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Original Research Article

TITLE: BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY TESTING OF MICRO-ORGANISM ISOLATED IN CASE OF NEONATAL SEPSIS

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Abstract

Background & Methods: Neonatal sepsis is a leading cause of morbidity and mortality in newborns (less than 1 month of age). The aim of the study is to study bacteriological profile & antibiotic susceptibility testing of micro-organism isolated in case of suspected neonatal sepsis. Neonatal sepsis was defined as neonates presenting with symptoms of fever, convulsions, lethargy, feeding or breathing problem, hypoglycemia, bulging fontanels, vomiting, and jaundice.

Results: Neonatal sepsis is more common in male child 98(59.3%) as compared to female 67(40.7%). Newborn in Early Onset Sepsis group-86(52.1%) had highest frequency of positive blood culture as compared to Late Onset Sepsis group -79(47.9%). Out of 165 cases of suspected neonatal sepsis 74(44.8%) showed positive blood culture. Out of positive cases Staphylococcus aureus (MRSA)- 44(59.4%) was most common species isolated. For Gram positive isolates most sensitive antibiotics were doxycycline, chloramphenicol and linezolid; and were resistant to azithromycin and ceftriaxone. Cotrimoxazole was most sensitive antibiotic for Gram Negative isolates. Antibiotics-amoxiclav, ampicillin, ceftriaxone, piperacillin-tazobactam, ceftazidime showed 100% resistivity for Gram Negative isolates.

Conclusion: Emergence of antibiotic resistance among bacterial isolates from neonatal sepsis is a major cause for treatment failure, higher morbidity and mortality. Proper antibiotic usage guidelines and its effective implementation could be milestone for revolution in the field of antibiotic resistance control. The epidemiology of neonatal sepsis, causative risk factors and antibiotic resistance pattern of pathogens may be used to develop guidelines for management of neonatal sepsis.

Keywords: Neonatal sepsis, Bacteriological profile, Antibiotic susceptibility
Study Design: Observational Study

Introduction

Neonatal septicemia is defined as a clinical syndrome characterized by systemic signs and symptoms of inflammatory response following the appearance or confirmation of infection during the first month of life [1]. It is the third most common cause of deaths among neonates, accounting for 2,25,000 deaths globally every year. The early symptoms and signs

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of neonatal septicemia infection are atypical and lack specificity, and are often similar to primary apnoea, simple febrile disease, feeding intolerance, anaemia and other non-infectious diseases, which are difficult to identify and easy to cause delay or overtreatment. Early screening of neonatal septicemia pathogens, early diagnosis, and reasonable use of antibiotics is very important [2].

Diagnosis of neonatal septicemia infection in the early hours offers better control and good patient's outcome. The standard method to determine whether a neonatal septicemia is in progress is a positive blood culture. It is found that the Klebsiella Pneumoniae was the most common bacteria causing neonatal septicemia, followed by Staphylococcus Aureus [3]. In the United States and Australia, Group B streptococcus was considered as the most common pathogenic bacteria causing neonatal septicemia. In recent years, due to frequent isolation, Acinetobacter is becoming increasingly important as a potential pathogen in neonatal septicemia. Some studies reported that bacterial infections associated with sepsis was prolonged ICU stay [4]. Because of long-term hospitalizations (mainly in ICUs), highly complex and invasive procedures, and the great need for broad-spectrum antibiotics, neonatal septicaemia generates enormous expenses for the health system [5]. Timely diagnosis of neonatal septicemia, reduction of antibiotic resistance, and evaluation of the influencing factors of ICU hospitalization days are of great significance for formulating appropriate treatment plans, shortening the NICU hospitalization time, and reducing the disease burden [6].

Material and Methods

The present observational study was conducted at ABVGMC Vidisha, M.P for 6 months duration, on 165 cases. Under aseptic conditions blood samples (1–2 ml) were collected from suspected neonates and inoculated directly into brain heart infusion (BHI) broth containing blood in a ratio of (1:5). Samples were transported to Microbiology laboratory at the earliest. Samples with mismatch patient details as per CRF; sample without case referral form (CRF); inadequate and leaked samples were rejected as per protocol. On reception culture bottles were incubated at 37 °C for 5–7 days and were examined daily for growth and turbidity. Following incubation subcultures were made on inhouse prepared MacConkey and Blood agar plates and incubated aerobically at 37 °C for 18-24 hours. The pure colonies obtained from subculture plates were processed as per standard microbiological techniques (colony morphology, direct microscopy, various biochemical reactions) for identification. Antibiotic susceptibility test was performed for identified isolate by Modified Kirby-bauer disk difusion method as Clinical and Laboratory Standards Institute (CLSI), United States guidelines.

Result

Table 1: Gender Distribution

S. No.	Gender	No.	Percentage	P Value
1	**Male**	98	59.3	.036165
2	**Female**	67	40.7	

Male newborns had the highest frequency 98(59.3%) of positive blood cultures as compared to female child which was 67(40.7%). The chi-square statistic is 0.2513. The *p*-value is .036165. The result is significant at $p < .05$.

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Table 2: Neonatal sepsis group

S. No.	Parameters	No.	Percentage	P Value
1	**EOS**	86	52.1	.046332
2	**LOS**	79	47.9	

Newborn in EOS group- 86(52.1%) had highest frequency of positive blood culture as compared to LOS group- 79(47.9%). The chi-square statistic is 0.9714. The *p*-value is .046332. The result is significant at $p < .05$.

Table 3: Bacteriological profile of neonatal sepsis from suspected neonates

Organism isolated	Number(n)	Percentage (%)	P Value
A. Gram Positive cocci			
1. Staphylococcus aureus (MRSA)	44	59.4	
2. Enterococcus spp.	05	6.8	
B. Gram Negative bacilli			
1. Acinetobacter spp.	05	6.8	< 0.00001
2. Citrobacter spp.	05	6.8	
3. Escherichia coli	04	5.4	
4. Klebsiella pneumoniae	02	2.7	
C. Candida spp.	09	12.1	
Total	**74**	**100**	

Out of 165 samples, 91(55.2%) samples showed NO growth of organism. Whereas 74(44.8%) samples showed positive growth of micro-organism. Out of 74 culture positive cases 49(66.2%) were Gram Positive cocci and 16(21.6%) Gram Negative bacilli. Staphylococcus aureus (MRSA)- 44(59.4%) was most common bacterial species isolated followed by Enterococcus spp.- 05(6.8%), Acinetobacter spp.- 05(6.8%), Citrobacter spp.- 05(6.8%) respectively. Other species isolated were Escherichia coli, Klebsiella pneumoniae. The chi-square statistic is 25.703. The *p*-value is < 0.00001. The result is significant at $p < .05$.

Table 4: Antimicrobial susceptibility pattern (Resistance %) of Gram Positive strains isolated from suspected cases of neonatal sepsis

Antibiotics	Staphylococcus aureus (MRSA) n=44 (%)	Enterococcus spp. n=05 (%)
Chloramphenicol	06 (13.6)	0
Clindamycin	33 (75)	05 (100)
Doxycycline	06 (13.6)	01 (20)
Gentamicin	26 (59.1)	N/A
Cotrimoxazole	20 (45.)	03 (60)

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Levofloxacin	23 (52.3)	05 (100)
Azithromycin	41 (93.2)	05 (100)
Ciprofloxacin	32 (72.7)	05 (100)
Penicillin	44 (100)	05 (100)
Ceftriaxone	41 (93.2)	05 (100)
Vancomycin	13 (29.5)	04 (80)
Linezolid	10 (22.7)	0

Among Gram positive isolates**,** Staphylococcus aureus (MRSA) was least resistant to chloramphenicol-6(13.6%) and doxycycline-6(13.6%) followed by linezolid-10(22.7%), vancomycin-13(29.5%) respectively. Making chloramphenicol and doxycycline as the most effective drug for treatment of Staphylococcus aureus (MRSA) in our study. Penicillin- 44 (100%) showed the highest resistivity followed by Azithromycin- 41(93.2%), Ceftriaxone- 41 (93.2%) for Staphylococcus aureus (MRSA). Most sensitive drug for Enterococcus spp. was linezolid, chloramphenicol, and doxycycline. Clindamycin, levofloxacin, azithromycin, Ciprofloxacin, penicillin, ceftriaxone were 100% resistive antibiotics against Enterococcus spp.

Table 5: Antimicrobial susceptibility pattern (Resistance %) of Gram Negative strains isolated from suspected cases of neonatal sepsis

Antimicrobials	**Acinetobacter spp. n=05 (%)**	**Citrobacter spp. n=05 (%)**	**Escherichia coli n=04 (%)**	**Klebsiella pneumoniae n=02 (%)**
Amikacin	04 (80)	05 (100)	01 (25)	02 (100)
Amoxiclav	05 (100)	05 (100)	04 (100)	02 (100)
Ampicillin	05 (100)	05 (100)	04 (100)	02 (100)
Ceftriaxone	05 (100)	05 (100)	04 (100)	02 (100)
Ciprofloxacin	04 (80)	05 (100)	04 (100)	0
Gentamicin	04 (80)	05 (100)	01 (25)	02 (100)
Tobramycin	04 (80)	05 (100)	03 (75)	02 (100)
Piperacillin-tazobactam	05 (100)	05 (100)	04 (100)	02 (100)
Ceftazidime	05 (100)	05 (100)	04 (100)	02 (100)
Meropenem	03 (60)	05 (100)	03 (75)	02 (100)
Doxycycline	03 (60)	01 (20)	0	02 (100)
Levofloxacin	03 (60)	03 (60)	02 (50)	0
Cotrimoxazole	03 (60)	01 (20)	01 (25)	0

Among Gram Negative bacilli, Acinetobacter spp. shows 100% resistance to amoxiclav, ampicillin, ceftriaxone, piperacillin-tazobactam, ceftazidime. Cotrimoxazole and doxycycline were the most sensitive antibiotics against Citrobacter spp. Escherichia coli was most sensitive to amikacin, gentamicin and cotrimoxazole whereas amoxiclav, ampicillin, ciprofloxacin, ceftriaxone, piperacillin-tazobactam, ceftazidime showed 100% resistivity. All isolates of Klebsiella pneumoniae showed 100% sensitivity to ciprofloxacin, levofloxacin,

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cotrimoxazole and amikacin, amoxiclav, ampicillin, ceftriaxone, gentamicin, tobramycin, piperacillin-tazobactam, ceftazidime, meropenem, doxycycline were found to be 100% resistive.

Discussion

Neonatal bacterial sepsis is a major cause of death in developing countries. The emergence of antibiotics resistant bacteria and its dissemination is exacerbated by inappropriate antimicrobial consumption and precarious living condition. The most common organisms associated with neonatal sepsis vary with time of infections and geographical location [7]. Therefore, information on bacteriological profile of neonatal sepsis and effective antimicrobials for its treatment are important to combat with neonatal morbidity and mortality issues. This study found 44.8% of neonates had microbiologically confirmed sepsis. The incidence rate of this study is in line with previous studies which have reported the incidence rate above 40% [8]. The high incidence rate is due to non-institutional delivery, low birth weight, underlying co-morbid infections, referral from other hospital and clinics, poor infection control practices, long ICU stays, overuse/unmonitored use of antibiotics. Most of these referred cases had the history of antibiotics therapy prior to referral. The finding of prevalence of positive blood culture in relation to different neonatal risk factor can be useful to determine the preventive measures for neonatal sepsis. The higher rate of growth positivity was observed in male compared to female neonates. This finding is in agreement with previous studies [9]. The prevalence of positive blood culture was found to be higher in 3 or above 3 days of age (late onset of sepsis) compared to below 3 days of age (early onset of sepsis). Most of the previous studies have shown similar pattern of high prevalence of neonatal sepsis in late onset of sepsis [10]. Prolonged use of invasive ventilator and catheter, failure of early breast feeding, longer use of parenteral nutrition, hospitalization, surgery, cardiovascular diseases, and respiratory infections lead to late onset of sepsis among neonates [11-12]. In our study Staphylococcus aureus (MRSA) was most common species isolated followed by Enterococcus spp., Acinetobacter spp., which is similar to the findings of Yadav NS et al., 2018 [13]. Our study has shown the frequency of isolation of Gram-Positive bacteria was higher compared to Gram-Negative bacteria. Studies by Yadav NS et al., 2018 and Jatsho J et al., 2020 showed Gram Negative bacteria isolated more frequently as compared to Gram Positive [13, 14]. This finding may be attributed to variation in geographical area, maternal vaginal flora, transmission from community, hospital environment, health care workers, family members. Our study shows Gram positive bacteria being most sensitive to doxycycline, chloramphenicol and linezolid; and were resistive to azithromycin and ceftriaxone. Cotrimoxazole was most sensitive antibiotic for Gram Negative isolates. Antibiotics like amoxiclav, ampicillin, ceftriaxone, piperacillin-tazobactam, ceftazidime showed 100% resistivity for Gram Negative isolates. These findings of antibiotic susceptibility pattern are in congruence with the studies of Yadav NS et al., 2018 and Pokhrel B et al., 2018 [13,5]. This emphasis on judicious, monitored and appropriate use of antibiotics to combat development of antimicrobial resistance among micro-organisms.

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Conclusion

Emergence of antibiotic resistance among bacterial isolates from neonatal sepsis is a major cause for treatment failure, higher morbidity and mortality. Proper antibiotic guidelines and its effective implementation could be milestone for revolution in the field of antibiotic resistance control. The epidemiology of neonatal sepsis, causative risk factors and antibiotic resistance pattern of pathogens may be used to develop guidelines for management of neonatal sepsis. This study summarized common pathogens and associated drug sensitivity, and factors influencing ICU stay length, prompting us to strengthen nosocomial infection control and formulate discriminating plans to prevent infections, reducing morbidity and length of ICU stay.

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