Research and development new symbiotic product and its clinical effect

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First stage: To study the gut microbiome

To determine gut microbiome types and to compare microbial composition of the gut microbiome in different aged Kazakhstani
Subjects were divided into 3 groups:

I group - to 44 years

II group – 50 – 70 years

III group - 90 years and above

Patients signed informed consent on the procedure, screening and collection of samples
1. General information (date and place of birth, weight, growth, gender, smoking habits, diet, nationality)

2. Information about diseases of gastro-intestinal tract (IBD, \textit{H. pylori}, dyspepsia, etc.)

3. General clinical information (blood group, antibiotics (last 2 months), birth, nutrition in infancy, stool frequency, regularity of the cycle, etc.)

4. Information about the diet (alcohol, probiotics, vegetables, dairy products)
16S rRNA approach
Sequence Scanner v 1.0

Data processing
It was observed that in all groups independently on age prevail two enterotypes

**Bacteroides enterotype**

**Firmicutes enterotype**
**Most numerous members of the gut microbiome**

<table>
<thead>
<tr>
<th></th>
<th>1 group (44 years)</th>
<th>2 group (50-75 years)</th>
<th>3 group (90 and more)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetanaerobacterium sp.</td>
<td>Red</td>
<td>Yellow</td>
<td>Orange</td>
</tr>
<tr>
<td>Acidovorax sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alistipes sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alloprevotella sp.</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Anaerostipes sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteroides sp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium sp.</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Eubacterium sp.</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ruminococcus sp.</td>
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</tbody>
</table>

- Less than 0.5%
- 0.5 – 10 %
- 10 – 25 %
- 25 – 35 %
- 35 – 45 %
- 45 – 60 %
Species’ variety in subjects of 30-70 years

Bacteroides xylanisolvens
Lactonifactor longoniformis
Akermansia muciniphila
Acetobacter rava
Catenibacterium mitsuokai
Clostridium cellulolyticum
Coprooccus catus
Eubacterium biforme
Lactobacillus delbrueckii
Lactobacillus mucosae
Lactobacillus plantarum
Lactobacillus rogosae
Leuconostoc mesenteroides
Prevotella salivae
Pyramidobacter piscicola
Citrobacter diversus
Clostridium indolis
Coprococcus comes
Salmonella typhi
Sarcina ventriculi
Prevotella melaninogenica
Species’ variety in subjects of 90 years and above

Alistipes indistinctus
Bacteroides ureolyticus
Clostridium hathewayi
Coprobacter fastidiosus
Eubacterium fiscicatena
Hespellia porcina
Mogibacterium timidum
Parabacteroides goldsteinii
Paraprevotella xylaniphila
Parasporobacterium paucivorans
<table>
<thead>
<tr>
<th>Functional characteristics</th>
<th>30-70 years</th>
<th>90 years and above</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant substrates degrading bacteria (cellulose)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mucin-degrading bacteria</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Butyrate-producing bacteria</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates converting bacteria (production of organic acids, acetate, ethanol, carbon dioxide, hydrogen)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein and starch degrading bacteria</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mucus-binding bacteria</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Infections causing pathogens</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
1. Two enterotypes of the gut microbiome: *Bacteroidetes* and *Firmicutes* independently on age of subjects

2. Significant differences in bacterial species between all 3 groups according to age

3. The richest diversity occurred in younger individuals and decreased in the elderly. It can be explained by the age factor

4. The percentage of bifidobacteria and lactobacilli is low in all research groups

5. More research is needed for better understanding of compositional structure of the gut microbiome of Kazakhstani population
A formula and a production technology were developed and a pilot batch of a symbiotic bio-product “НӘР” was produced (Dairy plant Astana – onim).

**Probiotic component**
- Prebiotic - inulin
- Plant fiber – pectin
- Fish collagen
- Milk

**Consortium content:**
- *Lactobacillus plantarum;*
- *Lactobacillus fermentum;*
- *Lactobacillus acidophilus;*
- *Bifidobacterium longum;*
- *Bifidobacterium bifidum*

NAR has been tested in JSC “Kazakh Academy of Nutrition”
The probiotic component is deposited in the National Depository of industrial microorganisms.
**Synbiotic “НӨP” NAR: properties**

Synbiotic activates primary immune response. First of all, synbiotic coming into an organism interacts with colon epithelium cells. Reaction of macrophages and dendritic cells of lamina propria is induced, and as a result induction of IL-6 (10080.0±238.0 pg/ml)

IL-6 promotes clonal expansion of IgA-lymphocytes, increase in quantity of IgA-producing cells and passing them through plasmatic cells in lamina propria of intestinal mucous (64.7±0.7).

In the analysis of induced cytokines, we observed significant increase in TNF-α and IFN-γ and regulatory cytokine IL-10

Induction of γ IFN along with increase in production of IL-12 (228.9±17.8) inhibits producing IL4 due to activation of signal pathway of NF-Kb and STAT

The listed changes bring Th1/Th2 balance towards Th1

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**Antioxidant activity**

Total antioxidant activity of the synbiotic product 67.4 mmol/ml

SOD is 1.42 U/mg

Glutathione reductase 0.06 U/ml

Damage index–0.60

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**Cholesterol lowering properties**

in MRS broth medium 22 %

in the presence of 0.2 % bovine bile 50 %.
## Preclinical and clinical study

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolation period (5 days)</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Control 1 (standart feeding)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Control 2 (standart+prebiotic)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Exp 1 1 dose NAR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Exp 2 probiotic component 1 dose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Exp 3 2 dose NAR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Exp 4 probiotic component 2 dose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>

1 stage of clinical trials - 70 patients: 35m and 35 f

Recruiting and Clinical - laboratory investigation were performed in two different Medical Center
Introduction

The aim of this study is to investigate the efficacy of a synbiotic in treatment of adult with metabolic syndrome.

Metabolic syndrome is an umbrella name for a collection of health risk factors — high blood pressure, high blood sugar, high triglycerides, low HDL cholesterol, high LDL cholesterol, and excess belly fat.
Study design

- Recruiting volunteers
- Initial clinical and laboratory examination
- Sampling: blood and stool
- Survey

Placebo group
Synbiotic group

Duration of synbiotic/placebo taking is 3 months

Repeated clinical and laboratory examination
Repeated collection of stool samples

Data analysis
Metagenome analysis of gut microbiome in normal and with metabolic syndrome, before and after receiving the symbiotic product NAR
SNP analysis on markers associated with metabolic syndrome
Randomisation and blinding

Random selection of patients from the database of Medical Center of President's Affairs Administration of Republic of Kazakhstan

The physician-researchers responsible for enrolling patients

Study leader is responsible for the distribution of participants by placebo-symbiotic groups

Placebo and synbiotic provided in identical packages

The physician-researchers collecting the reporting forms and performing the patients investigations were blind to the patient's treatment assignment
Participants and subject eligibility

Assessed for eligibility (n=180)

Declined to participate (n=13)

Enrollment (n=167)

Placebo group

Allocated to intervention (n=83)
Received allocated intervention (n=83)

Discontinued intervention (n=3)

Subjects completed (n=80)

Synbiotic group

Allocated to intervention (n=84)
Received allocated intervention (n=83)
Did not receive allocated intervention (n=1)

Lost to follow-up (n=2)

Subject completed (n=81)
We enrolled 161 adult 92 patients with metabolic syndrome 69 patients with no symptoms of metabolic syndrome

key criteria for inclusion:
1. No history of the use of probiotics or antibiotic for 3 months
2. Blood pressure: = 130/90 mmHg
3. Raised fasting plasma glucose (FPG): >100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes
4. Dyslipidemia: TG = 1.695 mmol/L; HDL-C = 0.9 mmol/L (male), = 1.0 mmol/L (female)
5. Central obesity: waist:hip ratio > 0.90 (male); > 0.85 (female), or body mass index > 30 kg/m2
Age categories
30-39; 40-49; 50-59; 60-69; 70-79

Before the start of the study, all patients were examined comprehensively, including the following list of clinical and laboratory examination:

- family history
- information on the use of antibiotics
- anthropometry
- characterization of the cardiovascular system
- characterization of the stool and its frequency
- characterization of immune status
- data for the blood glucose, ApoE, C-reactive protein, total cholesterol, HDL, LDL

Total about 200 questions
- my microbe
- Food Frequency Questionnaires (FFQ)
- SF Health Surveys
- Other questions about health
### Effect of synbiotic on function of Digestive tract

<table>
<thead>
<tr>
<th>average stool frequency</th>
<th>Synbiotic before</th>
<th>Synbiotic after</th>
<th>Placebo before</th>
<th>Placebo after</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;twice daily</td>
<td>14.9%</td>
<td>21.9%</td>
<td>15.3%</td>
<td>7.1%</td>
</tr>
<tr>
<td>once daily</td>
<td>14.2%</td>
<td>65.6%</td>
<td>3.8%</td>
<td>10.7%</td>
</tr>
<tr>
<td>once every 2 days</td>
<td>70%</td>
<td>12.5%</td>
<td>76.9%</td>
<td>82%</td>
</tr>
<tr>
<td>&lt;once every 2 days</td>
<td>0.9%</td>
<td>0</td>
<td>4%</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

**Bristol Stool Chart**
- **Type 1**: Separate hard lumps, like nuts (hard to pass)
- **Type 2**: Sausage-shaped but lumpy
- **Type 3**: Like a sausage but with cracks on the surface
- **Type 4**: Like a sausage or snake, smooth and soft
- **Type 5**: Soft blobs with clear cut edges
- **Type 6**: Fluffy pieces with ragged edges, a mushy stool
- **Type 7**: Watery, no solid pieces, Entirely Liquid
Clinical outcome

BMI Categories:
Underweight = <18.5
Normal weight = 18.5–24.9
Overweight = 25–29.9
Obesity = BMI of 30 or greater

34.3% no change in BMI
66.7% BMI decreased by 0.3 - 3.8

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight</th>
<th>BMI</th>
<th>Waist</th>
<th>BMI Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>713B053</td>
<td>158</td>
<td>62.5</td>
<td>53</td>
<td>25.03605</td>
</tr>
<tr>
<td>713A038</td>
<td>173</td>
<td>77</td>
<td>73.5</td>
<td>25.72756</td>
</tr>
<tr>
<td>713B065</td>
<td>164</td>
<td>88</td>
<td>86</td>
<td>32.71862</td>
</tr>
</tbody>
</table>

Waistline

synbiotic
42.86% - a decrease by 1-3 cm
40% - no changes
17.14% - an increase by 1 - 2 cm

placebo
14,2% - a decrease by 0,5-1 cm
63% - no changes
22,8% - an increase by 0,5 – 4 cm
## Effect of synbiotics on Lipid Profile

### mmol/L

<table>
<thead>
<tr>
<th>Data</th>
<th>Synbiotic</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
</tr>
<tr>
<td>Total Blood Cholesterol</td>
<td>4.92±0.25</td>
<td>4.58±0.23</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>3.37±0.28</td>
<td>2.98±0.27</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>1.09±0.08</td>
<td>1.20±0.07</td>
</tr>
<tr>
<td>TG</td>
<td>1.59±0.07</td>
<td>1.50±0.06</td>
</tr>
</tbody>
</table>
Effect of synbiotic on Inflammatory Markers

C-reactive protein (CRP)

Before 3,162±0,122 mg/L
After 1,987±0,124 mg/L

The erythrocyte sedimentation rate (ESR)

Before 11,19±1,00 mm/hour
After 9,88±0,85 mm/hour

Leukocytes (White Blood Cells)

Before 6,75±0,46 10⁹/L
After 6,16±0,39 10⁹/L

Low Risk: less than 1.0 mg/L
Average risk: 1.0 to 3.0 mg/L
High risk: above 3.0 mg/L
### Effect of synbiotic on HB

<table>
<thead>
<tr>
<th>Data</th>
<th>before</th>
<th>after</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>134±3</td>
<td>138±3</td>
</tr>
<tr>
<td>min</td>
<td>82</td>
<td>104</td>
</tr>
<tr>
<td>max</td>
<td>165</td>
<td>177</td>
</tr>
</tbody>
</table>

Bifidobacterium contributes to increasing permeability through the intestinal wall of the ions of calcium, iron, vitamin D.

Conclusion

Synbiotic NAR

- Improves motor function of the intestine
- Improves metabolism of macroorganism
  - Lowers Blood Cholesterol
- Possesses anti-Inflammatory effects

Synbiotic is proposed as an addition to the basic treatment, not an alternative to main therapy

- In the study group the percentage of colds were significantly lower than in placebo group
- The majority of patients reported an improvement in abdominal discomfort

Eat Probiotics foods, live healthy life
Acknowledgment

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Kozhakhmetov Samat PhD, senior researcher
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Shakhabayeva Gulnara PhD, senior researcher
Tynybayeva Indira
Baiskhanova Dinara

Prof. Francesco Marotta
Regenera Research Group

Medical Center of President's Affairs
Administration of Republic of Kazakhstan

USM NU

Dairy plant Astana - onim
Usenov KZh - collaborator of dairy plant
Baimenova BA - collaborator of dairy plant
Kozhentaeva ZT - dairy technologist
Zhabagenova A - microbiologist at the milk plant
So…..

Eat the “good bugs” every day………..

Invite them in…………

You will find they make very friendly houseguests.