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A Cross Sectional Study of Blood Culture, C Reactive Protein, Complete Blood Count in Neonatal Sepsis, in Tertiary Care Hospital, Salem

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A B S T R A C T

Neonatal sepsis is defined as a clinical syndrome of bacteremia with systemic signs and symptoms of infection in the first 28 days of life. Neonatal sepsis is classified as early onset sepsis (EOS) within 72 hours of birth and late onset sepsis (LOS) after 72 hours of birth. Neonatal sepsis is one of the major causes of mortality in neonates and it is responsible for 30 – 50 % of total neonatal death each year in developing countries. The emergence of antibiotic resistance to commonly used antibiotics lead to difficulty in the treatment of neonatal sepsis. Early diagnosis and treatment will lead to decrease in morbidity and mortality due to neonatal sepsis. The main aim and objectives of this stuy includes to estimate the prevalence of early and late onset neonatal sepsis in NICU. To study the bacteriological profile and its antibiotic susceptibility pattern of pathogens causing neonatal sepsis. Also to analyse correlation between CBC and CRP values along with blood culture among NICU patients. Most common organisms isolated in our institution by our study from total isolate of 120 are *Klebsiella pneumoniae* were 40 (33.30%), Coagulase negative *Staphylococcus* spp were 17 (14%). CRP is elevated in 40% of patients at the admission and 80 % after 72 hours of admission. Most of the patient have total leukocyte count more than 11000 – 72 (48%). 67 (44.6%) patient have more than 9000 of absolute neutrophil count in per mm3, which indicates bacterial infection. Antibiotic susceptibility pattern of Gram negative organism isolated in neonatal sepsis isolates show sensitivity in the order of 75 (83 %) Imipenem, 72 (79.50%) Piperacillin-tazobactum, 71 (78.30%) to Cefaperazone sulbactam, 68 (74.6%) to Gentamicin,, 67 (73.40%) to Amikacin,9(60%) to Doxycycline, 52 (59%) to Ciprofloxacin, 36 (38.5%) to Ceftazidime, 29 (37.3%) to Cotrimoxazole, 32 (36.10%) to Cefotaxime, 30 (33.70%) Amoxyclav. Neonatal sepsis is a major cause of death in developing countries which is largely preventable with rationale antimicrobial therapy. The most common pathogen associated with neonatal sepsis vary with time of infection and geographical location. Therefore, continuous monitoring of bacteriological profile in NICU and its antibiotic susceptibility pattern is needed. Early detection of the resistance pattern and strict follow up of antibiotic stewardship program and adherence to hospital antibiotic policy would reduce the emerging multidrug resistance, neonatal mortality and morbidity.

Introduction

Neonatal sepsis is defined as a clinical syndrome of bacteraemia with systemic signs and symptoms of infection in the first 28 days of life. Neonatal sepsis is classified as early onset sepsis (EOS) within 72hours of birth and late onset sepsis (LOS) after 72 hours of birth. Neonatal sepsis is one of the major causes of mortality in neonates and it is responsible for 30 – 50 % of total neonatal death each year in developing countries (Kumhar and Ramachandran, 2002; Meem, *et al.,* 2011). The emergence of antibiotic resistance to commonly used antibiotics lead to difficulty in the treatment of neonatal sepsis. Early diagnosis and treatment will lead to decrease in morbidity and mortality due to neonatal sepsis (Agarwal, *et al*., 2016; Haker, *et al.,* 2017).

The main aim and objectives of this study to estimate the prevalence of early and late onset neonatal sepsis in NICU. To study the bacteriological profile and its antibiotic susceptibility pattern of pathogens causing neonatal sepsis. And also to analyse correlation between CBC and CRP values along with blood culture among NICU patients.

Neonatal sepsis remains a significant cause of mortality in developing countries. Severe sepsis mortality ranges from 28% to 50%. Markers of inflammation such as C-RP, ESR, procalcitonin & CBC were still useful investigation for sepsis in NICU. CRP > 6 mg/l; Total leukocyte count<5000/mm are useful markers in diagnosing the both early onset sepsis & late onset sepsis. However blood culture remains the gold standard method for diagnosis of sepsis, but it is time consuming (Kayange, *et al.,* 2010). Hence timely diagnosis is usually a challenge in developing countries like India. In Early onset sepsis, signs and symptoms of sepsis appear within first 72 hours of life. In severe cases, the neonate may be symptomatic at birth. Usually neonates present with respiratory distress, pneumonia and meningitis. The source of infection is mostly of maternal in origin which was mostly acquired from mother prior to or during labour. In late onset sepsis, signs and symptoms of sepsis present after 72hours of life. The neonates usually present with septicemia, pneumonia or meningitis (Hornik, *et al*., 2012; Hornik, *et al*., 2017). The source of infection usually from the environment either nosocomial or community acquired. Suspected neonatal sepsis was considered if neonate had clinical symptoms of perinatal risk factors i.e. maternal pyrexia (within 1 week prenatal and/or 48 hours postnatal), prolonged rupture of membranes (18 hours), foul smelling vaginal discharge or/and maternal urinary tract infection diagnosed in last month. Neonates having unexplained hypothermia/hyperthermia, lethargy, irritability, poor feeding or milk intolerance, respiratory dysfunction evidenced by apnea (>10sec.), tachypnoea (>60 breaths/minute), cardiovascular dysfunction such as persistent tachycardia (>160 beat/min) or bradicardia (<100 beats/min), poor peripheral circulation, hypotonia or circumoral cyanosis or pallor were also included (Newman, *et al*., 2010; Sankar, *et al*., 2016). Culture positive sepsis is defined as laboratory confirmed blood stream infection ie.,pathogen is isolated from blood/CSF/sterile body fluids in neonates suspected to have sepsis based on clinical manifestations, risk factors and treatment with antibiotic therapy for septicemia. Positive sepsis screening is, consider as any two of the following parameters present in neonate. 1. Total leukocyte count (TLC) < 5000/mm3, 2. Immature to Total polymorph ratio (IT)> 0.2, 3. Absolute neutrophil count (ANC) <1800/ mm3, 4. C - reactive protein>6 mg/dl, 5. Micro ESR>15 mm. Presence of two abnormal parameters in a screen gives a sensitivity of 93-100% and specificity of 83%(13). Among this, most sensitive indicator is C reactive protein, Total Leucocyte count and Absolute Neutrophil count. CRP is a plasma protein which is synthesized in hepatocytes during infection and inflammation.CRP has best predictive value when it is measured within 24 to 48 hr of onset of infection. CRP has sensitivity of 41 to 96% and specificity of 72 to 100%. The increase in CRP value is better predictor than individual CRP value. Repeatedly negative CRP gives strong prediction against systemic bacterial

infection. This study was undertaken to determine the prevalence of the neonatal sepsis, to isolate the most common pathogen causing neonatal sepsis and their sensitivity pattern for framing the antibiotic policy for the NICU and to assess the correlation between CBC, CRP and blood culture.

Materials and Methods

Type of Study – Prospective & Cross-Sectional Study Period – 3 Months (August 2022–October 2022) Sample Size – 150 Inclusion criteria - < 28 days of NICU babies with signs and symptoms of sepsis and presence of risk factors Exclusion Criteria - > 28 days of NICU without signs and symptoms of sepsis Collection and processing of blood sample: Blood was collected from all neonates who were suspected to have sepsis before starting antibiotic therapy. Under strict aseptic precautions blood samples were collected. The venipuncture site was selected and skin on the site was cleaned thoroughly with 70% isopropyl alcohol in circular manner from centre to periphery. The same steps were repeated two times. The site was allowed to dry for 2 minutes (30). 1-2 ml of blood is collected and inoculated into 10-20 ml of Brain Heart infusion broth (BHI broth). It was incubated at 37℃ aerobically. The blood culture bottles were observed daily for macroscopic evidences of growth such as turbidity, hemolysis of red blood cells, gas bubbles, surface pellicle, clot formation. After 24 hours of aerobic incubation at 37℃, BHI blood culture broth was subcultured on Blood agar and MacConkey agar plates. Blood culture broth bottles were examined daily and subculture was done on solid media when there was a visible signs of growth. Blood culture broth bottles that did not show any visible signs of growth were sub cultured on solid media after 48hours, 5th and 7th day of incubation at 37℃. Blood culture was reported as negative if no growth was detected at the end of 7 days of incubation. Organisms were identified by colony appearance and further processed to biochemical identification test. 0.5 ml of blood is collected for CRP testing, serum was separated.CRP done by latex agglutination test (Kit Name). A second sample for determination of CRP was drawn 72 hours after the first one. Two CRP samples were taken, one at the time of admission and second one at 72 hours after the first one. CRP were read as negative when level was less than 5mg/dl and positive when level was more than 5mg/dl. Blood culture was followed for growth up to 7 days. Suspected neonatal sepsis patients were started on empirical antibiotic therapy on admission and first CRP and blood culture were sent for analysis. The first CRP is negative, continue antibiotic therapy and if the second CRP is also negative, the antibiotic therapy was discontinued. If the second CRP becomes positive, the antibiotic therapy is continued or changed, looking at the clinical symptoms of the neonate. If first and second CRP both were positive, the therapy was continued and the culture and sensitivity report were awaited for making decision regarding the antibiotic therapy. Antibiotics were given according to the culture and sensitivity report.

Results and Discussion

In this study, 150 patients with suspected neonatal sepsis were taken. Age and gender wise distribution: The study included age ranged from 1 up to 28 days. Males were 89(59.3%) of while 61(40.6%) were female patients. Male to female ratio 1.5:1 Neonatal age was divided into four categories out of which most presented in young age i.e. less than or equal to 5 days which was 79 (52.4%). Neonates of age 6-10 days were 35 (23.3%), 5 (3.3%) were of age range 11-15 days and presented at age more than 15 days 5(3.3%), ≥16-28 days is 1 (0.6%) Table 1 shows Out of 150 Neonates suspected had symptoms of neonatal sepsis 79 (52.4%) less than or equal to 5 days old, 35 (23.3%) of 6-10 days of old neonates, 5 (3.3 %) of 11 – 15 days of old neonates, 1(0.6%) of more than 16 days old- 28 days had symptoms of neonatal sepsis.

Table 1 shows, Out of 150, 75 (48%) were Male, 45 (30%) were Female Neonates suspected had symptoms of neonatal sepsis and blood culture came positive 35 out of 150 nenoates 14 (11%) male and 11 (9%) female showed no growth when blood culture was done. Table 2 shows, CRP was negative in 90 patients (60 %) at the time of admission, in 60

(40 %) patients CRP was found to be elevated at the time of admission. After 72 hours, CRP is found to be elevated in 120 (80 %) cases which indicates the sepsis, 30 (20%) cases were found to be negative in patients suspected of sepsis.

Above table 3 and chart shows out of 120 neonates most of the patient with blood culture positive neonatal sepsis had total leukocyte count more than 11000 is 72 (60 %). 42 (28%) had TLC in 4000-11000 range, only 6(4 %) of patients had counts less than 4000. 30 (20%) out of 150 neonates, have blood culture negative have total leckocyte count values of 4000- 11000 Above table 4 shows patient with absolute neutrophil count in per mm3 in 120 out of 150 blood culture positive neonatal sepsis, less than 1700 is about 16 (10.6 %), 35 (24.6%) patient have 1700-9000 Absolute Neutrophil count, 67(44%) patient have more than 9000. 30 (20%) out of 150 blood culture negative patients have Absolute Neutrophil count in per mm3 of 1700- 9000 Table 5 shows, Among120 isolates, *Klebsiella pneumonia* were 40 (33.30%), Coagulase negative *Staphylococcus* spp were 17(14%), Acinetobacter spp were 15 (12.50%), Klebsiella oxytoca were 12 (10%), *Pseudomonas aeruginosa* were 8(6.60%), *Staphylococcus aureus* were 7 (5.80%), *Escherichia coli* were 6(5%), *Candida* spp were 6 (5%), *Enterococcus* spp were 5 (4.10%), *Streptococcus* spp were 2 (1.60%), *Citrobacter* spp were 2(1.60%). Above table 6 shows the Antibiotic susceptibility pattern of Gram negative organism isolated in neonatal sepsis isolates show sensitivity in the order of 75 (83 %) Imipenem, 72(79.50%) Piperacillin-tazobactum, 71 (78.30%) to Cefaperazone sulbactam, 68 (74.6%) to Gentamicin, 67(73.40%) to Amikacin, 9 (60%) to Doxycycline, 52(59%) to Ciprofloxacin, 36(38.5%) to Ceftazidime, 29(37.3%) to Cotrimoxazole, 32 (36.10%) to Cefotaxime, 30(33.70%) Amoxyclav. Above table 7 shows antibiotic susceptibility pattern of gram positive organism in neonatal sepsis 31(100%) show sensitivity to Linezolid, 31(100%) to Vancomycin, 5(100%) to High level gentamicin, 17 (70.83%) to Doxycycline, 21 (67.7%) Ciprofloxacin, 20 (64.5%) Penicillin, 20 (64.5%) Ampicillin, 16 (62%) Clindamycin, 13 (54.17%) Cefoxitin, 15(51.7 %) Erythromycin, 11 (45.8%) Cotrimoxazole.

In our study there was slight male predominance, male to female ratio 1.5: 1. • Most common age group affected is less than or equal to 5 days which was 79 (62.4%) out of 120, indicates early onset sepsis. • CRP is elevated in 40% of patients at the admission and 80 % after 72 hours of admission which indicates repeat CRP level should be done after 72 hours of admission for neonatal sepsis screening. • Most of the patient have total leukocyte count more than 11000 – 72 (48%), 42 (28%) have TLC in 4000- 11000 range, only 6(4%) of patients have counts less than 4000. • 67(44.6%) patient have more than 9000 of absolute neutrophil count in per mm3, which indicates bacterial infection. • Most common organisms isolated in our institution by our study from total isolate of 120 are *Klebsiella pneumonia* were 40 (33.30%), Coagulase negative *Staphylococcus* spp were 17(14%). • Then organisms are isolated in the order of *Acinetobacter* spp were 15 (12.50%), *Klebsiella oxytoca* were 12(10%), *Pseudomonas aeruginosa* were 8(6.60%), *Staphylococcus aureus* were 7 (5.80%), *Escherichia coli* were 6(5%), Candida spp were 6 (5%), *Enterococcus* spp were 5 (4.10%), *Streptococcus* spp were 2 (1.60%), *Citrobacter* spp were 2(1.60%). • Antibiotic susceptibility pattern of Gram negative organism isolated in neonatal sepsis isolates show sensitivity in the order of 75 (83 %) Imipenem, 72 (79.50%) *Piperacillin- tazobactum*, 71(78.30%) to *Cefaperazone sulbactam*, 68 (74.6%) to Gentamicin, 67(73.40%) to Amikacin, 9(60%) to Doxycycline, 52(59%) to Ciprofloxacin, 36(38.5%) to Ceftazidime, 29(37.3%) to Cotrimoxazole, 32 (36.10%) to Cefotaxime, 30(33.70%) Amoxyclav. • Antibiotic susceptibility pattern of gram positive organism in neonatal sepsis 31(100%) show sensitivity to Linezolid, 31(100%) to Vancomycin, 5(100%) to High level gentamicin, 17 (70.83%) to Doxycycline, 21 (67.7%) Ciprofloxacin, 20 (64.5%) Penicliin, 20 (64.5%) Ampicilin, 16 (62%) Clindamycin, 13 (54.17%) Cefoxitin, 15 (51.7 %) Erythromycin, 11 (45.8%) Cotrimoxazole.

Table.1 Age and Gender Wise Distribution In Neonatal Sepsis

Characteristics In Days	**Neonatal sepsis suspected – blood culture done**		
	Positive	Negative	Total
≤ 5	79 (52.4 %)	15(10%)	94 (62.6%)
6 -10	35 (23.3%)	10(6.6%)	45 (30%)
11 – 15	5 (3.3 %)	1(0.6 %)	6 (4%)
≥16- 28 days	1(0.6%)	4(2.6%)	5 (3.3%)
Total	120	30	150
Gender			
Male	72 (48%)	17 (11.3%)	89 (59.3%)
Female	48 (32%)	13(8.6%)	61(40.6%)
Total	120	30	150

Table.2 CRP Values In Neonatal Sepsis

CRP at the time of admission		After 72 hours		Total (n= 150) 100%
Positive	Negative	Positive	Negative	
60 (40 %)	90 (60%)	120(80 %)	30(20%)	

CRP value (mg/dl)	Frequency
6-10	69 (57 %)
10-20	27 (18%)
20-50	16(13%)
50-60	8 (6.6%)
Total	120

Table.3 Total Leukocyte Count Values Correlation With Patient Suspected With Neonatal Sepsis

	Total leukocyte counts in per mm3	**Patients admitted in NICU**	**Percentage**
Blood culture positive (n=120)	Less than 4000	6	4%
	4000 –11000	42	28 %
	11000-20000	49	32.6 %
	20000-25000	23	15.3%
Blood culture negative (n= 30)	4000 –11000	30	20%
Total		150	100%

Table.4 Absolute Neutrohil Count Values Correlation With Patient Suspected With Neonatal Sepsis

	Absolute neutrophil count in per mm3	Patients admitted in NICU	Percentage
Blood culture positive (n= 120)	Less than 1700	16	10.6 %
	1700 –9000	37	24.6 %
	9000-12000	67	44.6 %
Blood culture negative (n=30)	1700 –9000	30	20 %

Table.5 Distribution of Microorganism Isolated from Culture Positive Neonatal Sepsis

Name of organism	Total number of Isolate	Percentage
Klebsiella pneumoniae	40	33.30%
Coagulase negative Staphylococcus spp	17	14%
Acinetobacter Spp.	15	12.50%
Klebsiella oxytoca	12	10%
Pseudomonas aeruginosa	8	6.60%
Staphylococcus aureus	7	5.80%
Escherichia coli	6	5%
Candida spp	6	5%
Enterococcus spp	5	4.10%
Streptococcus spp.	2	1.60%
Citrobacter spp	2	1.60%
TOTAL (n)	120	100%

Table.6 Antibiotic Susceptibility Pattern of Gram Negative Organism in Neonatal Sepsis

Organism / Drugs	G	AK	CTX	AMC	COT	CIP	PIT	CFS	CAZ	DO	IMP
Klebsiella pneumoniae (n=40)	32	33	15	20	15	29	35	34	14	NT	36
Acinetobacter Spp. (n=15)	11	10	8	NT	6	7	14	14	7	9	14
Klebsiella oxytoca (n=12)	10	10	5	7	6	8	10	10	6	NT	10
Pseudomonas aeruginosa (n=8)	7	6	NT	NT	NT	5	6	6	4	NT	7
Escherichia coli (n=6)	6	6	2	2	1	3	6	6	4	NT	6
Citrobacter spp (n=2)	2	2	2	1	1	0	1	1	1	NT	2
Total (n=83) / Percentage	68 (74. 60%)	67 (73.4 0%)	32 (36.1 0%)	30 (33.70 %)	29 (37.3 0%)	52 (59%)	72 (79.5 0%)	71 (78.3 0%)	36 38.50 %	9 60%	75 83%

Table.7 Antibiotic Susceptibility Pattern of Gram Positive Organism In Neonatal Sepsis

Organism	P	AMP	DO	CX	E	LZ	VA	CD	COT	CIP	HLG
Coagulase negative Staphylococcus (n=17)	10	10	12	10	7	17	17	9	8	9	NT
Enterococcus spp. (n=5)	5	5	NT	NT	5	5	5	NT	NT	5	5
Staphylococcus aureus (n=7)	3	3	5	3	3	7	7	5	3	5	NT
Streptococcus spp. (n=2)	2	2	NT	NT	NT	2	2	2	NT	2	NT
Total (n=31)	20	20	17	3	15	31	31	16	11	21	5
Percentage	64.51%	64.51%	70.83%	54.17%	51.72%	100%	100%	62%	45.83%	67.74%	100%

Fig.1 Chart Age and Sex Wise Distribution In Neonatal Sepsis

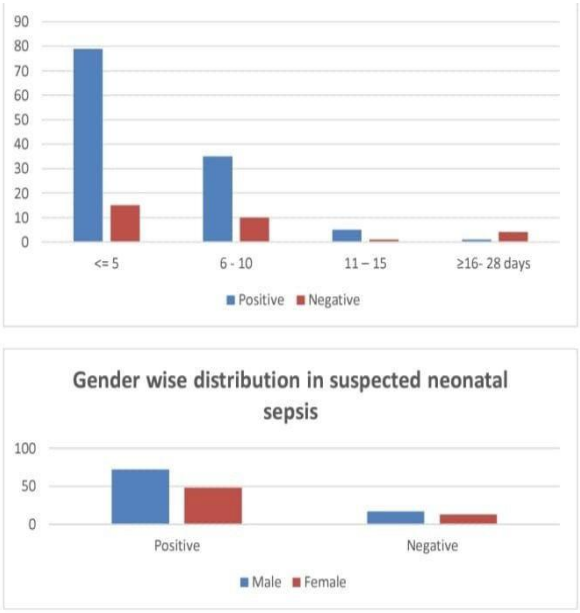


Fig.2 Chart CRP In Neonatal Sepsis

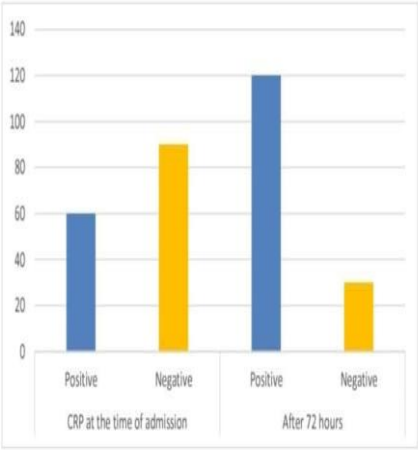


Chart.1 Total Leucocyte Count

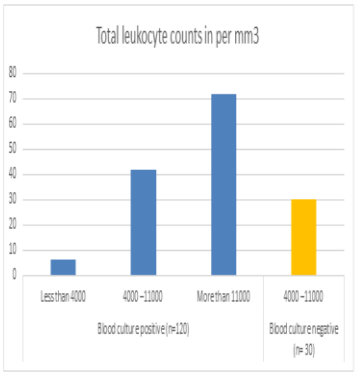


Chart.2 Absolute Neutrophil Count

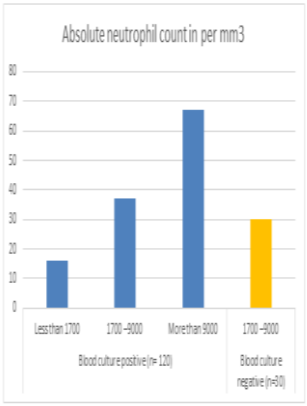


Chart.3 Total Isolate from Neonatal Sepsis

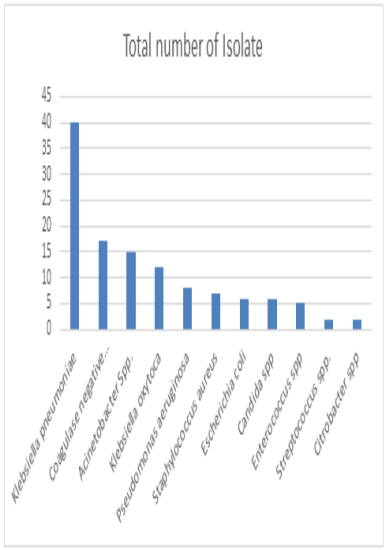


Chart.4 Antimicrobial Susceptibility Pattern of Gram Negative Organism

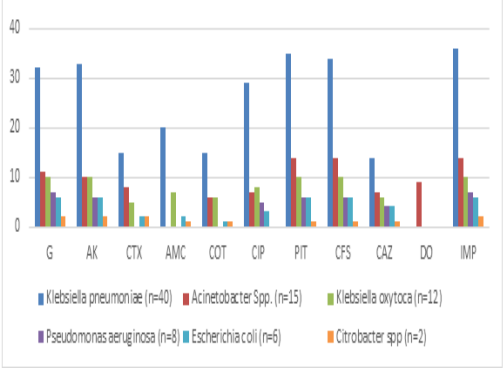
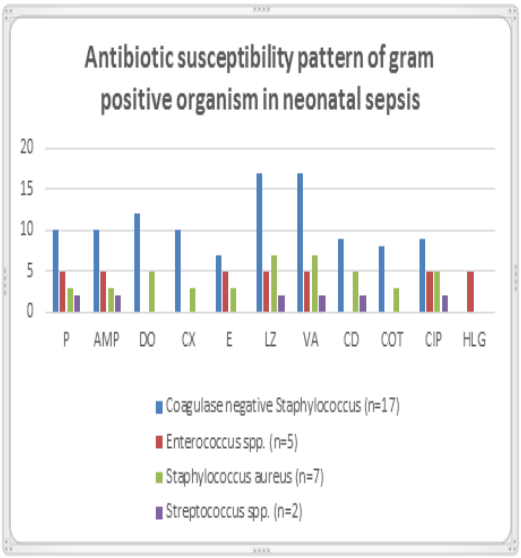


Chart.5 Antimicrobial Susceptibility Pattern of Gram Positive Organism



The delayed induction of the hepatic synthesis of CRP during the inflammatory response to infection lowers its sensitivity during the early phases of sepsis. The performance of serial determinations 24–48 h after the onset of symptoms is recommended, as it clearly improves diagnostic accuracy (Shah, *et al*., 2012; Satheesh Kumar *et al*., 2018). C-reactive protein has shown high performance in early diagnosing cases of neonatal sepsis. Its sensitivity, specificity, positive and negative predictive values were 95.7%, 82.4%, 70.2%, and 97.8%, respectively. Therefore, CRP may be useful in poor resource countries where blood culture is not available or while waiting for blood culture results. It may help deciding of initiation or discontinuation of the empiric antibiotic therapy (Shalini Tripathi, *et al*., 2010; Venkata, *et al*., 2018). CRP may, thus, help in the earlier recognition or diagnosing of neonatal sepsis whilst waiting for a blood culture report and lead to a subtle time reduction of the treatment, which in turn leads to judicious use of antibiotics undergoing CRP-guided therapy, making it one of the feasible parameters. Additionally, CRP recording at serial intervals could help modify antibiotic regimens accordingly and help manage the cases, thus shortening the hospital stay of neonates and preventing the emergence of resistance, decreasing treatment costs, reducing adverse effects, and less interference with the microbiome. Blood culture is the gold standard for the diagnosis of neonatal sepsis. However, its positivity rate is low and is affected by blood volume inoculated, prenatal

antibiotic use, level of bacteremia and laboratory capabilities. Gram-negative organisms (Klebsiella, Acinetobacter), CONS, and *S. aureus* are the leading cause of neonatal sepsis in this study, and most of them are resistant to multiple antibiotics. This study concludes that empiric therapy for suspected neonatal septicemia should cover both Gram-negative bacilli and Gram-positive cocci particularly *Klebsiella pneumoniae* and Staphylococcus spp. Ciprofloxacin and Amikacin, these two antibiotics can be included as empirical therapy for neonatal septicemia. Emergence of antibiotic resistance among bacterial isolates from neonatal sepsis is a major cause for treatment failure, higher morbidity and mortality. An effective infection-control programme, regular antibiotic susceptibility surveillance and evaluation, and the enforcement and periodic review of the antibiotic policy of the hospital as well as the encouragement of rational antibiotic use will reduce the rates of acquiring nosocomial infections and development of bacterial resistance.

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