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Microbiological Profile of Blood Culture in Suspected Cases of Neonatal Sepsis with Special Reference to MRSA and ESBLs in Tertiary Care Hospital, Belagavi, Karnataka

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Abstract

Background:
Neonatal sepsis is an infection with positive blood culture in the first 20 days of birth. It is one of the leading causes of neonatal mortality and morbidity. Staphylococcus aureus and Escherichia-coli were the important causative agents of neonatal sepsis but now CONS has also emerged as the predominant pathogen especially among premature and low birth weight infants. There is also the emergence of multi-drug resistant strains like MRSA and ESBLs. Therefore, this study was undertaken to know the spectrum of various causative organisms isolated from the blood culture of clinical suspected neonatal sepsis and their antimicrobial sensitivity profile, with special reference to MRSA and ESBLs in the medical college attached hospital in Belagavi.

Methods:
A retrospective study was conducted on blood cultures of clinically suspected cases of neonatal sepsis from September 2020 to August 2021. Samples were processed and organisms were speciated by conventional blood culture method and antimicrobial sensitivity was done by Kirby-Bauer disc diffusion method according to CLSI guidelines. Methicillin resistance was detected by the cefoxitin disc diffusion method and ESBLs production by a phenotypic method using a ceftazidime-clavulanic acid disc.

Results:
Out of 524 blood cultures, 161(30.7%) were positive. In positive cultures, early-onset septicemia (EONS) was seen in 134(50.3%) which was 5 times higher compared to late-onset septicemia (LONS)-27(16.7%). Staphylococcus aureus -82(50.93%) was the most common organism isolated followed by E. coli-30(18.63%), Klebsiella pneumonia-21(13.04%), CONS- 13(8.07%), Enterococcus-9(5.5%) and GNNF-6(3.7%). All the Gram-positive isolates were sensitive to Linezolid and Vancomycin. Methicillin resistance was seen in 32.92% of S. aureus isolated and ESBLs production was seen in 92.5% of Enterobacteriaceae isolates.

Conclusion:
The present study revealed that S. aureus and E. coli were the predominant organisms causing neonatal sepsis. CONS has also emerged as an important pathogen. Most of the isolates were resistant to routinely used antibiotics with a higher prevalence of multi-resistant strains like MRSA and ESBLs which is a cause of concern.

Keywords: Blood culture, Neonatal sepsis, Antibiotic susceptibility, MRSA, ESBLs

Introduction

Neonatal Sepsis refers to the presence of bloodstream infection during the first 28 days of life. Based on epidemiology and time of onset, it is classified into two types. Early-onset- neonatal sepsis (EONS) manifests within 72 hours of birth whereas late onset-

neonatal sepsis (LONS) manifests after 72 hours of birth[1]. Neonatal sepsis accounts for 16.4% of neonatal mortality and morbidity in India[2]. EONS is mainly caused by maternal genital flora and LONS is either nosocomial or community acquired. Group B streptococci (GBS) and E. coli predominate in the USA and Europe, whereas Staphylococci and Gram-negative bacilli are much more common in developing countries[3]. Coagulase-negative staphylococcus (CONS) is the most frequent cause of late-onset sepsis among new-born infants in neonatal intensive care units (NICU) worldwide. Incidences of up to 66% of late-onset sepsis have been reported. [16] CONS are a heterogeneous group of bacteria, consisting of approximately 40 species, of which, several species have been recognized as potential pathogens to humans.

Neonatal sepsis includes a spectrum of clinical symptoms which may be sometimes subclinical[4]. Isolation of organisms from blood culture remains the gold standard but many times the cultures remain negative. A negative culture does not rule out infection. Also, the growth of organisms like Coagulase Negative Staphylococcus (CONS) and others is not always indicative of infection. Factors like the number of blood cultures (repeated isolation), presence of risk factors, or underlying diseases help to confirm the infection. Furthermore, there are no sensitive markers for diagnostic purposes and the diagnosis often depends on clinical suspicion[5]. It is important to know about the causative agents and their resistance pattern as antibiotics used in the empirical therapy should have a spectrum of activity against common pathogens.

Therefore, this study was undertaken to determine the spectrum of causative agents and their resistance pattern in our tertiary care hospital.

Objectives:

1. To study the various isolated microorganisms and their antibiotic sensitivity pattern, from blood cultures of suspected cases of Neonatal sepsis from the NICU at teaching hospital.
2. To study the rate of ESBLs producers and MRSA in isolated organisms from blood cultures of suspected cases of Neonatal sepsis.

Methods:

A retrospective observational study was conducted in the Department of Microbiology of a Medical college attached hospital in Belagavi. Reports of blood cultures received from the NICU of suspected cases of Neonatal sepsis processed in the Microbiology department from September 2020 to August 2021 were analysed.

Blood samples were processed using the conventional blood culture method using Brain Heart infusion (BHI) broth. Then samples were cultured on MacConkey agar and Blood agar medium (Hi media PVT Ltd) which were incubated at 37^0C aerobically for 24 hours. Isolated organisms were identified by biochemical reactions and an antibiotic sensitivity test was done by Kirby Bauer disc diffusion method on Muller Hinton Agar (MHA) by CLSI guidelines[6].

The Gram-negative organisms were tested using, Ampicillin (10 □g), Amoxicillin-clavulanic acid (20/10µg), Amikacin (30µg), Ciprofloxacin (5µg), Ceftriaxone(30µg), Piperacillin- tazobactam (100/10µg), Imipenem (10µg), Aztreonam (30 µg).

The Gram-positive organisms were tested using a panel consisting of Penicillin (10 □g), Cefoxitin (30□g), Amoxicillin-clavulanic acid (20/10 µg), Amikacin (30µg), Gentamicin (30µg), Clindamycin (15□g), Ciprofloxacin (5 □g), Linezolid (30 □g) and Vancomycin (30

□g).

ATCC strain of *Staphylococcus aureus* 25923 and *Escherichia coli* ATCC 25922 were used as controls for antibiotic susceptibility tests. Ceftazidime and ceftazidime-clavulanic acid combined disc test was used to detect Extended Spectrum beta-lactamase (ESBL) for gram- negative isolates[6]. The pathogens were isolated, and their antibiotic resistance pattern was studied. Results of these culture reports were studied, and statistical analysis was done by descriptive analysis.

Results: A total of 524 blood samples from clinically suspected/diagnosed neonatal sepsis cases were received during the study period, September 2019-August 2020, in the Department of Microbiology. Blood culture was positive in 161 (30.7%), no growth in 332 (63.3%) as shown in Fig. A and contaminants in 31(5.9%).

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Among culture-positive cases, 80 (49.6%) were males and 81 (50.31%) females. The culture- proven sepsis included early-onset neonatal sepsis (EONS) - 134 (83.2%) and late-onset neonatal sepsis (LONS)- 27(16.7%) as shown in Fig B.

The culture-positive organisms include gram-positive organisms (104-64.5%) and gram- negative organisms (57-35.6%). Out of 161 isolates, Staphylococcus aureus was most common (82-50.93%) followed by *Escherichia coli (*30-18.63%), *Klebsiella pneumoniae* (21-13.04%), Coagulase-negative Staphylococcus (CONS) (13-8.07%), Enterococcus species (9-5.5%), and non-fermenter GNB (6-3.7%) as shown in Table I. The distribution of organisms isolated in both EONS and LONS is shown in Fig no. C.

The antibiotic sensitivity profile of Gram-positive isolates reveals that all the organisms were sensitive to linezolid and vancomycin and showed high resistance to Penicillin (77.8%), Amoxicillin-clavulanic acid (65.3%), Ciprofloxacin (55.5%) and comparatively lower resistance to amikacin (13.4%), Gentamicin (12.5%), Clindamycin (18.2%). as shown in fig D. Gram-negative isolates showed sensitivity to Amikacin (29.8%), Ciprofloxacin (29.8%), Piperacillin-tazobactam (19.2%), and Imipenem (19.2%) and high resistance to Ampicillin (96.6%), Amoxicillin-Clavulanic acid (98.2%), Ceftriaxone (94.7%), Aztreonam (92.9%). As shown in fig E. ESBL production was seen in 92.15% of Enterobacteriaceae isolates.

Methicillin resistance (MRSA) was seen in 32.92% of total Staphylococcus isolates and ESBLs production was seen in 92.15% of total Enterobacteriaceae isolates.

Fig A. Incidence of Sepsis based on Blood culture

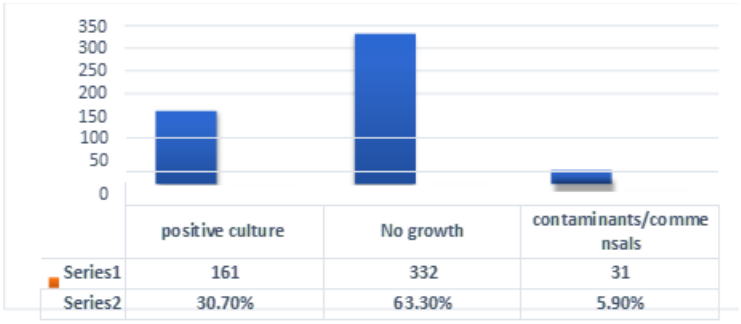

Fig. B. Types of Neonatal sepsis based on Microbiological culture

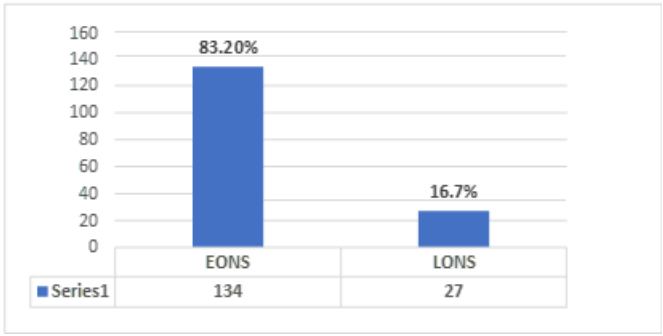

Table-I- Distribution of various organisms isolated

Name of organisms	Numbers (%)

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Staphylococcus aureus	82(50.93%)
Escherichia coli	30(18.63%)
Klebsiella pneumonia	21(13.04%)
CONS	13(8.07%)
Enterococcus	9(5.5%)
Gram-negative-non-fermenters	6(3.7%)

Fig-C Distribution of isolates in Early Onset Neonatal Sepsis (EONS) and Late Onset Neonatal Sepsis LONS).

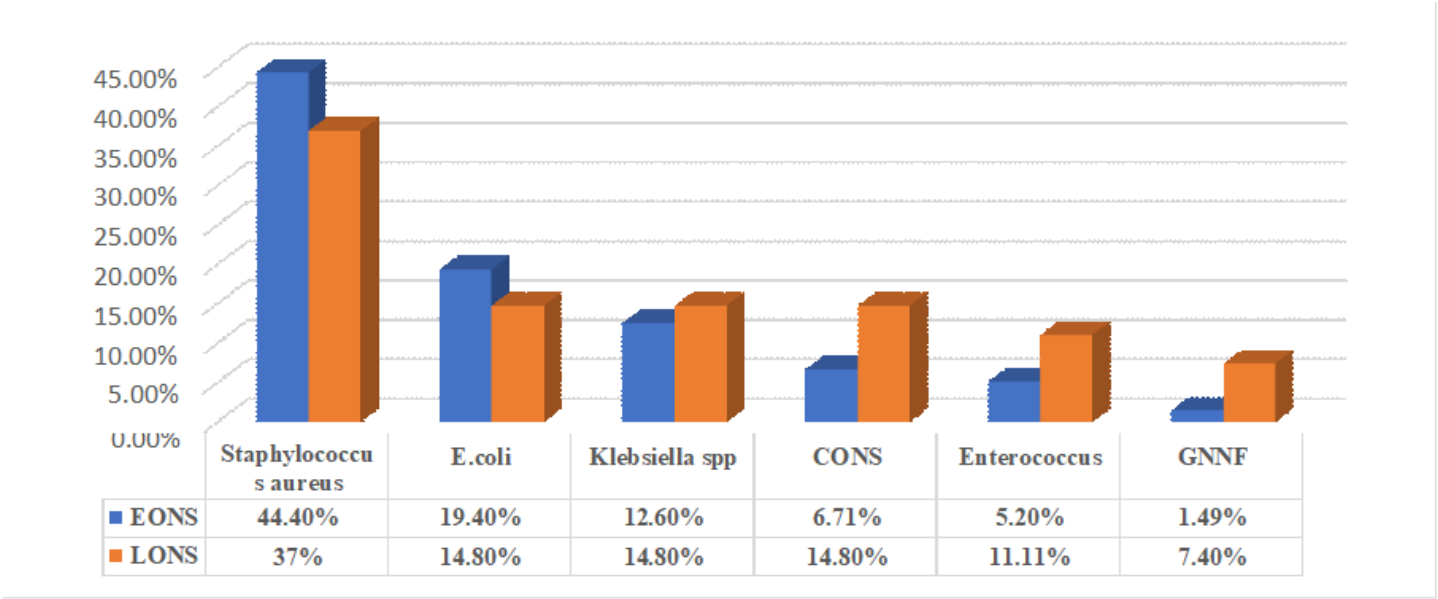

Fig D– Antibiotic sensitivity profile of gram-positive isolates

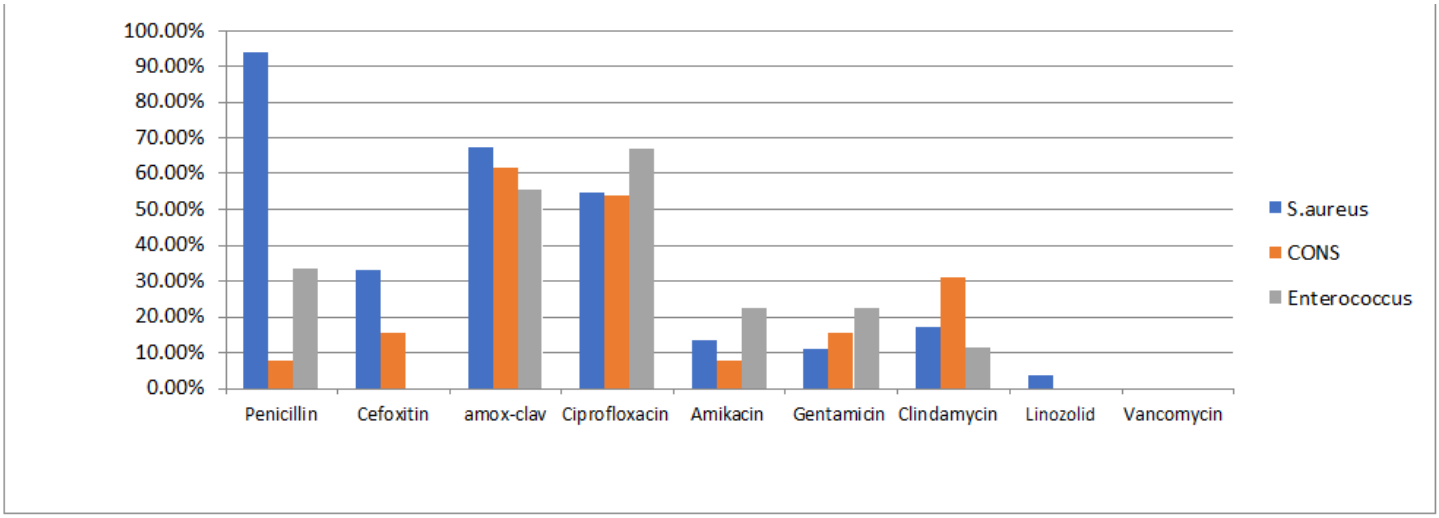

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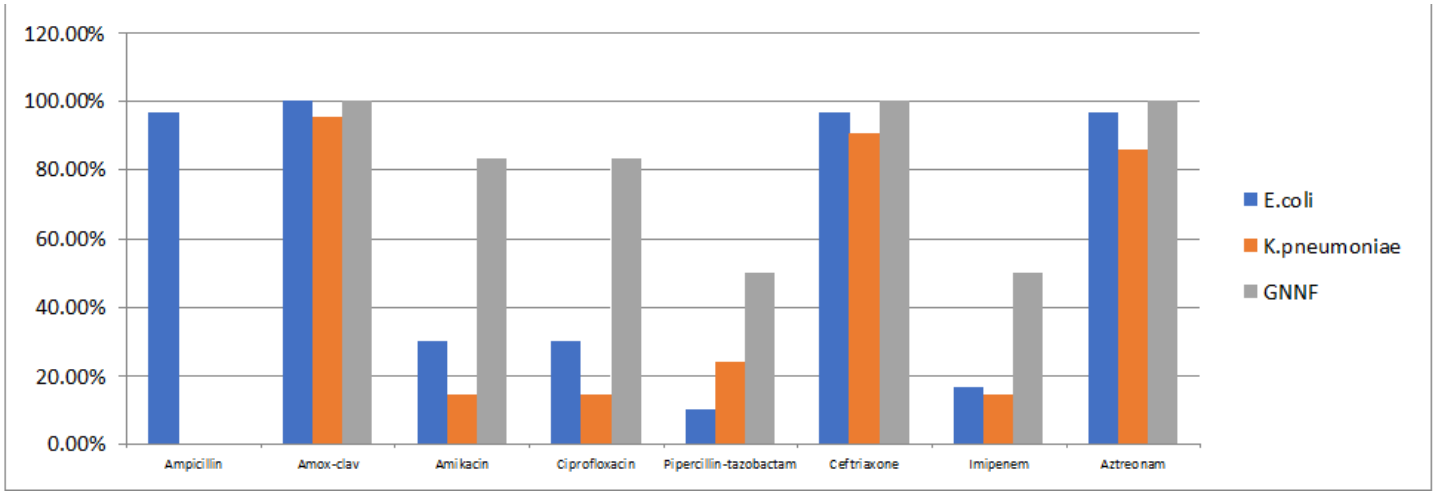
Fig. E- Antibiotic sensitivity profile of Gram-negative isolates.



Discussion:

Neonatal sepsis is a systemic bacterial infection with positive blood culture in the first 4 weeks of birth. Sepsis is the commonest cause of neonatal mortality in developing countries. The pattern of organisms causing neonatal sepsis has been constantly changing. The indiscriminate use of antibiotics has also resulted in the emergence of multidrug-resistant organisms including MRSA and ESBLs. Previously most common organisms causing neonatal sepsis were the Gram-negative organisms and Staphylococcus aureus. But in recent years along with these organisms, CONS has emerged as the predominant causative agent, which was mostly considered as a skin commensal previously[7,8].

This study was undertaken to determine the pattern of causative organisms isolated from the blood samples of clinically suspected cases of neonatal sepsis admitted in intensive care units and their antimicrobial resistance pattern and the prevalence of MRSA and ESBLs.

In the present study, the positivity rate was 30.7%. This is lower compared to the findings of other studies showing a positivity rate of 48%, 62.8%, 53.4% among cases of neonatal sepsis [9, 10, 11]. The difference may be due to variable incidence of neonatal sepsis from place to place and the use of conventional blood culture method in this study compared to automated blood culture methods.

In this study, both males and females were almost equally affected contributing to males 80(49.6%) and females 81(51.31%) of the total positive cultures.

In the present study EONS cases were 134(83.2%) and found to be 5 times higher than LONS 27(16.7%) of the total positive cultures. Similar results were reported by Nazir et al [11]. The higher rate of EONS may be due to the immaturity of immunological responses in the first few days of life and due to other factors like prematurity and low birth weight. Similar results were also shown by a study from North India, where culture-proven sepsis was found in 7.5/1000 live births, in that, 85% of neonates had EONS and 15% had LONS.[12] In a similar study from a secondary level rural hospital in South India, blood culture was positive in 26.2% cases and EONS was more common (94.4%) than LONS (5.6%) [13]. The present study showed a predominantly gram-positive isolate of 64.5% compared to Gram-negative isolates, 35.6% like the results reported by Thakur et al (60%), Bellot et. al (54.9%)[14,15].

In this study *Staphylococcus aureus*-82(50.93%) was the most common organism isolated followed by *Escherichia coli*- 30(18.63%), *Klebsiella pneumoniae*-21(13.04%), CONS- 13(8.07%), *Enterococcus*- 9(5.5%) and Gram-negative non-Fermenters- 6(3.7%). Previously CONS were often regarded as skin commensals. But de Leon.P. et.al stated that the presence of CONS in the blood cannot

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be taken as contamination, especially in patients of critical care units [16].

The antibiotic sensitivity profile of Gram-positive isolates shows sensitivity to linezolid and vancomycin. And showed high resistance to Penicillin (77.8%), Amoxicillin-clavulanic acid (65.3%), Ciprofloxacin (55.5%), and comparatively low resistance to Amikacin (13.4%), Gentamicin (12.5%), Clindamycin (18.2%).

In our study, Gram-Negative organisms showed high resistance to Ampicillin (96.6%), Amoxicillin-Clavulanic acid (98.2%), Ceftriaxone (94.7%), Aztreonam (92.9%), and low resistance rate to Amikacin (29.8%), Ciprofloxacin (29.8%), Piperacillin-tazobactam (19.2%),and Imipenem (19.2%).

Ampicillin, aminoglycoside, and third generation cephalosporins are regularly used to treat sepsis in a neonate. High resistance to these antibiotics among gram-negative bacteria has been reported from India[13, 17]. Pavan Kumar et al from Southern India reported 100%, 52.9%, and 31.2% resistance to ampicillin, gentamicin and third generation cephalosporins respectively, among Gram-negative bacilli[13]. Bhat et al from South India and Chandel et al in a multi-centric study reported similar findings[17].

In our study, Methicillin resistance was detected using Cefoxitin by disc diffusion method for Staphylococcus aureus and CONS. And Extended Spectrum beta-lactamase (ESBL) producing strains were identified by a phenotypic method using ceftazidime-clavulanic acid discs in Enterobacteriaceae isolates[6]. Methicillin resistance was seen in 32.15% of total Staphylococcus species and ESBLs production[22] was seen in 82.4%. Similar results were shown by Jyoti et al and Shivanna et al[18,19]. Vancomycin resistance was not seen in our study although it is common in Enterococcus species. This indicates an increase in the prevalence of methicillin resistance, ESBLs and an increase in the trend of multidrug-resistant isolates. Methicillin resistance is an important nosocomial pathogen especially in NICU and they are also a source and reservoir of resistance genes to other organisms in hospital settings [20,21].

Conclusion:

It is evident from this study that Staphylococcus aureus and Escherichia coli are the leading cause of neonatal sepsis and CONS are also emerging as predominant pathogens. Alarmingly, most of them are resistant to multiple, routinely used antibiotics. There is an increase in the prevalence rate of MRSA and ESBLs. As CONS are also skin commensals and a common contaminant, it is very important to differentiate the true infection from the contamination. Contamination rate in our study was also seen high. This is crucial to enhance the validity of blood culture so that the injudicious use of antibiotics is reduced. Regular antibiotic susceptibility surveillance and evaluation and enforcement of periodic review of the hospital's antibiotic policy are a must to reduce the rate of nosocomial infection and development of antibiotic resistance.

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