Research Article Open Access

Armenian fermented milk product Choratan and its influence on gut microbiota in health and pathology

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Submission Date: July 4th, 2018, **Acceptance Date:** September 28th, 2018, **Publication Date:** September 30th, 2018

Citation: Kalikyan Z., Avagyan V., Abrahamyan A., Vardanyan L., Selimyan A., Avagyan M. Armenian fermented milk product Choratan and its influence on gut microbiota in health and pathology. *Bioactive Compounds in Health and Disease* 2018; 1(5): 60-70. DOI: https://doi.org/10.31989/bchd.v1i5.562

ABSTRACT

Background: Choratan is an ancient Armenian fermented milk product which was recreated as a food supplement by A. Selimyan with the use of innovative technologies. Choratan contains large amounts of active *Lactobacillus acidophilus*, amino acids, and other bioactive compounds.

Aim: To investigate the impact of BAFS Choratan on gut microflora of the patients with different chronic disorders. Also, to evaluate the possibility using Choratan in addition to therapeutic schemes of chronic disorders for correction of microecological deviations.

Material and method: The influence of Choratan on gut microflora was studied in the frame of grant projects through the financing of State Committee of Science of Armenia. One hundred forty-one randomly chosen patients were included in the study with their informed consent. The patients divided into groups by diseases: gastrointestinal, allergic, and diabetes mellitus II type. Healthy patients were included in the fourth group. Bacteriological examinations of faeces were performed on 120 patients before and after Choratan was prescribed with an average dosage of 4-6g a day for 8 weeks.

Results: In all the groups, we observed improvements like the increase of normal microflora totally in 63.2%-68.3% and the decrease of conditionally pathogenic and pathogenic bacteria totally in 70.3%-100%. The mean amounts of all microorganisms were improved with statistically significant differences. 77.3% of patients noticed improvements in their conditions, which were more visible in healthy and allergic groups (87.1% and 82.4% respectively) and less impressive in patients with gastrointestinal diseases and diabetes (71.9% and 68.2% respectively).

Conclusion: From the results, we can conclude Choratan has positive influence on gut microflora and common health statuses. However, these changes depend on the nature of the disease and initial degree of dysbiotic deviations. Accordingly, we encourage more detailed placebo-controlled investigations.

Keywords: diseases; fermented foods; probiotics; bioactive food supplement, gut microflora; chronic diseases

BACKGROUND

The permanent influence of antimicrobial, antineoplastic, other drugs, technological food additives, industrial poisons, pesticides, radiation, and other stress agents on organisms results in a violation of the microecological system ("microorganism—gut microflora— environment") or dysbiosis of the bowel, which is accompanied with a variety of adverse effects: the spread of antibiotic-resistant strains, the expansion of the spectrum of diseases associated with the microbial factor, the increase of people with reduced resistance to the infections, and more [1]. Dysbiosis is always secondary and is a clinical and laboratory syndrome that develops in the case of some chronic diseases and pathologies. This is characterized by changes in the quantitative and qualitative composition of the intestinal microflora, as well as metabolism and immune system deviations that can be expressed by clinical symptoms. The main symptoms associated with intestinal activity include the following: diarrhea, constipation, flatulence, abdominal pains, etc. [2-4].

In recent years, numerous studies have been conducted on the role of gut microbiological deviations in various pathologies and the ways of their correction. Probiotics that contribute to the rehabilitation process are used in addition to the main treatment of chronic diseases [4-7]. Different effects of probiotic therapy on many gastrointestinal disorders, sexually transmitted infections, and immune disorders have been observed [8-11]. The effects of some probiotics with atopic diseases, rheumatoid arthritis, and regulation of cholesterol and glucose levels in blood have been investigated [5, 12-17]. There have been many probiotics widely used both in the composition of medicines and in the form of probiotic products, although the search for the most effective remedy is ongoing [3, 6, 7, 18].

There are also a few publications in Armenian scientific literature related to the use of probiotic methods. During the 20th century in Armenia, fermented milk product "Narine" was created. Narine was recommended by the World Health Organization (WHO) as a biologically active food supplement (BAFS), being widely used in the world for the past 50 years [5, 19, 20]. Choratan, known as Colostrum in the West is an ancient Armenian fermented milk product. At the beginning of the 21st century, Dr. Alexander Selimyan recreated Choratan as a food supplement using microbiological innovative processes and innovative technology [21]. Nevertheless, the available information is contradictory and not supported by scientific evidence.

MATERIAL AND METHOD

Choratan's content and properties

Choratan is a fermented milk bioactive food supplement developed from a dried concentrate of yogurt using the biotechnological method. Choratan is a yellow powder that contains *Lactobacillus acidophilus* with the total amount of $2.5*10^7$ per gram, essential amino acids with total amount of 1.6 grams in 100 grams of the substance and 2.96% of protein [21]. The milk product Choratan was exported from the laboratory of the National Institute of Standards of

Republic of Armenia (Protocol №767-10/26.07.2010) and received the Certificate of Compliance with Technical Requirements (№AST-A01.A-2233-2010).

The antibacterial properties of Choratan (its dried and liquid forms) have been tested with use of two known methods—agar diffusion and two-fold serial dilutions. The meat peptone stock (pH=7.2-7.4) and dried nutritious agar were used as media. Study by the *method of agar diffusion* was performed as the following: the agar was poured into Petri dishes with flat bottoms in two layers; uninoculated media were used for lower layer and agar medium with test culture—for the upper layer; the temperature of medium did not exceed 48-50°C; after solidification of the sown agar, the preparations of Choratan were placed on its surface; the Petri dishes were kept in the refrigerator for 24 hours and then placed in a thermostat (37°C) for 18 hours. The results were evaluated by the diameter of microbial growth inhibition zone (measured by a ruler). Study by the *method of two-fold serial dilutions* was performed as the following: the microbial bodies in amount of $2*10^6$ CFU/mL in medium were added to the test tubes containing media with different concentrations of Choratan preparations (1:2, 1:4, 1:8, and 1:16); the results were evaluated in accordance with turbidity after incubation at 37°C. The tests have been carried three times and then averaged. The following strains of bacteria were chosen: gram-positive *Staphylococcus aureus—209p, 91, 34,118* and gram-negative— *Salmonella typhi 31120-90, Proteus vulgaris, Shigella dysenteriae Flexneri 6858, and Eberthella typhi 79.*

Patients

Detailed clinical examination followed by appropriate laboratory analyses including mandatory bacteriological analysis of feces were performed to the patients with different pathologies (gastrointestinal, allergic, diabetes) who applied to the Centre of Traditional and Rehabilitative Medicine "AltMed". On the base of preliminary ("before") results of the bioanalysis of feces, a total of 141 patients with dysbiosis were included in our study with their informed consent. There were 42 males (29.8%) and 99 females (70.2%) with the mean age 36.2±1.75.

One hundred and ten patients with verified diagnoses of chronic diseases were distributed into the following main groups: (a) GI—gastrointestinal diseases (mainly associated with Helicobacter pylori: chronic gastritis/gastroduodenitis, gastro-esophageal reflux disease; irritable bowel syndrome), (b) AD—allergic diseases (mainly atopic dermatitis isolated or associated with bronchial asthma and/or allergic rhinitis; urticaria), (c) T2D—second type diabetes mellitus. The fourth was a healthy group (HG) composed of generally healthy patients with no specific clinical diagnosis but with some complaints of the discomfort in their digestion.

According to the current classification of microecological deviations and standards of normal composition of gut microflora [22], the degree of intestinal dysbiosis was determined: (a) I-II degree – decrease of the levels of normal microflora (lactobacilli ($\geq 10^6$CFU/g), bifidobacteria ($\geq 10^8$CFU/g) and *Esherichia coli* ($\geq 10^6$CFU/g)) or increase of the levels of pathogenic forms of *E. coli* (lactose negative or weakly fermentative active *E. coli* (≤10% from total amount of *E. coli*)); (b) III degree - decrease of the levels of normal microflora and increase of the levels of conditionally pathogenic microflora (*Clostridium* ($\leq 10^5$CFU/g); pathogenic forms of *E. coli*); (c) IV degree – decrease of the levels of normal microflora, increase of the levels of conditionally pathogenic microflora and presence of pathogenic microflora (*Candida* ($\leq 10^3$CFU/g); hemolytic *E. coli* (<0% from total amount of *E. coli*); other pathogens: *Clebsiella, Proteus, Enterobacter, Streptococcus, Staphylococus* et al. ($\leq 10^3$CFU/g)).

The appropriate therapeutic schemes were prescribed to the patients with clinical diagnoses and Choratan (purchased from manufacturer – "Croop" LLC) was added; Choratan was only prescribed to the patients without clinical diagnosis. The dosage depended on the patients' age, the level of dysbiosis, and the level of individual tolerance. Dosage was 2-3 times (4-6g) a day

for 8 weeks in average. During the treatment, the patients' conditions were monitored with regular intervals (after the first, second, fourth and eighth weeks). In the case of intolerance of Choratan, mostly expressed in a burning sensation, the dose was reduced.

The repeated ("after") bacteriological examinations of feces were performed on only 120 patients (response rate - 85.1%) due to interruption of treatment with the reason of worsening of condition (5 patients) or the refusal of further participation in the study (16 patients). There were 40 males (33.3%) and 80 females (66.7%) with mean age 35.9±1.94.

Statistical analysis was performed with use of Microsoft Office Excel. The reliability of results was provided with 95% probability at a critical significance level of p=0.05. Variables were expressed as the mean ± standard deviation or percentage (%). Student *t*-test and paired Student *t*-test were used for statistical analysis, $P < 0.05$ was considered to be significant and $P < 0.01$ highly significant.

The research was carried within the framework of the grant project by financing of the State Committee of Science of Republic of Armenia (№ 602/HK). The investigation was designed and implemented in accordance with the principles embodied in the Declaration of Helsinki of 1965. The main ethics aspects of our investigation were revised and approved by the Scientific Coordinatory Committee of National Institute of Health after S. Avdalbekyan (Approval № 02/15.02.2013).

RESULTS AND DISCUSSION

Choratan

On the base of testing of Choratan's antibacterial properties the following results were obtained (Table 1).

Table 1. The influence of Choratan on bacteria.

Choratan		***Bacterial strains***							
		Staphylococcus aureus				*S. Typhy*	*Proteus*	*Sh. Dysent*	*E*
		209p	*91*	*34*	*118*	*31120-90*	*vulgaris*	*Flexneri 6858*	*Typhi 79*
Liquid		*Bacteria-free zone diameter*							
	(mm)	17	18	13	15	18	15	16	17
		*Bacterial growth**							
Concentration	pure	-	-	-	-	-	-	-	-
	1:2	-	-	-	-	-	-	-	-
	1:4	-	-	-	-	-	-	-	-
	1:8	-	-	-	-	-	-	-	-
	1:16	+	+	+	+	+	+	++++	-
Dried		*Bacteria-free zone diameter*							
	(mm)	16	17	13	15	18	15	16	17
		*Bacterial growth**							
Concentration	pure	-	-	-	-	-	-	-	-
	1:2	-	-	-	-	-	-	-	-
	1:4	-	-	-	-	-	-	-	-
	1:8	++++	-	±	++	-	++	++++	-
	1:16	++++	++	++++	++++	++++	++++	++++	±

* «-» - no growth → «++++» - very intensive growth

The bacteria-free zones were approximately the same with both (dried and liquid) forms of Choratan—the differences were registered only in two samples of *Staphylococcuss aureus* (209p and 91), with the liquid form being favored. The bacterial growth was suppressed by exposure in both forms of Choratan but the liquid form was more active in less concentrated samples.

Patients

In the first stage of study among patients, the data on initial bacteriological examination (n = 141) was obtained. According to the data, the substantial quantitative deviations of separate components of intestinal microbiota were recorded. The most sufficient deviations were observed in the quantity of lactobacilli and bifidobacteria that decreased in 81.6% and 78.0% of patients respectively. The most prevalent deviation among conditionally pathogenic flora was the increase of *Clostridium* in 33.3% of patients. There were some differences between images of bacterial microflora depending on the pathology. Consequently, the decrease of bifidobacteria and the increase of *Clostridium* among patients of GI group (93.2% and 43.2% respectively), the decrease of lactobacilli and the increase of lactose negative *Escherichia coli* in the group of AD (97.1% and 29.4% respectively), and the increase of *Candida* in the group of T2D (31.3%) were the most expressive microbiotic deviations by comparison with other groups. Deviations of gut microflora were also registered in the group of almost healthy patients. In particular, the deviation of lactobacilli was prevalent in 71.0% of patients.

According to the classification of deviations of gut microflora and on the base of determined degrees of dysbiosis the structure of patients of different groups was defined (Figure 1).

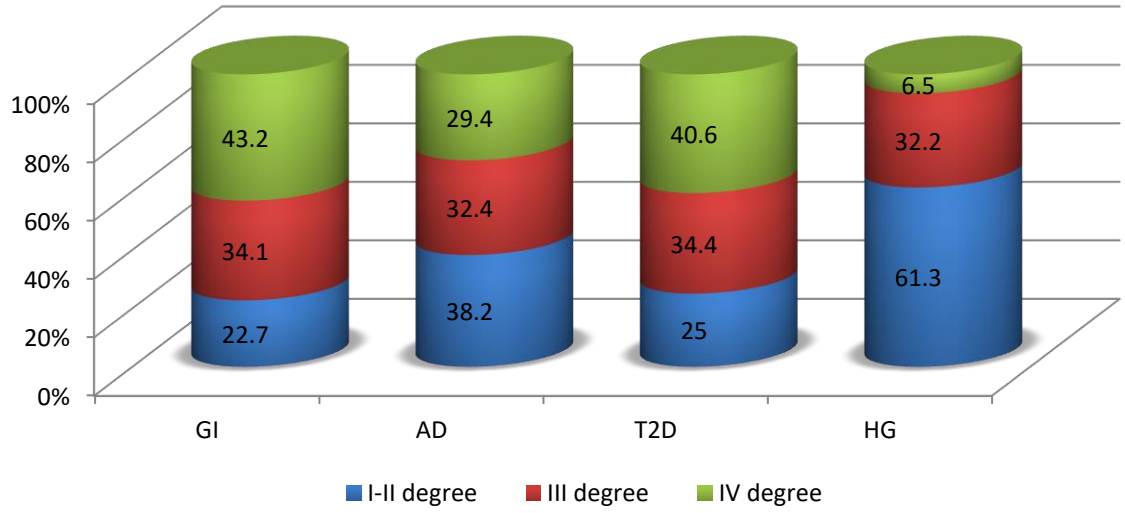


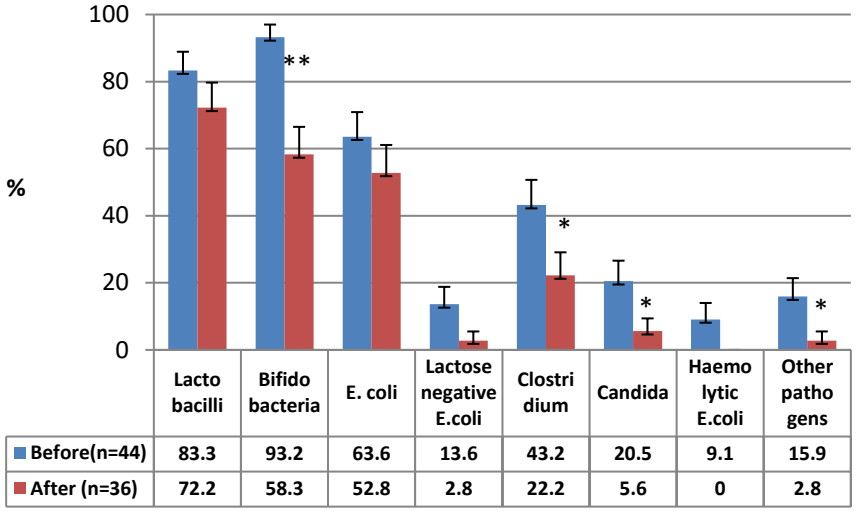
Figure 1. The structure of patients by degree of dysbiosis.

The weights of severe dysbiotic deviations predominate in T2D and GI groups were higher than 40%, while the weights of light deviations were lower than 25%. At the same time the weight of light dysbiotic deviations was particularly high in the group of patients without any

clinical diagnosis accounting for more than 60% appropriate to the other researchers' data [1-4]. The differences in the composition of dysbiotic deviations and degrees of dysbiosis between diseases may be related to their nature and severity. On the other hand, in most cases whether the disturbed microflora is causal or correlative with the disease remains unknown. But the possibility that disturbed microbiota may play a role in the onset or development of certain diseases is mentioned by several researchers [2, 6, 8].

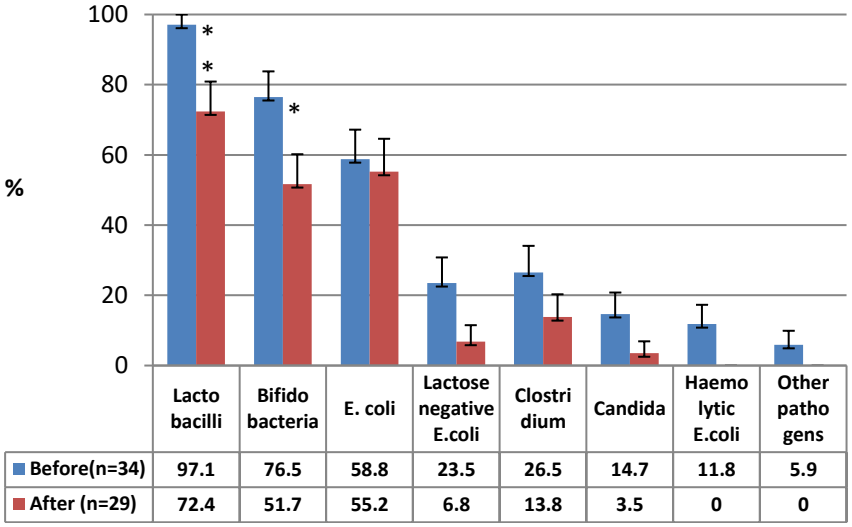
After Choratan administration, the repeated bacteriological examination of feces was performed to 120 patients. The prevalence of normal microflora deviations stayed higher than 50% among patients of all groups, which accounted for 60.8%, 54.2%, and 51.7% for lactobacilli, bifidobacteria, and *Escherichia coli* respectively. There were some differences between prevalence rates of some microorganisms' deviations among different groups of patients by comparison before and after administration of Choratan. Thus, the prevalence of deviations of Lactobacilli in the groups of AD and healthy patients, and bifidobacteria in the group of GI patients decreased with highly significant differences ($P<0.01$). At the same time the rates of *Clostridium, Candida* and other pathogens in the GI group and bifidobacteria in the AD group decreased with significant difference ($P<0.05$). All other rates decreased as well but the differences were insignificant $(P>0.05)$. The most expressive influence of Choratan intake on the prevalence of microflora deviations was registered in the group of patients with gastrointestinal diseases, while the group of T2D patients the changes were the least significant. The data on prevalence of main deviations of microflora among patients with different diseases before (n=141) and after (n=120) Choratan intake are presented in the Figure 2 (a, b, c, d).

According to the collected data of 120 patients all positive changes that are increasing (↑) of normal microflora and decreasing (↓) of conditionally pathogenic and pathogenic microflora (even without full correction of microbiota) considered as improvement. Thus, gut microflora generally improved. There were several significant changes in the quantity of different microorganisms after Choratan intake. The most improved rates were registered in conditionally pathogenic and pathogenic microflora such as *Clostridium, Candida*, pathogenic forms of *E. coli* et al. among all groups of patients and totally vary from 70.3% for *Clostridium* and 86.4% for *Candida* to 100.0% for others. At the same time, the levels of normal microflora (lactobacilli, bifidobacteria and *E. coli*) improved in 68.3%, 63.2%, and 67.1% of the patients respectively. There were some features in the data of participants with different diseases. In contrast to the other groups, the improvement of the level of lactobacilli was not higher (43.3%) among patients with gastrointestinal disorders, while the improvement of bifidobacteria and conditionally pathogenic *Clostridium* was lower among patients with diabetes (45.5% and 44.4% respectively), and E. coli - among patients with allergies (55.6%). The rates improved among patients of the healthy group (at least 72.7%) which is obviously due to the initial degree of dysbiosis.



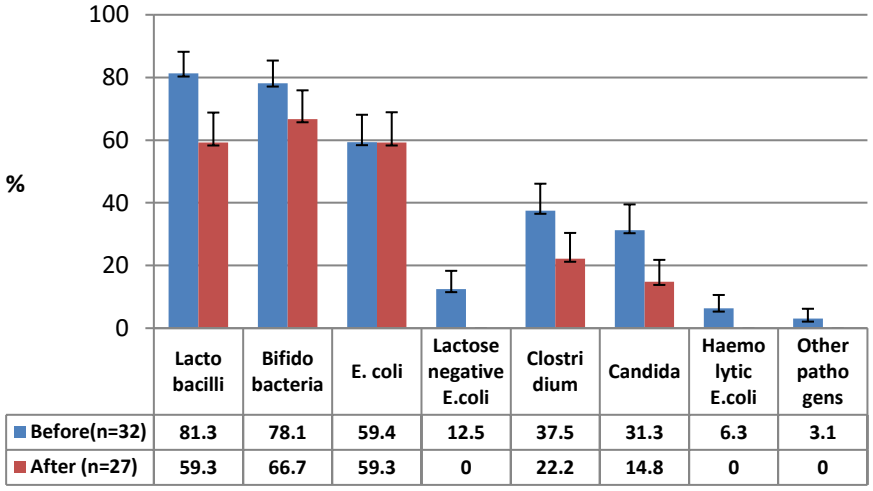
	Lacto bacilli	Bifido bacteria	E. coli	Lactose negative E.coli	Clostri dium	Candida	Haemo lytic E.coli	Other patho gens
Before(n=44)	83.3	93.2	63.6	13.6	43.2	20.5	9.1	15.9
After (n=36)	72.2	58.3	52.8	2.8	22.2	5.6	0	2.8

(a)Patients' group GI.



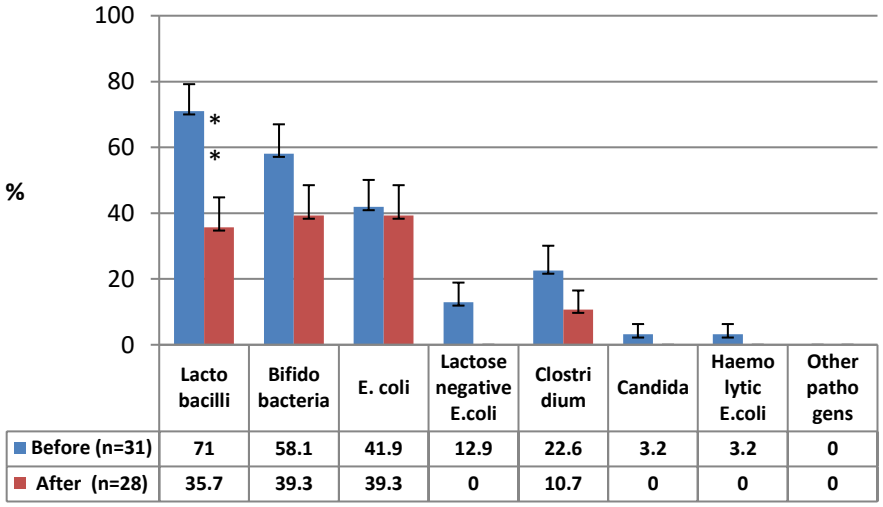
	Lacto bacilli	Bifido bacteria	E. coli	Lactose negative E.coli	Clostri dium	Candida	Haemo lytic E.coli	Other patho gens
Before(n=34)	97.1	76.5	58.8	23.5	26.5	14.7	11.8	5.9
After (n=29)	72.4	51.7	55.2	6.8	13.8	3.5	0	0

(b)Patients' group AD.



	Lacto bacilli	Bifido bacteria	E. coli	Lactose negative E.coli	Clostri dium	Candida	Haemo lytic E.coli	Other patho gens
Before(n=32)	81.3	78.1	59.4	12.5	37.5	31.3	6.3	3.1
After (n=27)	59.3	66.7	59.3	0	22.2	14.8	0	0

(c) Patients' group T2D.



	Lacto bacilli	Bifido bacteria	E. coli	Lactose negative E.coli	Clostri dium	Candida	Haemo lytic E.coli	Other patho gens
Before (n=31)	71	58.1	41.9	12.9	22.6	3.2	3.2	0
After (n=28)	35.7	39.3	39.3	0	10.7	0	0	0

(d) Patients' group HG.

Figure 2 (a,b,c,d). Prevalence of gut microflora deviations among patients with different diseases before and after administration of Choratan.

(Note: * The difference between prevalence of deviations before and after Choratan intake is significant (P<0.05)

** The difference between prevalence of deviations before and after Choratan intake is highly significant (P<0.01)

To understand the real improvement of gut microflora, mean amounts of the most important microorganisms were also calculated before and after Choratan administration, according to the accepted standards (Table 3).

Table 3. Amount of some intestinal microorganisms before and after administration of Choratan.

Microflora		Patients groups							
		GI (n=36)		*AD (n=29)*		*T2D (n=27)*		*HG (n=28)*	
		Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
Lactobacilli	m (lg/CFU/g)	5.17±0.28	5.72±0.3	4.66±0.24	5.66±0.31	5.04±0.31	5.9±0.34	5.61±0.34	6.57±0.34
	P		*<0.05**		*<0.01***		*<0.05**		*<0.01***
Bifidobacteria	m (lg/CFU/g)	5.67±0.23	7.06±0.32	6.24±0.4	7.14±0.38	6.11±0.37	6.7±0.41	6.57±0.47	7.54±0.41
	P		*<0.01***		*<0.01***		*>0.05*		*<0.05**
Esherichia coli	m (lg/CFU/g)	5.36±0.36	6.06±0.34	5.79±0.4	6.03±0.38	5.41±0.45	5.89±0.41	6.21±0.46	6.71±0.33
	P		*<0.05**		*>0.05*		*>0.05*		*>0.05*
Clostridium	m (lg/CFU/g)	4.17±0.3	3.72±0.25	3.76±0.39	3.35±0.32	4.0±0.39	3.67±0.36	3.21±0.44	2.71±0.36
	P		*<0.05**		*<0.05**		*>0.05*		*<0.05**
Candida	m (lg/CFU/g)	1.03±0.29	0.56±0.2	0.86±0.29	0.41±0.19	1.59±0.38	0.85±0.25	0.39±0.17	0.14±0.1
	P		*<0.05**		*<0.05**		*<0.05**		*>0.05*

The difference between mean amount of microorganisms before and after Choratan intake (↑ **or ↓) is significant (P<0.05)*

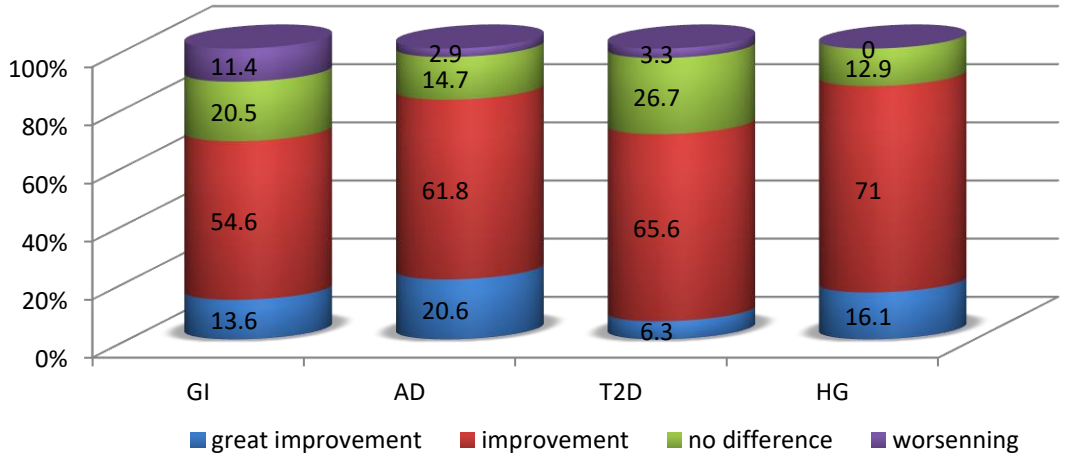
*** The difference between mean amount of microorganisms before and after Choratan intake (↑ **or** ↓) is highly significant (P<0.01)*

The amounts of all microorganisms were improved overall. However, there were some features in the levels of significance for different groups of patients. The mean number of lactobacilli improved with significant difference (*P<0.01*) in patients with allergic diseases and healthy patients and with significant difference (*P<0.05*)—in patients with gastrointestinal diseases and diabetes mellitus. Bifidobacteria was improved with highly significant difference in patients with gastrointestinal diseases, allergies, and in healthy patients. At the same time *E. coli* improved with statistically significant difference only in patients with GI disorders while the differences of mean amounts of *Clostridium* and *Candida* were not statistically significant (*P*>0.05) except for in patients with diabetes and the healthy group respectively. Thus, the lactobacilli was the most improved microorganisms from normoflora, which can be explained by the presence of *Lactobacillus acidophilus* in the content of Choratan.

Numerous studies tracking the impact of probiotics on microflora have concluded that probiotic survival through the intestine is dose-dependent and strain-dependent [2, 4, 8]. In the majority of cases, changes of fecal bacteria were not consistent among different studies and are dependent on factors that differ among studies, such as probiotic type, duration of intake, and time of sampling (during administration or during follow-up). On the basis of our investigation, trends of improvement of different microorganisms are also disease-dependent.

During and after the course of Choratan, the general condition of patients, particularly subjective changes related to the functioning of intestine, the correction of defecation, the elimination/reduction of pain, flatulence and other symptoms were evaluated. Thus, the majority of patients (77.3%) have demonstrated the positive impact of Choratan. Only 18% did not experience any difference and 5% had a worsening of condition due to the intake of Choratan in the form of a burning sensation. Figure 3 demonstrates the distribution of patients of different groups (n = 141) according to their condition.

Figure 2. The structure of patients by their subjective condition connected with intake of Choratan.



The most noticeable changes were demonstrated by patients of healthy group and allergy (87.1% and 82.4% respectively) and less with diabetes mellitus and especially those with gastrointestinal diseases (71.9% and 68.2% respectively), which is linked to the mechanisms of existing diseases (chronic and severe). Consequently, subjective symptoms' improvements are dependent on disease nature and severity of its course similarly to bacteriological changes. This outcome is appropriate for the opinion of some investigators who consider that there is great potential in further conduct of the studies to better elucidate microbiota changes that correlate with probiotic-induced improvements in health or symptom measures [2, 8, 12, 16].

CONCLUSIONS

There are positive changes (the majority of which are statistically significant) both in the bacteriological data of feces' analyses and subjective conditions of the patients. Although these changes depend on the nature and severity of disease and the degree of dysbiotic deviations, Choratan can be recommended as BAFS for correction of gut microbiota in given pathologies. Nevertheless, it is necessary to provide more detailed placebo-controlled clinical and laboratory investigations to the development of scientifically approved indications for the use of Choratan as a rehabilitative and preventive remedy in the complex treatment of chronic diseases.

List of Abbreviations: BAFS - biologically active food supplement; GI - gastrointestinal diseases; AD - allergic diseases; T2D - second type diabetes mellitus; HG - healthy group; E. coli - Escherichia coli.

Competing Interests: There was no conflict of interest.

Authors' Contributions: Z. Kalikyan designed the study, collected data, performed the statistical analysis, interpreted results, and wrote the manuscript. V. Avagyan, A. Abrahamyan, and L. Vardanyan have contributed to the data collection and interpretation of results. A. Selimyan contributed to the interpretation of results and revisions of the article critically for important intellectual content. M. Avagyan contributed to the design of the study, performance of statistical analysis and interpretation of results, revising the article critically for important intellectual content, and giving final approval of the version to be published.

Acknowledgements and Funding: The authors would like to thank everyone who contributed to the success of the study, especially the State Committee of Science of Republic of Armenia for funding of the project (№ 602/HK) and the leaderships of National Institute of Health after S. Avdalbekyan of Republic of Armenia and Yerevan State Medical University after Mkhitar Heratsi.

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