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Bacteriological profile and antibiotic sensitivity pattern of neonatal septicaemia in a tertiary care hospital in North India

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Abstract
Background: There is not much published literature on neonatal septicemia available for the North India Hence, we undertook this study to find out the bacteriological profile and antibiotic sensitivity pattern of neonatal septicemia in the neonatal Intensive Care Unit.
Material and Methods: Blood cultures were performed for all clinically suspected neonatal septicemia cases for 1-year. Identification of all pathogenic isolates was followed by antibiotic sensitivity testing.
Results: We did blood cultures for 450 neonates and 42% were culture positive. Early onset sepsis were 92 (49%) and 96 (51%) were late onset sepsis. Gram-positive isolates were 60% and 40% were Gram-negative. Staphylococcus aureus (40%), coagulase negative Staphylococcus species (16%), non-fermenter group of organisms (NFGOs) (15%), and Klebsiella pneumoniae (10%) were the main isolates. Nasal cannula 101 (54%), birth asphyxia 91 (48%), and prematurity 73 (38%) were the prominent risk factors associated with septicemia. Gram-positive organisms were highly resistant to penicillin (87%) whereas Gram-negative isolates showed high resistance to third generation cephalosporins (53–89%) and aminoglycosides (50–67%). The S. aureus isolates were methicillin-resistant in 41% whereas extended spectrum beta lactamase production was seen in 48% Gram-negative isolates.
Conclusion: Our study highlights the recent emergence of Gram-positive organisms as predominant cause of neonatal septicemia in this part of Kanpur. Though Gram-negative bacteria still remain the main cause of mortality in neonatal septicemia, we want to dispel the common notion among practitioners that they are the predominant isolates in neonatal septicemia.

Key words: *Blood culture, drug resistance, extended spectrum beta-lactamases, methicillin-resistant Staphylococcu aureus, neonates*

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I. Introduction

Blood infections are an extensive range of disorders that can vary from limited bacteraemia to fatal septicaemia[1]. If the presence of a bacterium is accompanied by its multiplication in the bloodstream, it is referred to as septicaemia[2]. Sepsis is one of the leading causes of mortality all over the world[3]. With delays in the diagnosis and treatment of sepsis, the mortality rate may increase up to 50% [4]. Despite the high costs of sepsis and its treatment, the risk of death remains very high compared to other diseases[3]. Disappointingly, in recent years, the number of sepsis cases have been rising significantly [5]. Studies show that a variety of microorganisms are capable of causing septicaemia, which usually depends on factors such as different geographical location. For example, a study on microorganisms isolated from blood cultures in India reported that S. aureus, coagulase-negative *Staphylococci*, and *Enterobacter* are the most common causes of bloodstream infections, while a similar study in showed that *Pseudomonas* and coagulase-negative *Staphylococci* were seen in blood cultures more often than others[1,6].

Multi-drug-resistant (MDR) infections in the paediatric age group, especially due to gram-negative bacteria (GNB) are increasing world over with higher mortality[7]. Overall mortality in Indian PICU due to Hospital-acquired infections (HAIs) has been estimated to be 26%. Bloodstream infections (BSI) are considered as the most serious infections in paediatric intensive care units (PICU) and carry the highest mortality with an estimated attributable mortality of 3% and crude mortality of 18% [8,9]. Many risk factors, especially in PICU, for Hospital acquired infections (HAIs) are common for adults and children which include exposure to invasive devices including intravascular catheters, intubation, hyper-alimentation, and other comorbidities like immune-suppression.

II. Material $ Method

500 sample from paediatric patient will be collected . from inpatients admitted to the ICU and NICU were collected from GSVM Medical college study was conducted from January 2021 to December 2021.

METHOD

All the Blood sample was collected from pediatric outpatient and inpatient were included and the study and data were collected in MacConkey agar, Blood agar from those yielding growth on culture. The bacterial isolate was identified with standard bio-chemical test Preliminary identification was done with the help of the following methods- Gram stain:-The suspected colonies were stained using gram stain method and their shape ,colour and arrangement were observed under light microscope. It was a cross-sectional study carried out in the NICU of a tertiary care medical college and hospital from Jan. 2021 to Dec. 2021. The various neonatal and maternal risk factors were also analysed. The neonatal risk factors studied were low birth weight (LBW), birth asphyxia, meconium staining, congenital anomalies, prematurity, central venous catheterisation >10 days, nasal canula usage, and continuous positive airway pressure use. The maternal risk factors included socioeconomic status, difficult delivery (caesarean, forceps, vacuum), premature rupture of membranes, prolonged rupture of membranes, maternal fever, recurrent abortions, prenatal care received, urinary tract infection, history of stillbirth, amniocentesis, cervical cerclage operation, and chorioamnionitis. A total of 450 suspected patients of neonatal septicaemia were included in the study. Neonatal septicaemia was suspected if one or more of the following signs and symptoms were present: Convulsions, respiratory rate >60/min, severe chest indrawing, nasal flaring, grunting, bulging fontanelle, pus draining from the ear, redness around umbilicus extending to the skin, temperature ≥37.5°C (99.5°F) or ≤36.4°C (97.52°F), lethargy, unconsciousness, reduced movements, not able to feed, not attaching to the breast, not suckling at all, crepitations in lungs, cyanosis, and reduced digital capillary refill time. Two millilitres of blood was collected from all neonates aseptically preferably before administration of the antibiotics and inoculated into 20 ml of brain heart infusion broth (Hi-Media, India) on the bedside. Blood culture bottles were transported immediately to the Microbiology Laboratory and were processed as per standard microbiological techniques and the isolates were identified.[10] Antibiotic sensitivity testing was performed on Mueller-Hinton agar plates by modified Kirby-Bauer disk diffusion method as per Clinical Laboratory Standard Institute guidelines.[11] E. coli ATCC 25922, S. aureus ATCC 25923, P. aeruginosa ATCC 27853 were used as standard strains. Screening for MRSA was done using a cefoxitin (30 µg) disc. [11] Resistance to ceftazidime (30 µg) disk was used as a screening method for detection of ESBL confirmed by double disk synergy test.[11]

Procedure of Gram Staining :- Gram Staining is a differential staining and it consist of four steps: Primary stain (crystal violet) for 1 minute. Mordant (gram,s iodine) for 1 minute. Decolourizer is – (acetone) for 5 sec. Counter stain (safranin) for 30 s

Inclusion Criteria : Paediatric patients with clinical infection and Blood sample will be collected.Social demographic date, medical history ,clinical history regarding age ,gender type of paediatric patient percentage and antibiotic therapy will be taken.

Exclusion Criteria : Repeat isolates from the same patient and from the same site/specimen all excluded.

Biochemical Test Catalase Test

Principle – This test demonstrates the presence of catalase an enzymes that catalyses the release of oxygen from hydrogen peroxide.

Procedure- Take a clean grease free slide. Divide it into two halves. One half serves at test and the other as control. Put one drop of 3% H202 each on the test and control halves Now, with the help of wooden stick a separate colony from the culture plate and touch on the surface of H2O2 marked as test. Look for the effervescence production. P.CATCC *Staphylococcus aurous* (25923)N.CATCC *Enterococcus faecal* is (29212)

Ethical Clearance

Ethical clearance will be obtained from the Institutional Ethical Committee of CSJM University, Kanpur.

Statistical Analysis

Statistical analysis was performed by using computer-based software, Statistical Package for Social Science (SPSS). Mean values of parameters were compared to determine.

III. Result

Of a total of 450 neonates investigated with blood culture, 188 (42%) were found to be positive for neonatal septicemia. Males were 117 (62%) and 71 (38%) females. Mean age at admission was 3.77 ± 5.02 days. Inborn neonates 111 (59%) were more common than outborn 77 (41%). Of 188 neonates, 106 (56%) and 82 (44%) were normal birth weight and LBW neonates, respectively, and 73 (39%) were preterm. Very LBW neonates, that is, weight below 1.5 kg were 19 (11%). EOS and LOS were 92 (49%) and 96 (49%) neonates, respectively. EOS was seen more common in inborn neonates (56%) compared tothe outborn (39%).

The common clinical presentations were respiratory distress secondary to birth asphyxia (48%), fever (20%), neonatal jaundice (8.5%), pneumonia (8%), decreased acceptance of feeds and lethargy (7%), and seizures or meningitis (5%). The neonatal risk factors such as birth asphyxia, use of a nasal cannula, and prematurity showed association with culture-proven neonatal septicemia ($P < 0.05$). None of the maternal risk factors were found to be significantly associated with neonatal septicemia. Of 188 (42%) positive blood cultures, the Gram-positive bacteria (GPB) and Gram-negative bacteria (GNB) accounted for (60%) and (40%). Respectively, Among Gram

-positive organisms, 66% isolates were S. aureus and among Gram-negative organisms, NFGO was the most common organism isolated (40%). Among NFGO, only Acinetobacter spp. (3%) could be identified. Other organisms isolated are summarised in Table 1. CONS and NFGO were considered as pathogens only if the temperature was >38°C or <36° C, history of initiation of antibiotic therapy or presence of the intravenous catheter. The resistance to penicillin, amoxicillin and clavulanic acid and azithromycin was 87%, 66% and 48% respectively among GPB and GNB [Figure 1]. Methicillin-resistance was detected in 29 (41%) of S. aureus. There were 30 (40%) MDR isolates among the total of 75 isolates of S. aureus. All the isolates were sensitive to vancomycin.

Table 1: Gram-positive and Gram-negative pathogens isolated from neonates

Gram-positive isolates (60%)	Gram-negative isolates (40%)
***Staphylococcus aureus* (66%)**	**Nonfermenter group of organisms (40%)**
***CONS spp* (31%)**	***Klebsiella pneumoniae* (24%)**
Group B Streptococcus (3%)	***Escherichia coli* (13.5%)**
	***Enterobacter spp.* (13.5%)**
	***Citrobacter spp.* (9%)**

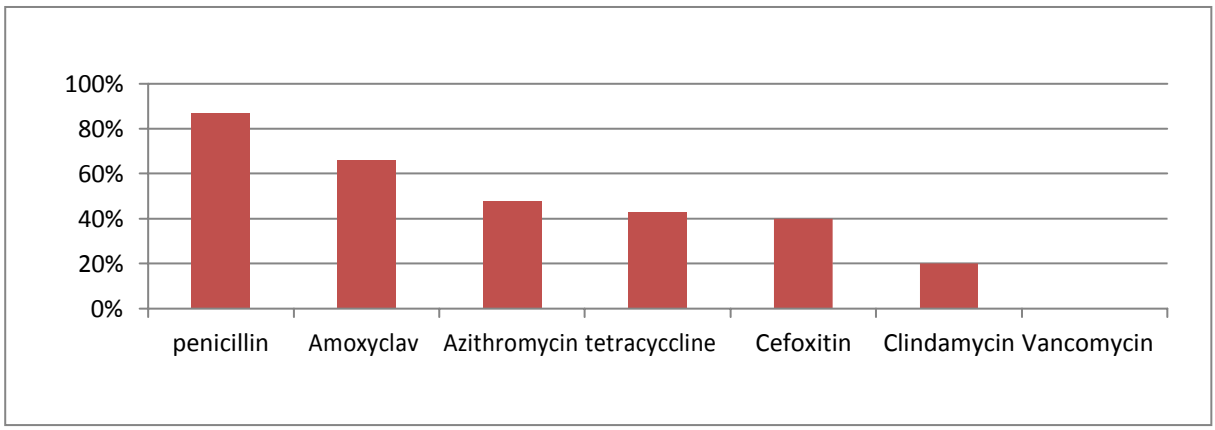

Figure 1: Antibiotic resistance pattern among Gram-positive isolates

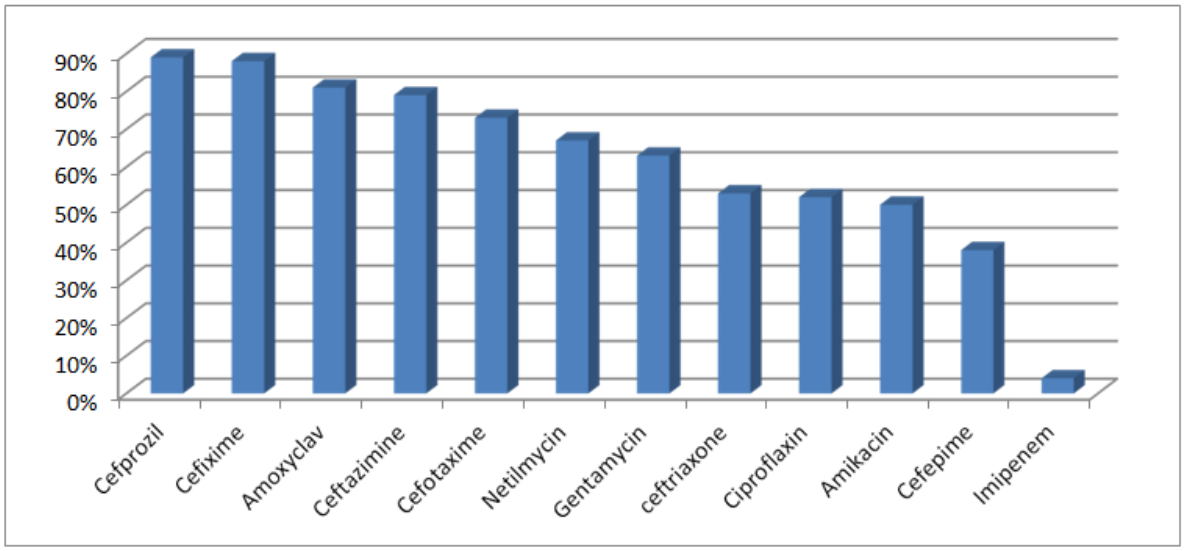

Figure 2: Antibiotic resistance pattern among Gram-negative isolates

Gram-negative organisms showed high resistance to the third generation cephalosporins. They also showed high resistance to amoxicillin and clavulanic acid combination (81%), netilmicin (67%) and gentamycin (63%). Resistance to ciprofloxacin and amikacin was observed in (52%) and (50%) respectively [Figure 2]. ESBL production was detected in 24 (48%) of GNB that included Klebsiella pneumonia 12 (50%), E. coli 6 (25%), NFGO 4 (16%) and Enterobacter spp. 2 (8%) High resistance against penicillin and amoxicillin and clavulanic acid was found for both GPB and GNB. The third generation cephalosporin showed weak activity against all tested bacteria. The considerable preponderance of amikacin activity over gentamicin and netilmicin was observed in GNB. Imipenem resistance was observed among 04% GNB. Out of the 45 Enterobacteriaceae isolates in our study 33 (73.3%) were MDR. All the Acinetobacter spp. were also MDR here were 22 (12%) mortality which was 17% among female neonates compared to 8.5% among male neonates. Highest mortality of neonates was seen with Klebsiella pneumoniae (27.7%) followed by NFGO (20%) and Enterobacter spp. (20%). The neonatal factors significantly associated with mortality were female sex, LBW, birth asphyxia, and nasal cannula ($P < 0.05$)

IV. Discussion

Septicemia remains a significant cause of morbidity and mortality in the newborn. The clinical diagnosis of neonatal septicemia is difficult as it presents with non-specific signs and symptoms. An early diagnosis of neonatal septicaemia is important to initiate appropriate and prompt treatment. The correct and timely identification of infectious agents and their antibiotic sensitivity patterns are essential to guide the clinicians regarding both the empirical and definitive treatment The bacteriological profile of septicemia keeps changing with the passage of time from region to region and hospital to hospital, in the same city or country. The emergence of resistant bacteria in NICU settings leads to failure in the treatment of neonatal septicemia. To supplement the management of septicemia in neonates, we need to do longitudinal surveillance of the NICUs and formulate periodic guidelines for empirical treatment. In recent years, there has been a lot of improvement in medical facilities and as a result, the survival rate of the preterm and LBW babies has improved. But at the same time, these neonates with immature immune defenses are exposed to NICU flora for a longer duration. Most of the neonatal septicemia cases now are either LBW or preterm. [12] In the present study, 44% were LBW and 39% were preterm neonates which has led to more LOS (51%) as compared to EOS (49%). Mhada et al. reported 23% of preterm neonates, in their study.[8] Among the maternal risk factors, the difficult delivery (32%) in the form of Caesarean, forceps or vacuum was found much higher risk factor in our study as compared to (14.88%) by Tallur et al.[13]

The bacteriological profile has changed worldwide from predominant Gram-negative to a predominant Gram-positive bacteria isolation.[14-15] Many recent studies have reported the emergence of some new emerging organisms such as CONS, NFGO, and Candida spp. as a cause of neonatal septicemia.[16-17] Our study showed a preponderance of Gram-positive isolates, 60% versus 40%, Gram-negative isolates. Ballot et al., Kaufman and Fairchild and Hoogen et al. reported the isolation of GPB in 54.9%, 68.2%, and 75%,

respectively, which is in concordance with the present study.[18,19,20] The colonisation of the skin and nasopharynx by CONS and S. aureus in health care workers, overcrowding in nurseries and NICU, and improper hand washing techniques may lead to transmission of Gram-positive organisms in neonates horizontally. There is a predominance of GPB Figure 1: Antibiotic resistance pattern among Gram-positive isolates Figure 2: Antibiotic resistance pattern among Gram-negative isolates

isolation in our study unlike other studies in the north india .[21,22] The reason could be due to overcrowding in nurseries/NICU and lack of knowledge about infection control measures among health care providers. Our study showed S. aureus (66%) and CONS (31%) as the most common Gram-positive organisms which is quite high as compared to studies conducted by Agnihotri et al. and Sundaram et al.[16,17] [Table 2]. NFGO and Klebsiella pneumoniae were the common Gram-negative isolates, which was comparable to the other studies in the region.[16,17] NFGO tend to colonize and proliferate in nurseries and are transmitted by cross infection. Both Gram- positive and Gram-negative isolates showed a high resistance to cephalosporins, penicillin, and amoxyclav in the current study, it was observed that antibiotic resistance among the Gram-positive isolates was highest to penicillin (87%) followed by amoxyclav (66%). Similar reports of high resistance to Ampicillin (71%) were reported by Bhat et al.[18] All the Gram-positive

isolates were sensitive to vancomycin similar to a study by Hoogen et al.[22] In the present study, 41% S. aureus isolates were found to be methicillin-resistant, compared to 11.1% reported by Kaistha et al.[25] Gram-negative isolates showed a high resistance to all cephalosporins which is similar to the resistance pattern reported by Agnihotri et al. And Bhat et al.[16,18] Klebsiella pneumoniae. showed resistance to all antibiotics tested except imipenem. In our study, the 48% were ESBL producers as compared to 72% as reported by Bhat et al.[18] This high resistance pattern could be attributed to the injudicious use of antibiotics in our region. In the present study, overall mortality was observed in 11.7%, whereas Chaudhary reported a mortality of 45.5% in their study, which is quite high as compared to our study.[23] This could be attributed to advancement in medical technology and better neonatal care in NICU.

V. Conclusion

We want to highlight the fact that Gram-positive organisms particularly S. aureus and CONS are now predominant organisms causing neonatal septicemia in our institution, as well as whole of north India as suggested by studies in past decade rom the predominance of Gram-negative organisms to Gram-positive organisms S. aureus in the past decade throughout the world, the reason for which is not clear.[10-15] Among Gram-negative bacteria (GNB), NFGO are one of the newly emerging groups of organisms with high mortality next only to Klebsiella spp. High resistance to cephalosporins is a cause of concern, as they are one of the most common prescribed antibiotic groups in the region. Urgent need for preventive measures including proper hand washing, barrier nursing, and judicious selection of antibiotics is recommended along with continuous surveillance of the neonatal septicemia.

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