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Original Research Article

Bacteriological profile and antibiotic susceptibility pattern of neonatal septicaemia in patients of neonatal intensive care unit, by BACTEC in a tertiary care hospital, Vadodara

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ABSTRACT

Background: Neonatal septicemia is the major cause of mortality and morbidity in developing countries like India. It is an ongoing major global public health challenge with major contribution from neonatal sepsis. Objective were to study the prevalent organisms and their antibiotic susceptibility pattern of neonatal septicemia in NICU by BACTEC and neonatal factors and maternal factors associated with neonatal sepsis.
Methods: A cross-sectional study was carried out from December 2020 to September 2021. Blood samples were taken from the suspected cases admitted to NICU for culture and antimicrobial susceptibility test. A total of 103 blood culture-proven neonatal sepsis patients were enrolled in this study.
Results: Out of 103 neonates having blood culture-proven sepsis, septicemia was most common in males (53.4%), preterm (73.4%), and low birth weight (61.2%), with vaginal mode of delivery (51.5%). Neonatal septicemia was predominantly caused by gram-positive methicillin resistant coagulase negative *Staphylococci* (CONS) (28.15%). Among the all-gram-positive organism, vancomycin was the most sensitive drug followed by linezolid. For the gram-negative organisms, piperacillin/ tazobactam was the most sensitive drug followed by meropenem. Turnaround time of an automated BACTEC system for reporting culture positivity along with antimicrobial susceptibility was most commonly within 3 days (55.34%).
Conclusions: A high level of suspicion is needed on clinical grounds for the diagnosis of neonatal septicemia. Early onset septicemia is more common which can be curtailed by clean vaginal deliveries. Prematurity and low birth remain the major presentation for admission to NICU followed by respiratory distress syndrome. Maternal risk factors are associated with an increase in the incidence of neonatal septicemia. Septic screen markers are important in the presumptive diagnosis of sepsis in neonates along with the correlation of clinical presentation. The yield of reporting culture-proven sepsis neonates is more rapid with an automated BACTEC system as compared to conventional blood culture methods.

Keywords: Neonatal septicemia, BACTEC, Neonatal factors, Maternal factors

INTRODUCTION

Over 140 million babies are born every year in the world (2020 estimate), and more than 2.4 million infants die every year with more than 1 million dying on the first day of life.[1] Over 25 million babies are born every year in India, which is one fifth of the world's annual childbirth. One of 25 million babies dies every minute in India.[2]

Occurrence of neonatal sepsis is 0.5 to 8.0/1000 births. Neonatal infections are responsible for approximately 33% of deaths and are the second leading cause of neonatal mortality.[3] The infant mortality rate is 40 deaths per 1000 live births and the under-5 mortality rate is 49 per 1000 live births.[3] Incidence of early neonatal bacterial sepsis varies from 0.9 to 1.5 per 1000 live births, while that of late neonatal sepsis varies from 3 to 3.7 per 1000 live births in developed countries. The incidence of clinically diagnosed neonatal sepsis in developing countries like ours is 49 to 170 per 1000 live births and culture-proven sepsis is 16 per 1000 live births.[4] Neonatal sepsis is defined as clinical pattern characterized by signs and symptoms of infections with/ without accompanying bacteremia in first month of life.[5] The common factors associated with neonatal sepsis are low birth weight, length of time spent in hospital, invasive procedure, colonization by bacteria from the environment, a significant proportion of these septicaemic babies are those who were born unattended outside the hospital in an unhygienic environment. Several factors like age and parity of mother, prenatal care, sex of newborn, gestational age, and associated congenital anomalies predispose newborns to neonatal bacterial diseases. Perinatal maternal complications such as abruption placenta, placenta previa, maternal toxemia, and premature rupture of the membrane increase the incidence of neonatal sepsis. The clinical manifestations of neonatal infections are influenced by the timing of exposure, inoculum size, immune status, and virulence of etiological agent.[6] Identification of organism causing septicaemia and its antibiotic susceptibility patterns reduces the morbidity and mortality in neonates. Early treatment and appropriate use of antibiotics along with hand hygiene practice would help in minimizing the risk of severe morbidity and mortality in sepsis and reducing the emergence of multi-drug resistant organisms in intensive care units. This study was undertaken to determine the prevalence of neonatal sepsis in tertiary care hospitals, a profile of microorganisms causing neonatal septicemia, and antibiotic sensitivity patterns of isolates.

METHODS

Study area and duration

A cross-sectional study was carried out in the department of microbiology, at S. S. G hospital and medical college Baroda, Vadodara from the ethical approval from December 2020 to September 2021.

Study sample

A total of one hundred and three patient of culture proven neonatal sepsis admitted in the neonatal intensive care unit of department of pediatrics at SSG hospital and medical college Baroda, Vadodara were included in this study.

Study population

All culture positive sepsis neonates aged 3 days to 28 days admitted in-intramural NICU (patient directly admitted in NICU, SSG hospital), extramural NICU (patient referred from other hospital).

Inclusion criteria

Blood culture samples collected prior to initiation of antimicrobial treatment from all the newborns in the age group of 0-28 days, who has clinical features suggestive of septicaemia (fever, not taking feed, respiratory distress, prematurity, low birth weight, convulsion, poor cry, meconium-stained liquor) admitted in the NICU of SSG hospital, Vadodara was included in the study.

Exclusion criteria

Neonates having severe congenital illness and Blood culture samples collected after initiation of antimicrobial treatment were excluded from the study.

Method of study

This study was carried out after obtaining the institutional ethics committee's approval. All relevant details like gestational age, sex, symptoms, birth history, weight at birth, usage of antibiotic were taken. All culture positive sepsis neonatal patient admitted in NICU and willing to participate during study period were included for study with informed assent, their blood sample received at department of microbiology, medical college Baroda. Blood culture bottle received was processed by automated blood culture system (BACTEC). Any blood culture bottle with red alert/ flag in instrument within 24-48 hours after insertion is considered positive and manual subculture was done on pre-dried plates of chocolate agar, brain heart infusion agar and MacConkey agar. The inoculated plates incubated aerobically in the incubator at 37°C for 24 hours, Chocolate agar plates were placed in CO_2 incubator at 35°-37°C in a 5% CO_2 atmosphere and the plates were observed for growth. Direct smear was prepared from the blood culture bottle and gram stain was carried out. Microscopic findings recorded and correlated with colony growth on culture plate next day. Growth on culture plates identified by colony characteristics, gram's stain of colony smear and standard biochemical tests. Blood culture bottles with no alert/green flag within 5 days of insertion in instrument was considered negative for bacterial growth and reported negative. Antibiotic sensitivity testing was performed on Mueller Hinton agar plates by modified Kirby Bauer disk diffusion method as per clinical laboratory standard institute guideline.

RESULTS

During study period, 103 neonates of clinically suspected and culture-proven sepsis were taken from the NICU of the paediatrics department of SSG hospital, Vadodara.

Table 1: Gender wise distribution in the septicaemia.

Gender	No. of neonates, N (%)
Male	55 (53.4)
Female	48 (46.6)

Neonatal septicemia was more common in male (53.4%) than in female (46.6%) neonates.

Table 2: Gestational age wise distribution in septicaemia.

Variables	No. of neonates, N (%)
Full term	40 (38.8)
Pre term	63 (61.2)
Extreme preterm	01
Very preterm	21
Moderate preterm	27
Late preterm	14

Neonatal septicemia were more common in preterm neonates (61.2%) than in the term neonates (38.8%).

Table 3: Birth weight wise distribution in septicaemia.

Birth weight (gm)	No. of neonates, N (%)
>2500	29 (28.1)
<2500	74 (71.9)
ELBW (<1000)	05
VLBW (<1500)	26
LBW (<2500)	43

Neonatal septicemia was more common in low birth weight (<2500 gm) babies (71.9%) than in (>2500 gm) babies (28.1%).

Table 4: Distribution of neonates according to onset of septicaemia, (n=103).

Onset	Neonates with blood positive culture, N (%)
Early onset septicemia (<72 hrs of life)	54 (52.4)
Late-onset septicemia (>72 hrs of life)	49 (47.5)

Early onset septicemia was more common (52.4%) in the neonates than late onset septicemia (47.5%).

Table 5: Mode of delivery wise distribution in septicemia, (n=103).

Mode of delivery	Neonates with blood positive culture, N (%)
Vaginal	53 (51.5)
Lower segment cesarean section (LSCS)	50 (48.5)

Neonatal septicemia was more common in babies delivered with vaginal mode of delivery (51.5%) than LSCS (48.5%).

Table 6: Distribution of maternal risk factors associated with septicemia, (n=103).

Maternal risk factor	No. of neonates, N (%)
Meconium-stained liquor (MSL)	16 (15.5)
Pregnancy-induced hypertension (PIH)	6 (5.8)
Oligohydramnios	6 (5.8)
Young primi mother	4 (3.9)
Leaking per-vaginal	4 (3.9)
Pre-eclampsia	4 (3.9)
Severe anemia	3 (2.9)
Non-progression of labour (NPL)	3 (2.9)
COVID-19 positive	2 (1.94)
Placenta previa, gestational DM,	1 (0.97)
Anhydroamnios, convulsion, perinatal	-
Depression, cord prolapsed, RPR positive etc (Others)	-

Meconium-stained liquor (MSL) (15.5%) and pregnancy induced hypertension (PIH) and oligohydroamnios (5.8%) were the commonest maternal risk factor associated with neonatal sepsis.

Table 7: Septic markers in neonates with septicemia, (n=103).

Septic markers	Neonates with blood positive culture, N (%)
C-reactive protein positive	97 (94.2)
C- reactive protein negative	6 (5.8)
Total leucocyte count (TLC) (<9000 cells/mm^3)	37 (35.9)
Platelet count (<1,50,000 cells/mm^3)	45 (43.7)

Out of 103 neonates with culture proven sepsis, majority of babies were positive for CRP screening (94.2%), only (5.8%) babies were tested negative for CRP screen. In the present study, 35.9% neonates with culture proven sepsis had total leucocyte count <9000 cells/mm^3, while 43.7% had platelet count <1,50,000 cells/mm^3.

Gram positive bacteria (54.4%) were the prominent pathogen causing septicemia followed by gram negative bacteria (45.6%).

Among the gram-positive bacteria methicillin resistant CONS (28.15%) was predominant followed by *Enterococcus faecalis* (12.6%).

Among the gram-negative bacteria, *Acinetobacter baumenii* (18.4%) was the predominant pathogen followed by *Klebsiella pneumonia* (13.6%) and *Escherichia coli* (10.7%).

Table 8: Organism isolates from blood culture.

Blood culture-positive organism	Culture positive cases, N (%)
MR CONS	29 (28.15)
Acinetobacter baumenii	19 (18.4)
Klebsiella pneumonia	14 (13.6)
Enterococcus faecalis	13 (12.6)
Escherechiae coli	11 (10.7)
CONS	8 (7.8)
Staphylococcus aureus	4 (3.9)
Pseudomonas spp.	3 (2.9)
MR Staphylococcus aureus	1 (0.98)
Streptococcus spp.	1 (0.98)
Total	103

Among the gram-negative organism isolated, the majority of strains are sensitive to colistin, piperacillin-tazobactam followed by meropenem. Among the gram-positive isolates vancomycin, and inezolid, were 100% sensitive. Among the isolated *Staphylococcus aureus* 01 strain was methicillin-resistant. Isolated *Enterococcus faecalis* was resistant to ampicillin and erythromycin.

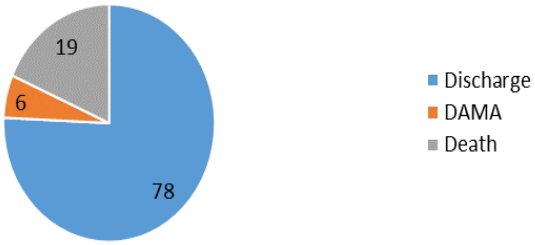

Figure 1: Distribution of outcomes of neonates with septicemia.

The overall mortality in the present study was 18.45% in culture proven cases of neonatal sepsis. The 78 neonates (75.7%) were discharged after complete cure from the hospital, while 6 neonates (5.8%) were either discharged against medical advice (DAMA) or absconded from the hospital in culture proven sepsis neonates.

DISCUSSION

Neonatal septicemia is an important cause of morbidity and mortality throughout the world and is a major concern in developing countries like India. As the disease may start with minimal and non-specific symptoms, early and accurate etiological diagnosis is not always easy. Though presumptive antibiotic therapy may result in overtreatment, it is necessary not to delay the treatment until clinical confirmation of septicemia is there, as it can lead to an increase in mortality in neonates. Therefore, more babies are evaluated and treated for sepsis than the number who have the condition.[7]

A male preponderance is noted in almost all the studies of neonatal sepsis.[5] In this study also Neonatal Septicaemia was more common in male (53.4%) than in female (46.6%) neonates.[8]

Prematurity is the prime important factor leading to infection. The preterm infants have a 3-10-fold higher incidence of infection than full-term infants. Premature babies have less immunogenic ability to resist and combat infections; also require prolonged intravenous access and other invasive procedures that impair barrier and clearance mechanisms, so they are at the highest risk of acquiring sepsis.[9,10]

Kumar et al showed 58.5% pre-mature neonates, which was quite comparable with the present study (61.2%) in having more premature neonates with sepsis. Neonatal sepsis is a recurrent complication of very low birth weight (VLBW) babies and it is an important cause of neonatal morbidity and mortality. It is clear that sepsis is inversely proportional to the birth weight of neonates and low birth weight increases the chances of sepsis. In a study by Waseem et al 51% of neonates with culture-proven sepsis had <2.5 kg birth weight, while 71.8% of neonates with culture-proven sepsis in the present study belonged to this group.[11] Roy et al study carried out in a tertiary care hospital of Lucknow, has reported 63.8% low birth weight babies in culture positive group.[12]

Various studies carried out in different part of the country at different time has shown gram negative organisms as the commonest one responsible for sepsis in India.[12-14] But the results show variation in a different geographical area. In the present study, the most common organism causing neonatal sepsis are gram-positive organisms. Isaacs et al has also reported CONS as a major causative organism of sepsis (57.1%).[15] Ozkanet al found CONS to be the most causative organism in sepsis in preterm neonates.[16] In our study most predominant organism isolated was coagulase-negative *Staphylococci*, followed by *Acinetobacter baumenii*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas species*, and *Streptococcus* species.

All gram-positive bacteria were sensitive to vancomycin and linezolid. And they possess a high degree of resistance to penicillin, cefoxitin, amoxyclave, and erythromycin. Shawl et al have also reported in their study a high degree of resistance in gram-positive organisms to most penicillin and cephalosporins, however, glycopeptides, and monobactams as effective antimicrobial agents.[17] Poonam et al also reported gram-positive organisms are highly sensitive to antimicrobial linezolid and vancomycin, and highly resistance to

penicillin and amoxyclav.[18] Pokhrel et al have also reported the same.[19] Out of isolated all *Staphylococcus aureus*, 1 strain was methicillin-resistant. In our study the result showed that isolated gram-negative organisms from blood culture were highly sensitive to colistin, piperacillin tazobactam followed by meropenam. Poonam et al have also reported the same, highly sensitive antimicrobial was carbapenems and piptaz.[18] Pokhrel et al have also reported the same.[19] Highly resistant drug was ampicillin and gentamycin.

Limitations encountered during the study are: 1) Single blood culture is collected although recommended is paired multiple blood culture collection. 2) Anaerobic culture is not performed.

CONCLUSION

Neonatal septicemia is the leading cause of mortality and morbidity in developing countries like India. Multiple antibiotic resistances among the isolates worsen the condition more. A high level of suspicion is needed on clinical grounds for diagnosis. Neonatal septicemia is more common in males, preterm, and low birth weight neonates. Early onset septicemia is more common which can be curtailed by clean vaginal deliveries. Prematurity and low birth remain the major presentation for admission to NICU followed by respiratory distress syndrome. Maternal risk factors are associated with an increase in the incidence of neonatal septicemia. Septic screen markers are important in the presumptive diagnosis of sepsis in neonates along with the correlation of clinical presentation. Blood culture remains the gold standard for diagnosis, provided it is collected before starting antibiotics and the proper amount of blood is added. The use of an automated BACTEC system increases the yield of positivity with a very minimal turnaround time (2-3 days). BACTEC made possible the early identification of organisms from the blood thus early reporting of susceptibility patterns to early initiate the antimicrobial treatment, which results in less mortality, early recovery, and minimize stay in the hospital. As gram-positive organisms were the commonest pathogens associated with neonatal septicemia, coagulase-negative *Staphylococci* were the commonest ones. Longitudinal surveillance should be carried out at regular intervals to describe the varied pathogens causing neonatal sepsis as well as their changing antibiotic susceptibility pattern and a local antibiogram should be formulated from the findings. Judicious use of antibiotics should be promoted. The importance of antenatal health checkups, institutional deliveries, and early and exclusive breastfeeding of newborns should be promoted. In the NICU, protocols for proper hand hygiene, barrier nursing, and rationalization of admissions and discharge from neonatal units are the key areas, where more importance should be given to decreasing the incidence of neonatal sepsis.

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