N.V. Gonchar[1,2], L.A. Lo Schiavo[3], A.N. Suvorov[4,5], N.P. Shabalov[6], A.S. Kolbin[7], A.P. Kasimova[7]
[1] Mechnikov North-Western State Medical University, Saint Petersburg, Russian Federation
[2] Research Institute for Pediatric Infections of the Russian Federal Biomedical Agency, Saint Petersburg, Russian Federation
[3] Municipal pediatric hospital No. 1, Saint Petersburg, Russian Federation
[4] Research Institute for Experimental Medicine, North-Western branch of the Russian Academy of Medical Sciences, Saint Petersburg, Russian Federation
[5] Saint Petersburg State University, Russian Federation
[6] Kirov Military Medical Academy of the Ministry of Defense of the Russian Federation, Saint Petersburg
[7] Academician Pavlov First Saint Petersburg State Medical University, Russian Federation

Clinical and Pharmacoeconomic Reasonability of Using Probiotic Enterococcus Strain for the Complex Developmental Care Program for Premature Infants

Author affiliation:
Gonchar Natal'ya Vasil'evna, PhD, Professor at the department of pediatrics and neonatology of the Mechnikov North-Western State Medical University; acting head of the department of intestinal infections at the Research Institute for Pediatric Infections of the Russian Federal Biomedical Agency (Federal State Budgetary Institution), senior research fellow
Address: 41 Kirochnaya Str., Saint Petersburg, 191015; **tel.:** +7 (812) 303-50-00; **e-mail:** nvgonchar@yandex.ru

Article received: 15.10.2014. **Accepted for publication:** 24.12.2014.

Introduction. *Possibilities of using probiotic enterococci in premature neonates undergoing inpatient antibacterial therapy remains understudied. The article is* ***aimed at*** *analyzing clinical and pharmacoeconomic reasonability of using probiotic Enterococcus faecium L3strain in premature infants with very low body weight in the framework of complex inpatient developmental care.* ***Patients and methods.*** *55 children randomized into 2 groups were observed: the control group (n = 26) was undergoing standard developmental care program, the primary group (n = 29) was introduced liquid probiotic Enterococcus faecium L3 strain (titer – 10^8 CFU/ml or more) (0.5 ml TID for 14 days) after attaining the enteral feeding volume of 5.0 ml.* ***Results.*** *Analysis of the clinical symptoms characteristic of non-smooth course of developmental care over premature infants helped to reveal higher frequency of infectious complications in the control group children than in the primary group (14 [53.8%] vs. 6 [20.7%]; $p < 0.05$). Acute food intolerance was observed less frequently in the primary group than in the control group (6 [20.7%] vs. 10 [38.5%], $p > 0.05$). The primary group's children featured significant decrease in the frequency of monocytosis, positive changes of intestinal microbiotic composition (increase in the amount of bifidum bacteria, lactobacilli, enterococci, decrease in the amount of Clostridium difficile and antibiotic-resistant clinical Klebsiella pneumoniae strains).* ***Conclusion.*** *Favorable outcome of developmental care over premature infants (absence of infectious complications) was less expensive in the primary group's children.*
Keywords: *premature infants, very low birth weight, antibiotic therapy, probiotic enterococcus strain, pharmacoeconomic reasonability.*

INTRODUCTION

According to the World Health Organization resolution A61/21 of May 2, 2015 passed at the 67th World Health Assembly, improvement of neonates' health is a national objective of utmost importance. The success of high technologies in medicine made developmental care over premature infants a lot more effective [1]. Infectious complications are significant in terms of

morbidity and mortality of premature infants with very low birth weight (VLBW). These complications develop in the setting of gastrointestinal immaturity, abnormal microbial colonization of the intestines, inappropriate enteral load, and other factors [2, 3]. When treating premature infants with VLBW, they receive parenteral feeding and prolonged antibacterial therapy, during which medicine groups and generations are changed multiple times; this facilitates a broad spectrum of adverse events including dysbiosis, weakened immunity, allergic responses, and metabolic disorders [4-6].

Use of probiotics to treat adults and children has demonstrated its effectiveness in terms of prevention and treatment of antibacterial therapy complications [7, 8]. Positive effects of probiotics have been confirmed and include the recovery of microbiocenosis both in the intestines and in the body in general, as well as anti-inflammatory activity and normalization of metabolism and digestion [9].

In recent years, probiotics have come to be successfully used to treat premature infants in order to reduce sepsis frequency as well as the prevalence and severity of necrotic enterocolitis [10-13].

Based on the results of clinical studies, it has been established that probiotic enterococcal strains are effective stimulators of local, humoral, and cellular immunity. They are capable of keeping broad spectrum cytokines at a level appropriate for normal functioning of the innate immunity system [14]. Scientific articles describe the use of Linex, a probiotic drug that contains *Enterococcus faecium*, to prevent infectious complications in premature neonates [15].

The Russian probiotic *W. faecium* L3 strain has been well-studied for safety and is an intense antagonist of pathogenic and opportunistic microflora. It does not contain pathogenic genes detectable by means of polymerase chain reaction (PCR) and is able to eliminate manifestations of dysbiosis [16].

What has not been well-studied yet are the possibilities of using probiotic enterococci to prevent infectious complications in premature neonates when they are undergoing inpatient developmental care.

According to the Guidelines on Optimal Pharmacotherapy, maximum effectiveness and safety of pharmaceuticals should be combined with satisfactory economic value. New effective treatment and prevention techniques can be tried and tested based on pharmacoeconomic studies among other things [17].

The objective of this article is to study clinical and pharmacoeconomic effectiveness of using probiotic *E. faecium* L3 strain to treat premature infants with very low birth weight when undergoing inpatient care.

PATIENTS AND METHODOLOGY

A prospective controlled clinical study has been carried out.

Patients. Overall, 55 premature infants with very low birth weight were monitored. These children were transferred from maternity hospitals and intensive care units to the specialized neonatal pathology unit of Saint Petersburg Pediatric Hospital No. 1 (headed by Prof. A.V. Kagan) to undergo further inpatient treatment in 2011-2012.

Inclusion criteria were as follows: VLBW (1,000-1,500 g), gestational age of 28-34 weeks at birth, and age of 3-21 days after birth at the time of admission.

Exclusion criteria were as follows: acute congenital developmental failures requiring surgical intervention during the neonatal period; severe perinatal pathologies of the central nervous system; mechanical lung ventilation at the intensive care unit for 10 days or longer.

The clinical study was reviewed and approved at a meeting of the S.M. Kirov Military Medical Academy Committee on Ethics.

Clinical-anamnestic methods of neonatal examination. When collecting the anamnestic data, antenatal and intranatal adverse factors were identified, including complicated obstetric-gynecological history of mothers, chronic intoxication, chronic somatic pathology, complicated and abortion-threatened pregnancy, and antibacterial treatment. Pyelonephritis and cystitis as

well as chronic ENT diseases were highlighted as the probable causes of intrauterine fetus infection. Term "chronic intoxication" included smoking, drug addiction, and continuous intake of pharmaceuticals. The nature of feeding and physical development parametrical dynamics were subject to evaluation. The researchers appraised the survived diseases, as well as organ and system pathologies. When monitoring the neonates at the inpatient facility, they would carry out a thorough daily system examination and evaluate dynamics of anthropometric parameters. The children were checked-up by surgeons, neuropathologists, ophthalmologists (and other specialists if necessary).

In-vitro methods. All the neonates under research would undergo general tests once in 10 days or more often if necessary. The tests included clinical blood test, general urine test, advanced stool test, biochemical blood test based on the standard methods with determination of the total protein level, bilirubin, transaminases (AlAT and AsAT), glucose, C-reactive protein, acid-base blood status, and the composition of electrolytes.

They studied the composition of microbiota by means of the bacteriological method and real time PCR (RT PCR) at a diagnostic laboratory headed by M. A. Suvorova (PhD in Medicine). The material was sampled for the tests three times: first when a child was admitted to the unit (Study 1), after that – once every fortnight (Studies 2 and 3). The bacteriological feces tests were carried out with due consideration of V. Krasnogolovets's guidelines (1999) and in compliance with the Industrial Standard "Patient Management Procedure. Intestinal Dysbacteriosis" (2003). The RT PCR with fluorescent detection was used to determine the ratio of DNA of obligatory and opportunistic microflora in fecal samples of the patients. The total nuclear DNA was extracted from fecal samples by means of the DNK-Express reagent kits mnufactured by Litech, Saint Petersburg. The amplification reaction was conducted in a MiniOpticon thermal cycler by Bio-Rad, USA. DNA-polymerase Taq by Silex, LLC, Moscow, was used for that purpose. The amount of identifiable microbial DNA was calculated on the basis of the values of the Ct threshold cycle using the $2^{\Delta Ct}$ method.

Antibiotic sensitivity of the clinical strains of opportunistic microflora (identifiable in the titer in the amount of 10^3 CFU/g or more) was studied by means of the disc-diffusion test using the standard discs and the Müller-Hinton agar. NCCLS (U.S., 1998) and MAKMAX tests (Russia, 1999) were used to evaluate results of the study.

Therapy methods. Premature infants with VLBW were randomly divided into two groups. Group 1, or the control group, n = 26, underwent standard developmental care. Group 2, or the primary group, n = 29, received 0.5 ml of liquid probiotic *E. faecium* L3 strain TID for 14 days after achieving a sustainable 5.0 ml volume of enteral nutrition.

The probiotic form of *E. faecium* L3 with a minimum titer of 10^8/ml CFU (No. RU.77.99.26.009.E.002272.02.11) is used as the culture for the production of non-dairy therapeutic nutrition (Bakfir, Laminolact). This probiotic form was either added to the formula or breastmilk bottle, or introduced into the feeding tube in case of tube nutrition.

The standard program for developmental care over premature infants with VLBW included appropriate respiratory therapy with the use of surfactant replacement at the first stage, transportation with preservation of temperature regimen, infusion therapy, complete or partial parenteral nutrition, and antibacterial therapy. The initial antibacterial therapy contained 2 medicine groups, penicillins (100 to 150 to 200 mg/kg/day of ampicillin intravenously) and aminoglycosides (4 to 5 mg/kg/day of netromycin, gentamycin intravenously), and would start at the maternity hospital. The antibacterial therapy would be changed after 10-14 days or earlier, if blood tests showed increased inflammatory changes. The second standard antibacterial therapy course comprised cephalosporins (50 mg/kg/day of cefuroxime and ceftazidime intravenously) together with aminoglycosides (10 to 15 mg/kg/day of amikacin intravenously). When blood tests showed inflammatory changes, the antibacterial therapy was continued using carbapenems (30-40 mg/kg/day of meropenem intravenously by drop infusion) and glycopeptides (20-30 mg/kg/day of vancomycin intravenously by drop infusion). When the medical history indicated viral intrauterine infection, antiviral medicines were prescribed (10-30 [up to 60] mg/kg/day of

acyclovir or 10 mg/kg/day of Cymevene), as well as specific human immunoglobulin drugs (1 ml/kg of NeoCytotect intravenously by drop infusion [No. 3]). Antifungal therapy was carried out with Diflucan (6 mg/kg/day). When the medical history featured infections, blood tests showed intense inflammatory changes, and inflammatory marker values (C-reactive protein and procalcitonin test) were high, human immunoglobulin drugs were prescribed (3 ml/kg of Pentaglobin intravenously by drop infusion [No. 3]). When apneustic syndrome developed, the medicines to prescribe were caffeine (0.5 ml intravenously and intramuscularly), Euphyllin (2.4 mg/kg intravenously by drop infusion), and inhalational therapy (Berodual and Pulmicort through nebulizer). The accompanying therapy prescribed in accordance with indications included neurotrophic drugs (0.3 ml of gliatilin twice a day intravenously and intramuscularly [No. 10]; 0.5 ml/day of Cerebrolysin; 0.5 ml/day of Actovegin intravenously and intramuscularly [No. 10]), diuretic drugs (Lasix, Verospiron), and vitamins B intravenously and intramuscularly (No. 10).

Infusion therapy was conducted in accordance with the Guidelines on Premature Infants with VLWB. The average daily fluid intake was 60-80-100 ml/kg on day 1 and 2, and 120-130-150 ml/kg/day after that. The glucose load was calculated based on the need. For children of 0-1 day of age, the dose was 8 mg/kg/min; for older children – 10-12 mg/kg/min. The glucose-saline solutions prepared at the inpatient pharmacy were used. Parenteral feeding included amino acid solutions (Aminoven Infant 10%; the amount of protein was calculated on the basis of a 2.5 g/kg daily dose) and lipid emulsions (Intralipid 20%; the amount was calculated on the basis of the lipid need, i.e. 0.5-1.0-2.5 g/kg/day).

Minimal trophic breastmilk nutrition was prescribed for premature infants if breastfeeding was feasible and mothers did not have acute and uncured infection sites, as dictated by the earliest enteral nutrition principle. When breastfeeding was not feasible, low osmolarity and high-calorie formulas were used. The initial enteral nutrition load was 1.0 ml per feeding. The amount of enteral nutrition would be increased gradually, and the children's status was under continuous monitoring. Follow-up monitoring of premature infants was used to trace signs of acute food intolerance: blotted abdomen, excessive vomiting, presence of residual volume in the gaster containing pathological impurities; stool delay; skin re-colorization, and respiratory failure.

If food intolerance developed, enteral nutrition would be suspended, and the child would then receive parenteral feeding only. The therapy was supplemented with metronidazole (7.5 mg/kg/day of metrogyl intravenously by drop infusion). When food intolerance symptoms were arrested, the children were reverted back to enteral feeding (the minimum amount).

The calorific value of parenteral and enteral feeding was calculated on a daily basis in compliance with the recommended norms. This value would be gradually increased from 60 kcal/kg/day to 120-130 kcal/kg/day. The amount of infusion therapy was reduced inversely-proportionally to the increase of the calorific value and the amount of enteral feeding.

Under conditions of a specialized inpatient unit, the premature infants with VLBW were placed into incubators maintaining permanent temperature and humidity of the environment (t 32−34 C^{o}; h 50-60-70%). When a child reached the body weight of 1,500 g, he/she would be laid on an open table warmed with a radiant heat source. The body temperature was subject to 24h/day control.

Premature infants were deemed ready to be discharged from the inpatient facility when the body weight was 2,000 g or more, and the children had an established sucking reflex and a satisfactory neurological status, as well as daily weight gain.

Evaluation of effectiveness of the developmental care of VLBW children

Effectiveness of the developmental care provided to premature infants with VLBW under monitoring was evaluated by the duration of parenteral feeding, the frequency of acute food intolerance, the frequency of infectious complications, the nature of hematologic parametrical changes, the duration of antibiotic therapy, the duration of inpatient care, and the dynamics of

intestinal microbiota composition. The infectious complications included diagnosed intrauterine infection (IUI), intra-amniotic infection (IAI), and necrotic enterocolitis (NEC).
The IUI group featured diseases caused by agents that were hematogenously transmitted from the infected mother to the fetus. The IUIs are characterized by placenta lesion with fetoplacental failure, fetus hypoxia, intrauterine development delay, and preterm birth as sequelae. Fetus IUI was understood as an infectious process emerging inside fetal shells. However, IUIs are not always clearly distinct from IAI in the real practice. The ICD-10 contains infections specific to the perinatal period (P35 to P39), including TORCH syndrome (herpes virus infection, chlamydia infection, congenital rubella, toxoplasmosis etc.), parasite and bacterial neonatal infections, and neonatal sepsis. All of these are conventionally believed to be IUIs. P39.2 Intra-amniotic infection of fetus is a separate section; it is not classified anywhere else. The common feature of IUIs and IAI is the infection source (which almost always is the mother). The discrepancy is the infection transmission pathway. IAI is characterized by either ascending or descending pathway if there is an infection site in the abdominal cavity or uterine adnexa. Thus, IAI was diagnosed if there were anamnestic data on the presence of vulvovaginitis, endocervicitis, and other chronic urogenital infections in the mother, especially when such diseases exacerbated during pregnancy or labor (chorioamnionitis). When the mother's pregnancy was complicated by acute respiratory viral infection or any other non-urogenital infection, the hematogenic infection transmission pathway was assumed. IUIs would be diagnosed in the event of transplacental transmission of the infectious agent confirmed by the fact that the mother's blood contained large amounts of causative agents, which served as the evidence of bacteremia or viremia and would facilitate development of fetal shell inflammation as well as the generalization of the infectious process.

Statistical processing of study results
The clinical and paraclinical parameters under research were adapted for mathematical processing and analyzed by means of a simple and multivariable statistical analysis using an Intel Celeron PC. The tool used to conduct computational experiments was Statistica for Windows v.7, a statistical analysis software package. The groups of premature infants were compared using the Student's t-test, and the Wilcoxon signed-rank test. Cross-tables were analyzed using the Pearson's χ^2 test, Yates' test, and Fisher's test (the results would be deemed relevant at $p < 0.05$).
Pharmacoeconomic analysis. To achieve objectives of this research, the average developmental care cost was calculated per premature infant. This cost included the direct medical costs (MC) of antibacterial, antiviral, antifungal, deintoxication, and symptomatic therapy, the costs of the probiotic strain, and the cost of adverse effects treatment. When carrying out the pharmacoeconomic analysis itself, the Cost-Effectiveness Ratio, or CER, was calculated using the following formula:
CER = MC / EF,
where MC is the average sum of costs and expenditures per patient, and EF is the share of patients with positive treatment results.
If effectiveness and cost of one of the programs under analysis exceeded another program's effectiveness and cost, incremental analysis supplemented by calculation of the ICER (incremental-cost-effectiveness) was required:
ICER = MC (method 1) – MC (method 2) / EF (method 1) – EF (method 2).
What is more, the NPT parameter (number of patients to treat in order to prevent one unfavorable outcome in the control group) used to compare pharmacoeconomic parameters of different types of treatment or prevention was calculated using the following formula:
NPT = 1 / RAR,
where RAR is the reduction of absolute risk or the difference between the frequency (share) of "insufficient effect" (unfavorable outcome) of treatment in the primary group and the control group.

RESULTS

The groups of premature infants with VLBW were comparable to each other in terms of initial parameters, i.e. sex, gestational age, birth weight and length, the APGAR score at birth, as well as age at admission (tb. 1).

Table 1. ***Peculiarities of the premature VLBW infant groups under research***

Parameters	**Group 1 (n = 26)**	**Group 2 (n = 29)**	**Significance level, p**
Boys (abs. n), %	11/42.3	14/48.3	> 0.05
Girls (abs. n), %	15/57.7	15/51.7	> 0.05
Gestaional age in weeks	28.9 ± 0.4	29.1 ± 0.4	> 0.05
Birth weight, g	1,197 ± 37	1,210 ± 40	> 0.05
Body length at birth, cm	37.0 ± 0.5	37.0 ± 0.5	> 0.05
Minute 1 APGAR Score (points)	5.3 ± 0.3	5.4 ± 0.4	> 0.05
Minute 5 APGAR Score (points)	6.5 ± 0.2	6.1 ± 0.3	> 0.05
Age at admission, days	3.6 ± 0.9	3.0 ± 0.4	> 0.05

Premature infants with VLBW had multiple adverse factors in the perinatal medical history. Complicated obstetric-gynecological history was identified in 49.1% of mothers, 45.5% had chromic somatic pathology, 14.5% underwent antibacterial therapy during pregnancy, 20.0% suffered chronic intoxication, 60.0% underwent therapy of threatened miscarriage; 41.8% of children were delivered via caesarean section, and 16.4% of mothers had fever during the labor.

The comparison of premature infant groups by the frequency of unfavorable perinatal history factors identified discrepancies only in terms of caesarean section frequency (55.2% in group 1, 28% in group 2; $p > 0.05$), which indicates that the control group children are at a higher risk of intestinal dysbiosis development.

Most patients of both groups were formula-fed. The total number of breast-fed and breast- and formula-fed children was 28.0% in group 1 and 37.9% in group 2 ($p > 0.05$).

Considering use of a central venous catheter for 10 days or longer, no significant difference between premature infants in groups 1 and 2 was observed (92.3% and 93.1%, respectively; $p > 0.05$). The same applies to antibiotic therapy for 10 days or longer (92% and 89.7%, $p > 0.05$), and antibiotic treatment change (80% and 82.8%, $p > 0.05$).

The frequent complications of preterm birth were respiratory distress syndrome (RDS) at the stage of developmental care at maternity hospital (61.8%); hypoxic-ischemic encephalopathy (81.8%), retinopathy in premature infants (34.5%), early anemia in premature infants (67.3%), and patent ductus arteriosus (25.5%).

Table 2. ***The frequency of subacute course of developmental care over immature infants with VLBW (abs./%)***

Parameters under research	**Group 1 (n=26)**		**Group 2 (n=29)**		**Pearson's χ^2 test**	**Significance level, p**
	no	yes	no	yes		
10+ days central venous access	3/11.5	23/88.5	2/6.9	27/93.0	0.02	> 0.05
10+ days antibiotic therapy	2/8.7	24/92.3	3/10.3	26/89.7	0.09	> 0.05
Acute food intolerance	16/61.5	10/38.5	23/79.3	6/20.7	1.88	> 0.05
IUI	20/76.9	6/23.1	26/89.7	3/10.3	1.62	> 0.05
IAI	19/73.1	7/26.9	26/89.7	3/10.3	2.53	> 0.05
NEC	24/92.3	2/7.7	100	0	2.08	> 0.05
Infectious complications	12/46.2	14/53.8	23/79.3	6/20.7	6.51	**< 0.05**

Note. IUI stands for intrauterine infection, IAI stands for intra amniotic infection, NEC stands for necrotic enterocolitis.

Infectious complications (tb. 2) were diagnosed in 20 (36.4%) premature infants with VLBW. IUIs were diagnosed in 9 (16.4%) children and verified in 3 (22.2%). Two of these children had IUI of the cytomegalovirus etiology; in one case, IUI had herpetic etiology (herpes virus type 1). IAI was diagnosed in 10 (18.2%) children and verified in 2. It was accompanied by streptococcal pulmonary lesion and staphylococcal gastrointestinal lesion. NEC was diagnosed in 2 (3.6%) children. The etiology was not identified in either case, but in one of them, NEC was combined with staphylococcal IAI. Neither case required surgical intervention.

When comparing groups 1 and 2 in terms of the average (M ± m) duration of parenteral feeding (19.8 ± 2.2 and 18.0 ± 1.9 days; $p > 0,05$), time before complete transition to enteral feeding (20.3 ± 2.2 and 18.8 ± 1.9 days; $p > 0.05$), the frequency of moderate abdominal swelling (25 and 15.4%; $p > 0.05$), the frequency of stool quality changes and defecation frequency increase (25 and 23.1%; $p > 0.05$); the frequency of early anemia in premature infants (72.4 and 65.4%; $p > 0,05$), the frequency of RDS identification, (55.2 and 65.4%; $p > 0,05$), frequency of bronchopulmonary dysplasia (13.8 and 7.7%; $p > 0,05$), and frequency of neonatal jaundice (17.2 and 7.7% in the groups 1 and 2, respectively; $p > 0,05$), no significant differences were identified by using Student's and Pearson χ^2 tests.

Analysis of clinical signs of the suboptimal course of developmental care over children with VLBW identified a significantly higher frequency of infectious complications in the control group (14, or 53.8%) patients as compared to the primary group (6, or 20.7%; $p < 0.05$).

Acute food intolerance was less frequent in the primary group children (6, or 20.7%). In the control group, 10 children had it (38.5%, $p > 0.05$). These conditions forced the developmental care personnel to revert back to complete parenteral feeding, which signified a significant disruption of the developmental care program.

The frequency of in vitro signs of suboptimal course of developmental care is given in tb. 3. Positive blood culture test combined with leukocytosis are the signs of systemic inflammatory response and are equally frequent in both groups. The control group children had a relevantly higher frequency of monocytosis (24 children, or 82.8% of the primary group; cf. to 26 or 100% of the control group; $p < 0.05$).

Table 3. ***Frequency of in-vitro signs of suboptimal course of developmental care over immature infants with VLBW (abs./%)***

Parameters under research	**Group 1 (n=26)**		**Group 2 (n=29)**		**Pearson's χ^2 test**	**Significance level, p**
	no	yes	no	yes		
Positive blood culture	25/96.2	1/3.8	27/93.1	2/6.9	0.24	> 0.05
Leukocytosis	14/53.8	12/46.2	17/58.6	12/41.4	0.06	> 0.05
Eoeosinophilia	12/46.2	14/53.8	18/62.1	11/37.9	1.15	> 0.05
Monocytosis	0/0	26/100	5/17.2	24/82.8	4.01	**< 0.05**
Anemia	4/15.4	22/84.6	4/13.8	25/86.2	0.26	> 0.05

A follow-up bacteriological study of feces of the primary group children established a highly significant rapid increase in the amount of bifidum bacteria, as well as delayed significant increase in the amount of lactobacilli. Delayed increase in the amount of bifidum bacteria amounts as well as the absence of lactobacilli fluctuations were observed in the control group children (tb. 4).

Table 4. ***Evaluation of intestinal microbiota composition dynamics based on bacteriological feces test results of premature infants with VLBW***

Qualitative indicators	**Quantitative indicators (CFU/g; M ± m) / Number of studies**			**Difference significance level, p**
	Study 1*	**Study 2***	**Study 3***	
The primary group (n = 29)				

Bifidobacterium	7.0×10^8 ± $8.4\times10^8/29$	7.0×10^8 ± $8.4\times10^8/29$	$1.0\times10^9\pm0.0/27$	**$_{1-2}$ < 0.001** **$_{1-3}$ < 0.001**
Lactobacillus	6.8×10^8 ± $9.0\times10^7/29$	7.3×10^8 ± $8.4\times10^7/29$	8.9×10^8 ± $6.1\times10^7/27$	**$_{1-3}$ < 0.05**
K. pneumoniae	$4.1\times10^5 \pm 2.7\times10^7$	$1.7\times10^6 \pm 2.0\times10^7$	$1.1\times10^6 \pm 2.0\times10^7$	> 0.05
The control group (n = 26)				
Bifidobacterium	6.9×10^8 ± $9.3\times10^7/25$	8.8×10^8 ± $6.9\times10^7/24$	9.2×10^8 ± $5.3\times10^7/26$	**$_{1-3}$ < 0.05**
Lactobacillus	5.2×10^8 ± $1.0\times10^8/25$	5.9×10^8 ± $1.0\times10^8/24$	6.6×10^8 ± $9.4\times10^7/26$	> 0.05
K. pneumoniae	$1.3\times10^6 \pm 3.1\times10^7$	$6.4\times10^7 \pm 2.4\times10^7$	$2.1\times10^6 \pm 2.2\times10^7$	> 0.05

Note. * **—** Study 1: at admission; Study 2: after 14 days; Study 3: after 28 days.

The Wilcoxon's test was applied to paired samples from the primary group; this resulted in identification of a relatively "late" increase in the amount of klebsiellae in the follow-up period based on bacteriological feces test ($p_{1-3} < 0.05$), whereas a relatively "early" increase was observed in the control group children ($p_{1-2} < 0.05$).

The RT PCR data showed a sigificant increase in the amount of enterococci in the primary group children during the second study ($p_{1-2} < 0.05$) as well as a significant reduction of *C. difficile* in the framework of studies 2 and 3 ($p_{1-2} <0.01$; $p_{1-3} < 0.01$). The control group did not have any significant changes of the intestinal microbiota composition (tb. 5).

Table 5. ***Evaluation of intestinal microbiota composition dynamics based on RT PCR test results of premature infants with VLBW***

Qualitative indicators	**Quantitative indicators (CFU/g; M ± m) / Number of studies**			**Difference significance level, p**
	Study 1*	**Study 2***	**Study 3***	
The primary group (n = 29)				
The total bacterial amount	6.4×10^{11} ± $4.3\times10^{11}/24$	9.4×10^{11} ± $3.4\times10^{11}/28$	1.5×10^{12} ± $4.1\times10^{11}/26$	> 0.05
B. fragilis	1.8×10^8 ± $6.4\times10^7/24$	2.8×10^9 ± $2.0\times10^9/27$	1.5×10^{11} ± $1.2\times10^{11}/26$	> 0.05
C. difficile	2.0×10^{10} ± $4.4\times10^9/3$	5.5×10^9 ± $2.2\times10^9/1$	1.9×10^9 ± $1.5\times10^9/8$	**$_{1-2}$ < 0,01** **$_{1-3}$ < 0,01**
E. coli	2.9×10^{10} ± $1.9\times10^{10}/24$	3.1×10^{11} ± $2.2\times10^{11}/27$	4.5×10^{11} ± $2.8\times10^{11}/26$	> 0.05
Enterococcus	1.1×10^{10} ± $4.1\times10^9/24$	6.1×10^{10} ± $2.5\times10^{10}/27$	2.4×10^{10} ± $1.2\times10^{10}/26$	**$_{1-2}$ < 0.05**
The control group (n = 26)				
The total bacterial amount	5.1×10^{11} ± $2.7\times10^{11}/18$	1.6×10^{11} ± $4.4\times10^{10}/15$	3.1×10^{11} ± $9.6\times10^{10}/15$	> 0.05
B. fragilis	3.9×10^{10} ± $3{,}8\times10^{10}/26$	4.0×10^9 ± $3.8\times10^9/26$	7.3×10^9 ± $4.5\times10^9/25$	> 0.05
C. difficile	5.0×10^9 ± $4.4\times10^9/1$	1.3×10^8 ± $2.5\times10^9/6$	2.6×10^8 ± $1.8\times10^9/4$	> 0.05
E. coli	4.5×10^{10} ± $3.8\times10^{10}/26$	2.5×10^{10} ± $1.1\times10^{10}/26$	7.7×10^{10} ± $4.1\times10^{10}/25$	> 0.05
Enterococcus	6.6×10^9 ± $2.6\times10^9/26$	5.4×10^{10} ± $3.5\times10^{10}/26$	2.7×10^{10} ± $9.5\times10^9/25$	> 0.05

Note. * **—** Study 1: at admission; Study 2: after 14 days; Study 3: after 28 days.

Opportunistic intestinal microflora of premature infants is mostly represented by klebsiellae identified in titers of 10^3 CFU/g or more. *K. pneumoniae* dominated the identified klebsiellae in premature infants of both groups during all the three studies: 38.2 ± 6.6; 49.1 ±7.4; 65.5 ± 6.4%, respectively ($p_{1-2} > 0.05$; $p_{1-3} < 0.01$).

The *K. pneumoniae* isolates were tested for sensitivity to antibiotics in the setting of the therapy (studies 1 and 2). The tests showed that in the premature infants of the primary group, the isolates had a relevantly increased sensitivity to norfloxacin (from 28.6 ± 8.4 to 59.1 ± 9.1%; $p < 0.05$) and ciprofloxacin (from 33.0 ± 8.7 to 71.4 ± 8.4%; $p < 0.01$). On the contrary, the control group had a relevantly reduced sensitivity to ampicillin/sulbactam (from 66.7 ± 9.2 to 0%; $p < 0.001$), norfloxacin (from 66.7 ± 9.2 to 35.7 ± 9.4%; $p < 0.05$), ciprofloxacin (from 66.7 ± 9.2 to 38.5 ± 9.5%; $p < 0.05$), and ceftazidime (from 30.0 ± 9.0 to 7.1 ± 5.0%; $p < 0.05$).

After 14 days since the cancellation of probiotics (study 3), not significant changes of klebsiella sensitivity to antibiotics under analysis were observed in the primary group children. An increased concentration of amoxiclav-sensitive strains (from 0 to 15 ± 7.0%; $p < 0.05$) and ceftazidime-sensitive strains (from 7.1 ± 5.0 to 30 ± 9.0%; $p < 0.05$) was observed in the control group.

The researchers have analyzed the pharmacoeconomic effectiveness parameters of developmental care programs for children with VLBW.

The result was as follows: the average developmental care cost was 39,344 ± 3,028.2 RUB in the primary group, and 37,344 ± 3,102.7 RUB in the control group. Thus, the developmental care cost difference was not significant.

However, pharmacoeconomic cost-effectiveness analysis that used the CER parameter helped to establish that effectiveness of developmental care in the primary group was significantly higher (CER_2 49,802.5) as compared to the control group (CER_1 81,182.6), which means that a positive outcome in the primary group requires less money:

CER_1 37,344/0.46 = 81,182.6 and CER_2 39,344/0.79 = 49,802.5,

where 0.46 and 0.79 are the share of children with no infectious complications.

Incremental cost analysis that used the ICER parameter (ICER = 39,344-37,344 / 0.79-0.46 = 6,060.6) helped to establish that despite the higher cost of the therapy using the same probiotic *E. faecium* L3 strain, achievement of the same outcome would require more money in the control group than in the primary group. The difference amounted to 6,060.6 RUB on the average. The NPT value (inversely proportional to the reduction of the absolute unfavorable outcome risk) was found to be 3.03 as a result of the calculation:

1 / (0.54-0.21),

where 0.54 and 0.21 are the shares of children with infectious complications of the control group and the primary group, respectively; 0.33, therefore, is the reduction of the absolute unfavorable outcome risk. It means that the number of patients to treat in order to prevent one unfavorable outcome is three when it comes to the control group.

DISCUSSION

Studies have shown that premature infants with VLBW had multiple unfavorable peculiarities of the perinatal anamnesis, nutrition, and treatment. Negative impact on the formation of intestinal microbiota was unavoidable [4, 5].

The developmental inpatient care program applied to premature infants with VLBW used probiotic *E. faecium* L3 strain and did not have any negative impact on the duration of parenteral nutrition, did not complicate the introduction of enteral nutrition, did not increase the frequency of such preterm birth complications as RDS and bronchopulmonary dysplasia, did not increase the frequency of early premature infant anemia, and neonatal jaundice. The study of subacute course signs in the setting of developmental care identified a higher frequency of monocytosis in the control group children. Monocytosis is known to indicate infection-caused inflammatory processes in infants and is connected to immunity stimulation by microbial antigens [18].

A significantly lower frequency of infectious complications was identified in the primary group children. Besides, there was a clear tendency to a lower frequency of acute food intolerance as compared to the control group. The obtained clinical outcomes were the consequence of positive changes of the indigenous and opportunistic intestinal microbiota as well as the corrective effect of such changes on the adaptive immunity. The changes included a significant increase in the amount of bifidum bacteria, lactobacilli, and enterococci; the change of opportunistic microflora was represented by reduction of *C. difficile* [7, 11].

A high frequency of klebsiella titers was identified in the composition of opportunistic microbiota in premature infants with VLBW. Application of probiotic *E. faecium* L3 strain to premature infants with VLBW facilitated reduction of antibiotic-resistant klebsiellae. However, the identified peculiarities of klebsiella isolate sensitivity to antibiotics did not persist after the probiotic strain was suspended. Meanwhile, an increased klebsiella isolate sensitivity to antibiotics was observed in the control group children; this indicated the formation of their own protective capacity. However, this effect would be achieved only after two weeks of inpatient developmental care or even later.

The average developmental care cost difference between the groups was not significant due to the relatively low cost of probiotic *E. faecium* L3 strain in use. The pharmacoeconomic cost-effectiveness analysis helped to find out that a positive outcome requires less money to achieve a positive outcome in the primary group of premature infants. Therefore, it has been shown that the maximum effectiveness of the programs for developmental care over premature infants with VLBW was in line with the optimal economic parameters [17].

CONCLUSION

The obtained results indicate it is clinically and economically reasonable to use probiotic *E. Faecium* L3 strain in the framework of the inpatient developmental care over premature infants with VLBW. Preventive application of probiotic *E. faecium* L3 strain in premature infants with VLBW facilitated a significantly lower frequency of infectious complications (cf. 6/20.7% in the primary group to 14/53.8% in the control group; $p < 0.05$) and a lower frequency of food tolerance (cf. 6/20.7% to 10/38.5%, respectively; $p > 0.05$), which was accompanied by a significant monocytosis frequency reduction, positive changes of intestinal microbiota, relevant increase in the amount of bifidum bacteria, lactobacilli, enterococci, as well as decrease in the amount of *C. difficile* and antibiotic-resistant *K. pneumoniae* clinical strains. The performed studies showed that use of probiotic *E. faecium* L3 strain as a part of developmental care over premature infants with VLBW provides for a strong protective potential of indigenous intestinal microbiota (bifidum bacteria, lactobacilli) against opportunistic microorganisms. It facilitates a significant reduction of infectious complication risks. Favorable outcome of developmental care over premature infants with VLBW (absence of infectious complications) was least expensive when the mixed therapy included probiotic *E. faecium* L3 strain as compared to the standard program.

CONFLICT OF INTEREST

The authors have indicated they have no financial support / conflict of interest relevant to this article to disclose.

REFERENCES

1. Surkov D.N., Ivanov D.O., Obolonskii A.I., Kapustina O.G., Volkov D.G., Petrenko Yu.V. Current strategies for nursing preterm infants. *Detskaya meditsina Severo-Zapada = Children's Medicine of Northwest*. 2012; 3 (1): 4–9.

2. Karavaeva S.A. Diagnosis and clinical features of necrotizing enterocolitis in children. *Vestnik khirurgii = Bulletin of surgery*. 2002; 161 (4): 41–46.

3. Bin-Nun A., Bromiker R., Wilschanski M. et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. *Pediatrics*. 2005; 147: 192-196.
4. Volyanyuk E.V. Features of antibiotic therapy in preterm infants. *Prakticheskaya meditsina = Practical medicine*. 2010; 40: 49–52.
5. Lysenko I.M., Barkun G.K., Zhuravleva L.N., Klisho V.E., Misnik O.M. Features colonization resistance neonatal pediatric ward for premature babies in pediatric regional clinical hospitals. *Okhrana materinstva i detstva = Maternity and child welfare service*. 2008; 1: 105–110.
6. Benis N.A., Samsonova T.V. Clinical and functional characteristics of preterm infants with extremely low and very low birth weight infants and different stages of gestation. *Detskaya meditsina Severo-Zapada = Children's Medicine of Northwest*. 2012; 3 (1): 26–29.
7. Zakharova I.N., Mazankova L.N. *Antibiotikassotsiirovannye diarei u detei: problema i reshenie. Uchebnoe posobie dlya vrachei* [Antibiotic-associated diarrhea in children: the problem and the solution. A manual for physicians]. Moscow, 2011. 48 p.
8. Sanders M. E., Akkermans L. M. A., Haller D. et al. Safety assessment of probiotics for human use. *Gut Microbes*. 2010; 1 (3): 164-85.
9. *Disbioz kishechnika. Rukovodstvo po diagnostike i lecheniyu. 2-e izd., ispr. i dop. Pod red. E.I. Tkachenko, A.N. Suvorova* [Intestinal dysbiosis.Guidelines for the diagnosis and treatment. 2nd ed., Rev. and add. Ed. EI Tkachenko, AN Suvorov]. St. Petersburg, InformMed, 2009. 276 p.
10. Deshpande G., Rao S., Patole S. Probiotics for prevention of necrotizing enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. *Lancet*. 2007; 369 (9573): 1614-20.
11. Lund B., Edlund C., Barkholt L. et al. Impact on human intestinal microflora of an Enterococcus faecium probiotic and vancomycin. *Scand J Infect Dis*. 2000; 32: 627-632.
12. Millar M., Wilks M., Costeloe K. Probiotics for preterm neonates? *Arch Dis Child Fetal Neonatal*. 2003; 88: F354-F358.
13. Schanler R. J. Probiotics and necrotising enterocolitis in premature neonates. *Arch Dis Child Fetal Neonatal*. 2006; 91: F395-7.
14. Bondarenko V.M., Suvorov A.N. *Simbioticheskie enterokokki i problemy enterokokkovoi opportunisticheskoi infektsii* [Symbiotic enterococci and problems enterococcal opportunistic infection]. Moscow, 2007.
15. Gorobchenko V., Kupryashina I., Miller Yu. Clinical and bacteriological efficacy of probiotic Linex in preterm infants. *Vrach = The Doctor*. 2008; 5: 70.
16. Tarasova E., Yermolenko E., Donets V. et al. The influence of probiotic enetrococci on the microbiota and cytokines expression in rats with dysbiosis induced by antibiotics. *Benef Microbes*. 2010; 1 (3): 265-70.
17. Kolbin A.S. *Kliniko-ekonomicheskie i farmakoepidemiologicheskie aspekty protivogribkovykh sredstv u detei s invazivnym mikozom. Avtoref. dis. …dokt. med. nauk* [Clinical, Economic and Pharmacoepidemiological Aspects of Antifungal Agents in Children with Invasive Fungal Infections. Author's abstract]. St. Petersburg, 2006. 42 p.
18. Shabalov N.P., Ivanov D.O., Shabalova N.N. Neonatal sepsis. *Novosti farmakoterapii = News of pharmacotherapy*. 2000; 7: 62–69.