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A STUDY OF MICROBIOLOGICAL PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN OF NEONATAL SEPTICEMIA IN NICU OF A TERTIARY CARE HOSPITAL IN WESTERN RAJASTHAN, INDIA

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ABSTRACT **Background:** Blood stream infections can lead to life-threatening sepsis and require rapid diagnosis and antimicrobial treatment. The objective of this study was to isolate pathogenic bacteria in neonatal septicaemia cases and study their antibiogram.**Materials and Methods:** 240 blood samples collected from clinically suspected cases of neonatal septicaemia obtained from Umaid Hospital, Dr S N Medical College, Jodhpur (Jan 24 to April 24) were studied by BACTEC automated blood culture systems and were identified as per standard phenotypic methods and antibiotic susceptibility was done by Kirby Bauer disc diffusion method (as per CLSI guidelines). **Result:** 92 (38.33%) positive blood cultures were obtained amongst 240 blood culture samples. Amongst these, 67 (72.83%) GNB, 24 (26.08%) GPC and 1(1.09%) Candida isolate was obtained. The predominant GNB isolated was Klebsiella (n=44) followed by Escherichia coli (n=9)and Acinetobacter(n=8), Citrobacter(3) and Pseudomonas (n=3).The most common pathogenic GPC isolated was Staphylococcus aureus (n=6) followed by Enterococcus spp (n=3) and CONS (n=15).Amongst GNB, Meropenem showed highest susceptibility (89%) followed by piperacillin-tazobactam(87.4%) and GPC showed 100% sensitivity to Linezolid and Vancomycin.**Conclusion:** Rapid isolation of pathogens by automated blood culture system provides early and appropriate treatment to seriously ill neonates leading to reduced mortality and duration of hospital stay.

KEYWORDS : Neonatal Septicaemia, Blood Culture, Bactec, Antibiotic Resistance

INTRODUCTION

Neonatal septicemia is responsible for approximately 25% of neonatal deaths worldwide, with the majority occurring in developing countries.[1,2]

The increasing prevalence of extended-spectrum beta-lactamase (ESBL)-producing organisms, methicillin-resistant Staphylococcus aureus (MRSA), and multidrug-resistant (MDR) strains is a major concern in Neonatal Intensive Care Units (NICUs) worldwide. Neonatal septicemia is broadly classified into early-onset sepsis (EOS) and late-onset sepsis (LOS).[3]

The microorganisms commonly associated with EOS include Group B Streptococcus (GBS), Escherichia coli, coagulase-negative staphylococci (CoNS), Haemophilus influenzae, and Listeria monocytogenes.[4]

In contrast, LOS is commonly caused by CoNS, Staphylococcus aureus, Escherichia coli, Klebsiella spp., Pseudomonas spp., Enterobacter spp., Candida spp., GBS, Serratia spp., Acinetobacter spp., and anaerobic organisms. Recent trends indicate an increase in infections caused by CoNS. Knowledge of the local bacteriological profile and antimicrobial susceptibility patterns is essential for selecting appropriate empirical antimicrobial therapy and improving outcomes among neonates with septicemia.[5]

Although extensive research on neonatal septicemia is available worldwide and in India, regional variations in the causative organisms and antimicrobial susceptibility patterns necessitate periodic local surveillance. Therefore, the present prospective cross-sectional study was undertaken to determine the microbiological profile of neonatal septicemia and assess the antimicrobial susceptibility pattern of isolates recovered from the NICU of a tertiary care hospital in Western Rajasthan.

Neonatal sepsis is a systemic inflammatory condition of bacterial, viral, or fungal origin occurring within the first 28 days of life and is associated with hemodynamic changes and clinical manifestations. It remains one of the leading causes of neonatal morbidity and mortality. Based on the age at onset, neonatal sepsis is classified as early-onset sepsis, occurring within the first 72 hours of life, and late-onset sepsis, occurring after 72 hours but within the first 28 days of life. Organisms acquired before or during delivery, including those associated with maternal–fetal infection, are mainly responsible for EOS, whereas organisms acquired after delivery from environmental, nosocomial, or community sources are generally responsible for LOS.[6]

According to the World Health Organization, approximately 1.6 million neonatal deaths occur globally every year, with a substantial proportion occurring in developing countries. Important risk factors associated with neonatal septicemia include premature rupture of membranes, prolonged rupture of membranes, prematurity, maternal urinary tract infection, poor maternal nutrition, low birth weight, birth asphyxia, and congenital anomalies. Various diagnostic investigations, including C-reactive protein (CRP), complete blood count (CBC), platelet count, and erythrocyte sedimentation rate (ESR), may support the diagnosis; however, blood culture remains the gold standard for the definitive diagnosis of neonatal septicemia. Epidemiological differences exist among regions and countries with regard to the incidence, risk factors, microbial profile, and antimicrobial susceptibility patterns of neonatal sepsis.[7]

AIM AND OBJECTIVES

Aim

To determine the microbiological profile and antimicrobial susceptibility pattern of isolates obtained from clinically suspected cases of neonatal septicemia admitted to the NICU of a tertiary care hospital in Western Rajasthan.

Objectives

To determine the blood culture positivity rate among clinically suspected cases of neonatal septicemia.

To identify the bacterial and fungal pathogens isolated from neonatal blood cultures.

To determine the antimicrobial susceptibility pattern of the bacterial isolates.

To provide local antibiogram data that may assist clinicians in selecting appropriate empirical antimicrobial therapy.

MATERIALS AND METHODS

Study Design: Prospective cross-sectional study.
Study Setting: Department of Microbiology, Umaid Hospital, Dr. S. N. Medical College, Jodhpur, Rajasthan, India.
Study Period: January 2024 to April 2024.

Sample Size: A total of 240 blood samples from clinically suspected cases of neonatal septicemia.

Inclusion Criteria:
Neonates with clinical features suggestive of systemic inflammatory response syndrome (SIRS) and no localizing source of infection.

Neonates presenting with poor activity, fever, refusal of feeds, lethargy, tachypnea, tachycardia, birth asphyxia, prematurity, or low birth weight.

Neonates delivered following premature rupture of membranes (PROM) or in the presence of foul-smelling liquor.

Exclusion Criteria:
Neonates with extreme prematurity (gestational age <30 weeks).
Neonates with birth weight <1000 g.
Neonates with gross congenital anomalies.
Neonates who had received antibiotics before admission.

Sample collection and processing:
The present study was carried out between January 2024 to April 2024 in the Department of Microbiology, Umaid Hospital, Dr S N Medical College, Jodhpur. Blood for culture was collected from 240 clinically diagnosed septicemia cases following strict aseptic Precautions. Two milliliter blood was collected and inoculated into BD BACTEC bottle. The BACTEC culture bottles were incubated in bactec machine and those bottle which was positive identified by machine and give indication to remove bottle from the machine. Then subculture was done onto Mac Conkey's agar, blood agar and Hi chrome agar. The growth obtained was identified by conventional biochemical tests like catalase test, coagulase test, oxidase test, tripal sugar iron agar test, citrate test, urease test, indole test etc.

Antibiotic susceptibility test:
The standard disk diffusion test for susceptibility to routine antibiotics was done by modified Kirby-Bauer method. Zone sizes were measured and interpreted according to CLSI standards. The drugs for disc diffusion testing(gram positive organisms) were in the following concentrations: Erythromycin(15µg), Amoxicillin/Clavulanic acid (30µg), Clindamycin (2µg), Ampicillin(10µg), Amikacin (30µg), Gentamicin (10µg), Vancomycin (30µg), Ciprofloxacin (5µg), Cefuroxime (30µg), Linezolid (30µg), Piperacillin/Tazobactam (110µg).

The drugs for disc diffusion testing (gram negative organisms) were in the following concentrations: Ampicillin10(µg), Amikacin (30µg), Gentamicin (10µg), Trimethoprim/Sulfamethoxazole (25µg), Cefotaxime (30µg),Ceftazidime (30µg), Meropenem (10µg), Amoxicillin /Clavulanic acid (30µg), Cefuroxime (30µg), Ciprofloxacin (5µg), Levofloxacin(5µg), Piperacillin/Tazobactam (110µg).

RESULTS

Out of 240 blood culture samples processed, 92 (38.33%) yielded positive blood culture growth, while 148 (61.67%) remained sterile. Among the 92 positive cultures, Gram-negative bacilli (GNB) were the predominant isolates accounting for 67 (72.83%) isolates, followed by Gram-positive cocci (GPC) with 24 (26.08%) isolates and Candida species with 1 (1.09%) isolate. The predominant Gram-negative isolate was *Klebsiella spp.* (n=44), followed by Escherichia coli (n=9), *Acinetobacter spp*. (n=8), *Citrobacter spp.* (n=3) and Pseudomonas spp. (n=3). Among Gram-positive cocci, coagulase-negative *staphylococci (CoNS)* were the most common isolates (n=15), followed by *Staphylococcus aureus* (n=6) and *Enterococcus spp*. (n=3). With regard to antimicrobial susceptibility, meropenem showed the highest sensitivity among GNB (89%), followed by piperacillin/tazobactam (87.4%) and levofloxacin (84.5%). Among GPC isolates, vancomycin and linezolid showed 100% sensitivity, followed by amikacin (95.7%).

Table 4.1: Distribution of blood culture results (n = 240)

Category	Number
Total blood samples	240
Positive blood cultures	92
Gram-negative bacilli (GNB)	67
Gram-positive cocci (GPC)	24
Candida/Yeast	1
Sterile cultures	148

A total of 240 blood culture samples were processed. Of these, 92 samples were culture positive and 148 were sterile. Gram-negative bacilli constituted the major group of positive isolates, followed by Gram-positive cocci, while Candida accounted for only one isolate.

Table 4.2: Antimicrobial susceptibility pattern of Gram-negative bacilli (n = 67)

Antibiotic	Resistant (%)	Sensitive (%)
Meropenem	11	89
Piperacillin/Tazobactam	12.6	87.4
Levofloxacin	15.5	84.5
Ampicillin	94	6
Gentamicin	68	22
Trimethoprim/Sulfamethoxazole	26.7	73.3
Cefotaxime	78.9	21.1
Ceftazidime	77.4	22.6
Amoxicillin/Clavulanic acid	84.9	15.1
Cefuroxime	77.5	22.5
Ciprofloxacin	76.9	23.1
Amikacin	73.7	22.3

Meropenem showed the highest sensitivity (89%) against Gram-negative isolates, followed by piperacillin/tazobactam (87.4%) and levofloxacin (84.5%). High resistance was observed against ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime and cefuroxime.

Table 4.3: Antimicrobial susceptibility pattern of Gram-positive cocci (n = 24)

Antibiotic	Resistant (%)	Sensitive (%)
Vancomycin	0	100
Linezolid	0	100
Amikacin	4.3	95.7
Amoxicillin/Clavulanic acid	46.4	53.6
Ciprofloxacin	57.4	42.6
Gentamicin	17	83
Piperacillin/Tazobactam	43.9	56.1
Erythromycin	80.6	19.4

Vancomycin and linezolid showed 100% sensitivity against Gram-positive cocci, followed by amikacin (95.7%). Erythromycin and ciprofloxacin showed relatively high resistance compared with other antibiotics.

Table 4.4: Distribution of Gram-negative bacterial isolates (n = 67)

Organism	Number
Klebsiella spp.	44
Escherichia coli	9
Acinetobacter spp.	8
Citrobacter spp.	3
Pseudomonas spp.	3

Klebsiella spp. was the predominant Gram-negative isolate recovered from blood cultures, followed by Escherichia coli and Acinetobacter spp. Citrobacter spp. and Pseudomonas spp. were isolated in smaller numbers.

Table 4.5: Distribution of Gram-positive coccal isolates (n = 24)

Organism	Number
Coagulase-negative staphylococci (CoNS)	15
Staphylococcus aureus	6
Enterococcus spp.	3

Coagulase-negative staphylococci (CoNS) were the most common Gram-positive cocci isolated, followed by Staphylococcus aureus and Enterococcus spp.

Overall, Gram-negative bacilli were the major pathogens isolated from bloodstream infections in the present study. Meropenem and piperacillin/tazobactam retained comparatively good activity against Gram-negative isolates, whereas vancomycin and linezolid remained highly effective against Gram-positive cocci. These findings indicate a substantial burden of antimicrobial resistance, especially among Gram-negative isolates, and emphasize the need for regular antibiogram surveillance to guide empirical therapy

Figure 4.1: Distribution of blood culture results

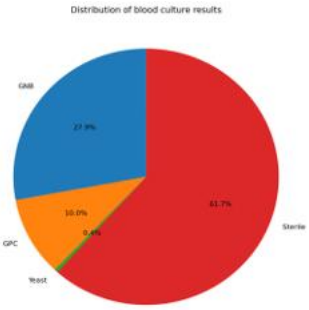


Figure 4.2: Sensitivity pattern of Gram-negative bacilli

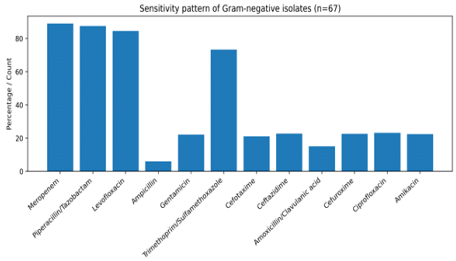


Figure 4.3: Sensitivity pattern of Gram-positive cocci

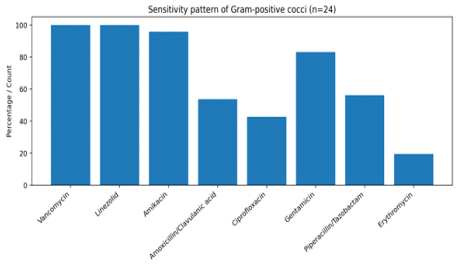


Figure 4.4: Distribution of Gram-negative isolates

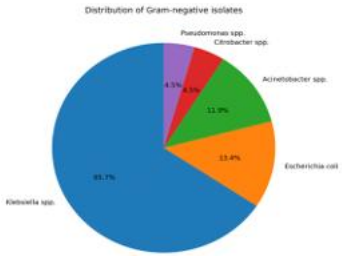
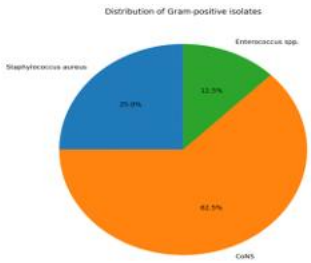


Figure 4.5: Distribution of Gram-positive isolates



DISCUSSION

Bloodstream infections remain an important cause of morbidity and mortality in hospitalized patients, particularly in critically ill individuals, neonates and patients with prolonged hospital stay. Timely blood culture processing and knowledge of local antimicrobial susceptibility patterns are therefore essential for appropriate management. In the present study, 92 out of 240 blood culture samples were positive, giving a positivity rate of 38.33%. This rate is somewhat higher than that reported by Nazir et al.[8] and Vasudeva et al.[9], who observed lower positivity rates in their respective studies. Such variation may be explained by differences in patient population, severity of illness, prior antibiotic exposure, blood culture practices and the proportion of hospital-acquired infections included in the study.

A striking finding of the present study was the predominance of Gram-negative bacilli, which accounted for 72.83% of all positive blood cultures. This suggests that Gram-negative septicemia continues to be a major problem in the present hospital setting. Similar findings have been reported in Indian studies where Gram-negative organisms formed the major bulk of bloodstream isolates.[9,10] The predominance of Gram-negative organisms in the present study may be attributed to the increasing burden of hospital-acquired infections, invasive procedures, device-associated infections and selective antibiotic pressure favoring resistant Gram-negative bacilli.

Among the Gram-negative isolates, Klebsiella spp. emerged as the predominant pathogen, followed by Escherichia coli and Acinetobacter spp. The predominance of Klebsiella is clinically significant because it is a well-recognized cause of healthcare-associated bloodstream infection and is often associated with multidrug resistance. Similar observations have been documented in Indian studies where Klebsiella and Escherichia coli were among the leading bloodstream pathogens.[9,11] However, some studies such as that by Nazir et al.[8] found Acinetobacter spp. to be more frequent, highlighting that the bacterial spectrum of bloodstream infection is strongly influenced by local epidemiology, patient profile and institutional infection control practices.

Among Gram-positive cocci, coagulase-negative staphylococci were the most common isolates, followed by Staphylococcus aureus and Enterococcus spp. This pattern is in agreement with the study by Nazir et al.[8], where coagulase-negative staphylococci were also the predominant Gram-positive isolate. CoNS are increasingly recognized as important pathogens in catheter-related bloodstream infection, neonatal sepsis and infections associated with prolonged hospitalization. At the same time, they may also represent skin contaminants during venepuncture. Therefore, their clinical significance should always be interpreted in relation to the patient's symptoms, repeat culture positivity and the presence of intravascular devices.

The antimicrobial susceptibility pattern of Gram-negative bacilli in the present study showed that meropenem was the most effective drug, with 89% sensitivity, followed by piperacillin/tazobactam and levofloxacin. In contrast, high resistance was observed against ampicillin, amoxicillin/clavulanic acid and third-generation cephalosporins. This finding is broadly in line with previous Indian studies that have reported poor susceptibility of Gram-negative bloodstream isolates to commonly used beta-lactams and cephalosporins, with better activity retained by carbapenems and higher-end combination drugs.[9,10] The high resistance to cephalosporins observed in the present study may reflect widespread empirical use of these antibiotics and the growing prevalence of extended-spectrum beta-lactamase-producing Enterobacterales.

The good activity of meropenem against Gram-negative isolates in the present study is encouraging, as it suggests that carbapenems still retain useful efficacy in this setting. However, this finding must be interpreted with caution because carbapenem resistance among Klebsiella pneumoniae and other Enterobacterales is being increasingly reported from India.[12,13] Manesh et al.[13] have highlighted the growing challenge posed by carbapenem-resistant Klebsiella bloodstream infections in South India. Therefore, although meropenem remains an important option for severe Gram-negative sepsis, its use should be carefully guided by culture reports and antimicrobial stewardship principles to avoid further resistance selection.

Another notable observation in the present study was the high sensitivity of Gram-negative isolates to piperacillin/tazobactam. This suggests that piperacillin/tazobactam may still serve as a useful carbapenem-sparing option in selected cases, provided susceptibility is known. Similar observations have been made in hospital-based Indian studies where beta-lactam/beta-lactamase inhibitor combinations retained activity against a proportion of bloodstream isolates despite widespread resistance to cephalosporins.[9,10] However, because local resistance trends change over time, empirical therapy should always be based on the institutional antibiogram rather than generalized assumptions.

With regard to Gram-positive cocci, vancomycin and linezolid showed 100% sensitivity in the present study, while amikacin also retained very good activity. This is consistent with previous Indian studies where vancomycin and linezolid remained the most effective agents against Gram-positive bloodstream isolates.[9,10] The preservation of

sensitivity to these agents is reassuring because they remain key drugs for the treatment of serious infections caused by resistant staphylococci and enterococci. Nevertheless, their use should be reserved for appropriate clinical situations to minimize the emergence of vancomycin- or linezolid-resistant organisms in future.

The present study also showed relatively high resistance among Gram-positive isolates to erythromycin and ciprofloxacin. This finding indicates that older commonly used agents may no longer be reliable for empirical coverage of suspected Gram-positive bloodstream infection. Similar resistance trends have been noted in earlier studies, where staphylococci isolated from blood cultures often showed reduced susceptibility to macrolides and fluoroquinolones while retaining sensitivity to vancomycin and linezolid.[8,9] These findings reinforce the need for routine susceptibility testing of all clinically significant blood culture isolates.

Candida species accounted for only one isolate in the present study, representing 1.09% of all positive blood cultures. This suggests that fungal bloodstream infection contributed only a small fraction of the overall septicemia burden in the study population. Although the proportion is low, candidemia remains clinically important because it is associated with prolonged hospitalization, broad-spectrum antibiotic use, central venous catheterization and high mortality. The low isolation rate in the present study may reflect differences in patient population, lower numbers of immunocompromised patients or lower prevalence of ICU-related fungal bloodstream infection compared with some tertiary care centres.

Taken together, the findings of the present study indicate that bloodstream infections in the study setting are predominantly caused by Gram-negative bacilli, especially Klebsiella spp., and are associated with substantial resistance to commonly used antibiotics. The predominance of Gram-negative pathogens, coupled with high resistance to ampicillin and cephalosporins, suggests that empirical treatment of septicemia in this hospital should be chosen carefully and should be guided as far as possible by the local antibiogram. At the same time, the preserved activity of meropenem, piperacillin/ tazobactam, vancomycin and linezolid provides important therapeutic guidance for clinicians managing severe bloodstream infections.

The present study therefore underlines the need for regular blood culture surveillance, periodic updating of hospital antibiograms, strict infection control practices and rational antimicrobial stewardship. Such measures are necessary not only to improve patient outcomes but also to limit the further emergence and spread of multidrug-resistant bloodstream pathogens. Overall, the findings of this study are broadly comparable with previously published Indian data, while also reflecting the specific microbiological profile and antimicrobial susceptibility pattern of the present institution.

CONCLUSION

Gram-negative bacilli, particularly Klebsiella spp., were the predominant pathogens causing neonatal septicemia. Meropenem and piperacillin/ tazobactam showed the highest activity against Gram-negative isolates, whereas vancomycin and linezolid showed 100% sensitivity against Gram-positive cocci. Early blood culture and antimicrobial susceptibility testing are essential for appropriate therapy. Periodic updating of the institutional antibiogram, strict infection-control practices and antimicrobial stewardship are recommended to limit the emergence of multidrug-resistant organisms.

LIMITATIONS

This was a single-centre study conducted over a limited four-month period; therefore, the findings may not be generalizable to other institutions or geographical regions. Clinical outcome data, detailed neonatal risk factors, differentiation between early- and late-onset sepsis, and molecular characterization of antimicrobial resistance mechanisms were not assessed. The small number of some individual isolates also limited organism-specific interpretation of antimicrobial susceptibility patterns.

RECOMMENDATIONS

Periodic surveillance of the microbiological profile and regular updating of the institutional antibiogram are recommended. Empirical antimicrobial therapy should be guided by local susceptibility data and modified according to individual culture and sensitivity results. Strict infection-prevention practices, rational antibiotic use, and effective antimicrobial stewardship should be strengthened to reduce the emergence and spread of multidrug-resistant organisms.

FUNDING

Nil.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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