

ETIOLOGY AND ANTIMICROBIAL PATTERNS OF NEONATAL SEPSIS

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Abstract

Background: In the world, sepsis is the third leading cause of neonatal mortality, accounting for almost 225,000 newborn deaths annually. Targeted and successful treatment and prevention efforts require information on the microbiological etiology of newborn sepsis and the antibiotic resistance profiles of the agents that cause it.

Objective: This study was conducted to determine the etiological agent of neonatal sepsis and their antimicrobial pattern.

Materials and Methods: This cross-sectional study was carried out from March 2023 to April 2024 by Pakistan institute of medical sciences Islamabad and Department Of Microbiology, Hazara University Mansehra. A total of 200 blood samples from patients of both gender were taken for this study. Blood culture and antibiotic sensitivity tests were performed for all the enrolled patients by using Kirby-Bauer disc diffusion method. SPSS version 24 was used for data analysis.

Results: A total of 200 neonates were enrolled in the current study. There were 70 (35%) male and 130 (65%) female neonates in our study. Amongst 200 samples, 95 (47.5%) were culture positive. Out of 95 positive blood culture, 73 (76.8%) had gram positive organisms and 20 (21.0%) had gram negative organisms; the remaining two (2.1%) tested positive for *Candida* spp. Coagulase negative staphylococci (n=35) accounted for the majority of gram-positive organism cases, then *Staphylococcus aureus* (n=25) and *Enterococcus* (n=7). *Klebsiella* (n=13) was the most common cause of gram-negative organism cases, followed by *E. coli* (n=4) and *Pseudomonas* (n=3). All the isolates in our study showed resistance to Amoxicillin, Cefixime, and Ampicillin. Amikacin and Vancomycin were susceptible against coagulase-negative staphylococci. Amikacin was completely effective against *Staphylococcus aureus*, while α -hemolytic *Streptococcus* was susceptible to Vancomycin, Amikacin, Piperacillin, and Tazobactam. All gram-negative organisms were resistant to ampicillin, cefixime, and Amoxicillin, whereas *Enterococcus* was sensitive to Amikacin and Vancomycin.

Conclusion: Our study concludes that gram-negative bacteria are less common than gram-positive bacteria. Among Gram-positive bacteria, coagulase-negative *Staphylococci* was isolated in majority of the cases. While in gram negative isolates *Klebsiella* was commonly observed. Majority of the isolates were resistant to commonly used antibiotics.

INTRODUCTION

Neonatal sepsis is a clinical condition caused by bloodstream infection that presents with non-specific systemic signs and symptoms in newborns [1]. The immaturity of the immune system and the necessity of many invasive operations make hospitalized newborns, particularly preterm neonates, susceptible to nosocomial infections [2–6]. Hemodynamic, post-inflammatory, and immunosuppressive alterations are its features, and they can result in significant morbidity and mortality [7]. Neonatal sepsis manifests clinically as fever or hypothermia, respiratory issues such as cyanosis and apnea, difficulty feeding, distension of the abdomen, vomiting, diarrhea, oliguria, lethargy, and irritability [7]. The classification of neonatal sepsis can be either early-onset sepsis (EOS) or late-onset sepsis (LOS), depending upon the development of symptoms. The EOS is vertically transferred and appears within the first 72 hours [8]. On the other hand, the LOS is primarily acquired horizontally from the environment and appears after 72 hours following childbirth [9]. Neonatal sepsis is the world's greatest cause of death, yet its frequency and rates of mortality are higher in low- and middle-income countries in comparison to high-income nations. This is likely because of inadequate infection control procedures and poor hygiene [10]. Although the neonatal mortality rate in Pakistan (per thousand live births) has decreased from 55 in 2013 to 42 in 2018 [11,12], it is still significantly higher than that of industrialized nations like the US, UK, and Canada, where it is <5 per 1000 live births. Compared to its neighbors, such as China (4/thousand live births), Iran (9 /thousand live births), and India (23 /thousand live births), Pakistan has an even higher newborn mortality rate [13]. Due to their weakened immune systems, it is very difficult for newborns to fight against bacterial illnesses. Group B *Listeria monocytogenes*, *Streptococci*, *Escherichia coli*, and *coagulase-negative Bacteria* are linked to neonatal sepsis while the other bacteria includes *Staphylococci* (CoNS), *Staphylococcus aureus*, *Enterococci*, *Klebsiella spp.*, *Enterobacter spp.*, *Pseudomonas spp.*, *Salmonella spp.*, and *H. Streptococcus pneumoniae*, *influenzae*, and *Neisseria meningitidis* [14–16]. Nevertheless, the pathogen profile varies by location. South Asia and sub-Saharan Africa have

low GBS prevalence and a predominance of gram-negative pathogens, while high-income countries have high GBS prevalence [17, 18, 19]. An estimated one-third of the four million neonatal deaths that occur globally each year are attributed to infections [20–22]. Sepsis and bacterial meningitis is still one of the leading causes of neonatal mortality, particularly for extremely low birth weight newborn infants [22]. Neonatal sepsis has a higher risk of death than other neonatal diseases, and bacterial infections constitute the primary cause of neonatal fatalities worldwide [23]. For neonatal sepsis patients to have a good prognosis, early antibiotic treatment is crucial. Therefore, it is a common practice to begin treatment before receiving of blood culture findings [24–29]. Due to the difficulty of obtaining sufficient blood samples for culture, the lengthy turnaround time for culture results, and the limited sensitivity of the blood culture approach, many doctors also choose empirical treatment for suspected neonatal sepsis [24,25]. According to WHO guidelines for the treatment of suspected neonatal infections, empirical treatment with ampicillin and gentamicin should be the first line of treatment. For patients who do not respond or whose drug-susceptibility testing of bacterial isolates shows resistance to first-line therapy, a third-generation cephalosporin should be used as the second line of treatment [30]. The bacterial infections that cause newborn sepsis and the patterns of antibiotic resistance vary by region and period [31], depending on the common antibiotics used in the neonatal department and the prevalent local pathogens [32, 33]. Antibiotic resistance is currently a common issue. This study was therefore conducted to determine the etiological agent of neonatal sepsis and their antimicrobial pattern.

Materials and Methods

This cross-sectional study was carried out from March 2023 to April 2024 by Pakistan institute of medical sciences Islamabad and Department Of Microbiology, Hazara University Mansehra. The current study included all neonates up to 28 days of age, weighted over 1500 grams, delivered to mothers who had a history of infection, and hospitalized due to suspected neonatal sepsis. The study excluded infants with congenital conditions including cystic

fibrosis, Down syndrome, and others that make them more vulnerable to infections, as well as those who have had recent surgical procedures. Our study was approved by the ethical committee of the institution. A total of 200 blood samples from patients of both gender were taken for this study. Before antimicrobial therapy, two milliliters of blood were aseptically taken and directly injected into Brain Heart Infusion (BHI) broth. The blood culture bottles were sent right away to the microbiology lab, where they were sub-cultured on blood agar, chocolate agar and MacConkey agar and incubated at 37°C for twenty-four hours. While chocolate agar and blood agar plates were incubated in moist environment enhanced with CO₂ using jar of candle for 2 days at 37°C. Aerobic incubation was used to incubate the inoculated McConkey agar plates. After seven days of incubation, blood culture bottles that showed no growth on the subculture were reported as negative. Standard microbiological techniques were used to process all of the blood samples that were collected for isolation and culture [34] as suggested by the Clinical Laboratory Standard Institute (CLSI), the Kirby-Bauer disc diffusion method criteria to test for antimicrobial susceptibility [35]. The antibiotic disks that were utilized were piperacillin/tazobactam (100/10 µg), teicoplanin (30 µg), erythromycin (15 µg), gentamicin (10 µg), meropenem (10 µg), nalidixic acid (10 µg), ofloxacin (5 µg), cefotaxime (30 µg), 30 µg, clindamycin (2 µg) ceftriaxone (30 µg), cotrimoxazole (25 µg), cloxacillin (5 µg), cefixime (5 µg), cefoxitin (30 µg), amikacin (30 µg), and vancomycin (30 µg). SPSS version 24 was used for data analysis.

Results

A total of 200 neonates were enrolled in the current study. Amongst 200 samples, 95 (47.5%) were culture positive. Out of 95 positive blood culture, 73 (76.8%) had gram positive organisms and 20 (21.0%) had gram negative organisms; the remaining two (2.1%) tested positive for Candida spp. (Table 1). There were 70 (35%) male and 130 (65%) female neonates in our study. We observed that 10 (5%) of the newborns were weighing less than 1500 grams, 80 (40%) were weighing between 1500 and 2500 grams, and the remaining newborns weighed more than 2500 grams. 80% of the cases involved a normal vaginal birth, 15 % involved LSCS, and the remaining instances involved vacuum aided delivery. (Table 2) *Coagulase negative staphylococci* (n=35) accounted for the majority of gram-positive organism cases, then *Staphylococcus aureus* (n=25) and *Enterococcus* (n=7). *Klebsiella* (n=13) was the most common cause of gram-negative organism cases, followed by *E. coli* (n=4) and *Pseudomonas* (n=3). *Coagulase-negative staphylococci* were the most frequent cause of early onset sepsis, whereas *Staphylococcus aureus* was the most frequent cause of late onset sepsis (Table 3). The Antibiotic susceptibility pattern shown in (Table 4-5). All the isolates in our study showed resistance to Amoxycillin, Cefixime, and Ampicillin. Amikacin and Vancomycin were susceptible against *coagulase-negative staphylococci*. Amikacin was completely effective against *Staphylococcus aureus*, while α -hemolytic *Streptococcus* was susceptible to Vancomycin, Amikacin, Piperacillin, and Tazobactum. All gram-negative organisms were resistant to ampicillin, cefixime, and Amoxycillin, whereas *Enterococcus* was sensitive to Amikacin and Vancomycin.

Table 1: Blood Culture Baseline Frequency

Blood cultures	Frequency	Percentages
Gram positive	73	(21.0%)
Gram negative	20	(76.8%)
Candida species	2	(2.1%).
Total	95	(100)

Table 2: Initial Features of Neonates and Their Mothers

Characteristics	Number	Percentage (%)
Gender		
Male	130	(65%)
Female	70	(35%)
Birth weights		
<1500	10	(5%)
>1500 to 2500	80	(40%)
>2500	110	(55%)
Delivery Method		
Vacuum	10	(5%)
Normal vaginal delivery	160	(80%)
Lower section caesarean section	30	(15%)
Onset of sepsis		
Early onset	135	(67.5%)
Late onset	65	(32.5%)

Table 3: Bacteriological Spectrum at Sepsis Onset

	Late onset sepsis (n=65)		Early onset sepsis (n=135)	
	N	Percentage	N	Percentage
Gram Negative				
<i>E.coli</i>	01	5%	03	15%
<i>Klebsiella</i>	06	30%	07	35%
<i>Pseudomonas</i>	0	0%	03	15%
Gram Positive				
<i>Staphylococcus aureus</i>	16	20.5%	09	12.3%
<i>Enterococcus</i>	06	8.2%	01	4.1%
<i>Coagulase-negative staphylococci</i>	10	12.3%	25	34.2%
<i>α-hemolytic Streptococcus</i>	02	1.3%	02	2.7%

Table 4: Gram-positive Organisms Isolated from Neonates and their Antimicrobial Susceptibility Profile.

Antibiotics	Ec			PDM			Kleb		
	R	I	S	R	I	S	R	I	S
Gentamicin	2	0	2	0	4	3	4	4	5
Cephalexin	3	0	0	6	0	0	11	0	0
Imipenem	0	0	3	6	0	0	0	0	11
Vancomycin	3	1	0	7	0	0	5	6	2
Ampicillin	3	1	0	6	0	0	11	0	0
Cefotaxim	3	0	0	6	0	0	10	0	0
Piperacillin+to	0	3	0	0	5	0	3	4	1
Cefixime	3	0	0	6	0	0	10	0	0
Amikacin	0	0	3	0	0	6	0	0	11
Amoxycillin	3	0	0	6	0	0	11	0	0
Ceftazidime	0	3	0	0	5	2	5	5	3
Ciprofloxacin	0	1	1	3	4	6	3	4	5
Ceftriaxone	0	3	0	6	0	0	11	1	0

Key; Ec: *E.coli*; PDM: *Pseudomonas*; Kleb: *Klebsiella*

Table 5: Gram-negative Organisms Isolated from Neonates and their Antimicrobial Susceptibility Profile.

Antibiotics	CoNS			Ent			SA			HS		
	R	I	S	R	I	S	R	I	S	R	I	S
Gentamicin	3	3	24	1	2	3	0	6	10	0	0	3
Cephalexin	26	0	0	5	0	0	15	0	0	3	0	0
Imipenem	0	8	20	19	0	4	0	4	13	0	3	0
Vancomycin	0	0	26	0	0	6	0	3	14	0	0	3
Ampicillin	26	0	0	6	0	0	16	0	0	3	0	0
Cefotaxim	0	2	11	9	2	30	4	0	7	1	2	0
Piperacillin+to	0	9	20	1	1	2	0	12	4	0	0	3
Cefixime	26	0	0	6	0	0	15	0	0	1	2	0
Amikacin	0	0	26	0	0	6	0	0	16	0	0	3
Amoxycillin	26	1	0	6	0	0	16	0	0	3	0	0
Ceftazidime	0	8	19	1	1	3	0	6	10	0	2	2
Ciprofloxacin	2	18	4	0	2	1	9	4	6	0	0	4
Ceftriaxone	20	6	2	6	0	0	15	2	0	3	0	0

Key; CoNS: *Coagulase-negative staphylococci*; Ent: *Enterococcus*; SA: *Staphylococcus aureus*; HS: *α-hemolytic Streptococcus*.

Discussion

Neonatal Sepsis is a major cause of death in developing nations. Insecure living conditions and inappropriate antimicrobial intake exacerbate the growth and spread of antibiotic-resistant bacteria.

The most common species linked to neonatal sepsis vary from one region to another region and time of infection [34]. Therefore, knowledge of the bacteriological profile of neonatal sepsis and effective antimicrobial agents for its treatment is crucial in

order to address neonatal morbidity and mortality issues. Neonatal sepsis is a leading cause of infant mortality and morbidity in our nation [35]. In this investigation, 200 blood samples were taken from neonates of both the gender. Amongst 200 samples, 95 (47.5%) were culture positive. Out of 95 positive blood culture, 73 (76.8%) had gram positive organisms and 20 (21.0%) had gram negative organisms; the remaining two (2.1%) tested positive for *Candida* spp. These results are consistent with earlier research conducted by Qadeer S. and Javed I. et al. [36]. A significantly higher rate of positive culture is also shown by other studies [37–38]. We found that sepsis was culture-proven in 47.5% of the neonates. In a previous study, Lamba et al. observed that 37.8% patients had culture positive [39]. According to our research, *Klebsiella* was the most prevalent gram-negative bacterium that caused early onset sepsis, whereas *coagulase-negative staphylococci* were the most prevalent gram-positive organism. *Staphylococcus aureus* was the most prevalent gram-positive bacterium in late-onset sepsis, whereas the only gram-negative organism was *Klebsiella*. If CoNS is separated from blood, it is typically regarded as a skin contamination. Since the clinical signs of CNS sepsis might vary, when this bacteria is found in the blood of severely ill newborns, it should be considered a major concern. Early onset sepsis was frequently caused by *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, according to Jajoo et al. [40]. Lamba et al. found that the most common bacterial isolate was *coagulase-negative Staphylococci*, followed by *Klebsiella* spp. According to a related study by Thapa et al., the most frequent isolate in early-onset sepsis was *Acinetobacter* species, but the most frequent isolate in late-onset sepsis was *Staphylococcus aureus* [41]. All the isolates in our study showed resistance to Amoxicillin, Cefixime,

and Ampicillin. Amikacin and Vancomycin were susceptible against *coagulase-negative staphylococci*. Amikacin was completely effective against *Staphylococcus aureus*, while α -hemolytic *Streptococcus* was susceptible to Vancomycin, Amikacin, Piperacillin, and Tazobactam. All gram-negative organisms were resistant to ampicillin, cefixime, and Amoxicillin, whereas *Enterococcus* was sensitive to Amikacin and Vancomycin. According to Jajoo et al., all gram-negative bacteria in their study were resistant to ofloxacin and least sensitive to amikacin (25%). They were also sensitive to meropenam (80%) piperacillintazobactam (75%), followed by chloramphenicol (50%) cefotaxime (50%) and imipenem (50%), Gram-positive bacteria in the Lamba et al. study were most sensitive to teicoplanin (88.57%), vancomycin (95.23%), and linezolid (97.15%), while ceftazidime (14.28%) was the least sensitive. The sensitivity of gram-negative organisms to, meropenam, imipenem and colistin was 89.89%, 86.43%, and 77.88%, respectively. Cephalosporin sensitivity was low. According to Thapa et al., *S. aureus* has the highest sensitivity rate to ofloxacin cotrimoxazole vancomycin, meropenem teicoplanin, amikacin, erythromycin and clindamycin, while *Enterobacteriaceae* had the greatest rate of sensitivity to gentamicin, ampicillin/sulbactam, piperacillin/tazobactam, amikacin, ofloxacin, and meropenem.

Conclusion

Our study concludes that gram-negative bacteria are less common than gram-positive bacteria. Among Gram-positive bacteria, *coagulase-negative Staphylococci* was isolated in majority of the cases. While in gram negative isolates *Klebsiella* was commonly observed. Majority of the isolates were resistant to commonly used antibiotics.

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