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# CHARACTERISTICS OF INTESTINAL MICROBIOME IN CHILDREN WITH AUTISM

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**ABSTRACT** — The aim of the study was to assess the prevalence of dysbiosis in children with autism spectrum disorders (ASD). The study was observational, cohort, comparative. All the patients included in it were divided into 2 groups. The first (main) group (n=46) consisted of children aged 4.92±1.57 years with an established diagnosis of ASD. The second (n=20, comparison group) included conditionally healthy children (1 and 2 health groups) aged 5.10±1.16 years, not suffering from ASD. It was stated that in children with autism a reduced content of lactobacilli (p=0.056), the practical absence of lactic acid streptococci (p=0.019), a rarer detection of individual strains of Enterobacter bacteria (Ent. cloacae; p=0.033), an increase of 2 or more times in the frequency in level of hemolytic Escherichia coli (E. coli; p=0.041), some strains of bacteria of the genus Klebsiella (Kl. Pneumoniae; p=0.080) and Citrobacter (Citr. freundii, p=0.015); Staphylococcus aureus (14% vs. 6%, p>0.050) and Candidae fungi (11% vs. 6%, p>0.050) were found more often than in healthy children. In addition, patients with autism have enough level of Bifidobacterium and Escherichia coli. The study showed the presence of shifts in the gut microbiome in children with autism spectrum disorders. At the same time, structure of microbiome differed significantly from that typical for healthy children.

**KEYWORDS** — autism, children, gut microbiota, dysbiosis.

## INTRODUCTION

According to the largest epidemiological studies in the world, there is a tendency to increase the prevalence of autism [4–6]. So, for 18 years, the prevalence rate has increased from 30.8 per 10,000 children in 2000 to 169 per 10,000 children in 2018 [3, 7]. One of the world's largest information and analytical systems Autism and Developmental Disabilities Monitoring (ADDM) of the Center for Disease Control and Prevention reports that the prevalence of autism among 8-year-olds in the United States of America in 2014 was 1.68%, which is 14% higher than in 2012 and 2010 [1, 4].

It is known that autism spectrum disorders (ASD) are characterized by a large number of comorbid conditions affecting various other organs and systems. At the same time, pathological conditions of the gastrointestinal tract (gastrointestinal tract) are described as the most common among concomitant diseases in patients with ASD (according to some data, almost 12% of people with ASD have concomitant gastrointestinal pathology) [1, 2, 6].

Separate studies show that the qualitative and quantitative composition of the intestinal microbiota in patients with ASD differs significantly from that typical for practically healthy people [1, 3, 5]. Thus, in patients with ASD, a smaller variety of microbial associations was noted, a decrease in the number of bacteria of the genera Prevotella, Corprococcus and Veilonellaceae, an increased ratio of Firmicutes / Bacteroidetes, high levels of Lactobacillus, Desulfovibrio, Sutterella; a high prevalence of microorganisms of the genus Clostridium among patients with ASD [1, 6]. Various types of abnormal RAS-associated intestinal metabolites have been studied. In particular, the number of reports of excessive production of short-chain fatty acids, para-cresol and ammonia has increased in recent years [3, 5, 6]. Based on this, a hypothesis has been put forward about the presence of pronounced shifts in the intestinal microbiome in ASD and the etiopathogenetic role of dysbiosis disorders in the development of the pathology under consideration [3, 5]. At the same time, there is not enough data in the literature to confirm it.

*The aim of the study* was to assess the prevalence of dysbiosis in children with autism spectrum disorders.

## MATERIAL AND METHODS

The study was observational, cohort, comparative. All the patients included in it were divided into 2 groups. The first (main) group (n=46) consisted of children aged 4.92±1.57 years with an established diagnosis of ASD. The second (n=20, comparison group) included conditionally healthy children (1 and 2 health groups) aged 5.10±1.16 years, not suffering from ASD. The diagnosis of ASD was verified according to the examination of a psychiatrist using ADOS and ADIR [1, 2, 6].

The examination of children of both groups included the collection of complaints, an objective examination by a pediatrician, a bacteriological study of

the qualitative and quantitative composition of the intestinal microflora, interviewing the mother /guardian of the child using a questionnaire *Studying the medical and social causes of the formation of health abnormalities and diseases in children*, the answers to which reflected the presence/absence of the child's biomedical risk factors of the mother's pregnancy and childbirth; risk factors of early childhood and risk factors associated with the child's living conditions.

This study was initiated with the approval of the Local Ethics Committee of the Volga Research Medical University of the Ministry of Health of the Russian Federation (Protocol No. 3 of 02/21/2020).

Statistical data processing was carried out using the Statistica 6.0. for Windows application software package. Parametric and nonparametric statistics were used. The analysis of the type of distribution of the trait was carried out using the Kolmogorov Smirnov method. Descriptive statistics included median (ME) and interquartile range [Q25–Q75]. Comparison of quantitative features in groups was carried out using the Wald-Wolfowitz criterion, used to compare two independent small samples; comparative analysis of qualitative features was carried out using the Pearson criterion22; the relationships between the features were evaluated by the gamma (γ) correlation analysis and the method of paired and/or multiple regression; the presence of statistical significance of differences in the groups was assumed at $p<0.05$.

## RESULTS

The results of the assessment of the state of intestinal microbiocenosis and the severity of its disorders are presented in Table 1. It was revealed that children with ASD were characterized by the most frequent detection of intestinal dysbiosis in general (p=0.019) and the detection of significant dysbiotic disorders in the form of intestinal dysbiosis of 3–4 degrees (p=0.049).

The qualitative and quantitative composition of the intestinal microbiota in patients with ASD differed significantly from that in children in the comparison group. Along with significant differences in the absolute content in the feces of individual representatives of the intestinal microbiota in children with ASD, there was a change in the frequency of their increase and/or decrease.

Thus, in children with autism a reduced content of lactobacilli (p=0.056), the practical absence of lactic acid streptococci (p=0.019), a rarer detection of individual strains of Enterobacter bacteria (Ent. cloacae; p=0.033), an increase of 2 or more times in the frequency in level of hemolytic Escherichia coli (E. coli; p=0.041), some strains of bacteria of the genus Klebsiella (Kl. Pneumoniae; p=0.080) and Citrobacter (Citr. freundii, p=0.015); Staphylococcus aureus (14% vs. 6%, p>0.050) and Candidae fungi (11% vs. 6%, p>0.050) were found more often than in healthy children. In addition, patients with autism have enough level of Bifidobacterium and Escherichia coli.

***Table 1.*** *Frequency of detection of intestinal dysbiosis in children with ASD (%)*

| | Healthy children (N=17) | Children with autism (N=37) | P-value |
|---|---|---|---|
| No dysbiosis | 41 | 3 | 0,001 |
| Dysbiosis of 1 degree | 18 | 32 | 0,250 |
| Dysbiosis of 2 degree | 35 | 35 | 0,144 |
| Dysbiosis of 3-4 degree | 6 | 30 | 0,049 |

## CONCLUSION

Thus, the study showed the presence of shifts in the gut microbiome in children with autism spectrum disorders. At the same time, structure of microbiome differed significantly from that typical for healthy children.

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