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Different Regimes of Multiprobiotic for Prevention of Immediate and Delayed Side Effects of Antibiotic Therapy In Children.

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ABSTRACT

In addition to diarrhoea, antibiotic-induced alterations in microflora composition increase suseptability to inflammatory diseases. In present study we tested the effectiveness of probiotic Symbiter® acidophilous (multiprobiotic) and the most optimal regime for its administration in children to prevent immediate and delayed side effects of antibiotic therapy. Forty children (3-17 years old) treated with ceftriaxone (7 or 14 days) were randomized to different regime of multiprobiotic ($1.0x10^{11}$ CFU/cm^3: *Lactobacilli*, *Lactococci*, *Bifidobacterium,* propionate-oxidising bacteria, acetic acid bacteria): immediately after antibiotic for 10 days; along with antibiotic and 10 days after; no probiotic supplementation. Feces and questionnaires were collected on day 0, immediately and 30 days after antibiotic therapy. Antibiotic therapy induced diarrhoea in 30.0% children. Profound dysbiotic changes were still observed in a month after therapy. Multiprobiotic immediately after antibiotic favored intestinal normoflora restoration but did not prevent diarrhea. Multiprobiotic along with antibiotic prevented the development of diarrhoea, dysbiotic changes and normalized sIgA levels; these effects became stronger, when multiprobiotic had been prolonged for 10 days. The most effective regime of multiprobiotic administration for the prevention of immediate and delayed antibiotic side effects is during all course of antibiotic therapy and at least 10 subsequent days after its withdrawal.

Keywords: multiprobiotic, antibiotic-associated diarrhea, sIgA, microflora

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INTRODUCTION

Broad spectrum antibiotics are frequently prescribed medicine in children. Moreover, consumption of penicillins and β-lactamase inhibitors are significantly increased over last years [5]. Antibiotic-associated diarrhea (AAD) is a common side effect of antibiotic treatment and ranges from 5 to 40 % incidents [2, 17, 19, 28]. Antibiotics cause diarrhea primarily through two mechanisms: by diminishing or eliminating bacterial species of the normal microflora that impairs vital microbial functions such as provision of nutrient short-chain fatty acids to colonocytes and metabolism of bile acids [3, 6]; or by creating a niche for the overgrowth of intestinal pathogens including *Clostridium difficile*, *Clostridium perfringens* type A, *Candida albicans*, *Klebsiella oxytoca*, and *Staphylococcus aureus* [1, 3, 10]. Most published randomized controlled trials and subsequent meta-analyses suggest benefit for probiotics in the prevention of AAD [19, 26]. The meta-analysis of 63 randomized, controlled trials involving almost 12 000 patients found that probiotics significantly reduced the relative risk of AAD (relative risk, RR: 0.58; 95% confidence interval, CI: 0.50–0.68) [8].

Recent epidemiological studies revealed that antibiotic therapy in early childhood has long lasting deleterious effect on microflora composition and its metabolic profile. These changes increase suseptability to inflammatory diseases such as inflammatory bowel diseas, asthma and psoriasis [11, 24, 29]. It was found that antibiotic-induced alterations in the intestinal microflora facilitate bacterial translocation via colonic epithelium, promoting inflammatory responses, and predisposing to increased disease in response to coincident injury [14]. These evidences pose new challenge for preventive medicine. It encourages to reevaluate regimes and type of probiotics in order to prevent AAD and later onset of inflammation.

Probiotic preparations may contain one bacteria or a number of different bacteria. A monostrain probiotic is defined as containing one strain of a certain species and consequently multistrain probiotics contain more than one strain of the same species or, at least of the same genus. The term multispecies probiotics is used for preparations containing strains that belong to one or preferentially more genera. Monostrain probiotics have a number of shortcomings: limited antagonistic activity of a strain, sensitivity to hydrochloric acid, bile and digestive enzymes, narrow spectrum of action, poor adhesive ability and growth in the intestine, dependence on the number of viable microbe cells It is thought that many probiotic bacteria work synergistically with each other to provide benefits that a single strain or species alone may. Different species of probiotic bacteria may be better able to colonise different parts of the gut, produce antibacterial agents against different pathogens or promote an immune response [27]. It was found that multispecies probiotics were superior for primary prevention of AAD [16, 18, 22, 25]. Moreover, multispecies probiotic *vs* monostrain was more effective both in inducing and maintaining remission in children with inflammatory bowel disease [4, 21].

Based on the published trials, it seems appropriate to start probiotic administration early, preferably simultaneously with antibiotic treatment, before modification of the gut microbiota and overgrowth of pathogens occur. It also seems appropriate to continue the administration of probiotic for the duration of antibiotic treatment [12, 26]. However, it remains unclear how long to continue probiotic after the cessation of antibiotic treatment to prevent delayed side effects of antibiotic.

The objective of present study was to test the effectiveness of multispecies probiotic Symbiter® acidophilous (multiprobiotic) and to explore the most optimal regime for its administration in children in order to prevent immediate and delayed side effects of antibiotic therapy (diarrhoea, fecals microflora composition and sIgA levels).

METHODS

The single center randomized controlled open label postmarketing trial was conducted in 40 children aged 3 to 14 years old, admitted for the acute infection diseases to the Clinical Department of Children's Infection Diseases, Bogomolets National Medical University. Informed written consent was obtained from the parents before enrollment of their children. The study was approved by the ethics committee of Bogomolets National Medical University (№ 2013). Our report is compliant with the CONSORT statement on randomized trials.

Inclusion and exclusion criterias

Inclusion criteria are were children from 3 to 14 years with moderate or severe acute bacterial infection diseases, prescribed short-term (≤14 days) parenteral antibiotic therapy. Exclusion criteria included a chronic gastrointestinal disorder, acute or chronic diarrhea, antimicrobial therapy administered during the previous 30 days, use of any prebiotic and/or probiotic pharmaceutical products within 30 days before the study, a severe bacterial infection with systemic involvement and an immunodeficiency state.

Information about patients was recorded using a case report form noting the following variables: gender, age, weight and height, medical history, diagnosis justifying antibiotic treatment, treatments prescribed during antibiotic treatment, and tolerability of treatment. Parents also completed a special daily diary describing intestinal function throughout the antibiotic treatment period and during following 4 weeks after antibiotic cessation (intervention period). AAD was defined by the daily production of at least 3 soft or liquid stools for at least 2 consecutive days.

Study design and Intervention

The study design was a prospective, randomized, controlled, open-label. The children were randomly assigned to two groups by using a computer-generated randomization list. The allocation schedule was fully concealed from the doctors working in the the Clinical Department of Children's Infection Diseases who recruited patients to the study. I group (n=20) was assigned to receive no additional probiotic supplement during all course of antibiotic therapy; II group (n=20) was assigned to receive multiprobiotic during all course of antibiotic therapy. After cessation of antibiotic treatment: patients of II group was assigned to receive multiprobiotic for 10 consequent days; patients of I group was split to two subgroups: Ia (n=10) and Ib (n=10). Patients of subgroup Ia didn't get any further treatment, while patients of subgroup Ib was assigned to receive multiprobiotic for 10 consequent days.

Patients were administered with non-lyophilized multiprobiotic "Symbiter® acidophilus concentrated" (multiprobiotic) (1 sachet/dose) once a day, at least 4 hr after antibiotic administration. One sachet/dose of multiprobiotic consists of active substance (symbiosis of live cells of (CFU/cm^3): *Lactobacilli* and *Lactococci*: $1.0x10^9$; *Bifidobacterium*: $1.0x10^8$; propionate-oxidising bacteria: $3.0x10^7$; acetic acid bacteria: $1.0x10^5$) and additive (skimmed fermented milk). Symbiter is manufactured by Research and Production Company "OD Prolisok" (Kyiv, Ukraine) and has been registered in Ukraine as a pharmaceutical product (Certificate of Registration No. UA/10146/01/01 if 22.10.2009, No. 763).

During the period of antibacterial therapy and intervention period, patients were not allowed to consume any other product that contained probiotics or prebiotics.

Fecal specimens were collected from all patients on admission (before antibiotic therapy), next day and in one month after cessation of antibiotic therapy for microbiological and biochemical examinations. Fresh specimens or within 1 hr after defecation were sent to the microbiology Lab for examination by bacteriological culture method and histology lab for microscopic detection of the leucocytes, muscle fibers, undigested carbohydrates, fats and mucus in fecal smears. Apart fecal samples were stored at −25°C until the fecal secretory IgA (sIgA) was performed. sIgA was measured by a commercially available sIgA immunoassay kit for biological fluids (Vector-Best, Russia). Briefly, 0.1-g aliquots of frozen feces were suspended in prediluted extraction buffer, vortex shaken, homogenized, and centrifuged. The supernatant was collected and assayed according to the manufacturer's instruction for the measurement of sIgA in fecal specimens. sIgA concentration was calculated in µg/g. The normal range for sIgA in feces was established and divided according age: for children up to 8 years old – 13.2 - 44.2 µg/g, for children >8 years old – 115.9-317.4 µg/g. The presence of pathogenic microflora in stool was detected in all patients with diarrhea.

Outcome Measures

The primary outcomes were the number of episodes of diarrhea during the period of antibacterial therapy and intervention period.

Secondary outcomes were microbiological and biochemical examinations of faeces, changes in level of fecal sIgA, the most optimal regime for administration of multiprobiotic.

Statistical analysis

Quantitative results are expressed as mean ± SD. The statistical significance was determined by the non-parametric Mann-Whitney U-test. Correlations were performed using Spearman's rank correlation (r_s) and Pearson's correlation coefficient (x^2). A *p* value of <0.05 was considered as a threshold for statistical significance in all analyses.

RESULTS AND DISCUSSION

Between March and November 2014, 41 children were eligible for the study, and 40 patients were randomized into 2 groups. On admission, children passed clinical examination and laboratory tests and were diagnosed with meningococcal disease, Lyme disease, acute bacterial tonsillitis or pseudotuberculosis according to Protocols for diagnosis and treatment of infectious diseases in children (Ministry of Health Care of Ukraine, 09.07.2004 № 354). According to Protocols for diagnosis and treatment of infectious diseases in children all children with meningococcal disease and pseudotuberculosis had severe illness, rest children had moderate severity of disease.

All patients were treated daily with antibiotic ceftriaxone (the 3rd generation of cephalosporin) for 7 or 14 days from the 1st day of hospitalization. Duration of antibiotic treatment dependent on diagnosis and severity of disease. No statistically significant differences were found between the groups in terms of age, sex, diagnose, severity of disease and duration of antibiotic treatment (Table 1). All 40 patients completed the antibiotic treatment period and intervention period.

Table 1: The distribution of patients by age, gender, diagnosis and duration of antibiotic therapy

Distribution criteria	Group I (abs, %)	Group II (abs, %)
Age		
≤ 8 years old	11 (55.0)	11 (55.0)
>8 years old	9 (45.0)	9 (45.0)
Gender		
Boys	9 (45.0)	8 (40.0)
Girls	11 (55.0)	12 (60.0)
Diagnosis		
Acute bacterial tonsillitis	17 (85.0)	16 (80.0)
Meningococcal disease: - *Meningococcemia* - *Purulent meningitis*	 0 1 (5.0)	 1 (5.0) 0
Pseudotuberculosis	1 (5.0)	1 (5.0)
Lyme disease	1 (5.0)	2 (10.0)
Severity of disease		
Mild	18 (90.0)	18 (90/0)
Severe	2 (10.0)	2 (10.0)
Duration of Antibiotic Therapy		
7 days	17 (85.0)	16 (80.0)
14 days	3 (15.0)	4 (20.0)

During the course of antibacterial therapy, the diarrhea was observed in 30% (n=6) children from the I group. Diarrhea appeared on the 3rd-6th day of antibiotic therapy, and lasted for 2 to 6 days. The frequency of loose stool ranged from 2 to 5 times per day. Microscopic examination of fecal smears revealed neutral fats and starch in 5% (n=1), undigested carbohydrates in 20% (n=4) and mucus in 25% (n=5) patients. Leukocytes and erythrocytes were not detected in feces. There was no significant deterioration of patients' condition with diarrhea. Their body temperature level and blood leucocytes number were in normal range. Stool of all patients with diarrhea were negative for pathogenic microflora. Antibacterial therapy was continued despite the appearance of diarrhea; but nutrition was corrected and the amount of drink was increased. Diarrhea was

not developed in children of the II group who received multiprobiotic along with antibiotic treatment. Similar effectiveness of probiotics to prevent AAD has been shown by others [7, 9].

Bacteriological assessment of feces next day after cessation of antibiotic therapy in patients from the I group revealed profound qualitative changes: increased number of *Candida spp*, lactose (-) and hemolytic *Escherichia coli* (*E. coli)* and appearance of *Klebsiella pneumoniae (K. pneumoniae)*. Multiprobiotic supplementation along with antibiotic therapy (the II group) prevented qualitative changes of microflora composition and increased the total number of *E. coli* ($p<0.05$) (Table 2).

Table 2: Fecal microflora composition at different time points of antibiotic therapy and the regimes of multiprobiotic supplementation

Microorganisms	Number of microorganisms (CFU/g)						
	On admission		***Next day after cessation of antibiotic therapy***		***In one month after cessation of antibiotic therapy***		
	I group (n=20)	II group (n=20)	I group (n=20)	II group (n=20)	I group (n=20)		II group (n=20)
					Ia (n=10)	Ib (n=10)	
Total number of E.coli	7.9±0.1	7.9±0.09	7.4±1.2	8.3±0.1*	7.6±1.2	7.9±0.8	8.2±0.1
Lactose (-) E.coli	-	-	3.0±2.5$^{\#}$	0*	2.3±2.5	0.8±1.9§	0§
Hemolytic *E.coli*	3%	3%	29.4%$^{\#}$	0*	29.4%	14%§	0$^{\S\&}$
Lactobacillus spp.	6.4±0.9	6.3±0.9	6.3±1.0	7.0±0.9	6.3±1.0	6.5±0.9	6.8±1.1
Bifidobacterium spp.	7.1±1.1	7.1±1.1	7.6±1.1	8.0±0.9	7.6±1.1	7.7±0.9	8.0±1.0
Candida spp.	2.0±0.4	1.2±0.4	4.3±2.5	3.0±2.1	4.7±2.8	3.9±2.1	3.0±2.1§
Staphylococcus aureus	0.9±2.4	0.6±3.5	1.8±2.1	0*	5.7±1.1*	2.3±2.1§	0.5±2.1$^{\S\&}$
Klebsiella pneumoniae	-	-	1.5±1.9$^{\#}$	0	1.5±1.9	0§	0§

*Legend: I group – no probiotic during all course of antibiotic therapy: Ia – no probiotic, Ib – probiotic for 10 days after cessation of antibiotic treatment. II group - probiotic during all course of antibiotic therapy plus for 10 days after cessation of antibiotic treatment. # - p<0.05 vs. number of microorganisms on admission; *- p<0.05 vs. I group next day after antibiotic therapy cessation; §- p<0.05 vs. Ia group; $^{\&}$ - p<0.05 vs. Ib group. Conventional culture method, M±SD.*

Since the normal range for fecal levels of sIgA is dependent on age, patients were compared *vs.* established normal range of sIgA levels for particular age group. As can be seen from Table 3, on admission 70% patients from the I group (≤8 years old - 16.9±4.6 µg/g; >8 years old – 215.6± 72.9 µg/g) and 65% patients from the II group (≤8 years old – 22.9±7.6 µg/g; >8 years old – 241.3±68.7 µg/g) had sIgA levels in normal range. 15% patients in both groups at age older than 8 years had sIgA levels lower than normal range (I group – 25.4±18.6 µg/g; II group – 64.5±27.5 µg/g). 15% patients from the I group (≤8 years old – 196.3±163.6 µg/g; >8 years old –469.2±143.6 µg/g) and 20% patients from the II group (≤8 years old – 166.6±186.6µg/g; >8 years old –392.7±33.6 µg/g) had sIgA levels higher than normal range. After cours of antibiotic without multiprobiotic (I group) number of children with normal sIgA levels decreased up to 35% *vs.* 70% ($p<0.05$) and increased number number of children with sIgA levels higher than normal levels up to 50% *vs.* 15% ($p<0.05$) patients. The combination of antibiotic with multiprobiotic (II group) increased the number of children with normal sIgA levels up to 95% *vs.* 65% ($p<0.05$) and decreased - with sIgA levels higher than normal up to 5% *vs.* 20% ($p<0.05$). The number of children with levels of sIgA lower than control was unchanged in the I group and equal zero in II group (Table 3).

We found positive correlation between elevated levels of sIgA and increased number of *K. pneumoniae* ($r_s=0.857$, $p<0.05$) and *Staphylococcus aureus (S. aureus)* ($r_s=0.l81$, $p<0.05$).

According to the literature [15, 23], the increased sIgA levels in faeces may be detected in children with intestinal dysbiosis, chronic pathology of gastrointestinal tract, allergic diseases and in frequently ill children. Increased sIgA level in faeces during antibiotic therapy indicates the development of non-specific immune reaction of the mucous membrane, which may be caused by dysbiotic changes in intestine: lowering

of normoflora (lactobacteria, bifidobacteria) and growth of opportunistic or pathogenic microorganisms [9]. In present study, antibiotic therapy increased number of patients with high fecal levels of sIgA. While, combination of antibiotic with multipriobiotic increased number of patinets with normal levels of sIgA. Therefore, we conducted correlation analysis between the levels of sIgA in faeces and the bacteriological profile of faeces. Detected correlation between sIgA level and the number of *K. pneumoniae* and *S.aureus* allowed us to consider sIgA level as a marker of dysbiotic changes in intestine.

Table 3 Fecal levels of secretory IgA (sIgA) at different time points of antibiotic therapy and the regimes of multiprobiotic supplementation

Levels of sIgA	Number of patients			
	On admission		Next day after cessation of antibiotic therapy	
	I group (n=20)	II group (n=20)	I group (n=20)	II group (n=20)
Normal range	14 (70.0%)	13 (65.0%)	7 (35.0%)*#	19 (95.0%)*
Higher normal range	3 (15.0 %)	4 (20.0%)	10 (50.0%)*#	1 (5.0%)*
Lower normal range	3 (15.0%)	3 (15.0%)	3 (15.0)	-

*Legend: I group – no probiotic during all course of antibiotic therapy. II group - probiotic during all course of antibiotic therapy. * - p<0.05 vs. particular group on admission; # - p<0.05 vs. II group on the next day after cessation of antibiotic therapy. sIgA ELISA kit for biological fluids, M±SD.*

Maintenance of low levels of sIgA may indicate lowered resistance of the organism and such children need thorough medical examination to conduct correcting immunotherapy [13]. Normalization of sIgA level after multiprobiotic application may be associated with immunomodulatory effect of the probiotic bacteria as described [23].

In one month after cessation of antibiotic therapy, we found further upregulation the number of *S. aureus* (2.5-fold, p<0.05) in group of children without multiprobiotic supplementation through all study period (Ia subgroup) *vs.* data on the next day after antibiotic cessation. The number of *Candida spp.,* lactose (-) & hemolytic *E. coli* and conditionally-pathogenic microflora were also increased (Table 2). Multiprobiotic supplementation started immediately after cessation of antibiotic therapy (Ib subgroup) decreased 2-fold number of *S. aureus,* lactose (-) *E. coli* (p<0.05 *vs.* Ia subgroup) and 2-fold number of children with hemolytic *E. coli* (p<0.05 *vs.* Ia subgroup); and disappearance of conditionally pathogenic microflora. Patients from the II group had composition of microflora similar to those before antibiotic therapy, with increased number of *Lactobacillus spp.* and *Bifidobacterium spp*. (Table 2).

Adverse events were not observed for either group, and the multiprobiotic were well tolerated.

CONCLUSION

We found that dysbiotic changes in intestine, associated with antibiotic therapy, did not disappear after the antibiotic withdrawal and became even deeper in dynamics due to the growth of opportunistic flora (mainly *Candida spp.* and *S. aureus*). Ten days multiprobiotic course, started immediately after antibiotic therapy withdrawal, favored restoration of intestine normoflora but did not prevent AAD. Application of the multiprobiotic along with antibiotic treatment prevented the development of dysbiotic changes in intestine, AAD and normalized the level of sIgA; moreover, this effect became stronger, when application of multiprobiotic had been prolonged for 10 days after antibiotic withdrawal. Taking into account these data, the most effective regime of multiprobiotic administration for prevention immediate and delayed antibiotic-induced changes in intestinal microbiocenosis is during all course of antibacterial therapy and at least 10 days after antibiotic withdrawal.

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Conflicts of Interest and Source of Funding

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