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Review Article

Probiotic Use in a Randomized Controlled Trial to Prevent Sepsis and/or Necrotizing Enterocolitis in Low-Birth-Weight Infants

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Abstract

Neonatal sepsis is a major problem throughout the world. Early detection of neonatal sepsis is difficult because the first signs of this disease may be minimal, especially in preterm infants. The clinical signs are similar to those of non-infectious processes. Definitive blood culture results not being immediately available may prove detrimental. As a result, many babies are evaluated and treated for presumed sepsis with parenteral antibiotics while awaiting microbial isolation. This study was undertaken to evaluate the efficacy of probiotics in prevention of Late onset sepsis and or NEC in low-birth-weight babies.

Keywords: Probiotic; Sepsis; Necrotizing**;** Enterocolitis; Low-Birth-Weight; Neonatal; Preterm.

Introduction

Neonatal Sepsis is a crucial infection worldwide. Sepsis is the main factor, which accounts for 30-50% of newborn deaths in developing nations [1,2].

Neonatal sepsis occurs 30 times per 1000 live births, citing data from the National Neonatal Perinatal Database (NNPD, 2002-2003). The database of 18 tertiary care neonatal units in India, which accounts for 19% of all neonatal deaths, identifies sepsis as one of the most common causes of infant mortality [3]. Sepsis associated mortality is predominantly avoidable with pre-detection, rational antimicrobial therapy and aggressive supportive therapy and infection control measures on the unit.

Early detection of neonatal sepsis is difficult because the first signs may be minimal,

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especially in preterm infants. Clinical signs are similar to non-infectious processes. It may be detrimental to not have immediate access to definitive blood culture results.

As a result, many babies are evaluated and treated for presumed sepsis with parenteral antibiotics while awaiting microbial isolation. It is unfortunate that only a small percentage of these babies actually merit sepsis diagnosis, and all others incur unnecessary hospitalization and expense. There is still a need for a trustworthy test because there is currently no laboratory test with 100% specificity and sensitivity.

The gold standard for confirming a diagnosis has historically been blood culture, however the test's results are only available after 48-72 hours. Additionally, the findings of recent research have highlighted the worry over potential culture-negative sepsis, particularly in the context of rising maternal antibiotic use.

Therefore, broad-spectrum antibiotics are used to treat newborns who have "risk factors" for neonatal sepsis, which necessitates a lengthy hospital stay.

Neonatal sepsis is the most prevalent condition leading to neonatal mortality in poor nations, especially in low-birth-weight infants [4,5].

Due to a number of factors, including weakened immunity in the form of low immunoglobulin, low complement and phagocyte capabilities, low mucosal and skin barrier integrity, and abnormal gut colonization, preterm and SGA infants are particularly susceptible to sepsis [6]. BW babies usually require a prolonged stay in ICU.

This involves using antibiotics, limiting maternal microbiota exposure, and potentially compromising infection control measures like washing one's hands and sterile feed. As a consequence, there is a decrease in exposure and colonization by typical commensal microorganisms.

A healthy adult's fecal ecology may include 400 different bacterial strains, as opposed to no more than 20 in a preterm in the NICU. These include yeast, *Klebsiella*, *enterococci*, *clostridia*, and *staphylococcus aureus* [7]. Necrotizing enterocolitis (NEC) and sepsis may develop as a result of this atypical pattern of colonization, a lack of microbial diversity in the intestine, immature local defense mechanisms, and immaturity of the gut mucosa. This also leads to the acquisition of antibiotic resistant strains.

Therefore, colonization with desirable microflora may help LBW newborns experience less sepsis/NEC. Probiotic bacteria supplementation is one way to promote healthy intestinal colonization with desired flora.

The purpose of this research was to determine whether probiotics can help prevent late onset sepsis and/or NEC in LBW babies.

Aim and objectives

Aim of the study

To study the effect of oral *Bacillus clausii* on prevention of Late onset Sepsis and (or) NEC in low-birth-weight babies.

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The study has the same aim and objectives as the previous one. However, the study population is different and there are not many studies done on the topic in Indian scenarios.

Except for one study by Samanta and colleagues [8] that enrolled 186 eligible preterm (<32 weeks) and VLBW children in a NICU in India over a 6-month period, all studies conducted to date have been on non-Indian cohorts.

In order to modify the practice, this study was conducted in researcher's setting. Only 4 studies (Study by Dani, et al., study by Miller, et al., study by Mihatsch, et al. and Romeo, et al.) done so far had the objective of nosocomial sepsis. It is for this reason that this study is chosen, especially among Indian population.

Review of literature

History of septicaemia [9]

Infection as an extremely serious hazard to the premature infant was recognized soon after Louis Pasteur's (1822-1895) revolutionary achievements. Gueniot was one of the first to recognize infection dangers in the premature infant. Gueniot pointed out in 1872, the need for proper feeding and temperature regulation and also for stringent attention to the infant's skin to prevent infection.

In the early 1880's, Jacques Grancher (1843-1907), a french pediatrician and a close associate of Pasteur developed a plan to isolate infants in the wards of the hospital by instituting a rudimentary form of cubicle construction. Around the same time, Stephane Tarnier's efforts to reduce the mortality of premature infants delivered at the Maternite de Paris Hospital led to the introduction of the first closed incubator and to the practice of gavage feedings.

At the hospital des Enfants Malades in Paris, Budin introduced sterilization of utensils, disinfection of linen and floors, use of surgical gowns and careful scrubbing of the attendants' hands before examining all infants.

Budin showed the need to disinfect the incubator every other day. Budin and Tarnier used a solution of antiseptique forte (carbolic acid 5/100; Mercury bichloride 1/2000) as a disinfectant.

Budin was persuaded of the necessity of setting aside room for the isolation of diseased newborns in 1896 by a major epidemic of respiratory tract illness among premature infants at the maternite. This realization led Budin to design a special unit for premature infants, the first of its kind in the world.

In 1960, Alexander J. Schaffer coined the word "Neonatology" referring to the study of all infants from the birth up to 4 weeks of life.

McCracken coined the term "Sepsis Neonatorum" in 1981, to describe a disease that affects infants less than one month old, who are clinically ill, and have positive blood cultures.

Epidemiology of neonatal sepsis

Septicemia incidence is influenced markedly by intrauterine life quality, host factors and environmental factors. The incidence of

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septicemia varies from 2.7/1000 live births in developed countries [10] to 38/1000 live births in developing countries. There is no bench marking for late-onset sepsis in India and no consensus on the incidence. It varies from place to place and depends on the environment and local unit practices. Neonatal septicemia incidence quoted in various studies is given in the Table 1 below.

Serial no.	Study	Incidence of neonatal septicemia/1000 live births
1	Freedman, et al. [11]	0.85
2	Bhakoo, et al. [12]	1.2
3	Kuruvilla, et al. [13]	9.8
4	Khatua, et al. [14]	6.55
5	Moses, et al. [15]	0.5

Table 1: Incidence of neonatal septicemia in various studies.

The occurrence fluctuates depending on several circumstances such as preterm, LBW, IUGR, sex, etc. Sepsis incidence varies between various settings, such as community care centers and tertiary care centers as well as various categories, such as LBW, VLBW, and ELBW. In the community, 36 villages with a population of 36,613 had a 65% prevalence of newborn sepsis that was discovered based on clinical suspicion, according to a study by Abhay Bangh at Ghadchiroli [16].

Sepsis prevalence based on birth weight [17]

- 401-750g=43%
- 751-1000g=28%
- 1001-1250g=15%
- 1251-1500g=7%

The prevalence of late-onset sepsis was highest in developing nations [18]

- <1000g=46.4%
- <1249g=30.6%
- <1500g=16.8%

Sepsis can occur often in impoverished nations for a number of causes, including:

1. The majority of infants are underweight and preterm (33%) [19].
2. The majority of deliveries are made by inexperienced dais at homes [20].
3. Variable use of aseptic precautions at the time of delivery (5 cleans viz; clean hands, clean surface, clean cord, clean blade, and clean cord tie).
4. Traditional practices include [20,21] colostrum deprivation, applying cow dung to the cord, delaying the start of breastfeeding, and delivering pre-lacteal meals.
5. A higher prevalence of cross infections could result from understaffing and NICU overcrowding [18].

6. Inadequate infection prevention techniques because of inadequate resources.

Sex incidence

In Khatua, et al., [22] studies, 70% of infants with neonatal septicemia were males.

Washburn, et al., [23] found a ratio of 2:1 with male predominance and proposed a genetic origin to explain the difference in sepsis incidence between males and females.

The reason is thought to be that the Gamma globulin production regulators are most likely found on the X chromosome.

Microbiology

Although the prevalence of neonatal septicemia has not decreased throughout decades, there have been significant changes in the organisms that cause it [24]. The main pathogens for early-onset neonatal sepsis, as reported in western literature, are Group B *Streptococcus* (GBS) Type 1a, 1b, 1a/c, 2, 3, *Escherichia coli* (E. coli), *Klebsiella*, *Listeria monocytogenes*, *Netreococci*, and *Hemophilus influenzae*, whereas the main pathogens for late-onset sepsis are GBS Type 3, E.

In India, the most frequent causes of both early and late sepsis are *Klebsiella*, *E. coli*, *Pseudomonas*, and *Staphylococcus aureus*.

Blood culture isolates in neonatal sepsis (n=837), based on NNPD[3]		
1	*Klebsiella pneumonia*	29.70%
2	*Staphylococcus aureus*	14.70%
3	*Escherichia coli*	13.90%
4	*Pseudomonas aeruginosa*	9.20%
5	*Enterobacter sp.*	7.90%
6	*Streptococcus albus*	7.20%
7	*Candida sp.*	4.80%
8	*Acinetobacter*	2.40%
9	*Streptococcus viridians*	1.40%
10	Others	8.70%

Table 2: Spectrum of bacterial pathogens in neonatal sepsis, based on NNPD.

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The Table 2 displays the range of bacterial infections analyzed from hospital-based data gathered by the National Neonatal Perinatal Database (NNPD) network from various facilities across the nation.

It's crucial to keep in consideration that the bacterial flora is dynamic, differs from region to region, and progresses gradually in the same region.

To develop a logical antibiotic policy that is best for the nursery at any given time, it is crucial to closely monitor the bacterial flora of the NICU and the pathogens' patterns of antibiotic susceptibility.

Neonatal sepsis types [25]

Sepsis as defined in various settings is as follows.

Bacteremia

Presence of bacteria in blood [26].

Occult Bacteremia

It is transient invasion of blood stream without an obvious focus of infection [26].

Early onset sepsis (EOS)

It can be described as sepsis occurring within the initial 72 hours of life. The newborn may exhibit symptoms upon birth in serious circumstances. This is brought on by organisms prevalent in the vaginal tract of pregnant women.

Pneumonia and respiratory distress are the most common symptoms of EOS in infants. A higher risk of EOS has been correlated to some maternal and neonatal disorders.

The following characteristics are linked to an increased risk of early-onset sepsis:

- Low birth weight (LBW) (<2500 grams) or prematurity (<37 completed weeks of gestation).
- Within two weeks of delivery, maternal febrile illness with evidence of bacterial infection.
- Alcohol with a foul odor or meconium stains.
- Premature or Prolonged Rupture of Membranes (PROM) >24 hours.
- Single unclean or >3 sterile vaginal examinations during labor.
- Prolonged labor (sum of 1st and 2nd stage of labor >24 hours).
- Perinatal asphyxia (Apgar score <4 at 1min).

Late-onset sepsis (LOS)

It is defined as sepsis presenting after 72 hours of age. LOS infections are either nosocomial (associated with hospitals) or community-acquired, and neonates typically present with septicemia, pneumonia, or meningitis. The risk of late onset sepsis (hospital associated) is influenced by a variety of factors which include:

- Low birth weight or prematurity.
- Admittance to Neonatal intensive care unit(NICU).
- Mechanical ventilation.
- Invasive procedures.
- Intake of parenteral fluids and utilization of stock solutions.

Poor cleanliness, inadequate cord care, bottle feeding, and pre-lacteal meals are some

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factors that could increase the risk of LOS acquired in the community.

Clinical manifestations [27]

Non-specific features

The early indications of sepsis are often subtle and non-specific; indeed, for early diagnosis a high index of skepticism is essential.

There are a number of symptoms and signs that neonates may exhibit with sepsis, including:

- Hypothermia or fever (former is more common in preterm and low birth weight babies).
- Lethargy, poor cry, refusal of feeds.
- Poor perfusion, prolonged capillary refilling time.
- Hypotonia, absent neonatal reflexes.
- Bradycardia, tachycardia.
- Hypoglycemia or hyperglycemia.
- Metabolic acidosis.

Infection in the newborn may present with non-specific and often subtle clinical findings.

The alteration of behavior of the baby and change in the established feeding pattern in the neonate are the early features in early neonatal sepsis.

A baby who previously sucked normally become lethargic, unresponsive, and unwilling to do so.

The other non-specific clinical features are vomiting, grunting, abdominal distension, apnea, cyanotic spells, poor perfusion, shock, petechiae, purpura or unexplained jaundice.

Specific characteristics of multiple systems

Central nervous system (CNS)

High pitched cry, excessive irritability, seizures, neck retractions, vacant stare, bulging anterior fontanel, stupor, coma.

Cardiovascular system

Poor perfusion, prolonged capillary refilling time, hypotension, shock.

Gastrointestinal system

Feed intolerance, vomiting, diarrhea, abdominal distension, necrotizing enterocolitis.

Hepatic

Direct hyperbilirubinemia and unexplained prolonged hyperbilirubinemia, hepatomegaly.

Renal

Acute renal failure, metabolic acidosis.

Hematological

Bleeding, petechiae and purpura, thrombocytopenia.

Skin changes

Sclerema, mottling, umbilical discharge and periumbilical erythema.

Temperature instability

Hypothermia is the commonest feature of early onset neonatal sepsis. Preterm and low birth weight babies with sepsis commonly have subnormal, irregular fluctuation of

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temperature. Cold peripheries with warm body may be an early indication of sepsis. Hyperthermia may be the first indication in late onset disease. Sepsis will occur in ten percent of full-term neonates with fevers (>37.8 ℃) unrelated to environmental factors.

Septic shock

The organisms which cause septic shock are *E.coli, Kiebsiella, Pseudomonas* and *Serratia*. The principal triggering factor is the polysaccharide component of bacterial cell wall. This will initiate the generation of kinins, activation of Hageman factor and complement cascade of activation of kinins, resulting in hypotension.

Bone and joint manifestations [28,29]

The epiphyseal plate is crossed by several small vessels that provide immediate association between the epiphysis and metaphysic of long bones, during the neonatal period and infancy. The infection of a metaphyseal site can therefore spread to the epiphysis via the growth plate. Commonest etiological agent is *Staphylococcus aureus*. Initial signs and symptoms are non-specific. Physical examination reveals swelling, raised local temperature and tenderness. Diagnosis is by blood culture and sensitivity, gram staining and culture of intra-articular fluid. Radiographs may be normal initially, but later on may show widening of joint space in septic arthritis. Ultrasound and MRI are helpful in diagnosis.

Urinary tract infection [29]

Escherichia coli is the commonest etiological agent, *Klebsiella* and *Pseudomonas* being encountered less frequently. Clinical features are nonspecific and the golden criterion for diagnosis is by urine culture and sensitivity of suprapubic aspirate.

Necrotizing enterocolitis

Necrotizing enterocolitis is the most frequent gastrointestinal emergency in a newborn.

Risk factors for NEC [30,31]

1. Factors affecting host susceptibility:
    - levels of secretory IgM.
    - levels of platelet activating factor-acetyl hydrolase.
    - Hypoxic or ischemic injury.
    - Enterocyte maturity.
2. Presence of bacteria in the GIT.
3. Abnormal bacterial colonization of GIT:
    - Propensity of Enterobacteriaceae to translocate.
    - Lack of competing micro flora.
    - Impact on mucosal differentiation.
4. Lack of antenatal steroids for mucosal maturity.
5. Lack of breast feeding:
    - lack of mucosal barrier.

Clinical manifestations of NEC

Detection of sepsis

It is based on:

1. Clinical features.
2. Laboratory investigations (CRP, micro ESR, total WBC count and differential

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count, absolute neutrophil count and band cells).

3. Blood culture.

Stage	Systemic signs	Intestinal signs	Radiological signs
IA-suspect NEC	Temperature Instability, apnea, bradycardia, lethargy	Elevated pregavage residuals, mild abdominal distention, emesis, guaiac positive stool	Normal or intestinal dilation, mild ileus
IB-suspect NEC	Same as above	Bright red blood per rectum	Same as above
IIA definite NEC-mildly ill	Same as above	Same as above, and absent bowel sounds	Intestinal dilatation, ileus, pneumatosis intestinalis
IIB-Definite NEC moderately ill	Same as above, plus mild thrombocytopenia	Same as above and signs of gen. peritonitis, marked tenderness, abdominal cellulitis, or tight lower quadrant mass	Same as II A and portal vein gas doubtful ascites
IIIA-advanced NEC-bowel intact	Same as II B plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, DIC, neutropenia	Same as above and signs of gen. peritonitis, marked tenderness and distention of abdomen	Same as II B and definite ascites
IIIB-advanced NEC-bowel perforated	Same as III A	Same as III A	Same as II B and pneumoperitoneum

Table 3: Modified bells' staging for NEC.

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Clinical

Probable sepsis

Clinical sepsis with any one of the following parameters: CRP >10mg/dL, micro ESR >10 mm, TLC: <5000 or >15000 cells/cumm, ANC: <1800 cells/cumm; platelets: <1,00,000 cells/cumm.

Definite sepsis (Microbiologically proven sepsis)

Culture positive cases.

C-reactive protein

This is a globulin molecule that is produced in the liver as a result of IL-1 and IL-6 activation during any generalized inflammatory event. Viral infections may not increase CRP, however systemic bacterial and fungal infections cause a substantial increase. Serial measurement of CRP is a more accurate predictor of progress of the infection than the WBC indices. Level of more than 8 microgram/ml is considered as positive [32]. Laser nephelometry, single radioimmuno diffusion assay [33], semi-quantitative latex agglutination technique can be used to quantify CRP accurately. Positive predictive value of CRP is high but the negative predictive value is very low.

Micro ESR

Micro ESR measurement is an easy and affordable approach, albeit it is not a highly reliable indicator of neonatal sepsis. It is specific but has less sensitivity due to delay in rising and the long term required for normalization after clinical recovery. During the first three days of life, the normal value is up to 6mm in the first hour, while by the end of the month, the maximum fall may be up to 11mm/hr. Value more than 10mm/hr is suggestive of neonatal sepsis.

Blood count

Total leukocyte count has a low predictive value for sepsis because of the wide range of normal count from 8000 to 20,000 cells/cumm.

Leukopenia: Total count less than 5000cells/cumm.

Absolute neutropenia: Less than 1500cells/cunun [32].

Band neutrophil count more than 20% and band cell to total neutrophil ratio more than 2 is considered as sepsis [23].

Platelet count less than 100000/cumm is considered positive.

When at least one of the above-mentioned criteria are satisfied, sepsis screen is considered positive. It has 93% sensitivity and 88% specificity [32].

Blood culture

When it comes to detecting sepsis, blood cultures are considered to be the gold standard. Antibiotics should be initiated after a blood culture has been performed. In clinical practice this may not always be possible. Hence blood culture is needed to:

1. Document the presence of infection.
2. Decide duration of therapy.
3. Decide change of antibiotic based on organism sensitivity.

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4. Prognosticate.

Skin preparation and technique of drawing blood:

1. Clean the skin with alcohol swabs three times or until the swabs appear free of surface dirt and allow drying.
2. Apply 2% chlorhexidine in 70% alcohol solution and swab three times in centrifugal circles over anticipated site of venipuncture and allow drying for at least 30 seconds.
3. Draw blood from peripheral prominent veins like those of the arms legs or scalp.

Collection from catheter or along with other blood samples is not recommended. Volume of blood collected depends on the magnitude of septicemia and the age of the patient. Neonates usually have a high magnitude of bacteremia and therefore a minimum of 0.2ml blood should be collected. In order to obtain a high yield of organisms, 1-2ml of blood should be drawn. There are a number of substances in human blood that are capable of inhibiting the bacterial growth. Therefore, blood should be mixed with five to ten folds of culture media to maximize diagnostic yield. Turnaround time for blood culture: It is the time required for the growth of organisms. Conventional blood culture methods need 5-7 days but recent advanced automated culture systems give quicker reports usually within 3-4 days and a few studies can detect a positive culture as early as 12-24 hours. 90% of the reports including sensitivity reports can be given within 30 hours. In recent years several new methods of blood culture have been developed which are automated and involve continuous monitoring like BACTEC.

The parameters to label a positive culture as true infection are:

1. Identification of an organism.
2. Isolation of the same organism from a normally sterile site.

Identifying the significance of coagulase negative staphylococci is the most common clinical problem related to blood culture practice. In a large blood culture study, it was found that only 5-7% of coagulase negative staphylococcal cultures are significant.

Probiotics

History

The probiotic concept was born following the suggestion by Ilia Ilitch Metchnikov (Nobel Prize Winner, 1908) in the early 20th century, that the consumption of fermented milk products contributed to Bulgarian peasants' long lives. The bacteria do not necessarily harm man, but on the contrary, can play an important part in human well-being.

Definition

Probiotics are live microbial supplements that improve the microbial balance of the host [34].

Criteria for probiotic

Before a microorganism can be classified as a probiotic, several criteria must be met [35].

These include:

1. Human ancestry.

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2. Lack of pathogenicity.
3. Resistance to technological advancement (delivery vehicle viability).
4. Resilience in bile and acid.
5. Target epithelial tissue adhesion.
6. Longevity inside the gastrointestinal tract.
7. Manufacturing of antimicrobial agents.
8. The capacity to influence immunological function.
9. The capacity to affect metabolic processes.

Probiotic microorganisms

Bifidobacterium sp. and *Lactobacillus*, two types of lactic acid bacteria, are the most widely utilized probiotics.

Others include *Streptococcus sps.*, *Ecoli;* nonbacterial organisms like nonpathogenic yeast such as *Saccharomyces boulardi* have also been used.

Generic Name	Species	Strain	References
Lactic acid bacteria			
1. *Lactobacillus*	*L. rhamnosus* *L. reuteri* *L. acidophilus* *L. bulgaricus*	ATCC 53103 GG DSM 12245 LB Not specified	34-44 45,46 47,48 49-51
2. *Bifidobacterium*	*B. bifidum* *B. infantum* *B. longum* *B. thermophilum* *B. lactis*	Not specified	49-51
3. *Streptococcus*	*Streptococcus thermophilus* *Enterococcus faecium* *Streptococcus intermedieus* *Streptococcus alfa-emoliticus*	Not specified	49-52
Bacillus-gram negative	*Escherichia coli*		
Bacillus-gram positive	*Bacillus clausii*		
Non lactic acid bacteria			
1. Saccharomyces	*S. boulardi*	Not specified	53

Table 4: Classification of probiotics.

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Beneficial effects of probiotics

The use of probiotics in preterm infants may have several advantages, including [36]:

1. A decrease in the number of pathogenic organisms in the bowel reservoir.
2. Enhanced enteral nutrient intake.
3. Less reliance on intravenous nourishment.
4. Improved gut mucosal defense against bacteria and bacterial byproducts.
5. Higher immune system defense.

Probiotic usage has the potential to enhance nutrition, lower the risk of sepsis, reduce the need for antibiotics, and avoid NEC.

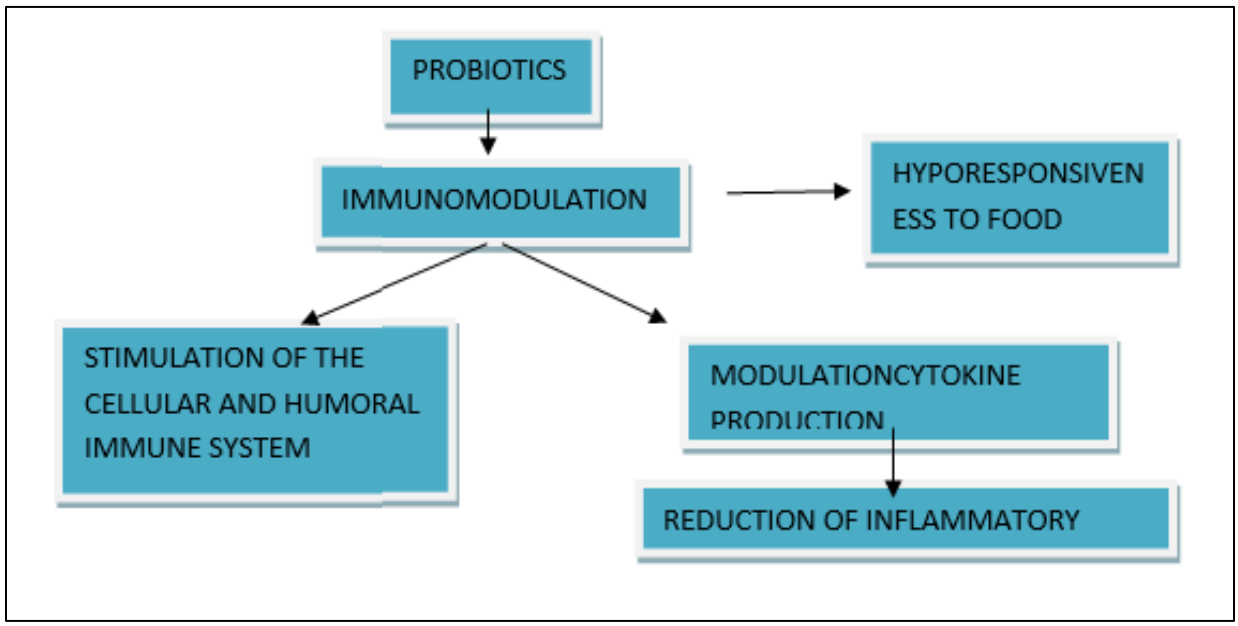

Figure 1: Mechanisms via which probiotic use may be predicted to lower the prevalence of infection in premature infants.

Mechanisms by which preterm newborns are given probiotics may be expected to lower the risk of infection

1. Increased mucosal barrier to bacterial and bacterial byproduct translocation [37-45].
2. Decreased prevalence of suspected or confirmed newborn NEC [46-50].
3. An improvement in enteral nutrition [51] that reduced the need for IV feeding.
4. Alterations in the pattern of GIT colonization that may reduce the number of enterococci, a potential pathogen, that are present in preterm newborns and perhaps increase the presence of streptococcus salivaricus, a beneficial micro flora [52].
5. Up regulation of immune responses [53-58].
6. Increased anti-inflammatory cytokine production.

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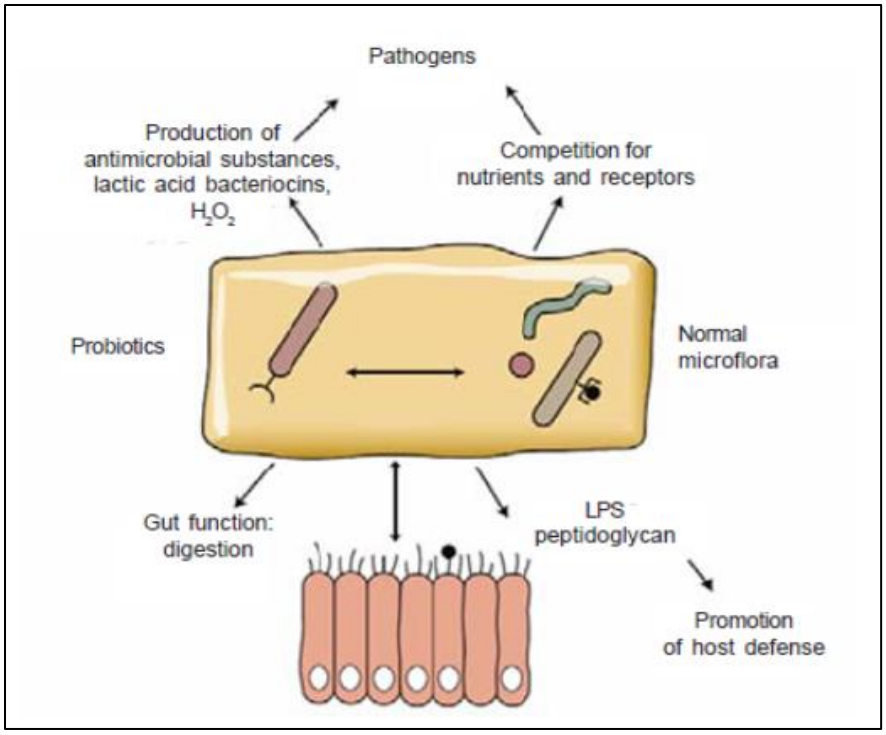

Figure 2: Potential ways that probiotics can prevent NEC.

Potential ways that probiotics could prevent NEC [36]

1. Lessening of potential pathogens' ability to colonize the mucosa.
2. Increased mucosal barrier to bacterial and bacterial product translocation.
3. Potential pathogens are excluded through competition.
4. Altering how the host reacts to microbial products.
5. Improving enteral nutrition.

Dosage

Minimum effective dosage: 10^6-10^9 CFU (colony forming units)/day [59].

Side effects

Probiotics are thought to be secure and well tolerated.

Recently, numerous review articles analyzed potential dangers.

To summarize, three categories of potential negative effects might occur [60,61]:

1. Systemic infections, particularly in a host with impaired immune system.
2. Excessive immunological stimulation in people with weak immune systems.
3. The exchange of genes for bacterial resistance.

Probiotics' applications in clinical practice

1. Sepsis or NEC in LBW babies.
2. Urinary tract infection.
3. Vaginal infections.
4. Helicobacter pylori infections.
5. Bacterial diarrhea.

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6. Antibiotic associated diarrhea.
7. Inflammatory bowel disease.
8. Traveler's diarrhea.

Index probiotics

In the study, *Bacillus clausii* was used.

Microbial characteristics of *Bacillus clausii*

It is an alkalophilic, Gram-positive, aerobic, non-pathogenic, ubiquitous bacteria, able to form spores, and highly resistant to both physical and chemical factors.

As the studies so far have used different probiotics, *Bacillus clausii* was used because of its cost effectiveness and it is available in liquid form which is easy to administer in babies.

In order to determine whether probiotic (*Bacillus clausii*) use can prevent late-onset sepsis and/or NEC in LBW newborns, this study was carried out.

A few studies have been done to determine whether probiotics are effective at preventing sepsis and/or NEC in low-birth-weight infants.

In 1999, Hoyos and colleagues [62] conducted a largely retrospective study on 2519 newborns in Bogota, Columbia, which sparked curiosity about using probiotic supplements to prevent NEC. Hoyos [62] examined *Bifidobacterium infantis* and *Lactobacillus acidophilus* in a prospective cohort trial with historical controls to see if incidence of NEC may get reduced. Throughout a year, a probiotics mixture was given to 1237 neonates (mean gestational age 35 weeks; mean birth weight 2040g). During the treatment year, NEC's primary outcome decreased (3% versus 6.6%; P=0.002), and NEC-related mortality decreased (37.8% versus 41.2%; P=0.005). There was no difference between the two groups in terms of nosocomial sepsis (5.4% versus 5.5%).

An increasing body of research, including numerous recent meta-analyses, has emerged since that time.

Deshpande and associates carried out a meta-analysis of 11 randomized controlled trials (RCTs) in 2010 [63,64]. It was discovered that probiotic prophylaxis decreased all-cause mortality (relative risk [RR] 0.42] and NEC rates from 6.56% to 2.37% (placebo versus probiotic, respectively), both of which were statistically significant advantages. Even though there was no difference in length of stay, the scientists came to the conclusion that further research was "unnecessary if a suitable probiotic product is available" [63,64].

A more recent meta-analysis including 15 RCTs was carried out by Mihatsch and colleagues [65] utilizing stricter criteria [65]. Studies with the main or secondary goal of reducing NEC were incorporated in the study by Mihatsch, et al. Ten of the trials examined by Deshpande and colleagues were included in that analysis, along with five additional studies, two of which had the biggest baseline NEC rates (5%). Probiotics were not statistically found to be beneficial for any outcome (NEC, death, sepsis, or TFF) in the authors' analysis; however, the aggregate data for risk reduction was not supplied for any analysis carried out. Additional analyses by

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the authors that stratified the data by probiotic strain revealed no impact on NEC, mortality, sepsis, or TFF. Unfortunately, the categorization resulted in fewer patients, which weakened the conclusions [65]. Due to the differing characteristics of the included studies and results, as well as the various statistical methods that were performed, the conclusions from those two analyses varied drastically.

The authors concurred that the baseline NEC rate was a significant factor that determines the potential effect of probiotic supplementation in a population despite discrepancies in the meta-analyses' outcomes.

A NICU in Israel with a prestudy NEC incidence rate of 15% and 145 very low birth weight (VLBW) neonates (1500g) were included in the study by Bin-Nun and colleagues [66]. Neonatals were randomly assigned to receive either unsupplemented milk or a once-daily supplement of a probiotic blend made up of *Bifidobacterium infantis*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum* (ABC Dophilus, Solgar, Leonia, NJ). In both groups, over 60% of the newborns solely got mother's expressed breast milk (EBM), whereas the remaining neonates received infant formula (Similac Special Care, Abbott Laboratories, Abbott Park, IL) due to a lack of EBM or other circumstances. Between the probiotic group and the control group, the average birth weight (BW) and gestational age (GA) were comparable. In the probiotic supplemented group, the rate of NEC was lower than in the control group (4% vs. 16.4%, respectively, p=0.03). The number needed to treat (NNT) to avert one case of NEC was 8, which resulted in a 12.4% decrease in the incidence of NEC overall. The probiotic group had decreased NEC-related mortality, although this was not statistically significant. The manufacturer of ABC Dophilus funded the study [66].

A RCT examining the effectiveness of *Lactobacillus sporogenes* for NEC prevention in a total of 221 newborns from a NICU in Turkey was published by Sari and colleagues in 2010 [67]. Although the inclusion criteria for the study also included preterm newborns with a GA of less than 33 weeks, it was done on VLBW infants. While the control group simply received the meals, infants in the study group received EBM or formula supplemented with *L. sporogenes* once day, beginning at the first feed and continuing until discharge. There was no difference in the incidence of NEC between the study group and the control group (5.8% vs. 9%, respectively; p=0.515). One newborn in the control group (1%) and none of the infants in the study group (1%) perished from NEC. Furthermore, it is noteworthy to highlight that in determining the sample size, the authors underestimated the baseline NEC rate at 32% (based on current NEC rates at the institution), which would have made it more difficult for the study to demonstrate a statistically significant probiotic impact [67].

Samanta and colleagues [68] recruited 186 eligible preterm (32 weeks) and VLBW infants during the course of a 6-month experiment at a NICU in India. From the start of feedings until discharge, the study group got a probiotic combination of *Bifidobacterium infantis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Lactobacillus*

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acidophilus twice daily, while the control group only received breast milk. The incidence of NEC was considerably lower in the study group than in the control group (5.5% vs. 15.8%, respectively, p=0.032). The usage of probiotics reduced NEC by 10.3%, with the result that the NNT to stop one case of NEC was 10. It is necessary to provide more information about this trial's elements such breastfeeding rates and the incidence of NEC at baseline. The 15.8% NEC rate may not accurately reflect the institution's typical NEC rates [68].

In a recent investigation, Awad and colleagues [69] found that probiotics had remarkable impacts in an Egyptian unit with a high baseline NEC rate of 31%. In that study, stage 2 or higher NEC was reduced using *L acidophilus* that had been killed, *L acidophilus* that was still alive, or a placebo. In that single-center prospective trial, a total of 150 neonates were enrolled, 89 of them were preterm. The preterm infants had a considerably lower overall rate of NEC (0%, 2.7%, and 31.3%, respectively), but there were no discernible variations in the sepsis rates. With live probiotics, the NNT to stop one case of NEC in premature newborns was 3. Unfortunately, neither the infants' delayed intake nor the sort of feeds received were thoroughly discussed. It was unclear how the preterm population was stratified; the procedure seems to have been done posthoc because there was no information supplied in the methodology sections [69].

The most accurate portrayal of US NEC incidence rates comes from two Taiwanese investigations carried out by Lin and colleagues [70,71]. The studies were partially blinded which means breast milk team was aware of the group assignments in both of the investigations that Lin and colleagues did. Infloran, Swiss Serum and Vaccine Institute, Berne, Switzerland, supplemented *L. acidophilus* and *B. infantis* to the breast milk of VLBW infants receiving NICU care in single-center research conducted in 2005 whereas the control group received solely breast milk feeds. With an NNT of 24, the probiotics group had a considerably lower incidence of NEC than the control group (1.1% vs. 5.3%, respectively; p=0.03) [70].

In 2008, Lin and associates released a multicenter trial with a similar design as a follow-up to this study. In that study, the same kind of probiotic was administered to a larger patient sample (434 newborns) at seven NICUs in Taiwan [71]. Notably, the manufacturer of Infloran, Laboratorio Farmaceutico, Italy, was acquired by another company after the conclusion of the initial trial, and the formulation was subsequently modified to incorporate *B bifidum* rather than *B infantis* [71]. When EBM was insufficient in both groups of infants in that trial, extra formula feedings were added; over 60% of neonates received only EMB. Again, the study's findings showed that, with an NNT of 21 to prevent one instance of NEC, there was a lower rate of NEC in the group being investigated compared to the control (1.8% vs. 6.5%, respectively, p=0.02). Although NEC rates of both of these studies were lower than expected (23% in the 2005 study and 25% in the 2008 study) by around 4-fold, NEC incidence in the United States is closely resembled by the NEC rates observed (~5%-7%) in these studies [34,29]. Strong evidence

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supporting the potential advantages of probiotics for avoiding the occurrence of NEC in the United States was provided by the findings of the research by Lin, et al., [70,71].

Conversely, probiotics' effectiveness tends to decline in studies with extremely low NEC rates. One of the studies most commonly cited for the prevention of NEC is the experiment done by Dani and colleagues [72]. It is the largest ongoing prospective study, involving 585 newborns in 12 Italian NICUs with GA <33 weeks or BW <1500 g. In the study by Dani, et al., infants were fed breast milk or formula once daily beginning at the first feeding, either supplemented or not with Lactobacillus rhamnosus GG (Dicoflor, Dicofarm, Rome, Italy). While the remaining newborns either received formula or mixed feeding, 63% of the infants received EBM alone. Compared to the control group, the incidence of NEC was lower in the study group (1.4% vs. 2.8%, respectively), but the distinction was not statistically noteworthy. The incidence of NEC in the study was significantly lower than the predicted rate of 10%, which may account for the lack of relevance. In contrast to the control group, which experienced a 25% mortality rate related to NEC, there were no NEC-related deaths in the probiotic group. Future research ought to concentrate on regions with a higher prevalence of NEC, according to Dani and colleagues' findings.

In a NICU in northeast Brazil, Braga and colleagues [73] registered 231 preterm infants weighing between 750 and 1499g at the time of delivery. The sample size was predicated on an institutional basal NEC rate of 10% at the time of the investigation, similar to the study by Dani, et al., [43]. Randomised Infants were given human milk (Yakult LB, So Paulo, Brazil) either with or without probiotic supplementation, including *Lactobacillus casei* and *Bifidobacterium breve*. The study's findings indicated that the incidence of NEC was lower in the study group than in the control group (0% vs. 3.6%, respectively); however, the statistical relevance of this finding was not discussed in the study. The fact that study infants only drank breast milk may have contributed to the low risk of NEC. There was a trend towards a decreased incidence of NEC, even though the total benefit of probiotics was declared statistically negligible in these two investigations.

When deciding whether to utilize probiotics in the NICU to avoid NEC, NEC rate shall be one of the initial considerations taken into account. Probiotics can dramatically diminish the rate of NEC in units with an elevated incidence, according to the evidence that is currently available. Unfortunately, the impact drastically declines as NEC incidences diminish. Before proving a benefit in areas with lower NEC rates, more research will be needed. Future studies may be constrained by the large sample sizes needed to prove probiotic benefit. For example, Mihatsch, et al., [65]. With a 5% NEC prevalence, it was calculated that to estimate 50% reduction rate at least 714 newborns would be needed per group (0.05, 0.2) [65]. There are currently no experiments aiming a sample size that big, despite larger clinical trials being conducted.

One of the early research projects on probiotics in neonates was published by Millar, et al., [74]. Twenty premature babies

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were randomly assigned to receive milk feeds or milk feeds with Lactobacillus GG twice every day for two weeks starting on the day of the beginning of feeds. The two groups did not differ in terms of sepsis or NEC. In an RCT by Mihatsch, et al., [65] 183 VLBW infants under 30 weeks of gestation were randomized to receive *Bifidobacterium lactis* or a placebo supplemented milk feeding for the first 6 weeks of life.

The main result was the "incidence density" of nosocomial infections, which is defined as the periods of increased C-reactive protein (>10 mg/L) from day 7 following the start of breast feedings to the 42nd day of life that is the ratio of number of nosocomial infections to total number of patient days. When it came to the incidence density of nosocomial infections and the actual number of nosocomial sepsis, there was no discernible difference between the two groups. Surprisingly, *Bifidobacterium lactis* did not grow in any of the blood cultures.

A recent study by Romeo, et al., [75] on 249 preterm newborns examined the effectiveness of probiotics in preventing intestinal *Candida* colonization and late-onset sepsis.

The newborns were randomly divided into three groups: the control group received no supplements, the group supplemented with *Lactobacillus reuteri*, and the group supplemented with *Lactobacillus rhamnosus*. The average gestation period was 33 weeks. In comparison to the probiotic groups, the control groups had considerably more *Candida* in the stools. On the contrary the two infants in the LGG group, the nine infants in the control group and only one newborn in the LR group experienced nosocomial sepsis.

Probiotics for preterm infants: Published systematic reviews/meta-analyses for the prevention of sepsis and NEC

Probiotics for preterm newborns have been the subject of two systematic reviews and four meta-analyses [63,64,76-79]. Five RCTs utilizing probiotics to prevent NEC in preterm children were included in the systematic review by Barclay, et al., [76]. Due to the study design's substantial heterogeneity, no meta-analysis was carried out. Probiotics may be helpful in decreasing the severity of NEC. Deshpande, et al., [63] published the first meta-analysis involving 7 RCTs. This meta-analysis showed a lower risk of NEC ≥ stage 2 (relative risk [RR] 0.36, 95% confidence interval [CI] 0.20-0.65, number needed to treat [NNT] 25, 95% CI 17-50) in the probiotic group compared to control. Statistically, there was no significant difference in sepsis risk (6 trials, 1355 participants, RR 0.94, 0.74-1.20). Probiotic supplements lower overall mortality (RR 0.47, 95% CI 0.30-0.73), but not death from sepsis and NEC. The modified meta-analysis by the same group of authors in 2010 has included four additional study reports [64]. In the probiotic group, the risk of NEC was considerably decreased (RR 0.35, 95% CI 0.23-0.55). In probiotic group incidence of nosocomial sepsis did not get diminished (RR 0.98, 95% CI 0.81-1.18).

16 trials with 2842 randomized infants were included in the Cochrane review on probiotics [77]. The trials had a wide range of enrollment requirements, including birth

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weight and gestational age, baseline NEC risk in the control groups, timing, dosing, probiotic composition, and feeding procedures. Enteral probiotics supplementation substantially decreased the risk of severe NEC ≥ stage 2 (RR 0.35, 95% CI 0.24-0.52) and all-cause death (RR 0.40, 95% CI 0.27-0.60), according to the meta-analysis. No indication of a substantial decrease in nosocomial sepsis was observed (typical RR 0.90, 95% CI 0.76-1.07). The scholars came to the conclusion that enteral probiotic supplementation protects preterm newborns from severe NEC and all-cause death. More research is required to determine the best formulation and dosage to use for newborns with extremely low birth weights (<1000gms).

In order to reduce mortality and prevent NEC and sepsis in preterm newborns, Mihatsch, et al., [78] conducted a systematic evaluation of the level of evidence for routine use of probiotics. There were 15 trials in total; 2 of them were of the level of evidence-1b (LoE), while the other 13 were level 2b LoE. The investigations were very different from one another.

According to the authors, some probiotics are effective in decreasing severe NEC (2b LoE) and decreasing mortality (2b LoE). Probiotics do not hasten the progress of feeding (1b and 2b LoE). In terms of preventing nosocomial sepsis, there were no convincing advantages.

The authors come to the conclusion that there is inadequate data to suggest routine probiotic administration to preterm newborns. However, there is promising data (2b LoE) that supports additional research into the effectiveness and security of particular probiotics in situations when there is an elevated local prevalence of severe NEC.

20 trials were included in the most recent meta-analysis by Wang, et al., [79]; 4 of those studies were Published in Chinese biomedical literature. In preterm VLBW newborns, probiotic supplementation was linked to a significantly lower risk of NEC (RR 0.33, 95% CI, 0.24-0.46). The probiotic group had a considerably lower chance of mortality (RR 0.56, 95% CI, 0.43-0.73). Sepsis risk was equivalent for both the probiotic group and the placebo group (RR 0.90, 95% CI 0.71-1.15). The ideal probiotic supplement and its long-term effects, according to the authors, require more research.

In conclusion, multiple meta-analyses have demonstrated that enteral probiotics supplementation can lower the risk of NEC and all-cause mortality; however, probiotics supplementation does not improve the risk of nosocomial sepsis in preterm newborns.

Limitations of probiotic meta-analyses in preterm infants

RCTs that met a variety of inclusion criteria were included in the various meta-analyses. The majority of the RCTs included preterm infants of <34 weeks [64,79] and one RCT <37 weeks [77]. The RCTs contained in the meta-analysis have methodological variations which include various entry requirements for gestational age, the kind and strain of probiotics employed, and the dosage and length of the intervention. Most meta-analyses involved at least ten distinct probiotic strains. NEC is the main result in the majority of RCTs. None of the meta-

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analyses have taken the information pertaining to individuals who were exclusively breastfed, were born very prematurely, or had extremely low birth weights.

Out of all studies so far, all of them have been done on non-Indian cohorts. There is only one study by Over the course of six months, Samanta and colleagues [68] enrolled 186 eligible preterm (<32 weeks) and VLBW newborns in an Indian NICU.

Hence, this study was chosen to be performed in researcher's setting so that the practice can be modified.

Only 4 studies (Study by Dani, et al., study by Miller, et al., study by Mihatsch, et al., and Romeo, et al.) so far have done with the objectives of nosocomial sepsis.

Hence the other reason for choosing this study especially in Indian population.

Material and methods

Study design

A Hospital based Prospective single blinded Randomized control trial study.

Study area

In tertiary care Neonatal Intensive Care Unit, (NICU), NICE Hospital for Women, Newborn & Children Hyderabad this study was conducted.

This is an 80 bedded tertiary care neonatal unit. Most of the patients admitting in this Hospital are from Hyderabad, Secunderabad and from nearby districts.

Study population

Patients admitted to Neonatal Intensive Care were used in the study.

Sample size

$$\frac{N = Z^2 P (1-P)}{E^2}$$

Where,

Z is percentage point corresponding to significance level.

For a significance of 5% Z is 1.96.

Prevalence of late onset sepsis in the unit during the study period was 15%.

E corresponds to maximum likely error is 0.1 (10%).

Therefore, the minimum sample size N is 49.

Out of all admissions made during the research period, only 69 newborns were qualified.

Study period: March 2013-March 2014.

Sampling technique

Consecutive patients satisfying inclusion criteria were enrolled in the study after obtaining informed consent.

Inclusion Criteria

Babies are eligible for study if fulfill all the following criteria:

1. Birth weight <2.5kgs.
2. Gestational age <37 weeks.
3. No signs of Sepsis at 24hrs of life.
4. Hemodynamically stable.

Consent

Informed consent [Annexure I] was obtained from the parents (or) legal guardian, for the additional blood sampling. The study was approved by Hospital ethical committee [Annexure II].

Exclusion criteria

1. Major congenital Malformations, like gastroschisis, omphalocele, congenital diaphragmatic hernia.
2. Severe Birth Asphyxia.
3. Early onset sepsis.
4. Sepsis at the time of admission.

After the inclusion criteria were met, infants were randomized.

Randomization

The study group and control group were randomly assigned to the eligible infants.

In order to randomize, random sequences created by computers were used.

Method

The study included all neonates who met the inclusion standards. The details were collected and entered in the proforma (See Annexure III). Babies in the study group received oral *Bacillus clausii* twice day for seven days at a dose of 1 billion. Babies in Control group did not receive anything.

Following precautions were taken for the prevention of cross contamination:

1. The prescription was given by the principal investigator for all the eligible babies on a separate drug sheet which was kept in the shift incharge cupboard.
2. Only shift in charge nurses were involved in administering the medication to the babies.
3. Principal investigator was responsible for overseeing the administration of medication.
4. Set number of each medication was given by the principal investigator and the number was tallied according to the number of prescriptions by the principal investigator on regular basis.

Outcome

- Primary outcome: Incidence of Late onset Sepsis/NEC.
  If a baby showed two or more clinical symptoms in addition to one positive sepsis screen, sepsis was assumed to be present.
  If a newborn showed abdominal distension, gastrointestinal aspirates greater than 20% of the feed volume, and stool samples that tested positive for occult or frank blood, NEC was assumed to be present.
  X-ray depicting dilated bowel loops, thickened bowel walls or pneumatosis intestinalis.
- Mortality, fluid consumption time, daily weight loss, feed intolerance, and length of hospital stay were considered secondary outcome indicators. The prevalence was measured of late-onset sepsis and NEC in relation to prophylactic use of probiotics, and NEC on the unit was a rare incidence and so death was not taken as primary outcome.

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Monitoring

All enrolled infants had clinical monitoring for sepsis every day at intervals of six hours.

When a newborn displayed symptoms of sepsis or NEC, the following tests were performed as per clinical judgement.

Complete blood picture, Absolute neutrophil count, CRP, micro ESR, Blood Culture, X-ray abdomen.

Management

The infant was handled in accordance with unit protocols.

All babies received Expressed breast milk as the 1st milk and trophic feeds were started on day 1 of life at the rate of 10-20ml/kg/day for all babies.

When the infant could tolerate 100 ml/kg of oral meals per day, the feeds were increased by 20ml/kg per day, and the IV fluids were stopped.

Statistical analysis

Data analysis

Microsoft Excel was used to input the data into the computer, and the Statistical Package for Social Sciences (SPSS) version 22 was used for the study. For qualitative variables, statistical test Chi-square was used and for quantitative variables, statistical test.

The sepsis groups were compared using a one-way ANOVA, and a p-value of <0.05 was considered statistically significant.

The traditional formulas were used to compute sensitivity, specificity, positive predictive value, and negative predictive value, using "blood culture" as the gold standard.

Results

69 infants in all were chosen to participate in the trial throughout the study period. 34 of the newborns were placed in the control group, while 35 were placed in the *Bacillus clausii* group.

Sex	**Cases (n-35)**	**Control (n-34)**	**Total**
Male	18 (51.4%)	18 (52.9%)	36
Female	17 (48.6%)	16 (47.1%)	33

Table 5: Gender Distribution of the study population-Out of the 69 neonates, 18 (51.4%) were males and 17 (48.6%) were females in study population. In control group, males were 18 (52.9%) and females were 16 (47.1%).

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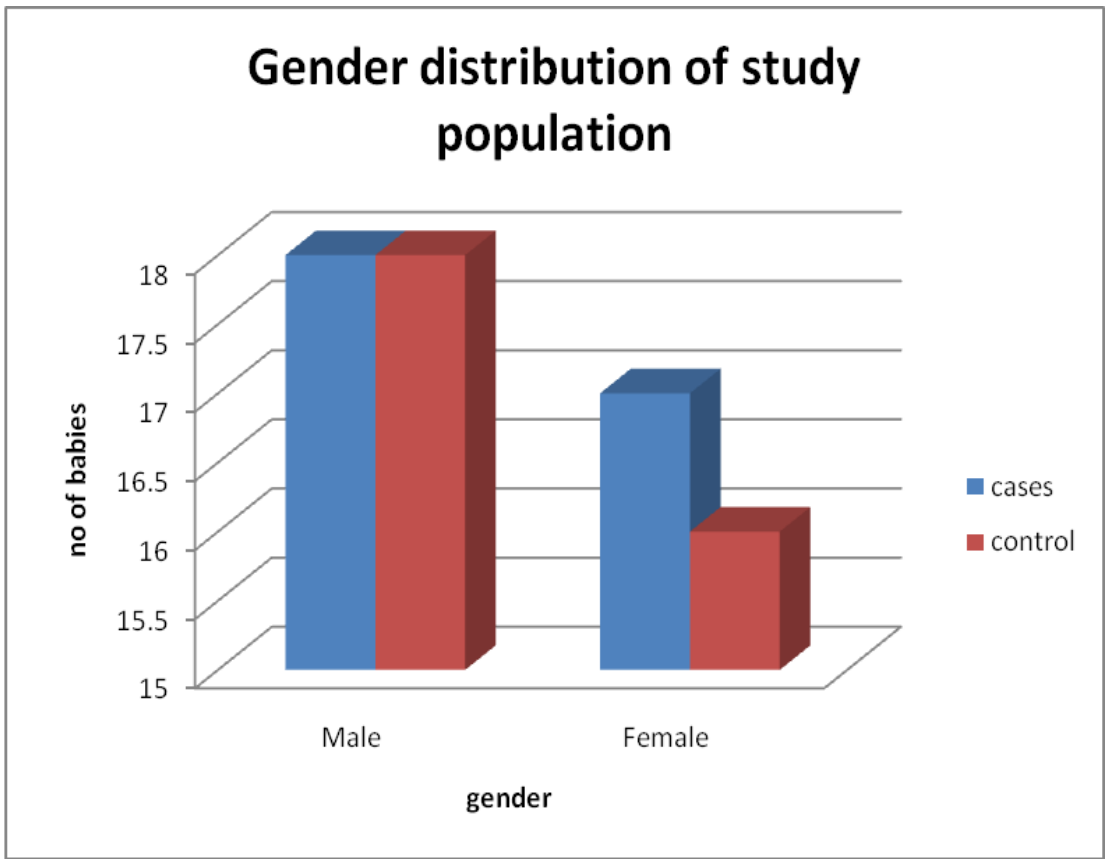

Figure 3: Distribution of gender of study population.

Gestational age	Cases (n-35)	Controls (n-34)	Total
<30 weeks	1 (2.85%)	2 (5.88%)	3
30-34 weeks	25 (71.4%)	26 (76.4%)	51
>34 weeks	9 (25.7%)	6 (17.6%)	15

Table 6: Gestational age-wise distribution of study population-In the study, 1 (2.85%) neonate was <30 weeks, 25 (71.4%) was between 30-34 weeks and 9 (25.7%) were above 34 weeks. In control group, 2 (5.88%) neonates were <30 weeks, 26 (76.4%) were between 30-34 weeks, 6 (17.6%) were >34 weeks. P-value of 0.708. Mean gestational age was 33.28 ranging from 28 weeks to 36 weeks.

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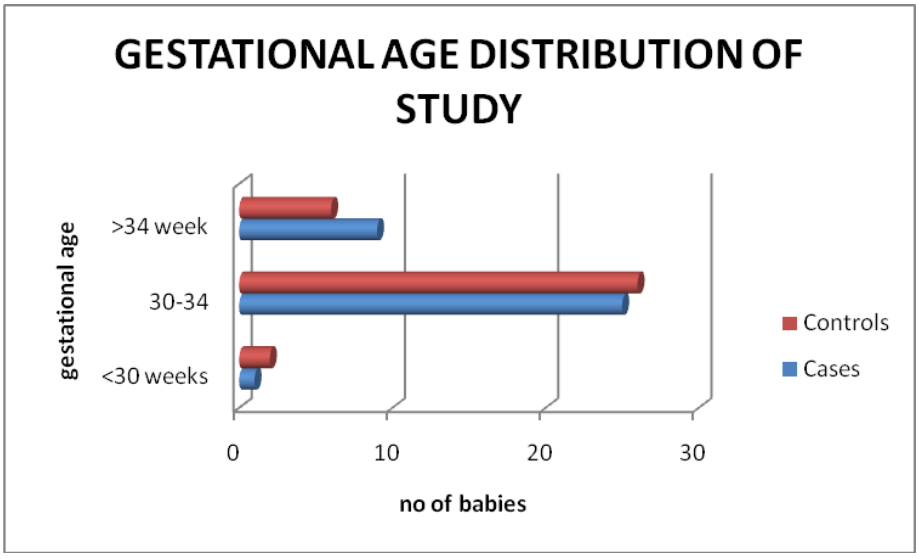

Figure 4: Gestational age distribution of the study population.

Weight	Cases (n-35)	Controls (34)	Total
<1.5kg	16 (45.7%)	11 (32.35%)	27 (39.13%)
>1.5kg	19 (54.28%)	23 (67.64%)	42 (60.86%)

Table 7: Birth weight-wise distribution of study population-In the study, 16 (45.7%) of the neonates had a birth weight of <1.5kg and 19 (54.28%) neonates had a birth weight of 1.5kg or more in the study group. In control group, 11 (32.35%) was <1.5kgs and 23 (67.64%) were >1.5kgs. Mean birth weight was 1534grams ranging from 760gm to 1940grams.

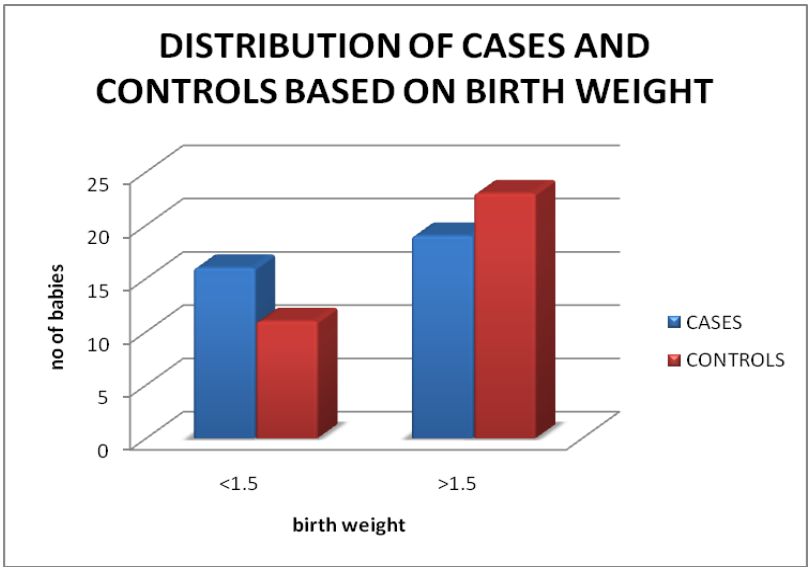

Figure 5: Birth weight-wise distribution of study population.

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	Cases(n-35)	Controls (n-34)	P value
	Mean ± SD	Mean ± SD	
Gestational Age (33.28)	33.37 ± 1.85	33.21 ± 1.81	0.708
Birth weight 1.536	1.522	1.546	0.689
Males	18 (51.4%)	18 (52.9%)	
Females	17 (48.6%)	16 (47.1%)	

Table 8: Baseline characteristics of neonates under study and control groups.

Out of the 35 neonates in study population, 18 (51.4%) were males and 17 (48.6%) were females. In control group, males were 18 (52.9%) and females were 16 (47.1%).

One way ANOVA had a p-value of 0.91 indicating that there was no change that is statistically significant among the study groups in terms of sex distribution.

The mean age of presentation in study group was 33.37 ± 1.85. In control group,mean gestational age 33.21 ± 1.81. There was no statistically significant difference among the study groups (P-value 0.708). In the research group, the mean birth weight was 1.522, while in the control group, it was 1.546. There was no statistically significant difference between the study groups, according to the p-value of 0.689.

The prevalence of NEC on the unit has been very low. Hence, the study was extended to see the effect of probiotics on how frequently late-onset sepsis occurs and that was the reason for selection of babies less than 2.5kgs although the incidence of NEC is rare in this population.

Mode of delivery	Cases (n-35)	Control (n-34)
LSCS	17 (48.6%)	13 (38.2%)
NVD	18 (51.4%)	21 (61.76%)

Table 9: Comparison of mode of delivery in study and control groups-In study group, 17 deliveries were LSCS, and 18 by NVD. In control group, 13 was LSCS, and 21 were NVD. There was no statistically evident difference between the study groups, according to the p-value of 0.39.

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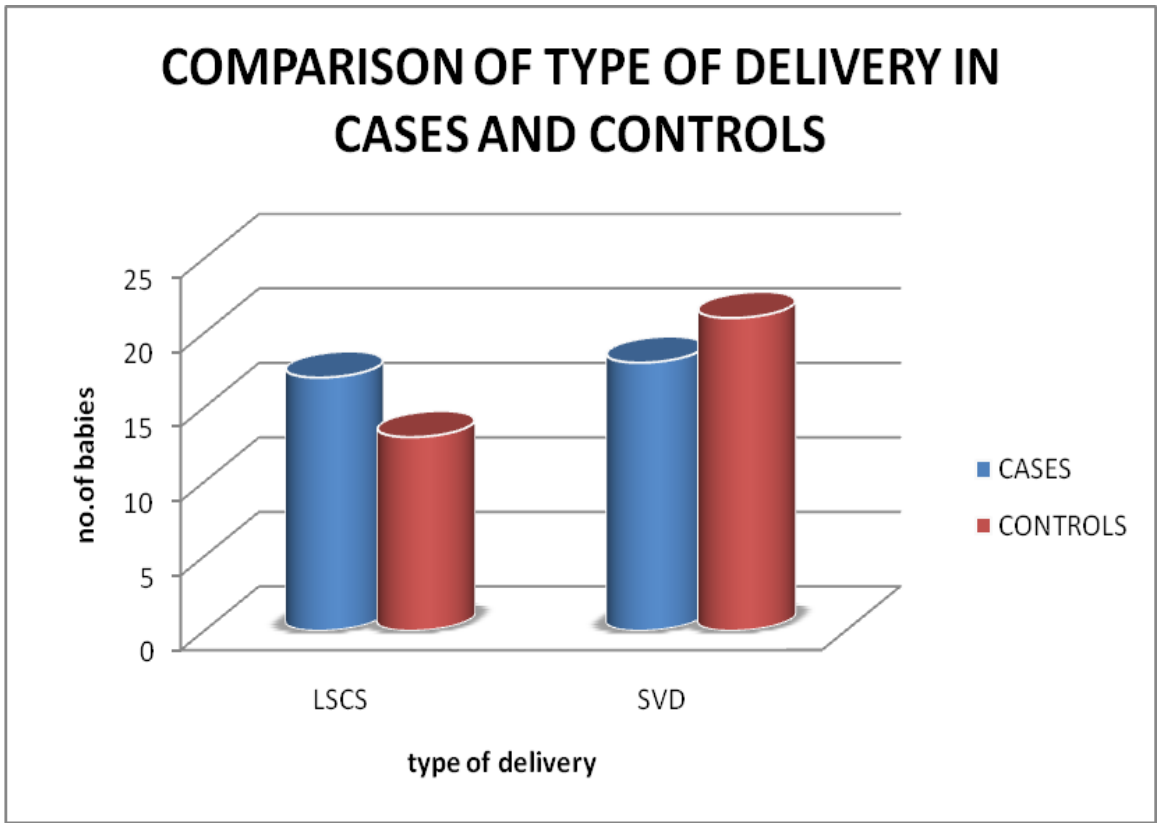

Figure 6: Comparison of type of delivery in cases and controls.

Maternal risk factors	Cases (n-35)	Controls (n-34)	Total
Oligohydramnios (6) -8.69%	3	3	6
PROM	1	0	1
PIH	15	12	27

Table 10: Comparison of maternal risk factors in study and control groups.

In study population, 15 mothers had PIH, 3 members with oligohydromnias and 1 had PROM. In control group, 12 had PIH, and 3 members had oligohydromnias.

In terms of gestational age, birth weight, gender distribution, delivery method, and maternal risk factors, the study and control groups were equivalent.

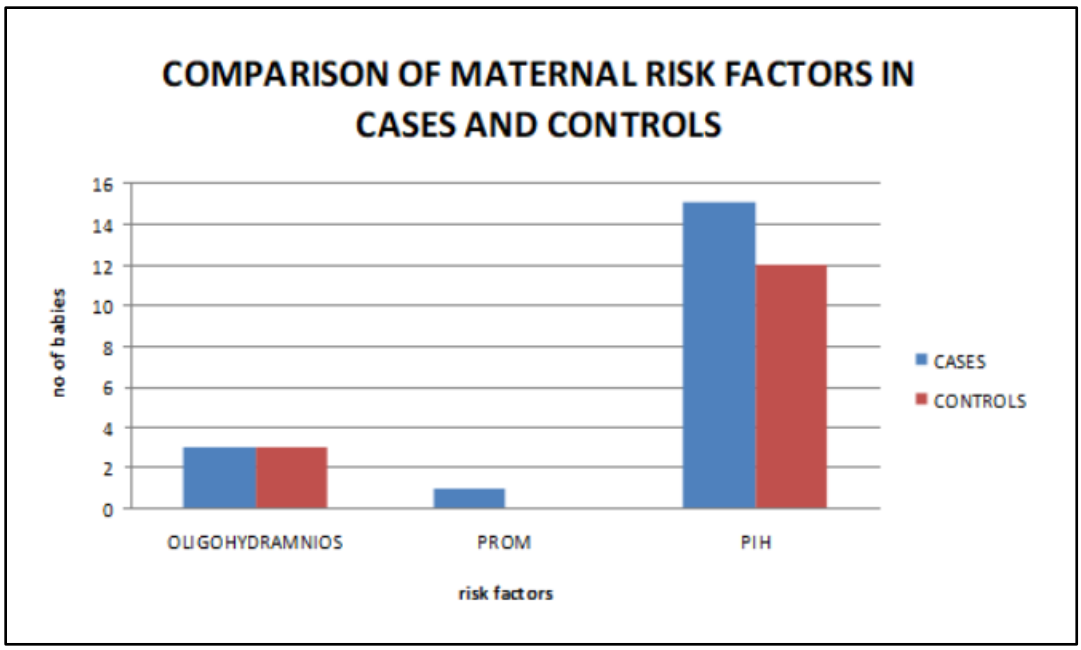

Figure 7: Comparison of maternal risk factors in cases and controls.

All babies both in cases and control groups have received expressed breast milk as the 1st milk and trophic feeds were started on day 1 of life at the rate of 10-20ml/kg/day for all babies. There is no way to study or establish that the gut is colonized with bacteria. It was assumed that by 1 week it would be colonized. More over all the studies that have been done so far worldwide also have not done any tests to look for colonization.

Clinical features	Cases with sepsis (n-3)	Controls with sepsis (n-3)
Lethargy	3	3
Increased CRT	3	1
Apnea	0	1
Tachycardia	2	2
Sclerema	1	1

Table 11: Comparison of clinical features in cases and controls.

Most of the neonates had lethargy and dullness as most common symptom. Other symptoms were increased capillary refill time, tachcardia, tachypnoea, apnea. 2 babies developed sclerema.

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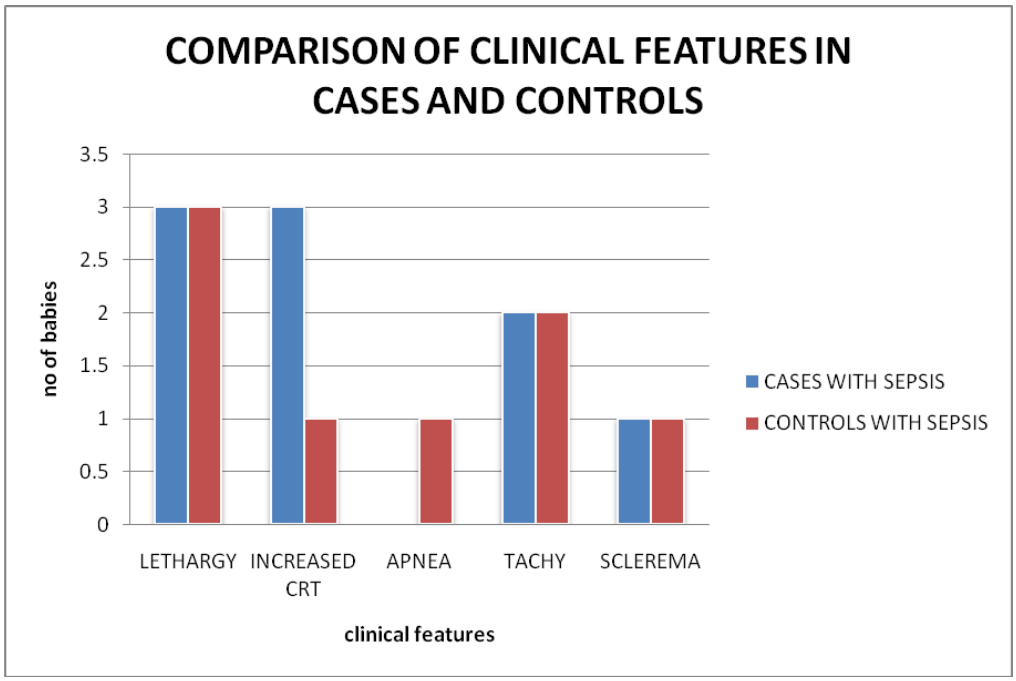

Figure 8: Comparison of clinical features in study and control groups.

Blood culture positive	Cases	Controls	Total
Klebsiella	1	1	2

Table 12: Bacteriological profile of sepsis in study and control groups-Out of 3 cases with positive sepsis, 1 was culture positive. Organism being *Klebsiella pneumoniae*. In control group, 1 culture positive and organism was *Klebsiella pneumoniae*.

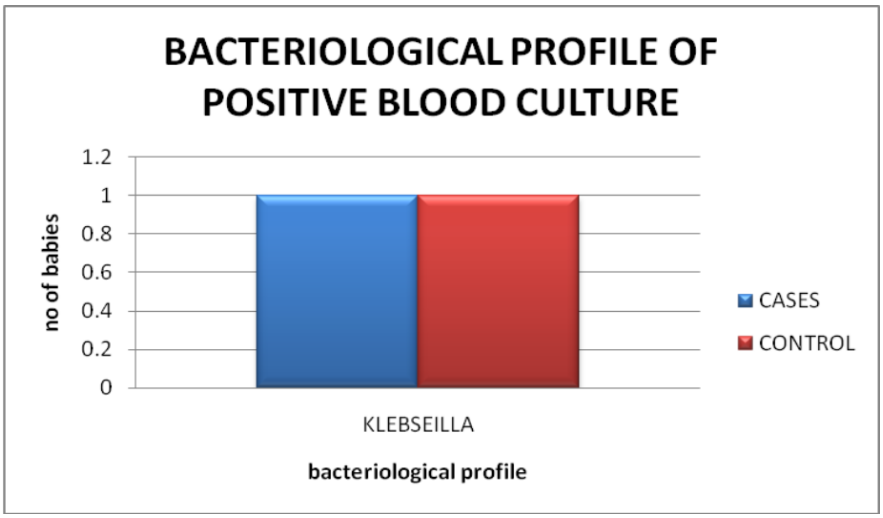

Figure 9: Bacteriological profile of sepsis in study and control groups.

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	Cases (n=35)	Control (n=34)	P-Value
Sepsis	3	3	0.7
NEC	0	0	

Table 13: Comparison of primary outcome in cases and controls.

Out of the 35 neonates in study population, 3 babies had sepsis. In control group, 3 babies had sepsis. Between the study groups, there was no statistically significant difference (p-value 0.70).

The prevalence of sepsis is comparable between the two groups.

Neither group's infants lacked NEC.

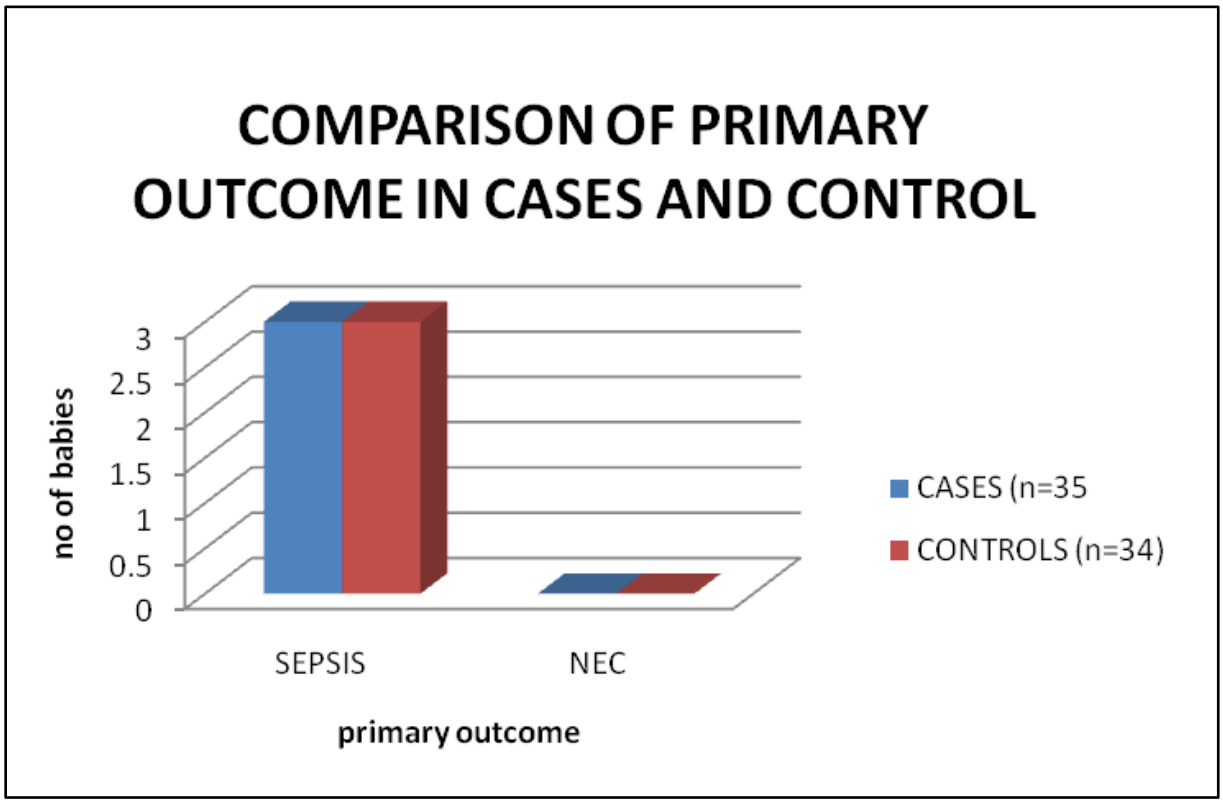

Figure 10: Comparison of primary outcome in cases and controls.

	Cases	Controls
Probable sepsis	2	2
Definitive sepsis	1	1

Table 14: Comparison of type of sepsis in study and control group.

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Out of 3 sepsis positives in study group, 2 hadprobable sepsis with no bacterial growth and 1 was *klebsiella* positive . In control group, 2 hadprobable sepsis and 1 was culture positive (*Klebsiella pneumoniae*).

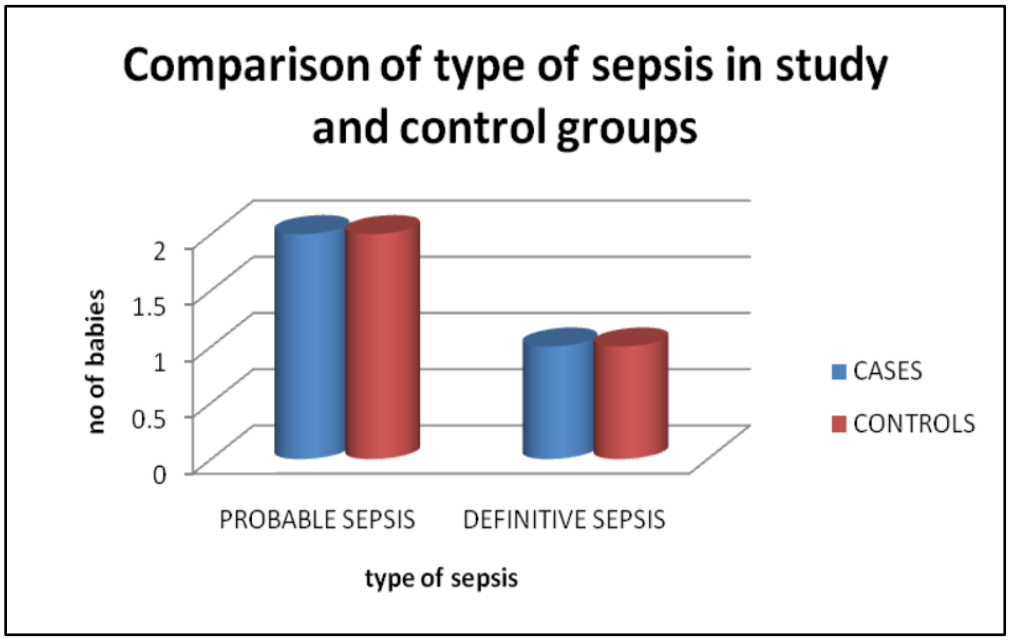

Figure 11: Comparison of the study's sepsis type with that of the control group.

Secondary outcome	Cases (n-35)	Controls (n-34)	P-value
Mortality	3 (8.6%)	3 (8.8%)	
Duration of IV fluids (in days)	3	3.6	
Duration of hospital stay (in days)	6.97 ± 0.89	6.94 ± 1.4	0.91
Weight loss per day (in grams)	0.03	0.032	0.445
Feed intolerance	0	0	

Table 15: Comparison of secondary outcome in study and control groups.

In the study group, 3 babies died. Out of 3 babies, 2 were died with sepsis. In Control group, out of 3 babies, 1 was died with sepsis. 3 babies in each group died of sepsis. 0^{th} NEC and feed intolerance were absent in any baby in each group. Both groups received IV fluids for about the same amount of time. In the study group, the length of hospitalization was 6.97 ± 0.89 days, whereas in the control group, it was 6.94 ± 1.41 days (P=0.91).

Average daily weight reduction in the *Bacillus clausii* group was 30gm, compared to 32gm in the placebo group.

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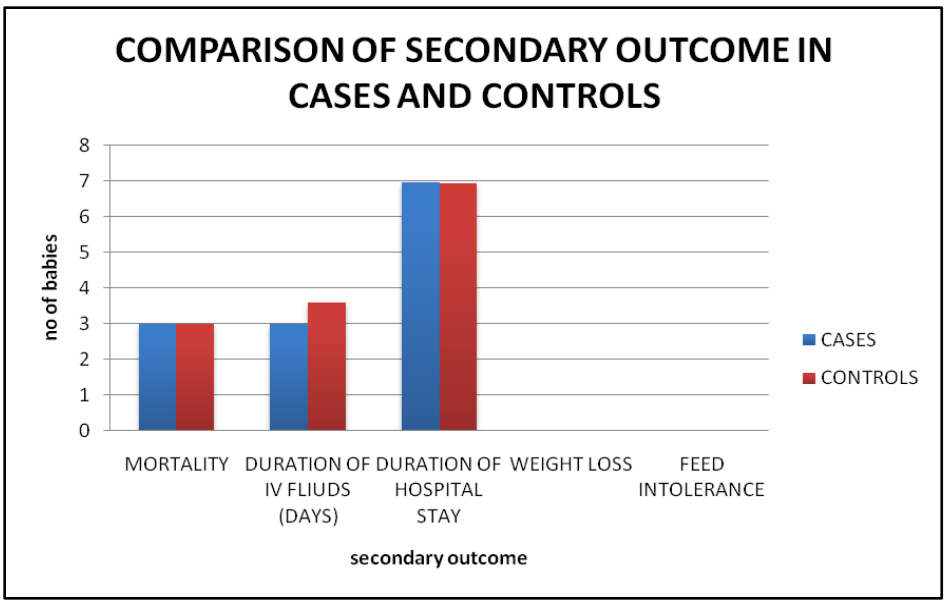

Figure 12: Comparison of secondary outcome in study and control groups.

	Clnical features	CRP	Blood culture	Cause of death
***Bacillus clausii* group**				
1	Lethargy, tachycardia, prolonged CFT	48	No bacterial growth	Sepsis
2	Apnea			Respiratory failure
3	Dull, prolonged CFT, sclerema	30	*Klebsiella*	Sepsis
Control group				
1	Respiratory distress	Neg		HMD
2	Lethargy, tachycardia, prolonged CFT	36	no bacterial growth	Sepsis
3	Apnea			Respiratory failure

Table 16: Details of babies died in study and control group.

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Out of 35 babies in study group, 2 babies died with sepsis . In control group, 1 baby died with sepsis.

Discussion

Of the 69 infants that participated in this study, most of the babies were between 30-34 weeks of gestation, mean gestational age was 33.28, p-value of 0.708.

Most of the babies were above 1.5kg birth weight and the mean birth weight was 1.53.

In another study by Carlo Danni, et al., [72], 585 babies were enrolled, in which all babies were below 33 weeks of gestation and birth weight was <1.5kgs. The study was conducted in 12 NICUs.

In a study conducted by Hung Chin Lin, et al., all the babies enrolled were below <1.5kgs.

The study by Bin, et al., [66], enrolled 145 babies. Mean gestational age 26.8, mean birth weight 949gm.

In the study, 8.51 percent of the 69 enrolled newborns got sepsis, compared to 8.82 percent in the control group.

Neither group's infants possessed NEC. The incidence of sepsis or NEC is not statistically different between the study and control groups.

This study by Carlo Danni, et al., appears to be similar since 4.7% of babies in the study group and 4.1% of babies in the control group experienced sepsis, whereas NEC occurred in 1.4% of study group babies and 2.8% of control group babies, both of which were not statistically significant.

In the study by Hung Chin Lin, et al., [70], the incidence of sepsis and NEC was significantly lower in the probiotic group compared to the control group (p-value 0.03 for both conditions).

The incidence of NEC was statistically significantly reduced with oral probiotic (p-0.0002) therapy in research by Hoyos AB [62].

In the study, mortality was 8.6% in cases and 8.8% in controls. There was no feed intolerance in both the groups. Hospital stay was 6.97 in cases and 6.94 in controls with p-value of 0.91. There was no statistical significance in duration of hospital stay in both the groups.

According to a study by Danni, et al., [72], probiotics may delay the onset of this condition. Probiotics may also be advantageous in lowering the incidence of NEC-related deaths in high-risk populations with shorter gestational times.

In the Samanta, et al., trial [68], there was lessened NEC incidence in VLBW infants, a shorter hospital stays, and a quicker time to complete feeds. There was a decreased incidence of death or NEC in VLBW preterm newborns in 2005 study by Lin, et al., [70].

Previous studies showed that use of probiotics is useful in circumvention of NEC and sepsis in premature and LBW babies, but this study did not show any conclusive evidence for or against it.

Randomized controlled studies with larger samples are needed to know the efficacy of *Bacillus clausii*.

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Large multi-centric double blind randomized controlled trials are needed to conclusively prove the efficacy of *Bacillus clausii* or other probiotics.

Summary and conclusion

Probiotics' effectiveness in preventing Late-onset sepsis and/or NEC in low-birth-weight infants was investigated in this study. A single blind randomized control trial was done with 69 babies.

All neonates fulfilling the inclusion criteria were enrolled in study. The patient was randomized after inclusion. The study group and control group were randomly assigned to the eligible infants.

In order to randomize, random sequences created by computers were used. The study included all neonates who met the inclusion requirements. The details were collected and entered in the Study proforma. Babies in the trial group received oral *Bacillus clausii* twice day for seven days at a dose of 1 billion. All enrolled infants had clinical monitoring for sepsis each day at intervals of six hours.

The conclusions of the study are as follows:

- 69 infants in all were recruited in the trial throughout the study period.
- 34 other infants were placed in the control group, while 35 were assigned to the *Bacillus clausii* group. Out of the 69 neonates, 18 (51.4%) were males and 17 (48.6%) were females in study population. In control group, males were 18 (52.9%) and females were 16 (47.1%).
- Mean gestational age was 33.28 ranging from 28 weeks to 36 weeks
- Mean birth weight was 1534grams ranging from 760gm to 1940gms
- Out of the 35 neonates in study population, 3 babies had sepsis.
- In control group,3 babies had sepsis.
- Between the study groups, there was no statistically significant difference (p=0.708).
- Both groups of babies lacked NEC.
- In the study, mortality was 8.6% in cases and 8.8% in controls.
- There was no feed intolerance in both the groups.
- There was no statistical significance in duration of hospital stay in both the groups.

Previous studies showed that use of probiotics is useful in prevention of NEC and sepsis in premature and LBW babies, but this study did not show any conclusive evidence for or against it. Randomized controlled studies with larger samples are needed to know the efficacy of *Bacillus clausii*.

Limitations of the study

- Smaller size of study population.
- EBM was aggressively fed to all of the infants (all infants received exclusively EBM). Hence, probably did not have any feed intolerance and there was low incidence of sepsis.
- Since the average length of IV fluids was only 3 days, the duration of IV fluids were aggressively reduced as the enteral feeding was aggressive.
- 61% of babies were >1.5Kg.

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The study did not show any conclusive evidence for or against the effectiveness of *Bacillus clausii* for circumvention of late onset sepsis or NEC.

Recommendations

1. Large multicentric double blind study is needed to prove the efficacy of the probiotics.
2. Large sample size needed to prove or disprove the effects of probiotics.
3. A different type of probiotic other than *Bacillus clausii* either on its own or in combination could be used for the study.
4. The duration used was one week in the study but a longer duration could be used.
5. It was recommended that a study with a large sample size could be done to look at the effects of *Bacillus clausii* on other aspects, other than sepsis and NEC, like feed tolerance. In larger study, adverse effects of the probiotics, if any, could be studied.

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Annexure 1

Consent form

Patient name: Age: Sex:

Hospital no.: IP no.:

I ..... father/mother of ..... aged ..... do hereby give my consent to do the necessary investigations that would help in diagnosis of my child's illness.

I have been explained about the illness and investigations required for diagnosis/management and also the academic study undertaken by the doctor.

I fully understand the nature of the study and I agree to be part of the study.

I have been informed that the medical details of my child would be confidential.

I have signed this consent voluntarily without any form of compulsion.

Name and signature of the parent/guardian:

Name & signature of the medical officer:

Relationship with the child:

Name & Signature of the witness:

Place:

Date:

Annexure II

Approval of ethical committee and its composition: Approved

S. No.	Name	Affiliation
1	Mr. Lakshman K	Chairperson (Advocate, Bar council of AP).
2	Dr. Murthy P S	Former Professor, HOD-Gandhi Medical college.
3	Dr. Manoj Malviya	Divisional Director-Neonatology.
4	Dr. Kaza Prabhavathi	Consultant Anesthetist.
5	Dr. Gowri Reddy M	Child Psychologist, Niloufer Hospital.
6	Dr. Kirti Khetan	Consultant Pathologist.

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Approval of scientific committee and its composition: Approved

S. No.	Name	Affiliation
1	Dr. M Reddy Padmanabh	HOD & Head of the Institution
2	Dr. Alladi Venkateswarlu	Former Professor, HOD, KMC, Manipal
3	Dr. Manoj Malviya	Divisional Director-Neonatologist
4	Dr. Durga Bhavani K	Consultant Neonatologist
5	Dr. Suresh Kumar P	Consultant Pediatrician
6	Dr. Kirti Khetan	Consultant Pathologist

This study was approved by Institutional Scientific Committee.

Annexure III

Proforma

Name: **Date of birth:**

Age: **Time of birth:**

Sex: M/F **IP no:**

History:

Address:

Antenatal history:

G P L A D

H/o PIH/APH/gestational diabetes, UTI, foul smelling liquor, fever, other.

Perinatal history

H/o PROM, MSL

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Mode of delivery: LSCS-indication for LSCS:/Vaginal/Forceps

Duration of labor

APGAR 1min: 5min:

Examination of the baby

Head to toe examination for congenital anomalies.

Pallor, cyanosis, icterus, adenopathy, edema, petechiae, bleeding from any site.

Passage of urine and meconium.

Vital data:

Heart rate:

Resp. rate:

CFT:

Temperature:

BP:

Systemic examination:

CVS:

Respiratory:

GIT:

CNS:

Investigations

CBP: WBC: N: L: M: E: B: Platelets:

ANC:

CRP:

Micro ESR:

Chest X-ray/abdominal X-ray:

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RBS:

S. Creatinine: Blood urea: S. Electrolytes:

CSF:

Blood Culture: Organism:

Monitoring and treatment given

	D1	D2	D3	D4	D5	D6	D7
Oxygen							
IV fluids							
Antibiotics							
Feeds: Type							
Volume							
Method							
Other drugs							

D-Day of life.

Outcome

- Alive and discharged/deaths
- Duration of hospital stay
- Sepsis
- NEC
- Weight loss per day in grams.
- Duration of fluids

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