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Bacteriological Profile and Antibiotic Sensitivity and Resistance Pattern of Neonatal Sepsis in Tertiary Care Teaching Hospital in North Karnataka, India- A Prospective Observational Study

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ABSTRACT

Introduction: Pediatricians always face a constant challenge in treating the neonatal sepsis due to the changing patterns of the microbial flora. Appropriate microbiological surveillance and assessment of antimicrobial resistance is a key component in reducing the rate of neonatal sepsis and its associated mortality.

Material and Methods: This prospective observational study was carried out in NICU of Shri B M Patil Medical College Hospital and Research Centre, Vijayapura for a period of 1 year. For all clinical suspected cases of neonatal sepsis, laboratory screening for sepsis was done along with Blood culture and antibiotic susceptibility testing. The data was analyzed using descriptive statistics.

Results: Out of 1250 admissions in NICU during the study period, 475 neonates were suspected with clinical septicemia. 180 (37.9%) neonates had culture positive sepsis. Among 180 newborns enrolled, 64% cases had early onset sepsis, while 36% had late onset sepsis. Most common presentation was respiratory distress in the form of hurried breathing; chest in drawing (37%). Of 180 positive blood cultures, 97 (54%) were Gram negative bacteria, 72(40%) Gram positive bacteria and 11(6%) were Fungal Sepsis. The most common organism isolated was Klebsiella (31%) followed by staphylococcus aureus (21%). Gram negative organisms were sensitive to Meropenem (68%), followed by Piperacillin-Tazobactam (52%), Netilmicin (48%), Amikacin (38%) and Gentamicin (32%). Gram positive organisms showed good sensitivity to Vancomycin (69.4%), Linezolid (52%) and Amoxicillin (35%). Gram negative organisms were resistant to Ampicillin (59.1%), followed by Cefotaxim (48.2%). Gram positive organisms were resistant to Ampicillin (66.8%), followed by Amikacin (47%). Fungal organisms were sensitive to Amphotericin B (67%), followed by Fluconazole (33%).

Conclusions: Major cause of mortality was by Gram Negative sepsis followed by Fungal and Gram Positive sepsis. Antimicrobial stewardship program should be implemented to rationalize antibiotic usage to reduce neonatal mortality due to sepsis.

Keywords: Neonatal Sepsis, Blood Culture, Antibiotics, Sensitivity, Resistance

INTRODUCTION

Neonatal Sepsis presents a dynamic challenge to pediatricians worldwide. In developing countries, it is one of the major causes of morbidity and mortality among the newborns.[1,2] The major causes of neonatal death are prematurity (28%), sepsis (26%), and asphyxia (23%) worldwide.[3] Of the total sepsis related neonatal deaths in 2019, 38.9% occurred in South Asia.[4]

Nearly, 84 million neonates died in India in 2019.[4] The current Indian neonatal mortality rate is 23 per 1000 live births and in Karnataka , it is 16 per 1000 live births.[5] Neonatal Sepsis can be defined as "a clinical syndrome characterized by systemic signs and symptoms of bacteraemia during the first 28 days of life". It is labeled as "early onset (first 72 hours of life)" and "late onset" (beyond 72 hours of life)

sepsis.[6] This classification has clinical importance, as early onset neonatal sepsis is generally acquired from pathogens of maternal genital tract, where as late onset sepsis has its origin either from the community or from hospital. About 62% of the infections in South Asia occur in the first 72 hours of life, roughly translating into an incidence of 9.8 per 1000 live births.[7]

Gram Negative Organisms are most commonly encountered organisms from developing countries followed by Gram Positive organisms. Multidrug antibiotic resistance is a rising concern in NICUs particularly in developing countries. The reasons for this resistance are irrational use of antibiotics and unsuccessful infection control in maternity centers.[1]

Pediatricians who manage NICU always face a constant challenge in treating the neonatal sepsis due to the changing patterns of the microbial flora. Appropriate microbiological surveillance and assessment of antimicrobial resistance is a key component in reducing the rate of neonatal sepsis and its associated mortality. Thus regular monitoring and updates on the causes of neonatal sepsis and the antimicrobial sensitivity pattern is important for cost effective treatment and prevention of neonatal sepsis.

This study was conducted to assess the clinical spectrum, bacteriological profile and antibiotic sensitivity pattern of neonatal sepsis in NICU in a tertiary care hospital, so that suitable antimicrobial policy could be made for cost effective management of neonatal sepsis.

MATERIAL AND METHODS

This study was conducted at Level II B NICU of Shri B M Patil Medical College Hospital and Research Center, Vijayapura, Karnataka, India. **Study Duration:** The study was conducted from July 2019 to June 2020 after obtaining Institutional Ethics Committee approval. **Study Design:** Prospective Observational Study. **Inclusion Criteria:** Neonates admitted to NICU with suspected neonatal sepsis were included in the study. **Exclusion Criteria:** Neonates with antibiotic usage prior to hospitalization, neonates who died before reports of blood culture sensitivity were excluded from the study.

Sepsis was suspected in neonates with the maternal history of urinary tract infection, vaginitis and early membrane rupture and clinical or histological chorioamnionitis in last trimester and clinical presentation of one or more of the symptoms like respiratory distress (tachypnea, grunting and chest retractions), refusal of feeds, lethargy, fever, neonatal seizures, vomiting, neonatal jaundice, hypothermia, cyanosis, apnea, and excessive crying, etc. For all clinical suspected cases of neonatal sepsis, laboratory screening for sepsis was done with Complete blood count with Total leucocytes count, I-T ratio, Absolute neutrophil count, C-reactive protein (CRP), micro ESR along with Blood culture and antibiotic susceptibility testing. Samples for blood culture were obtained before the commencement of antibiotics under strict asepsis, and were sent to the microbiological laboratory. Blood samples were collected from a peripheral vein with proper aseptic precautions before starting any antibiotic therapy. All blood cultures were incubated at 37°C and observed for 72 hours for growth of micro-organism. Antimicrobial susceptibility of bacterial isolates was done by disc diffusion method. The neonates with suspected sepsis were started empirically on antibiotics, which were changed according to the sensitivity pattern once the culture report was available. Neonates were classified into two groups according to the timing of sepsis diagnosis: EOS diagnosed ≤ 72 hours of life and LOS diagnosed > 72 hours of life. Demographic, clinical and laboratory data were documented in structured proforma for all included neonates.

STATISTICAL ANALYSIS

The collected data was analyzed using descriptive statistics using the SPSS version 23.0 (SPSS Inc. NY, USA). Association and correlation of qualitative data were tested by chi-square test and Fischer's exact test was applied in quantitative data. A P value <0.05 was considered statistically significant.

RESULTS

Out of 1250 admissions in NICU during the study period, 475 neonates were suspected with clinical septicemia. 180 (37.9%) neonates had culture positive sepsis. Out of these 180 neonates enrolled, 77 (43%) were females and 103 (57%) were males. 73(40%) newborns were having normal birth weight, 51 (28%) were having low birth weight, 39 (22%) were having very low birth weight and

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17(10%) were extremely low birth weight.54 (30%) newborns were delivered via normal vaginal delivery, 17(9%) via assisted NVD and 109(61%) via lower section caesarean section respectively. 124(69%) newborns were between 34-40 weeks gestation, 40(22%) were 28-34weeks gestation, 16(9%) were <28 weeks gestation respectively. 123(68%) newborns were Appropriate for gestational age, 45(25%) newborns were Small for gestational age, 12(7%) were large for gestational age. Baseline Characteristics of 180 neonates enrolled in the study showed no statistically significant difference among the study groups. (Table-1)

Most common presentation was respiratory distress in the form of hurried breathing; chest in drawing (37%), followed by lethargy and refusal of feeds (21%), fever (14%), neonatal seizures (11%). Persistent neonatal jaundice, apnea, abdominal distension and excessive cry were the other presenting symptoms (17%).

Among 180 newborns enrolled, 116(64%) cases had early onset sepsis, while 64 (36%) had late onset sepsis. Of 180 positive blood cultures, 97 (54%) were Gram negative bacteria, 72(40%) Gram positive bacteria and 11(6%) were Fungal Sepsis. Among 98(54%) gram negative organisms, *Klebsiella pneumonia* was seen in 55(56%), *Pseudomonas* 14(14%), E. Coli 12 (12%) and *Acinetobacter* 10(10%). Among 72 (40%) gram positive organism MRSA was the commonest 38 (53%) followed by *Enterococcus* 17 (23%) and Coagulase negative *staphylococci* 9(13%). Among 10 (6%) Fungal Sepsis, C*andida sp.*10 (100%) isolated. (Table2).

Mean birth weight in gram positive organism was 1.95±0.76, in gram negative organism was 2.05±0.97and in fungal sepsis it was 1.45±0.87 (p= 0.112). Average gestational age in gram positive organism 36.78±2.45, in gram negative 35.89±4.56 and in fungal sepsis 35.38±3.18 (p=0.247).

There was no statistically significant difference in terms of gestational age, premature rupture of membranes, maternal fever, meconium stain, Necrotizing enterocolitis, Gastro intestinal bleed and pulmonary hemorrhage among different groups of organisms. (Table-3)

Gram negative organisms were sensitive to Meropenem (68%), followed by Piperacillin-Tazobactam (52%), Netilmicin (48%), Amikacin (38%) and Gentamicin (32%). Gram positive organisms showed good sensitivity to Vancomycin (69.4%), Linezolid (52%) and Amoxicillin (35%). Gram negative organisms were resistant to Ampicillin (59.1%), followed by Cefotaxim (48.2%). Gram positive organisms were resistant to Ampicillin (66.8%), followed by Amikacin (47%). Fungal organisms were sensitive to Amphotericin B (67%), followed by Fluconazole (33%).

Among gram negative organisms, Klebsiella pneumonia showed good sensitivity to Meropenem (61.3%) followed by Piperacillin-Tazobactam (48%) and high resistance was observed to Ampicillin (59%). Among Klebsiella, 5 (9%) cases (Carbapenem Resistant klebsiella) were multi drug resistant, Sensitive only to Colistin (100%). Acinetobacter Sep. showed sensitivity to Meropenem (80%), Piperacillin-Tazobactum (60%) and Netilmicin (50%). *Pseudomonas aeruginosa* showed good sensitivity to Meropenem (57%), Netilmicin (50%) followed by Linezolid (43%). E. Coli was sensitive to Meropenem (75%) and Piperacillin-Tazobactam (58%) and high resistance was observed to Ampicillin (49%). Among gram positive organisms, Staphylococcus aureus showed good sensitivity to vancomycin (68.4%), Linezolid (55%), and high resistance was observed to Ampicillin (57%). CONS were sensitive to Vancomycin (77%), Linezolid (56%) and resistance to Ampicillin (44%) (Table 4).

Klebsiella pneumoniae 24 (60%) was the leading cause of death in the newborns with sepsis followed by Pseudomonas spp. 5 (13%), E- coli 2 (5%), Staph. aureus 6 (15%), Candida sepsis 3(7.5%). (Table 5).

DISCUSSION

Neonatal Septicemia requires rapid approach along with prompt treatment for intact survival and normal neurodevelopment outcome. Antibiotic resistance has become a global concern. Reports of multidrug-resistant organism causing neonatal sepsis in developing countries are rising. There is a constant change of bacterial flora and sensitivity patterns from point in time. For effective management of neonatal septicemia, study of

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bacteriological profile along with the antimicrobial sensitivity pattern plays a crucial role to guide the pediatricians regarding both the empirical and definitive management.

Out of 1250 admissions in NICU during the study period, 475 neonates were suspected with clinical septicemia. 180 (14.4%) neonates had culture positive sepsis. Percentage of neonatal septicemia is similar to study done by Shrestha S et al. (13.6%).[8] Studies have shown differing results ranging as low as 6.1% in study by Shrestha NJ et al. to as high as 21% by Barbara Js et al, 46.6 % by G Eyesus et al. and 56.6% by Jain NK et al respectively.[9, 10, 11, 12.] The wide variation observed in these studies could be due to differences in the lab facilities and influenced by the practice of using antibiotics prior to the hospital arrival.

Out of these 180 neonates enrolled, 43% were females and 57% were males which are similar in comparison to study by Heena et al (63.4%) and Pramila et al (58.6%).[13,14]

Baseline Characteristics of 180 neonates enrolled in the study showed no statistically significant difference among the study groups. 40% newborns were having normal birth weight, 28% were having low birth weight, 22% were having very low birth weight and 10% were extremely low birth weight. Heena et al observed that low birth weight (76.20%) newborns were high.[13] Vikram et al stated similar finding in their research data.[15] In this study, 68% neonates were appropriate for gestational age, 25% neonates small for gestational age and 7% neonates large for gestational age which was similar to Pramila et al study.[14]

Most commonly, it is the gram negative organisms responsible for neonatal sepsis in developing countries.[16] In the present study, blood culture positivity in cases of neonatal sepsis was 37.9%. Khante SV et al[17], reported blood culture positivity of 36.2% in neonatal sepsis similar to the present study. Prevalence rate of blood culture positivity in cases of neonatal sepsis of 39.6% and 35% was reported by Sharma CM et al[18] and Gandhi S et al[19] respectively.

Most common presentation was respiratory distress in the form of hurried breathing; chest in drawing (37%), followed by lethargy and refusal of feeds (21%) in the present study which was similar to studies done by Khante SV et al[17] and Satyamurthi et al.[20]

Among 180 newborns enrolled, 116(64%) cases had early onset sepsis, while 64 (36%) had late onset sepsis. Peterside O et al[21] and Muley VA et al[22], found early onset sepsis in 66% and 66.7% cases respectively and late onset sepsis in 34% and 33.3% cases respectively in accordance with the present study.

In the present study, the Gram negative organisms, Gram positive organisms and Fungal organisms accounted for 54%, 40% and 10% respectively. Studies done by Tak SK et al[23], Shrestha et al[8], Rajana R et al[24] also showed that gram-negative organisms were more frequent causes of neonatal sepsis. Ten of the isolates were candida albicans (100%) which was similar to the study done by Gandhi S et al.[19]

The most common organism isolated was Klebsiella (31%) followed by staphylococcus aureus (21%) which are similar to the studies done by Tak sk et al[23], Shrestha et al[8] and Natasha Sawhney et al.[25]

In our study, 54% cases are due to gram negative organisms followed by 40% gram positive organisms and 6% fungal sepsis. Among 54% gram negative organisms isolated, Klebsiella pneumonia was seen in 56%, Pseudomonas 14%, E. coli 12% and Acinetobacter 10%. Among 40% gram positive organisms, MRSA was the commonest 53% followed by Enterococcus 23% and Coagulase negative staphylococci 13%. Among 6% Fungal Sepsis, Candida sp. 100% isolated. Rehman et al., reported gram negative organisms as the commonest cause of neonatal sepsis with E.coli (36%) accounting for most of cases.[26] Khassawneh et al., from Jordan also reported gram negative organisms as the commonest cause of neonatal sepsis.[27]

"NNPD study" observed that Klebsiella pneumoniae (32.5%) was most common isolated organism followed by Staphylococcus aureus (13.6%) and Escherichia coli (10.6%).[28] Heena et al study also found that Klebsiella (54%) was commonest, followed by Pseudomonas (15.9%) and Escherichia coli (11.1%).[13] It is also similar to Charoo et al, Parvez et al and Swarnkar et al study, where Klebsiella pneumoniae (48.1%) was commonest

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organism followed by Pseudomonas spp (18.5%) and Acetobacter (14.8%).[29,30,31]. On the contrary study by Torkman et al, stated that Enterobacter spp (39.6%) was the commonest organism and Tripti et al, study showed Pseudomonas spp(40%) was the commonest pathogen.[32,33] A study by Kathleen et al found Group-B Streptococci (59.8%) was the commonest organism followed by E-coli (40.2%).[34] Pramila et al were also found Staph. aureus (58.62%) was the most common followed by Klebsiella (16.09%) coagulase negative Staphylococcus (6.89%).[14]

In this study, MRSA was the second most common organism responsible for 21% of all cases. Rehman et al., and Kurein et al., also reported 29% and 13% cases of Staphylococcus aureus responsible for neonatal sepsis respectively.[26,35].

Venkateshan S et al., had reported 5-6% incidence of CONS in neonatal sepsis.[36] In developed countries CONS is the major causative organism of late onset sepsis. Sanghvi K P et al., had reported CONS in 61% cases of neonatal sepsis. CONS were isolated less commonly in this study (5%).[37]

In this study, other than bacterial pathogens, fungal organism isolated were 6 % (Candida spp), similar to Pramila et al., (2.29%).[14] Venkateshan S et al., and Guida et al., had reported 11% and 8% of fungal sepsis in their respective studies.[36,38] In recent study (2009) by Bhat et al., reported that 8.5% of septic VLBW neonates were having fungal sepsis.[39] Calveros T et al., in 2007, have reported incidence of fungal sepsis in 1% of VLBW neonates.[40]

Mortality in neonates admitted with sepsis in our study was 22%. Our centre is the tertiary referral centre in north Karnataka with higher rates of complicate deliveries and admissions, which substantiates the higher mortality rate. Among gram negative sepsis, mortality was 32% while gram positive sepsis had 8% mortality and fungal sepsis had 30% mortality. (P<0. 001) Study by Khassawneh et al., and Akarsu et al., had found similar high mortality in gram negative sepsis.[27,41] Venkateshan S et al., had documented 42% mortality in neonatal sepsis in 1996 which gradually decreased to 20% in 2006.[36] Klebsiella pneumoniae 60% was the leading cause of death in the neonates with sepsis followed by Pseudomonas spp. 13%, Staph. aureus 15%, Candida sepsis 7.5%, E- coli 5%. Vergnano et al also attributes Klebsiella and Pseudomonas to high case fatality rates in his study.[42]

The Gram-negative organisms showed good sensitivity to Meropenem, Piperacillin-Tazobactam, Netilmicin, amikacin and high resistance to ampicillin. This finding is similar to another study done by Satyamurthi et al[20], Pooja et al[43], Pokhrel B et al[44.] The Gram-positive organisms showed good sensitivity to Vancomycin, Linezolid, Teicoplanin and high resistance to Ampicillin. Studies done by Gandhi s et al[19,] Pooja et al[43], and Pokhrel B et al[44] also showed similar findings.

CONCLUSION

Neonatal sepsis contributes to leading cause of mortality in neonates admitted in NICU. Major cause of mortality was by Gram Negative sepsis followed by Fungal and Gram Positive sepsis. Gram negative organisms were the commonly isolated organisms. This study emphasizes that empirical therapy for suspected neonatal septicemia should cover both Gram-negative and Gram-positive organisms particularly Klebsiella pneumoniae and Staphylococcus aureus which were more prevalent in this region. There is also need for regular periodic surveillance of the causative organisms of neonatal sepsis as well as their antibiotic susceptibility patterns to curtail the inappropriate use of antibiotics and emergence of resistant strains and review the hospital antibiotic policy from time to time. There is a need to implement Antimicrobial stewardship program to rationalize antibiotic usage to reduce neonatal mortality due to sepsis. Early detection of sepsis and judicial use of antibiotics are useful to decrease neonatal mortality and the emergence of multidrug resistant bacteria.

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FIGURE 1: ORGANISMS ISOLATED IN NEONATAL SEPSIS

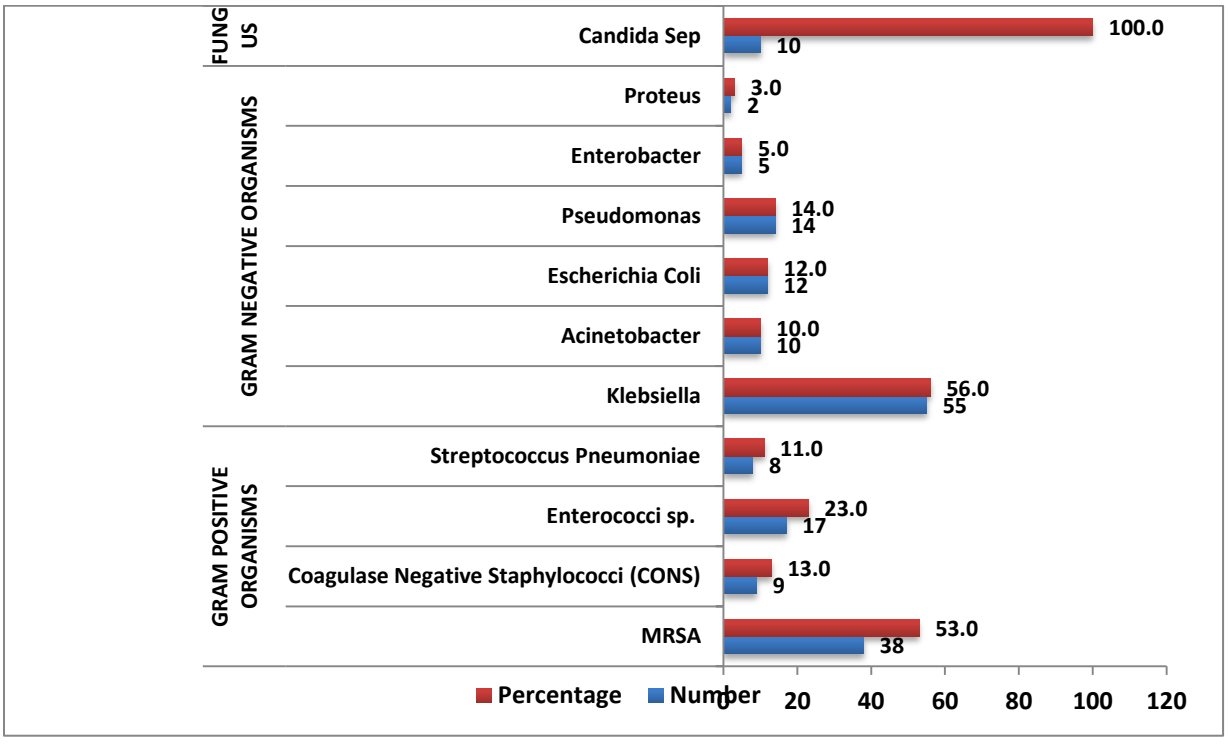

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TABLE 1: BASELINE PARAMETERS IN NEONATAL SEPSIS.

BASELINE PARAMATERS		Gram negative (n=98)	Gram Positive (n=72)	Fungus (n=10)	p value
GENDER	Male	56	40	07	0.688
	Female	42	32	03	
BIRTH WEIGHT (KG)	<1	09	05	03	0.178
	1-1.5	22	14	03	
	1.5-2.5	29	20	02	
	>2.5	38	33	02	
GESTATIONAL AGE	<28wks	09	05	02	0.305
	28-34wks	22	14	04	
	34-40wks	67	53	04	
MODE OF DELIVERY	NVD	30	20	04	0.742
	ASSISTED	07	09	01	
	LSCS	61	43	05	
APPROPRAIATE FOR GESTATIONAL AGE	AGA	69	48	06	0.894
	SGA	22	20	03	
	LGA	07	04	01	

TABLE 2: ORGANISMS ISOLATED IN NEONATAL SEPSIS.

ORGANISMS		Frequency	Percentage %
GRAM POSTIVE ORGANISMS (n= 72)	MRSA	38	53
	Coagulase Negative Staphylococci (CONS)	9	13
	Enterococci sp.	17	23
	Streptococcus Pneumoniae	8	11
GRAM NEGATIVE ORGANISMS	Klebsiella	55	56%
	Acinetobacter	10	10%

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(n=98)	Escherichia Coli	12	12%
	Pseudomonas	14	14%
	Enterobacter	5	5%
	Proteus	2	3%
FUNGUS (n=10)	Candida Sep	10	100%

TABLE 3: VARIOUS PARAMETERS IN NEONATAL SEPSIS.

	Gram Negative (n=98)	**Gram Positive (n=72)**	**Fungus (n=10)**	**P VALUE**
BIRTH WEIGHT	2.05±0.97	1.95±0.76	1.45±0.87	0.112
GESTATIONAL AGE	35.89±4.56	36.78±2.45	35.38±3.18	0.247
PROM	24	16	02	0.912
Maternal Fever	15	11	00	0.842
Meconium Stained Liquor	20	19	00	0.441
Necrotizing Enterocolitis	19	14	03	0.718
Pulmonary Hemorrhage	09	07	02	0.552
G I BLEED	29	22	02	0.789

Note: * significant at 5% level of significance ($p<0.05$)

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Table-4: Antibiotic Sensitivity Pattern of Isolates.

Antibiotics	Gram Negative organisms (%)				Gram Positive organisms (%)		Fungus
	Klebsiella pneumonia (55)	**Acineto Bacter species (10)**	**Pseudomonas aerugonisa (14)**	**E. Coli (12)**	**Staphylococcus aureus (38)**	**Coagulase Negative Staph (09)**	**Candida Species (6)**
Ampicillin	10(18%)	0(0)	0(0)	2(17%)	4(11%)	1(11%)	0 (0)
Gentamicin	18(33%)	5(50%)	4(29%)	5(42%)	5(13%)	2(21%)	0 (0)
Amikacin	21(38%)	4(40%)	6(43%)	6(50%)	7(18%)	2(21%)	0 (0)
Meropenem	34 (61.3%)	8(80%)	8(57%)	9 (75%)	20(53%)	4(45%)	0 (0)
Piperacillin Tazobactam	26 (48%)	6(60%)	5(36%)	7(58%)	17(45%)	4(45%)	0 (0)
Netilmicin	28(51%)	5(50%)	7(50%)	5(42%)	10(26%)	3(33%)	0 (0)
Vancomycin	0 (0)	0 (0)	0 (0)	0 (0)	26(68.4)	7(77%)	0 (0)
Linezolid	0 (0)	0 (0)	6 (43%)	0 (0)	21(55)	5 (56%)	0 (0)
Fluconazole	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2(33%)
Amphotericin B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4(67%)

Table 5- Outcome of Sepsis with Different Organisms Isolated.

Organisms	**DEATH (n=40)**
Klebsiella	24(60%)
MRSA	06(15%)
Pseudomonas	05(13%)
Candida Sep	03(7.5%)
Escherichia Coli	02(5%)

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