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Characteristics of the intestinal microbiota in patients with colorectal cancer

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ABSTRACT *AIM: to evaluate the intestinal microbiota and identify its characteristic features in patients with colorectal cancer.*
PATIENTS AND METHODS: the composition of the intestinal microbiota in patients with colorectal cancer was analyzed, while the comparison group consisted of patients with non-inflammatory bowel diseases.
RESULTS: previous studies have shown the possible role of enterococci and some types of clostridia in stimulating oncogenic processes and, on the contrary, the vital role of lactobacilli and bifidobacteria in intestinal homeostasis maintaining. The proportion of enterococci was increased in patients with colorectal cancer (12.7% in the study group and 7.6% in the control group) on the contrary the proportion of bifidobacteria and obligate anaerobes was decreased (1.5% in the study group and 4% in the control groups) and (3.3% of the study group and 9.7% in the control group) respectively. Decrease of the microbiota biodiversity was observed for the patients with colorectal cancer that was calculated by the Shannon diversity Index (4.46 in the study group and 4.8 in the control group), also two-fold increase of Pseudomonas aeruginosa (2.2% in the study group and 1.1% in the control group) was found for this cohort, Clostridium septicum was isolated only from patients with colorectal cancer.
CONCLUSION: this study results suggest the diagnostic significance of the diversity of the intestinal microbiota.

KEYWORDS: *colorectal cancer, microbiota, anaerobes, butyrate-producing bacteria, lumen microbiota, wall microbiota*

CONFLICT OF INTEREST: *The authors declare no conflict of interest*

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INTRODUCTION

According to a study published by Catherine de Martel et al., 2020 [1] in 2018, approximately 2.2 million cases of cancer associated with infection were diagnosed worldwide, which as per ASIR (age-standardized incidence rates) is equal to 25.0 cases per 100,000 person-years. Among the main etiological agents, *Helicobacter pylori* (810,000 cases, ASIR 8.7 cases per 100,000 person-years), human papilloma virus (HPV) (690000, 8.0), hepatitis B virus (HBV) (360,000, 4.1) and hepatitis C virus (HCV) (160000, 1.7). As for colorectal cancer (CRC), at the moment there is no convincing data on a positive correlation between the presence, absence or persistence of a certain infectious agent and the development of colorectal cancer, which is obvious for stomach, cervical and liver cancers (HVP, *H. pylori* and HCV, respectively). Some researchers point to non-infection-related cancers (breast cancer, prostate cancer, colorectal cancer), although there are studies for breast cancer suggesting a link with an infectious agent similar to Murine Mammillary tumor virus (MMTV), which causes breast cancer in mice [2,3].
At the same time, colorectal cancer with 1.8 million new cases and 881,000 deaths in 2018 [4] remains a global problem, being the third most

common oncological disease and the second most fatal malignant neoplasm [5]. According to forecasts, 2.2 million new cases and 1.1 million deaths are expected in the world by 2030 [6]. At the same time, the etiology of colorectal cancer is very complex and includes both genetic and environmental factors [7]. The twin method and family studies have demonstrated that only a small part of colorectal cancer is associated with a genetic predisposition, namely familial adenomatosis, hereditary non-polypous colorectal cancer, Peitz-Jaegers syndrome and other rare diseases [8–10]. Also, many cases of colorectal cancer are recognized as sporadic [11]. At the same time, more and more data indicate that the intestinal microbiota plays an important role in the occurrence, progression and metastasis of colorectal cancer [12].

In the process of modeling colorectal cancer in animal experiments, a change in bacterial communities is shown. In particular, rats with colorectal cancer showed a significant decrease in butyrate-producing bacteria, such as *Roseburia* and *Eubacterium*, and a decrease in the number of probiotic species, such as *Ruminococcus* and *Lactobacillus* [13]. When analyzing scientific publications on the topic of colorectal cancer, attention is drawn to the availability of data on the association of enterococcal infection and malignant neoplasms of the large intestine. For example, in a study by Kajihara et al. [14], a high comorbidity of cancer and enterococcal infection, defined as a monomicrobial culture, was demonstrated, while the study did not consider cases of colonization by enterococci. A study by Amarnani et al. [15] explicitly indicates the possible role of intestinal enterococci in the development of large intestine cancer, which is also reflected in a study by Li et al. [16]. Possible molecular mechanisms of carcinogenesis associated with enterococcal infection are described in studies by Ruiz, P.A., et al. and Wang, X., et al. [17,18]. Among obligate anaerobes, *Clostridium septicum*, which is a gram-positive spore-forming obligate anaerobic bacterium, is considered as a microorganism, and also possibly associated with the development of CRC. It has been suggested that the lack of oxygen and the acidic environment of the large intestine tumor provides favorable conditions for the germination of *C.Septicum* spores [19]. It remains a debatable question whether the presence of *C.Septicum* is the cause of cancer or whether the bacterium contributes to the further development of the tumor.

Separately, it is worth noting the role of bifidobacteria, which are one of the main colonizers of the intestine. In general, it is believed that their interaction with the host begins shortly after birth, although recent studies have questioned the dogma of sterility of the intrauterine environment, providing evidence of the presence of microorganisms in the placenta, amniotic fluid and umbilical cord in a healthy full-term pregnancy [20–22]. It is assumed that some representatives of the genus *Bifidobacterium* have various positive effects for the macroorganism at the local and systemic levels, for example, limiting colonization/invasion of pathogenic flora or affecting the immune system through changes in innate and/or adaptive immune responses. Thus, studies have revealed a number of secreted or surface-associated molecules that act as mediators for establishing a dialogue between bifidobacteria and the host immune system and allow interaction with immune cells associated with the mucosal layer [23]. In addition, by-products of carbohydrate metabolism of bifidobacteria act as vectors that directly and indirectly trigger the host's immune response, the latter by stimulating the growth of other commensal microorganisms, such as bacteria producing propionate or butyrate [24]. Among the short-chain fatty acids produced in the human large intestine, butyrate plays a key role in maintaining intestinal health and is the preferred source of energy for intestinal epithelial cells. As a result, the consumption of butyrate improves the integrity of the intestinal epithelial cells of the host, promoting close contacts, cell proliferation and mucin production by goblet cells [25,26]. There is also evidence of the role of butyrate as an anti-inflammatory agent [27,28].

AIM

To evaluate intestinal microbiota and to identify its features in patients with colorectal cancer.

PATIENTS AND METHODS

Patients. The main group — patients with colorectal cancer (regardless of TNM) — 100 patients.
Comparison group — patients with hemorrhoids, anal fissure, rectal fistulas — 76 patients.
Patients underwent inpatient treatment at the Center in the period from January 2021 to December 2022.
Biomaterial under study. Samples of parietal (biopsies of the large intestine mucosal layer) and lumen (feces) microbiota were studied in each patient. In patients with colorectal cancer, biopsy material from the tumor was additionally studied. In total, 604 (300 samples in patients with CRC and 304 samples in patients of the comparison group) biosimilars were included in the study. The microbiological study was carried out using an expanded range of nutrient media (25 types of nutrient media for primary sowing of biomaterial), with incubation in aerobic, microaerophilic and anaerobic conditions. Identification of isolated microorganisms was carried out using mass spectrometry on the MALDI-TOF platform. The study of the cultural properties of microorganisms, growth conditions, colony morphology was carried out using standard microbiological methods.
Statistical data processing was carried out using the Statistics program. The data were evaluated separately for the lumen faeces and for the wall flora. The contribution of each microorganism was expressed as a fraction of the microbiota, based on the analysis of the entire data set, the Shannon biodiversity index was calculated using the formula $H = -\Sigma\ pi * \ln(pi)$. The Shannon Index allows us to take into account both species richness and quantitative differences between species.

RESULTS

The results obtained in the study of the parietal and lumen microbiota were combined into the microbiota of the large intestine. The total number of isolates isolated in patients with colorectal cancer was 1,381 isolates, while 1,813 isolates were isolated in the comparison group.
The data on the spectrum of microorganisms in samples obtained from patients with colorectal cancer are shown in Figure 1, from patients of the comparison group — in Figure 2.
Figures 1 and 2 show the combined number of isolated strains from the corresponding species along the Y axis. The species composition is represented on the X — axis. The microbiota of patients with colorectal cancer is characterized by a poor species composition, in contrast to the comparison group. Attention is drawn to the dominance of such groups of microorganisms as *Enterococcus* with a clear decrease in the content of *Bifidobacterium*. A total of 236 species of microorganisms were isolated in samples obtained from 100 patients. The Shannon index for the microbiota of patients with colorectal cancer was 4.47.
In patients of the comparison group, attention is drawn to several pronounced peaks in the content of microorganisms such as *E. coli, E. faecalis, S. epidermidis,* and *Bifidobacterium longum*. In general, we can note a pronounced biodegradation of the microbiota in patients without CRC. A total of 280 species of microorganisms were isolated in samples obtained from 76 patients. The Shannon index for the microbiota of patients in the comparison group was 4.8.
In the group of patients with CRC, 236 species of microorganisms were isolated, of which 4 species had the highest incidence of occurrence (more than 40 isolated strains): *E.coli, Klebsiella pneumoniae, Enterococcus faecalis* and *Enterococcus faecium*. 280 microorganisms were isolated in the comparison group, 6 species had the highest incidence of occurrence (more than 40 isolated strains): *E. coli* (together with *E.coli* hem +), *Proteus mirabilis, K.pneumoniae, E.faecalis, Staphylococcus epidermidis, Bifidobacterium longum*. As a result of studying the species composition of the microbiota in patients with CRC relative to the comparison group, the following types of microorganisms were not isolated in the CRC group: *Vibrio ezurae, Listeria grayi, Dermacoccus nishinomiyaensis, Moraxella osloensis, Rothiaamarae, Dermacoccus nishinomiyaensis, Paeniglutamici bacter psychrophenolicus, Paracoccusversutus, Veillonellaspp, Clostridium butyricum, Megasphaeraelsdenii, Sutterella wadsworthensis, Magnusiomycescapitatus, Kazachstaniapintolopesii, Wickerhamomycesanomalus*.

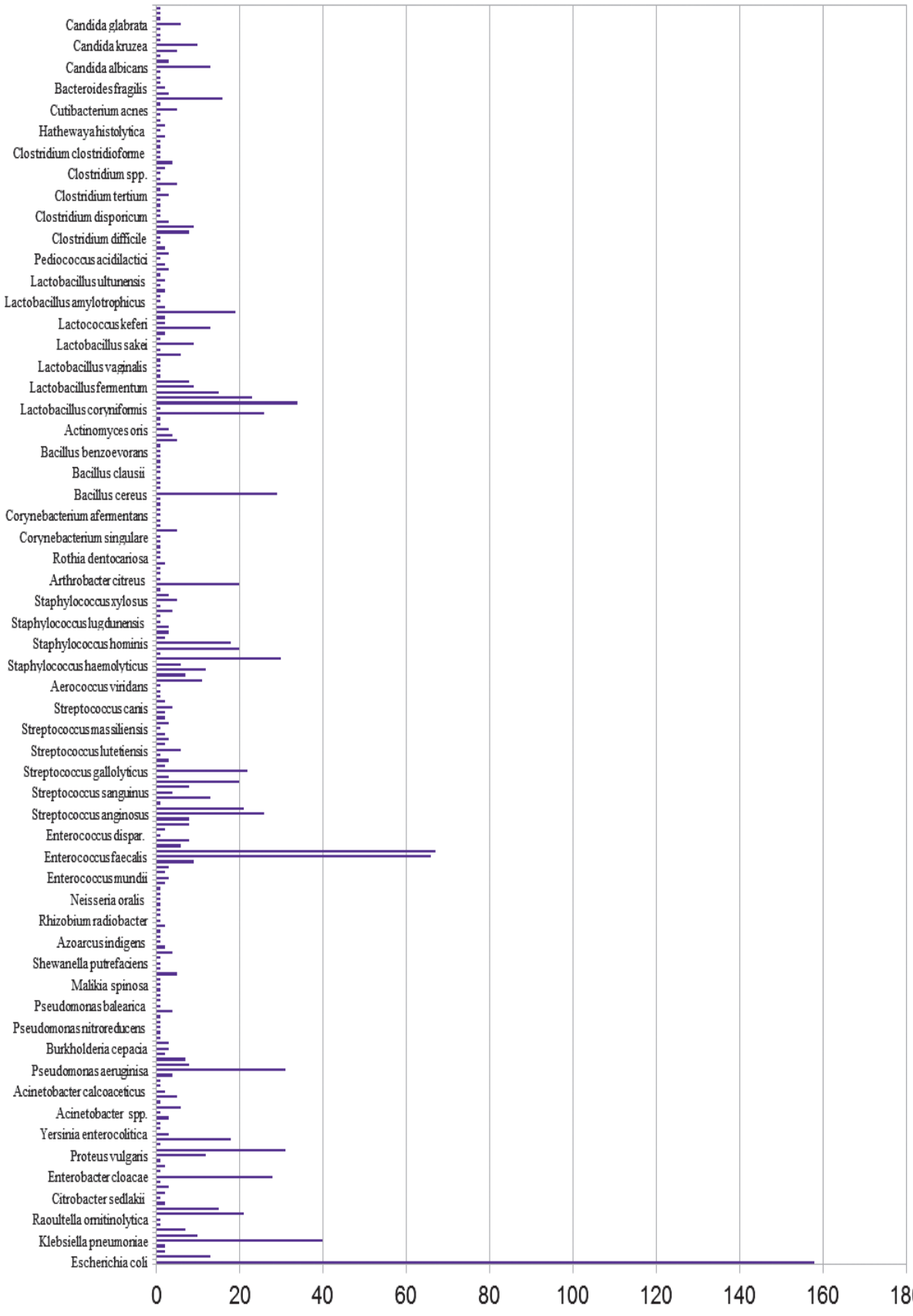

Figure 1. *Spectrum of microorganisms in samples of patients with colorectal cancer*

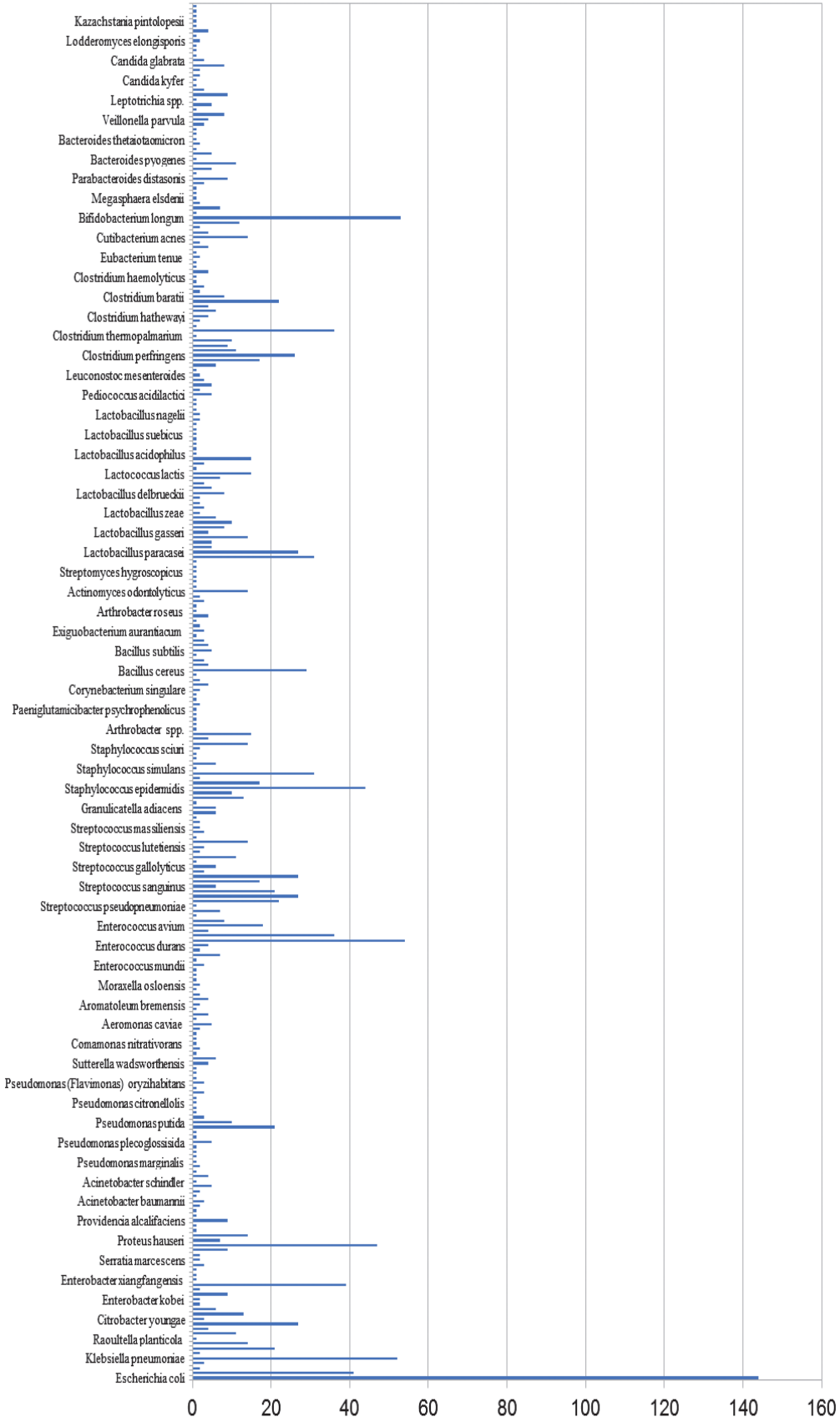

Figure 2. *Spectrum of microorganisms in samples of patients with diseases of the rectum and anus*

At the same time, *Filifactorhathewaya, Blautiacoccoides, Propionibacterium avium, Clostridium septicum* were found only in the group of patients with CRC. The representation of *Pseudomonas aeruginosa* was higher in the group of patients with colorectal cancer by 2.2% of the microbiome, in the comparison group the incidence of Pseudomonas aeruginosa was exactly two times lower and amounted to 1.1%.

To assess the distribution of microorganisms depending on taxonomic affiliation, microorganisms were grouped into taxonomic groups; the data are shown in Figure 3. Particular attention should be paid to the coincidence of the representation of enterobacteria, as well as a significant difference in the representation of gram-negative obligate anaerobes, obligate spore anaerobes, bifidobacteria and enterococci. Thus, in the group of obligate gram-negative anaerobes in patients with CRC, only 4 types of gram-negative anaerobes were isolated: *Bacteroides fragilis* was isolated in two patients, and *Bacteroides vulgatus, Veillonelladispar, Thaueraaminoaromatica* were detected only once, while 21 types of obligate gram-negative anaerobes with fairly widely represented species of *Vellionells spp., Bacteroides spp.* and *Parabacteroides spp.* were isolated in the comparison group patients.

In the group of obligate spore anaerobes, a similar pattern was observed in terms of representation: 19 clostridium species in patients with CRC and 21 species in the comparison group.

The dominant species isolated in patients with CRC was *Clostridium perfringens*, and in patients of the comparison group *Clostridium innocuum*. *C.septicum* was found only in the biomaterial of patients with CRC. In general, 46 species belonging to the genus *Clostridium spp*. were isolated in these patients, while 175 species were isolated in the comparison group. Among the bifidobacteria in patients with CRC, only 3 species were identified: *Bifidobacterium bifidum, Bifidobacterium longum* and *Bifidobacterium breve*. The total number of isolated isolates of bifibodacteria was 20 strains, while 75 strains belonging to five types of bifidobacteria were isolated in the comparison group: *Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum* and *Bifidobacterium adolescentis*.

Among enterococci in patients with CRC, 175 strains of 11 species were isolated, and in the comparison group 138 strains belonging to 10 species. The contribution of enterococci in patients with CRC was 12.7%, the most common species were *E.faecalis* and *E.faecium*, with a share in the microbiota of 4.8% each. In the comparison group, the contribution of enterococci was 7.6% *E.faecalis* and *E.faecium* with a share in the microbiota of 3.0% and 2.0%, respectively.

In the group with colorectal cancer, the representation of bifidobacteria was significantly reduced (1.5%), while in the comparison group, bifidobacteria occupy 4.0% of the microbiome. Also noteworthy is the decrease in the spectrum of obligate spore anaerobes in the group of patients with CRC (3.3%), while in the comparison group obligate spore anaerobes occupy almost 10% (9.7%) of the microbiome. On the contrary, enterococci are more widely represented in patients with CRC (12.7%) than in the comparison group (7.6%).

To assess the representation of microorganisms by types of respiration, microorganisms were grouped into 4 groups: obligate aerobes, obligate anaerobes, microaerophiles (capnophiles) and facultative anaerobes. The data by groups are shown in Figure 4.

In the representation of microorganisms, according to their type of respiration, there was no statistically significant difference between the three groups — obligate aerobes, microaerophiles and facultative anaerobes. On the contrary, in the group of obligate anaerobes, there is a drop in the number of bacteria. Thus, only 4 types of gram-negative anaerobes were detected in the group of patients with colorectal cancer, while 21 types of gram-negative anaerobic microorganisms were detected in the control group. In general, the proportion of obligate anaerobes in patients with CRC did not exceed 7% (6.9%), while in the comparison group obligate anaerobes are the second largest group of microorganisms — more than 20.9% of the microbiome.

In general, it should be noted the preservation of a harmonious relationship between groups of microorganisms and, as expected, the leading group turned out to be facultative anaerobes, mainly represented by the order *Enterobacteriales*. At the

same time, a statistically significant difference was found in the group of obligate anaerobic microorganisms — the representation of obligate anaerobes in the group of patients with CRC is lower than in the comparison group: 7% vs. 21%, obviously achieved due to the differences described above in the groups of gram-negative and spore-forming anaerobes.

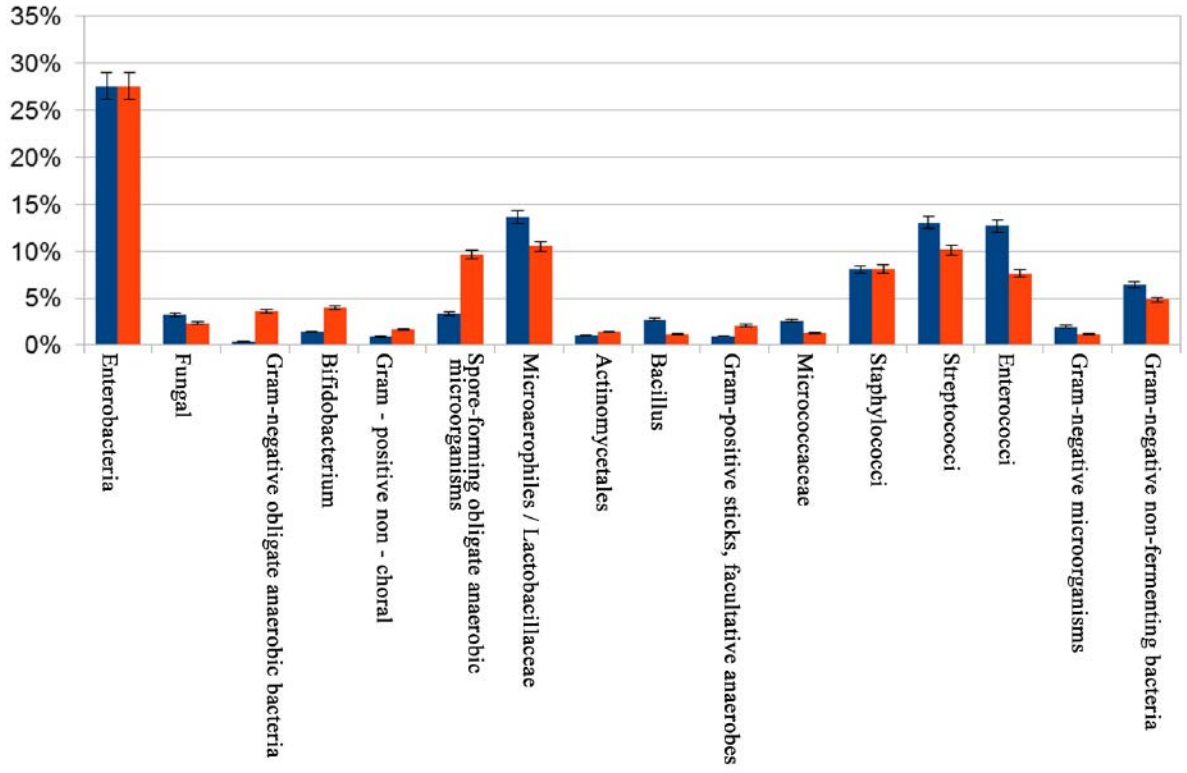

Figure 3. *Distribution of microbiota by taxonomic groups*

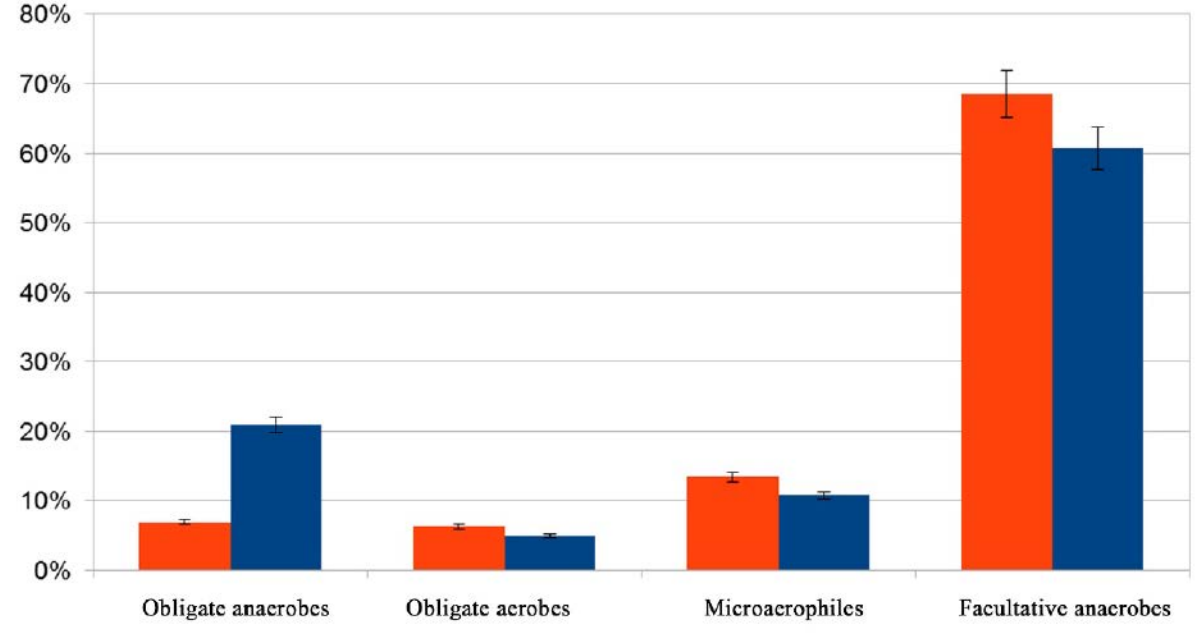

Figure 4. *Distribution of isolated microorganisms by types of respiration*

Table 1. *Ratios within the indigenous microflora*

	CRC	Comparison Group
Lactobacilli/Bifidobacteria	9.4	2.6
The proportion of lactobacilli	13.6%	10.5%
The proportion of bifidobacteria	1.5%	4.0%
Spore/non-sporemicroorganisms	3.8	5.8

The assessment of the presence of indigenous (autochthonous) microflora in relation to the total microbial number (the total number of isolated isolates of microorganisms) in the studied groups of patients is shown in Table 1.

Attention is drawn to the significant difference in the ratios of lactobacilli and bifidobacteria. The data obtained indicate a decrease in the number of bifidobacteria in patients with CRC (1.5%), which causes a threefold difference in the ratio of bifidobacteria/lactobacilli.

The study revealed a decrease in the proportion of bifidobacteria in the group of patients with CRC (1.5%), while in the comparison group bifidobacteria occupy 4% of the microbiome. The high peak of B. longum in patients of the comparison group, clearly visible in Figure 2, looks especially bright, while the representation of this species alone is almost 3% (2.9%) of the entire microbiota. In patients with CRC, *B. longum* also dominates, but in the overall picture of the microbiota, bifidobacteria are lost; the number of *B. longum* in patients with CRC is about 1% of the total microflora (1.1%). The ratio between spore and non-spore obligate anaerobes is also noteworthy, since in the CRC group it is 3.8, while in the comparison group it is 5.8, which is due to the high rate of occurrence of *Clostridium spp.* in patients of the comparison group (46 isolates in the CRC group and 175 in the comparison group).

DISCUSSION

The human intestine is a biocenosis with numerous connections both between the macroorganism and the microbiota, and within the microbial community. The data obtained demonstrate significant changes in the microbiota in patients with colorectal cancer. When grouping microorganisms by type of respiration, the most pronounced changes occur in the group of anaerobic microorganisms — the proportion of anaerobes in patients with colorectal cancer was less than 6.9%, and in the comparison group — 20.9%.

At the same time, there is a decrease in the level of microbial diversity, expressed by the Shannon biodiversity Index.

In patients with colorectal cancer, the Shannon index was 4.46; at the same time, the total number of isolated microorganisms was 236, while 280 species of microorganisms were isolated in a smaller control group and the Shannon biodiversity index was 4.8. We especially clearly see a decrease in the representation of bifidobacteria in patients with CRC. The proportion of bifidobacteria in the group of patients with colorectal cancer was 1.5% of the microbiome, while in the comparison group it reached 4.0%. The species composition of bifidobacteria in patients with CRC was represented by three species: *B.bifidum, B.longum, B.breve*, while in patients with benign disease of the rectum and anus, the species composition of bifidobacteria is represented by five species: *B.bifidum, B.longum, B.catenulatum, B.pseudocatenulatum, B.adolescentis*. In the group of patients with colorectal cancer, the proportion of spore gram—positive anaerobes amounted to 3.3%, while in the comparison group, spore anaerobes were a significant component of the microflora with a proportion of almost 9.65%. However, potentially oncogenic *Clostridium septicum* was found only in patients with colorectal cancer. The representation of enterococci was higher in the CRC group; 12.7% of the total microbiome, *E.faecalis* and *E.faecium*were among the most common bacteria, with a share of 4.8% each in the microbiota. Given the data on the potential oncogenicity of *Enterococcus*, further studies of the role of *Enterococcus* in the carcinogenesis of colorectal cancer seem appropriate. *Pseudomonas aeruginosa* was not widely represented in both groups, but given the propensity of *P.aeruginosa* to colonize damaged tissues, the high incidence in cancer patients (2.2% in the CRC group and 1.1% in patients without cancer) does not seem accidental.

In further studies of marker microorganisms associated with colorectal cancer, attention should be paid to the presence of the following microorganisms: *Clostridium septicum, Enterococcus faecalis* and *Enterococcus faecium, Pseudomonas aeruginosa*, as well as to the ratio of microorganisms between groups of bifidobacteria and lactobacilli, spore and non-spore anaerobes and the representation of anaerobic microorganisms in general.

CONCLUSION

In patients with colorectal cancer, the composition and diversity of the intestinal microbiota changes: the biodiversity of the intestinal microflora decreases, the representation and incidence of occurrence of bifidobacteria and spore gram-positive anaerobes decreases, the incidence of occurrence of enterococci increases, potentially oncogenic microorganisms such as *Clostridium septicum* are isolated.

Whether the observed changes are the cause or consequence of colorectal cancer remains to be found out in further studies, which could potentially reveal both the molecular nature of the interactions of indicator microorganisms with macroorganism tissues, and approach the question of the diagnostic significance of the specific fullness of the intestinal microbiome.

AUTHORS CONTRIBUTION

Concept and design of the study: *Marina A. Sukhina, Sergey I. Achkasov, Sergei M. Yudin*

Collection and processing of the material: *Marina A. Sukhina, Matvey H. Stavtsev*

Statistical processing: *Marina A. Sukhina, Matvey H. Stavtsev*

Writing of the text: *Matvey H. Stavtsev*

Editing: *Marina A. Sukhina, Sergey I. Achkasov, Sergei M. Yudin*

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