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RESEARCH ARTICLE
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EARLY VS LATE ONSET SEPSIS IN NEONATES- TIME TO SHIFT THE PARADIGM?

Mr Birendra Kumar Behera[1*], Dr Hara Prasad Panigrahy[2], Dr Seema Mishra[3], Dr Priyadarshini Debata[4], Dr Siba Sankar Mahapatra[5]

[1*]MSc Microbiology (Berhampur University), Microbiology trainee, IMS and SUM III Berhampur virendrabehera44@gmail.com
[2]PG resident, Dept of Community Medicine MKCG Medical College and Hospital Berhampur, mail id - miki.rocky27@gmail.com
[3]Assistant professor Dept of Microbiology, IMS & SUM III Berhampur, seemam14@gmail.com
[4]Assistant professor, Dept of Microbiology, PGIMER & Capital hospital Bhubaneswar, E mail id: priyadebata4@gmail.com, Phone no :7978479097
[5]DNB Medical oncology resident Apollo Hospitals Navi Mumbai, mahapatra.shiv4@gmail.com

***Corresponding author:** Mr Birendra kumar Behera
*MSc Microbiology (Berhampur University), Microbiology trainee, IMS and SUM III Berhampur virendrabehera44@gmail.com

ABSTRACT

Neonatal sepsis is defined as a clinical syndrome characterized by systemic signs of infection in an infant of 28 days or younger and accompanied by isolation of bacterial pathogens from the blood stream. Nearly one-third of neonatal mortality in India. Pathogens such as *Staphylococcus aureus* and *Klebsiella spp.* are the most common bacteria responsible for neonatal sepsis in India**.**

METHODOLOGY: -The total of 111 samples were collected in (SNCU) ward of MKCG Medical college,Berhampur, Odisha from January 2024 to march 2024. All neonates with blood culture positive sepsis were included in this study and then immediately transported to the laboratory diagnosis. The study revealed that both gram-positive and gram-negative bacteria were associated with neonatal septicemia. Mostly gram-negative bacteria are predominant than gram-positive bacteria.

AIM AND OBJECTIVE OF THE STUDY: To isolate and identify the organisms causing neonatal septicemia & to study the antimicrobial susceptibility pattern of the isolated organisms.

MATERIALS AND METHOD:

Inclusion criteria: Neonates with clinical sign and symptoms of sepsis at the time of admission.
Exclusion criteria: Neonates without sign and symptoms of sepsis. Prior antibiotic administration.
Approximately (1-3ml) of blood was collected from neonates using proper aseptic precaution and inoculated immediately into (30ml) of brain heart infusion broth & sub cultured after overnight incubation on MacConkey agar and 5% sheep blood agar. Any growth was identified by colony

characteristics & standard biochemical test. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method.
DISCUSSION: Among the culture positive samples predominant pathogen isolates were gram negative (56.5%) followed by gram positive (43.47%). *Klebsiella spp.* (38.6%) was most common organism isolates causing neonatal septicemia. Among gram positive organisms *Staphylococcus aureus* was the predominant pathogen.
CONCLUSION: During the neonatal period, septicemia remains an important cause of morbidity and mortality. High level of culture positive neonatal sepsis is of great concern in tertiary health care set up. In this study gram negative bacteria were the predominant organisms causing septicemia. It is important to isolate and identify various organisms causing sepsis in neonates and to study their antimicrobial sensitivity pattern so that proper treatment can be given for better clinical outcome.

INTRODUCTION:
Neonatal sepsis is defined as a clinical syndrome characterized by systemic signs of infection in an infant of 28 days or younger and accompanied by isolation of bacterial pathogens from the blood stream.[1,2] Blood stream infection have been quoted as the most common infections in this age group. A very wide spectrum of organisms has been described for cases of neonatal septicemia and this spectrum is subjected to geographical alteration[3] Based on the timing of clinical presentation, neonatal sepsis is often differentiated into early onset sepsis (**EOS**) and late onset sepsis (**LOS**). **EOS** is defined as any infection presenting in the first 72 hours of life, whereas **LOS** occurs after 72 hours of postnatal life. Globally sepsis is still one of the major causes of morbidity and mortality in neonates, in spite of recent advances in health care units.[4]
Nearly one-third of neonatal mortality in India is due to neonatal sepsis and death occurs in 30% of culture is positive neonates.[5,6] Neonates are particularly vulnerable to infection because of weak immune barrier. EOS occurs usually due to pathogen present in the genital tract of the mother whereas LOS occurs due to pathogen acquired either from the hospital or from the community. There is a gradually increasing trend of multi drug resistant (MDR) pathogens in the tertiary care hospital.[7]
The incidence of sepsis in hospital-based studies is 30 per 1000 live births and in community-based studies, the incidence is (2.7-17%) of all live births. [8,9] Prolonged hospitalization of very low birth weight neonate, use of central lines, catheter and respiratory support for their survival lead to nosocomial infection. Pathogens such as *Staphylococcus aureus, Klebsiella spp., Acinetobacter baumannii, Pseudomonas spp*., are the most common cause of neonatal sepsis in India and southern Odisha.[10]
In spite of great advances in antimicrobial therapy, neonatal life support measures and the early detection of risk factors, septicemia continues to be a major cause of mortality and morbidity among neonates around the world.[11].

AIM AND OBJECTIVE OF THE STUDY:
- To isolate and identify the organisms causing neonatal septicemia.
- To know the MRSA prevalence among neonates with septicemia.
- To study the antimicrobial susceptibility pattern of the isolated organisms.

Blood Stream Infection: A blood stream infection, also known as a bacteremia, blood poisoning or septicemia, is an infection caused by bacteria entering the bloodstream. It occurs when a bacterial infection somewhere in the body, such as in the lungs, intestines, urine or skin, enters the bloodstream.[18]

Symptoms: Fever with chills & rigor, nausea vomiting, Shortness of breath, fast heart rate & confusion.

Sepsis: Sepsis occurs when your body has a strong immune response to the infection. This leads to widespread inflammation throughout the body.

Septic shock: One complication of septicemia is a serious drop in blood pressure, which is called septic shock. Toxins released by the bacteria in the bloodstream can cause extremely low blood flow, which may result in organ or tissue damage.

Acute respiratory distress syndrome (ARDS): This is a life-threatening condition that prevents oxygen in your lungs from reaching your blood. It often results in some level of permanent lung damage. It can also damage your brain, leading to memory problems.

Neonatal Sepsis: Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first month of life. It is a life-threatening medical condition characterized by the presence of a systemic infection in a newborn infant, typically occurring within the first 28 days of life. This condition arises due to the invasion and proliferation of pathogenic microorganisms, including bacteria, viruses, or fungi, within the bloodstream.[11]

Classification of neonatal sepsis: Neonatal sepsis is broadly divided into two types according to age of onset: Early-onset sepsis (before 7days life of born baby) & Late-onset sepsis (after 7-28days of baby)

Early onset sepsis (EOS): Early onset neonatal infections are typically transmitted vertically from the mother to the infant. Organisms can be acquired transplacental via infected amniotic fluid, or by direct contact in passage through the birth canal. (EOS) is defined as onset of features of sepsis within 72hrs of life. [12,13] Most babies with EOS present with respiratory distress and it is associated with substantial morbidity and mortality.

Late onset sepsis (LOS): Late onset sepsis are usually due to organisms acquired from the environment as well as from the caregivers.[14] Late-onset sepsis (LOS) is sepsis occurring after 72 h in NICU infants and 7 days of life in term infants, has been variably defined as occurring up to the age of <90 or 120 days, and may be caused by vertically or horizontally acquired pathogen. Globally, the most frequent bacterial culprit is *Staphylococcus aureus*, followed by *Klebsiella spp.*

MATERIALS AND METHOD:

This is a Prospective observational study. The study was conducted at MKCG, Medical College, Berhampur. Blood samples were collected from neonates admitted at SNCU with clinical evidence of septicemia. The study period was Three months from (Jan 2024-march 2024).

METHODOLOGY:

Sample collection: Approximately (1-3ml) of blood was collected from neonates using proper aseptic precaution and inoculated immediately into (30ml) of brain heart infusion broth with 0.025% Sodium polyanethol sulfonate as anticoagulant agent (Micro express Tulip diagnostic (P) Ltd., Goa). The broth was sub cultured after overnight incubation on MacConkey agar and 5% sheep blood agar. A negative result was followed up by examining the broth daily and doing a final subculture at the end of 7days or appearance of turbidity whichever was earlier. Any growth was identified by colonial characteristics and standard biochemical test.[12] Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method as per the (CLSI) Clinical and Laboratory Standards Institute Recommendation.[13]

Sample Processing: Brain Heart Infusion broth was labeled with (Name, Date and Time of collection) and the culture bottle was brought to the laboratory and then culture bottle was incubated at 37℃ for(18-24hr) after which sub culturing onto the following plates were done using sterile technique:

Mac Conkey agar plate:
Plates were incubated at 37℃ for(18-24hr) aerobically and the growths in the culture plates were observed. The colony size, shape, edge, margin and consistency were noted.
Broth was examined for any turbidity, pellicle formation and deposit. The organisms were identified by colonial morphology, Gram stain and Biochemical test.
Colony Identification:
The colonies in conventional Blood culture were identified as below:

- Small (1-3mm in diameter), circular, low convex opaque colonies and pink colored colonies on MacConkey agar were identified as *Staphylococcus aureus.* They were confirmed by slide and Coagulate, Catalase test.
- Small, circular, low-convex, opaque colonies and greyish or yellowish colonies are usually surrounded by zone of hemolysis on blood agar and were identified as Methicillin Resistance *Staphylococcus aureus*. They were confirmed by slide and Coagulase test, Catalase test.
- Colonies are (2-3 mm in diameter), domed, mucoid, and no pigmented. On MacConkey agar *Acinetobacter baumannii* colonies appeared as small, pale yellow to pink and non/polarity lactose fermented, but the colonies were red colour after short period (2-3) weeks. They may be presumptively identified as aerobic, gram-negative, catalase positive, oxidase-negative, nonmotile, nonfermenting coccobacilli.
- Large, convex, translucent, mucoid with Lactose Fermenting (Pink) mucoid colonies on MacConkey Agar were identified as *Klebsiella spp.* Biochemical tests Indole, Motility, Citrate, Urease, and TSI confirmed the identification of the organism as *Klebsiella spp.*
- Large, flat and spreading, translucent with serrated edge and NLF colonies on MacConkey Agar were identified as *Pseudomonas spp.* Positive Catalase and Oxidase test and TSI with Alkaline Butt and Alkaline slant (K/K) confirmed the identification. Gram stain was done for all the samples.

Tests for pure culture of Gram-negative bacilli: Oxidase test, Indole test, Citrate test, Triple-sugar-iron test (TSI test)

Test for pure culture of Gram-positive cocci: Catalase test, Coagulase test:
MRSA Detection: MRSA strains are detected by using 30µg of Cefoxitin disc as per the CLSI guidelines.

Antimicrobial Susceptibility Testing:

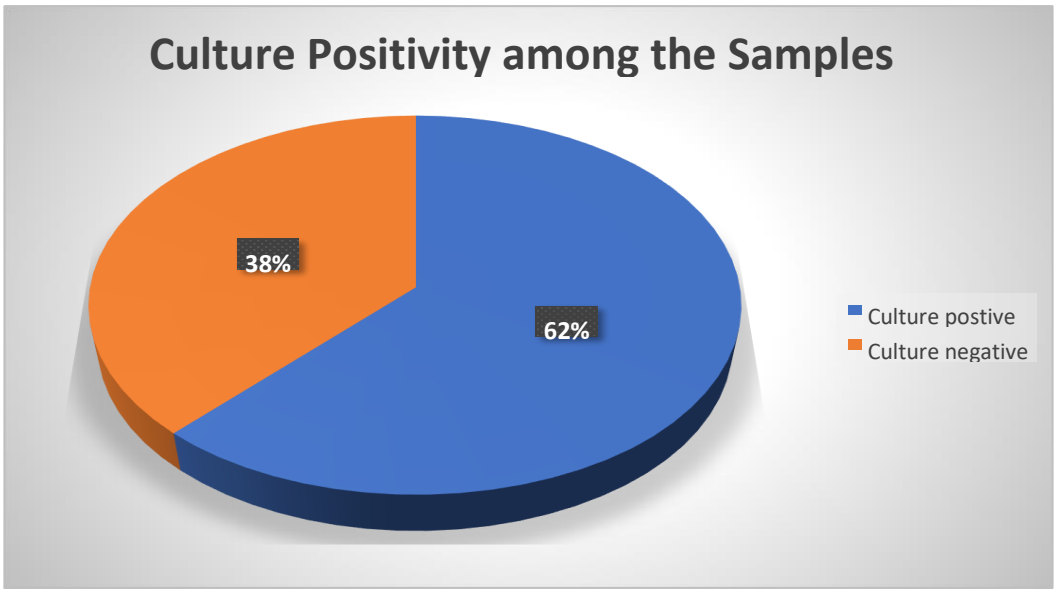
i. Materials required for antibiogram:

A. Muller Hinton agar.
B. Antibiotic disc.

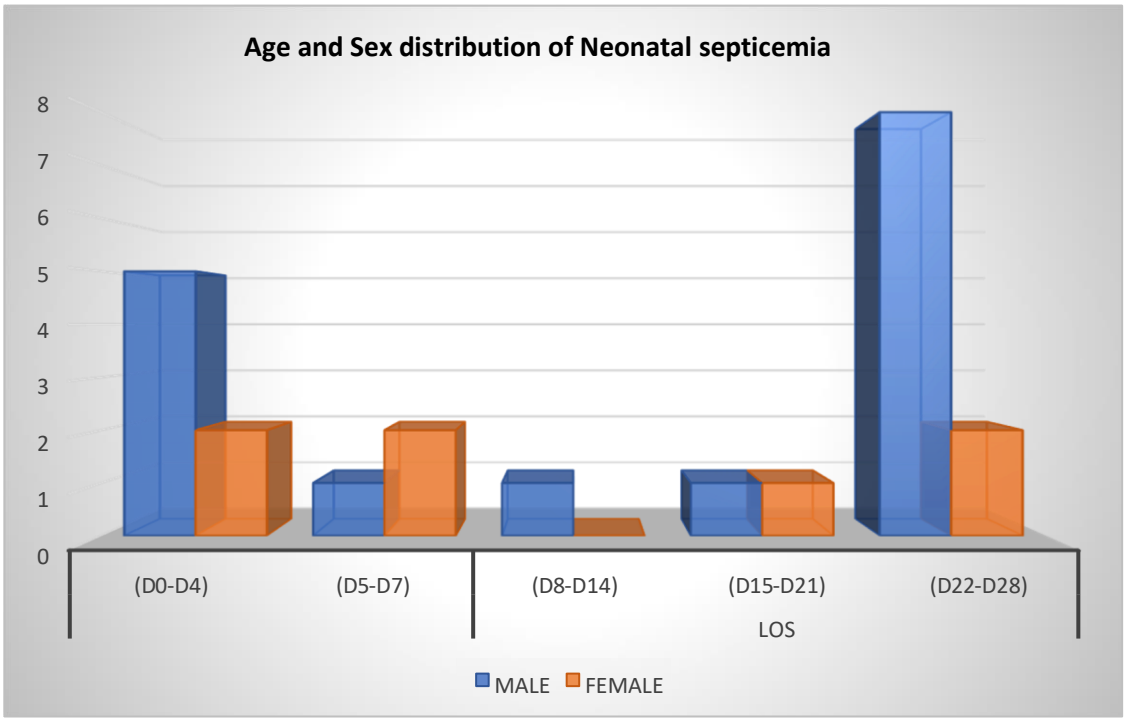
ii. Media preparation (Muller Hinton agar for 100ml):
Muller Hinton agar (3.8gm) (Hi media) was dissolved in 100ml of distilled water, then autoclaved at 121℃ for 15 minutes and was cooled to 52℃ and then about 14ml was poured in the petri dish in a laminar air floor cabinet. Allowed to set, labelled and then stored in the refrigerator.

iii. Procedure of inoculum preparation for Kirby-Bauer disc diffusion testing:
Standard inoculums adjusted to 0.5 Mc Farland was swabbed on Muller Hinton Agar and was allowed to soak for 2 to 5 minutes.
After that antibiotic disc (as per CLSI guideline) were placed on the surface of the media and pressed gently.
Muller Hinton agar plates were then incubated at 37^{o}c for 24 hours.
After 24 hours, the inhibition zones were measured and interpreted by the recommendations of clinical and laboratory standards.

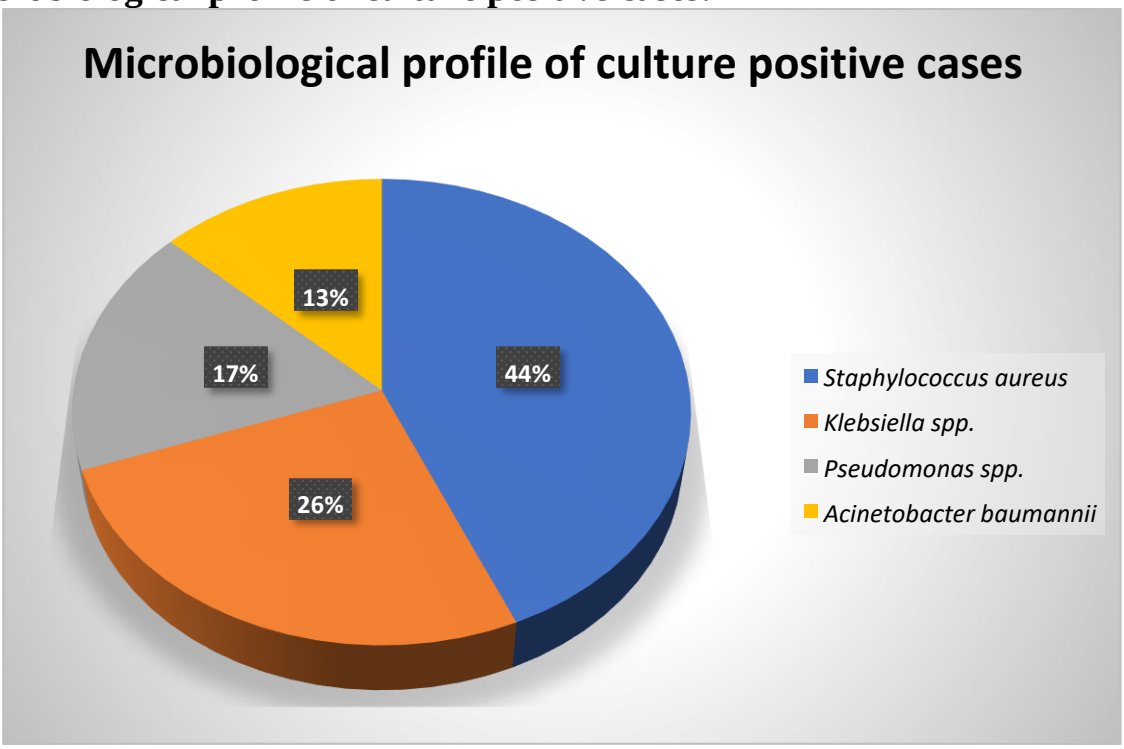
Culture positivity among the samples:

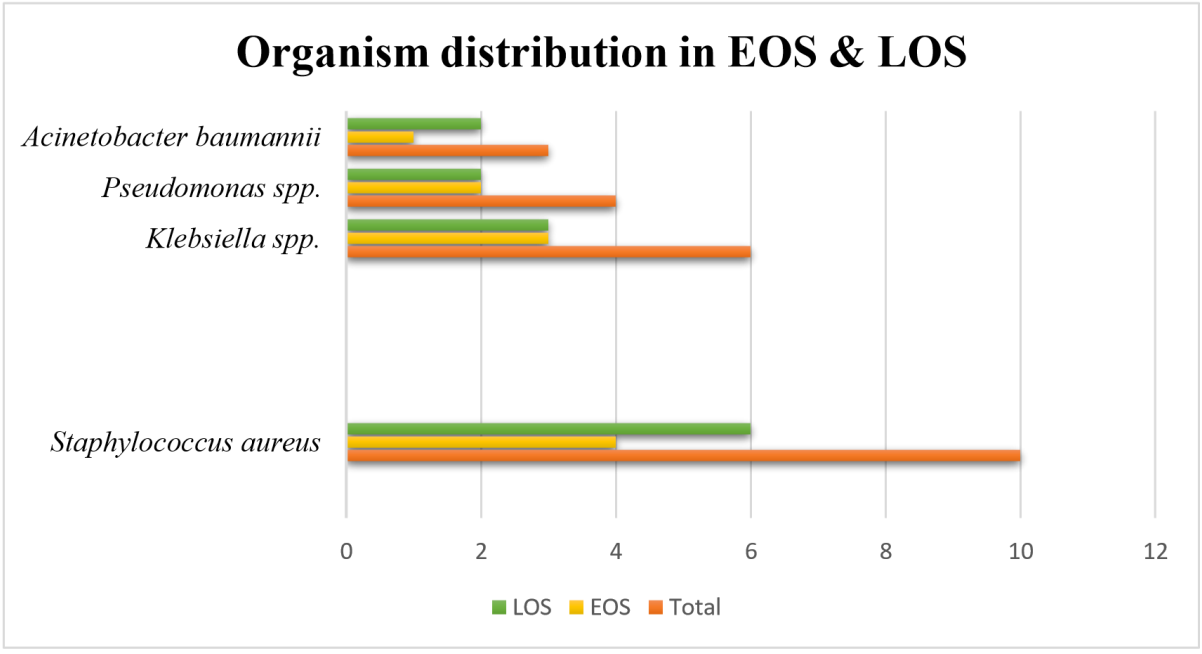


Age and sex distribution of Neonatal septicemia:



Microbiological profile of culture positive cases:





Total number of *Staphylococcus aureus* organism:

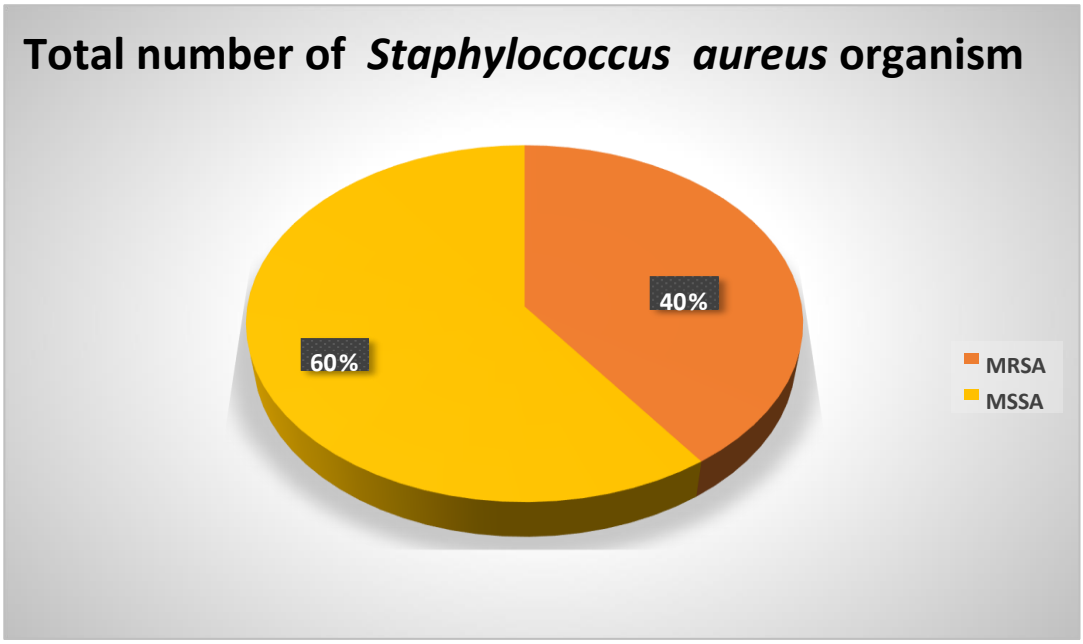


Table:2 Antibiogram of *Staphylococcus aureus*: -

Antibiotic	***Staphylococcus aureus* (MRSA) (n=8)**	
	Sensitive	**%**
Co-trimoxazole (COT)	5	62.5%
Gentamicin (GEN)	4	50%
Linezolid (LZ)	8	100%
Cefoxitin (CX)	3	37.5%
Vancomycin (VA)	8	100%
Erythromycin (E)	1	12.5%
Clindamycin (CD)	3	37.5%
Penicillin-G (P)	2	25%
Levofloxacin (LE)	3	37.5%

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Table:3 Antibiogram of *Klebsiella spp.*: -

Antibiotic	***Klebsiella spp.* (n=6)**	
	Sensitive	**%**
Co-trimoxazole (COT)	5	(83.3%)
Gentamicin (GEN)	4	(66.6%)
Meropenem (MRP)	1	(16.6%)
Cefotaxime (CTX)	1	(16.6%)
Ampicillin (AMP)	1	(16.6%)
Levofloxacin (LE)	4	(66.6%)
Amikacin (AK)	3	(50%)
Cefepime (CPM)	1	(16.6%)
Piperacillin (PIT)	2	(33.3%)

Table:4 Antibiogram of *Pseudomonas spp.:* -

Antibiotic	*Pseudomonas spp.* (n=4)	
	Sensitive	%
Aztreonam (AT)	3	(75%)
Gentamicin (GEN)	3	(75%)
Meropenem (MRP)	2	(50%)
Ceftazidime (CAZ)	1	(25%)
Cefepime (CPM)	1	(25%)
Levofloxacin (LE)	3	(75%)
Piperacillin (PIT)	1	(25%)

Table:5 Antibiogram of *Acinetobacter baumannii: -*

Antibiotic	*Acinetobacter baumannii* (n=3)	
	Sensitive	%
Ampicillin/Sulbactam (A/S)	2	(66.6%)
Gentamicin (GEN)	1	(33.3%)
Meropenem (MRP)	2	(66.6%)
Ceftazidime (CAZ)	1	(33.3%)
Cefepime (CPM)	1	(33.3%)
Levofloxacin (LE)	2	(66.6%)
Piperacillin (PIT)	2	(66.6%)
Co-trimoxazole (COT)	2	(66.6%)
Cefotaxime (CTX)	1	(33.3%)

DISCUSSION

This study presents microbiological profile of neonatal septicemia. This study was carried out at MKCG MCH microbiology department, Berhampur for a period of 3months from (Jan-2024 to march-2024). During this study period a total of 111 patients blood sample was isolated from neonatal septicemia.

In our study culture positivity among the blood sample was (62.16%) which is similar to study by Purva Mukherjee et al. in which the culture positive was (67.02%).

According to our study the prevalence of LOS & EOS was 65.2% & 34.7% respectively. which is comparable to study by Prativa Biswas et al. who had a similar prevalence of LOS (73.3%) & EOS (26.6%).

According to our study 37.5% of patients belonged to the age group 0-7 days. which is concordant to the result seen the study by Kausik kumar Sarangi et al. showing 50.1% cases belonging to the age group 0-7 days.

Among the culture positive samples predominant pathogen isolates were gram negative (56.5%) followed by gram positive (43.47%). Of the gram-negative isolates *klebsiella spp.* (26.08%) was the predominant isolate followed by *Pseudomonas spp.* (17.3%), *Acinetobacter baumannii* (13.04%), which is almost similar to the result of the study by Dipti Patnaik et al. showing predominantly gram-negative isolates (63.1%) followed by gram positive (36.9%) out of theses, *Klebsiella spp.* (38.6%) was most common organism isolates causing neonatal septicemia.

In EOS cases the prevalence of gram-negative bacteria was 46.15% & gram-positive bacteria was 25%, thus finding is similar to the result seen in a study by Sanghamitra Satapathi et al. in which a prevalence of gram-negative bacteria was 73.3% & gram-positive bacteria was 26.6% respectively.

Among gram positive organisms Staphylococcus aureus was the predominant pathogen. The antibiogram of the isolates showed that majority of isolates were sensitive to Linezolid (100%) and Vancomycin (100%). Soumini Ratha et al. also showing similar sensitive pattern.

Among gram negative organisms *Klebsiella spp.* was the predominant pathogen. The antibiogram of the isolates showed that majority of isolates were sensitive to Co-trimoxazole (83.4%) and Levofloxacin (66.7%). Santosh Kumar Panda et al. also showed sensitive to Co- trimoxazole (80%) and Levofloxacin (70.3%).

In our study maximum number of *Pseudomonas spp.* were sensitive to Aztreonam that is (75%) followed with Gentamicin (73.4%). Prativa Biswas et al. which shows similar sensitive pattern.

In our study showed maximum numbers of antibiotics shows sensitivity to *Acinetobacter baumannii* Ampicillin/Sulbactam (66.7%) followed with Meropenem (65%), Piperacillin (64.5%). Surya Narayan Mishra et al. also showed sensitive to Ampicillin/Sulbactam (68%) followed with Meropenem, Piperacillin.

Among the 10 isolates of Staphylococcus aureus 4 were Methicillin resistance *Staphylococcus aureus* (40%) and this result in concordance with the results of the study by Hare Krishna Tiwari et al. which is (34.8%).

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