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Original Research Article.

Bacteriological Profile and Antibiotic Susceptibility Patterns in Neonatal Septicaemia in Tertiary Care Hospital

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ABSTRACT

Introduction: Neonatal septicaemia has great role in morbidity and mortality among neonates. Neonatal mortality rate has been reported in India as 17 per 1000 live births as per 2016-17 data. Neonatal septicaemia may be of early onset or late onset depending of the age of the neonates. The most common bacterial agents involved are Group B Streptococcus, Klebsiella pneumoniae, CoNS, Streptococcus pneumoniae, Haemophilus influenzae etc. Diagnosis is done by many methods but the most important and absolute mode of diagnosis is blood culture.

Aims and Objectives: The present study is done for the detection of bacteriological profile and their antibiotic susceptibility pattern in case of neonatal septicaemia. Early diagnosis and specific treatment can save the lives of many neonates who are suffering from neonatal septicaemia.

Materials and Methods: The material used for the diagnosis is venous blood of the suspected neonates. Blood culture method is used for the diagnosis of Neonatal septicaemia. Repeated subculture is done on Blood agar, Nutrient agar, and MacConkey agar plates. Confirmation of organism is done through different biochemical tests. The antibiotic susceptibility testing was performed on Muller Hinton agar (MHA) by Kirby-Bauer disc diffusion method for bacterial isolates, as per clinical and laboratory standards institute (CLSI) guideline.

Results: Total 206 cases of suspected neonatal septicaemia were investigated in which 142 cases are found positive. Most common organism isolated was Klebsiella pneumoniae (39.44%) than Staphylococcus aureus (33.8%), other organisms are Escherichia coli (9.86%), CoNS (8.48%), Pseudomonas (5.63%), Enterococcus (2.82%) etc. overall incidence of Gram negative organism (54.93%) was more than Gram positive organism (45.07%). As far as antibiotic sensitivity pattern was concerned most of the organism were 100% sensitive to imipenem, meropenem and colistin B and resistant to Ampicillin.

Conclusion: Gram negative isolates were more common than Gram positive as the causative agents of neonatal sepsis. The most common causative organism was Klebsiella pneumoniae. The other organisms isolated were Pseudomonas aeruginosa, Staphylococcus aureus, CoNS, etc. Most of the Gram negative isolates were sensitive to Amikacin, Gentamycin, Ofloxacin and Ciprofloxacin but were highly susceptible to Meropenem, Imipenem and Collistin-B. The Gram positive isolates were better sensitive to Amikacin, Cephalosporin, Ciprofloxacin and Clindamycin but were less sensitive or resistant to Ampicillin and Erythromycin. They showed high susceptibility to Ticoplanim, Linezolid, Vancomycin and Methicillin.

Keywords: CoNS- Coagulase Negative Staphylococcus, CLSI-Clinical and Laboratory Standards Institute, MHA- Muller Hinton Agar, EONS- Early Onset Neonatal Sepsis, LONS- Late Onset Neonatal Sepsis.

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INTRODUCTION

Neonatal sepsis is a significant cause of morbidity and mortality among neonates worldwide.[1] Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteraemia occurring in first month of life. According to National Neonatal Perinatal database 2016-17 collected from various parts of India, Neonatal mortality rate have been reported to be 17 per 1000 live births.[2] Incidence of nosocomial septicaemia in neonate from India ranges from 1.5 to 37%.[3] Neonatal sepsis can be classified into two relatively distinct syndromes based on the age of presentation, early onset and late onset neonatal sepsis. Early onset neonatal sepsis (EONS) occurs within 72 hrs of life, while late onset neonatal sepsis (LONS) occurs between 72 hrs to 90 days of life. Common bacterial agents involved in early onset sepsis include Group B Streptococcus, Escherichia coli, Klebsiella pneumoniae, Coagulase negative Staphylococcus, Haemophilus influenzae, Listeria monocytogen, and Streptococcus pneumonia.[4]

Organism commonly associated with late onset sepsis includes CoNS, Klebsiella pneumonia, Staphylococcus aureus, and Streptococcus pyogenes. In developing countries, Escherichia coli, Klebsiella species and Staphylococcus aureus are the most common pathogens of EOS. Whereas S. aureus, CoNS, Streptococcus pyogenes are the most commonly reported organism in LOS. Group B Streptococcus is most important cause of neonatal sepsis in Europe and North America. Neonatal sepsis is more common in premature and low birth weight baby. Various laboratory tests are used to diagnose neonatal sepsis like total leucocytes count, C- reactive protein level, erythrocyte sedimentation rate, acridine orange stained buffy coat smear examination, Procalcitonin level etc. The advantages of these tests are that they are sensitive indicator of sepsis, less expansive and rapid test. However these tests neither tell us about the aetiology of neonatal sepsis and whether the sepsis is unimicrobial or polymicrobial nor their antimicrobial susceptibility pattern. In India sepsis has been reported as a cause of neonatal death in 20-50% of cases in community based studies.[5-7] The Gold standard for diagnosis of neonatal sepsis is isolation of bacterial agent from blood culture.[8] Both Gram negative as well as Gram positive bacteria has been isolated from blood samples. The type of organism that predominates in neonatal septicaemia depends upon the time and place. Knowledge of bacteriological profile of neonatal sepsis and its antibiotic susceptibility pattern in geographical areas provide us the guidance to initiate empirical antibiotic treatment which is the mainstay of management of neonatal sepsis.

AIMS AND OBJECTIVE

The present study was aimed at determining the bacteriological profile and their antimicrobial susceptibility pattern in neonatal septicaemic patients. Following a rational antibiotic therapy, we could minimize the risk of neonatal morbidity and mortality as well as decrease the development of multidrug resistant bacteria.

MATERIALS AND METHODS

The present study was conducted in the department of Microbiology, Nalanda Medical College & Hospital, Patna from December 2016 to May 2017. The ethical committee of NMCH had given their permission and approval for this study. During this period blood samples from clinically suspected cases of neonatal septicaemia had been collected from newborn, admitted to department of paediatric and Neonatal Intensive care unit.

Inclusion Criteria

All new born babies aged 0-28 days presenting with one or more clinical features suggestive of septicaemia and having one or more risk factor like low birth weight, prematurity, birth asphyxia, premature rupture of membranes, prolonged labour, home delivery were included in present study.

Neonates presenting with signs and symptoms such as refusal to feed, lethargy, fever, hypothermia, vomiting, diarrhoea, abdominal distension, jaundice, respiratory distress seizures, irritability,, cyanosis, bulging of anterior fontanel, pustules on skin, apnoea, conjunctival discharge etc. were taken up for study. History and clinical findings of suspected neonatal were recorded. Blood samples from referred cases were collected from peripheral vein with proper aseptic precautions preferably before starting any antibiotic therapy. Consent was taken from parents of these neonates for participation in the study after explaining the study protocol to them in language that they understood best.

Exclusion Criteria

Neonates having extreme prematurity (<30 wks of gestational age) birth weight less than 1000 grams, gross congenital anomalies, age more than 28 days at the time of diagnosis was excluded from study.

Sample Collection

1 ml of blood for culture was drawn in sterile syringes after skin preparation by two step processes with 70% alcohol and povidone Iodine application then dried for 1 minute. Blood was collected aseptically and incubated in blood culture bottle containing 10 ml of sterile glucose broth and bile broth thus making a dilution of 1 in 10 to nullify the natural bacteriostatic / bactericidal activity of blood. These bottles were incubated at 37^0c temperature under aerobic conditions in incubator maximum for 7 days. Subculture done on culture media like blood agar, chocolate agar, MacConkey agar on 2nd, 3rd, 5th and 7th day.

If the growth was observed further subcultures were not done. Growth if any was processed according to standard microbiological techniques which includes Gram staining, colony characteristics and biochemical properties described in Mackie and McCartney practical medical microbiology and Bailey and Scott diagnostic microbiology. Blood culture broth which showed no microbial growth after 7 days were reported as culture negative.[8,9]

The Antibiotic susceptibility testing was performed on Muller Hinton agar (MHA) by Kirby-Bauer disc diffusion method for bacterial isolates, as per clinical and laboratory standards institute (CLSI) guideline. Staphylococcus aureus (ATCC 25923), E.Coli (ATCC 25922) and P. aeruoginosa (ATCC 27853) was used as quality control throughout the study for culture and antimicrobial susceptibility testing. 'Commercially available discs (Hi-media Co. Mumbai India) were used. Concentration of discs used were erythromycin (15 micro g) co-trimoxazole (25 micro g) ciprofloxacin (5 micro g) ampicillin (30 micro g) piperacillin + tazobactum (100/10 micro g) cftazidime (30 micro g) amikacin (30 micro g) cefoperozone (30 micro g) oflaxacin (5 micro g) vancomycin (30 micro g) linezolid (30 micro g) and imipenem (10 micro g) meropenem(10 micro g) cefotaxime (30 micro g) ceftriaxone (30 micro g) colistin (10 micro g).[10]

Table 1: Bacteriological profile of microorganisms isolated and identified from blood culture of septicaemia. (142)

Organism	No. Of growth	(%)of growth	Gram+ve/Gram-ve
Staph. Aureus	48	33.80	
CoNS	12	08.45	45.07%
Enterococcus	04	02.82	
K.pneumoniae	56	39.44	
E.coli	14	09.86	54.93%
Pseudomonas	08	05.63	

Table 2: Antiboitic sensitivity pattern of isolated Gram negative organism

	Klebsiella(56)	E.coli(14)	Pseudomonas(8)	Total(78)
Ampicillin	6 (10.71%)	2 (14.28%)	1 (12.5%)	9
Piperacillin& Tazobactum	25 (44.64%)	8 (57.14%)	3 (37.5%)	36
Ceftriaxone	28 (50%)	8 (57.14%)	3 (37.5%)	39
Ceftazidime	30 (53.57%)	9 (64.28%)	6 (75%)	43
Cefoperazone	26 (46.42%)	8 (57.14%)	3 (37.5%)	37
Cefotaxime	27 (48.21%)	9 (64.28%)	3 (37.5%)	39
Amikacin	34 (60.71%)	12 (85.71%)	5 (62.5%)	51
Ciprofloxacin	38 (67.85%)	10 (71.54%)	4 (50%)	50
Ofloxacin	38 (67.85%)	11 (78.57%)	5 (62.5%)	54
Meropenem	54 (96.4%)	14 (100%)	8 (100%)	76
Imipenem	56 (100%)	14 (100%)	8 (100%)	78
Collistin-B	56 (100%)	14 (100%)	8 (100%)	78

Table 3: Antibiotic sensitivity pattern of Gram positive organism

	S.aureus (48)	CONS (12)	Enterococcus (4)	Total (64)
Amikacin	32 (66.67%)	7 (58.33%)	0 (0%)	39
Ceftriaxone	35 (72.92%)	7 (58.33%)	1 (25%)	43
Erythromycin	15 (31.25%)	5 (41.67%)	1 (25%)	21
Amoxy-clavunate	26 (54.16%)	6 (50%)	0 (0%)	32
Clindamycin	29 (60.42%)	7 (58.33%)	3 (75%)	39
Cefoperazone	28 (58.33%)	7 (58.33%)	2 (50%)	37
Methicillin	42 (87.50%)	9 (75%)	3 (75%)	54
Ciprofloxacin	29 (60.42%)	8 (66.67%)	2 (50%)	39
Cefotaxime	34 (70.83%)	7 (58.33%)	1 (25%)	42
Vancomycin	42 (87.5%)	11 (91.67%)	4 (100%)	57
Ampicillin	07 (14.58%)	02 (16.67%)	1 (25%)	10
Linezolid	46 (95.83%)	12 (100%)	4 (100%)	62
Ticoplanin	48 (100%)	12 (100%)	4 (100%)	64

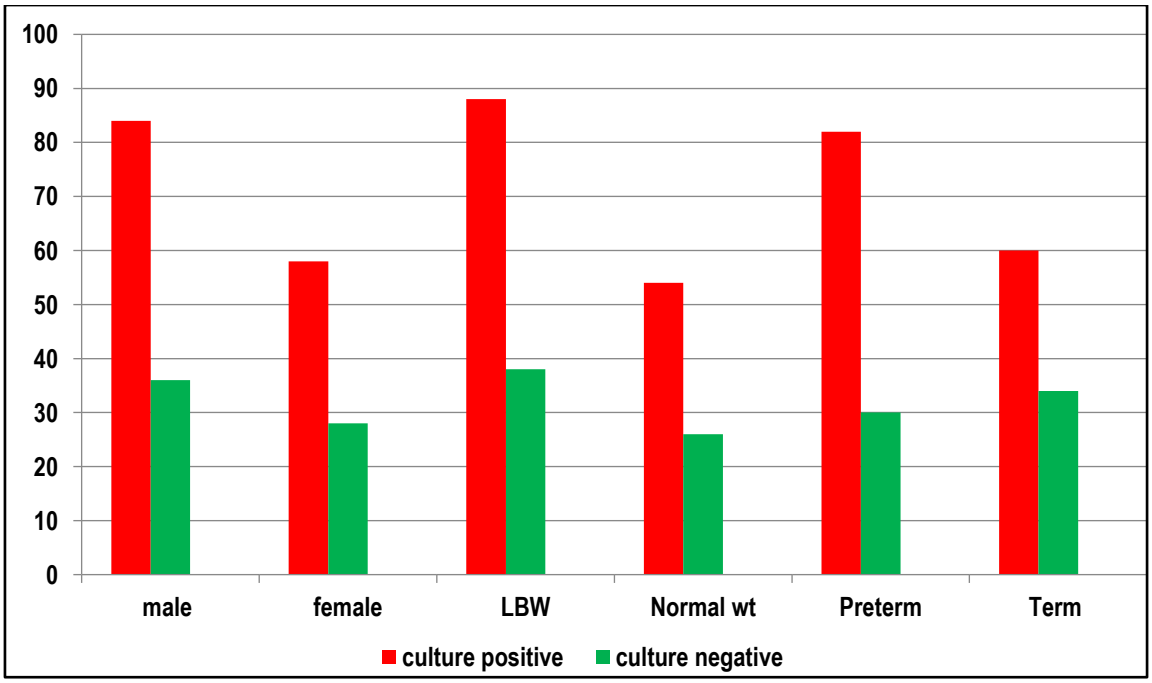

Fig 1: Relation between neonatal risk factors and blood culture.

Table 4: Neonatal risk factor

	Male	Female	LBW	Normal Birth wt.	Preterm	Term
Culture Positive	84	58	88	54	82	60
Culture Negative	36	28	38	26	30	34

RESULTS

Total 142 cases were found positive out of 206 suspected neonatal cases. So, Incidence of neonatal septicaemia was 68.93%. The Microbiological profile of neonatal septicaemia was found in following order as shown in table 2.

Klebsiella pneumoniae 56 (39.44), Staphylococcus aureus 48 (33.80) were major isolates. Other Isolates include Escherichia coli 14(9.86) Coagulase negative staphylococcus 12(8.48) Pseudomonas 8(5.63) Enterococcus 04(2.82) etc. Incidence of Gram positive organism (45.07) is less in comparison to Gram negative organism (54.93) in our study. In our study Klebsiella pneumoniae is the predominant organism followed by Staphylococcus aureus, Escherichia coli and Coagulase negative staphylococci respectively. In all the case of Gram negative organism, Klebsiella Pneumoniae were 100% sensitive to imipenem and colistin B, 96.4% were sensitive to meropenem ,67.85% were sensitive to ciprofloxacin and ofloxacin, 60.71% were sensitive to amikacin. Sensitivity pattern of Klebsiella pneumonia to ceftazidime (53.57%), ceftriaxone (50%) Piperacilline & Tazobactum (44.62%), Cefotaxime (48.21) cefoperazone (46.42%) respectively. Klebsiella Pneumoniae was least sensitive to ampicillin. Regarding E.coli species 100% sensitivity was seen to meropenem, Imipenem, Colistin-B, 78.57% sensitivity was seen to ciprofloxacin and ofloxacin, 85.71% sensitivity was seen to amikacin, E.coli was least sensitive to ampicillin also (14.28%). Regarding Pseudomonas species 100% sensitivity was seen to meropenem, Imipenem, Colistin B 62.5% sensitivity was seen to amikacin and 75% sensitivity was seen to ofloxacin. Sensitivity pattern of Pseudomonas to Piperacillin/ Tazobatum (37.5%), Ceftriaxone (37.5%), cefoperazone (37.5%) Cefotaxime (37.5%). Pseudomonas was least sensitive to ampicillin (12.5%). In case of Gram negative organisms, overall sensitivity was maximum seen with Colistin- B, Imipenem and Meropenem and least sensitivity with ampicillin. In all cases of gram positive organism, Staphylococcus aureus was 100% sensitive to Ticoplanin 95.83% were sensitive to linezolid.. Vancomycin and methicillin both sensitivity were 87.5% Sensitivity pattern of staphylococcus aureus to cefotaxime (70.83%), Ceftriaxone (72.92%), Clindamycin (60.42%), ciprofloxacin (60.42%), amikacin (66.67%). Least sensitivity was seen among ampicillin (14.58%) and Erythromycin (31.25%). Regarding Coagulase negative staphylococcus, 100% sensitivity was found to ticoplanin and linezolid. Vancomycin was sensitive to 91.67%. Sensitivity pattern of CoNS to Ceftriaxone (58.33), Cefotaxime (58.33), Clindamycin (58.33), ciprofloxacin (66.67), amikacin (58.33). Least sensitivity was seen with ampicillin (16.67). Regarding Enterococcus 100% sensitivity were found to ticoplanin and linezolid. Enterococci were least sensitive to amikacin (0%) amoxy-clavunate (0%). In case of Gram positive organism sensitivity was maximum seen with Ticoplanin & Linezolid and least with ampicillin.

In our study neonatal sepsis was more common among pre terms, low birth baby, male baby. Out of 206, 142 cases were culture positive and 64 were culture negative. Among culture positive cases we had seen that male baby (59.15%) were affected more than female. Our study also showed that neonatal sepsis was more common among low birth wt. (<2500gm) baby (61.97%) in culture positive cases. Among culture positive cases neonatal sepsis was also more common among preterm (57.75%) baby.

Results of Blood Culture in Neonatal Septicaemia

Total no. of suspected samples for neonatal septicaemia are 206. Blood culture was done for all these samples.

Total no. of Positive growth for microorganism= 142 (68.93)

Total no. of no growth = 64 (31.07)

DISCUSSION

During the study period, 206 neonates are clinically diagnosed with septicaemia. Among them 142 (68.93%) were blood culture positive. This was in concordance with other studies by Roy et al and Kayange n et al. Incidence of neonatal septicaemia is variable and differs from place to place. Neonatal septicaemia depends on various factors like gestational age, fetal birth weight, maternal nutrition, peri-natal care and hygienic conditions, child health care facilities etc. In the present study, males were more affected than female and the male to female ratio was 142:86. This is comparable to other studies by Begum S et al and Shrestha NJ et al.[11,12] The reason for male preponderance is unknown, but this could be due to sex-dependent factors. The synthesis of gamma globulin is probably regulated by X-linked immunoregulatory genes and as males are having one X chromosome they are more prone for neonatal septicaemia than females. The organisms causing neonatal septicaemia differ from area to area also change with time even in the same area which may be due to different geographical and living conditions. Gram negative bacterial isolates (54.93%) were more common than gram positive isolates (45.07%) in our study. This is in contrast to developed countries where gram positive bacteria were more commonly reported. This was in concordance with National Neonatal Perinatal Database (NNPD) Aletayeb SMH et al. and Agnihotri N et al.[13,14] In this study the most frequent isolate was klebsiella pnemoniae 56 (39.44%). This was in accordance with other Indian Studies NNPD, Agnihotri N et al. and Kayange N et al.[14,15] Escherichia coli was second most common Gram negative organism followed by Pseudomonas aeruginosa. Staphylococcus aureus 48 (33.80) was the commonest Gram positive organism and was second most common organism among all isolates. Among the isolates a considerable percentage (12%) was CoNS as pathogen which could be due to immature immune system and a large population of premature and debilitated infants. All Gram negative isolates were having considerable sensitivity to amikacin, gentamicin, ofloxacin and ciprofloxacin but were highly susceptible to meropenem, Imipenem and Collistin-B. Our study findings correlated well with the finding of others viz Aletayeb SMH et al, Waseem R et al, Mane Ak et al.[13,16,18] The Gram positive isolates, having better susceptibility to amikacin, cephalosporin, ciprofloxacin, clindamycin but were more resistant to Ampicillin and Erythromycin in the present study. They showed high susceptibility to Ticoplanim, linezolid, vancomycin and methicillin. Our findings correlated with studies by Aletayeb SMH et al, Roy I et al, Mane AK et al.[13,17,18]

CONCLUSION

From our study we noticed that gram negative bacteria were more common cause of septicaemia in neonates and Klebsiella pneumoniae was the predominant pathogen. We also noticed that these gram negative bacteria were resistant to routinely used antibiotics; hence their resistant pattern should be considered essential before deciding the empirical treatment. The higher

antibiotics such as Colistin-B, meropenem and imipenem should be reserved for multidrug resistant gram negative bacteria whereas Linezolid, Ticoplanin and Vancomycin should be reserved for drug resistant gram positive isolates.

The positive blood culture with antibiotic sensitivity of isolated organism (s) is best guide to antimicrobial therapy as resistance to antibiotics is worldwide problem that cause ineffectiveness of empirical treatment. However it will be important to continue the surveillance of neonatal septicaemia in order to closely follow changes in trends and identify risk factor to obtain information for empirical antibiotic therapy and to act rapidly in case of major changes in susceptibility pattern.

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