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Original Research Article

Biochemical Changes and Microbial profile of Neonatal Sepsis at Tertiary Care Hospital

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Abstract:

Objectives: The present study was to evaluate the biochemical changes and microbial profile of neonatal sepsis at tertiary care hospital.

Methods: Blood culture sample included a single sample collected from a peripheral vein or artery under aseptic conditions. The local site was cleansed with 70% alcohol and povidone iodine (1%), followed by 70% alcohol again. Blood cultures were done in a brain heart infusion biphasic medium. Isolate was identified by their characteristic appearance on their respective media, Gram staining and confirmed by the pattern of biochemical reactions using the standard method. Biochemical data including urea, creatinine, total bilirubin, direct aminotransferase aminotransferase protein (CRP) were recorded.

Results: A total of 546 newborns with clinical sepsis were included. Blood culture reports were positive in 102 cases (18.68%). Male-to-female ratio of 1.75:1. 78(76.47%) had early-onset sepsis and 24(23.53%) had late-onset sepsis. Gestational age of all 102 neonates was 34.56±5.12 weeks. 1 minute and 5 minutes APGAR score was 7.64±3.36 and 8.75±2.64 respectively. Mean urea of all neonates were 55.86±40.17 mg/dl. Mean CRP level was 8.32±9.16 mg/dL. Gram-negative bacilli and Gram-positive cocci were 66/102(64.71%), 36/102(35.29%) respectively. Highest sensitivity among Gram-negative isolates was to imipenem (96.83%), followed by amikacin (51.52%) and netilmicin (40.35%). Gram-positive isolates had the highest sensitivity of 94% to linezolid, 69.39% to tetracycline, 65.31% to piperacillin/ tazobactam, and 54.17% to ciprofloxacin.

Conclusions: Klebsiella spp. and coagulase-negative staphylococci (CONS) are the most common organisms isolates in neonatal sepsis. Highest sensitivity among Gram-negative isolates was to imipenem followed by amikacin and netilmicin and gram-positive isolates are to linezolid followed by tetracycline, piperacillin/ tazobactam and ciprofloxacin. The gold standard for diagnostic sepsis is blood culture and the CRP is also particularly useful for monitoring the response to treatment and guiding antibiotic therapy. Hence, the elevated CRP concentrations is indicator of sepsis.

Keywords: Neonatal sepsis, Biochemical parameter, Microbial profile, Antibiogram.

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Introduction

Neonatal sepsis is a systemic infection in the first 28 days of life, encompasses with bloodstream infections, pneumonia and meningitis. It is the third most common cause of deaths among neonates [1,2]. In India about one-third of neonatal mortality is due to neonatal sepsis and death occurs in 30% of culture positive. Neonates [3,4]. Based on the onset of illness, Neonatal sepsis is classified as early onset sepsis (EOS) (72 h), occurs due to pathogens acquired either from the hospital or from the community [3].

As per the Centers for Disease Control and Prevention (CDC) guidelines Multi-drug resistance was defined as the acquired resistance to at least one agent in three or more antimicrobial categories. Strict antibiotics stewardship program will enable us to counteract multi drug resistance patterns of emerging pathogens [4,5]. The prevalence of organism in SNCUs, differ from tertiary care NICUs in our country and also different from that of the Western world. Pathogens such as Klebsiella pneumoniae and Escherichia coli are the most

common cause of neonatal sepsis in India and South Asia [6,7].

Newborns are more vulnerable to bacterial infections than older children due to their immature immune systems. They have a weak inflammatory response to infection, low antibody levels, and reduced complement activity [8]. In addition, newborns in the Neonatal Intensive Care Unit (NICU) are most liable to multidrug-resistant (MDR) bacterial infections due to many factors, including low birth weight, small gestational age, weak immunity, invasive procedures, prolonged antibiotic therapy, prolonged hospital stays (≥ 5 days), and low adherence to infection control measures. Neonatal sepsis caused by MDR organisms was linked to a high rate of mortality [9].

The distribution of pathogenic agents causing neonatal sepsis varies over time from one country to another, among different locations within the same country, and even within the same setting from time to time. This variation is mainly attributed to changes in antibiotic prescribing practices, the emergence of antibiotic-resistant bacteria, and alterations in lifestyle and healthcare activities [10]. The causative bacteria of neonatal sepsis became more resistant to the commonly used empirical antibiotic agents, making the empirical treatment of these infections much more difficult [11].

It is critical for each NICU to regularly assess the local etiology of sepsis, update antibiotic resistance patterns, and compare findings with clinical profiles to identify the possible risk factors. Such practices will ensure the proper selection of empirical antibiotic therapy and support the development of effective management strategies [12]. Objective of the present study was to evaluate the biochemical changes and microbial profile of neonatal sepsis at tertiary care hospital.

Material & Methods

The present study was conducted in the Department of Biochemistry with the collaboration of Department of Microbiology and Department of Pediatrics of Sri Krishna Medical College and Hospital, Muzaffarpur, Bihar, India during a period from January 2025 to November 2025.

An analysis was conducted on all blood culture reports obtained from newborns admitted to the Department of Pediatrics and the Neonatal Intensive Care Unit (NICU) Sri Krishna Medical College and Hospital, Muzaffarpur, Bihar,

A total of 546 neonates blood culture was done for all neonates suspected to septicemia.

Methods: Blood culture sample included a single sample collected from a peripheral vein or artery under aseptic conditions.

The local site was cleansed with 70% alcohol and povidone iodine (1%), followed by 70% alcohol again. Blood cultures were done in a brain heart infusion biphasic medium. Approximately, 3 ml of blood was inoculated into the brain heart infusion broth and incubated at 37˚C. Subcultures were done on sheep blood agar and MacConkey agar at the earliest visual detection of turbidity or blindly on days 1, 4, and 7 if the bottles did not show turbidity. Isolate was identified by their characteristic appearance on their respective media, Gram staining and confirmed by the pattern of biochemical reactions using the standard method[32]. Members of the family enterobacteriaceae were identified by indole production, H2 S production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization tests, and other tests. For Gram-positive bacteria, coagulase, catalase, bacitracin and optochin susceptibility tests and other tests were used. Blood culture broth that showed no microbial growth within seven days was reported as culture negative, only after result of routine subculture on blood, MacConkey, and chocolate agar [33]. Antimicrobial susceptibility testing was performed for all blood culture isolates by Kirby–Bauer disc diffusion method as recommended in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines.[11] The drugs for disc diffusion testing were in the following concentrations: Ampicillin (10 µg), cloxacillin (1 µg), lomefloxacin (10 µg), amoxiclav (20/10 µg), cephalexin (30 µg), cefuroxime (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), (30 µg), penicillin (10 units), tetracycline (30 µg), co-trimoxazole (1·25 µg trimethoprim/23·75 µg sulfamethoxazole), amikacin (30 µg), ofloxacin (5 µg), sparfloxacin (5 µg), pefloxacin (5 µg), cefoperazone (75 µg), netilmicin (30 µg), imipenem (10 µg), piperacillin/ tazobactam (100/10 µg), azithromycin (15 µg), and linezolid (30 µg). The discs were obtained from Himedia (India) Laboratories.

General Data: Day of life that the blood sampling was performed (0-7 days of life), gender, Apgar score (1 and 5 minute), GA, BW.

Biochemical data including urea, creatinine, total bilirubin, direct aminotransferase aminotransferase protein (CPR) was analysed and recorded.

Results

A total of 546 newborns with clinical sepsis were included. Blood culture reports were positive in 102 cases (18.68%). Among the culture positive cases, there were 65 (63.72%) male and 37(36.27%) female neonates with the male-to-female ratio of 1.75:1. Out of 102 cases, 78(76.47%) had early-onset sepsis and 24(23.53%) had late-onset sepsis.

Gestational age OF all 102 neonates was 34.56±5.12 weeks. Birth weight was 2510±1070 kilograms. 1 minute and 5 minutes APGAR score was 7.64±3.36 and 8.75±2.64 respectively. Temperature at blood collection of all neonates was 35.85±0.54 °C. Mean urea of all neonates were 55.86±40.17 mg/Dl Creatinine level was 0.81±0.21 mg/dL. Total bilirubin level was 8.12±5.24 mg/dL. Direct Bilirubin level was 0.79±0.62 mg/dL. GOT level of all neonates was 61.62±49.56 U/L. GPT level was 36.28±47.29 U/L. and mean CRP level of all neonates was 83.25±91.63 mg/L.

Table 1: Description and Biochemical parameters of neonatal sepsis.

Parameters	Mean ± S.D.
Gestational Age(weeks)	34.56±5.12
Birth weight(grams)	2510±1070
1 min APGAR score(102)	7.64±3.36
5 min APGAR score (102)	8.75±2.64
Temperature at blood collection (°C, 102)	35.85±0.54
Urea (mg/dL)	55.86±40.17
Creatinine (mg/dL)	0.81±0.21
Total Bilirubin (mg/dL)	8.12±5.24
Direct Bilirubin (mg/dL)	0.79±0.62
GOT (U/L)	61.62±49.56
GPT (U/L)	36.28±47.29
CRP (mg/L)	8.32±9.16

Out of 102 isolates, Gram-negative bacilli and Gram-positive cocci were 66/102(64.71%), 36/102(35.29%) respectively. Klebsiella spp. and coagulase-negative staphylococci (CONS) were the most common Gram-negative and Gram-positive organisms respectively.

Table 2: Distribution of isolated organisms.

Organism	Frequency of isolation (%)	Early onset	Late onset
Klebsiella	34(33.33%)	24(30.77%)	10(41.67%)
Coagulase-negative staphylococci	31(30.39%)	23(29.49%)	8(33.33%)
Acinetobacter	10(9.80%)	7(8.97%)	3(12.5%)
Staphylococcus aureus	8(7.84%)	7(8.97%)	1(4.17%)
Citrobacter	4(3.92%)	4(5.13%)	0
Others (Escherichia coli, Pseudomonas, Candida, Enterococcus, and Streptococcus)	15(14.71%)	13(16.67%)	2(8.33%)
Total	102(100%)	78(76.47%)	24(23.53%)

Highest sensitivity among Gram-negative isolates was to imipenem (96.83%), followed by amikacin (51.52%) and netilmicin (40.35%).

Table 3: Antibiotic susceptibility of Gram-negative organisms.

Antibiotics	Resistant (%)	Sensitive (%)
Ampicillin	63(95.45%)	3(4.55%)
Amoxiclav	61(93.84%)	4(6.15%)
Sparfloxacin	59(90.77%)	6(9.23%)
Cefuroxime	38(58.46%)	27(41.54%)
Tetracycline	44(65.67%)	23(34.33%)
Gentamicin	45(68.18%)	21(31.82%)
Co-trimoxazole	54(88.52%)	7(11.47%)
Ciprofloxacin	41(64.06%)	23(35.94%)
Cephalexin	57(91.93%)	5(8.06%)
Amikacin	32(48.48%)	34(51.52%)
Lomefloxacin	56(91.80%)	5(8.19%)
Ofloxacin	41(66.13%)	21(33.87%)
Netilmicin	34(59.65%)	23(40.35%)
Imipenem	2(3.17%)	61(96.83%)

Gram-positive isolates had the highest sensitivity of 94% to linezolid, 69.39% to tetracycline, 65.31% to piperacillin/ tazobactam, and 54.17% to ciprofloxacin.

Table 4: Antibiotic susceptibility of Gram-positive organisms.

Antibiotics	Resistant (%)	Sensitive (%)
Penicillin	46(90.19%)	5(9.81%)
Erythromycin	32(66.67%)	16(33.33%)
Tetracycline	15(30.61%)	34(69.39%)
Cephalexin	34(73.91%)	12(26.09%)
Cloxacillin	40(86.96%)	6(13.04%)
Pefloxacin	31(68.89%)	14(31.11%)
Piperacillin/tazobactam	17(34.69%)	32(65.31%)
Cefoperazon	30(63.83%)	17(36.17%)
Gentamicin	25(56.82%)	19(43.18%)
Ciprofloxacin	22(45.83%)	26(54.17%)
Amoxiclav	38(79.17%)	10(20.83%)
Cefuroxime	32(66.67%)	16(33.33%)
Azithromycin	29(70.73%)	12(29.27%)
Linezolid	03(6%)	47(94%)

Discussions

Neonatal sepsis is defined as a life-threatening bloodstream infection that occurs within the first 4 weeks after birth [13]. In developing countries, it represents a significant cause of illness and death among newborns [14] and requires intense management due to multi-system affection [15].

In the present study, 0ut of the 546 clinically suspected cases of sepsis, 102 neonates were culture positive with a blood culture positivity rate of 18.68%. The incidence of Gram-negative and Gram-positive organisms was 64.71% and 35.29.3%, respectively. There were 76.47% isolates from early onset septicemia cases, while 23.53% were from late-onset illness.

In the present study, a male predominance with male-to-female ratio was 1.75:1, which agrees with previous reports. This might be because of the importance given to the male infants and also because of more number of male infants born compared to female infants born. Culture-positivity for aerobic organisms in neonates vary from 25% to 60% [16,17]. In the present study, blood culture-positivity rate was 18.68%. This finding was comparable with other reports.[18] However, a high blood culture-positivity rate in septicemic children (56%) had been reported by Sharma et al.[19] and Jain et al.[20].

A low blood culture isolation rate could be due to administration of antibiotic before blood collection from the primary centers or the possibility of infection with anaerobes. A negative blood culture does not exclude sepsis and about 26% of all neonatal sepsis could be due to anaerobes [18].

The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. Overall, Gram-negative organisms are more common and are mainly represented by Klebsiella, Escherichia coli, Pseudomonas, and Salmonella. Of the Gram-positive organisms, Staphylococcus aureus, CONS, Streptococcus pneumonia, and S. pyogenes are most commonly isolated [21].

In the present study, Gram-negative and Gram-positive septicemia was encountered in 64.71% and 35.29% of the culture-positive cases, which was comparable to a study conducted by Agnihotri et al., 22] which reported that Gram-negative and Gram-positive organisms were responsible for 59% and 41% of the septicemia cases, respectively. Similar observations were made by other workers[23,24].

The report of the National Neonatal-Perinatal database showed Klebsiella as the predominant (29%) pathogen [16] .

In the present study, Klebsiella spp.(33.33%) was the predominant Gram-negative species isolated, which agrees with previous reports [25,18]. Out of total 102 cases of neonatal sepsis, 98 (76.47%) were early-onset sepsis, which was comparable to previous studies [22,24].

Antibiotic resistance is today a global problem. Reports of multi-resistant bacteria causing neonatal sepsis in developing countries are increasing. The wide availability of over-the-counter antibiotics and the inappropriate use of broad-spectrum antibiotics in the community may explain this situation. It is difficult to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable [21].

Antibiotic susceptibility pattern was studied for all isolates causing neonatal sepsis. The analysis of drug resistance pattern showed that, among Gram-negative isolates, maximum numbers (97%) were resistant to ampicillin and lowest to imipenem (7%).

Resistance was observed to be against commonly used antibiotics such as ampicillin, amoxiclav, cephalexin, and co-trimoxazole. Among Gram-positive isolates, high resistance was seen to penicillin (90%), cloxacillin (84%), and amoxiclav (76%). Least resistance was seen to linezolid (9%), followed by tetracycline (32%), and piperacillin/tazobactam (36%). The greater prevalence of resistance to commonly used antibiotics has also been reported by other studies [25,23]. Among aminoglycosides, amikacin was found to have an edge over netilmicin and gentamicin in Gram-negative septicemia, with sensitivity of 52%, 41%, and 33%, respectively. Similar observations have been made by previous group of workers [26].

In the present study, maximum sensitivity (96.83%) was observed in imipenem and linezolid (94%). Sensitivity to imipenem and linezolid was much higher than that to other antibiotics, but these two drugs should not be used indiscriminately and be kept as a reserve drug, otherwise resistance to these drugs may develop, thereby threatening the treatment.

CRP is an acute phase reactant, a protein synthesized and secreted by the liver in response to inflammatory cytokines, specifically IL-6[27] and is commonly used for bacterial sepsis detection in neonates. Still it is not useful as an early phase infection marker and it lacks specificity [28]. All neonates in our study had a high CRP level, the mean was 8.21 mg/dl. In their study, Zhou et al. [29] have found a CRP level >0.8 mg/dl in neonates (39.1%) with positive blood culture results and 45.3% of them died within 7 days after birth, a higher prevalence than us (20.96%) [29]. Also, Mannan et al. (2010) [30] found that CRP was raised in 72% of cases of neonates with positive blood culture and only in 4% of control cases, and their study concluded that CRP is the most sensitive method (93%) in culture proven sepsis, 79% in suspected sepsis and its positive predictive value in suspected sepsis amounts to 88%. Hofer et al. (2012) [31] found that a growing body of evidence suggests a link between GA and CRP kinetics with lower baseline CRP values and a lower CRP response to infection in preterm compared to term newborns. All correlations between all independent variables that we studied (1 and 5 min Apgar score, GA, BW) and CRP are negatively correlated, a high value of independent variable is associated with a low CRP value, but it is not statistically significant. Hofer et al. (2012) [31] concluded that CRP has the best diagnostic accuracy when combined with another infection marker like PCT, IL-6, and IL-8, that provides a higher sensitivity during the early phases of sepsis.

Conclusion

The present study concluded that the Klebsiella spp. and coagulase-negative staphylococci (CONS) are the most common organisms isolates in neonatal sepsis. Highest sensitivity among Gram-negative isolates was to imipenem followed by amikacin and netilmicin and gram-positive isolates are to linezolid followed by tetracycline, piperacillin/ tazobactam and ciprofloxacin. The gold standard for diagnostic sepsis is blood culture and the CRP is also particularly useful for monitoring the response to treatment and guiding antibiotic therapy. Hence, the elevated level of CRP concentrations is indicator of sepsis.

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