffmail.com)

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### ABSTRACT

**Background:** Sepsis presents a potentially life-threatening medical condition marked by a systemic inflammatory response throughout the body, triggered by various pathogenic organisms found in the bloodstream. Neonatal sepsis stands as the third primary contributor to neonatal mortality. Blood culture methods have evolved significantly from conventional techniques involving bottles of nutrient broths cultured in incubator and subsequent subculture based on visual assessments to modern automated systems. These automated instruments, which are continuously monitored and enhanced the efficiency of blood cultures. One of the widely employed blood culture systems in numerous diagnostic microbiology laboratories is the BacT/ALERT 3D system developed by BioMérieux Inc.

**Methods:** This is a hospital-based observational study we were trying to evaluate the time to positivity (TTP), identification and antimicrobial susceptibility patterns of causative organism in blood culture. One thirty-nine matched pairs of blood cultures from neonates were evaluated by the BacT/ALERT 3D system.

**Results:** Out of 139 blood samples that were processed 40 (28.77%) were positive. The median time to detection in the BacT/ALERT 3D system for isolates was found to be on an average of 11.8 hours in our study. Gram-negative isolates were found most common cause of sepsis, accounting for 75%, compared to gram-positive isolates, which accounted for 25%. Among the identified isolates, *Klebsiella pneumoniae* and *Escherichia coli* were the most prevalent, responsible for 25% each.

**Conclusions:** Neonatal septicemia poses a life-threatening emergency. The BacT/ALERT 3D system is more sensitive, rapid, higher efficiency in detecting prevalent bloodstream pathogens.

**Keywords:** Automated blood culture system, BacT/ALERT 3D, Bloodstream infection, Early-onset neonatal sepsis, Late-onset neonatal sepsis, Neonatal sepsis

## INTRODUCTION

Neonatal mortality causes a significant global public health challenge, particularly in bridging child health inequalities between developed and developing nations. The majority of the approximately 4 million neonatal deaths each year occur in low and middle-income countries. Three primary conditions drive this staggering figure: Infections, Birth asphyxia and complications arising from Premature birth.<sup>1</sup> Of these, severe infections account for over one-third of neonatal deaths, while the clinical syndrome of neonatal sepsis, pneumonia is responsible for a quarter. Alarming, case fatality rates for

neonatal infections remain obstinately high, whether among hospitalized infants or those within communities. Tackling these main problems is very important to reduce newborn deaths and ensure all children have a fair chance at good health around the world.<sup>2</sup> Neonatal sepsis is typically divided into two primary categories: Early-onset neonatal sepsis (EONS) and Late-onset neonatal sepsis (LONS), depending on the timing of infection, mode of transmission and causative organisms. EONS refers to vertically transmitted infections within the first three days of life (72 hours), while LONS denotes horizontally transmitted infections occurring after 72 hours of life, often attributed to microorganisms prevalent in hospital

settings.<sup>3</sup> Additionally, a third classification, known as very late-onset neonatal sepsis (VLOS), has been identified by some researchers. VLOS primarily affects infants hospitalized in neonatal intensive care units (NICUs) and typically manifests after 30 days of hospitalization until discharge.<sup>4</sup>

Early diagnosis plays a crucial role in mitigating the morbidity and mortality associated with neonatal septicemia. While isolating bacterial agents from conventional blood cultures stands as the gold standard for diagnosis.<sup>5</sup> Nevertheless, this approach has certain drawbacks, such as prolonged reporting times and a notable rate of contamination.<sup>6</sup> Blood culturing techniques have evolved significantly from the traditional method of using nutrient broths in conventional incubators, with subsequent subculturing based on visual assessments, to the contemporary automated systems. The advent of continuously monitored automated instruments has undeniably enhanced the efficiency of blood cultures.<sup>7</sup>

The BacT/ALERT 3D, developed by BioMérieux in Marcy l'Etoile, France, represents an automated blood culture system renowned for its efficiency in saving both time and labor. It offers advantages such as reduced risk of contamination, shorter incubation periods and increased rates of isolating pathogens compared to traditional blood culture methods.<sup>8,9</sup>

Each bottle in the BacT/ALERT 3D system is equipped with a CO<sub>2</sub> sensor securely affixed to its base, with a semi-permeable membrane acting as a barrier between the sensor and the liquid broth. As microorganisms within the broth generate CO<sub>2</sub>, it diffuses through the membrane, dissolving in water and triggering the release of hydrogen ions. This process leads to a reduction in pH levels, causing the CO<sub>2</sub> sensor to transition in colour from blue or dark green to lighter green or yellow. Consequently, the reflected red light increases. The BacT/ALERT system incubates and periodically scans for CO<sub>2</sub> production every 10 minutes, utilizing a computerized database management system to record and report the obtained results.<sup>10</sup> TTP was defined as the time between when the incubation started and when the machine first showed a signal that growth was detected in the culture bottle.

Hence, early detection of pathogenic organisms is crucial for guiding accurate antibiotic therapy and minimizing the use of unnecessary antibiotic administration to the patients.

## METHODS

A hospital based observational study was conducted at Government Medical College Akola from October 2022-November 2023. A total of 139 paired blood samples were collected and processed. The study received approval from the Institutional Ethics Committee (IEC approval no. 221/2026). Aseptically, 0.5-1 ml of venous blood was drawn as per standard microbiological procedures and

inoculated into BacT/ALERT PF Plus blood culture bottles (BioMérieux, France).<sup>11</sup> Two blood samples from two different sites were obtained simultaneously. All samples were processed using the BacT/ALERT 3D system and culture status (positive or negative) was monitored up to the end of day 7 at 37°C of incubation.<sup>12,13</sup> As soon as growth detected by machine, the corresponding bottle removed from the system and the sample subjected to Gram staining and cultured on Blood agar and MacConkey agar and incubated at 37°C for 18-24 hours.<sup>14</sup>

The findings from the Gram stain smear were informed to the physicians to initiate preliminary management of the cases. Growth observed on the culture plates was identified using standard microbiological methods. Antimicrobial sensitivity testing was performed utilizing the Vitek 2 Compact system and the susceptibility profile of the organisms was determined using the guidelines published by the Clinical and Laboratory Standards Institute (CLSI).<sup>15</sup> The study included neonates aged  $\leq 28$  days who were admitted to the NICU or neonatal wards during the study period. Neonates evaluated for suspected sepsis and from whom blood culture samples were obtained for microbiological investigation were included. Eligibility was restricted to blood samples processed exclusively using the BacT/ALERT 3D automated blood culture system.

Blood cultures from patients older than 28 days, those received from non-neonatal units such as paediatric or adult wards, samples processed using manual blood culture systems and blood culture specimens with inadequate volume, improper labelling or leakage or transport-related errors were excluded from the study. Pathogens were identified when the same organism with matching sensitivity patterns was isolated from paired blood cultures. Conversely, if the organism was found in only one of the paired blood culture bottles or if mixed growth was detected, it was reported as contaminant.<sup>16</sup>

## RESULTS

Blood culture was positive in 40 (28.77%) cases and 99 (71.22%) were negative. The majority of positive blood cultures (77.5%) were identified within the initial 24 hours. Among these, 12.5% were detected within the first 12 hours, while 30% were identified between 12 and 18 hours, 35% between 18 and 24 hours, 17.5% of growth was observed between 24 and 48 hours, with a remaining 5% detected after 48 hours (Figure 1).

Out of a total of 40 clinically significant isolates, 11 (27.5%) belonged to early onset septicemia, while 29 (72.5%) cases were between the ages of 3 days to 28 days, indicating late onset septicemia (Table 1). Out of 40 isolates 31 (77.5%) were isolated from males and 9 (22.5%) were isolated from females. Of the 40 isolates, 30 (75%) were Gram-negative bacteria and 10 (25%) were Gram-positive bacteria. The most frequently encountered

Gram-negative isolates were *Klebsiella pneumoniae* and *Escherichia coli* 10 (25%) each, followed by 7 (17.5%) *Pseudomonas aeruginosa*, 3 (7.5%) *Proteus mirabilis*. In case of Gram-positive isolates 9 (22.5%) were *Staphylococcus aureus* and 1 (2.5%) *Enterococcus faecalis* (Figure 2).

11 cases of EONS, Gram-negative bacilli accounted for 9 cases (81.81%), while Gram-positive cocci accounted for 2 cases (18.18%). Among these, *Klebsiella pneumoniae* was the most frequently isolated pathogen, identified in 5 cases (45.45%). In 29 cases of LONS, Gram-negative bacilli were predominant, comprising 21 cases (72.41%), compared to Gram-positive cocci which accounted for 8 cases (27.58%). The most common pathogens identified in LONS cases were *S. aureus* and *Escherichia coli*, each found in 7 cases (24.13%) (Table 2).

Among the Gram-negative isolates, the highest antibiotic sensitivity was noted with Imipenem and Meropenem (93.3%), followed by Amikacin and Tobramycin (75.7%) and Ciprofloxacin (73.3%). Gentamicin showed a sensitivity rate of 58.3% in *E. coli* and Amikacin showed 77% sensitivity rate in *Pseudomonas aeruginosa*. Notably, the predominant isolate, *Klebsiella pneumoniae*, exhibited complete resistance (100%) to ampicillin (Figure 3). All Gram-positive pathogenic isolates exhibited sensitivity to vancomycin, linezolid and teicoplanin.

Additionally, approximately 88% to 90% of the isolates demonstrated sensitivity to penicillin, cefoxitin, ciprofloxacin, sulfamethoxazole/trimethoprim, as well as amoxicillin/clavulanic acid. The incidence of MRSA among the *S. aureus* isolates was 10-12% and inducible clindamycin resistance was seen in 5%. Sensitivity rates were lower for gentamicin, erythromycin, clindamycin and amikacin, in descending order (Figure 4).

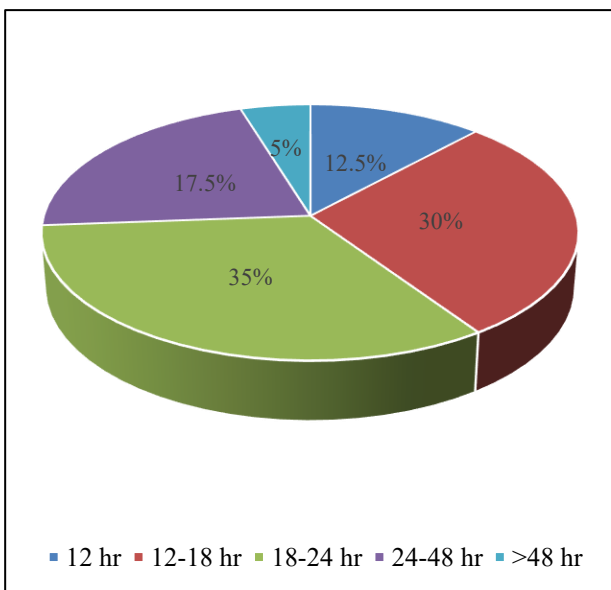


Figure 1: Distribution of time of positivity by BacT/ALERT 3D.

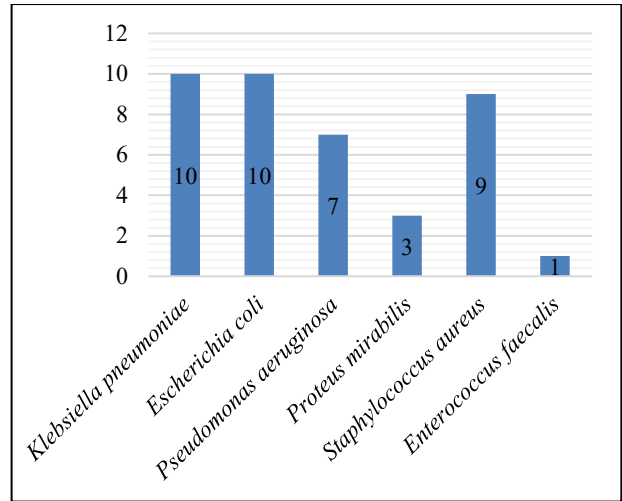


Figure 2: Distribution of positive culture on the basis of bacterial isolates.

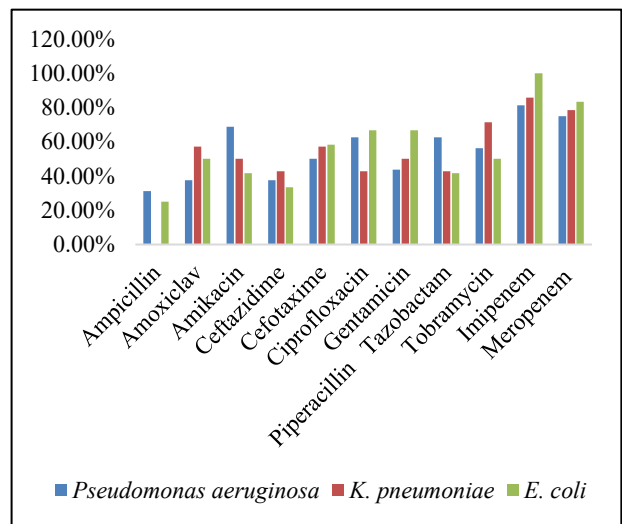


Figure 3: Antibiotic sensitivity pattern of Gram-negative Bacteria.

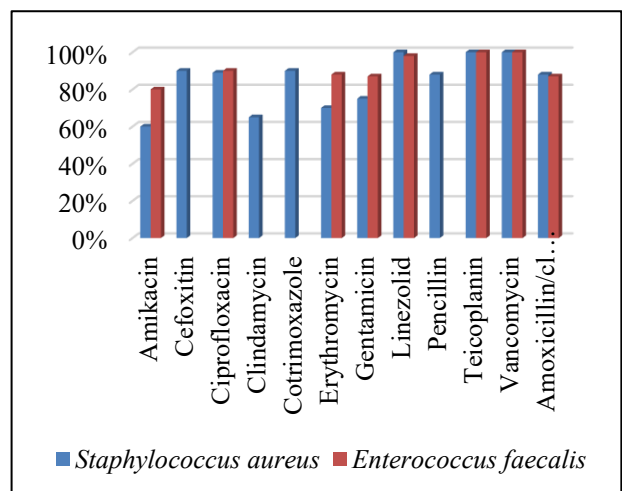


Figure 4: Antibiotic sensitivity pattern of Gram-positive cocci.

**Table 1: Age wise distribution of culture positive cases (n=40).**

Age (in days)	No. of cases	Total (%)
0-3	11	27.5
4-6	04	
7-9	02	
10-12	05	
13-15	04	72.5
16-18	05	
19-21	02	
22-24	04	
25-28	03	
0-28	40	100

**Table 2: Distribution of organisms as per the onset of septicaemia.**

Isolates	EONS (%)	LONS (%)	Total (%)
<b>Gram negative organisms</b>	9 (81.81)	21 (72.41)	30 (75)
<i>Klebsiella pneumoniae</i>	5 (45.45)	5 (17.25)	10 (25)
<i>Escherichia coli</i>	3 (27.27)	7 (24.13)	10 (25)
<i>Pseudomonas aeruginosa</i>	1 (9.1)	6 (20.70)	7 (17.5)
<i>Proteus mirabilis</i>	0 (0)	3 (10.35)	3 (7.5)
<b>Gram positive organisms</b>	2 (18.18)	8 (27.58)	10 (25)
<i>Staphylococcus aureus</i>	2 (18.18)	7 (24.13)	9 (22.5)
<i>Enterococcus faecalis</i>	0 (0)	1 (3.44)	1 (2.5)
Total	11 (100)	29 (100)	40 (75)

## DISCUSSION

A timely and accurate diagnosis of septicaemia is crucial for effectively managing suspected cases. However, traditional blood culture results are typically delayed by 48-72 hours leads to delayed initiation of appropriate antibiotics.<sup>17</sup> BacT/ALERT 3D and similar fully automated systems provide numerous benefits compared to manual blood cultures for detecting microbes in body fluids. These systems are self-contained, eliminating the need for repeated handling of bottles and thereby reducing workload and potential errors.<sup>10,18</sup> The analysis shows the majority of positive blood cultures (77.5%) were identified within 24 hours of incubation, Specifically, 12.5% of these cultures showed growth within the initial 12 hours, followed by 30% between 12 and 18 hours and 35% between 18 and 24 hours of incubation.

Janjindamai et al conducted a study in Thailand investigating the time to blood culture positivity using the BacT/ALERT system in 75 newborn infants suspected of sepsis. They observed that the positivity rates, considering definite and possible bacterial pathogens, were 71% at 24 hours, 95% at 36 hours and 97% at 48 hours.<sup>19</sup> These findings closely align with a separate retrospective assessment by Kumar et al which analyzed over 400 cultures and the positivity rates for definite and probable bacterial pathogens were 89% at 36 hours and 97% at 48 hours.<sup>20</sup> The median TTP for bacterial isolates in our study

found to be 11.8 hours which is similar TTP of 11.5 hours was reported by Hasan et al.<sup>6</sup> The positivity rate of 28.77% aligns with rates observed in studies conducted by Muley VA et al and Kante et al, Sarah Magdy Abdelhamid et al, reported positivity rate of 10.29%, Jardine et al, documented a detection rate of 6%.<sup>14-17</sup> In contrast, Vinod Kumar et al, observed a positivity rate exceeding 50%.<sup>20</sup>

In the current study, the most common isolates were *K. pneumoniae* and *E. coli*, accounting for 25%, followed by *S. aureus* with 22.5%. Which seems to be aligned closely with those reported by Muley et al, *K. pneumoniae* was identified as a predominant pathogen in the NNPD Report.<sup>14,21</sup> 2002-2003, as well as in similar studies conducted by Kante et al, Chelliah et al, Iregbu et al and Tankhiwale et al.<sup>15,22-24</sup> A concerning observation from this study is that *E. coli* and *S. aureus* were identified in 7 cases (24.13%) of LONS.

In few studies gram-positive bacterial isolates were more commonly associated with LOS and gram-negative isolates were associated with EONS.<sup>16</sup> These findings have significantly helped in therapy and infection control. *K. pneumoniae*, *E. coli* and *S. aureus* have the ability to persist in the environment for extended periods and are widely distributed within hospital settings. Consequently, these pathogens pose a risk of transmission from contaminated environments to patients, especially through lapses in infection control practices. *S. aureus* isolates

demonstrated highest sensitivity to Vancomycin, Linezolid and Teicoplanin. Susceptibility was also observed with Penicillin, Cefoxitin, Ciprofloxacin, Sulfamethoxazole/Trimethoprim, Amoxicillin/Clavulanic acid, Gentamicin, Erythromycin, Clindamycin and Amikacin. Findings correlated with the studies by Aletayeb et al, Mane et al, Kausik et al and Mustafa et al.<sup>25-28</sup> Among the gram-negative isolates, the highest antibiotic sensitivity was observed for Imipenem and Meropenem, followed by Amikacin, Ciprofloxacin, Tobramycin and Gentamicin. Similar findings were reported in a study by Mustafa et al. Another study conducted by Mane et al also demonstrated high sensitivity to Imipenem (100%) and Ciprofloxacin (66.6%), with notable resistance observed against Ampicillin (81.5%) and Gentamicin (85.2%).<sup>26,28</sup>

In summary, achieving a shorter time to positivity reduces costs and minimizes medication errors. While the upfront investment in automated systems like BacT/ALERT 3D system may seem high for clinical laboratories in developing countries, these costs are balanced over time through decreased labour expenses, shorter hospitalizations and reduced morbidities.

### Limitation

This was a single-center observational study, which may limit the generalizability of the findings and precludes establishing causality. Additionally, clinical outcomes and cost-effectiveness of BacT/ALERT 3D-based automation were not evaluated.

### CONCLUSION

This study demonstrates that the BacT/ALERT 3D system offers several advantages over manual blood culture methods for the detection of microbial pathogens, with greater efficiency and faster identification of common bloodstream organisms. Considering its potential to improve clinical outcomes in infected neonates and to facilitate earlier discontinuation of antibiotics in non-infected infants, the higher laboratory costs associated with automated blood culture systems often limiting their adoption in developing countries may be justified.

The findings further suggest that empirical therapy for suspected neonatal septicemia should provide coverage against both gram-negative bacilli and gram-positive cocci, particularly *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*. Additionally, regular and periodic surveillance of the etiological agents of neonatal sepsis and their antimicrobial susceptibility patterns is essential to reduce inappropriate antibiotic use, prevent the emergence of resistant strains and enable timely revision of hospital antibiotic policies.

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