NAME OF SPONSOR: **Lek Pharmaceuticals d.d.** **Verovškov 57** **SI-1526 Ljubljana** **NAME OF FINISHED PRODUCT:** **Linef Forte, trde kapsule** **NAME OF ACTIVE INGREDIENTS:** **Lactic acid bateria (LA-5 and BB-12)**	INDIVIDUAL STUDY SYNOPSIS **LINPT01** **EudraCT No.2009-010713-69**
Title of the Study:	Randomized, placebo controlled, double-blind, parallel group, multicentric Phase IV study on the clinical efficacy of lactic acid bacteria in form of hard capsules (capsule contains not less than 2 x 10^9 CFU of liophilisated lactic acid bacteria *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium animalis subsp. lactis* (BB-12) in ration 1:1) as prevention of antibiotic associated acute diarrhea in adults.
Investigator:	MULTICENTER MAIN: Assoc. Prof. Janez Tomažič, PhD. MD. UMC Ljubljana, Clinic for Infectious Diseases and Febrile Illnesses, Japljeva ulica 2, 1000 Ljubljana, Slovenia OTHER: Katarina Vukelič, MD, Renata Verboten Kopriva, MD, Snežana Ulčar Kostič, MD
Study Center:	• University Medical Centre Ljubljana, Clinic for Infectious Diseases and Febrile Illnesses, Japljeva ulica 2, 1000 Ljubljana, Slovenia • Community Health Centre Grosuplje, Pod gozdom cesta I/14, 1290 Grosuplje, Slovenia • Private Practice for Internal Medicine Renata Kopriva, MD, Partizanska pot 81, 1270 Litija, Slovenia • Pulmonary Practice Ulčar, MD, Ljubljanska cesta 21 C, 1241 Kamnik, Slovenia
Publication(s):	NO
Studied Period:	The first subject first visit was on 3rd September 2009 and the last subject last visit was on 28th January 2010 followed by a follow up telephone call on 4th February 2010.
Development Phase:	Post-marketing trail (IV phase)
Objectives:	<u>Primary objective:</u> • To demonstrate clinical efficacy of new Linex® Forte hard capsules in comparison with placebo regarding frequency of occurrence of diarrhea in treatment with antibiotics. <u>Secondary objectives:</u> • To determine the duration of diarrhea. • To observe microflora change from beginning to the end of treatment. • To determine efficacy in *Clostridium difficile* diarrhea.

Methodology/Study design	This is a phase IV, randomized, double-blind, placebo controlled, parallel-group, multicentric study designed to evaluate clinical efficacy of Linex® Forte hard capsules as prevention of antibiotic-associated acute diarrhea in adults. Study participation lasted approximately 2-3 weeks, subjects were initiated study treatment at beginning of their antibiotic therapy and continue with study treatment for one week after completion of antibiotic therapy. Stool samples were collected from patients experiencing diarrhea and analyzed for presence of *C. difficile*. Duration of diarrhea was compared between the groups using data from diary cards. Microflora changes were observed from beginning to the end of treatment in subgroup of 50 patients.
Number of Subjects:	The study included 200 subjects initially, 100 subjects in the Linex group and 100 subjects in the placebo group. Study ended 96 subjects in the Linex group and 90 in the placebo group.
Diagnosis and Main Criteria for Inclusion:	Male and female subjects 18 to 70 years of age receiving for any reasons amoxicillin-clavulanate oral therapy for at least 7 days to 2 weeks .
Test Product: **Dose:** **Mode of Administration:**	**Linex Forte hard capsules** 1 cps 2-times daily during the antibiotic treatment (7-14 days) and additional one week after the completion of antibiotic therapy, together 14-21 days. (Each capsule contains not less than 1 x 10E9 CFU of *Lactobacillus acidophilus* (LA-5) as a lyophillisat and not less than 1 x 10E9 CFU of *Bifidobacterium animalis subsp. lactis* (BB-12) as a lyophilisat.) oral
Reference Product: **Dose:** **Mode of Administration:**	PLACEBO match the size, appearance and taste of Linex® Forte 1 cps 2-times daily during the antibiotic treatment (7-14 days) and additional one week after the completion of antibiotic therapy, together 14-21 days. oral
Duration of Treatment:	From 14 to 21 days: during the antibiotic treatment (7-14 days) and additional one week after the completion of antibiotic therapy
Criteria for Evaluation:	The incidence of diarrhea and other adverse events. Microbiological analysis of microflora changes by colony counting in units of grams of the stool bacteria and quantification analysis of real-time PCR.

Safety	Advers events
Statistical Methods:	Nonparametric Mann-Whitney test , Fisher's Exact Test and Wilcoxon Signed Ranks Test The limit for statistical significance was 0.05, but for the interpretation we also considered the level of the significance 0.1, where a difference between larger samples might prove to be statistically significant.
Results	

Primary objective*:*

The incidence of diarrhea

Detailed analysis of the incidence of diarrhea within the age groups also showed no significant differences between the Linex group and the placebo group. According to the age group up to 50 years, the incidence of diarrhea was 7.5% (4 of 53) in the Linex and 8.3% (4 of 48) in the placebo. The differences between proportions were not proved (p>0.1). Among subjects in the age group above 50 years the incidence of diarrhea in Linex group was 7.0% (3 of 43) and 16.7% (7 of 42) in the placebo. The differences were not statistically proved (p> 0.1).

			Group		Total
			Linex	Placebo	
Diarrhea	yes	N	7	11	18
		%	7,30%	12,20%	9,70%
	no	N	89	79	168
		%	92,70%	87,80%	90,30%
Total		N	96	90	186
		%	100,00%	100,00%	100,00%
Fisher's Exact Test		(sig. 0,323)			

Secondary objective*:*

- **Duration of diarrhea (based on subsample of those subjects who had diarrhoea)**

Group	Mean	Median	Minimum	Maximum	Std. Deviation	N	KV
Linex	4,429	4	2	7	1,9024	7	0,43
Placebo	3,00	2	1	13	3,5497	11	1,18
Total	3,556	3	1	13	3,03358	18	
Mann-Whitney test	Z=-2,076 (sig: 0,038)						

The average duration of diarrhea among the 18 subjects was 3.56 days. The minimum duration of diarrhea was 1 day and the maximum of 13 days. The mean duration of diarrhea in the Linex group was 4.43 days and in the placebo group 3.00 days. Based on the nonparametric Mann-Whitney test, the statistically significant differences could be confirmed. The duration of diarrhea in Linex group was significantly longer than in the placebo group (p <0.05).

- **The results of colony counting units from different groups of bacteria in a gram of stool**

Bacteria of the genus *Bifidobacterium content after the first and after the second measurement*

In the first measurement the lowest measured value was less than 15E+03 or below detection limit and occurs in both groups of the subjects. To include these values into the statistical analysis, we fixed them to a value 1. Such value did not affected the results of nonparametric tests. The maximum counted value in the first measurement was 8.9E+08 and it was noted in the placebo group where was also higher dispersion of data (CV=4.957) than in Linex group. In the second measurement the minimum value of 1 occurs in both groups to lead a peak in the placebo group (5.40E +08).

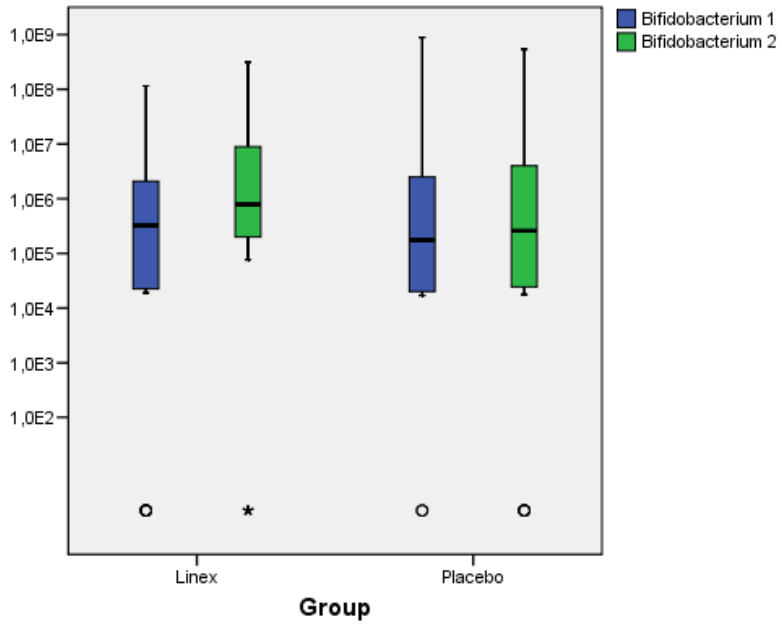
Group		*Bifidobacterium* 1	*Bifidobacterium*2
Linex	Mean	9,42E+06	1,83E+07
	Median	3,25E+05	7,90E+05
	Minimum	1,00E+00	1,00E+00
	Maximum	1,15E+08	3,14E+08
	Std. Deviation	2,52E+07	6,49E+07
	CV	2,672	3,546
	N	23	23
Placebo	Mean	4,65E+07	3,50E+07
	Median	1,75E+05	2,60E+05
	Minimum	1,00E+00	1,00E+00
	Maximum	8,90E+08	5,40E+08
	Std. Deviation	1,93E+08	1,20E+08
	CV	4,159	3,440
	N	21	21
Total	Mean	2,71E+07	2,63E+07
	Median	2,40E+05	7,08E+05
	Minimum	1,00E+00	1,00E+00
	Maximum	8,90E+08	5,40E+08
	Std. Deviation	1,34E+08	9,46E+07
	CV	4,957	3,604
	N	44	44
Mann-Whitney test		Z=-0,012; sig. 0,991	Z=-0,907; sig. 0,364
Wilcoxon Signed Ranks Test (Linex)		Z=-1,456; sig. 0,145	

Wilcoxon Signed Ranks Test (placebo)	Z=-0,226; sig. 0,821

In the first measurement, the median in the group Linex was 3.25E+05 and in the placebo group 1.75E+05. In the second measurement the median in both groups increased to 7.9E+05 in the Linex group and in the placebo group 2,6 E+05.

Using the Mann-Whitney test for independent samples, statistically significant differences in mean ranks could not be confirmed neither in the first nor in the second measurement (p>0.05).

Wilcoxon rank test showed, that in none of the group could not be confirmed statistically significant differences between the first and second measurement.



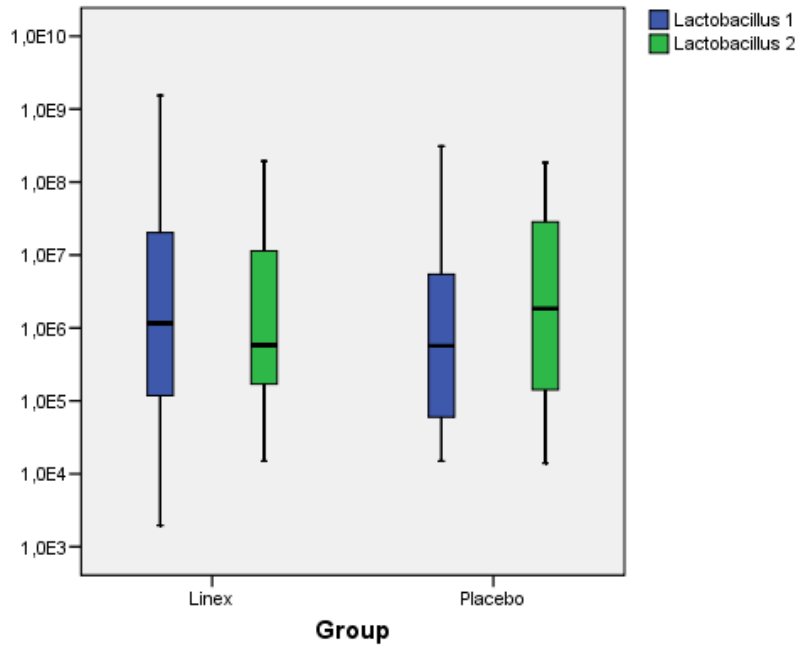
Bacteria of the genus *Lactobacillus content after the first and after the second measurement*

In the first measurement, the values ranged from 1.96 E+03 to 1.54E+09; the both of extremes were in the Linex group. In the second measurement, the values ranged from 1.4E+04 (placebo) to 1.94 E+08 (Linex). Dispersion of the values in both groups declined in the second measurement.

Group		*Lactobacillus* 1	*Lactobacillus* 2
Linex	Mean	1,18E+08	2,15E+07
	Median	9,45E+05	6,15E+05
	Minimum	1,96E+03	1,50E+04
	Maximum	1,54E+09	1,94E+08
	Std. Deviation	3,36E+08	4,79E+07
	CV	2,841	2,233
	N	23	22

Placebo	Mean	2,17E+07	2,89E+07
	Median	5,70E+05	1,84E+06
	Minimum	1,50E+04	1,40E+04
	Maximum	3,10E+08	1,85E+08
	Std. Deviation	6,82E+07	5,31E+07
	CV	3,137	1,837
	N	21	21
Total	Mean	7,22E+07	2,51E+07
	Median	9,38E+05	9,47E+05
	Minimum	1,96E+03	1,40E+04
	Maximum	1,54E+09	1,94E+08
	Std. Deviation	2,49E+08	5,00E+07
	CV	3,456	1,994
	N	44	43
Mann-Whitney test		Z=-1,001; sig. 0,317	Z=-0,547; sig. 0,584
Wilcoxon Signed Ranks Test (Linex)		Z=-0,991; sig. 0,322	
Wilcoxon Signed Ranks Test (placebo)		Z=-1,408; sig. 0,159	

In the first measurement, the median in the Linex group was 9.45E+05 and in the placebo group 5.7E+05. Based on the Mann-Whitney test for independent samples, the differences of the mean ranks between the two groups could not be statistically confirmed (p>0.05). In the second measurement, comparing to the first one, the median in Linex group decreased to 6.15E+05 but in the placebo group it increased up to 1.84E+06. Even in this measurement the differences between the groups were not statistically significant (p> 0.05). Wilcoxon rank test shows that neither in the Linex group nor in the control group, the statistically significant differences between first and second measurement could not be confirmed.



Staphylococcus content after the first and after the second measurement

Group		*Staphylococcus* 1	*Staphylococcus* 2
Linex	Mean	3,93E+05	5,47E+05
	Median	1,00E+00	1,00E+00
	Minimum	1,00E+00	1,00E+00
	Maximum	3,15E+06	6,40E+06
	Std. Deviation	8,24E+05	1,43E+06
	CV	2,095	2,616
	N	23	23
Placebo	Mean	9,78E+05	8,41E+05
	Median	1,00E+00	6,00E+03
	Minimum	1,00E+00	1,00E+00
	Maximum	2,02E+07	1,53E+07
	Std. Deviation	4,40E+06	3,41E+06
	CV	4,502	4,052
	N	21	20
Total	Mean	6,73E+05	6,84E+05
	Median	1,00E+00	1,00E+00

	Minimum	1,00E+00	1,00E+00
	Maximum	2,02E+07	1,53E+07
	Std. Deviation	3,08E+06	2,52E+06
	CV	4,572	3,684
	N	44	43
Mann-Whitney test		Z=-0,834; sig. 0,404	Z=-0; sig. 1,000
Wilcoxon Signed Ranks Test (Linex)		Z=-0,284; sig. 0,776	
Wilcoxon Signed Ranks Test (placebo)		Z=-1,538; sig. 0,124	

The proportion of samples with detected presence of the bacteria of the genus *Staphylococcus* according to the Linex/placebo group - 1 measurement

			Group		Total
			Linex	Placebo	
Staphylococcus 1	not detected	N	12	12	24
		%	52,20%	57,10%	54,50%
	detected	N	11	9	20
		%	47,80%	42,90%	45,50%
Total		N	23	21	44
		%	100,00%	100,00%	100,00%
Fisher's Exact Test		(sig. 0,771)			

The proportion of samples with detected presence of bacteria of the genus Staphylococcus by group - 2 measurement

			Group		Total
			Linex	Placebo	
Staphylococcus 2	not detected	N	12	10	22
		%	52,20%	50,00%	51,20%
	detected	N	11	10	21
		%	47,80%	50,00%	48,80%
Total		N	23	20	43
		%	100,00%	100,00%	100,00%
Fisher's Exact Test		(sig. 1,00)			

Comparison of proportions of samples with the reported presence of bacteria of the genus Staphylococcus from 1st and 2nd measured separately for the Linex and placebo group

As already shown in tables 14 and 16, there were almost no differences in the proportion of subjects between 1st and 2nd measurement within the Linex and placebo groups. The proportion of subjects with incidence of bacteria *Staphylococcus* has not significantly changed from 1st and 2nd measuring both in Linex and placebo group.

Group				Parallel		Total
				1	2	
Linex	*Staphylococcus*	not detected	N	12	12	24
			%	52,20%	52,20%	52,20%
		detected	N	11	11	22
			%	47,80%	47,80%	47,80%
	Total		N	23	23	46
			%	100,00%	100,00%	100,00%
Placebo	*Staphylococcus*	not detected	N	12	10	22
			%	57,10%	50,00%	53,70%
		detected	N	9	10	19
			%	42,90%	50,00%	46,30%
	Total		N	21	20	41
			%	100,00%	100,00%	100,00%
	Fisher's Exact Test (Linex)		(sig. 1,00)			
	Fisher's Exact Test (placebo)		(sig. 0,758)			

Enterococcus *content after the first and after the second measurement*

The median in the Linex group was 7.70E+04 and in placebo group 1.28E+04. The Mann-Whitney test showed that the difference between the groups was on the limit of statistical significance ($p<0.1$). In the second measurement the median in Linex group decreased slightly and differences on the basis of Wilcoxon rank test could not be confirmed as statistically significant ($p>0.05$). In the placebo group, the median significantly increased up to 7.68E+04 ($p <0.05$).

Group		*Enterococcus* 1	*Enterococcus* 2
Linex	Mean	3,47E+06	2,92E+06
	Median	7,70E+04	5,95E+04

	Minimum	1,00E+00	1,00E+00
	Maximum	5,55E+07	3,75E+07
	Std. Deviation	1,16E+07	8,23E+06
	CV	3,343	2,821
	N	23	23
Placebo	Mean	1,28E+06	5,89E+06
	Median	1,28E+04	2,35E+05
	Minimum	1,00E+00	1,00E+00
	Maximum	2,56E+07	5,70E+07
	Std. Deviation	5,58E+06	1,40E+07
	CV	4,371	2,373
	N	21	21
Total	Mean	2,43E+06	4,34E+06
	Median	1,86E+04	7,68E+04
	Minimum	1,00E+00	1,00E+00
	Maximum	5,55E+07	5,70E+07
	Std. Deviation	9,20E+06	1,13E+07
	CV	3,796	2,607
	N	44	44
Mann-Whitney test		Z=-1,670; sig. 0,095	Z=-0,799; sig. 0,424
Wilcoxon Signed Ranks Test (Linex)		Z=-0,179; sig. 0,858	
Wilcoxon Signed Ranks Test (placebo)		Z=-3,061; sig. 0,002	

Bacteria *Enterobactericeae* Group *content after the first and after the second measurement*

In the Linex group, the median in the first measurement was 2.40E+05, while in the second measurement decreased to 1.25E+04. In the placebo group, the median was 1.25E+04 in the first measurement, and latter increased slightly to 1.50E+04.

At the first measurement the Mann-Whitney test for two independent samples indicated for placebo group lower median but statistically not significant ($p < 0.1$), at the second measurement, however there were no differences between groups.

Based on the Wilcoxon rank test the medians between the first and second measurements did not significantly differ in both groups of subjects.

Group		*Enterobacteriaceae* 1	*Enterobacteriaceae* 2
Linex	Mean	1,57E+06	1,24E+06
	Median	2,40E+05	1,25E+04
	Minimum	1,00E+00	1,00E+00
	Maximum	1,99E+07	1,25E+07
	Std. Deviation	4,30E+06	3,26E+06
	CV	2,732	2,624
	N	23	23
Placebo	Mean	1,29E+06	6,67E+05
	Median	1,25E+04	1,50E+04
	Minimum	1,00E+00	1,00E+00
	Maximum	2,16E+07	1,11E+07
	Std. Deviation	4,74E+06	2,41E+06
	CV	3,665	3,608
	N	21	21
Total	Mean	1,44E+06	9,67E+05
	Median	7,70E+04	1,38E+04
	Minimum	1,00E+00	1,00E+00
	Maximum	2,16E+07	1,25E+07
	Std. Deviation	4,46E+06	2,86E+06
	CV	3,101	2,962
	N	44	44
Mann-Whitney test		Z=-1,839; sig. 0,066	Z=-0,087; sig. 0.931
Wilcoxon Signed Ranks Test (Linex)		Z=-1,157; sig. 0,247	
Wilcoxon Signed Ranks Test (placebo)		Z=-0,057; sig. 0,955	

Yeast fungus content after the first and after the second measurement.

In the Linex group, the median of the first measurement was 1.75E+04 but in the placebo group it was 1. This means that more than half of the samples were below the limit of detection. The Mann-Whitney test and the Wilcoxon rank test were not appropriate for such data. The data were recoded into two ranks: a) below the limit of detection and b) above the limit of detection. With Fisher's exact test it was

examined whether there were differences between the groups and between measurements. The same procedure was done for the second measurement since the medians of both groups were below the limit of detection.

Group		yeast 1	yeast 2
Linex	Mean	3,72E+05	1,70E+05
	Median	1,75E+04	1,00E+00
	Minimum	1,00E+00	1,00E+00
	Maximum	2,90E+06	1,73E+06
	Std. Deviation	8,62E+05	4,07E+05
	CV	2,318	2,389
	N	23	23
Placebo	Mean	1,09E+04	3,82E+04
	Median	1,00E+00	1,00E+00
	Minimum	1,00E+00	1,00E+00
	Maximum	6,85E+04	5,20E+05
	Std. Deviation	2,11E+04	1,12E+05
	CV	1,943	2,945
	N	21	21
Total	Mean	1,99E+05	1,07E+05
	Median	1,00E+00	1,00E+00
	Minimum	1,00E+00	1,00E+00
	Maximum	2,90E+06	1,73E+06
	Std. Deviation	6,43E+05	3,08E+05
	CV	3,223	2,875
	N	44	44
Mann-Whitney test		Z=-2,08; sig. 0,038	Z=-0,087; sig. 0,258
Wilcoxon Signed Ranks Test (Linex)		Z=-0,170; sig. 0,865	
Wilcoxon Signed Ranks Test (placebo)		Z=-1,304; sig. 0,192	

Safety results	

Adverse events of the antibiotic treatment associated with indigestion (without the incidence of diarrhea)

The incidence of adverse events of antibiotic therapy was around 16.1% in both groups and thus the difference between the groups was not statistically significant (p> 0.1).

			Group		Total
			Linex	Placebo	
Adverse events	yes	N	16	14	30
		%	16,70%	15,60%	16,10%
	no	N	80	76	156
		%	83,30%	84,40%	83,90%
Total		N	96	90	186
		%	100,00%	100,00%	100,00%
Fisher's Exact Test		(sig. 0,845)			

Adverse events of antibiotic treatment associated with indigestion (including diarrhea)

Above one fifth (21.0 %) of subjects reported the adverse events associated with indigestion, including diarrhea (21.9% in Linex, and 20.0% in the placebo group). The Fisher's exact test showed no statistically significant differences (p> 0.1).

			Group		Total
			Linex	Placebo	
Adverse events (including diarrhoea)	yes	N	21	18	39
		%	21,90%	20,00%	21,00%
	no	N	75	72	147
		%	78,10%	80,00%	79,00%
Total		N	96	90	186
		%	100,00%	100,00%	100,00%
Fisher's Exact Test		sig.: 0,857			

Comparison of proportions of the samples with detected yeast fungus during the 1st and 2nd measurement, shown separately for the Linex and the placebo group

In the Linex group, the percentages of samples with detected yeast fungus reduced from the first to the second measurement from 56.5% to 47.8%, but the difference could not be statistically proved (p> 0.1). In the placebo group, the proportion increased from 28.6% to 38.1%, but the difference also

was not statistically significant (p> 0.1).

Group				Parallel		Total
				1	2	
Linex	yeast fungus	not detected	N	10	12	22
			%	43,50%	52,20%	47,80%
		detected	N	13	11	24
			%	56,50%	47,80%	52,20%
	Total		N	23	23	46
			%	100,00%	100,00%	100,00%
Placebo	yeast fungus	not detected	N	15	13	28
			%	71,40%	61,90%	66,70%
		detected	N	6	8	14
			%	28,60%	38,10%	33,30%
	Total		N	21	21	42
			%	100,00%	100,00%	100,00%
	Fisher's Exact Test (Linex)		(sig. 0,768)			
	Fisher's Exact Test (placebo)		(sig. 0,744)			

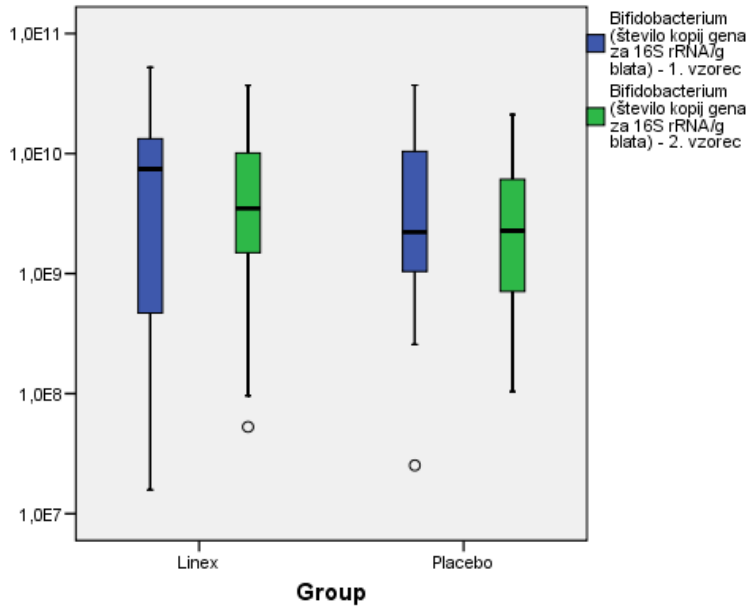
- **Results of quantification of each genus of bacteria in the stool of subjects with a quantitative analysis of DNA (Real Time PCR)**

Bacteria of the genus *Bifidobacterium content after the first and after the second measurement and the difference between measurements*

In the first measurement, the lowest value for the number of copies was recorded in the placebo group (2.53 E+07), but the highest in Linex group (5.22 E+10). Variation of the values was quite similar. Based on a higher value of coefficient of variance, the higher dispersion of data appeared in the first measurement.

Group		*Bifidobacterium* (Number of copies of 16S rRNA gene / g stool) –	*Bifidobacterium* (Number of copies of 16S rRNA gene / g stool) –	*Bifidobacterium difference between the first and the second measurement* (Number of copies of 16S

		1. Measurement	2. Measurement	rRNA gene / g stool) – 2. Measurement -- 1. Measurement
Linex	Mean	1,01E+10	8,38E+09	-3,98E+09
	Median	4,62E+09	3,49E+09	-2,52E+08
	Minimum	1,58E+07	5,29E+07	-3,59E+10
	Maximum	5,22E+10	3,92E+10	1,51E+10
	Std. Deviation	1,44E+10	1,13E+10	1,17E+10
	CV	1,42	1,34	-2,94
	N	21	21	19
Placebo	Mean	8,01E+09	5,43E+09	-2,98E+09
	Median	2,14E+09	2,28E+09	-1,16E+08
	Minimum	2,53E+07	1,04E+08	-3,42E+10
	Maximum	3,71E+10	2,11E+10	1,45E+10
	Std. Deviation	1,16E+10	7,03E+09	1,14E+10
	CV	1,45	1,30	-3,84
	N	20	19	19
Total	Mean	9,08E+09	6,98E+09	-3,48E+09
	Median	2,68E+09	3,42E+09	-2,16E+08
	Minimum	1,58E+07	5,29E+07	-3,59E+10
	Maximum	5,22E+10	3,92E+10	1,51E+10
	Std. Deviation	1,30E+10	9,49E+09	1,14E+10
	CV	1,43	1,36	-3,29
	N	41	40	38
Mann-Whitney test		Z=-0,104; sig. 0,919	Z=-0,555; sig. 0,592	Z=-0,0482; sig. 0,630
Wilcoxon Signed Ranks Test (Linex)		Z=-0,328; sig. 0,184		-
Wilcoxon Signed Ranks Test (placebo)		Z=-0,563; sig. 0,573		-



In the first measurement a higher median was reported in the Linex (4.62E+09) than the placebo group (2.14E+09). In the second measure the median of copies in both groups decreased (Linex: 2.52E+08, placebo: 1.16E+08), but much more in Linex than in the placebo group.

Based on Wilcoxon rank test (for paired samples) the differences between measurements could not be statistically confirmed (p> 0.05) in any group.

Mann-Whitney test showed that the median of the amount of the copies for the colonies of bacteria *Bifidobacterium* not significantly differed between the two groups in both measurements (p>0.05). The difference between first and second measurement for both group did not appear significantly (p> 0.05).

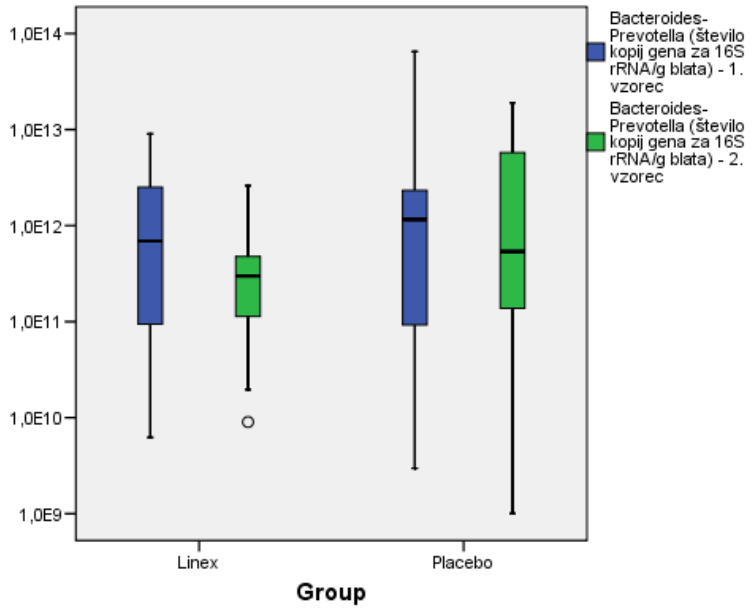
Bacteria *Bacteroides-Prevotella* genus *content after the first and after the second measurement and the difference between measurements*

In the first measurement, the number of the copies of *Bacteroides-Prevotella bacteria* ranged from 3.95E+08 (Linex) to 6.5E+13 (placebo). Variation of the values was very high (CV=3.25), especially in the placebo group (CV=2.97).

In the second measurement, the values ranged between 1.01E+09 and 1.89E+13. Both extremes were in the placebo group, where was also higher variation of the results (CV=1.45) than in the Linex group (CV=1.35).

Group		Bacteroides-Prevotella (Number of copies of 16S rRNA gene / g stool) – 1 Measurement	Bacteroides-Prevotella (Number of copies of 16S rRNA gene / g stool) – 2 Measurement	Bacteroides-Prevotella difference (Number of copies of 16S rRNA gene / g stool) – 2. Measurement -- 1. Measurement

Linex	Mean	1,45E+12	4,36E+11	-1,22E+12
	Median	4,43E+11	2,60E+11	-5,71E+11
	Minimum	3,95E+08	9,01E+09	-8,11E+12
	Maximum	9,02E+12	2,60E+12	8,89E+11
	Std. Deviation	2,21E+12	5,87E+11	2,07E+12
	CV	1,52	1,35	-1,69E
	N	22	20	19
Placebo	Mean	4,71E+12	3,86E+12	-8,52E+11
	Median	1,16E+12	5,37E+11	1,70E+11
	Minimum	2,97E+09	1,01E+09	-4,61E+13
	Maximum	6,50E+13	1,89E+13	1,51E+13
	Std. Deviation	1,40E+13	5,59E+12	1,11E+13
	CV	2,97	1,45	-13,2
	N	21	21	21
Total	Mean	3,04E+12	2,19E+12	-1,03E+12
	Median	8,47E+11	3,30E+11	-3,89E+09
	Minimum	3,95E+08	1,01E+09	-4,61E+13
	Maximum	6,50E+13	1,89E+13	1,51E+13
	Std. Deviation	9,91E+12	4,33E+12	8,08E+12
	CV	3,25E	1,98E	-7,86E
	N	43	41	40
Mann-Whitney test		Z=-0,911; sig. 0,362	Z=-1,761; sig. 0,078	Z=-0,909; sig. 0,056
Wilcoxon Signed Ranks Test (Linex)		Z=-2,173; sig. 0,030		-
Wilcoxon Signed Ranks Test (placebo)		Z=-0,539; sig. 0,590		-



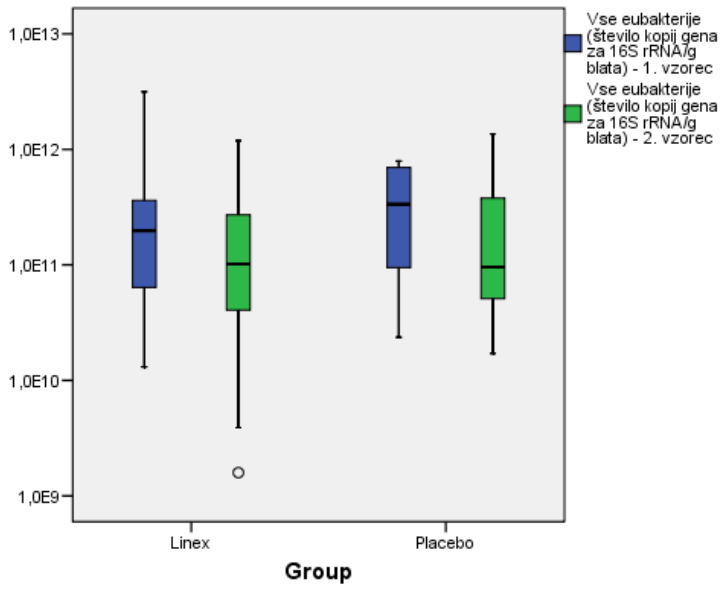
In the Linex group the median was 4.43E+11 in the first measurement and in the second, 1.16E+12. In the placebo group the median was in both measurements higher than in the Linex group (first: 1.16E+12, second: 5.37E+11). The median therefore declined in the second measurement, in both groups.
Based on the Mann-Whitney test for independent samples it can be confirmed that in the first measurement there were no statistically significant differences in mean rank between the two groups ($p>0.05$). In the second measurement the mean rank of the placebo group was higher, but the difference was just above the limit of statistical significance ($p<0.1$).
Wilcoxon rank test proved that in the Linex group the mean rank of number of copies in the second measurement significantly declined ($p<0.05$), but in the placebo group the difference between the measurements could not be confirmed ($p> 0.01$).

Eubacterias *content after the first and after the second measurement and the difference between measurements*

The minimum value is recorded in the Linex group in both measurements (first measurement: 1.31E+10, second measurement: 1.59E+09). The highest value in the first measurement was noted in the Linex group (3.16E+12), but in the second measurement in the placebo group (1.36E+12). In both measurements, the highest variation of the values was in Linex group, since the variation coefficient in this group was higher than in placebo.

Group		(Number of copies of 16S rRNA gene / g stool) – 1 Measurement	(Number of copies of 16S rRNA gene / g stool) – 2 Measurement	(Number of copies of 16S rRNA gene / g stool) – 2. Measurement -- 1. Measurement
Linex	Mean	4,47E+11	2,76E+11	-1,71E+11
	Median	1,98E+11	1,02E+11	-3,42E+10

	Minimum	1,31E+10	1,59E+09	-1,97E+12
	Maximum	3,16E+12	1,19E+12	6,55E+11
	Std. Deviation	7,12E+11	3,84E+11	5,14E+11
	CV	1,59	1,39	-3,01
	N	23	23	23
Placebo	Mean	3,83E+11	2,77E+11	-1,06E+11
	Median	3,35E+11	9,58E+10	-6,70E+10
	Minimum	2,36E+10	1,71E+10	-7,05E+11
	Maximum	7,94E+11	1,36E+12	1,33E+12
	Std. Deviation	2,87E+11	3,60E+11	4,68E+11
	CV	0,75	1,30	-4,43
	N	21	21	21
Total	Mean	4,16E+11	2,77E+11	-1,40E+11
	Median	2,65E+11	9,89E+10	-3,67E+10
	Minimum	1,31E+10	1,59E+09	-1,97E+12
	Maximum	3,16E+12	1,36E+12	1,33E+12
	Std. Deviation	5,46E+11	3,68E+11	4,88E+11
	CV	1,31	1,33	-3,49
	N	44	44	44
Mann-Whitney test		Z=-0,881; sig. 0,378	Z=-0,294; sig. 0,769	Z=-0,247; sig. 0,805
Wilcoxon Signed Ranks Test (Linex)		Z=-1,582; sig. 0,114		-
Wilcoxon Signed Ranks Test (placebo)		Z=-1,512; sig. 0,131		-



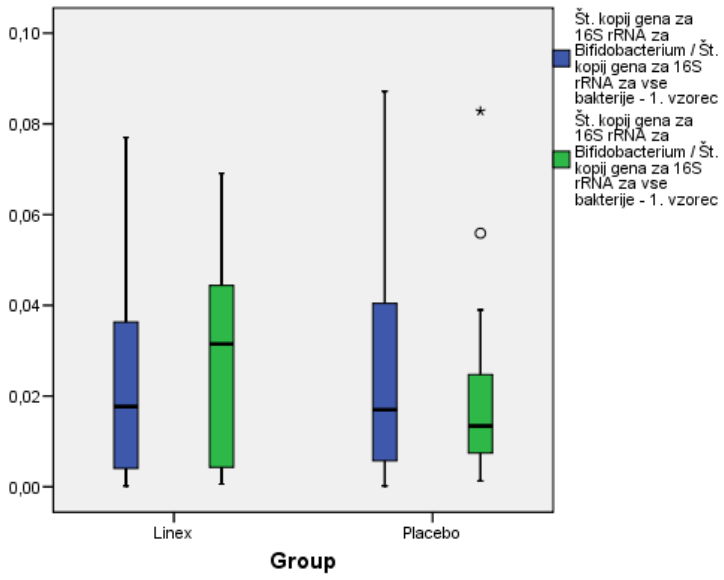
In the first measurement the median was higher in the placebo group (3.35E+11) than in Linex group (1.98E+11). In the second measurement the median was more similar (Linex: 1.02E+11, placebo: 9.58E+10).
Mann-Whitney test for independent samples showed that the experimental and the control group did not differ in mean rank both in first and second measurement (p>0.05); also the difference between the first and second measurement, regarding the group membership was not significant (p> 0.05).
In both groups the median decreased in the second measurement, but the Wilcoxon rank test did nor confirmed the significant differences (p> 0.05).

- **Results of quantification of *Bifidobacterium* in the stool of the subjects, normalized to data obtained for all bacteria**

The minimum share *Bifidobacterium/all bacteria* amounted in the first measurement 0.0002 (in both groups) and the maximum share was 0.174 (placebo group). The largest proportion of analyzed bacteria in the placebo group was 0.174 and 0.144 in the Linex group. Dispersion of the rates in the placebo group was higher than in the Linex. In the second measurement the minimum rate was in the placebo group 0.0013 and in the Linex group 0.006. The maximum value was in the Linex group 1.000 and in the placebo group 0.581.

Group		NO. copies of 16S rRNA gene of Bifidobacterium / No. copies of 16S rRNA gene of all bacteria - 1 sample	NO. copies of 16S rRNA gene of Bifidobacterium / No. copies of 16S rRNA gene of all bacteria - 2 sample	NO. copies of 16S rRNA gene of Bifidobacterium / No. copies of 16S rRNA gene of all bacteria – 2.-1. sample
Linex	Mean	0,026	0,100	0,029
	Median	0,017	0,032	0,005
	Minimum	0,0002	0,0006	-0,1411
	Maximum	0,144	1	0,3251
	Std. Deviation	0,034	0,225	0,107

	CV	1,31	2,26	3,63
	N	21	21	19
Placebo	Mean	0,030	0,049	0,017
	Median	0,016	0,013	0,002
	Minimum	0,0002	0,0013	-0,1599
	Maximum	0,174	0,581	0,4938
	Std. Deviation	0,0408	0,130	0,124
	CV	1,35	2,66	7,15
	N	20	19	19
Total	Mean	0,028	0,076	0,023
	Median	0,017	0,018	0,002
	Minimum	0,0002	0,0006	-0,1599
	Maximum	0,174	1	0,4938
	Std. Deviation	0,0368	0,186	0,114
	CV	1,32	2,46	4,89
	N	41	40	38
Mann-Whitney test		Z=-0,143; sig. 0,886	Z=-0,989; sig. 0,323	Z=-0,978; sig. 0,328
Wilcoxon Signed Ranks Test (Linex)		Z=-1,006; sig. 0,314		-
Wilcoxon Signed Ranks Test (placebo)		Z=-0,362; sig. 0,717		-



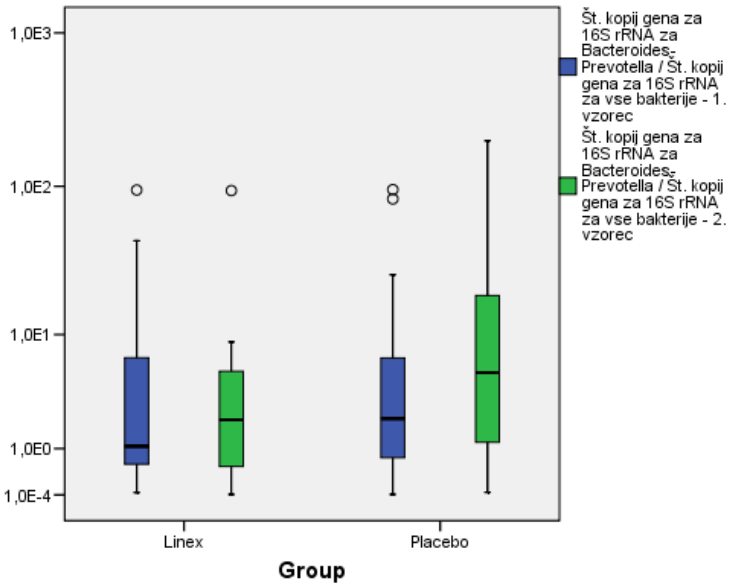
The median of the ratio of *Bifidobacterium/all bacteria* was in the first measurement in the Linex group 0.017 and it is comparable to the ratio in the placebo group (0.016). In the second measurement the median of this ratio in Linex group rose up to 0.032 but in the placebo group it slightly decreased to 0.013. The Mann-Whitney test for two independent samples showed that the experimental group did not differ from the control group in mean rank ratios as in the first and the second measurements ($p>0.05$). According to the Wilcoxon rank test the medians of the ratios of *bifidobacteria* did not significantly differ between the two measurements in any of the studied groups ($p> 0.05$).

- **Results of quantification of Prevotella in subjects's stool, normalized to data obtained for all bacteria**

In the first measurement the share of bacteria *Prevotella*/all bacteria ranged between 0.01 (both groups of subjects) and 95.140 (placebo group), the coefficient of variance was higher in the Linex group. In the second measurement values ranged from 0.01 (Linex group) to 198.14 (placebo group). The coefficient of variation was again higher in the Linex group.

Group		NO. copies of 16S rRNA gene of Bacteroides-Prevotella / No. copies of 16S rRNA gene of all bacteria - 1 sample	NO. copies of 16S rRNA gene of Bacteroides-Prevotella / No. copies of 16S rRNA gene of all bacteria - 2 sample	NO. copies of 16S rRNA gene of Bacteroides-Prevotella / No. copies of 16S rRNA gene of all bacteria - 2.-1. sample
Linex	Mean	10,2547	7,5917	-3,7811
	Median	0,923	3,135	0,463
	Minimum	0,010	0,010	-85,710
	Maximum	94,550	93,700	50,380
	Std. Deviation	22,572	20,442	25,053
	CV	2,201	2,693	-6,626
	N	22	20	19
Placebo	Mean	12,1596	28,1437	15,9841
	Median	2,124	5,219	2,594
	Minimum	0,010	0,040	-20,650
	Maximum	95,140	198,240	103,110
	Std. Deviation	26,219	51,224	34,136
	CV	2,156	1,820	2,136
	N	21	21	21
Total	Mean	11,185	18,1184	6,5957
	Median	1,874	4,389	1,0095

	Minimum	0,010	0,010	-85,710
	Maximum	95,140	198,240	103,110
	Std. Deviation	24,146	40,232	31,419
	CV	2,159	2,221	4,764
	N	43	41	40
Mann-Whitney test		Z=-0,899; sig. 0,369	Z=-1,408; sig. 0,159	Z=-1,855; sig. 0,064
Wilcoxon Signed Ranks Test (Linex)		Z=-0,161; sig. 0,872		-
Wilcoxon Signed Ranks Test (placebo)		Z=-2,103; sig. 0,035		-



Based on the Mann- Whitney test for independent samples, we could not confirm the statistically significant differences between the groups neither in the first nor in the second measurement (p>0.05). Although it is clearly shown in upper chart that in the second measurement the differences between the two groups have increased due to a much higher median in the placebo group.

As shown in Table 31. The mean rank of the difference between the measurements regarding the group was just above of statistical significance (p<0.1). The share of bacteria *Prevotella* has in the second measuring more increased in the placebo group than in the Linex group.

The similar conclusions could be done using the Wilcoxon rank test for dependent samples. In the Linex group there was no difference between the first and the second measurement. In the placebo group the mean rank of the numbers of copies significantly increased in the second measurement (p <0.5).

- **The results of microbiological analysis**

Subjects who experienced diarrhea were requested to provide stool samples for analysis regarding

presence of *Clostridium difficile* . Enzyme immunoassay tests were performed for the presence of *Clostridium difficile* toxin antigen A/B and the cultivation of *Clostridium difficile*. Additionally samples were used for the cultivation of coproculture and it's identification.
Of all 18 patients who experienced diarrhea during the trial, only 11 patients sent the stool sample for microbiological analysis.

Percentage of subjects with detected *Clostridium difficile* toxin antigen A/B (enzyme immunoassay) - according to the group

			Group		Total
			Linex	Placebo	
Enzyme immunoassay of Clostridium difficille toxin A / B	negative	N	3	5	8
		%	100,00%	62,50%	72,70%
	pozitive	N	0	3	3
		%	0,00%	37,50%	27,30%
Total		N	3	8	11
		%	100,00%	100,00%	100,00%
Fisher's Exact Test		sig.: 0,491			

The results of enzyme immunoassay analysis were done on samples of 3 subjects in the Linex group and 8 subjects from the placebo group. In any stool sample of the Linex group the *Clostridium difficile* toxin antigen A/B was not detected, but in the placebo group there where 3 of them (37.5%). According to the Fisher's exact test the difference of shares in groups were too small to be statistically confirmed (p> 0.1).

Percentage of subjects with the presence of *Clostridium difficile* culture - according to the group

			Group		Total
			Linex	Placebo	
Clostridium difficile culture	negative	N	3	7	10
		%	100,00%	87,50%	90,90%
	pozitive	N	0	1	1
		%	0,00%	12,50%	9,10%
Total		N	3	8	11
		%	100,00%	100,00%	100,00%
Fisher's Exact Test		sig.: 1			

The results of the analysis are based on the 3 samples from the Linex group and 8 samples from the placebo group. In the Linex group from none of the stool sample *Clostridium difficile* was cultivated, but it was cultivated from 1 stool sample in the placebo group. According to the Fisfer's exact test there were no significant differences between the groups.

Percentage of subjects with the presence of individual coprocultures - by group

			Group		Total
			Linex	Placebo	
coprocultures	none	N	2	7	9
		%	66,70%	87,50%	81,80%
	Bacillus cereus	N	0	1	1
		%	0,00%	12,50%	9,10%
	Yeast fungus	N	1	0	1

		%	33,30%	0,00%	9,10%
Total		N	3	8	11
		%	100,00%	100,00%	100,00%
Likelihood Ratio		sig.: 0,187			

The results are based on the analysis of 3 samples from the Linex group and 8 samples from placebo group. In the Linex group there were 2 stool samples (66.7%) who had no presence of coprocultures. In the placebo group, however, there were 7 (87.5%) of them. In 1 stool sample from the placebo group the *Bacillus cereus* was detected. Also in 1 stool sample from the Linex group the fungi yeasts were detected. Based on the »Likelihood Ratio« test the differences between the two groups could not be statistically confirmed.

Conclusion according to primary endpoint:	Overall incidence of diarrhea in the study during the antibiotic treatment with amoxicillin-clavulanic acid was below the expectations and was totally 9.7%. Totally 18 cases (9.7%) of study subjects experienced diarrhea, 7 cases (7.3%) in the Linex group and 11 cases (12.2%) in the placebo group. Occurrence of diarrhea was lower in Linex group (difference 4.9%) , although according to Fisher's exact test the difference between the proportions of subjects was too small to show statistically significant differences (p>0.1).
Conclusion according to secondary end points	The average duration of diarrhea among the 18 subjects was 3.56 days. The mean duration of diarrhea in the Linex group was 4.43 days and in the placebo group 3.00 days. Based on the nonparametric Mann-Whitney test, the difference in duration of diarrhea between the groups was significant (p <0.05) (shorter in placebo group). Microbiological analysis of the 11 diarrheal samples (3 from Linex and 8 from placebo group) detected the presence of Clostridium difficile toxin antigen A/B in 3 samples from the placebo group and in none from the Linex group. Clostridium difficile was cultivated from one stool sample in placebo group and from none in Linex group. According to Fisher's exact test the difference in results regarding Clostridium difficile tests between groups is not statistically significant. (p> 0.1) . Coprocultura confirmed no presence of Salmonella, Campylobacter, Shigella, Yersinia and Staphylococcus aureus. The Bacillus cereus was detected in 1 stool sample from the placebo group and yeasts fungi in 1 stool sample from the Linex group. The differences between the two groups could not be statistically confirmed. Microflora analyses showed an extremely high level of variability of the microbiota within the studied group. Noticed was a decrease in the median number of colony units of the Enterobacteria group (Enterobacteriaceae) in the final samples in the Linex group, compared to the baseline samples. Since this trend could not be seen in the placebo group, the reduction in the number of Enterobacteria (although not confirmed as statistically significant) may be due to the use of the probiotic.

	As the use of *Bifidobacterium animalis* subsp. *lactis* BB-12 and Lactobacillus acidophilus LA-5 did not cause a significant increase in the number of cultivable Bifidobacteria and Lactobacilli in the faeces of the trial subjects. The DNA quantification showed that in the placebo group (but not in the Linex group) the share of bacteria of the Bacteroides-Prevotella group in the final faecal samples was higher than in the initial samples, while in the Linex group the average ranking of the number of copies of 16S rRNA genes of the Bacteroides-Prevotella group in the final samples was lower compared to the initial samples. The above stated observations nevertheless suggest that the use of a probiotic had a certain effect on the microbiota in terms of an increase in the number of bacteria of the Bifidobacteriumgenus and a decrease in the number of bacteria of the Bacteroides-Prevotellagroup, yet due to a high degree of variability between individual subjects the changes did not prove to be significant. In the present study, the probiotic therapy did not have significant effect on the change of population level of the bacterial groups studied. This implies that probiotics tested did not affect the activity of these specific groups which predominate in the gut. However, impact on the normal gut flora is not required by probiotics; specific effects can be expressed also without significantly effecting the gut microbiota. The results of the study showed positive trends in the prevention of antibiotic associated diarrhea with the use of Linex® Forte. Positive trends are seen in three points; in the reduced frequency of diarrhea occurrence, in the reduced frequency of Clostridium difficile diarrhea and in the changes of the gut microflora. Since in the study the medium daily dose of Linex® Forte (2 capsules daily) has been used it can be assumed that the use of a maximum daily dose of Linex® Forte (3 capsules daily) would reinforced this positive trends and further contribute to the successfully prevention of antibiotic diarrhea.
Date of the Report:	24-08-2010