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RESEARCH ARTICLE

CLINICO-BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NEONATAL SEPTICEMIA IN A TERTIARY CARE CENTRE IN RAJASTHAN

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ABSTRACT

Background: Neonatal septicaemia in India is a major cause of morbidity and mortality worldwide, particularly in the developing countries. Early diagnosis and appropriate antimicrobial therapy for these cases are essential for improving outcomes. **Objectives:** To evaluate the clinico-bacteriological profile and antimicrobial susceptibility pattern of neonatal septicemia among neonates admitted to a tertiary care hospital. **Methods:** This hospital-based prospective observational study was conducted over one year in the NICU of Jhalawar Medical College, Jhalawar. A total of 250 neonates with clinically suspected sepsis were included. Blood cultures were performed, and isolates were identified using standard microbiological techniques. Antibiotic susceptibility testing was done according to CLSI guidelines. **Results:** Out of 250 suspected cases, 115 (46%) were culture-positive. *Staphylococcus aureus* was the most common isolate (36.52%), followed by *Acinetobacter* species (17.39%). MRSA constituted a significant proportion. Prematurity and PROM were significantly associated with culture-positive sepsis ($p < 0.05$). Respiratory distress was the most common clinical presentation. High resistance was observed against commonly used antibiotics, while Linezolid, carbapenems, and Piperacillin–Tazobactam showed better sensitivity. **Conclusion:** Neonatal septicaemia is increasingly associated with multidrug-resistant organisms. Local antibiogram-based antibiotic policies and strict infection control practices are essential to reduce neonatal morbidity and mortality.

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INTRODUCTION

Neonatal sepsis is one of the most serious and life-threatening conditions affecting newborns worldwide. Despite remarkable advances in obstetric care, neonatal resuscitation, antimicrobial therapy, and improvements in neonatal intensive care technology, septicaemia continues to be a leading cause of neonatal morbidity and mortality, particularly in developing countries (1). The neonatal period, defined as the first 28 days of life, is characterized by an immature immune system, making neonates highly vulnerable to infections. Neonatal sepsis is defined as a clinical syndrome characterized by systemic signs of infection with or without isolation of a pathogen from sterile body fluids (2). Based on the onset of illness, neonatal sepsis is classified into: Early-onset sepsis (EOS within 72 hours of life), and Late-onset sepsis (LOS after 72 hours). Globally, neonatal infections account for nearly one-third of neonatal deaths. The incidence of neonatal sepsis varies widely, ranging from 1–5 per 1000 live births in developed countries to 7–38 per 1000 live births in developing regions. In India, neonatal sepsis continues to be a major cause of NICU admissions and mortality (3). The bacteriological profile of neonatal septicaemia varies geographically. Group B Streptococcus predominates is more prevalent in developed countries while Gram-positive organisms such as *Staphylococcus aureus* and Gram-negative bacilli are more common in developing settings (2). Increasing antimicrobial resistance has further complicated management. Therefore, region-specific data on causative organisms and antibiotic sensitivity patterns are crucial for guiding empirical therapy. (4) This study was conducted to evaluate the clinico-bacteriological profile and antimicrobial susceptibility pattern of neonatal septicemia in a tertiary care center in Rajasthan, where limited data is available.The research question of this study was "What is the clinico-bacteriological profile and antimicrobial susceptibility pattern of neonatal septicaemia among neonates admitted to the NICU of Jhalawar Medical College and Hospital ?"

AIM: To study the clinic-bacteriological profile and antimicrobial susceptibility pattern of neonatal septicaemia among neonates admitted to the NICU.

PRIMARY OBJECTIVE

To determine the bacteriological profile of neonatal septicaemia in neonates admitted in NICU.

SECONDARY OBJECTIVE

- To isolate the causative bacteria from patient of neonatal septicaemia by aerobic incubation.
- To determine the antibiotic susceptibility pattern of bacteria.
- To analyse and assess the clinical presentation of neonatal septicaemia.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Microbiology in collaboration with the NICU at Jhalawar Medical College, Jhalawar over a period of one year.

Study Population: A total of 250 neonates with clinical suspicion of septicaemia were included in the study. Neonates presenting with signs and symptoms suggestive of sepsis were enrolled after obtaining informed consent.

Inclusion Criteria: Clinically suspected neonates with at least one of the following sign and symptom were included in study. Eg. Abnormalities of body temperature, either exceeding 99°F or falling below 95°F, Increased respiratory rate, defined as more than 60 breaths per minute, Poor feeding, irritability or reduced activity, T Respiratory rate more than 60 per minute, apnea, cyanosis, abnormal cry and not accepting feed, Drowsy or unconscious or septic focus on skin or umbilicus. (1)

Exclusion Criteria: Neonates already on prolonged antibiotic therapy for more than 48 hours before admission.

SAMPLE SIZE CALCULATION

A study was conducted with a total of 250 neonates with clinical features suggestive of septicaemia who were enrolled in the study. Blood samples were obtained from all selected cases for further analysis. The sample size was calculated according to the prevalence of neonatal septicaemia. $n = t^2 \times P (1-P) / e^2$ (P was 8.5%).

Sample Collection and Processing: Blood samples were collected under aseptic precautions before initiation of antibiotic therapy. Approximately 1–2 mL of blood was inoculated into culture media and incubated aerobically. (6)

Sample Processing: The inoculated blood culture bottles were maintained at 37°C under humid conditions and were inspected after 24 hours for evidence of microbial growth, such as turbidity, discoloration, or clot formation. Than subculture was performed on Blood Agar and Mac Conkey agar, and a Gram stain smear was prepared. If no growth found in the initial subculture, the culture bottles were re-incubated and monitored twice daily for signs of growth up to the 5 day. A final subculture was performed on the fifth day of incubation using the same culture medium, along with repeat Gram staining. (6) bacteria was identified on the basis of microscopy , colony formation and biochemical reaction.

Antimicrobial Susceptibility Testing: Following isolation and definitive identification of the organisms, antimicrobial susceptibility testing was carried out using the Kirby–Bauer disk diffusion methodThe assay was performed on Mueller–Hinton agar employing commercially prepared antibiotic discs. A preselected panel of antimicrobial discs, comprising Doxycycline (30 µg) , Cefoxitin 30µg), Erythromycin (15 µg), Clindamycin (2 µg), Linezolid (30 µg), Levofloxacin(5 µg), Amoxycillin-clavulenic acid (20/10µg), Piperacillin-Tazobactam (100/10µg), Ceftriaxone(30µg),Meropenem (10 µg) Gentamycin (10µg), Imipenem (10µg), Tobramycin (10 µg), Ampicillin(10µg), gentamicin (10µg), cotrimoxazole (1.25/23.75µg), ciprofloxacin(5µg), cefotaxime (30µg), amikacin(30µg), and , Cefepime (30µg) , Ceftazidime(30 µg),Ceftazidime-Clavulunic (30/10 µg) acidwas applied to the surface of the inoculated Mueller–Hinton agar plates (7). The results were interpreted in accordance with CLSI guidelines and reported as susceptible, or resistant.

Controls used with each batch: Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923 (7)

Data Analysis: Clinical, laboratory, and microbiological data were recorded and analyzed statistically. Chi-square test was used to determine significance, and p-value < 0.05 was considered significant.

RESULTS

The study was performed during April 2025 to Jan 2026 at Department of Microbiology, Jhalawar medical college & Hospital. The study group included 250 cases of suspected neonatal septicemia. The blood samples were taken prior to antibiotic administration. Regarding the onset of sepsis, early-onset sepsis (EOS) was observed in 142 (56.8%) clinically suspected cases and 68 (59.1%) culture-confirmed cases. Late-onset sepsis (LOS) was seen in 108 (43.2%) suspected cases and 47 (40.9%) culture-positive cases. In terms of mode of delivery, 137 (54.8%) of clinically suspected cases were delivered by normal vaginal delivery (NVD) and 113 (45.2%) by lower segment caesarean section (LSCS). Among culture-positive cases, 70 (60.9%) were delivered by NVD and 45 (39.1%) by LSCS.

A statistically significant association was observed between gestational age and culture positivity ($p = 0.0238$), indicating that preterm neonates had a significantly higher proportion of culture-confirmed sepsis compared to term neonates. The association between PROM and culture positivity was found to be statistically significant ($p = 0.0191$), indicating that neonates born to mothers with PROM had a higher likelihood of developing culture-proven sepsis. Maternal fever or urinary tract infection (UTI) was documented in 23 (9.2%) of clinically suspected cases and in 14 (12.2%) of culture-positive cases. Although a slightly higher proportion of culture-confirmed cases had a history of maternal fever/UTI. A history of multiple per vaginal examinations was observed in 142 (56.8%) clinically suspected cases and 63 (54.7%) culture-positive cases. Hypoglycemia was documented in 106 (42.4%) clinically suspected cases and in 59 (51.3%) culture-positive cases,

Table 1. Demographic characteristics of suspected cases and culture positive sepsis cases

Variables		Clinical sepsis (n=250)		culture positive sepsis (n=115)		P value
		Freq(n)	Percent (%)	Freq(n)	Percent (%)	
Place of delivery	Outborn	195	78.00%	94	81.70%	0.4138
	Inborn	55	22%	21	18.30%	
Gender	Male	133	53.20%	68	59.10%	0.29
	Female	117	46.80%	47	40.90%	
Onset of sepsis	EOS	142	56.80%	68	59.10%	0.6756
	LOS	108	43.20%	47	40.90%	
Mode of delivery	LSCS	113	45.20%	45	39.10%	0.2769
	NVD	137	54.80%	70	60.90%	

Table 2. Description of neonatal characteristics between clinical suspected sepsis cases and culture-positive sepsis cases

Variables		Clinical sepsis (n=250)		culture positive sepsis (n=115)	
		Freq(n)	Percent(%)	Freq(n)	Percent(%)
Gestational age	Term	134	53.60%	47	41.90%
	Preterm	116	46.40%	68	59.10%
Birth weight	NBW	127	50.80%	61	53%
	LBW	123	49.20%	54	47%

Table 3. Description of maternal charicteristics between clinical suspected cases and culture positive sepsis cases

Variables		Clinical sepsis (n=250)		culture positive sepsis (n=115)		P value
		Freq(n)	Percent(%)	Freq(n)	Percent(%)	
PROM	Yes	117	46.8	69	60	0.0191
	No	133	53.2	46	40	
Maternal fever/UTI	Yes	23	9.2	14	12.2	0.1577
	No	194	77.6	96	83.5	
Multiple vaginal exam	Yes	142	56.8	63	54.7	0.1619
	No	71	28.4	21	18.2	

Table 4. Baseline distribution according to clinical presentationbetween clinical sepsis and culture positive sepsis

Variables	Clinical sepsis (n=250)		culture positive sepsis (n=115)	
	Freq (n)	Percent (%)	Freq (n)	Percent (%)
Hypothermia	89	35.6	59	51.3
Hypoglycemia	106	42.4	59	51.3
Respiratory distress	148	59.2	83	72.2
Refusal to feed	136	54.4	77	67
Fever	133	53.2	55	47.8
Seizures	32	12.8	22	19.1
Jaundice	38	15.2	21	18.3

Table 5. Distribution of culture positive cases according to their microbiologicalprofile

ORGANISM	FREQUENCY	PERCENTAGE
Staphylococcus aureus	42	36.52
Acinetobactersp	20	17.39
Klebsiellasp	19	16.52
Escherichia coli	8	6.96
Pseudomonas sp	4	3.48
Citrobactersp	4	3.48
CONS	4	3.48
MRSA	14	12.17
TOTAL	**115**	**100**

suggesting that it was relatively more frequent in neonates with microbiologically proven infection. A total of 115 culture-positive isolates were obtained from neonates with confirmed sepsis. The distribution of bacterial isolates is presented in the table. *Staphylococcus aureus* was the predominant pathogen, followed by *Acinetobacter baumannii* and *Klebsiella pneumoniae,* highlighting the significant contribution of both gram-positive and gram-negative organisms in neonatal sepsis in the present study. Good susceptibility was found for Linezolid and levofloxacin. Imipenem and meropenem, piperacillin tazobactam showed good sesitivity across most isolates.

Among aminoglycosides, tobramycin showed high sensitivity across organisms, followed by amikacin and gentamicin, although gentamicin demonstrated comparatively higher resistance rates. Fluoroquinolone (ciprofloxacin) susceptibility was variable, with a considerable proportion of isolates demonstrating resistance. Third generation cephalosporins such as ceftriaxone and ceftazidime showed higher resistance rates among most Gram-negative isolates.

DISCUSSION

In the present study out of 250 cases, 46 % were culture positive. This relatively high proportion reflect high burden of neonatal

Table 6. Antibiotic Susceptibility profile of Gram Positive organisms

Antibiotics	Staph aureus	MRSA
Doxycillin	30(71.4%)	7(50%)
Cefoxitine	42(100%)	1(7.1%)
Erythromycin	29(69%)	0
Cotrimoxazole	32(76%)	2(14%)
Clindamycin	28(66.7%)	3(21.4%)
Linezolid	40 (95.2%)	10(71.4%)
Levofloxacin	34(81%)	8(57.1%)

Table 7. Antibiotic susceptibility profile of Gram Negative Bacilli

Antibiotics	AcinetoBacter (n-20)	KLEBSIELLA (n-19)	E.COLI (n-8)
Cotrimoxazole	5(25%)	4(21%)	22(25%)
Ciprofloxacin	9(45%)	7(36.8%)	0(%)
Amoxiclav	6(30%)	4(21%)	6(75%)
Piperciline-Tazobactum	16(80%)	18(94%)	7(87.5%)
Ceftriaxone	4(20%)	2(10.5%)	7(87.5%)
Gentamycin	7(35%)	3(15.8%)	2(25%)
Imipenem	18(90%)	18(94.7%)	7(87%)
Tobramycin	15(75%)	17(89.5%)	8(100%)
Amikacin	7(35%)	16(84.7%)	6(75%)
Cefepime	6(30%)	6(31.6%)	2(25%)
Meropenam	16(80%)	17(89.5%)	8(100%)
Ceftazidime	13(65%)	12(63%)	7(87.5%)
Ceftazidime-Clavulanic acid	4(20%)	12(63%)	8(100%)

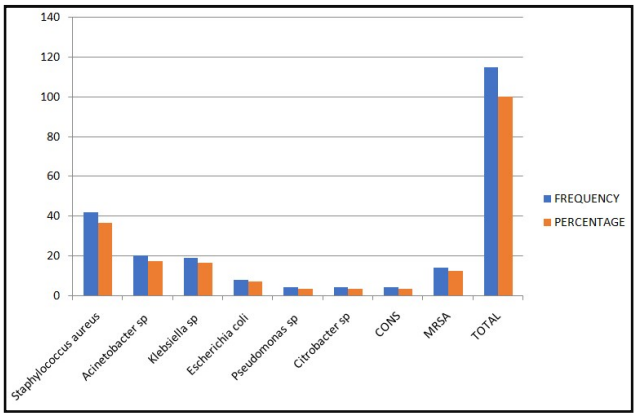

Fig. 1. Distribution of culture positive cases according to their microbiologicalprofile

infections in the study setting. A large proportion of neonates in this study were born outside the health care settings. While a slightly higher number of culture-positive cases were seen among these outborn neonates, this difference was not statistically meaningful. But still, this finding is important, as outborn baby may face delays in referral, suboptimal hygiene practices at birth, and additional risks during transportation, and all of these factors can contribute to infection vulnerability. In present study higher proportion of male neonates were observed although it is not statistically significant but in male infants are often brought to medical attention more promptly in many settings in most of rural areas (5). The early-onset cases of neonatal septicaemia were somewhat more frequent, this suggests that both maternal factors and postnatal environmental factors both contribute to high rate of neonatal infections. These findings reinforce the idea that neonatal sepsis does not depend on a single factor but rather arises from a complex interplay of multiple influences (8). In the present study one of the most important findings was the strong association between prematurity and culture-positive sepsis. Premature infants have underdeveloped immune systems, receive fewer maternal antibodies, and often require prolonged hospitalization with multiple interventions. All of these risk factors increases the susceptibility to infection. In contrast, low birth weight neonates did not show a significant relationship with culture positivity. This suggests that gestational age may be a more decisive factor than neonatal weight itself (9). Among maternal risk factors, premature rupture of membranes (PROM) is a important risk factor A notable number of neonates with confirmed sepsis had a history of PROM, highlighting its role in allowing pathogens to ascend and infect the fetus (10). The causative bacterial profile revealed a predominance of *Staphylococcus aureus*. It is a Gram-positive organism. When methicillin-resistant strains were included, this pattern raises concerns about hospital-acquired infections, especially in neonatal intensive care units where invasive procedures and close contact are common. It also suggests that infection control practices require continuous monitoring and strengthening. In present study MRSA show multidrug resistance, similar to Patel et al and Sharma et al. observed that Clindamycin resistance was rising among Staphylococcus isolates, likely due to inducible MLSB resistance. (11,12) This correlates with the relatively lower sensitivity to Clindamycin (66.7%) in the present study. Verma et al. reported high prevalence of MRSA in NICU settings, with multidrug resistance patterns similar to those observed in this study. These findings suggest that Linezolid is not a reserve drug anymore. (13) Karthikeyan & Premkumar, 2020 reported lower MRSA prevalence: ~25–30% in contrast to present study (66.6 %). (14) The high resistance to commonly used antibiotics underscores the challenge of managing MRSA infections in neonatal settings. Present study also reported the high prevalence of multidrug resistant organism in Gram negative bacilli. These organisms are well known for their ability to survive in hospital environments and develop resistance to multiple antibiotics. The high burden of Acinetobacter and klebsiella points toward a growing burden of healthcare-associated infections.

The antibiotic resistance patterns observed in this study is a cause for concern for health care settings. Gram-positive organisms, particularly *Staphylococcus aureus*, remained largely sensitive to drugs like Linezolid and Cefoxitin. However, resistance to commonly used antibiotics such as erythromycin and clindamycin was noticeable in this study. This indicates evolving resistance mechanisms. MRSA isolates, in particular, demonstrated resistance to multiple drug classes, leaving only a few reliable treatment options (4). High resistance to third-generation cephalosporins in Gram negative bacilli suggests widespread production of extended-spectrum beta-lactamases. While carbapenems and certain combination antibioticsshowed little effectiveness, early signs of resistance to these last-resort drugs are emerging. This emerging resistance trend reflects a broader shift toward multidrug-resistant infections, which significantly complicates treatment decisions (12). These findings are consistent with several Indian studies but contrast with data from developed countries, where antimicrobial resistance is comparatively lower. The results highlight the urgent need for antimicrobial stewardship and strict infection control practices in neonatal intensive care units. Similar to this study Viswanathan et al. (India) reported high resistance in Klebsiella and Acinetobacter (4). Sharma et al. says Increasing resistance to aminoglycosides and cephalosporins and Piperacillin–Tazobactam are effective to the organism 24. In contrast to result of this study an investigator from USA reported predominance of E. coli and Gram positive streptococcus, and better antibiotic sensitivity

and low MDR rates. Tsering et al.from India reported higher sensitivity to Gentamicin and Ceftriaxone and Lower resistance rates (15). The persistence of effectiveness of a few key antibiotics offers some reassurance, but it is not a long-term solution. Without careful use, these drugs too may lose their efficacy. Therefore, continuous monitoring of resistance patterns, rational prescribing practices, and regular updating of treatment guidelines are essential.

Limitation: Although the present study provides important data regarding the clinic-bacteriological profile and antimicrobial susceptibity pattern but it has some limitations it is single centre study based on hospital population which may limit the generalizability of findings. Microbial profile and resistance pattern vary across the global region. This study primarily focuses on aerobic bacterial isolates only, fungal pathogen and anaerobic bacteria were not studied.

CONCLUSION

In summary, this study highlights a significant burden of neonatal septicemia, with prematurity and PROM emerging as key risk factors. While demographic and perinatal characteristics showed certain trends, they did not independently predict culture-confirmed infection. The dominance of *Staphylococcus aureus,* along with multidrug-resistant Gram-negative organisms, underscores the growing impact of hospital-acquired infections. Improving infection control measures, ensuring early identification of vulnerable neonates, and promoting judicious use of antibiotics are critical steps toward better outcomes. Future studies using advanced analytical methods may help identify independent predictors more clearly and support targeted interventions in neonatal care.

Conflict of Interest: Nil

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