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Original Article

Paediatrics Section

Bacterial Isolates in a Neonatal Intensive Care Unit-A Rural Perspective

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ABSTRACT

Introduction: Bacterial sepsis is one of the most common causes of mortality and morbidity in neonates. The spectrum of bacteria that cause neonatal sepsis varies and antibiotic resistance is an increasing problem for these bacteria.

Aim: 1) To study the bacteriological profile in the neonates admitted in the Neonatal Intensive Care Unit (NICU) of a tertiary care teaching hospital. 2) To determine the antibiotic sensitivity pattern of the same so that appropriate antibiotics can be chosen to improve the treatment and asepsis.

Materials and Methods: This was a retrospective observational study which was conducted in the NICU of a tertiary teaching hospital. All symptomatic neonates up to 28 days old admitted in NICU were included in this study. A total of 118 samples were sent for cultures. The data was obtained from the Neonatal ICU registers, Medical Department Records and Microbiology laboratory records.

Results: In this study, 118 neonates were considered and depending upon the inclusion criteria those having neonatal sepsis and were admitted to the NICU were taken. The risk of having growth in preterm neonates is 2.27 times more than that in full term neonates. Prevalence of Microbial growth was highest in neonates who were less than 2 kg in birth weight i.e. very low birth weight babies. Microbial growth in neonates admitted within 3 days of life was 1.273 times more than that in neonates who were admitted after 3 days of life. Positive co-relation was seen between microbial growth and who stayed in the hospital for more than seven days. Out of 118 isolates, 80 had no growth, 13 showed MRSA positive, 9 were positive for Staphylococci, 8 were for *Klebsiella*, 3 Gram negative bacilli, 2 *Citrobacter* and 1 remaining.

Conclusion: In view of the changing spectrum of the causative agents of neonatal septicaemia and antibiotic sensitivity and resistance pattern from time to time, a positive blood culture and the antibiotic sensitivity and resistance pattern testing of the isolates are the best guide to the antimicrobial therapy which would be beneficial to the best outcome of the disease.

Keywords: Antibiotics, Bacteria, Blood culture and sensitivity, Microbial growth, Neonatal sepsis, Septicaemia

INTRODUCTION

Neonatal septicaemia is a one of the leading causes of neonatal morbidity and mortality in India [1].The term “septicaemia” indicates the presence of actively multiplying bacteria and their toxins circulating in the blood stream. Neonatal septicaemia is a clinical syndrome which is characterised by systemic signs of infection and accompanied by bacteraemia in the first 28 days of life [2]. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes [3]. Current neonatal mortality rate in India is 27/1000 live births [4].

Many studies have been published at both national and international platforms highlighting the bacterial isolates and their culture patterns. However, much research has not been done on the same in a rural set up. Neonatal ICU with resource limited setting, which created a need to conduct this project.

Clinically, neonatal sepsis may present as hypo/hyperthermia, lethargy, poor cry, refusal of feeding, poor perfusion, hypotonia, absent neonatal reflex, respiratory distress and bradycardia / tachycardia. The source of infection is either nosocomial or community acquired [5].

Current study was undertaken to identify the common bacterial pathogens and their antimicrobial susceptibility and resistance pattern in neonates with sepsis in a tertiary care hospital in rural setting providing neonatal intensive care services. Knowledge of likely causative organisms and their antimicrobial sensitivity pattern could help in instituting early and effective antibiotic therapy for neonatal sepsis.

MATERIALS AND METHODS

As per WHO criteria, a newborn infant, or neonate is a child under 28 days of age [6]. Early onset sepsis presents within the first 72 hours of life. The source of infection is generally the maternal genital tract. On the other hand, late onset sepsis usually presents after 72 hours of age and is either hospital acquired or community acquired [7]. Hence, all symptomatic neonates up to 28-day-old admitted in NICU of tertiary teaching hospital suggestive of neonatal septicaemia were included in our study. At Type 1 error α=0.1 and Type 2 error β=0.2 (i.e., power of test is 80%), the estimated sample size is 118. Data of 118 samples sent for cultures from the period between October 2016 to March 2018. It was an observational retrospective study conducted only on the neonates; thus, the maternal data was not taken into consideration.

Inclusion criteria: All neonates with clinical history, signs and symptoms of sepsis admitted in the NICU of a tertiary care hospital.

Exclusion criteria: 1. Babies more than 28 days of life. 2. Babies not admitted in the NICU.

As the study mainly focuses on the microbiological aspect and the sepsis outcomes, we have not taken into review the clinical risk factors of the neonate and the outcomes.

The data was obtained from the Neonatal ICU registers and Medical Department Records. Microbiology laboratory records was also taken into consideration to find out the antimicrobial sensitivity pattern. At least 3 mL of blood was collected from any peripheral vein after proper cleaning of the venepuncture site (area of 5 cm) with spirit and povidone iodine from where the sample was to be collected. Blood sample was collected on the day of appearance of signs of sepsis.

Glucose broth was used for collection of samples. Semi-automated BACTEC was used for diagnosis. Liquid Thioglycolate media was used for collection, if anaerobic bacteria were suspected to be the organism. Isolation and Identification was also done manually in most of the cases. Three subcultures were done on alternate days at 37°C. Disk diffusion was done by Kirby Bauer's Method. It takes approximately 3-8 days to identify sepsis. The result was taken as no growth if negative results were observed for 5 days. Summarisation of data was done with one-way and two-way tables. Appropriate Statistical methods using chi-square method was applied [8,9].

Institutional Ethics Committee approval was taken. Ethical Reference Number is IEC/493.

RESULTS

In this study, 118 neonates were considered and depending upon the inclusion criteria, those having neonatal sepsis and admitted to the NICU were taken.

Out of total 118 cases, males (57.62%) outnumbered females (42.38%) in our study, out of which 33.89% had positive growth and in them 57.5% were males and 42.5% were females [Table/Fig-1]. Since Odds Ratio is <1 there is no risk or association between gender and growth in this study.

	Male	Female	Total
Growth	23	17	40
No growth	45	33	78
Total	68	50	118

[Table/Fig-1]: Microbial growth pattern depending on the gender of the neonates.
Odds ratio= 0.992 and Chi square= 0.0004

Out of 118 neonates, 55 were preterm and 34 were full term. The growth in preterm neonates is 2.27 times more than that in full term neonates. Therefore, there is positive association between bacterial culture growth and preterm babies and they are more prone to be culture positive as they are more vulnerable than full term babies [Table/Fig-2].

	Full Term	Preterm	Total
Growth	16	24	40
No growth	47	31	78
Total	34	55	118

[Table/Fig-2]: Microbial growth pattern depending on the gestational age of the neonates.
Odds ratio= 2.27 and Chi square= 3.84

Out of 118 neonates, 50 babies were low birth weight, 37 babies were above 2 kg but less than 2.5 kg and 31 babies were >2.5 kg. Hence the prevalence of growth is highest in neonates who are less than 2kg in birth weight i.e., very low birth weight babies (57.5%) which is again a pointer towards more vulnerability [Table/Fig-3].

	<2 kg	>2.5 kg	2-2.5 kg	Total
Growth	23	9	8	40
No growth	27	28	23	78
Total	50	37	31	118

[Table/Fig-3]: Microbial growth pattern depending on the weight of the neonates.
Chi square= 8.78; Since chi-square is more than $p_{0.05}$=5.99, there is a positive association between birth weights and growth; $P_{<2}$=56% $P_{2\text{-}2.5}$=25.8 $P_{>2.5}$=24.32%

Hence the risk of having growth in neonates admitted within 3 days of life is 1.273 times more than that in neonates who are admitted after 3 days of life. Microbial growth in neonates

admitted within 3 days of life (EOS) is 1.273 times more (32.5%) than that in neonates who are admitted after 3 days of life (IOS) which was 12.5%. Highest prevalence of sepsis (55%) is seen in neonates who stayed in the hospital for more than 7 days (LOS) [Table/Fig-4].

	≤3 days	>3 days	Total
Growth	35	5	40
no growth	66	12	78
Total	101	17	118

[Table/Fig-4]: Microbial growth pattern depending on the day of life of admission of the neonates.
Odds Ratio= 1.273 and Chi Square= 0.1784

Since Chi-square is less than $P_{0.05}$=5.99, there is no association between the total duration/ days stayed at the hospital and microbial growth. However the highest prevalence of sepsis is seen in neonates who stayed in the hospital for more than 7 days. This could be due to I/V Cannula sepsis and exposure to procedures and blood sampling [Table/Fig-5].

	<3	>7	3.0-7.0	Total
Growth	13	22	5	40
No growth	27	29	22	78
Total	40	51	27	118

[Table/Fig-5]: Microbial growth pattern depending on the number of days stayed in the hospital by the Neonates.
Chi square= 3.19; $P_{<5}$= 32.5% P_{5-10}= 18.51% $P_{>10}$=43.13% .

Since Odds Ratio is <1 there is no risk or association between delivery type and growth in this study [Table/Fig-6].

	LSCS	Vaginal	Total
Growth	18	22	40
No growth	44	34	78
Total	62	56	118

[Table/Fig-6]: Microbial growth pattern depending on the type of delivery of the neonates.
Odds ratio= 0.632 Chi square= 1.380

Since chi-square is less than $P_{0.05}$=5.99, there is no association between the outcome and growth. Out of total 118 cases, 107 babies were discharged and went home, 1 went DAMA and 10 babies died where cause of death was sepsis and co-morbidities [Table/Fig-7].

	DAMA	Death	Discharge	Total
Growth	0	5	35	40
No growth	1	5	72	78
Total	1	10	107	118

[Table/Fig-7]: Microbial growth pattern depending on the outcome of the neonates.
Chi square= 1.1476; P_{DAMA} =0% P_{DEATH} =50% $P_{DISCHARGE}$=32.710%

Out of 118 isolates, 80 had no growth. 13% showed MRSA Positive, 9 were positive for Staphylococci, 8 were for *Kleibsella*, 3 Gram negative bacilli, 2 *Citrobacter* and remaining all 1 [Table/Fig-8].

Organism	Number of isolates	Percentage
No growth	80	66.66
MRSA	13	10.83
Staphylococcus	9	7.50
Klebsiella	8	6.66
E. coli	1	0.83
MSSA	1	0.83
Citrobacter	2	1.66
Pseudomonas aureus	1	0.83
Gram negative Bacilli	3	2.5
Acinobacter	1	0.83
Micrococci	1	0.83

[Table/Fig-8]: Overall microbial growth pattern.

DISCUSSION

As no previous study on bacterial isolates has been done in this region, there was a need to conduct this study. The study helped in finding the most prevalent growth which would further help in preparing an antibiotic usage policy for this region.

In the present study 118 isolates were studied from the Neonatal Intensive care unit suspected to be having sepsis.

About 40 neonates (47.2%) showed positive growth almost same to Vaniya HV et al., [10] which was (51%) but lower than Amin AJ et al., [5] which was (62%) but higher than Srinivasagam M et al., study in Tamilnadu [11] which was 21.54% only, and 78 neonates (52.8%) had no growth. There is no risk or association between gender and growth in this study. Males (57.62%) outnumbered females (42.38%) which were similar in almost all studies. However the growth in preterm neonates is 2.27 times more than that in full term neonates which was same as Karachi study [12] but contradictory to Vaniya HV et al., study [10] where full terms outnumbered preterms. And also the prevalence of growth is highest in neonates who are less than 2 kg in birth weight i.e., very low birth weight babies (57.5%) similar to Srinivasagam study [11] and Karachi Study [12].

Microbial growth in neonates admitted within 3 days of life (EOS) is 1.273 times more (32.5%) than that in neonates who are admitted after 3 days of life (IOS) which was 12.5%. Highest prevalence of sepsis (55%) is seen in neonates who stayed in the hospital for more than 7 days (LOS). The present study findings were contradictory to studies done by JJ group of hospitals [5] and study done by Srinivasagam M et al., [11] where EOS was more. These findings could be due to more out born admissions in NICU who were referred late or IV Cannula sepsis.

Out of 118 isolates, 80 had no growth,13 showed MRSA Positive, 9 were positive for Staphylococci, 8 were for *Kleibsella*,

3 Gram negative Bacilli, 2 Citrobacter and 1 remaining. Maximum isolates showed no growth which goes in favour of good handwashing practices in NICU or the late referrals must have been already on some antibiotics to which they had already responded. Maximum studies [12-23] reported gram negative growth which was not seen in this study. It has reported that Methicillin Resistant Staphylococci (MRSA) as maximum growth. Neonates are exposed to *Staphylococcus aureus* shortly after birth and can become colonised quickly after contact with adult skin or their environment. Between 30-70% of humans are carriers of *Staphylococcus aureus*, so neonates have a very high likelihood of exposure during the immediate period after birth. Most common sites of colonisation with *S. aureus* include the umbilical cord, skin, nasopharynx, and gastrointestinal tract. For MRSA, the nares and umbilicus are the most common sites of initial colonization [24].

Neonates can become colonized by MRSA in a number of different ways. Traditionally, MRSA has been demonstrated to spread horizontally by healthcare-associated transmission, i.e., via contact with healthcare workers or the hospital environment. Recently, vertical transmission of MRSA from mothers to their infants has been described. There is no risk or association between delivery type and microbial growth in this study. There is no association between the outcome of neonate and microbial growth.

LIMITATION

The antibiotic sensitivity pattern has not been studied as it was intend to continue this research to study the pattern in future.

CONCLUSION

Neonatal septicaemia is still a leading cause of morbidity and mortality in developing countries like India. Additional factors, such as overcrowding and understaffing in the NICU, have been associated with increased risk of healthcare-associated transmission and colonisation, which may lead to epidemics of MRSA infection. In view of the changing spectrum of the causative agents of neonatal septicaemia and antibiotic sensitivity and resistance pattern from time to time, a positive blood culture and the antibiotic sensitivity and resistance pattern testing of the isolates are the best guide to the antimicrobial therapy which would be beneficial to the best outcome of the disease.

This study has discussed the most prevalent growth in this region and thus can be used as a future reference to find out the antibiotic sensitivity to these growths which will further help in formulating an antibiotic policy to provide the most suitable and appropriate treatment.

The study can be used further to find out the maternal co-relation with neonatal sepsis to find out the prevalence of early onset sepsis and its repercussions on the neonate.

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