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Bacteriological Profile and Antibiotic Susceptibility Pattern in View of Neonatal Septicemia in a Tertiary Care Hospital in Western Maharashtra

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ABSTRACT

Introduction: Neonatal septicemia is a generalized bacterial infection that occurs during the first 4 weeks of life and is a leading cause of neonatal mortality in India. This study aims to determine the bacteriological profile and antibiotic sensitivity patterns of isolates from blood cultures of suspected septicemic neonates.
Material & Methods: Blood samples from 943 neonates suspected of sepsis from NICU of GMCH, Miraj, were enrolled in the study. Blood was collected with aseptic precaution. Subcultures were made on Blood agar and MacConkey agar plates every alternate day till 5th day of incubation. Organisms were followed according to standard microbiological techniques.
Results: Out of 943, 268 blood cultures of suspected cases, (28.41%) were culture positive. Coagulase negative Staphylococci (CONS) (34.32%) was the most common isolate followed by *Klebsiella species* (21.64%), *Enterococcus species* (14.55%) and *Citrobacter freundii* and Non-fermenter GNB 4.85% each. Linezolid was found to be 100% effective in CONS and *Enterococcus species.* Imipenem was found to be 100% effective in GNB isolates. All the Gram-negative bacteria were 100% resistant to Ampicillin. Also, all the GNB showed high resistance to 1st, 2nd, 3rd and 4th generation Cephalosporins.
Conclusion: The present study shows the predominance of CONS as the causative agent of neonatal sepsis followed by *Klebsiella species* suggesting the nosocomial infection. Antimicrobial resistance is emerging in bacteria causing neonatal septicemia. Strict antibiotic stewardship has to be practiced to prevent impending treatment failure.
Keywords: Septicemia, neonates, antimicrobial resistance, Coagulase negative Staphylococci

INTRODUCTION

"A clinical syndrome of bacteremia with systemic signs and symptoms of infection in the first four weeks of life" is called as neonatal septicemia (Sepsis neonatorum). [1] It comprises various systemic infections of the newborn such as meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections. [2] Globally, neonatal sepsis is associated with high rates of morbidity and

mortality. [3] In developing nations, it is the main cause of newborn mortality. Between 1 to 8 incidences of culture-proven sepsis are reported for every 1000 live newborns. [4] It is estimated that 36–55% of neonates in India suffer from sepsis and the rate of neonatal sepsis case fatalities varies from 25% to 65%. [5,6]

Neonates are highly vulnerable to sepsis due to their immature immune system, reduced phagocytic activity of white blood cells, and incompletely developed epidermal barriers. The gold standard for diagnosing sepsis is blood culture, which takes at least 48 hours to produce preliminary results, necessitating the start of an empirical antibiotic selection process. [7]

Depending on the postnatal day the condition manifests, neonatal sepsis is divided into two categories: early and late onset. Early-onset neonatal sepsis (EONS) happens within the first 72 hours of life while late-onset neonatal sepsis (LONS) develops between 72 hours and 28 days of life.[8] In EONS, maternal genital tract is the source of ascending infection. Premature rupture of membranes (PROM), chorioamnionitis, peripartum fever, urinary tract infection within two weeks prior to delivery, extended rupture of membranes (more than eighteen hours), multiple gestations, and cesarean sections are among the maternal risk factors linked to an increased incidence of EONS. Prematurity, prolonged invasive interventions such as intravascular catheterization and mechanical ventilation, failure to initiate early enteral feeding with breast milk, prolonged parenteral nutrition, hospitalization, surgery, and underlying respiratory and cardiovascular diseases are risk factors linked to LONS.[9]

The neonate may show symptoms in utero (fetal tachycardia, poor beat to beat variability) or within a few hours after birth in extreme cases of EONS.[10]

Neonatal sepsis has vague signs and symptoms. These include fever or hypothermia, breathing difficulties (including cyanosis and apnea), feeding issues, irritability or lethargy, hypotonia, seizures, bulging fontanel, inadequate perfusion, bleeding issues, distended abdomen, hepatomegaly, and unexplained jaundice.[11]

Several conditions including prematurity, birth asphyxia, transitory tachypnea, hypoglycemia, and other physiologic abnormalities may be considered as due to infections and treated with antibiotics as bacteremia due to overlap in their clinical presentation during the first few days of life. [12] Seven clinical indicators have been identified by the World Health Organization (WHO), including difficulties in feeding, convulsions, movement only when prompted, respiration rate greater than 60 beats per minute, severe chest indrawing, and an axillary temperature >37.5 °C or <35.5°C. [13]

The causative organisms of neonatal sepsis include *Group B streptococci, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Coagulase-Negative Staphylococci (CONS), Enterococci, Klebsiella spp., Enterobacter spp., Pseudomonas spp., Salmonella spp., H. influenzae, Neisseria meningitidis, Streptococcus pneumoniae* etc. [14] Overall, it has been seen that gram negative bacteria are more prevalent and are primarily represented by *Klebsiella, Escherichia coli and Pseudomonas*. Gram-positive bacteria that have been isolated most frequently are *Staphylococcus aureus, Coagulase Negative Staphylococci (CONS) and Group B Streptococcus*.[15]

The microorganisms most frequently linked to EONS are *Group B Streptococcus, CONS, Escherichia coli,* and *Haemophilus influenzae*. The organisms that cause LONS are *CONS*, *Staphylococcus aureus, Klebsiella spp., Escherichia coli, Enterobacter spp., Pseudomonas spp., Candida spp., Group B Streptococcus, Serratia spp., Acinetobacter spp.,* and the anaerobes.[16] *Group B Streptococcus* and *CONS* are the most frequent causes of EONS and LONS in developed nations, respectively but developing nations have a

whole distinct range of microorganisms. [17] *Klebsiella, Escherichia coli, Enterobacter*, and other common bacterial species are the main causes of neonatal septicemia in developing nations including India, Bangladesh, Nigeria, and Pakistan. [18]

Neonatal sepsis has high mortality and delay in diagnosis and treatment with inappropriate antibiotics may have unfavorable outcomes. Periodic evaluation of organisms responsible for neonatal sepsis is essential to identify the common pathogens of the disease as well as the antimicrobial susceptibility profile of the pathogens in a particular area, for the appropriate management of neonates and reducing the emergence of multidrug resistant organisms.

The objectives of the current study were to determine the bacteriological profile of blood samples from suspected cases of neonatal septicemia and to study their antimicrobial susceptibility pattern in a tertiary care hospital.

MATERIAL AND METHODS

Study design

This is a retrospective observational study. The study period was from December 2023 to May 2024, Department of Microbiology, Government Medical College and Hospital, Miraj.

Sample collection

All the blood samples received in the Department of Microbiology from clinically suspected cases of neonatal septicemia admitted under the Neonatal Intensive Care Unit of GMCH, Miraj, and PVPGH Sangli were included in the study. A total of 943 blood samples were received in six months study duration.

About 1-2 ml of blood was drawn under strict aseptic precautions before starting antimicrobial therapy and directly inoculated into Brain Heart Infusion broth (BHIB) in a ratio of blood: BHIB of 1:5. The blood culture bottles were immediately sent to the microbiology laboratory.

Sample Processing

Conventional blood culture bottles (BHIB) were incubated at 37◦ C for 24 hours. Then sub-cultures were made on MacConkey agar, blood agar, and chocolate agar plates on alternate days till the 5th day of incubation. Plates were incubated aerobically overnight at 37°C after inoculation. Positive growths were processed accordingly for identification while the blood culture was reported as negative if subcultures done after 5th day of incubation does not show any bacterial growth.

Bacterial identification and speciation were done by standard microbiological techniques procedures by using colony morphology, Gram staining and battery of biochemical reactions.[7] Members of the family Enterobacterales were identified by oxidase test, indole production, methyl red test, Voges Proskauer test, citrate utilization, urease test, carbohydrate utilization test, H2S production, and other tests. For Gram-positive bacteria, coagulase and catalase tests were done.

Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method on Muller Hinton agar plates. Antibiotic disks (Hi-Media) were used and after overnight incubation of plates at 37°C, the diameter of the clear zone around the antibiotic disc was measured and interpretation of susceptibility was done as recommended by Clinical Laboratory Standard Institute (CLSI) 2023/2024 guidelines. For quality control of antimicrobial susceptibility testing, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 were used.

Statistical analysis

Microsoft Excel was used for analysis of distribution of different bacterial isolates, their sensitivity pattern and interpretation of results.

RESULTS

During the study period, a total of 943 blood samples were received in the Microbiology laboratory from the suspected cases of neonatal septicemia. Among the 943 samples, culture positive samples were 268 (28.41 %) whereas 675 (71.58 %) samples were reported culture negative.

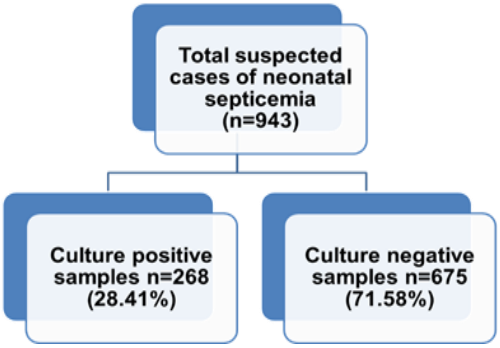

Fig 1: Distribution of samples with positive and negative blood culture.

Table 1. Distribution of culture-positive organisms among suspected cases of neonatal septicemia.

Gram-positive Isolates	Gram-negative Isolates
169 (63.05%)	99 (36.94%)

Table 1 reveals distribution of culture-positive organisms among suspected cases of neonatal septicemia. Out of 268 culture-positive bacterial isolates, 169 (63.05%) were Gram-positive isolates and 99 (36.94%) were Gram-negative isolates.

Table 2. Sex-wise distribution of culture-positive bacterial isolates.

Male	Female
165 (61.56%)	103 (38.43%)

Table 2 shows sex-wise distribution of culture-positive bacterial isolates.
Out of 268 neonates, 165 (61.56 %) were male and 103 (38.43 %) were female neonates.

Table 3. Frequency and percentage of Gram-Positive Cocci in neonatal septicaemia in our study.

Gram Positive Isolates	Frequency (n = 268)	Percentage (%)
***Coagulase Negative Staphylococci* (CONS)**	**92**	**34.32**
Enterococcus spp.	39	14.55
Streptococcus spp.	21	7.83
Staphylococcus aureus	17	6.34

Table 4. Frequency and percentage of Gram-Negative Bacilli in neonatal septicaemia in our study.

Gram Negative Isolates	Frequency (n = 268)	Percentage (%)
Klebsiella spp.	**58**	**21.64**
Citrobacter freundii	13	4.85
Non-fermenter GNB	13	4.85
Escherichia coli	10	3.73
Acinetobacter spp.	4	1.49
Enterobacter spp.	1	0.37

Table 3 and 4 shows frequency and percentage of Gram-positive cocci and Gram-negative bacilli in our study.

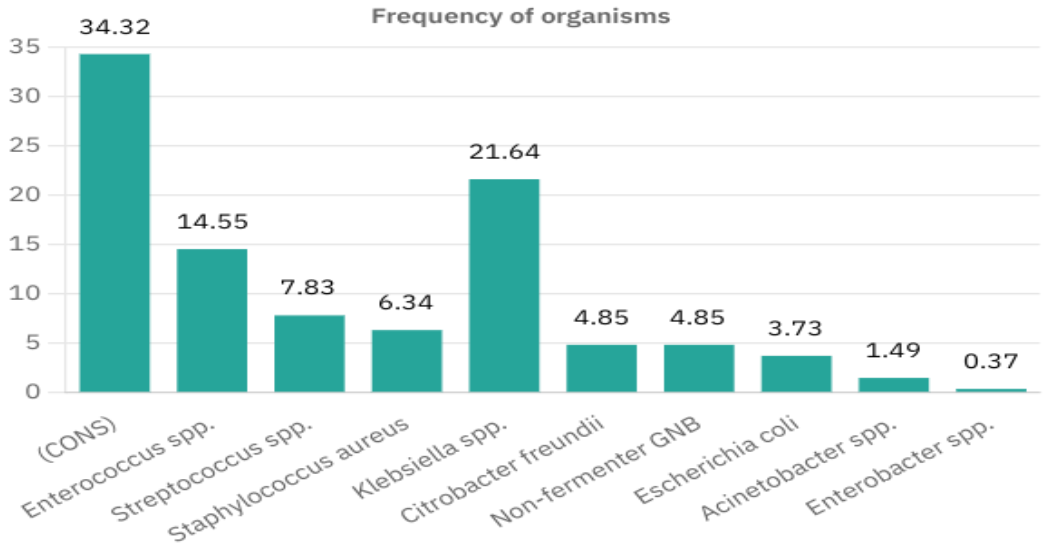

Fig 2. Frequency of organism from neonatal septicemia.

Coagulase Negative Staphylococci (CONS) was the predominantly isolated organism in 92 patients (34.32 %) followed by *Klebsiella spp.* in 58 (21.64 %) blood culture proven cases. The third most common causative agent of neonatal sepsis found was *Enterococcus spp.* 39 (14.55 %).

Table 5. Antibiotic resistance pattern of Gram positive isolates.

Antimicrobials	**CONS (n=92)**		***Enterococcus spp.* (n=39)**		***Streptococcus spp.* (n=21)**		***Staphylococcus aureus* (n=17)**	
	(n)	**(%)**	**(n)**	**(%)**	**(n)**	**(%)**	**(n)**	**(%)**
Penicillin	92	100	35	89.74	20	95.23	17	100
Erythromycin	90	98	35	89.74	20	95.23	15	88.23
Clindamycin	70	76	NT	NT	NT	NT	13	76.47
Cefoxitin	48	52	NT	NT	NT	NT	10	58.82
Ciprofloxacin	44	48	33	84.61	18	85.71	7	41.17
Gentamicin	24	26	NT	NT	NT	NT	4	23.52
Cotrimoxazole	22	24	NT	NT	NT	NT	5	29.41
Linezolid	0	0	0	0	0	0	0	0
Vancomycin	NT	NT	8	20.5	4	19.04	NT	NT
High level Gentamicin	NT	NT	12	30.76	5	23.80	NT	NT
Ampicillin	NT	NT	35	89.74	20	95.23	NT	NT

NT = Not Tested

Table 5 shows resistance pattern of Gram-positive cocci. *Coagulase Negative Staphylococci* (CONS) were found 100% resistant to Penicillin and 98% resistant to Erythromycin. 48 isolates (52%) were Methicillin resistant (MRCoNS). *Enterococcus species* were 89.74% resistant to Penicillin and Erythromycin. *Staphylococcus aureus* showed 100% resistance to Penicillin and 88.23% resistance to Erythromycin. 58.82% isolates were MRSA. Gram positive isolates were 100 % sensitive to Linezolid. Out of 39 isolates of *Enterococcus spp.* 8 (20.5%) were Vancomycin resistant (VRE).

Table 6. Antibiotic resistance pattern of Gram-negative isolates

Antimicrobials	*Klebsiella spp.*(n=58)		*Citrobacter freundii* (n=13)		Non fermenter GNB (n=13)		*Escherichia coli* (n=10)		*Acinetobacter spp.* (n=4)	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Ampicillin	58	100	13	100	NT	NT	10	100	4	100
Cefazolin	56	97	12	92.30	NT	NT	10	100	4	100
Cefuroxime	56	97	10	76.92	NT	NT	10	100	4	100
Ceftriaxone	46	79	8	61.53	NT	NT	6	60	4	100
Cefepime	40	69	6	46.15	6	46.15	6	60	3	75
Gentamicin	54	93	12	92.30	8	61.53	7	70	3	75
Amikacin	52	90	12	92.30	8	61.53	7	70	3	75
Piperacillin-Tazobactam	22	38	10	76.92	2	15.38	1	10	3	75
Imipenem	0	0	0	0	0	0	0	0	0	0
Meropenem	NT	NT	4	30.76	NT	NT	0	0	1	25
Ciprofloxacin	NT	NT	4	30.76	3	23.07	5	50	1	25
Cotrimoxazole	NT	NT	6	46.15	NT	NT	2	20	2	50
Aztreonam	NT	NT	NT	NT	5	38.46	NT	NT	NT	NT
Ceftazidime	NT	NT	NT	NT	4	30.76	NT	NT	NT	NT
Tobramycin	NT	NT	NT	NT	10	76.92	NT	NT	NT	NT

NT= Not Tested

Table 6 shows resistance pattern of Gram-negative bacilli. All GNB were resistant to Ampicillin (100% resistance). *Klebsiella species, Citrobacter freundii, Escherichia coli and Acinetobacter spp.* showed high resistance to 1st, 2nd, 3rd and 4th generation Cephalosporins.

All the *Acinetobacter spp.* were 100% resistant to Ampicillin, Cefazolin, Cefuroxime and Ceftriaxone. The higher sensitivity of *Acinetobacter spp.* was observed to Imipenem (100%), Meropenem (75%) and Ciprofloxacin (75%).

All the GNB were 100% sensitive to Imipenem (100% sensitivity) while all the GNB showed higher sensitivity to Meropenem. [*Escherichia coli* (100% sensitive), *Acinetobacter spp.* (75% sensitive), *Citrobacter freundii* (70% sensitive).

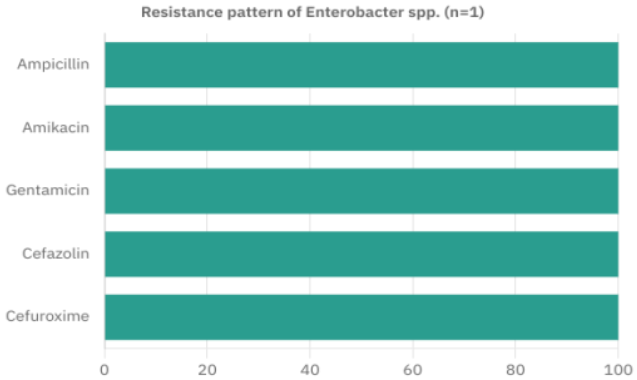

Fig 3. Resistance pattern in *Enterobacter spp.*

Single isolate of *Enterobacter spp.* was obtained which was found resistant to Ampicillin, Gentamicin, Amikacin, Cefazolin and Cefuroxime and sensitive to Imipenem, Cefepime, Ceftriaxone, Ciprofloxacin, Cotrimoxazole, Piperacillin-Tazobactam and Meropenem.

DISCUSSION

Neonatal sepsis is a major cause of morbidity and mortality in developing

countries such as India despite considerable progress in hygiene, introduction of new antimicrobial agents and advanced measures for early diagnosis and treatment.[19] World Health Organization has estimated that 1.6 million deaths occur globally every year because of neonatal infections and 40% of all neonatal deaths occur in developing countries.[20] In this study, an attempt has been made to know the bacteriological profile of causative agents of neonatal septicaemia and their antimicrobial susceptibility pattern in a tertiary care hospital.

The blood culture positivity rate among suspected cases of neonatal septicaemia in our study is 28.41% which is similar to the study by Soni et al 28.09%. [1] Other studies have shown positivity rate varying from 2.5% to 82%. The possible reason of varied positivity could be due to differences in sample size, prior antibiotic usage, and inclusion of fungal isolates.[5] While bacteria are the most frequent cause of newborn sepsis, other organisms such as enterovirus, adenovirus, coxsackievirus, rubella virus, candida species, and toxoplasma species can also cause the condition resulting in only a small percentage of blood cultures showing evidence of pathogenic organisms.[21] Anaerobic causes of infection cannot be ruled-out as anaerobic culture was not performed in this study. Chow et al. reported that 26% of all neonatal septicaemia was caused by anaerobes.[22]

Male preponderance was seen in this study. Out of 268 culture positive samples,165 samples (61.56 %) were from males and 103 samples (38.43 %) were from female neonates. According to the "male disadvantage hypothesis," male neonates are at a higher risk of sepsis as they are more susceptible to unfavorable perinatal and postnatal environmental conditions and are more likely to be preterm and have lower birth weights, increasing their risk of neonatal sepsis. [23] This is in concordance with study by Kumar GV et al. [24] while Chacko et al. [25] found no notable variation in the occurrence of neonatal sepsis by sex.

In this study predominance of Gram-positive cocci (63.05 %) over Gram-negative bacilli (36.94 %) was observed which is comparable to the study by Thakur et al. [16] but in contrast to the study by Viswanathan R *et al* [26] and Gosalia E *et al.* [27] where Gram-negative bacilli were predominant organisms responsible for 68% and 80.65% cases respectively.

CONS were the predominant pathogen in our study 34.32 % followed by *Klebsiella spp.* 21.64%. This is in accordance with the study by Bhattacharya et al. [4] *Klebsiella* was the predominant pathogen in 29% of cases as reported by National Neonatal Perinatal Database.[28] Most of the studies have reported *Klebsiella* or *E. coli* as predominant pathogen in neonatal sepsis in developing countries [29,20,30] which is in contrast to our study.

The colonization of skin and nasopharynx by CONS in HCWs, overcrowding in NICU, and improper handwashing are stated to be responsible for horizontal transmission in neonates resulting in a preponderance of CONS. [16] Even though CONS is typically thought of as a skin contaminant, its presence in the blood of severely ill neonates, especially in the second week of life, should be taken seriously and treated, particularly if it is isolated repeatedly.[31] Neonates may be exposed to Gram-negative organisms through humidification apparatus, resuscitation equipment, or articles used in daily care. Lack of normal flora and lack of serum bactericidins against Gram-negative bacteria, and recent extensive use of antibiotics in both mother and infants are some of the causes of increase in resistant Gram-negative organisms. The main source of *Klebsiella spp*. is the gastrointestinal tract and its transmission occurs through the hands of hospital personnel and its spread is facilitated by immature immunity of neonates, virulence factors of *Klebsiella spp*., and hospital environment.[7]

In our study, Gram-negative bacilli showed high resistance to ampicillin (100%), gentamycin (92%) and cefuroxime (91%) which is higher than in a study by Joshi SG *et al.* who have shown predominance of gram-negative bacteria exhibiting a range of resistance to cephalosporins (25-75 %) and aminoglycosides (23-69 %).[32]

Gram-positive cocci showed high resistance to penicillin (98%) and erythromycin (89%) which is similar to the study by Gyawali N *et al*. which also showed resistance to penicillin 98%. [33] Probably, prolonged antepartum exposure to beta-lactams (ampicillin and penicillin) may be the reason for this high level of resistance. [34]

The high-level antibiotic resistance pattern in our study may be due to the inappropriate use of these antibiotics.

Limitations

Current study was conducted at a single centre over a short period of time (6 months). Fungal and anaerobic isolates were not included in this study. Besides, this was a retrospective study. A prospective, multicentric study will probably be more helpful in understanding wider spectrum of neonatal sepsis, antimicrobial sensitivity pattern and will help us deciding the appropriate antibiotic policy in newborns.

CONCLUSION

The present study showed the predominance of *Coagulase negative Staphylococci* (CONS) as the causative agent of neonatal sepsis followed by *Klebsiella spp.* which may probably be an indication of nosocomial infection.

The higher antibiotic resistance percentage shown by Gram negative bacilli in the present study draws attention to the possibility of high number of MDR stains in the present study. It needs a further evaluation in this direction.

The microbial etiology of neonatal septicaemia is diverse and varies from place to place which necessitates the periodic study of microbial spectrum and their resistance pattern in order to design a specific empirical antibiotic regimen for the neonatal sepsis. Effective guidelines for the control and prevention of nosocomial infections should be implemented to reduce the incidence, morbidity, and mortality of neonatal sepsis. Judicious use of antibiotics, strict implementation of infection control practices and implementation of antimicrobial stewardship are necessary to reduce emergence of antimicrobial resistance in bacteria causing neonatal sepsis.

Declaration by Authors

Ethical approval: Ethical approval was not required to carry out this work as the bacterial isolates were collected as part of routine patient care investigation in the Government Medical college & Hospital, Miraj.

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Conflict of Interest: The authors declare no conflict of interest.

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