Microbiological Study of Neonatal Sepsis Using Automated Blood Culture System at a Tertiary Health Care Centre

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ABSTRACT

Introduction: Sepsis in neonates is responsible for highly considerable mortality, especially in developing countries like India, where it is contributing 19% of neonatal deaths. Present study was undertaken to study aerobic bacterial profile of neonatal septicemia by automated blood culture systems, using BacT/ALERT 3D 60 blood culture system and miniAPI bacterial identification and antibiotic susceptibility testing system, considering the importance of urgent and accurate identification of the pathogen and to reduce the unnecessary therapeutic load on patients as well as clinicians.

Aim: To isolate and identify bacterial pathogens in neonatal sepsis and to know their antibiotic susceptibility pattern using BacT/ALERT and miniAPI systems.

Material and Methods: 157 blood samples from neonates suspected to be in sepsis were studied during the study period using BacT/ALERT 3D 60 systems for blood culture and miniAPI system for identification and antibiotic susceptibility of isolates.

Results: 70 blood cultures were flagged positive by BacT/ALERT. E.coli was the most common i.e 16 (22.85%); followed by Coagulase negative Staphylococcus (CoNS), isolated in 15 (21.42%), followed by Staphylococcus aureus isolated in 14 (20%).

Conclusion: Total blood culture positivity rate was 44.58%. E. coli was the most common pathogen isolated. By using BacT/ALERT 3D 60, 65.71% blood culture positivity was observed within 24 hours incubation and bottles flagged positive within 48 hours were 59 (84.28%). Both, the BacT/ALERT 3D 60 and the miniAPI systems, were efficient, less time consuming and labor saving which is essential for the clinician and more importantly for the patient.

Key words: Neonatal sepsis, Bacterial sepsis, automated blood culture, BacT/ALERT, miniAPI

INTRODUCTION:

Neonatal sepsis is responsible for 30-50% of the total neonatal deaths in developing countries. It is estimated that up to 20% of the neonates develop sepsis and approximately 1% die of sepsis related causes. According to the data from National Neonatal Perinatal Database, sepsis is found to be one of the commonest causes of neonatal mortality in India contributing to 19% of all neonatal deaths.

There is an urgent need to know whether the baby has sepsis to institute treatment as quickly as possible. Confirmation of the diagnosis may take time, and diagnostic tests are used to obtain a rapid
indication of the infection status. These tests are not perfect. Some real cases of infection will produce negative test results, whereas some babies without infection will test positive.

The gold standard for diagnosis of septicemia is the isolation of bacterial agents from the blood culture. Newer methods of detecting bacteremia through blood culture studies have emerged and are proven to be most promising with better sensitivity and specificity; while not sacrificing time. Continuous monitoring demonstrates that most positive blood cultures are detected in a relatively short period of time, the majority within 24 hours.

BacT/ALERT 3D is an automated culture system, which continuously monitors for any growth in every 10 min in each bottle independently. The equipment works on the principle of colorimetry and gives a signal as soon as any trace of growth is encountered based on inbuilt set of algorithms.

The API system includes 32 wells containing dehydrated reagent that corresponds to various biochemical reactions using which most of the microorganisms can be processed using rapid strips in just 4-5 hours.

Adjusting antibiotic therapy according to the results of blood culture findings leads to a narrowing of antibiotic therapy. Thereby, the emergence of antibiotic resistance can be avoided or delayed. Antibiotic resistance may be reversed if the antibiotic use is decreased.

Hence, present study was undertaken to study aerobic bacterial profile of neonatal septicemia by automated blood culture systems, using BacT/ALERT 3D 60 blood culture system and miniAPI (Figure 1) bacterial identification and antibiotic susceptibility testing system, both by Biomerieux.
MATERIAL AND METHODS:

Study Design:

Blood culture sample of neonates were subjected for processing under automated blood culture system BacT/ALERT 3D 60 and positive blood culture samples were further processed by using miniAPI system for bacterial identification and antibiotic susceptibility testing of isolates.

This Observational study was conducted in the microbiology laboratory of a tertiary care hospital over a period of one and a half year (January 13 to June 14). Blood samples from clinically diagnosed or suspected neonates with septicemia (0-28 days of age) of both the sexes during the study period were collected and processed.

1-2 ml of blood sample from each neonate was collected and inoculated in special pediatric blood culture media bottle - The BacT/Alert PF bottle containing 20 ml broth. Collection of blood was done using all standard aseptic precautions. After blood was collected in the syringe using the needle; same needle was used to inoculate BacT/Alert bottles.

The Bact/Alert bottles possess a plastic top cap which is to be removed by breaking just before inoculation. Under the plastic cap, there is a rubber septum which is not sterile and hence was cleansed with 70% alcohol or iodine solution and allowed to dry before inoculation. Blood was introduced into the bottle only after the drying of rubber septum.

The inoculated bottles were loaded into the BacT/Alert 3D/60 system incubator and incubated for a maximum period of 5 days. The bottles contain media and atmosphere which promote the recovery of a wide variety of microorganisms without venting. Standard BacT/Alert software provided with the system was used for recording the results. Bottles flagged as negative after the maximum period of 5 days were unloaded from incubator and subcultured for a final confirmation.

Bottles flagged as positive were unloaded from the system and processed further as follows - Appropriate amount of fluid was aspirated from the bottles with sterile precautions. This fluid was used for smear preparation for Gram staining and subculture; for which Nutrient agar, 5% Blood agar, MacConkey agar were inoculated and incubated. Based on colony morphology of the growth on subcultured media plates, Gram staining of the smear from colony, Catalase test and Oxidase test; selection and processing of miniAPI strips for identification and antibiotic susceptibility of that organism was done as per manufacturer's instructions. (Figure 2)

Figure 2. For selection of miniAPI strips.
The miniAPI strips are basically made up of plastic having 32 wells or depressions; called as cupules, in each strip. The ID strips are for the identification of the organism and ATB strips are for the antibiotic susceptibility of that isolate. ID strips are provided with biochemicals and other reagents in dried and preserved form in the cupules. ATB strips are provided with the antibiotics in dried preserved form in the cupules.

After the selection, the ID strips as well as the ATB strips were inoculated according to the guidelines provided by the manufacturer.

The miniAPI strips used are

**Identification strips (ID strips)**
Rapid ID 32 E, ID 32 GN, ID 32 STAPH, rapid ID 32 STREP.

**Antimicrobial susceptibility testing strips (ATB strips)**
Rapid ATB E 4, ATB PSE 5, ATB STAPH 5, ATB STREP 5, ATB ENTEROC 5.

The strips were then read using the miniAPI system.

**OBSERVATION AND RESULTS:**

Out of total 157 blood samples, 99 (63.05%) samples were collected from male and 58 (36.94%) were from female neonates. Out of them, 70 (44.58%) were flagged as positive by BacT/ALERT 3D 60 system and 87 (55.41%) were negative. (Figure 3)

**Figure 3. Blood culture report by BacT/ALERT**

As far as Time duration taken by BacT/ALERT 3D 60 is concerned, of the total 70 culture positive samples, 46 (65.71%) were flagged positive within first 24 hours of incubation. Additional 13 (18.57%) were flagged between 24-48 hours and 11 (15.71%) samples were flagged positive after 48 hours. In total, 59 (84.28%) positive blood samples were detected within 48 hours of incubation. (Figure 4)

**Figure 4. Time taken to flag the bottles as positive by BacT/ALERT 3D 60 system**

Sex wise distribution of culture positive cases revealed positivity more in male, i.e. 44 (62.85%) out of 70; while Female neonates comprised of 26 (37.14%). The male to Female ratio observed was 1.69:1. (Figure 5)

**Figure 5. Sex wise distribution of culture positive cases**

Of the total 70 isolates, *E.coli* was the most common i.e 16 (22.85%); followed by *Coagulase*...
negative Staphylococcus (CoNS), isolated in 15 (21.42%), followed by Staphylococcus aureus isolated in 14 (20%), Klebsiella pneumoniae in 10 (14.28%), Pseudomonas aeruginosa in 6 (8.57%), Enterococcus faecalis-3 (4.28%) and Streptococcus pyogenes in 2 (2.85%). (Figure 6)

**Figure 6. Organism wise distribution of isolates from positive blood samples**

[Bar chart image]

**Antibiotic susceptibility patterns:**

Among Coagulase negative Staphylococci (CoNS), maximum susceptibility was found to the Quinupristin-Dalfopristin (100%) and Vancomycin (100%) followed by Minocycline (93.33%) and Levofloxacin (86.66%). Least susceptibility by CoNS was shown to Erythromycin (40%) and to Penicillin (13.33%).

Staphylococcus aureus showed maximum susceptibility to the Quinupristin-Dalfopristin (100%) and Vancomycin (100%) followed by Tetracycline (85.71%) and Rifampicin (78.57%). Cotri-moxazole and Penicillin were the drugs to which Staphylococcus aureus isolates were least susceptible, 42.85% and 21.42%, respectively.

E.coli isolates were most susceptible to Piperacillin + Tazobactum (93.75%) and Nitrofurantoin (93.75%), followed by Ticarcillin + Clavulanic acid (87.5%). Least susceptibility of E.coli isolates was observed to Cefepime (18.75%) and Ampicillin (12.50%).

Among Klebsiella pneumoniae isolates, maximum susceptibility was shown to Piperacillin + Tazobactum (90%) and Piperacillin (80%) followed by Ciprofloxacin (70%) and Ceftriaxone (70%). Least susceptibility was shown to Ampicillin (20%), Cefoxitin (20%) and Cefazolin (10%).

*Pseudomonas aeruginosa* were most susceptible to Imipenem (100%), Meropenem (100%) and Piperacillin + Tazobactum (83.33%) followed by Amikacin (66.66%) and Gentamicin (66.66%). These isolates were least susceptible to Cefepime (33.33%) and Ticarcillin (16.66%)

Among the 70 neonates who were diagnosed to be having septicemia by positive blood cultures, 58 (82.85%) improved and were discharged from the wards. 12 (17.14%) neonates succumbed to their illness.

**DISCUSSION:**

Globally, an estimated 4 million babies die in the first 4 weeks of life (the neonatal period) every year\(^1\) and half of them die in their 1st 24 hours. It accounts for 40% of under 5 mortality. 98% of these deaths occur in developing countries.\(^2\)

In India, the Sample Registration System estimates of neonatal mortality for the year 2010 is about 25 per 1000 live births in early neonatal period (0-7 days) with 28 for rural areas and 15 for urban areas.\(^3\)

In our study, out of 157 blood samples, 44.58% i.e. 70 blood samples were confirmed as positive. Ghanshyamet al\(^4\) (2002) in their study, observed 42.00% positivity in blood cultures.
Ananthakrishnan et al (2009) in their study concluded that 40.6% samples were positive in blood culture. According to Gandhi S et al (2013) out of 238 samples studied, blood culture was positive in 76 cases (32%).

In the study by Wadud A et al (2009), out of 4915 sample, 707 (14.38 %) were positive by BacT/ALERT system. In the study by Kennedy GT et al (1995), Four thousand bottles were analyzed, of which 477 (11.92%) were positive by BacT/ALERT system. The percentage of positivity in our study is higher as compared to the above mentioned studies where BacT/ALERT system was used.

Out of 70, for 11 (15.71%) samples the BacT/ALERT system needed more than 48 hours to detect as positive while 59 (84.28%) positive samples were detected within 48 hours of incubation.

Kennedy GT et al (1995) mentioned in their study that, 29% positive blood cultures were detected in less than 12 hours, 68% by 24 hours, 81% by 48 hours, and 87% by 72 hours.

Our observations about detecting 84.28% positive samples in less than 48 hours duration is comparable with the study by Kennedy GT et al.

In the study by Jyothi P et al, among the culture positive cases, there were 86 (65.5%) male and 45 (34.5%) female neonates with the male-to-female ratio of 1.9:1.

In the study by Haseeb M et al (2014), analysis with respect to sex of the baby showed that 65 cases (61.9%) were males and 40 cases (38.09%) were females with a male: female ratio of 1.62: 1.

Gheibiet al (2008) observed the male to female ratio as 1.67:1 in a study of 227 cases. Our observation is similar to that of Gheibiet al.

Khatauaet al postulated that the factors regulating the synthesis of gamma globulins are probably situated on the X chromosome. Presence of one X chromosome in the male infant thus confers less immunological protection compared to female counterpart. Singh stated that male infants are around 4 times at increased risk to develop sepsis compared to females. Term male infants have an approximately two fold higher incidence of septicemia than term females.

According to the study by Jyothi P et al, etiology of the 131 isolates included Gram-negative bacilli (73/131, 55.7%) and Gram-positive cocci (58/131, 44.3%). Klebsiella spp. (30.5%) and coagulase-negative Staphylococci (CONS) (27.5%) were the most common Gram-negative and Gram-positive organisms respectively.

Gandhi S et al studies observations are that E.coli (21.25%) was the most common isolate, Staphylococcus aureus (20%) and Klebsiella pneumoniae (20%) were isolated in equal proportion. The next isolate was CoNS (11.25%).

The observations of present study correlate well with the study by Gandhi S et al. Freeman et al reported a rise in CONS septicemia in neonates from 25% to 68.8% during 1975-1982 at their hospital. Data from a tertiary care hospital in India for year 2001-2002 revealed that CONS were the commonest gram positive bacteria isolated from cases of neonatal septicemia.

Although in a study by Baumgart S et al, CONS were found to be the most frequent blood contaminant; Hammerberg et al stated that after the careful cleaning of venipuncture site, the growth of CONS in blood culture of specimens of premature neonates indicates bacteremia rather than the skin contaminant in the vast majority of cases.
In the present study, 6 (8.57%) strains of *Pseudomonas aeruginosa* (*P. aeruginosa*) were isolated. Guha et al. and Movahedian et al. isolated 16.2% and 36% *P. aeruginosa* in their studies; respectively. Pseudomonas infection is rarely perinatally acquired however, it is among the more common gram-negative organisms causing nosocomial sepsis in NICU patients.

Prior to antibiotic era, the mortality from septicemia was 90% but it declined to 24%-58% after antibiotics came into use. The varying microbiological pattern of neonatal septicemia warrants the need for an ongoing review of causative organisms and their antibiotic sensitivity pattern. The inability to be certain of infection, coupled with non-specific signs of life threatening illness in neonates have resulted in widespread use of antibiotics, aggravating the problem of antibiotic resistance.

Jyothi P et al. observed that Gram-positive isolates had sensitivity of 91% to Linezolid, 68% to Tetracycline, 64% to Piperacillin / Tazobactam, Erythromycin, and 52% to Ciprofloxacin.

Ghanshyam et al. reported that 50% of the *Klebsiella* and *E. coli* isolates were sensitive to Cefotaxime. A study by Movahedian et al. revealed a very high degree of resistance in gram negative organisms not only to commonly used antibiotics, but also predominantly to broad spectrum cephalosporins. Gheibie et al. reported high resistance to Cefotaxime (67.5%), Ceftriaxone (65.3%) and Ceftazidime (64.3%) among gram negative organisms.

Jyothi P et al. observed that best overall sensitivity among Gram-negative isolates was to Imipenem (93%), followed by Amikacin (52%) and Netilmicin (41%).

Although positive blood culture is the gold standard in the diagnosis of neonatal sepsis; in the absence of proof of sepsis, many clinicians and even some neonatologists feel obliged to continue antibiotics for a full 10 days course. If the baby is not infected he or she is being subjected to unnecessary treatment. There is also danger of removing useful or susceptible organisms and encouraging resistant ones. If this occurs we shall reach a stage to go on to use more expensive antibiotics, or we have no more chance to use new drug combinations.

Strict infection control in neonatal units, hand washing combined with judicious policy for antibiotic therapy are the main solution to this problem. It will be important, however, to continue surveillance of neonatal sepsis in order to follow closely changes in trends and risk factors, to obtain information for empiric antibiotic therapy and to react rapidly in case of major changes in susceptibility patterns and occurrence of outbreaks.

**LIMITATION OF THE STUDY:**

This is an observational study. As the study group was restricted to neonates only, sample volume for blood culture was a crucial issue. Neonates admitted in the hospital were subjected to various investigations under biochemistry, microbiology and pathology (hematology) lab. As sample collection is not that easy in neonates, mostly due to small size of blood vessels and most neonates being investigated are underweight. Multiple pricking is not advisable hence blood collected in single prick was distributed for three labs as biochemistry, microbiology and pathology (hematology). As sample received in microbiology was less in volume, it was subjected only for automated blood culture system; conventional blood culture system being excluded from the study. And for the very same reason, comparison of blood culture by conventional and modern system was not possible.
CONCLUSION:

Total blood culture positivity rate was 44.58%. By using BacT/ALERT 3D 60, 65.71% blood culture positivity was observed within 24 hours incubation and bottles flagged positive within 48 hours were 84.28%.

All 70 positive blood cultures were monobacterial. Amongst gram positive bacteria CoNS and in Gram negative bacteria *E.coli* were predominant. *E.coli* isolates showed maximum susceptibility to Piperacillin + Tazobactum combination and least susceptible to Ampicillin. CoNS were maximum susceptible to Vancomycin and Quinupristin - Dalfopristin while least susceptible to Penicillin.

Time for detection of positivity by BacT/ALERT in blood cultures was short. Both these systems, the BacT/ALERT 3D 60 and the miniAPI, were efficient, less time consuming and labor saving which is essential for the clinician and more importantly for the patient.

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