Original Research Article

Microbiological profile of Neonatal Septicemia by automated blood culture system (BacT/ALERT) at a tertiary care hospital

ABSTRACT:

Background: This study was carried out to do rapid diagnosis of neonatal septicemia so as to prevent septicemia related complications and neonatal morbidity and mortality. **Materials and Methods:** This study was carried out in a tertiary care hospital from a period of 1/3/2014 to 31/8/2015. This is a non-randomized, prospective study in which one hundred and nine cases of suspected neonatal septicemia on the basis of antenatal high risk factors and signs and symptoms of sepsis were studied. Blood samples were collected from neonates in whom septicemia was suspected usually before antibacterial agents were given and both positive and negative samples were detected by automated system BacT/ALERT. **Result:** Out of 109 samples tested, 69 samples were positive and 40 samples were negative. The incidence of neonatal septicemia due to gram positive organisms (62.85%) was found to be high. Among them *Coagulase negative staphylococci* (CONS) (27) (38.57%) was most common organism followed by *Staphylococcus aureus* (11) (15.71%), *Enterococcus spp.* (7.14%) and *Streptococcus spp.* (1.43%). Rate of gram negative organisms was quite low (32.85%). Out of them *Acinetobacter spp.* (17.14%) was most common followed by *Klebsiella spp.* (7.14%), *E.coli* (5.71%) and *Pseudomonas spp.* (2.86%). **Conclusion:** Early detection of neonatal sepsis along with broad spectrum antibiotics lead to reduced mortality rate.

Key words: Neonatal septicemia, BacT/ALERT, Gram positive organisms, Gram negative organisms.

INTRODUCTION:

Neonatal septicemia is a clinical syndrome that occurs due to acute invasion of micro-organisms and their active multiplication in the blood stream that has been documented by positive blood culture during the first month of life [13].

It is one of the four leading causes of neonatal demise in our country. It is estimated that 20% of all neonates develop septicemia [9,6] and it is responsible for 30-50% of total deaths during the first month of life in developing countries [9,2]. Neonatal septicemia is the commonest cause of neonatal mortality in the developing countries like India [14], so prompt detection of micro-organisms through blood culture and appropriate antimicrobial therapy with the help of other laboratory investigations and supportive care are the key determinants for positive outcome in this serious pediatric emergency.

The neonates particularly those born prematurely are compromised hosts. During the first month of life, they are subjected to infections by a unique list of pathogens including coagulase positive and negative *staphylococci*, coliform organisms, group–B *streptococci*.

The fulminent nature of this illness makes it necessary to review the relative frequency of various bacteria responsible for this serious condition so that appropriate antibiotic treatment and patient care can be instituted as early as possible [18].

The reported incidence of neonatal septicemia in developed countries varies from 1 to 10 per 1000 live birth .It is difficult to assess the true incidence in our country because of two reasons:

1. The data from community setting is not available.
2. Even in hospitals the diagnosis is often not based on microbiological studies but on clinical grounds alone.

Incidence of neonatal septicemia in hospital born infants in India is in range of 11.0-24.5 per 1000 live births [23].

The purpose of this study is to find out the etiological agents present in our hospital as early as possible by the automated blood culture system named BacT/ALERT .

MATERIAL AND METHODS:

This study was carried out in a tertiary care hospital from a period of 1/3/2014 to 31/8/2015 after clearance of IRB. This is a non-randomized, prospective study in which one hundred and nine cases of suspected neonatal septicemia on the basis of antenatal high risk factors and signs and symptoms of sepsis including maternal fever, prolonged rupture of membranes, foul smelling lochia, temperature instability, feeding difficulty, respiratory distress, jaundice, convulsions, autonomic disturbances during this period were studied. Blood samples were collected from neonates in whom septicemia was suspected usually before antibacterial agents were given.

Inclusion criteria:

1. Neonates (age – 0 to 28 days).
2. Neonates having signs and symptoms of septicemia such as maternal fever, feeding difficulty, convulsions and respiratory distress syndrome.
3. Premature infants.
4. Only blood samples of neonates and not any other fluid.

Exclusion criteria:

1. Infants and children above the age of 28 days and adults and elderly patients.
2. Any other body fluid except blood.

Clinical history, birth history including treatment given was noted in all cases. All the details were taken within a proforma.

Blood samples were collected with disposable syringe and needle under strict asepsis. Usually samples were collected from peripheral vein. Skin was painted with 70% alcohol and allowed to dry. Then approximately 2 ml of blood was collected and immediately transferred to BacT/ALERT PF PLUS aerobic blood culture bottles. The bottles were incubated in BacT/ALERT system and patient's clinical data was introduced in the computer.

PRINCIPLE OF THE TEST:

The BacT/ALERT microbial detection system utilizes a colorimetric sensor and reflected light to monitor presence and production of carbon dioxide (CO_2) dissolved in culture medium. If microorganisms are present in test sample, carbon dioxide is produced as the microorganisms metabolize the substrates in culture medium. When growth of the microorganisms produces CO_2, the color of gas permeable sensor installed in the bottom of each culture bottle changes from bluegreen to yellow. The lighter color results in an increase of reflectance units monitored by the system. Bottle reflectance is monitored and recorded by the instrument every 10 minutes.

Bottles that do not become positive remain in the system for 5 days. After 5 days, the computer prints out a list of negative bottles and illuminates the light adjacent to each well containing a negative bottle. These bottles are removed from the system and discarded and declared negative. The positive bottles are unloaded and subcultures are made on mac Conkey's medium and blood agar plates. Organisms are identified from their colony characteristics, appearances in smear, biochemical reactions, appearance on mannitol salt agar and bile esculin agar. This study has been approved by ethical committee.

RESULT: A total 109 samples were tested out of which 69(63.30%) were positive and 40(36.70%) were negative for neonatal septicemia. Out of 109 total cases, 69(63.3 %) had been suspected of neonatal septicemia between 0-3 days of age and 40 (36.7%) had been suspected between 4-28 days of age[Table 1]. Out of 109 cases of septicemia, 66 (60.55%) cases were being suspected in male neonates, while 43(39.45%) were being suspected in female neonates[Table 2]. Out of 69 positive cases, 44 (63.8%) cases were noted positive between 0-3 days of age while 25 (36.2%) cases were noted positive between 4-28 days of age[Table 3]. 45 (65.22%) were male neonates while 24 (34.78%) were female neonates[Table 4].

Out of 69 positive cases, in one positive case two organisms were isolated. So total organisms isolated were 70. Out of 70 organisms isolated, Gram positive cocci are 44 (62.85%), Gram negative bacilli are 23 (32.85%) and *candida non-albicans* are 3 (4.30%)[Table 5]. Amongst gram positive bacteria, 27 (38.57%) of coagulase negative *staphylococci* (CONS), 11 (15.71%) of *staphylococcus aureus*, 5(7.14%) of *enterococcus spp*. and 1(1.43%) of *streptococcal spp*. isolated from blood cultures. Amongst gram negative bacteria, 12 (17.14%) of *acinetobacter spp.*, 5(7.14%) of *klebsiella spp*., 4(5.71%) of *E.coli*, 2(2.86%) of *pseudomonas spp.* and amongst candida, 3(4.30%) of *candida nonalbicans* isolated from blood culture samples[Table 6].

DISCUSSION:

Neonatal septicemia has remained one of the most common causes of child death in the early stage of life span. Varieties of microorganisms like gram positive and gram negative bacteria and fungi like candida are responsible for neonatal septicemia. The most difficult tasks faced by neonatologist is to differentiate between septicemic and non-septicemic cases, this situation is complicated by conditions like birth asphyxia, hypoglycemia, hypothermia, intracranial hemorrhage. For definitive diagnosis of septicemia causative microbes are required to be isolated from blood culture which takes minimum time of 48 hours. It also provides correct guideline to institute rational antibiotic therapy.

Present study is undertaken with the idea to obtain scientific information regarding prevalent etiological agents and their rapid detection by BacT/ALERT system.

Neonatal septicemia must be treated urgently and specifically to reduce the mortality. Suspected infection based on clinical criteria need to be supported by microbial investigations to find causative organisms and their antibiotic sensitivity pattern.

In present study rate of gram positive organisms were 62.85% which is comparable with the study of Kavitha Prabhu *et al*(64.19%) [11]and Abhishek Mehta *et al*(67.8%)[1] and Gram negative organisms were 32.85% which is comparable with the study of Kavitha Prabhu *et al* (34.56%)[11] and Abhishek Mehta *et al* (28.7%)[1] and *Candida spp.* were 4.30 % comparable with the Abhishek Mehta *et al* (2.87%)[1] and R. Nandana *et al* (6.25%)[20].

A total number of 109 neonates with clinically suspected neonatal septicemia were studied. 69 (63.3%) cases out of them were blood culture positive which is comparable with the study of Izeta softic *et al* [8]. Maximun number of neonatal septicemia was between 0-3 days of age (63.8%) which is similar to study done by Vrishali *et al* [24]. Neonatal septicemia occurs more in male patients (65.22%) than female patients (34.78%) which is similar to study done by Navodeep Saha *et al* [17] . Early sepsis was more commonly associated with *coagulase negative staphylococci, acinetobacter species* and *staphylococcus aureus* whereas late sepsis was more commonly associated with *coagulase negative staphylococci*. In present study all samples show pure growth of organism. The incidence of neonatal septicemia due to gram positive organisms (62.85%) was found to be high. Similar findings of high positivity by gram positive organisms were seen in studies of Galhotra S and Van den hoogen A [22, 5].where as some researchers (Muley *et al* 2015, Jyothi *et al* 2013) reported Gram negative organisms as predominant

pathogens[15, 10]. Among them *Coagulase negative staphylococci* 27 (38.57%) was most common organism followed by *Staphylococcus aureus* 11 (15.71%), *Enterococcus spp.* 5(7.14%) and *Streptococcus spp*. 1(1.43%) which is similar to study done by Bipin gupta *et al* [4], Ballot DE *et al* [3]. In studies done by other researchers [7, 21] also CONS were the most common organisms isolated in neonatal septicemia cases. The increasing prevalence of (CONS) infections is due to their increasing antibiotic resistance as reported by some researchers [19] . Rate of gram negative organisms was quite low (32.85%). Out of them *Acinetobacter spp.* (17.14%) was most common followed by *Klebsiella spp.* (7.14%), *E.coli* (5.71%) and *Pseudomonas spp.* (2.86%).in other studies, other gram negative bacteria have been found to be most common gram negative bacteria e.g. *Klebsiella spp*. [16, 12].

CONCLUSION: In summary, neonatal septicemia is more common during first 3 days of life and it is more common in male neonates. The Gram positive organisms are the frequent cause of neonatal septicemia. Early detection of neonatal sepsis along with broad spectrum antibiotics lead to reduced mortality rate. Better management of sepsis related complications have also reduced the mortality. Practice of hand washing, asepsis during resuscitation procedures of neonates, frequent microbial surveillance, fumigation of nursery and management of babies with neonatal sepsis in isolated chamber have controlled the spread of infection to other neonates. Promotion of breast feeding and discouraging top feeding can also significantly reduce neonatal mortality rate.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1: Distribution of cases according to age of onset of septicemia

AGE	TOTAL NO. OF CASES	PERCENTAGE
0 - 3 days of age	69	63.3%
4-28 days of age	40	36.7%
TOTAL	**109**	**100%**

Table 2: Distribution of cases according to sex

SEX	TOTAL NO. OF CASES	PERCENTAGE
Male	66	60.55%
Female	43	39.45%
TOTAL	**109**	**100%**

Table 3: Distribution of positive cases according to age of onset of septicemia

AGE	POSITIVE CASES	PERCENTAGE
0-3 days of age	44	63.8%
4-28 days of age	25	36.2%
Total	**69**	**100%**

Table 4: Distribution of positive cases according to sex

SEX	POSITIVE CASES	PERCENTAGE
Male	45	65.22%
Female	24	34.78%
Total	**69**	**100%**

Table 5: Organism wise distribution of positive cases of neonatal septicemia

ORGANISM	NO. OF CASES	PERCENTAGE(%)
Gram positive cocci	44	62.85%
Gram negative bacilli	23	32.85%
Candida nonalbicans	3	4.30%
Total	**70**	**100%**

Table 6: Distribution of positive cases according to individual organism

ORGANISM	NO. OF CASES	PERCENTAGE
GRAM POSITIVE ORGANISM		
1.*Coagulase negative staphylococci*	27	38.57%
2.*Staphylococccus aureus*	11	15.71%
3.*Enterococcus spp.*	5	7.14%
4.*Streptococcus spp.*	1	1.43%
Total	**44**	**62.85%**
GRAM NEGATIVE ORGANISM		
1.acinetobactor spp.	12	17.14%
2.klebsiella spp.	5	7.14%
3.E.coli	4	5.71%
4.pseudomonas spp.	2	2.86%
Total	**23**	**32.85%**
CANDIDA		
1.*Candida nonalbicans*	3	4.30%
TOTAL	**70**	**100%**