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ASSESSMENT OF THE CULTURE POSITIVITY OF BLOOD PATHOGENS AND CLINICAL OUTCOME OF NEONATAL SEPTICAEMIA PATIENTS ADMITTED IN INTRAMURAL NICU OF A TERTIARY CARE REFERRAL HOSPITAL.

Microbiology

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ABSTRACT

Summary: Septicemia has been found to occur in 2.3% of intramural live births.2 It is estimated that 20% of all neonates develop sepsis3 and is responsible for 30-50% of total neonatal death in developing countries. Moreover, the causative organisms of EOS and LOS sepsis are similar, especially in a hospital setting in a developing country. This cross-sectional, observational study was conducted from August 2021 to August 2022 in the Department of Microbiology and Pediatrics at the UCMS & GTBH Delhi. A total of 1250 patients having clinical suspected neonatal sepsis admitted in intramural NICU of tertiary Hospital with a birth weight of ≥ 1000 gm and/or birth gestation of 28+0 to 41+6 weeks were included in this study. A blood culture sample was processed according to standard guidelines. Out of the 1250 suspected septicemia newborns patients, bacterial blood culture positive in 350 (28%) patients. Although 72% (900) patients blood culture samples were sterile after 48 hours incubation and 5th day subculture. Of the 350 bacterial cultures-positive septicemia patients, 250 (71.4%) patients had been diagnosed with a case of early-onset sepsis (EOS). Whereas, Late-onset sepsis (LOS) was three times lesser than EOS 28.5%. Present study Gram-negative 233 (66.57%) bacterial blood culture isolates were more than Gram-positive blood culture bacterial isolates 117 (33.42%).The AST of bacterial blood culture isolates were analyzed. Our study indomitable the burden; demographic, clinical and laboratory parameters; etiology; phenotypic profile of AMR, and risk factors for NICU associated neonatal sepsis in intramural newborns. High burden of neonatal sepsis and associated antimicrobial resistance were documented.

KEYWORDS

Blood culture, Neonatal septicemia, Culture positivity, Antimicrobial susceptibility.

INTRODUCTION

Septicemia in neonates hospitalized in the Neonatal Intensive Care Unit (N.I.C.U.) is a global problem and a significant contributor to neonatal morbidity and death. World Health Organization (WHO) estimates about 3.3 million neonatal deaths a year, the majority of them occurring in developing countries.[1] Septicemia has been found to occur in 2.3% of intramural live births[2]. It is estimated that 20% of all neonates develop sepsis[3] and is responsible for 30-50% of total neonatal death in developing countries[4].Neonatal septicemia is a clinical syndrome of bacteremia characterized by systemic signs and symptoms in the first month of life. A high index of suspicion is important in the management of neonatal septicemia because the diagnosis is delayed and at times difficult due to paucity or delayed clinical manifestation. However, it is imperative that treatment is instituted early and timely for a favorable outcome in these cases.

Sepsis occurring in the first 72 hours of life is defined as early-onset sepsis (EOS) and that occurring beyond 72 hours as late-onset sepsis (LOS). [5] Despite advances in health care, neonatal sepsis, especially that caused by Gram-negative rod bacteria, is a significant cause of morbidity and mortality among neonates[6]. An increase in sepsis caused by Gram-negative organisms has been reported in recent years from Nepal[7]. Neonatal sepsis caused by Gram-negative microorganisms is responsible for 18%–78% of all neonatal sepsis. [8,9,10] In the developing world, *Escherichia coli* (*E. coli*), *Klebsiella species,* and *Staphylococcus aureus* (*S. aureus*) are the most common pathogens of EOS, whereas *S. aureus, Streptococcus pneumoniae*, and *Streptococcus pyogenes* are the most commonly reported organisms in LOS. [11,12] Moreover, the causative organisms of EOS and LOS sepsis are similar, especially in a hospital setting in a developing country.[13]Microorganisms implicated in neonatal septicemia have developed increased drug resistance to commonly used antibiotics and thus making treatment extremely difficult.[14,15]Thus, the knowledge of both the common pathogens causing septicemia in neonates and their antimicrobial susceptibility is essential in order to select appropriate antimicrobial treatment. Moreover, antimicrobial susceptibility patterns of pathogens vary geographically and are temporally dependent on local pathogens and patterns of antibiotic use. Hence, the present study was conducted for the assessment of the culture positivity of blood pathogens and clinical outcomes of neonatal septicemia patients admitted in the Intramural NICU of a tertiary care hospital. This study also identified an existing correlation between the clinical presentation & high-risk factors in a newborn with the microbiological profile in patients with neonatal septicemia.

MATERIALS & METHODS:

This cross-sectional, observational study was conducted from August 2021 to August 2022 in the Department of Microbiology and Pediatrics at the University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi. A total of 1250 patients having clinical suspected

neonatal sepsis admitted in intramural NICU of GTB Hospital with a birth weight of ≥ 1000gm and/or birth gestation of28+0 to 41+6 weeks were included in this study. Neonates who had undergone surgery because of risk of wound infection, congenital anomalies, acute bilirubin encephalopathy, severe perinatal asphyxia, neonates born to mothers who have received antibiotics before delivery, birth weight less than 1000 gms and age more than 28 days at the time of diagnosis were excluded from the study.

Case definition: As per the criteria by Standard Treatment Protocol for management of common newborn conditions in hospitals (Adapted from WHO Guidelines).[15]

1) Breathing difficulty (RR>60/min) AND Severe chest drawing OR grunting Or any two of the following signs are present
2) Fast breathing (RR>60/min)
3) Convulsions
4) Unconscious or Lethargic (no spontaneous movements)
5) Abnormal body temperature (axillary temperature <36.5° or >37.5°C)
6) No feeding or feeding poor after having fed well.
7) Abdominal distension and/or vomiting

Or

When the age of the newborn is 3 days or less with any of the following two maternal risk factors: maternal fever, foul smelling or purulent amniotic fluid, or prolonged rupture of membranes more than 18 hrs.

Sample collection:

A blood sample was collected before starting antimicrobial therapy. In patients already on antibiotic therapy, a blood sample is usually taken just before the next dose of antimicrobial agents. Out of 3 ml of blood sample was drawn using a sterile syringe, 0.5-1 ml of the blood sample was inoculated aseptically into a blood culture bottle containing 10 ml of Brain heart infusion broth in a ratio of 1:10. Rest 2 ml of the blood was allowed to clot in a sterile bottle to collect serum for the estimation of serological test like C-reactive protein (CRP) and Procalcitonin (PCT) were tested from quantitative Enzyme-Linked Immunosorbent Assay (ELISA) and semi-quantitative rapid lateral flow assay. The bottles were immediately brought to the Microbiology laboratory for incubation at 37°C. The bottles were incubated for five days before being reported as negative. A subculture on blood agar and MacConkey agar were performed manually from each blood culture bottle at 24 hours, 48 hours incubation at 37°C and 5th day subculture also. After incubation at 37ºC the bacterial isolates were identified by gram staining, colony characteristics, and a battery of biochemical tests, following a standard protocol.[6]Antimicrobial susceptibility testing was performed for all the bacterial isolates as per the latest CLSI guidelines 2021 by modified Kirby Bauer method. For all the tests, positive and negative controls were kept. Every batch of Mueller Hinton agar and antibiotic discs was tested by using ATCC control strains. One patient was considered for one blood culture isolate. A repeat sample of the same patient was not considered in the present study.

This study has been approved from the institutional ethical committee (IEC No: GTBHEC/APVL/2021/456-72).

Statistical analysis:

Descriptive variables and prevalenceareexpressed as percentages. The data accrued on all neonatal sepsis was analyzed using SPSS latest version. The Chi-square test was used in assessing the associations between categorical variables. A p-value of 0.05 or less was considered statistically significant.

RESULTS:

During the study period, A total of 1250 patients having suspected neonatal sepsis admitted in intramural NICU of GTB Hospital with a birth weight of ≥ 1000 gm and/or birth gestation of 28+0 to 41+6 weeks were enrolled. Out of the 1250 suspected septicemia newborns patients, bacterial blood culture positive in 350 (28%) patients. Although 72% (900) patients blood culture samples were sterile after 48 hours incubation and 5th day subculture. Of the 350 bacterial cultures-positive septicemia patients, 250 (71.4%) patients had been diagnosed with a case of early-onset sepsis (EOS). Whereas, Late-onset sepsis (LOS) was three times lesser than EOS 28.5%. In this study females (59%) outnumbered male septicemia intramural newborn patients (41%).

Amongst the culture-positive patients, there were 144 (41%) male and 206 (59%) female neonates. Present study Gram-negative 233 (66.57%) bacterial blood culture isolates were more than Gram-positive blood culture bacterial isolates 117 (33.42%). The most common risk factor associated with septicemia suspected in intramural newborns was perinatal birth asphyxia in 67 patients (19.4%) followed by preterm birth in 54 (15.4%) newborn septicemia patients. Of the 350 positive blood cultures isolated patients, 315 (90%) had CRP more than 0.8 mg/dl whereas PCT was found to be reactive in 41% of cases. (Tab. 1)

Out of 350 blood culture positive neonatal septicemia patients, the *Klebsiella species* (78%) was the most common bacterial blood culture isolates among Gram-negative bacteria. Among Gram-positive bacterial blood culture coagulase-negative *Staphylococcus* (CONS-59%) and *Staphylococcus aureus* (46%) were frequently isolated. (Figure 1)

Antibiotics susceptibility pattern in Gram-positive cocci blood culture bacterial isolates:

In Gram-positive cocci of intramural neonatal septicemia patients Methicillin-resistant *Staphylococcus aureus* (MRSA) was 46% (53/117=46%)) among *Staphyloccocusaureus* isolated patients. Methicillin susceptible *Staphyloccocusaureus* (MSSA) isolates were 64% (74/117=64%)) of *Staphyloccocusaureus*-isolated septicemia patients. Out of 53 (46%), MRSA *Staphylococcus aureus* isolates, none of the isolates was vancomycin resistant *S. aureus* (VRSA) and or vancomycin-intermediate *Staphylococcus aureus* (VISA). Out of 59% (69 isolates) of CONS isolated from intramural septicemia patients, 35% (40/117=35%) were Methicillin resistant CONS (MR-CONS) while 65% (76/117=65%) were isolated Methicillin sensitive CONS (MS-CONS). Among 46% isolated MRSA isolates; all were 100 % susceptible to vancomycin and linezolid. The antimicrobial susceptibility pattern of *Staphyloccocus aureus* and CONS blood culture septicemia isolates were resistant to amoxicillin (68%), ciprofloxacin (75%), and erythromycin (60%) respectively. *Enterococcus spp*. blood culture septicemia isolates were 30-40% resistant to erythromycin. All bacterial isolates were susceptible to vancomycin and linezolid. The zone of inhibition was measured for the linezolid and vancomycin disk. One sample was tested for a single patient. All antibiotics were not tested for all bacterial isolates. Cefoxitin antibiotic was tested for screening the MRSA among *staphylococcus* blood culture isolates. (Tab. 2)

Antibiotics susceptibility pattern in Gram-negative blood culture bacterial isolates:

The Gram-negative blood culture septicemia causative *Escherichia coli* showed susceptibility to Third generation cephalosporins were approximately 20-30%. *Klebsiella spp. Citrobacter spp.* and *Enterobacter spp.* showed susceptibility from 20-40 % with 3rd generations of cephalosporins. Amongst aminoglycosides (amikacin & gentamicin) 20-50% of isolates including *Escherichia coli, Klebsiellaspp, Citrobacter spp*., and *Enterobacter spp*. were susceptible, although *Acinetobacterbaumannii* were 60-80% susceptible to aminoglycosides group of antibiotics. Ciprofloxacin were 20-40% susceptible to gram- negative bacilli. Colistin was 100% susceptible to all gram-negative blood culture isolates by the disk diffusion method. Carbapenem were susceptible to 25-40% to *Escherichia coli, Klebsiellaspp, Citrobacter spp*. and *Enterobacter spp. Acinetobacterbaumannii* was 60-80% susceptible with imipenem and meropenem antibiotics. (Tab. 3) Of the 350 blood culture positive newborns septicemia patients, 51% of patients had highly raised CRP level more than and equal to 3.2mg/dl and 31.4 % (110 patients) had CRP levels ranging from 0.8-1.5 mg/dl. 206 (59%) septicemia patients have reactive procalcitonin markers. CRP & PCT were statistically significant from chi-square test (p 0.05). (Tab. 4). We did not checked the CRP levels and PCT marker in blood culture negative newborns septicemia patients.

DISCUSSION

In the present study, a total of 1250 suspected septicemia newborn patients, bacterial blood culture were isolated in 350 (28%) patients which were comparable to another study conducted in Gujrat in which bacterial blood culture was isolated in 35% of patients. [16]It was observed that females (59%) were more predominated than males (41%) in newborn patients. Contradicting the results of the current study, AletayebS et al[17], Celicia C et al [18], Rabia S et al [19] and Ahmad A et al [20] have reported a higher number of male neonatal septicemia cases than female neonatal septicemia cases. The gender

bias in our society's treatment of male infants may be the cause of female predominance.[21] In this existing study, EOS, and LOS were noted at 71.4% and 28.5 % respectively. Our finding coincides with the various published studies by Jain AK et al [22] and Aletayeb et al [17] in which EOS (64.70%) and LOS (35.30%) were documented. Newborns demonstrate a deficiency in phagocytic migration to the site of infection, as well as suboptimal complement activation and a possible deficiency in the newborn's defense system against viral pathogens.[23]

This study isolated, gram-positive bacterial isolates 117 (33.42%) whereas gram-negative bacterial isolates 233 (66.57%) among intramural neonatal septicemia patients. (Tab.2 and 3) Studies reported globally from Kathmandu, North India, and the Philippines showed that gram-negative comprised 76.66% and gram-positive blood culture bacterial isolates were 23.34% of neonatal septicemia patients.[17, 18]Aletayeb S et al. reported that gram-negative bacterial isolates were 92.8%, whereas, gram-positive bacterial isolates was 7.2% isolated amongst neonatal septicemia patients.[17] As the prevalence of bacterial blood culture isolates varies geographically wise, the findings of different studies on microbial isolates conducted by a variety of published studies from various authors were inconsistent. It also depends on the multiple maternal and baby factors such as; the mother's hygiene, the delivery method, and the locationetc. The blood samples of suspected neonatal septicemia patient's cultures negative could be attributable to non-bacterial growth, the presence of viral agents, fastidious organisms, and anaerobic causes, as well as the ratio of the volume of blood taken to the liquid broth and prior antibiotic therapy.[24, 25] Furthermore, none of the studies we compared our data took into account circumstances where there was no bacterial growth. As a result, their percentage is higher than in our study. The risk factors among intramural suspected neonatal septicemia patients in the present study were perinatal birth asphyxia 67 (19.4%) followed by IUGR which was 16.5%. Our finding was regressing to the study published by Assudani H J et al, in which the perinatal asphyxia rate was 0.86%.[16] The present study shows that *Klebsiella spp.* (78%) was the most prevalent isolate amongst intramural neonatal septicemia. *Klebsiella spp.* (46.40%) was the most prevalent isolate in the study published by Aletayeb S et al. While various authors have published *pseudomonas spp.* as the most prevalent isolate (43.20%).[18] *E. coli* was listed as the most frequent isolate studied by Rahman S et al (36.60%) and Rizvi F et al (16%).[26, 27] According to Tsering et al. the most typical isolate was *S. aureus* (41.18%).[28] The cleanliness of the mother, the method of delivery, and the location all affect whether a gram-positive or gram-negative bacterial isolate predominates.[20,21,22]

CRP and positive blood culture results were statistically significantly correlated in this study (Chi-square test, p-value 0.0001) (Tab. 4). Elevated CRP levels were linked to patients with positive cultures in research by Caldas JPS et al.[29], where the results were similar (Chi-square test, p-value 0.05). This demonstrates the significance of CRP as a key indicator of septicemia.

Neonatal septicemia is a serious medical emergency, and prompt antibiotic therapy is crucial for a successful outcome. Ampicillin and aminoglycoside are the traditional first-line (empiric) medications for neonatal sepsis and meningitis. But many institutions have revised their empiric antimicrobial strategy for newborn sepsis after the introduction of third-generation cephalosporins. Amoxicillin/ampicillin resistance was often observed in the isolates of S. aureus and Gram-negative bacteria in the current study, suggesting that using these medications alone may not be successful. The best drugs against S. aureus were discovered to be vancomycin and linezolid. The most effective drug against gram-negative isolates was discovered to be netilmicin. However, resistance to this drug grew over the course of the five-year trial. The drug shown to be most effective against non-fermenters is amikacin. Our study reveals that, based on in vitro susceptibility results, aminoglycosides, third-generation cephalosporins, glycopeptides, and polymyxins are the most effective medications for the treatment of neonatal septicemia.

CONCLUSION:

Our study indomitable the burden; demographic, clinical and laboratory parameters; etiology; phenotypic profile of AMR, and risk factors for NICU associated neonatal sepsis in intramural newborns. High burden of neonatal sepsis and associated antimicrobial resistance were documented. Further, it was evidenced that majority of sepsis episodes were late-onsetand hospital acquired with the odds of sepsis being raised with prolong insertion several invasive devices of neonatal care. It also emphasizes the need to conduct infection surveillance and improve infection control measures. The accommodation of extensive environmental investigation to pinpoint the source and interventional approach to further validate preventable risk factors are warranted in further studies.

Table 1: Risk factor, laboratory parameters and outcome association of Intramural culture positive newbornssepticemiain the study group. (n=350)

Category	Intramural culture positive newborns septicemia	N=350 (%)
Intramural culture positive newborns septicemia with associated risk factors	Perinatal birth asphyxia	67 (19.4)
	Intra uterine growth retardation (IUGR)	58 (16.5)
	Preterm birth	54 (15.4)
	Neonatal Jaundice	50 (14.2)
	Hypoxic Ischemic Encephalopathy (HIE)	25 (7.1)
	Meconium stained liquor (MSL)	24 (6.8)
	Hypoglycemia/Hypothermia	20 (5.7)
	Meconium Aspiration syndrome /Resp distress	20 (5.7)
	Small for gestational age (SGA)	35 (10)
	Thrombotic thrombocytopenic purpura (TTP)	17 (4.8)
Maternal risk factors		
	Mode of Delivery	
	Assisted vaginal delivery	5 (1.4)
	Home delivery	2 (0.5)
	Lower segment cesarian section (LSCS)	161 (46)
	Normal vaginal delivery (NVD)	182 (52)
Laboratory parameters		
	C- Reactive protein (CRP) ranges	
	<0.8 mg/dl	35 (1)
	0.8-1.5 mg/dl	25 (7.1)
	1.6-3.1 mg/dl	180(51)
	>3.2 mg/dl	110(31.4)
	Procalcitonin (PCT)	
	Reactive	144 (41)
	Non reactive	206 (59)
Patient outcome	Survived	192(54.85)
	Death	100(28.85)
	Transferred	50(14.28)
	Status unknown	08 (4)

Table 2: Antimicrobial susceptibility spectrum of Gram-positive cocci isolates in neonatal septicemia patients. (n=117)

Antibiotics discs	No of susceptible isolates of GPC (n=117)		
	Staphylococcus aureus n=43 S (%)	Enterococcus sppn=14 S (%)	Coagulase Negative Staphylocccocus n=60 S (%)
Cefoxitin (30µg)	23 (54%)	ND	33(55%)
Ampicillin (10 µg)	29 (68%)	8(58%)	42 (70%)
Clindamycin ((2 µg)	15 (35%)	ND	27 (45%)
Erythromycin (15 µg)	25(60%)	9 (70%)	33 (55%)
Gentamicin (10 µg)	27 (65%)	ND	36 (60%)
Ciprofloxacin (5 µg)	23 (55%)	ND	21(35%)
Amikacin (30 µg)	30(70%)	ND	34 (58%)
High level Gentamicin (120 µg)	ND	8(60%)	ND

Vancomycin (MIC)	43(100%)	14 (100%)	60(100%)
Linezolid (30 µg)	43(100%)	14 (100%)	60(100%)
Teicoplanin (30µg)	ND	14 (100%)	ND
Amoxycillin-Clavulanic acid (23.75/1.25 µg)	30 (70%)	ND	36 (60%)
Trimethoprim-Sulfamethoxazole (1.25/23.75 µg)	30(70%)	ND	39(65%)

ND: Not done (antibiotic did not tested for particular organism)
All antibiotics were not tested for all bacterial blood culture isolates.

Table 3: Antimicrobial suscpetibility spectrum of Gram-negative bacilli isolates in intramural newborns speticemia patients. (n=233)

Antibiotic discs	No of susceptible blood culture isolates of GNB (n=233)				
	Escherichia coli n=39 S (%)	Klebsiella spp n=181 S (%)	Enterobacter spp. n=116 S (%)	Citrobacter spp. n=48 S (%)	Acinetobacterbaumannii n=151 S (%)
Amoxicillin (20µg)	14(36%)	45(25%)	46(40%)	21(45%)	ND
Ceftriaxone (30µg)	11 (28%)	36(20%)	44(38%)	12(25%)	ND
Cefotaxime (30µg)	10(26%)	54(30%)	52(45%)	9(20%)	ND
Amikacin (30µg)	17(43%)	36(20%)	29(25%)	10(22%)	111(74%)
Gentamicin (10µg)	17(43%)	54(30%)	32(28%)	14(30%)	105(70%)
Netilmicin (30µg)	31(80%)	130(72 %)	78(68%)	36(75%)	98(65%)
Ciprofloxacin (5µg)	9(23%)	36(20%)	25(22%)	18(38%)	54(36%)
Chloramphenicol (30µg)	23(60%)	135(75 %)	40(35%)	33(70%)	ND
Imipenem (10µg)	11(30%)	27(15%)	23(20%)	10(22%)	90(60%)
Meropenem (10µg)	9(25%)	32(18%)	32(28%)	14(30%)	102(68%)
Colistin	39(100%)	18(100 %)	116 (100%)	48(100 %)	151(100%)
Piperacillin+ Tazobactem (100/10µg)	14(38%)	81(45%)	64(56%)	14(30%)	90(60%)
Amoxycillin-clavulanic acid (20/ 10µg)	21(55%)	72(40%)	ND	ND	ND

ND: Not done (antibiotic did not tested for particular organism)
All antibiotics were not tested for all bacterial blood culture isolates.

Table 4: Correlation of C - reactive protein and Procalcitonin with blood culture positivity from intramural neonatal septicemia patients.

Category	C-Reactive Protein (CRP)				Procalcitonin (PCT)	
	<0.8 mg/dl (normal) n(%)	0.8-1.5 mg/dl (abnormal) n(%)	1.6-3.1 mg/dl (abnormal) n(%)	≥3.2 mg/dl (abnormal) n(%)	Reactive	Non reactive
Blood culture positive (n=350)	35 (1%)	25 (7.1%)	180 (51%)	110(31.4%)	206 (59%)	144 (41%)
Blood culture negative (n=900)	700 (78%)	200 (22%)	0	0	101 (11.2%)	799 (89%)
p-value*	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

***p value of less than 0.05 is considered as significant.**

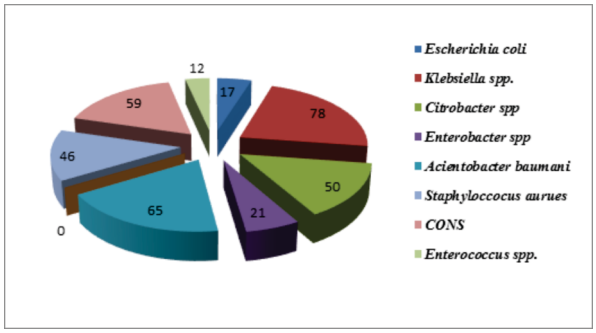

Fig 1: Percentage-wise distribution of blood culture positive bacterial isolates in neonatal septicemia in the study group (n=350).

- *CONS: Coagulase Negative Staphyloccoccus; S.aurues: Staphyloccocus aureus; E.coli: Escherichia coli*

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