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RESEARCH ARTICLE

Microbiological profile of neonatal septicaemia in a tertiary care hospital at Dehradun.

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Abstract

Introduction: Incidence of neonatal septicaemia varies from place to place and from on setup to other, which continuously changes with time and usage of antibiotics. Different groups of organisms have been implicated for causing early onset and late onset septicaemia. The majority of for neonatal septicaemia may be due to transplacental transmission, contaminated instruments, respirators used for resuscitation and inadequate handwashing by health care personnel. **Material & methods**: This prospective study was carried out on suspected cases of neonatal septicaemia by analyzing their blood cultures and determination of antibiotic sensitivities on the isolates. **Results**: A total of 335 neonates were studied on clinical suspicion of septicaemia. 227/335(67.76%) blood cultures were sterile while 108/335 (32.24%) were positive by automated blood culture system. Culture positive cases when analysed showed growth of bacteria in 69/108 (63.89%) and fungi in 39/108 (36.11%). *Staphylococcus aureus* (11/34, 32.35%), *Klebsiella pneumoniae* (14/35, 40%) and *non albicans Candida* (30/39, 76.92%) were the predominant isolates amongst Gram positive, Gram negative and yeast –like organisms respectively. **Conclusion**: Periodic surveillance of isolates from suspected cases of neonatal septicaemia must be carried out for the effective management of cases and formulating antibiotic policy at hospital settings.

Introduction

Introduction

Sepsis is defined as SIRS (systemic inflammatory response syndrome) resulting from a suspected or proven infectious etiology. The clinical spectrum of sepsis begins when a systemic (eg. bacteremia, rickettsial disease, fungemia, viremia) or localised (eg. meningitis, pneumonia, pyelonephritis) infection progresses from sepsis to severe sepsis. SIRS is an inflammatory cascade that is initiated by the host response to an infectious or non-infectious trigger (Nelson et al., 2012).

Though rare but, apparent or inapparent maternal bacteraemia can produce fatal infection by transplacental route (Kaufman D et al., 2004; Hervas JA et al., 1993). Prolonged labour and excessive manipulation increases the risk of contamination of amniotic fluid. Rarely amniotic infection occurs in the presence of intact membrane (Kaufman D et al., 2004; Ayengar V et al., 1991). The most common organisms found in amniotic fluid and vagina are *Escherichia coli, Streptococcus faecalis* and *Staphylococcus aureus* (Ng PC et al., 1997). Clinical diagnosis of sepsis in new born infants is not easy because symptoms and signs are nonspecific. There is no laboratory test with 100% specificity and sensitivity. Diagnosis of neonatal septicaemia is ultimately based on the positive blood culture which is the gold standard. However, at least 48-72 hours is needed. The neonates with "risk factors" for neonatal sepsis are thus treated with broad-spectrum antibiotics and require prolonged hospitalization (Tripathi S et al., 2010). Positive blood culture may not always establish diagnosis of neonatal septicaemia. It may be positive due to contamination or transient bacteraemia (Siegel JD et al, 1981).

With this background, the current study was undertaken to investigate the incidence of neonatal sepsis, bacteriological profile and antibiotic sensitivity patterns of the organisms isolated from the clinically suspected cases of neonatal sepsis admitted in our hospital (Shri Mahant Indiresh Hospital, Dehradun).

Study design:-

In the present prospective study, blood samples from 335 neonates admitted for suspected neonatal septicaemia, in the NICU in Sri Mahant Indiresh Hospital, Patel Nagar, Dehradun were collected, from January 2012 to December 2012.

Selection criteria for Subjects:-

Inclusion criteria:-

Neonates of both sexes were included in this study. Neonates presenting with signs and symptoms such as refusal to feed, lethargy, fever, hypothermia, vomiting, diarrhoea, abdominal distension, jaundice, respiratory distress, seizures or any external evidence of sepsis like umbilical cord infection, skin infection etc. were taken up for study. A sample showing the growth of organisms of low pathogenicity, a repeat blood sample was taken and on isolation of the organism on repeat culture, it was included in this study. Development of signs and symptoms of sepsis within 72 hours of birth was considered as Early Onset Sepsis (EOS) and after 72 hours was considered as Late Onset Sepsis (LOS).

Exclusion criteria:-

Neonates with absence of signs of sepsis were excluded from this study. Low pathogenic organisms like Coagulase negative *Staphylococcus* (CoNS), *Candida* spp. unless grown on repeat culture were excluded.

Material & Methods:-

BacT/ALERT automated culture system was used for blood culture. Blood cultures were considered negative only after 5 days of incubation at 37ºC. Positive culture bottles were promptly removed and a smear was made for Gram staining. Then a subculture was done on Columbia blood agar and MacConkey Agar and incubated aerobically at 37°C for 16-18 hrs.

After 18 hours, plates were observed for growth and the processing was done for further identification of organisms as per standard laboratory protocol (Winn W Jr et al., 2006; Collee JG et al.,1996) and Kirby Bauer's disc diffusion method was followed as per CLSI guidelines using commercially available discs for antibiotic sensitivity (Rex JH et al., 2012).

Statistical analysis:-

Results were analysed using Chi square method for their statistical significance.

Results:-

A total of 335 neonates were studied for clinically suspected septicaemia. With 213/335 (63.55%) males and 122/335 (36.41%) females, male preponderance was observed. Rate of culture positivity was also higher in the males as compared to females.

When the pattern of onset of symptoms of septicaemia in neonates was critically analysed, early onset septicaemia was observed clinically in 159/335 (47.46%) neonates, of which 36/159 (22.64%) were culture positive. While 176/335 (52.53%) had a late onset of septicaemia and 72/176 (40.90%) were culture positive. Statistically this finding was highly significant with a p value of 0.0004. (Table 1) The results of blood cultures of the clinically suspected cases of neonatal septicaemia show that 227/335(67.76%) blood cultures were sterile while 108/335 (32.24%) were positive. Culture positive cases when analysed, showed growth of bacteria in 69/108 (63.89%) and fungi in 39/108 (36.11%).

Among the various Gram positive bacteria isolated in this study, *Staphylococcus aureus* (MSSA) was the predominant isolate accounting for 11/34 (32.35%) cases while, *Streptococcus pyogenes* was isolated only in 1/34 (2.94%)cases.(Table 2) On the other hand, *Klebsiella pneumoniae* with 14/35 (40%) cases, dominated amongst the

Gram negative isolates. (Table 3) Of the various fungal isolates in this study, *Candida albicans* was isolated in 9/39 (23.08%) cases and Non *albicans Candida* species were isolated in 30/39 (76.92%) cases. All the fungal isolates were yeast-like only.

All the 34 Gram positive isolates were analysed for their resistance pattern against various antibiotics. *Staphylococcal* isolates were predominantly resistant to cotrimoxazole, erythromycin and clindamycin. 2/34 (5.8%) of the CoNS were found to be 100% resistant to amoxycillin-clavulanate, cefuroxime, cotrimoxazole, erythromycin and clindamycin. (Table 4) Among 35 Gram negative isolates, all the strains of *Klebsiella pneumoniae* 14/35 (40%) isolates were resistant to amoxycillin-clavulanate, cefotaxime, ceftazidime and gentamicin while isolates of *Escherichia coli* were found to be 100% resistant to cefotaxime, aztreonam, gentamicin, amikacin and levofloxacin. Among the nonfermenters, 3 isolates each of *Acinetobacter spp*. and *Pseudomonas spp*. were found to be resistant to almost all the antibiotics except polymyxin B. (Table 5)

Discussion:-

Neonatal sepsis is the most common cause of neonatal mortality despite treatment. Neonatal sepsis can be classified into two subtypes depending upon onset of symptoms. In the present study 335 neonates clinically suspected of having neonatal sepsis were studied. Of these, 213 were males and 122 were females. 72/213(33.33%) males and 36/122(29.51%) females were found to be culture positive. Male preponderance observed in this study was similar to certain other studies (Tallur SS et al., 2000; Joshi SG et al., 2000; Jaswal RS et al., 2003).

The high incidence of culture positivity in extramural cases could be due to unhygienic conditions, untrained dai deliveries and premature deliveries being referred to our hospital from far off places. The culture positivity in intramural deliveries owed to associated multiple risk factors and possible nosocomial infections. Various workers have reported EOS ranging from 24%-77.6% and LOS ranging from 22.4%-76% which simulates our study (22.64% in EOS and 40.90% in LOS) (Venkataseshan S et al., 2009; Christo GG et al., 1990; Sriram R, 2011).

Findings of our study demonstrate a culture positivity of 32.24% (108/335) which coincides with the range (18.2% to 47.5%) of culture positivity in other studies (Nwadioha SI et al., 2012; Roy I et al., 2002). Among the culture positive samples, bacterial isolates accounted for 63.89% and fungi in 36.11%.Probable contaminants like CoNS when isolated were considered as pathogens only when isolated on repeat culture. Otherwise considered as contaminants and excluded from the study. The spectrum of organisms causing neonatal sepsis is quite different in developed and developing countries. Both bacterial and fungal organisms are implicated in neonatal sepsis.

Analysis of various studies establish the fact that *Klebsiella* species and *Staphylococcus aureus* still continue to be the major contributors of neonatal septicaemia. Our study was in agreement with a number of published studies (Joshi SG et al., 2000; Venkataseshan S et al., 2009; Sriram R, 2011; Chugh K et al., 1988; Vaidya U et al., 1991; Sharma A et al., 1993; Banerjee M et al., 1993; De A et al., 1998).

Among all Gram positive isolates, *Staphylococcus aureus* was the predominant pathogens accounting for 14 isolates out of which 3 were MRSA. These MRSA though resistant to a number of antibiotics were still sensitive to vancomycin, linezolid and teicoplanin which are last respite for treatment. 9/34 (26.47%) isolates were *Enterococcus* species of which 3 were Vancomycin Resistant Enterococci (VRE) indicating their rising trend. All enterococcal isolates were 100% resistant to ampicillin, levofloxacin and erythromycin.

Majority of *Klebsiella* species were found to be resistant to commonly used antibiotics like amoxycillin-clavulanate, cefotaxime, gentamicin and cotrimoxazole. It was seen that polymyxin B and piperacillin-tazobactam were the two most effective drugs against *Klebsiella* species. 11/107 isolates of *Escherichia coli* were prominently found to be multi drug resistant owing to the vigorous treatment on empirical basis and its presence as a common resident flora. Few non-fermenters (6/35) found in our study included *Acinetobacter* species and *Pseudomonas* species which were resistant to almost all the drugs tested except polymyxin B.

The association between prematurity and blood borne candidial infections has been recognised for many years in the past. Over the same period of time the incidence of candidemia has escalated from 25 to 123 cases per 10,000 NICU admissions. Isolation of *Candida* species from a blood culture should never be regarded as a contaminant and should prompt an immediate search for evidence of dissemination, which occurs in approximately 10% of premature newborns with candidemia (Kit P et al., 1988).

National nosocomial infection surveillance data show the *Candida* species to be the fifth leading cause of blood stream infections in hospitals and the fourth in the intensive care units (Jarvis WR et al., 1991). Critical analysis of our data with the other workers suggests increasing importance of *Candida* species in causing neonatal sepsis and upsurge of cases due to *non albicans Candida* (de Haan TR et al., 2013; Oberoi JK et al., 2012; Singh RI et al., 2011; Ma CF et al., 2013). Despite the fact that fungal isolates are often missed or considered contaminants, they still play an important role. In our study fungal isolates contributed to about 35.5%, of which *Candida albicans* was isolated in 21.05% and *Non albicans Candida* species were 78.94%. All the fungal isolates were yeast-like only. A rising trend in pathogenicity of *Non albicans Candida* is observed in our study.

Antibiotic resistance which is now a global problem has increased due to wide availability of over the counter antibiotics and inappropriate use of broad spectrum antibiotics in the community. Reports of multidrug resistant bacteria causing neonatal sepsis in developing countries are increasing, particularly in ICUs. Spread of resistant organisms in hospitals is commonly encountered, although babies admitted from the community may also carry resistant pathogens.

TABLE 1: Distribution of Early and Late onset sepsis (N=335)

Onset Of Sepsis	Culture negative	Culture positive	Total
Early onset sepsis	123	36	**159**
Late onset sepsis	104	72	**176**

Chi square = 3.338, p =0.0004 (highly significant)

TABLE 2: Distribution of Gram positive isolates

Gram positive isolates	Numbers
Staphylococcus aureus	11 (32.35)
MRSA	3 (8.82)
CoNS	8 (23.52)
MRCoNS	2 (5.88)
Streptococcus pyogenes	1 (2.94)
Enterococcus species	9 (26.47)
Total	**34**

Figures in parentheses are percentages.

TABLE 3: Distribution of Gram negative isolates

Gram negative isolates	Numbers
Escherichia coli	11 (31.42)
Klebsiella pneumonia	14 (40)
Klebsiella oxytoca	1 (2.85)
Acinetobacter species	3 (8.57)
Pseudomonas species	3 (8.57)
Enterobacter species	2 (5.71)
Citrobacter freundii	1 (2.85)
Total	**35**

Figures in parentheses are percentages.

TABLE 4. Resistance pattern of Gram positive isolates (N=34)

Antibiotics	*Staphylococcus aureus* (N=11)	MRSA (N=3)	CoNS (N=8)	MRCoNS (N=2)	*Strep pyogenes* (N=1)	*Enterococcus spp*. (N=9)
Ampicillin	1	3	3	2	0	9
Amoxy-Clav.	1	3	1	2	0	8
Cefuroxime	6	3	2	2	0	0
Cefotaxime	0	0	0	0	0	0
Cefepime	1	0	0	0	0	3

Cotrimoxazole	9	3	1	2	1	8
Levofloxacin	0	0	1	0	0	9
Erythromycin	8	3	5	2	0	9
Clindamycin	8	3	3	2	0	0
Vancomycin	0	0	0	0	0	3
Linezolid	0	0	0	0	0	0
Teicoplanin	0	2	0	2	1	0

TABLE 5. Resistance pattern of Gram negative isolates (N=35)

Antibiotics	***Esch coli* (N=11)**	***Klebsiella pneumoniae* (N=14)**	***Klebsiella oxytoca* (N=1)**	***Enterobacter spp.* (N=2)**	***Citro freundii* (N=1)**	***Acineto spp.* (N=3)**	***Pseudo spp.* (N=3)**
Amoxy-Clav	8	14	1	2	1	3	3
Pip-Taz	1	8	0	1	1	1	3
Cefotaxime	11	14	1	2	1	3	3
Ceftazidime	9	14	1	2	1	3	3
Meropenem	3	11	0	1	0	1	1
Aztreonam	11	5	0	2	1	3	3
Gentamicin	11	14	1	1	1	3	3
Amikacin	11	2	1	1	1	3	3
Polymyxin B	0	0	0	0	0	0	0
Levofloxacin	11	5	0	2	0	3	3
Cotrimoxazole	10	11	1	1	0	3	3

Conclusion:-

Neonatal septicaemia is one of the leading causes of mortality and morbidity in developing countries like India. In neonates, sepsis is difficult to diagnose clinically. So, if there is even a remote suspicion of sepsis, neonates are frequently treated with antibiotics empirically until cultures are sufficiently proven to be negative. For the effective management of neonatal septicaemia cases, the bacteriological profile with their antibiotic sensitivity pattern must be reviewed. A surveillance of the nosocomial pathogens for resistograms in a given set up is needed in order to guide appropriate selection of empiric therapy. Every hospital should have its individual antibiotic policy since the standard antibiotic policy may not hold true for every area.

The present study emphasizes the importance of periodic surveys of microbial flora encountered in particular neonatal settings to recognize the trend. It is recommended that on the basis of periodic surveillance of the isolates (especially from critical care units) up to species level as well their antibiograms at regular intervals, critical analysis of such data and formulation of a rotational antibiotic policy at hospital level should be adopted. Since a sizeable number of blood culture specimens were negative by aerobic culture, the possibility of infection by anaerobes must be entertained and therefore, anaerobic culture should be performed routinely in cases of neonatal septicaemia.

Note that, in resource-limited countries, hand washing or hand hygiene program is recommended as the most effective evidence-based strategy that will reduce the rates of nosocomial infection in NICUs.

References:-

- Ayengar V, Madhulika, Vani SN. Neonatal sepsis due to vertical transmission from maternal genital tract. IJ P 1991; 5 8(5):661-4.
- Banerjee M, Sahu K, Bhattacharya S, Adhya S, Bhowmick P, Chakraborty P."Outbreak of neonatal septicemia with multidrug resistant Klebsiella pneumoniae". Indian J Paediat. 1993; 60: 25-27.

- Christo GG, Mathai J, Nalini B, Baliga M, Venkatesh A. Neonatal Citrobacter sepsis: clinical and epidemiological aspects. IJP1990; 57(6):781-4.
- Chugh K, Aggarwal BB, Kaul V K, Arya S C. Bacteriological profile of neonatal septicaemia. IJP 1988, 55; 6:961-965.
- De A, Saraswathi K, Gogate A, Raghavan K. C-reactive protein and buffy coat smear in early diagnosis of childhood septicemia. Indian J Pathol Microbiol. 1998; 41(1):23-6.
- de Haan TR, Beckers L, de Jonge RCJ, Spanjaard L, van Toledo L, et al. (2013) Neonatal Gram Negative and Candida Sepsis Survival and Neurodevelopmental Outcome at the Corrected Age of 24 Months. PLoS ONE 8(3): e59214. doi:10.1371/journal.pone.0059214.
- Hervás JA, Alomar A, Salvá F, Reina J et al. Neonatal Sepsis and Meningitis in Mallorca, Spain, 1977–1991. Clin Infect Dis. (1993) 16 (5):719-724. doi: 10.1093/clind/16.5.719.
- Jarvis WR, Edwards JR, Culver DH et al. Nosocomial infection rates in adult and paediatric intensive care units in the United States, 1980-1989. Am J Med 1991;91(suppl 3B):185S-91S.
- Jaswal RS, Kaushal RK, Goel A and Pathania K. Role of C-Reactive Protein in deciding duration of antibiotic therapy in Neonatal septicaemia. Ind. Paedtr.2003; 40:880-84.
- Joshi SG, Ghole VS, Niphadkar KB. "Neonatal Gram negative bacteraemia." IJP 2000; 67: 27: 32.
- Kaufman D, Fairchild KD. Clinical Microbiology of bacterial and fungal sepsis in very- low- birth – weight infants. Clin. Microbial. Rev.2004:17(3): 638-680. Doi: 10.1128/CMR.17.3.638-680.2004.
- Kite P, Millar MR, Gorham P, Congdon. Comparison of five tests used in diagnosis of neonatal bacteraemia. Archives of diseases in Childhood. 1988; 63: 639-643.
- Ma CF, Li FQ, Shi L, Hu Y, Wang Y, Huang M and Kong Q. Surveillance study of species distribution, antifungal susceptibility and mortality of nosocomial candidemia in a tertiary care hospital in China. BMC infectious Diseases 2013; 13: 337.
- Mackie, McCartney. Practical Medical Microbiology. ColleeJG, Fraser AG, Marmion BP, Simmons A,. 14th edt. New York: Churchill and Livingstone; 1996
- Nelson textbook of Paediatrics, 19th ed. Philadelphia; Saunders publication, 2012. Thomson Press India Ltd.
- Ng PC, Cheng SH, Chui KM, Fok TF et al. Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecule and C-reactive protein in preterm very low birth weight infants. Arch. Dis. Child Fetal Neonatal Ed 1997;77(3):F 221-7.
- Nwadioha SI, Nwokedi EOP, Kashibu E, Odimayo MS and Okwori EE. A review of bacterial isolates in blood cultures of children with suspected septicaemia in a Nigerian tertiary hospital. Afr. J. Microbiol. Res. 2010; 4(4): 222-225.
- Oberoi JK, Wattal C, Goel N, Raveendran R, Dutta S and Prasad K. Non- albicans Candida species in blood stream infections in a tertiary care hospital at New Delhi, India. IJMR 2012; 136: 997-1003.
- Rex JH, Ferraro MJ, Anderson NL, Body BA, Forbes BA Fritsche TR et al. CLSI: Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second Informational Supplement. CLSI document M100-S22.
- Roy I, Jain A, Kumar M et al. Bacteriology of neonatal septicaemia in a tertiary care hospital of northern India. IJMM 2002;20(3): 156-159.
- Siegel JD, McCracken GH. Sepsis Neonatorum. N Engl J Med 1981; 304:642-647March 12, 1981DOI: 10.1056/NEJM198103123041105.
- Sharma A, Kutty CVK, Sabharwal U et al. Evaluation of sepsis screen for diagnosis of neonatal septicaemia. IJP 1993; 60: 559-563.
- Singh RI, Xess I, Mathur P, Behera B, Gupta B and Misra MC. Epidemiology of candidaemia in critically ill trauma patients: experiences of a level I trauma centre in North India. JMM 2011;60: 342-348.
- Sriram R. Correlation of blood culture results with the sepsis score and the sepsis screen in the diagnosis of neonatal septicaemia. Int.J.Biol. Med. Res 2011;2(1):360-368.
- Tallur SS, Kasturi AV, Nadgir SD and Krishna BV. Clinico-bacteriological study of neonatal septicaemia in Hubli.IJP 2000;67(3): 169-74.
- Tripathi S and Malik GK. Neonatal Sepsis: past, present and future; a review article. Internet Journal of Medical Update 2010 July; 5(2):45-54.
- Vaidya U, Bhave S, Hegde V, Pandit AN. "Neonatal Septicemia: A reapprasial with special reference to use of Cefotaxime". Indian Paediatrics: 1991; 28: 1265-70.
- Venkataseshan S, Kumar P, Dutta S et al. Blood cultures confirmed bacterial sepsis in neonates in a north Indian tertiary care centre: changes over last decade. Jpn. J. Infect. Dis 2009, 62; 46-50. .

- Winn W Jr, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P,et al.(2006) Nonfermenting Gram negative bacilli. In: Koneman's color Atlas and textbook of Diagnostic Microbiology. 6th ed. USA: Lippincott Williams and Wilkins Company; 2006. p. 305-91