BACTERIOLOGICAL PROFILE OF NEONATAL SEPSIS AND ANTIBIOGRAM PATTERN OF THE ISOLATES IN NATIONAL MEDICAL COLLEGE AND TEACHING HOSPITAL, BIRGUNJ.

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ABSTRACT

Introduction: Neonatal sepsis continues to be a major cause of neonatal mortality and morbidity. A very wide spectrum of organisms and their susceptibility pattern has been described for cases of neonatal sepsis and these spectrums is subject to geographical alterations.

Material and Methods: A prospective hospital based study was carried out with the objective to study the etiological agents of neonatal sepsis and their antibiogram pattern. Blood culture was performed from all the suspected cases of neonatal sepsis from National Medical College and Teaching Hospital. Antibiotic susceptibility test, ESBL test and Methicillin-resistance test were performed according CLSI guidelines (2014). SPSS version 19 software was used for data analysis.

Results: In total 255 blood samples were studied, out of which 79 (31%) were culture positive. The organisms isolated were *Staphylococcus aureus* followed by CONS and *Enterococcus faecalis*. The most common risk factors associated with neonatal sepsis were low birth weight and preterm delivery, whereas clinical features were fever, respiratory distress and seizure. Meropenem, levofloxacin and amikacin was found most effective antibiotic against all isolates. Among total isolates 89.9% (71/79) multi drug resistant organisms were isolated. Among Gram-negative pathogen, 68.5% (13/19) were ESBL producers and among Gram-positive, 77.3% (34/44) *Staphylococcus* species were methicillin resistant.

Conclusion: Staphylococcal sepsis is common in both community and hospital acquired sepsis. The multidrug resistant strains make the treatment difficult and grave sequel. Knowledge of sensitivity patterns is essential when local polices on the uses of antibiotics are being devised.

Keywords: *Antibiogram pattern, Bacteriological profile, Neonatal sepsis.*

INTRODUCTION

"Neonatal period" is defined as the first 28 days of life and "sepsis" is defined as systemic inflammatory response syndrome (SIRS); an inflammatory cascade that is initiated by the host in response to infection. Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first 28 days of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infection.[1] Neonatal sepsis remains as an important cause of morbidity and mortality among infants in the world. About 45% of neonatal deaths account for all deaths among children under age five and 22 per 1000 live birth in Nepal. [2]

The neonatal sepsis can be categorized into two types; Early-onset neonatal sepsis (EOS) and late onset neonatal sepsis (LOS). EOS has been variably defined based on the age at onset, with bacteremia occurring at ≥72 h in infants hospitalized in the neonatal intensive care unit (NICU), and LOS ≤72 h of birth.[3] Various maternal and fetal risk factors are associated with sepsis.[3,4] It is caused by both Gram-negative and Gram-positive bacteria sometimes yeasts. Most common etiological agents are *E-coli, Pseudomonas, Klebsiella, Proteus, Staphylococcus aureus*, CONS. However Group B *Streptococci, Acinetobacter, Citrobacter, Enterobacter, Haemophilus influenza, Serretia, Listeria monocytogenes*, and anaerobes can also cause neonatal sepsis.[3-5]

Infection occurs either in fetal life, during birth or after birth, can be acquired either from the community or at the nursery in the hospital. Although neonatal sepsis can be treated with suitable antibiotics, nowadays due to emergence of multidrug resistant (MDR) organisms, treatments of neonatal sepsis becomes challenging for the medical officers. So identification of the proper causative agent and suitable antibiotic for treatment of the infection is very important for the favorable outcome.

The main objectives of this study is to isolate causative agent of neonatal sepsis from blood cultures of neonates admitted in NICU suspecting sepsis and analyze the antibiogram pattern of common isolates along with to find the correlation of clinical features (fever, respiratory distress, poor feeding, poor cry, jaundice, cyanosis, seizure, vomiting) and risk factors (birth weight, gestation age, place of delivery, mode of delivery, sex of baby) with culture proven sepsis.

MATERIAL AND METHODS

A prospective hospital based study was conducted at National Medical College and Teaching Hospital (NMCTH), Birgunj, Parsa, Nepal between January to June 2019. Ethical clearance was taken from Institutional Review Committee of NMCTH Birgunj. A total 255 neonate admitted in NICU and premature baby care unit (PBU) with suspicion of sepsis were investigated. Babies more than 28 days and not clinically suspected for sepsis were excluded. Information about the suspected neonates was collected directly by interviewing parents, interaction with nurse and pediatricians by using semi-structured questionnaire. 1 ml blood sample was drawn from the antecubital or femoral vein either by doctors or trained nurses under aseptic condition from suspected neonates, dispensed into screw capped bottle of Brain Heart Infusion Broth (BHI), transported within an hour to the laboratory. The culture bottle was labeled with laboratory identification number and incubated at 37°C for 7 days. The culture bottle was observed and examined daily for any visible growth such as turbidity, hemolysis of red blood cells, formation of gas bubbles and clot formation of discrete colonies. The broth cultures were subcultured on Blood agar, Mac-conkey agar and Chocolate agar plates as laboratory standard method.[6] Culture plates were observed for bacterial growth after 24 hrs incubation.

Identification of significant isolates was done depending upon morphological appearance of the colonies, Gram staining reactions, and biochemical properties. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method recommended by guidelines.[7] Primarily the antibiotics were chosen on the basis of the use in hospital, however, most antibiotics were selected according Clinical and Laboratory Standard Institute (CLSI) guidelines.[7]

ESBL testing was performed (in case of Gram negative isolates) by double disk diffusion method and methicillin resistant strains are isolated by disc diffusion method among *Staphylococcus* species as described in CLSI.[7]

Data were analyzed by SPSS version 19 software and P value less than 0.05 was considered to be significant.

RESULTS

In total, 255 blood cultures from neonates admitted in NICU and PBU of NMCTH on suspicion of sepsis were studied. Among 255 sample studied, 79 (31%) of blood samples showed positive growth of organism. Gram-positive isolates were predominated over Gram-negative isolates. Most common isolates were *Staphylococcus aureus* followed by CONS, *Enterococcus faecalis, Klebsiella pneumoniae* as shown in table 1. Among total sample, 30% (60/200) and 34.54% (19/55) were proven sepsis in EOS and LOS respectively. Different risk factors and their correlation with culture proven sepsis were studied which was shown in table 4. Preterm and very low birth weight (VLBW) of neonates showed high growth positivity.

All 255 neonates, showed different sign and symptoms due to which they were admitted and suspected for sepsis. The most common and prevalent clinical features and their association with neonatal sepsis was shown in table 5. Among these respiratory distress, fever and poor cry were most common clinical features.

Antibiotics susceptibility pattern of all isolates showed high sensitivity with meropenem, tetracycline, chloramphenicol, levofloxacin. Sensitivity patterns of Gram-positive and Gram-negative isolates were shown in table 2 and 3 respectively. Among total 89.9% (71/79) of the isolates were multidrug resistant. Among 60 Gram-positive isolates 53 (88.3%) and among 19 Gram-negative isolates 18 (94.7%) were multidrug resistant respectively.

Methicillin resistant strains were detected among *Staphylococcus* species. 24 (85.7%) strains of *S. aureus* and 10 (62.5%) strains of CONS showed resistance against methicillin. Overall representation of methicillin resistant strains is shown in figure 1. Among Gram-negative isolates, most of the *P.aeruginosa* strains were ESBL producers. Comparision of ESBL producers among Gram-negative isolates were shown in figure 2.

Table 1 Bacterial etiological agents isolated from blood culture in neonate suspected with sepsis

Table 2

Etiological agents	EOS No (%)	LOS NO (%)	Total No (%)
S. aureus	21 (35)	7 (36.8)	28 (35.5)
CONS	13 (21.7)	3 (15.8)	16 (20.3)
E. faecalis	12 (20)	4 (21.1)	16 (20.3)
K.pneumoniae	7 (11.7)	3 (15.8)	10 (12.7)
P.aeruginosa	4 (6.7)	2 (10.5)	6 (7.5)
E. coli	2 (3.3)	0	2 (2.5)
P. mirabilis	1 (1.7)	0	1 (1.2)
Total	**60 (100.1)**	**19 (100.0)**	**79 (100)**

Antibiotic susceptibility pattern of Gram-positive isolates

Antibiotics	*S. aureus* No (%)	CONS No (%)	*E. faecalis* No (%)
Penicillin	1(3.6)	0	0
Azithromycin	14 (50)	4(25)	Not Tested
Ceftriaxone	4(14.3)	3(18.8)	1(6.3)
Linezolid	19(67.9)	11(68.8)	13(81.3)
Tetracycline	22(78.6)	11(68.8)	10(62.5)
Vancomycin	21(75)	14(87.5)	14(87.5)
Levofloxacin	18(64.3)	13(81.3)	2(12.5)
Chloramphenicol	24(85.7)	15(93.8)	13(81.3)
Amikacin	19(75)	12(87.5)	2(18.7)
Gentamicin	19(67.9)	12(75)	2(12.5)
HLG(high level gentamicin)	Not Tested	Not Tested	7(43.8)
Cefixime	3(10.7)	2(12.5)	0
Meropenem	24(85.7)	15(93.8)	7(43.8)

Table 3 Antibiotic susceptibility pattern of Gram-negative isolates

Antibiotics	*K. pneumoniae* No (%)	*P. aeruginosa* No (%)	*E. coli* No (%)	*P. mirabilis* No (%)
Ampicillin	0	0	0	1(100)

Amikacin	7 (70)	5(83.3)	100	1 (100)
Gentamicin	3(30)	4(66.7)	1(50)	1(100)
Cefepime	0	0	1 (50)	1(100)
Cefoxitin	0	0	0	1(100)
Ceftriaxone	0	0	1(50)	0
Levofloxacin	6 (60)	6(100)	2(100)	100
Meropenem	10(100)	5(83.3)	2(100)	100
Piperacillin	4(40)	1(16.7)	0	0
Chloramphenicol	8(80)	6(100)	2(100)	100
Tetracycline	8(80)	5(83.3)	2(100)	0

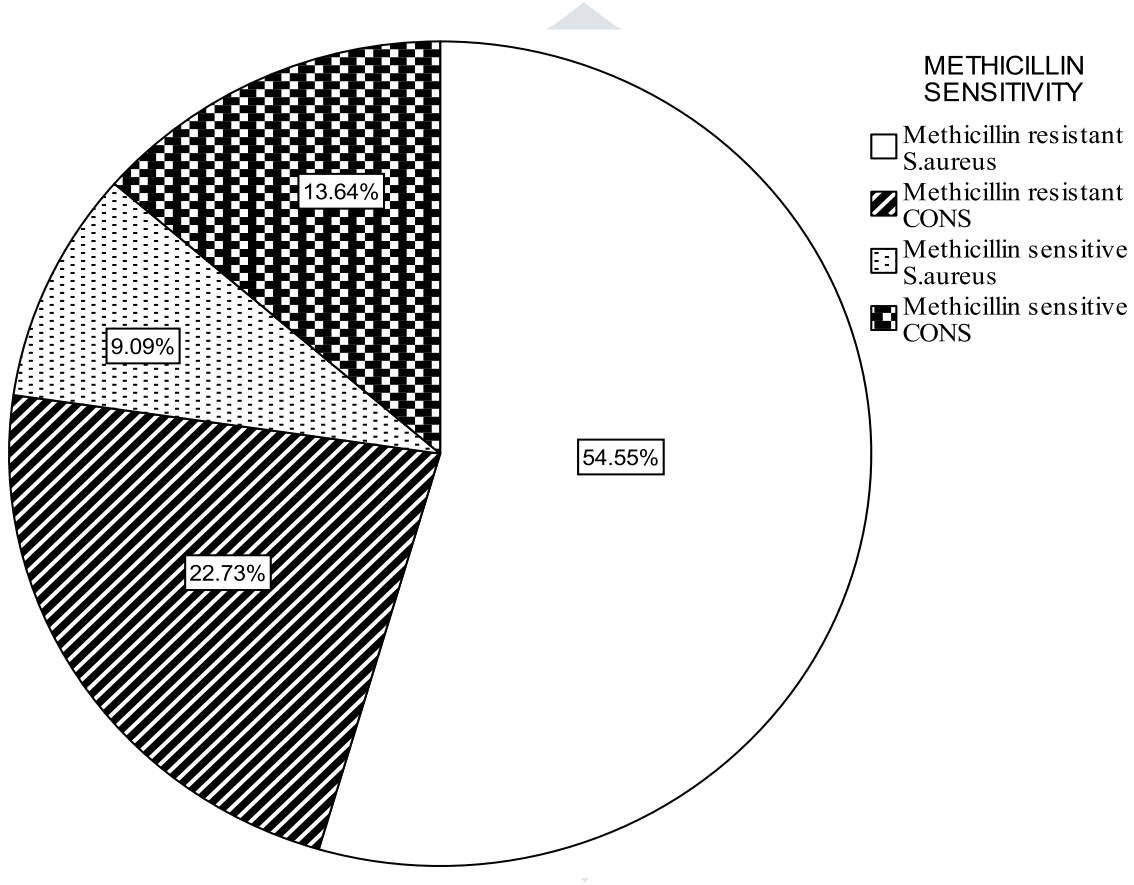

Figure 1 Distribution of methicillin susceptibility pattern among *Staphylococcus* species

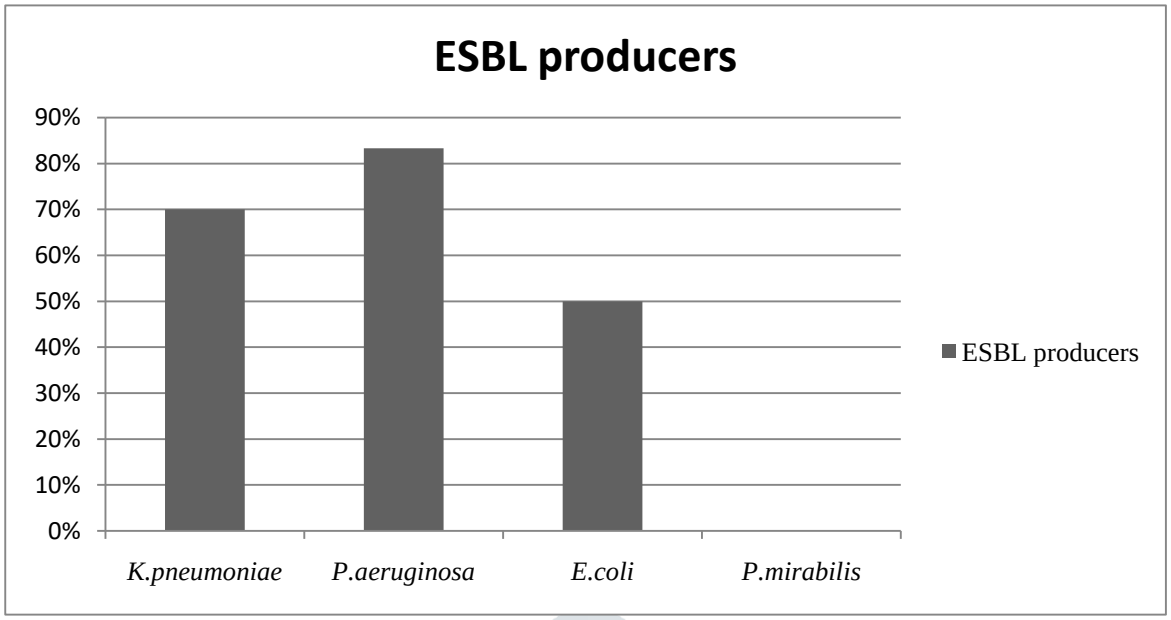

Figure 2 Comparison of ESBL producers among Gram-negative isolates

Table 4 Association between predisposing risk factors with culture proven sepsis

Characteristics	Growth of organisms No (%)	Total No (%)	P value
Mode of delivery			
Normal	58(30.9)	188(73.7)	
Cesarean	17 (27.9)	61(23.9)	>0.05
Vaccum	4(66.7)	6(2.3)	
Place of delivery			
Hospital	54(27.1)	199(78.0)	
Home	0(0)	2(0.78)	>0.05
Other Hospital	25(46.3)	54 (21.2)	
Gestation age			
Preterm(<37 weeks)	46(41.4)	111(43.5)	
Term(37-42 weeks)	32 (23.9)	134(52.5)	<0.05
Post term(>42 weeks)	9(10)	10(3.9)	
Birth weight			
VLBW (>1500g)	11(47.8)	23 (9)	
LBW (1500-2500g)	35(44.9)	78(30.6)	<0.05
Normal (2500-4000g)	33(21.6)	153(60)	
Overweight (>4000g)	0(0)	1(0.4)	
Gender			

Male	53(29)	183(71.8)	>0.05
female	26(36.1)	72(28.23)	

Table 5 Common clinical features among neonates suspected for sepsis

Sign and symptoms	Culture positive No (%)	Culture negative No (%)	P- value
Respiratory distress			
Yes	62 (38.5)	99 (61.5)	0.001
No	17 (29.1)	77 (64.9)	
Poor cry			
Yes	27 (25.7)	78 (74.3)	0.128
No	52 (34.7)	98 (65.3)	
Poor feeding			
Yes	30 (33.7)	59 (66.3)	0.490
No	49(29.5)	117 (70.5)	
Cyanosis			
Yes	5 (17.9)	23 (82.1)	0.111
No	74 (32.6)	153 (67.4)	
Fever			
Yes	55 (53.9)	47 (46.1)	0.000
No	24 (15.7)	129 (84.3)	
Seizure			
Yes	36 (66.7)	18 (33.3)	0.000
No	43 (21.4)	158 (78.6)	
Jaundice			
Yes	10 (22.2)	35 (77.8)	0.161
No	69 (32.9)	141 (67.1)	
Vomiting			
Yes	6 (26.1)	17 (73.9)	0.595
No	73 (31.5)	159 (68.5)	

DISCUSSION:

During the study period, 79 (31%) blood culture reports were positive. The isolation rate of bacteria in this study was comparable to previous study.[8-10] The incidence of neonatal septicemia varies different countries as well as different places in same countries. This rate is high comparing with previous study carried out at other

institute within the country.[5,11] This may be due to the fact that most of the infected neonates were referred from other hospitals and health centers, where they already go through antibiotic treatments.

In present study, EOS cases are most common than LOS but culture positivity was high in LOS which is similar to previous study.[12] This is due to that culture positive cases were mostly referred from other hospitals; similarly the preterm babies acquire infection during hospital stay.

The frequency of isolation of Gram-positive and negative bacteria from blood culture in this study was 60 (75.95%) and 19 (24.05%) respectively and most common isolates was *S. aureus* similar type of results were reported from previous studies.[4,8,11] *S. aureus* is the common hospital acquired organisms which accounts for most of the infections and there is high risk of the transmission of *S. aureus* to neonates from health care workers and relatives. CONS in similar way emerging one of the common causative agent of sepsis, as it is normal flora of human skin, during catheritization, vein puncture or other instrumentation, get chance to enter into blood and cause sepsis. Among Gram-negative, most commonly *K. pneumoniae* and *Pseudomonas aeruginosa* were isolated.

Among total samples, 183 samples from male and 72 samples from female neonates were investigated. Most of studies showed growth rate is higher in male than female neonates but there was no any statistical significance with sex of neonates and sepsis.[8,11,13] In this study growth positivity was high in female neonates similar with previous study. [14-15] This may be the fact that female neonates were smaller in number and premature than their male counterparts and therefore at greater risk for complications. Preterm and low birth weight neonates showed highly significant association with sepsis, this is due to low immune power and incomplete growth of the neonates. The incidence of neonatal sepsis is higher in preterm babies than full term and normal birth weight neonates.[10,14,16]

Regarding place of delivery, blood culture positivity was high in other hospital born neonates because they were referred from after infection for tertiary care. Statistically the place of delivery and sepsis showed insignificant association.[13] In this study growth positivity was high in normal vaginal delivery this may be due to poor hygienic practice but statistically there was no association between the mode of delivery and growth of the organisms, similar as other studies.[14,16]

There is several clinical manifestation of neonatal sepsis, some common clinical features studied in this study and their associations with sepsis were showed in table 5. Significant association was found between respiratory distress, fever and seizure with blood culture positive result, similar with previous studies.[4,10] Some neonates showed other features like poor feeding, cyanosis, jaundice and vomiting with culture positive result but statistically they had not significant association, similar as other studies.[4,14]

Among the total isolated organisms most of them were MDR organisms. Gram-negative organism showed more resistance than Gram-positive isolates similar with previous study.[11] Gram-positive organisms showed high sensitivity with vancomycin, tetracycline, chloramphenicol, meropenem, intermediate sensitivity with linezolid, levofloxacin, amikacin, gentamicin, and low sensitivity toward azithromycin, penicillin, cefixime, ceftrioxones. Similar types result was reported by other studies where vancomycin becomes drug of choice.[8-9] Among Gram-positive organisms, *E. faecalis* showed high resistance against the selected antibiotics. Only 43.8% (7/16) was sensitive with HLG (gentamicin) which is specific antibiotics for highly resistant *Enterococci* species.

In this study, 85.7% (24/28) MRSA and 62.5% (10/16) MRCONS were isolated. Emergence of methicillin resistant *S. aureus* and CONS is increasing day by day and arises as big problem for health professionals as they are resistant to all β-lactamses including cephalosporins and carbapenems.[4,9] Some new generations of cephalosporins and carbapenems are sensitive to them, so to overcome the problem vancomycin was discovered, but vancomycin resistant strains are also emerges day by day.

Gram-negative organisms showed high sensitivity with meropenem, amikacin, levofloxacin, tetracycline, chloramphenicol, intermediate sensitivity with piperacillin, gentamicin, and low sensitivity with cefepime, cefotaxime ceftazidime, ceftriaxone, cefoxitin. Several studies reported that most of the Gram-negative organisms were ESBL positive that is, they are resistant to many antibiotics like penicillins, 3rd generation

cephalosporins, monobactams and sensitive to cephamycins and carbapenems. In this study 68.4% (13/19) Gram-negative isolates were ESBL producers. Due to these MDR and ESBL producing characters, meropenem and amikacin is used as drug of choice against Gram-negative organisms.

According to WHO, 1st line drug used for neonatal sepsis is ampicillin along with aminoglycosides and third line drug is levofloxacin, cefotaxime, amikacin but due to high resistance to these drugs, now commonly used antibiotics for treatments are carbapenems, vancomycin, levofloxcin and amikacin in NMCTH, Birgunj, which is comparable with other studies.[9,11] In this study, although chloramphenicol and tetracycline showed highest sensitivity with almost all isolates but due to its selective uses if patients are allergic to primary drug and its side effects, these drugs were not recommended as well as not used routinely but can be preserve for future use.

Development of sepsis in a neonate is a medical emergency and generally the clinicians do not wait for microbiology report and start treatment empirically. If the infant is not infected he or she is being subjected to unnecessary treatment, this may also remove susceptible organisms and encourage resistant ones, which become one of cause to using more expensive, high dose or new combination of antibiotics. Therefore, an urgent evaluation and development of antibiotic policies and protocols for neonatal sepsis is important for management of neonatal sepsis along with maintaining personal hygiene, using gloves, proper cleanliness of wards is important to overcome nosocomial spread.

CONCLUSION

Staphylococcal sepsis is common in both community and hospital acquired sepsis. Premature and low birth weight neonates were more prone to infection. The multidrug resistant strains, ESBL producers, methicillin resistant strains make the treatment difficult and grave sequel which is life threatening. Due to an alarmingly high degree of antibiotic resistance the local database should be prepared, monitored and reviewed regularly regarding the etiological agent and their antibiotic susceptibility pattern, which guide the clinicians for empirical treatment.

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