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### Spectrum Of Bacterial Pathogens Isolated From Neonatal Sepsis In An NICU And Their Sex Ratios In Causing Septicemia From A Tertiary Care Centre.

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#### Abstract

**Background:** Neonatal septicemia is a clinical condition characterized by systemic signs and symptoms due to bacteremia in the first month of the life. Neonatal septicemia is considered one of the leading causes of neonatal mortality globally. The risk factors those may be associated with neonatal septicemia are premature rupture of membrane, prolonged rupture, prematurity, urinary tract infection, poor maternal nutrition, low birth weight, birth asphyxia, and congenital anomalies. The spectrum of organisms causing neonatal septicemia shows variation in different countries and even varies in hospitals of the same region. In developed countries, gram-negative organisms are the most common organisms of neonatal septicemia. The present study objective was to study the spectrum of bacterial isolates from blood culture and their sex ratios in causing septicemia in neonates.

**Materials and Methods:** A Total of 140 blood samples were screened for sepsis in new borns less than 28 days old suspected on clinical grounds. 2ml of blood collected and inoculated in 10 ml of Brain Heart Infusion Broth(BHI). Subculture was done on blood agar. The growth from mac conkey or blood agar was noted and the organism was identified by standard characteristic tests, further subculture from BHI was done on 3<sup>rd</sup> and 7<sup>th</sup> day of incubation.

**Result:** Among the 140 patients, 26 showed Gram Positive organisms, 52 showed Gram negative organisms, 11 Candida /yeasts like were isolated, and 51 showed No Growth after 3 times subculture. Among the 26 Gram Positive organisms, Staphylococcus aureus were 15(57.69%) and enterococcus were 11 (42.31%). Among the 52 Gram Negative organisms, Escherichia coli were 3(5.76%), Klebsiella pneumonia 23(44.24%) , NFGNB were 14 (26.92%), Citrobacter spp 4(7.68%).

**Conclusion:** Positive blood culture is the gold standard in the diagnosis of neonatal sepsis. Early identification of organisms causing neonatal sepsis and appropriate use of antibiotics to minimize the morbidity and mortality and emerging resistance in bacteria is essential to control neonatal sepsis in neonatal intensive care units.

**Keywords:** Gram stains, Agar Medium, Blood culture, Bacteria.

## Introduction

Neonatal bacterial sepsis remains an important cause of mortality among neonates. Neonatal sepsis is defined as a clinical syndrome of bacteria with systemic signs and symptoms of infection in the first 4 weeks of life<sup>1</sup>. 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). Usually EOS is due to vertical transmission of pathogen and LOS is due to horizontal transmission of pathogen from care givers. Risk factors for early onset sepsis include prolonged ruptured membranes (>18hrs), fetal distress, preterm delivery, history of Group B streptococcus (GBS) infection in previous infant, GBS bacteriuria in current pregnancy. LOS occurs due to prolonged hospitalization, eg preterm infant in a NICU, presence of foreign bodies eg intravenous catheters, endotracheal tubes etc cross infection by staff and parents, malformations such as urinary tract anomalies eg vesicoureteral reflux or neural tube defects<sup>4</sup>. The present study conducted to study the spectrum of bacterial isolates from blood culture and their sex ratios in causing Septicemia in neonates [1,2,3].

## Materials and Methods

A retrospective study was conducted in neonates admitted to NICU multiple medical institution in India. A Total of 116 blood samples were screened for sepsis in new borns less than 28 days old suspected on clinical grounds. 2ml of blood collected and inoculated in 10 ml of Brain Heart Infusion Broth (BHI). Subculture was done on blood agar, MacConkey agar after 24 hours of incubation with sterile inoculation loop (4mm). The growth from MacConkey or blood agar was noted and the organism was identified by standard characteristic tests like Gram stain, Colony morphology, Catalase, Coagulase test and the IMVIC test. If first subculture did not show any growth, further subculture from BHI was done on 3<sup>rd</sup> and 7<sup>th</sup> day of incubation

## Result

Out of 140 patients admitted during the study period, 88 were male and 62 were female neonates with ratio of 1.1:1. Among the 140 patients, 26 showed Gram Positive organisms, 52 showed Gram negative organisms, 11 *Candida* / yeasts like were isolated, and 51 showed No Growth after 3 times subculture. Among the 26 Gram Positive organisms, *Staphylococcus aureus* were 15 (57.69%) and enterococcus were 11 (42.31%). Among the 52 Gram Negative organisms, *Escherichia coli* were 3 (5.76%), *Klebsiella pneumoniae* 23 (44.24%), NFGNB were 14 (26.92%), *Citrobacter spp* 4 (7.68%).

## Discussion

Neonatal sepsis is a syndrome of clinical manifestations of inflammatory responses due to a spectrum of systemic infections, such as Septicemia, pneumonia, bone-related infections, and meningitis. Clinical manifestations of sepsis are non-specific and resemble many non-infection-related disorders. However, despite this dilemma, consensus definition and global guidelines are still lacking owing to variability among different studies and clinical practices, as well as epidemiologic differences. Although blood culture is considered the gold standard in the diagnosis of neonatal sepsis, low rates of positivity constitute a real management challenge [4].

The clinical presentations of neonatal sepsis are nonspecific. This includes symptoms like fever, respiratory distress, lethargy/irritability, convulsions, bulging fontanel, refusal to feed, jaundice, bleeding, abdominal distension, and temperature dysregulation. Early-onset sepsis (EOS) presents within 72 hrs of life, and late-onset sepsis (LOS) presents beyond 72 hours of life. EOS presents where the maternal genital tract is the source of ascending infection. Maternal risk factors like premature rupture of membranes (PROM), chorioamnionitis, peripartum fever, urinary tract infection within 2 weeks prior to delivery and prolonged rupture of membranes > 18 hours, multiple gestations, and caesarean sections are associated with increased risk of EOS. LOS occurs as a result of postnatal nosocomial infections or community-acquired infections. The risk factors associated with LOS are prematurity, prolonged invasive interventions like mechanical ventilation and intravascular catheterization, failure of early enteral feeding with breast milk, long duration of

parenteral nutrition, hospitalization, surgery, and underlying respiratory and cardiovascular diseases. The spectrum of microbiological pathogens implicated in neonatal sepsis, in developing countries, differs from those occurring in developed countries, with majority contributed by gram-negative organisms in resource-poor areas [5]. The present study conducted to study the spectrum of bacterial isolates from blood culture and their sex ratios in causing Septicemia in neonates.

The study of Mohammad N et al[4] conducted in seventy blood cultures reported, (31.7%) were bacteria-positive: 45.3% for Klebsiella, 22.7% for coagulase-negative staphylococci (CoNS), and for Acinetobacter (10.7%), methicillin-resistant Staphylococcus aureus (MRSA) (9.3%), Pseudomonas (5.3%), Enterobacter (4%), and streptococci (2.7%). High resistance to all cephalosporins, B-lactamase combinations, penicillin, carbapenems, and aminoglycosides was observed. All Gram-negative Enterobacteria showed the highest sensitivity to levofloxacin, whereas Pseudomonas and Acinetobacter were highly sensitive to polymyxinB. Gram-positive samples were sensitive to vancomycin and linezolid. Streptococci were slightly sensitive to vancomycin and highly sensitive to macrolides and cefotaxime. Latsho et al[5] reported in their study, incidence of culture positive neonatal sepsis was 19 per 1000 admissions with a blood culture positivity rate of 14%. 54.5% had culture-positive early-onset sepsis (EOS). Prematurity ( $p = 0.012$ ), APGAR  $< 6$  ( $p = 0.018$ ), low birth weight ( $p < 0.001$ ), and maternal intrapartum antibiotics ( $p = 0.031$ ) significantly increased risk for culture-positive EOS. Prematurity ( $p < 0.001$ ), low birth weight ( $p = 0.001$ ), and parental nutrition ( $p = 0.007$ ) were significantly associated with increased risk of culture-positive late-onset sepsis. A positive screen had sensitivity of 81.8% and negative predictive value of 87.7%. Gram-negative organisms were most commonly isolated (64.6%). Coagulase-negative Staphylococci (31%) were the commonest isolate followed by Klebsiella pneumoniae (27%) and Acinetobacter (18.8%). Ninety percent of Acinetobacter were carbapenem resistant. Gram-negative sepsis had mortality of 88.9%. This study concluded preterm, low birth weight, low APGAR scores, intrapartum antibiotics, and parental nutrition were significantly associated with neonatal sepsis. Coagulase-negative Staphylococci, Klebsiella pneumoniae, and Acinetobacter were the principal causative organisms. Gram-negative organisms had high resistance to commonly used antibiotics.

The Study conducted by Hassan DM [6] reported, out of the 371 neonates, 137 (37%) had positive blood culture results, of whom 49% died versus only 7.7% of neonates with a negative blood culture ( $P$ -value  $< 0.0001$ ). Low birth weight, prematurity, and the duration of hospital stay were considered as positive blood culture risk factors. Meanwhile, among 85 cultures that yielded Gram-negative pathogens, 16 isolates were identified as ESBL producers with Klebsiella pneumoniae being the most frequently encountered isolate (19.7%). Of the neonates inflicted with ESBL-sepsis, 62.5% died versus 11.6% with non-ESBL sepsis. The study of Nkoyo O Uwe[7] et al, two hundred and ninety neonates were recruited during the study period. Seventy-three (25.2%) neonates had culture-proven sepsis. One (0.3%) neonate had meningitis and no neonates (0%) had confirmed urinary tract infection. Of the 73 neonates with positive blood cultures, 56 (76.7%) had early-onset sepsis and 17 (23.3%) had late-onset sepsis. Gram-negative bacilli accounted for 60.3% of all isolates. Predominantly isolated pathogens were Staphylococcus aureus (20.5%), CoNS (19.2%) and Klebsiella pneumoniae (13.7%). The isolates were most susceptible to levofloxacin and amikacin. This study concluded maintaining regular antibiotic surveillance for appropriate empirical antibiotics is important as part of neonatal care.

The study of Thapa S et al[8] Out of 516 specimens, bacterial growth was obtained in 56 specimens (10.8%). Prevalence of early onset sepsis was higher 35 (62.5%) in neonates compared to late onset sepsis 21 (37.5%). Majority of neonatal Septicemia were caused by gram-negative isolates 39 (69.6%). Acinetobacter species 18 (32.1%) was most commonly isolated organism followed by Staphylococcus aureus 11 (19.6%). The predominant isolate in early onset Septicemia was Acinetobacter species 18 (32.1%) and Staphylococcus aureus 9 (16%) and in late onset Septicemia was Staphylococcus aureus 11 (19.6%) and Acinetobacter species 5 (8.9%). Staphylococcus aureus and coagulase-negative Staphylococci displayed highest susceptibility towards vancomycin, amikacin, teicoplanin, and meropenem. Gram-negative isolates

showed susceptibility towards amikacin, piperacillin/tazobactam, meropenem, ofloxacin, and gentamicin. This study concluded *Acinetobacter* species and *Staphylococcus aureus* remain the most predominant organisms responsible for neonatal Septicemia in a tertiary care setting and demonstrate a high resistance to the commonly used antibiotics [9]. Above all, since the rate of *Acinetobacter* species causing sepsis is distressing, inspiring interest to control the excess burden of *Acinetobacter* species infection is mandatory.

### Conclusion

Among the bacterial isolates Gram negative Septicemia was found to be more predominant. Males were found to be more commonly associated with neonatal sepsis in our centre. *Klebsiella pneumoniae* was the most commonly isolated pathogen followed by *Staphylococcus aureus* and NFGNB, 3<sup>rd</sup> most commonly isolated was *Enterococcus*, *Candida* and *Escherichia coli* then followed by *Citrobacter* sp.

Positive blood culture is the gold standard in the diagnosis of neonatal sepsis.<sup>2</sup> Early identification of organisms causing neonatal sepsis and appropriate use of antibiotics to minimize the morbidity and mortality and emerging resistance in bacteria is essential to control neonatal sepsis in neonatal intensive care units.

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