ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 03 | March - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN OF ORGANISMS ISOLATED FROM NEONATAL SEPSIS PATIENTS AT TERTIARY CARE HOSPITAL.

Microbiology

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ABSTRACT

INTRODUCTION: As neonatal septicemia is a life threatening emergency and delays in diagnosis and treatment with appropriate antibiotics may have devastating consequences, surveillance is needed for an ongoing review of the causative organisms and their antibiotic susceptibility pattern. **AIMS AND OBJECTIVES:** To identify the common bacterial pathogens associated with neonatal sepsis and their antibiotic susceptibility pattern. **MATERIALS AND METHODS:** 124 blood samples were collected from neonates with clinically suspected cases of neonatal sepsis admitted in neonatal intensive care unit (NICU) and special care newborn unit (SCNU), MGM Medical College and Hospital Jamshedpur between the periods of November 2019 to November 2020. The blood sample was inoculated into Brain Heart Infusion Broth Supplemented with 0.05% SPS culture vial. Positive vials were subjected to gram staining, subculture, and antimicrobial susceptibility test. **RESULTS:** 53 cases were culture positive among 124 suspected cases. Among culture positive cases there was male predominance (59.7%). Most of the culture positive neonates were preterm i.e. born before 37 weeks (56%). Among the culture positive cases early onset sepsis (64%) was more. Gram negative bacteria were most common isolate being responsible for 67.9% of cases of septicemia. *Klebsiella pneumonia* was found to be the most common isolate among gram negative bacilli and CONS (*coagulase negative Staphylococcus)* was the most common isolate among gram positive cocci. **CONCLUSION:** The antibiotic abuse has resulted in confusion in diagnosis and the emergence of drug resistant bacterial strains in the nurseries with grave sequelae. Proper hand washing and aseptic obstetric procedures, high incidence of suspicion, prompt diagnosis, aggressive management of sepsis and timely identifying high risk factors can help to prevent neonatal sepsis.

KEYWORDS

neonatal septicemia, early onset sepsis, *Klebsiella pneumonia, coagulase negative Staphylococcus.*

INTRODUCTION:

Neonatal septicemia refers to a clinical syndrome characterized by systemic signs and symptoms due to generalized bacterial infection with a positive blood culture in the first four weeks of life.[1] Neonatal sepsis is a significant cause of morbidity and mortality among neonates worldwide[2,3]. World Health Organization has estimated that 1.6 million deaths occur globally every year due to neonatal infections and 40% of all neonatal deaths occur in developing countries [4].

Bacterial infections are the commonest cause for neonatal septicemia. Fulminant and fatal course of infection may result from complications such as shock, disseminated intravascular coagulation and multi-system organ failures. Most of the neonatal sepsis related deaths are preventable if suspected early and treated with appropriate antibiotics. Thus, the early diagnosis of this life threatening condition, is mandatory for a timely treatment and a favourable outcome.[1,5,6] Based on the timing of the infection neonatal sepsis has been classified into early onset sepsis (EOS) and late onset sepsis (LOS). Early onset sepsis is defined as onset of sepsis within 72 hours of birth and late onset sepsis is defined as onset of sepsis after 72 hours of birth.[7]

Early onset infections are caused by organisms prevalent in the maternal genital tract or in the delivery area. The associated factors for early-onset sepsis include low birth weight, prolonged rupture of membranes, foul smelling liquor, multiple per vaginal examinations, maternal fever, difficulty or prolonged labour and aspiration of meconium. Early onset sepsis manifests frequently as pneumonia and less commonly as meningitis.

Late onset septicemia is caused by the organisms thriving in the external environment of home or the hospital. The infection is often transmitted through the hands of the care-providers. The onset of symptoms is usually delayed beyond 72 hours after birth and the presentation is that of septicemia, pneumonia or meningitis. The associated factors of late onset sepsis include low birth weight, lack of breastfeeding, superficial infections (pyoderma ,umbilical sepsis),aspiration of feeds , disruption of skin integrity with needle pricks and use of intravenous fluids.

MATERIALS AND METHODS:

A cross sectional study was conducted in the Department of Microbiology at MGM Medical College and Hospital Jamshedpur, Jharkhand, between the period of November 2019 to November 2020. 124 blood samples were collected from neonates with clinically suspected cases of neonatal sepsis admitted in neonatal intensive care unit (NICU) and special care newborn unit (SCNU), MGM Medical College and Hospital Jamshedpur,

INCLUSION CRITERIA :

All the blood samples which came for culture from the neonatal ICU with clinical suspicion of sepsis (i.e neonates with poor activity, fever, refusal of feed ,lethargy , tachypnea , tachycardia ,birth asphyxia ,prematurity ,low birth weight etc) were taken into study group except for ones which come under the exclusion criteria.

EXCLUSION CRITERIA:

Neonates with age more than 28 days or neonates on antibiotics prior to collection of blood were excluded from the study.

Sample collection and processing:

The blood sample was taken from peripheral vein under aseptic conditions. The local site was cleansed with 70% alcohol and providine iodine(1%) followed by 70% alcohol again. Approximately, 1-2 ml of venous blood was collected using blood collection set. The blood sample was inoculated into 20ml Brain Heart Infusion Broth culture vial and the septum was swabbed with alcohol before inoculation. The inoculated BHI Broth was brought to the Department of Microbiology, MGM Medical College for further processing.

The inoculated vials were incubated aerobically at 37°C for 7 days. Subculture was done on sheep blood agar and MacConkey agar routinely after 48 h and 7 days. Subculture was also done in between.

Positive vials were subjected to gram staining, subculture, and antimicrobial susceptibility test. The vials were declared negative after seven days of aerobic incubation.

For subculture, the fluid was inoculated on MacConkey agar and Blood agar plates and incubated at 37^{0}C for 18-24 hrs. After 24 hrs, the

plates were observed for any growth. The colonies were identified by gram's staining, motility test and standard biochemical tests.

The antimicrobial susceptibility testing of isolates was done by the standard disc diffusion method (Kirby Bauer method) using commercial discs according to CLSI guidelines (2019).

RESULT:

53 cases were culture positive among 124 suspected cases. Among culture positive cases there was male predominance (59.7%) and most of them were preterm i.e. born before 37 weeks (56%). It was seen that among the culture positive cases early onset sepsis (64%) was more. Respiratory distress secondary to birth asphyxia ,poor feeding and hypothermia was the most common clinical presentation . Gram negative bacteria were most common isolate being responsible for 67.9% of cases of septicemia. *Klebsiella pneumonia (33.9%)* was found to be the most common isolate followed by *Escherichia Coli* (28%). Among gram positive Cocci CONS (*coagulase negative Staphylococcus)* was the most common isolate followed by *Staphylococcus aureus* accounting for 18.9% and 13.2% cases respectively.

Table 01: Sex Wise Distribution Of Cases

Sex	Suspected cases		Culture positive cases	
	Number	Percentage	Number	Percentage
Males	70	59.7	34	64.1
Females	54	40.3	19	35.9
Total	124	100	53	100

Table 02: Distribution Of Cases According To Gestational Age.

Gestational age	Suspected cases		Culture positive cases	
	Number	Percentage	Number	Percentage
Preterm (<37 weeks)	70	56	32	60.6
Term (≥37 weeks)	54	44	21	39.4
Total	124	100	53	100

Table 03: Bacterias Isolated By Blood Culture

Sl no.	Name of organisms	EOS	LOS	Total
1	*Klebsiella pneumoniae*	14	04	18
2	*Escherichia Coli*	12	3	15
3	*CONS*	3	7	10
4	*Staphylococus Aureus*	3	4	7
5	Others	0	3	3
	Total	32	21	53

Table 04: Antibiotic Susceptibility Pattern Among Gram Positive Organism

Sl. No.	Antibiotics	Sensitive	%	Resistant	%
1.	Amoxicillin	5	29.4	12	70.6
2.	Ciprofloxacin	12	70.6	5	29.4
3.	Chloramphenicol	9	52.9	8	47.1
4.	Cefoxitin	13	76.5	4	23.5
5.	Clindamycin	14	82.4	4	17.6
6.	Erythromycin	5	29.4	12	70.6
7.	Gentamicin	9	52.9	8	47.1
8.	Linezolid	17	100	0	0
9.	Tetracycline	11	64.7	6	35.3
10.	Vancomycin	17	100	0	0

Table 05: Antibiotic Susceptibility Pattern Among Gram Negative Organism

Sl. No.	Antibiotics	Sensitive	%	Resistant	%
1.	Amikacin	25	69.4	11	30.6
2.	Ampicillin	4	11	32	89
3.	Cefotaxime	18	50	18	50
4.	Cefoperazone	17	47.2	19	52.8
5.	Colistin	36	100	0	0
6.	Ceftazidime	14	38.9	22	61.1
7.	Ceftriaxone	19	52.8	17	47.2
8.	Imipenem	36	100	0	0
9.	Levofloxacin	18	50	18	50
10.	Piperacillin + Tazobactam	29	80.5	7	19.5

DISCUSSION:

In present study neonatal septicemia was more common in males (59.7%) as compared to females (40.3%). The male to female ratio in our study was 1.48:1 which correlated with the studies of S Thakur et al[8] and M. Mustafa et al[9] who have also reported male preponderance in their study. In present study 56% preterm neonates and 44% term neonates were blood culture positive. In the study conducted by E.M.R.S. El. Din et al[10], 58.9% of neonates were preterm who developed neonatal septicemia. This shows that preterm neonates are more prone to develop sepsis because of their immature immune system.

In present study, maximum culture positive cases were seen in neonates less than 72 hours of age (early onset septicemia) i.e. 64% as compared to neonates aged more than 72 hours (late onset septicemia) i.e. 36%. This correlated with the studies done by V. Vijayvergia et al[11] and V. A. Muley et al[12] who reported a higher proportion of early onset septicemia cases.

In present study respiratory distress secondary to birth asphyxia, fever, poor feeding and hypothermia was the commonest clinical presentation which was consistent with the finding of S. Thakur et al[8] , T. V. Mhada et al[13] and B Satyamurthi et al[16] .

In present study, out of 124 suspected cases of neonatal septicemia, 53 cases were blood culture positive i.e. 42.7%. which correlates with the studies of V. Vijayvergia et al[11], S. Thakur et al[8], M. Mustafa et al[9] and A.J.Shah et al[14].

In present study gram negative bacilli were more frequently (67.9%) involved in causing neonatal septicemia than gram positive cocci (32.1%) which was consistent with the studies done by M. Mustafa et al[9], V. Vijayvergia et al[11], V. A. Muley et al[12] and P. Jyothi et al[15].

In present study out of 53 culture positive cases, the most predominant isolate was *Klebsiella pneumoniae* (33.9%). This finding was in concordance with the study carried out by M. Mustafa et al[9](35.4%) and P. Jyothi et al[15] (30.5%).*Escherichia Coli* (28%) was the second most common isolate found, this finding was very similar with the study of M. Mustafa et al[9] (22.5%).

Among gram positive cocci, *Coagulase negative staphylococcus* (CONS) was the most common isolate found followed by *Staphylococcus aureus* (18.9%) and (13.2%). This finding correlates with the studies of Mythri B.A. et al [17] (20.13%) and (9.1%) and B. Satyamurthi et al[16] (13.98%) and (18.1%).

All strains of gram positive cocci were 100% sensitive to Linezolid and Vancomycin, 82.2% were sensitive to Clindamycin, 71.4% and 76.6% were sensitive to Ciprofloxacin, and Cefoxitin . Resistance was maximum to Erythromycin (70.6%) and Amoxicillin (70.6%) , followed by Gentamicin (48.8%). Organism were sensitive to Cephalosporins to a higher degree than to Penicillin. V. Vijayvergia et al[11] and M. Mustafa et al[9] in their study also reported 100% sensitivity to Linezolid, Vancomycin and maximum resistance to Erythromycin.

All strains of gram negative isolates were 100% sensitive to Colistin and Imipenem. 80.5% were sensitive to Piperacillin + Tazobactum and 69.4.2% were sensitive to Amikacin. Maximum resistance was seen to Ampicillin (88.4%) followed by third generation Cephalosporins . 44.4% of *Klebsiella pneumonia* and 40 % of Escherichia Coli are ESBL producers .This finding was consistent with M. Mustafa et al[9] and S . Thakur et al[8]. M. Mustafa et al[9] study also reported 100% sensitivity to Colistin and Imipenem. M. Mustafa et al[9] and P. Jyothi et al[15] in their study reported maximum resistance to Ampicillin.

CONCLUSION :

Health education should be given to the populations on the dangers of indiscriminate use of antibiotics and thereafter emergence of antibiotic resistance which is currently considered to be a major problem in our society and which has been responsible for the ineffectiveness of most commonly used antibiotics. This study shows that antimicrobial resistance is a universal problem .Various measures are taken to decrease the emergence antibiotic resistance are implementing infection control policy in hospital, restricting the use of the broad spectrum antibiotics, rational use of antibiotics, prescribing only specific antibiotic therapy after evaluating the antibiotic sensitivity test of isolates. The best pathway for the prevention of neonatal sepsis, are safe and clean deliveries, hand hygiene practice, barrier nursing, exclusive breast feeding, avoidance of unnecessary invasive

procedures and rationalization of admissions to and discharge from neonatal units.

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