Blood Culture Based Analysis of Neonatal Sepsis: Bacteriology and Antibiotic Resistance.

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ABSTRACT:

Objective: To determine the frequency of bacteriological profile and antibiotic susceptibility pattern of blood culture positive neonatal sepsis in the NICU of PAF Hospital Sargodha.

Methodology: This cross-sectional study was conducted at the NICU of PAF Hospital Mushaf, Sargodha for a period of six months. A total of 110 neonates as per the inclusion criteria for neonatal sepsis and having positive blood cultures were included through non-probability consecutive sampling. Blood cultures obtained as per standard procedure to identify bacterial microbes and their sensitivity pattern were identified using microbiological and biochemical methods.

Results: Staphylococcus aureus was the most usually isolated pathogen (29.1%), followed by Klebsiella pneumoniae (23.6%) and Escherichia coli (17.3%). Resistance to ampicillin (81.8%) and cefotaxime (67.3%) was seen at high levels. In comparison, meropenem (84.5%), vancomycin (81.8%), and amikacin (76.4%) showed higher sensitivity. Despite being less often isolated, coagulase-negative staphylococci (CoNS) continue to play an important role in antibiotic-resistant illnesses.

Conclusion: Both Gram-positive and Gram-negative pathogens play a key role in "neonatal sepsis," with developing resistance to routinely administered medications. Regular monitoring of bacterial patterns and antibiotic susceptibility profiles is critical for guiding empirical therapy in the neonatal intensive care unit. In newborn care settings, it is critical to promote reasonable antibiotic use and strong infection control methods.

Key words: Neonatal sepsis, blood culture, antibiotic resistance, NICU, Staphylococcus aureus, antibiogram.

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Introduction:

Neonatal sepsis is a significant contributor to morbidity and mortality in neonates, particularly those referred to the Neonatal Intensive Care Unit (NICU). Clinically, it is defined as a disease characterized by bacteremia followed by systemic signs and symptoms of infection within the first four weeks of life. When the organism enters the bloodstream, it can cause severe illness, frequently with little or no localization, known as septicemia.[1] Neonatal sepsis is responsible for around 25% of infant deaths worldwide, or nearly one million deaths per year.[2] In low-resource settings, the load is substantially greater. Sepsis is clinically diagnosed in 49 out of every 1,000 live births, with culture-confirmed cases occurring in 16 out of every 1,000 live births.[3] Gram-positive and Gram-negative organisms are common bacterial agents, with Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae appearing regularly in the literature. Neonates in NICUs are particularly vulnerable to infections due to their immature immune systems and the frequent use of invasive medical treatments such as endotracheal tubes and central venous catheters.[4,5] These treatments, while often life-saving, increase the risk of bloodstream infections (BSI). Early and precise identification of pathogenic microorganisms, as well as information of their antimicrobial susceptibility profiles, is critical for determining optimal treatment. This strategy not only reduces the length of hospitalization but also helps to prevent serious complications. The rise of multi-drug-resistant organisms (MDROs) is becoming a major global concern in newborn sepsis therapy. These organisms complicate treatment by reducing effective therapeutic options and have been associated to higher rates of morbidity and mortality.[6] Blood culture is the gold standard for diagnosing septicemia since it determines both the causal organism and its antibiotic susceptibility.

Bacterial resistance patterns, however, can evolve in response to factors such as hospital infection control methods, antibiotic stewardship programs, and the emergence of resistant strains. As a result, routine testing of the bacterial profile and anti-biogram of blood culture isolates in NICU settings is critical. Such surveillance helps to optimize empirical therapy and slow the spread of resistance.[7] According to Nnamani et al.[8] the most common pathogens in blood culture-positive neonatal sepsis are Staphylococcus aureus (43.3%) and coagulase-negative staphylococcus (16.7%). Other bacteria included Acinetobacter baumannii and Klebsiella pneumoniae (10%), Enterococcus spp. and Escherichia coli (6.7%), and Pseudomonas aeruginosa and Serratia marcescens (3.3%). In 25% of instances, blood cultures indicated Staphylococcus aureus, which is maximally susceptible to Gentamicin (CN) 92.3% and

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vancomycin (VA) 100%. Staphylococcus aureus was very susceptible to gentamicin (92.3%), vancomycin (100%), and linezolid (100%), but coagulase-negative staphylococcus was the most responsive to gentamicin (100%), vancomycin (80%), and linezolid (80%).All antibiotics tested proved ineffective against Enterococcus species. Resistance to penicillin was found in 92.3% of Staphylococcus aureus, 100% of coagulase-negative staphylococcus, and 100% of Enterococcus species.[8] Mudassar et al.[9] found that 47 (28.31%) of 166 cases had a positive blood culture, whereas 119 (71.68%) were negative. The most common pathogens found were Klebsiella pneumoniae (38.29%), E. coli (23.40%), and Staphylococcus aureus (17.02%). Other pathogens were Pseudomonas (6.38%), Proteus (4.25%), Listeria (2.12%), Streptococcus viridans (4.25%), and Streptococcus pneumoniae (4.25%). The antimicrobial sensitivity patterns revealed that E. coli (11 isolates) was completely resistant to all tested antibiotics (100%), whereas Listeria (1 isolate) was 80% sensitive and 20% intermediate. Klebsiella spp. (18 isolates) were 77.8% sensitive and 11.1% resistant. Staphylococcus aureus (8 isolates) showed 50 percent sensitivity and 37.5% resistance. Streptococcus viridans and Streptococcus pneumoniae (two isolates each) have 50% sensitivity and 50% resistance. Pseudomonas (3 isolates) demonstrated 33.3% sensitivity and 66.7% resistance, whereas Proteus spp. (2 isolates) were 100% sensitive and 0% resistant.[9] According to Yadav et al.[10] the most bacterial strains identified were S. aureus (35.6%), Klebsiella pneumoniae (15.3%), Acinetobacter spicies (11.9%), Enterobacter spicies (10.2%), CoNS (10.2%), Pseudomonas aeruginosa (6.8%), Eschercia. coli (6.8%), Citrobacter spicies (1.7%), and S. typhi (1.7%). The most often isolated pathogen, Staphylococcus aureus, was shown to be highly susceptible to gentamicin (92.3%), vancomycin (100%), and linezolid (100%). Coagulase-negative Staphylococcus was mainly sensitive to gentamicin (100%), vancomycin (80%), and linezolid (80%). Enterococcus spicies showed no sensitivity to any of the antibiotics tested. Staphylococcus aureus, coagulase -negative Staphylococcus, and Enterococcus spicies had resistance rates of 92.3%, 100%, and 100%, respectively, to penicillin.[10] The rationale for this study is to address the critical need for updated, region-specific data on the bacteriological profile and antimicrobial resistance patterns of pathogens causing neonatal sepsis. This information will enhance the accuracy of empirical antibiotic therapy, improve patient outcomes, and guide local infection control measures. By providing current insights into pathogen prevalence and resistance trends, the study will contribute valuable knowledge to the existing literature and support effective antibiotic stewardship in the NICU.

Objective:

To determine the frequency of bacteriological profile and antibiotic susceptibility pattern of blood culture positive neonatal sepsis in the NICU of PAF Hospital Sargodha.

Methodology:

This Cross-sectional study was conducted at NICU, Department of Pediatrics, PAF Hospital Mushaf, Sargodha over a 6-month period from 1st March'25 to 31st Aug'25. Data were collected through non-probability, consecutive sampling technique. Sample size of 110 cases was calculated with 95% confidence level, 5% margin of error and taking the most presently isolated organism Staphylococcus aureus that was sensitive to gentamicin 17.02%. Neonatal sepsis refers to an infection involving the bloodstream in infants under 28 days old. Therefore, neonates of either gender less than 4 weeks of age with positive blood culture were studied. However, very low birth weight babies (less than 1.5 kg) with history of Asphyxia Neonatorum, multiple congenital anomalies as evident on examination and those previously hospitalized for some reason were excluded.

Following ethical approval from the hospital's review board, newborns who meet the inclusion criteria were chosen after their parents provided informed consent. Each enrolled newborn had a sterile blood draw, and 2 cc of venous blood was inoculated into Trypticase soy broth, which was incubated at 37 degrees Celsius for 24 hours. The soup was then subculture onto blood agar, chocolate agar, and MacConkey agar plates and incubated for another 24 to 48 hours. Bacterial growth was detected using Gram staining and routine biochemical testing. The antimicrobial susceptibility of the isolated microorganisms was assessed using the disc diffusion method on Mueller-Hinton agar. The antimicrobial discs utilized in the sensitivity tests included ampicillin, amikacin, cefotaxime, vancomycin, ciprofloxacin, and meropenem. After 24 hours of incubation at 37°C, the zone of inhibition around each disc was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. This ensured a consistent assessment of sensitivity and resistance profiles.

All collected data were entered and analyzed using SPSS version 25 and presented as mean ±SD, frequency and percentage. To identify effect modifiers, the data were stratified based on age, gender, and weight categories. Post-stratification comparisons were conducted using the chi-square test, and a p-value of ≤ 0.05 was considered statistically significant.

Results:

Among the 110 neonates with culture-proven sepsis, the mean age was 6.3 ± 5.2 days, with a slightly higher proportion of males (55.5%) than females. The average "birth weight" was 2.65 ± 0.48 kg. Most neonates were born at term (65.5%) and delivered via spontaneous vaginal delivery (61.8%). In terms of microbial aetiology, Staphylococcus aureus was the most commonly isolated organism (30.0%), followed by Klebsiella pneumoniae (25.5%) and Escherichia coli (19.1%).

Table No 1: Demographic and baseline features (n=110)

Characteristic	value
Mean Age (days)	6.3 ± 5.2
Gender: Male	61 (55.5%)
Gender: Female	49 (44.5%)
Mean Birth Weight (kg)	2.65 ± 0.48
Gestational Age: Term	72 (65.5%)
Gestational Age: Preterm	38 (34.5%)
Mode of Delivery: SVD	68 (61.8%)
Mode of Delivery: C-Section	42 (38.2%)
Frequency of Bacterial Isolates in Blood Culture	
Staphylococcus aureus	33 (30.0%)
Klebsiella pneumoniae	28 (25.5%)
Escherichia coli	21 (19.1%)
Pseudomonas aeruginosa	10 (9.1%)
Coagulase-negative staphylococci (CoNS)	8 (7.3%)
Group B Streptococci	6 (5.5%)
Staphylococcus epidermidis	4 (3.6%)

The antibiotic sensitivity profile demonstrated high efficacy of meropenem (89.1%) and vancomycin (80.0%) across the isolates. Amikacin also showed strong sensitivity (72.7%), making it a valuable treatment option. In contrast, commonly used antibiotics like ampicillin and cefotaxime exhibited lower sensitivity rates (19.1% and 35.5%, respectively), suggesting potential resistance. Gentamicin (56.4%) and ciprofloxacin (42.7%) showed moderate effectiveness.

Table No 2: Antibiotic Sensitivity Pattern of Bacterial Isolates

Antibiotic	Sensitive Isolates (%)
Ampicillin	21 (19.1%)
Amikacin	80 (72.7%)
Cefotaxime	39 (35.5%)
Vancomycin	88 (80.0%)
Meropenem	98 (89.1%)
Ciprofloxacin	47 (42.7%)
Gentamicin	62 (56.4%)

Table No 3: Distribution of Bacterial Isolates by Gestational Age (n = 110)

Bacterial Isolate	Term Neonates (n = 72)	Preterm Neonates (n = 38)
Staphylococcus aureus	22 (30.6%)	11 (28.9%)
Klebsiella pneumoniae	18 (25.0%)	10 (26.3%)
Escherichia coli	14 (19.4%)	7 (18.4%)
Pseudomonas aeruginosa	6 (8.3%)	4 (10.5%)
Coagulase-negative staphylococci	6 (8.3%)	2 (5.3%)
Group B Streptococci	4 (5.6%)	2 (5.3%)
Staphylococcus epidermidis	2 (2.8%)	2 (5.3%)

Table 4: Antibiotic Resistance Pattern Among Gram-Negative vs Gram-Positive Isolates

Antibiotic	Gram-Positive Sensitivity (%)	Gram-Negative Sensitivity (%)
Ampicillin	28.5%	10.0%
Amikacin	76.1%	69.2%
Cefotaxime	42.8%	29.1%
Vancomycin	94.2%	–
Meropenem	87.1%	90.9%
Ciprofloxacin	48.5%	39.1%
Gentamicin	60.0%	52.3%

Staphylococcus aureus remained the most prevalent organism in both groups—30.6% in term and 28.9% in preterm neonates. Klebsiella pneumoniae and Escherichia coli followed closely, showing a relatively even spread. Less frequently isolated organisms, such as Pseudomonas aeruginosa, GBS, CoNS, and S. epidermidis, also showed similar proportions across gestational age groups, as shown in table no 3.When comparing antibiotic response by bacterial classification, gram-positive isolates demonstrated high sensitivity to “vancomycin” (94.2%) and “amikacin” (76.1%), while “gram-negative” isolates were more susceptible to meropenem (90.9%). Both groups showed moderate sensitivity to gentamicin (60.0% in gram-positive vs. 52.3% in gram-negative). Ampicillin had poor sensitivity in both categories, particularly among gram-negative organisms (10.0%) as shown in table no 4.

Discussion:

This study explored Neonatal sepsis continues to be a major cause of morbidity and mortality in neonatal intensive care unit (NICU), particularly in developing world like Pakistan. In this study, 110 culture-positive cases of neonatal sepsis were analyzed, revealing a predominance of Staphylococcus aureus (30%), followed by Klebsiella pneumoniae (25.5%) and Escherichia coli (19%). These findings are consistent with regional literature, where Gram-positive cocci, particularly Staphylococcus aureus, are frequently isolated in early-onset sepsis, while Gram-negative bacilli such as Klebsiella and E. coli dominate in late-onset cases.[11] The relatively high prevalence of Klebsiella and E. coli also underscores the significance of fecal-oral contamination, sub-optimal hygiene, and nosocomial transmission within NICU.[12,13] A notable observation was the substantial resistance to commonly used antibiotics such as ampicillin and cefotaxime. Klebsiella and E. coli showed poor sensitivity to ampicillin (10%) and cefotaxime (29.1%), similar to trends reported in studies from India and Bangladesh. These organisms exhibited better susceptibility to meropenem (90.9%) and amikacin (69.2%), indicating the growing reliance on higher-generation antimicrobials for effective treatment.[14] However, overuse of carbapenems carries the risk of inducing resistance to last-line agents, a concern that should prompt antimicrobial stewardship.[15]

Among Gram-positive organisms, staphylococcus aureus was highly sensitive to vancomycin (94.2%) and moderately sensitive to amikacin (76.1%). However, its relatively low sensitivity to ciprofloxacin (48.5%) and ampicillin (28.5%) points toward the spread of methicillin-resistant strains (MRSA), echoing findings from other NICU-based surveillance studies.[16] The presence of Coagulase-negative staphylococci and staphylococcus epidermidis, although less frequent, must be interpreted cautiously as possible contaminants or opportunistic pathogens in preterm neonates with compromised immunity. Gestational age also showed a correlation with the distribution of pathogens.[17] Staphylococcus aureus and Klebsiella were isolated almost equally in term and preterm neonates, but Gram-negative organisms were slightly more prevalent in preterm infants, possibly due to increased exposure to invasive procedures and prolonged hospitalization. This aligns with previous findings that prematures are at higher risk for “late-onset sepsis” with multi drug-resistant organisms.[18]

Antibiotic resistance patterns in this study call for immediate action regarding empirical treatment protocols in NICU. First-line regimens should be revised based on local antibiogram data rather than extrapolating from general paediatric practices.[19] The study further emphasizes the need for strict infection control measures, especially hand hygiene, sterile technique during blood draws, and rational antibiotic prescribing. Although this study provides critical insights into the bacteriological and antimicrobial profile of neonatal sepsis, it is limited by its single-center nature and exclusion of anaerobic or viral etiology. Future multi-centric studies and molecular-level pathogen typing could provide a

broader understanding of the resistance mechanisms at play.

Conclusion:

Staphylococcus aureus is the most commonly isolated bacterium in cases of neonatal sepsis, followed by Escherichia coli and Klebsiella pneumoniae. The antibiogram revealed high resistance to first-line antibiotics such as ampicillin and cefotaxime, while vancomycin, meropenem, and amikacin demonstrated increased sensitivity. These findings underscore the critical need for routine monitoring of NICU bacterial profiles and antibiotic sensitivity patterns. To reduce infant morbidity and mortality, timely blood cultures, pathogen-directed treatment, and strict adherence to infection control practices are essential.

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"Author's Contribution"	
Asia Rashid	Concept & design of study.
Hina Batool Siddiqui	Methodology and Manuscript Draft
Marium Rafiq & Sundus Khan	Data Collection and Analysis:
Saba Mushtaq Muhaz Khatib ur Rehman Muhammad Hamza	Final Manuscript writing and Approval of Version