Neonatal sepsis and resistance pattern of isolates in Tertiary level neonatal unit: Time to evaluate the empirical antibiotics selection

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Abstract

Objective: To find out the most common organisms involved in neonatal sepsis origination and observe the pattern of antibiotic sensitivity and resistance of bacterial isolates.

Materials and Methods: This descriptive cross-sectional study was conducted at the Department of Paediatrics Izzat Ali Shah Hospital, Wah Cantt. Out of 420 patients admitted with sepsis in NICU, 19.5% of patients with positive blood cultures were included in the study. A consecutive, non-probability sampling technique was used.

Results: Out of 82 positive blood cultures gram-positive bacteria were observed in 19 patients (23.2%) and gram-negative bacteria were seen in 63 patients (76.8%). The most common gram-negative pathogens isolated were Acinetobacter (29.3%) and Klebsiella (24.4%). Staphylococcus aureus (12.2%) was the commonest gram-positive organism isolated. Gram-negative organisms showed maximum sensitivity to Tigecycline and Colistin and were resistant to Cefixime, Aztreonam, Amoxicillin, and Ceftriaxone. Gram-positive bacteria were sensitive to Teicoplanin, Linezolid, and Vancomycin while resistance was shown to penicillin and amoxicillin.

Conclusion: The current study showed that gram-negative bacteria were the major contributors to sepsis in the respective setup and showed resistance to first-line antibiotics such as Penicillins and Cephalosporins. Strict infection control measures need to be implemented to avoid the emergence of resistant strains of pathogens in NICUs. This will help to reduce the incidence of neonatal sepsis leading to mortality.

Keywords: Neonatal sepsis, Resistance, Antibiotics.

Introduction

Neonatal sepsis is defined as a systemic infection occurring within the first 28 days of life, manifested by clinical signs and symptoms of infection, with or without isolation of bacterial pathogen in blood culture. Neonatal sepsis is one of the major causes of morbidity and mortality in neonates. The death rate of neonates is 225,000 every year. Three fourth of these deaths occur in neonates with an age of less than a week and among these 99% take place in undeveloped and underdeveloped countries due to infections.[1,2]

Neonatal sepsis is classified as early-onset sepsis (EOS) and late-onset sepsis occurring before and after 72 hours of life respectively.[3] Group B streptococci emerged as the leading cause of EOS in the past. Increased use of intra-partum antibiotic prophylaxis resulted in a marked reduction of early-onset group B Streptococcus sepsis. On the other hand, prolonged and frequent exposure to broad-spectrum antibiotics in the mother resulted in the colonization of neonatal skin and gastrointestinal tract with resistant bacteria.

Neonatal sepsis is caused by many gram-negative and gram-positive bacteria. Many of these bacteria are normal commensal of human body surfaces and gain entry into the body through indwelling foreign devices such as intravenous catheters. This makes hospitalized patients susceptible to deadly infections caused by this bacteria.[4]

Clinically, the diagnosis of neonatal sepsis is difficult due to nonspecific signs and symptoms. The gold standard test for diagnosis is blood cultures but it takes time for its results to get finalized.[5] In the meantime neonates are given empirical antibiotic therapy. The diversity of etiological agents has changed over time due to changing patterns of antibiotics use and modifications in lifestyle. The reasons for changing patterns of antibiotic resistance are multi-factorial but most important is the excessive use of antibiotics.

The effectiveness of empirical therapy is reduced due to changes in the microbiological pattern of neonatal sepsis and increased resistance of bacterial isolates to commonly used antibiotics. Monitoring the emergence of resistance is important as it guides empirical antimicrobial therapy against the most common pathogens.

In developing countries like ours emerging bacterial resistance is a new potential threat for neonates and management of such patients is becoming a challenge. So the need of the hour is to study the bacterial pathogens responsible for neonatal sepsis and their susceptibility patterns so as to renew our empirical therapy and management plan for neonatal sepsis. The rationale of our study is to characterize the bacteriological profile and pattern of antibiotic sensitivity and resistance.

Materials and Methods

Objectives: To find out the most common organisms involved in neonatal sepsis origination and observe the pattern of antibiotic sensitivity and resistance of the bacterial isolates.

Study design: Descriptive cross-sectional study

Study population: All neonates admitted to Neonatal Intensive Care Unit, Izzat Ali Shah Hospital, Wah Cantt. from September 2016 to August 2020.

Setting: This study was conducted at the Department of Paediatrics, Izzat Ali Shah Hospital, Wah Cantt. and approved by the Research and Ethical committee of the institute.

Sample size: Out of 420 patients admitted to NICU during the specified period of time, 82 patients had positive blood cultures and were included in the study.

Inclusion criteria: All neonates of either gender irrespective of gestational age, weight, and mode of delivery admitted in NICU via outpatient department, labor room, and emergency department was included. The neonates with clinically suspected sepsis showing signs of fever, lethargy, and reluctance to feed, seizures, irritability, etc., and their laboratory parameters like complete blood count, C-reactive protein (CRP), etc. were included in the study. Later they had blood cultures with positive growths.

Exclusion criteria: Neonates whose blood cultures revealed no growth or those that were contaminated were excluded. Neonates having the history of antibiotic administration 48 hours prior to admission.

Study duration: 4 years (2016-2020)

Data collection technique: Consecutive, non-probability sampling technique was used. Blood samples were collected from the patients satisfying the inclusion criteria and sent to a private lab in culture bottles provided by the laboratory. Bactec 9240 blood culture system was used by the laboratory for incubation. Isolates were identified by the standard bacteriological techniques. Antibiotics sensitivity was performed by modified Kirby and Bauer disc diffusion methods. Discs of Amoxicillin, Ceftriaxone, Cefixime Cefipime, Ciprofloxacin, Levofloxacin, Imipenem, Meropenem, Amikacin, Gentamicin, Cefoxitin, Cefoperazone, Salbactam, piperacillin-tazobactam,

trimethoprim-Sulphamethoxazole, Cefotaxime, ampicillin Sulbactam, Ceftazidime, Cefopirome, Teicoplanin, Doxycycline, Penicillin, Chloramphenicol, Colistin, Tigecycline, Aztreonam were used for susceptibility testing. Concurrently quality control was applied as per CLSI guidelines.

These antibiotic sensitivity results were further interpreted as sensitive, intermediate, and resistant based on the size of the 'zone of inhibition', according to CLSI.

Data collection tool: A structured data collection proforma was developed for the collection of data.

Data entry and analysis: Data was analyzed using SPSS version 25. Mean and standard deviation was taken out for numerical variables such as age. Frequencies of gram-positive and gram-negative bacteria were calculated and their susceptibility pattern with different antibiotics was observed. Early-onset of disease was considered as disease occurring within the first 72 hours of life and late-onset as septicemia occurring after 72 hours of life. The Association of age and gender with the onset of disease was calculated by applying the Chi-square test-taking P-value<0.05 as significant.

Results

The study included 82 neonates with positive blood culture reports. The mean age was 8.95 ± 9.076 days. Patients having early onset of septicemia that is before 72 hours of age were 29 (35.4%) and 53 (64.6%) patients developed sepsis after 72 hours of age. More patients were admitted with late-onset sepsis as compared to early-onset and there was no significant association between age and the onset of disease (P-value=0.69). Gender distribution revealed 47 (57.3%) males and 35 (42.7%) female patients. The incidence of septicemia was more marked in male neonates than females. The onset of sepsis was not significant to gender in our study (P-value=0.77).

Out of 82 positive blood cultures gram-positive bacteria were observed in 19 patients (23.2%) and gram-negative bacteria were witnessed in 63 patients (76.8%). Among the patients suffering from infection with gram-positive bacteria, 6 (31.5%) had early onset of disease, and 13 (68%) patients suffered late-onset. Out of 63 cultures with gram-negative bacteria, 23 (36.5%) patients were reported with early onset of sepsis and 40 (63.5%) patients had late onset of septicemia (Figure 1).
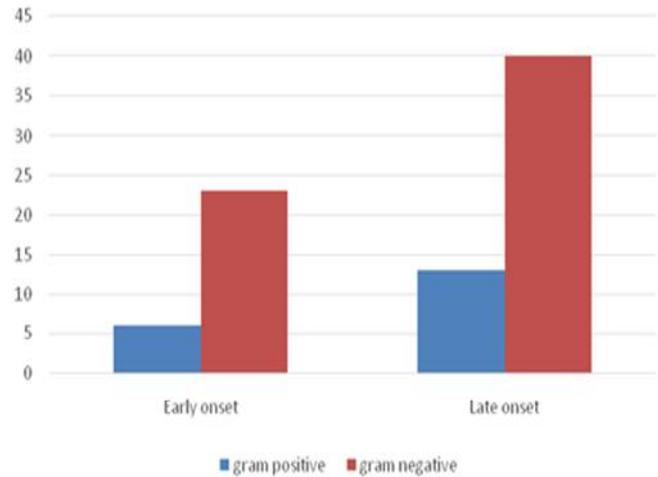

Figure 1: Gram reaction of bacteria isolated and their relationship with the onset of disease

Gram-negative bacteria contributed to most of the septicemia in neonates. The most common gram-negative pathogens isolated were Acinetobacter (29.3%) and Klebsiella (24.4%). Acinetobacter was the most common pathogen responsible for causing the early onset of disease in neonates. It was most sensitive to Colistin and Tigecyclin. Resistance for Acinetobacter was shown by Levofloxacin, Meropenem. Amikacin, Aztreonam, Cefixime, and Amoxicillin. Whereas Klebsiella responsible for late-onset septicemia showed maximum sensitivity to Tigecyclin least to Meropenem, Levofloxacin, and Ciprofloxacin. It was resistant to Cefixime, Ceftriaxone followed by Amoxicillin. Staphylococcus aureus was the commonest (12.2%) and MRSA (8.5%) was the second most common gram-positive organism isolated and was mostly involved in late-onset of disease in neonates (Figure 2). It was most sensitive to Teicoplanin, Linezolid, and Vancomycin and showed resistance to Penicillin, Amoxicillin, and Ceftriaxone. The current study showed that the pathogens isolated were highly resistant to first and second-line antibiotics such as Penicillins and Cephalosporins. Figures 3 and 4 show the sensitivity and resistance pattern of different gram reactive bacteria in relation to the antibiotics tested.
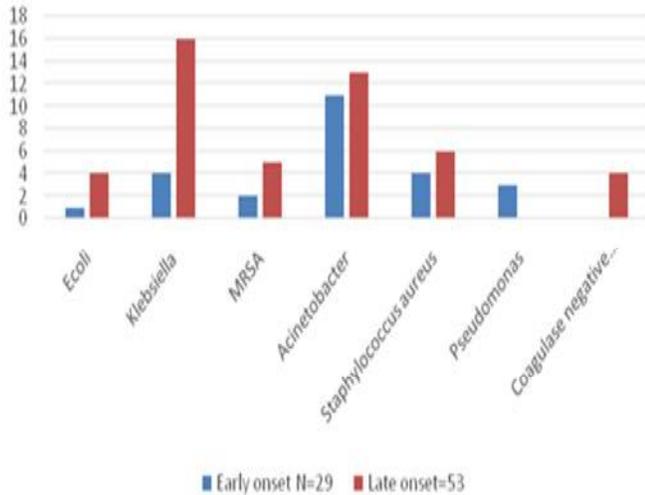
Figure 2: Antibiogram profile of isolated bacteria in relation to disease inception
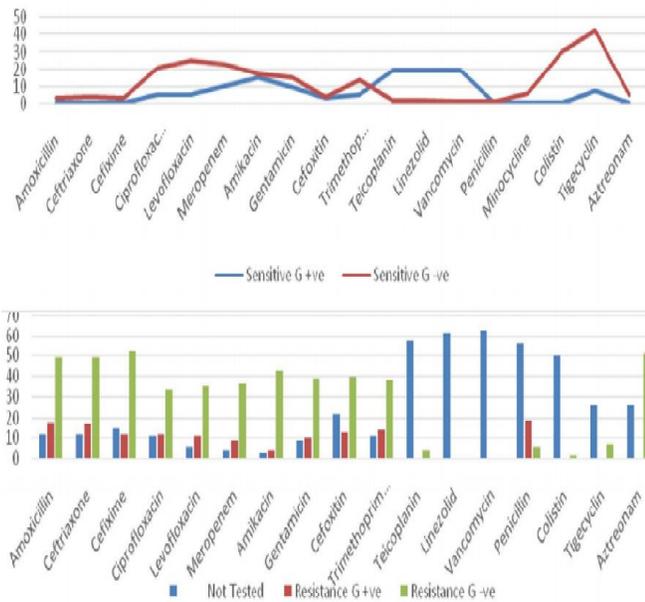

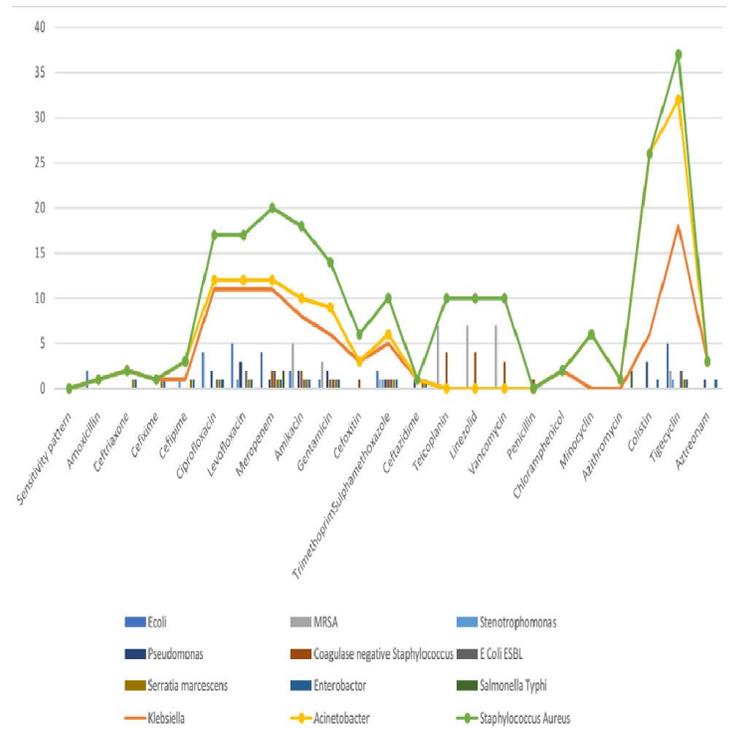**Figure 3: Resistance and sensitivity pattern of Gram-Positive and Gram-negative bacteria**

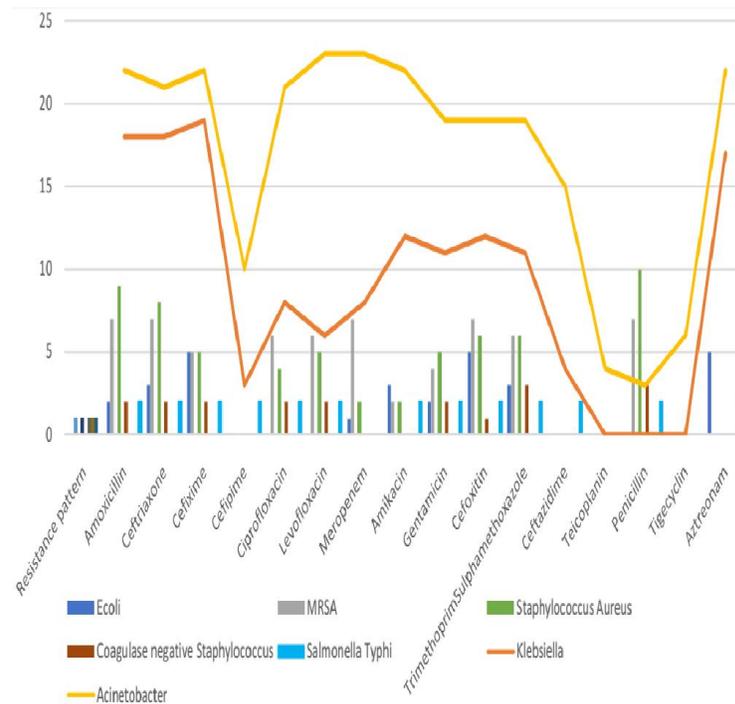**Figure 4(a): Sensitivity pattern of isolated pathogens in relation to the antibiotics tested**

Figure 4(b): Resistance pattern of isolated pathogens in relation to the antibiotics tested

Discussion

Sepsis is an important cause of morbidity and mortality in neonates. Infection in neonates can arise from the mother's uterus, during passage through the birth canal, or from the hospital environment.[4] Dealing with septicemia is an unavoidable emergency and the right choice of drugs is the key to a good prognosis and management.[2] We have a death rate of 49/1000 live births accounting for 7% of worldwide neonatal deaths.[6]

Gold standard investigation for diagnosis of septicemia is blood culture. For effective management one should have a sound knowledge of the antibiotic susceptibility pattern of the respective NICU.[7] In the current study out of the 82 neonates with positive blood cultures, 29 (35.4%) developed sepsis before 72 hours of age, and 53 (64.6%) patients had disease onset after 72 hours of life. Males were affected more as compared to females which is similar to a 4-year study conducted in Peshawar which showed a higher frequency of late-onset of disease in males.[8]

Current study showed 63 (76.8%) gram-negative and 19 (23.2%) gram-positive isolates (P-value=0.001). This finding is quite similar to the studies conducted by Obaid et al and Bhamre et al.[8,9]

Gram-negative organisms isolated in our study include Ecoli, Klebsiella, MRSA, Acinetobacter, Stenotrophomonas, Pseudomonas, E Coli ESBL, Serratia marcescens, Enterobactor, and Salmonellatyphi. Among these Acinetobacter (29.3%) and Klebsiella (24.4%) are the commonest pathogens involved in causing septicemia in neonates in our setup. Klebsiella and Staphylococcus aureus have been mentioned as the commonest organisms involved in the community and hospital-acquired infections in developing countries.[2] Early-onset infection was caused predominantly by Acinetobacter (29.3%) while Klebsiella (24.4%) caused the late onset of disease. In a study conducted in POF hospital Wah Cantt, Acinetobacter and Klebsiella were the common causative agents responsible for septicemia in neonates.[10] Whereas similar research carried out in the same setup later, revealed Klebsiella followed by coagulase-negative Staphylococci as the most common pathogens isolated.[11] Similar patterns of pathogenic isolations were seen in a study in Islamabad including dominant isolation of Klebsiella followed by Acinetobacter, Ecoli, and Pseudomonas.[12] Klebsiella was also the principal pathogen isolated from positive blood cultures of neonates in India.[13] In a study conducted in Sialkot, Klebsiella pneumonae and E-coli

were gram-negative bacteria, and Staphylococcus aureus (12.2%) causing the late onset of disease was the most common gram-positive organism isolated. Ecoli was isolated in similar studies conducted in Peshawar, Hyderabad, and Karachi[6].

The current study revealed that Gram-negative organisms showed maximum sensitivity to Tigecycline and Colistin followed by Levofloxacin and Meropenem. Acinetobacter was the most common gram-negative pathogen isolated. It was most sensitive to Colistin and Tigecycline. Sensitivity to the latter was also shown by Klebsiella. Haritha et al also observed the sensitivity of gram-negative organisms to Carbapenems and Tigecyclin and gram-positive organisms were found to be sensitive to Linezolid and Vancomycin[14]. Resistance for Acinetobacter was shown by Levofloxacin, Meropenem. Also, Amikacin, Aztreonam, Cefixime, and Amoxicillin showed equal resistance to Acinetobacter. Klebsiella, the second commonest pathogen isolated in the current study showed sensitivity to Ciprofloxacin, Levofloxacin, and Meropenem while resistance to Cefixime and Aztreonam followed by Amoxicillin and Ceftriaxone was observed. The efficacy of Meropenem for Klebsiella was also proved in another study in Rawalpindi[15]. Cultures with the growth of gram-positive organisms including Staph aureus and Coagulase-negative Staphylococcus showed the best sensitivity to Teicoplanin, Linezolid, and Vancomycin. Two studies from Faisalabad and Iran showed maximum sensitivity to Linezolid (100%) and Vancomycin(100%) by gram-positive organisms and resistance to Penicillin, Amoxicillin, and Ceftriaxone. This is strongly in agreement with our results[16, 17]. Infection control measures must be firmly implemented and efficient use of antibiotics such as combination therapies in NICUs according to culture reports may be carried out. This will help to minimize the emerging resistance to first-line drugs [18].

In the last few years, this NICU has been upgraded from level I to level III NICU with long-term ventilation and neonatal surgeries therefore the reasonable number of Klebsiella and Acinetobacter culture-positive patients were ventilator-dependent for a long time. As studies have proved that Acinetobacter and Klebsiella live in lines and fluids, therefore, the unit has targeted infection control in these particular areas and will keep reviewing the spectrum of microorganisms in the future.

Conclusion

The current study showed that gram-negative organisms are more common than gram-positive organisms in cases of neonatal sepsis. Most of the organisms showed resistance to first-line antibiotics such as Penicillins and Cephalosporins. Strict infection control measures need to be implemented to avoid the emergence of resistant strains of pathogens in NICUs. This will help to reduce the incidence of neonatal sepsis leading to mortality. It is important to keep a track of organisms growing in the NICU so the selection of antibiotics. It is suggested that in critically ill patients of septicemia with blood cultures revealing no growth and showing no improvement with 1st and 2nd line antibiotics combination of Tigecyclin or Colistin (to cover gram-negative organisms) can be used with either Linezolid, Vancomycin, and teicoplanin (to cover gram-positive organisms).

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