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The human gastroinstestinal tract microbiota in health – current knowledge summary

Monika Prylińska-Jaśkowiak[1], Marcin Kożuchowski[1]

1. Department of Physiology of Physical Activity and Functional Anatomy, Faculty of Health Sciences, Collegium Medicum im. Ludwik Rydygier in Bydgoszcz, Nicolaus Copernicus University in Toruń

ABSTRACT

The health of the human body is influenced by many external and internal factors, but one of the most important of them is the rich microbiota of the human body. The number of microorganism cells inhabiting the human body exceeds the number of cells that make up the human body. Several different ecosystems coexist in the human body, incl. microbiota of the skin, eye, respiratory tract, urogenital tract and gastrointestinal tract. The composition of microbiota in various parts of the human body, as well as in various parts of a given system or organ, differs significantly from one another. Detailed knowledge of the composition of the human microbiota, as well as its functions for the host organism, is a starting point for further considerations on the meaning of dysbiosis, i.e. changes in the composition of the microbiota, for changing the functioning of the microbiota-host relationship and the development of disease states. It seems that further scientific research on this topic will lead to the recognition of microbiota as a marker and diagnostic tool, and possibly a past therapeutic target for some diseases. This publication attempts to organize and summarize basic information on the human gastrointestinal tract microbiota (GIT microbiota) in health.

KEYWORDS

human microbiota, gut microbiota, gastrointestinal tract microbiota, health, homeostasis, gut health, metagenomic studies, 16S rRNA sequencing

1. INTRODUCTION

Human health is conditioned by many external and internal factors. One of them is the rich world of microorganisms functioning in the human body [1]. The number of microbial cells colonizing various systems of the human body together exceeds the total number of cells that make up the human body. Bacteria constitute a significant part of the composition of human microbiota, but viruses, fungi, parasites, archaea and some unicellular eukaryotes are also present here [1]. Many of these microorganisms are yet to be identified or named.

The term microbiota is used to define all microorganisms inhabiting a given environment [2, 3]. Microorganisms inhabiting the interior of the human body and its surface constitute the human microbiota [2]. The concept of microbiota is also associated with the often confused concept of the microbiome, ie a set of genes that make up the microbiome [2,4]. Metagenomics is a branch of molecular research that studies the complexity of microbiome [2].

For several years, with the development of research methods and the emergence of the possibility of sequencing the genetic material of microorganisms, the interest in microbiota has been growing steadily, which can be seen in the growing number of scientific publications on microbiota appearing recently. This publication

summarizes the current knowledge about the microbiota of the digestive tract of a healthy human body, as well as the existing relationships between the human body and intestinal microorganisms. The current metagenomic methods for studying the human microbiota were also reviewed. Understanding the composition of the microbiota and the interactions between microbes in the gastrointestinal tract in a healthy organism is crucial for further scientific research on dysbiosis and its impact on the human body.

2. MATERIALS AND METHODS

This publication is based on a scientific literature review, which was accomplished by searching the Google Scholar and PubMed scientific databases. The following keywords were searched for: human microbiota, gut microbiota, gastrointestinal tract microbiota, health, homeostasis, gut health, metagenomic studies, 16S rRNA sequencing. Based on the found publications, the current knowledge about human gastrointestinal tract microbiome in health has been summarized and the main techniques now available for metagenomic studies have been reviewed.

3. MICROBIOTA - GENERAL CHARACTERISTICS

The human body consists of 10^{13} cells that communicate with each other and form an inseparable system [1]. Disruption of communication between cells can cause organ dysfunction and lead to disease development. The human organism is inhabited by about 1000 species of microorganisms, and their total number is even up to 10^{14} [1]. This means that there are up to 10 bacterial cells per 1 cell of the human body. It is estimated that the number of genes in the genetic material of microorganisms is approximately 150 times greater than the number of genes in the human genome [1]. Thus, after the nuclear and mitochondrial genome, the set of microbial genes can be referred to as the third human genome. The sum of human and microorganism genes creates a unique mix of metabolic features that are impossible to obtain without the information contained in the genetic material of microorganisms. For example, some of the intestinal microorganisms code for proteins involved in functions important to the host's health, such as enzymes required for the hydrolysis of undigested food compounds and the synthesis of vitamins [2]. Such a system: man-microorganisms was called a superorganism [8]. Due to the development of very complex and efficient signals and communication pathways between cells, the microorganism and the host constantly interact on many levels, including genetic, metabolic and immunological. If this interaction is undisturbed between a healthy host organism and a normal microbiota, a unique, dynamic homeostasis is created, which is a prerequisite for the proper functioning of the human organism.

The colonization of the host organism with microorganisms begins at the time of childbirth and undergoes numerous changes during human life [9, 10, 11, 12]. Despite the variety of external and internal stimuli to which the human microbiota is subjected, namely diet, physical activity, travel, diseases, hormonal cycles and others, as well as the large inter-individual variability of the microbiota, the basic intestinal microbiome was identified, the composition of which is practically constant in healthy people adults and was called the core microbiome [13]. The composition of the core microbiome in individual age groups is presented in Table 1. [2, 19]. Microbiota actively participates in maintaining a healthy state of the organism in adulthood, and its quantitative and qualitative changes may lead to dysbiosis and disease development [2, 13]. To date, a number of functions have been associated with the core microbiome, including polysaccharide digestion, immune system development, protection against infection, vitamin synthesis, fat storage, angiogenesis regulation and behavioral development [14, 15, 16, 17]. Interestingly, the genes encoded by the human core microbiome encode proteins necessary for the survival of the host but absent from the human genome, this discovery led to the definition of the microbiome as "our forgotten organ" [18].

Table 1. Composition of human intestinal microflora throughout life. Under healthy conditions, the diversity and richness of microbes increases with age, reaching the greatest complexity in adulthood. Despite the inter- and intra-individual differences, the gut microbiome is practically stable in healthy adults. In the elderly, as in infants, the gut microbiome is more unstable and has less variation from adults. [2, 19]

Age	Phylum level microbial composition (from the most to the less represented)	Modyfing factors
Infants (up to 2-3 years old)	*Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes*	• Vaginal vs caesarian delivery • Gestational age • Infant hospitalisation • Breast vs formula feeding • Age of solid food introduction • Malnutrition • Antibiotic treatments
Adults	*Firmicutes, Bacteroidetes,*	• Diet

	Actinobacteria, Proteobacteria	• Hormonal cycles • Travel • Therapies • Illness
Elderly	*Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria*	• Lifestyle changes • Nutritional changes • Increased susceptibility to infections and inflammatory diseases • Use of more medications

The quantitative and qualitative composition of microorganisms inhabiting individual parts of the human body is different. The following microbiota are distinguished: intestinal, eye, skin, respiratory, and genitourinary [2]. In the human intestinal microbiota, bacteria of the Firmicutes, Bacteroidetes and Actinobacteria types are most frequently represented, while Proteobacteria, Fusobacteria, Cyanobacteria and Verrucomicrobia are less represented [15].

Firmicutes batteries play an important role in maintaining the proper functioning of the intestinal barrier and the immune balance in the digestive tract. The types of bacteria of the Firmicutes type include Clostridium, Eubacterium, Faecalibacterium, Ruminococcus, Lactobacillus, Enterococcus, Streptococcus.

The second dominant type of intestinal bacteria are Bacterioidetes, which can account for up to 40-50% of the intestinal microbiota. They are fermenting bacteria, i.e. bacteria involved in the metabolic process aimed at producing energy. Bacteroidetes species also play an important role in protein metabolism. The types of bacteria of the Bacterioidetes type are Bacterioides, Prevotella and Parabacteroides.

Actinobacteria are a slightly smaller group of intestinal bacteria. They play a key role in the maintenance of intestinal homeostasis. Microorganisms belonging to this type, especially Bifidobacteria, are widely used as probiotics that modify the composition of the intestinal microbiota and have beneficial effects in many pathological conditions, gastrointestinal diseases and systemic diseases. Examples of bacteria of the Actinobacteria type include, but are not limited to, Bifidobacterium, Atopobium, Collinsella, Adlercreutzia.

Proteobacteria make up about 2% of the intestinal microbiota. Their hypertrophy is most often observed in metabolic diseases, such as type 2 diabetes, obesity, non-alcoholic fatty liver disease (NAFLD), and nonalcoholic steatohepatitis (NASH). Proteobacterial overgrowth also appears to be involved in cardiovascular disease. Proteobacteria overgrowth can therefore be treated as a marker of dysbiosis and microbiota instability and predispose to the development of disease states. Examples of Proteobacteria include Escherichia, Enterobacter, Citrobacter, Bilophila, among others.

A small group of intestinal microorganisms are also bacteria of the Fusobacteria type, which can constitute up to 2-3% of the intestinal microbiota composition. There are scientific reports that the overgrowth of Fusobacterium bacteria with the development of colorectal cancer, irritable bowel syndrome or autoimmune diseases, e.g. inflammatory bowel disease.

Table 2. shows the composition of the microbiota inhabiting individual parts of the human body.

Table 2. The composition of the human microbiota in selected parts of the human body [2].

Human microbal habitats	Microbiota composistion		
	Bacteria phyla	% of bacteria abundance	Number of species
Oral cavity	*Firmicutes* *Bacteroides* *Proteobacteria* *Actinobacteria* *Fusobacteria*	36,7 11,9 17,1 11,9 5,2	>500
Skin	*Actinobacteria* *Firmicutes* *Proteobacteria* *Bacteroides*	52,0 24,4 16,5 6,3	około 300
Airways	*Actinobacteria* *Firmicutes* *Proteobacteria* *Bacteroides*	55,0 15,0 8,0 3,0	>500
Gut	*Firmicutes* *Bacteroides* *Actinobacteria*	38,8 27,8 8,2	>1000

	Proteobacteria	2,1	
Urogenital tract (mailny female)	*Firmicutes* *Bacteroidetes* *Actinobacteria*	83,0 3,0 3,0	około 150

Individual sections of the gastrointestinal tract differ in terms of motility, conditions (eg pH), the type of secreted digestive enzymes, as well as the secretion and absorption of various substances, including bile [1]. There is no microbiota in the light of the initial sections of the upper gastrointestinal tract (mouth, throat, esophagus). This is due to the conditions prevailing in these parts of the digestive tract, as well as the fact that the consumed food stays in these parts of the digestive tract for a short time, therefore microorganisms mainly colonize the oral mucosa and the esophagus. A very rich microbiota is found on the teeth, gums, tongue and tonsils. Due to the low pH of gastric juice, its microbiota is sparse and not very diverse. The duodenum and the initial part of the small intestine are characterized by a low pH, rapid passage of the chyme and the presence of a large amount of bile acids, which is also not conducive to the diversity of the intestinal microbiota. The free passage of food in the distal parts of the gastrointestinal tract enables bacterial colonization of both the mucosa and the intestinal lumen. In the final section of the large intestine, bacteria of the intestinal microbiota reach a concentration of up to 100 billion bacteria in 1 gram of intestinal content, which makes this section of the gastrointestinal tract one of the most densely colonized ecosystems in the world [5]. The intestinal microbiota is responsible for about 1-2 kg of the body weight of an adult [20].

4. THE GASTROINTESTINAL TRACT MICROBIOTA - DETAILED CHARACTERISTICS

4.1. ORAL MICROBIOTA

The oral cavity is the starting point of the digestive tract. It contains soft and hard tissues, and each part of the oral cavity has different pH conditions, oxygen availability or nutrients, therefore the composition of the microbiota is different in each of them [6, 7].

The mucous membranes of the cheeks and palate are areas of low microbiological diversity, while the tongue, thanks to its papillary structure, creates good conditions for the development of both anaerobic and aerobic bacteria, and its microbiota is very diverse. On the surface of the teeth, significant amounts of microorganisms accumulate and form a type of biofilm called plaque. In periodontal pockets and gingival grooves, there are good conditions for the growth of anaerobic bacteria, such as Porhyromonas, Fusobacterium, Prevotella, and Treponema. The presence of microbiota has been demonstrated even on tooth roots [7, 41]. When taking samples for examination from the oral cavity, it is very important to determine the place of collecting the material in order to know exactly which part of the oral cavity the obtained results relate to [7]. Bacteria present in a healthy mouth are of 6 types: Firmicutes, Actinobacteria, Proteobacteria, Fusobacteria, Bacteroidetes and Spirochaetes. The composition of the oral microbiota is summarized in Table 3. [42].

Table 3. Oral microbiota bacteria [42].

Part of oral cavity	Bacteria of oral microbiota
The surface of the teeth	*Streptococcus mutans* *Actinomyces sp.* *Eubacterium sp.* *Peptostreptococcus sp.*
Dental plaque	*Actinomyxes sp.* *Rothia sp.* *Kocuria sp.* *Arsenicicoccus sp.* *Microbacterium sp.* *Propionibacterium sp.* *Mycobacterium sp.* *Dietzia sp.* *Turicella sp.* *Corynebacterium sp.* *Bifidobacterium sp.* *Scardovia sp.* *Parascardovia sp.*
Tounge	*Veillonella atypica* *Porphyronas gingivalis*

	Selenomonas sp. *Actinobacillus actinomycetemcomitans* *Prevotella intermedia* *Capnocytophaga sp.* *Streptococcus faecalis* *Eikenella corrodens*
The gingival fissure	*Fusobacterium sp.* *Prevotella sp.* *Porhyromonas sp.*
Oropharyngeal part	*Stretococcus salivarius* *Stretococcus mutans* *Stretococcus anginosus* *Stretococcus pyogenes* *Stretococcus pneumoniae*
Tonsil	*Stretococcus viridans* *Neisseria sp.* *Haemophilus influenzae* gronkowce

The saliva in the oral cavity is a buffer that maintains a beneficial pH for microbiota, removes unnecessary fermentation products and bacteria not associated with the structures of the oral cavity. Additionally, it contains antimicrobial substances and distributes the substrates needed by microbiota bacteria for growth and development throughout the oral cavity [43]. It is therefore recognized that the analysis of the composition of saliva can help determine the overall composition of the oral cavity. No correlation was found between the amount of bacteria in saliva, plaque or in the mouth. On the other hand, the composition of the tongue microbiota is similar to that of saliva [7]. The high variability of the conditions inside the oral cavity contributes to the large diversity of this microbiota [7]. Bacteria affect the metabolism and functioning of the human immune system. Similarly, the presence of diseases in the host organism affects the microbiota, which justifies changes in the composition of the oral microbiota in people with diabetes, bacteremia, endocarditis, cancer or autoimmune diseases [44].

Bacteria are the most abundant part of the oral microbiota, but apart from them, there are also fungi, viruses, phages and a group of small bacteria (Candidate Phyla Radiation, CPR). CPR microorganisms were first described in 2016. by Hug et al. So far, approx. 35 types of these microorganisms have been identified, but it is estimated that their number may constitute even 15% of the dominant bacteria. CPR is characterized by small size, the presence of specific genes and the absence of genes encoding proteins involved in the tricarboxylic acid cycle, amino acid biosynthesis pathways and ribosomal subunits. Therefore, CPR is not able to independently carry out many metabolic processes, including protein biosynthesis and are unable to live independently. Therefore, it has been suggested that they may be bacterial parasites. There is currently no information on their functioning and the role they play in the microbiota [45].

In the microbiota of the oral cavity of a healthy person, there are up to 75 species of fungi, most often Candida, Cladosporium, Aureobasidium, Aspergillus, Malassezia. One of the fungal representatives in the oral microbiota is Candida albicans, which interacts with bacteria [45]:

• C. albicans and Streptococcus oralis - this interaction increases the production of calpain, a proteolytic enzyme that breaks down cadherin that builds connections between the cells of the oral epithelium. This increases the risk of oral candidiasis.

• C. albicans and Streptococcus mutans - the presence of C. albicans enhances the expression of S. mutans virulence factors. It has been suggested that such an interaction could lead to tooth decay.

• C. albicans and Fusobacterium nucleatum - the effect of this interaction is the inhibition of C. albicans growth and its ability to produce hyphae, in addition, this interaction reduces the production of pro-inflammatory cytokines by macrophages and reduces the risk of such an immune system reaction that occurs in the presence of pathogenic bacteria .

• C. albicans and Staphylococcus aureus - the simultaneous presence of both these microorganisms reduces the susceptibility of staphylococci to antimicrobial agents [46].

The interactions between the human body and the microbiota can be called symbiosis. On the one hand, the host organism provides the microbes with a nutrient-rich environment at a constant temperature. On the other hand, microbiota protects the host's organism against external microorganisms, has an immuno-modulating effect, and reduces the intensity of the processes leading to the inflammatory reaction against commensal microorganisms. In addition, bacteria of the oral microbiota take part in the processes of lactic acid metabolism, reduction of nitrates, and the production of compounds increasing the pH (including ammonia) [43, 46].

The oral microbiota is influenced by many factors, including age, diet, oral hygiene, smoking, drinking alcohol, ethnicity, geographic region. There are also theories that gender may determine the composition of the oral microbiota [43, 45].

4.2. ESOPHAGUS MICROBIOTA

The esophagus, which connects the oral cavity with the stomach, is another part of the gastrointestinal tract and is lined on the inside with a mucosa covered with a single-layer squamous epithelium. There is a valve at the end of the esophagus that allows food to pass freely towards the stomach while preventing it from flowing back into the esophagus.

The contact of the food content is short, saliva is responsible for the hydration of the esophageal mucosa, hence the pH inside the esophagus is similar to the oral cavity (pH = 7) [47]. In individual sections of the esophagus, the conditions may be variable - in the upper section, they are similar to those in the oral cavity, in the lower section, they are similar to the conditions in the stomach, and in the middle, they are mixed [47]. Therefore, it is likely that the composition of microbiota in individual sections of the esophagus may differ [47].

Various research studies that have been conducted to date to determine the composition of the esophageal microbiota have used different methods of sampling for testing (including sampling by aspiration or biopsy), as well as different methods of sample analysis (methods of culture, 16S rRNA sequencing). Regardless of the method of sampling and the selected method of their analysis, it has been proven that Streptococcus is present in large amounts in the esophagus, which allows the conclusion that it is the dominant bacterium in the esophagus of healthy people [47]. In addition, bacteria such as Prevotella and Veillonella were often identified, but in smaller numbers [47]. The bacteria identified in the esophageal microbiota prove that the composition of the esophageal microbiota is strongly determined by the bacteria of the oral cavity, which are resistant to the conditions prevailing in the esophagus. However, it should be noted that several non-oral bacteria have been identified in the esophagus. Therefore, it can be said that the microbiota of the oral cavity affects the microbiota of the esophagus, but they are still two separate ecosystems [47].

There are not many data available to draw conclusions regarding the factors influencing the composition and functions of the esophageal microbiota. The following factors are most often mentioned in the literature: esophageal diseases (gastroesophageal reflux, Barret's esophagus, neoplastic diseases), age, diet, use of proton pump inhibitors (PPI) [47].

4.3. STOMACH MICROBIOTA

The stomach is the next part of the gastrointestinal tract, as it is a heterogeneous organ with various conditions. Due to the structure of the epithelium, the stomach can be divided into the cardia, body and pylorus (antrum). These areas also have different pH values due to the fact that hydrochloric acid and pepsinogen are produced in the stomach body. There are also different antibacterial substances that are secreted in different parts of the stomach. It has been suggested that there are diseases specific to a given area of the stomach and that gastric microbiota may have a different composition in different parts of this organ [48].

The composition of the gastric microbiota is strongly determined by the presence of hydrochloric acid. Acid-resistant bacteria mainly come from the mouth and food. They include: Streptococcus, Neisseria, Lactobacillus. The amount of bacteria in the stomach is estimated at <103 CFU / ml [49]. Compared to the esophagus microbiota in the stomach, ob. Less Streptococcus and more Rothia mucilaginosa, Porphyromonas and Lachnospiraceae are served (this applies to the analysis of biopsy samples). However, in the duodenum, i.e. the first segment of the small intestine, there is a further reduction in the amount of Streptococcus and the maintenance of the trend of increasing the amount of Rothia mucilaginosa, Porphyromonas and Lachnospiraceae bacteria. This means that in order to determine the influence of the stomach environment on its microbiota, individual bacteria should be considered individually [48]. However, the differences in the composition of the esophagus, stomach and duodenum microbiota confirm the thesis that the role of the stomach is, among others, selecting bacteria that enter the lower gastrointestinal tract, which is to reduce the risk of infection and inflammation of further parts of the gastrointestinal tract, as well as ensure proper work and fulfillment of its functions. This means that any interventions that may change the composition of the gastric microbiota (including operations in the stomach, taking medications that reduce gastric acid secretion) may increase the risk of dysbiosis in further parts of the gastrointestinal tract [48].

Research suggests that the composition of the gastric microbiota is strongly influenced by the bacterium Helicobacter pylori. In people without H. pylori infection, 5 types of bacteria were found in the gastric microbiota: Proteobacteria, Firmicutes, Actinobacteria, Bacteroides and Fusobacteria, the most numerous of which were Actinobacteria and Firmicutes [48]. Firmicutes are believed to be the most transcriptionally active bacteria in the gastric microbiota [48]. In people infected with H. pylori, the diversity of gastric microbiota is significantly lower, and 75-99% of all bacteria that make up the microbiota of this segment are Proteobacteria.

The composition of this microbiota is comparable to that of Barret's esophagus [48]. Infection with H. pylori is followed by colonization with bacteria other than H. pylori, which may contribute to the development of gastric diseases. This may be due to the fact that their structural elements and products of metabolism may enhance the immune response induced by H. pylori infection and cause gastritis due to mechanisms independent of the presence of H. pylori bacteria [49].

An important factor influencing the modification of the composition of the gastric microbiota is the action of drugs from the group of proton pump inhibitors (PPI), which are now commonly used among patients and very often overused. PPI have been shown to be bacteriostatic against H. pylori bacteria and increase gastric pH, which in turn leads to bacterial overgrowth in the stomach [50]. In one of the publications it was shown that the use of PPI leads to a 200-fold increase in the number of bacteria in the gastric microbiota, and the scale of bacterial overgrowth is significantly higher in H. pylori infected individuals compared to uninfected individuals [50, 51]. Additionally, in the gastric microbiota of people treated with PPI, e.g. bacteria of the Bifidobacteriaceae family that occur in the mouth. This may mean that the use of these drugs creates conditions enabling the growth of bacteria from the higher parts of the gastrointestinal tract [52]. Such dysbiosis may lead to the patient feeling nausea, bloating, and may also increase the risk of gastrointestinal infections of the etiology of Salmonella, Campylobacter, Shigella, or Clostridioidum difficile [48].

4.4. GUT MICROBIOTA

The intestines are colonized by over 1000 species of bacteria, and the vast majority of them are strictly anaerobic, later relatively anaerobic and aerobic [5]. Among these bacteria, the most numerous are the types: Firmicutes, Bacteroidetes (these 2 types can constitute up to 90% of the intestinal microbiota composition) and Actinobacteria, while the smaller amounts are found in the Proteobacteria, Fusobacteria, Cyanobacteria and Verrucomicrobiota types [2, 5].

Bacteroidetes are Gram-negative, absolutely anaerobic microorganisms. They produce lipopolysaccharides (LPS), proteases, neuraminidases, fibrinolysin, and collagenases, which then participate in the processes of food digestion, signal transmission, environmental control, and inhibition of the growth of other microorganisms in the intestine. Moreover, these bacteria ferment various bicarbonates and cause the formation of short-chain fatty acids (SCFA, including butyric acid) and are involved in the metabolism of bile acids [5].

The Firmicutes type contains several thousand species of gram-positive bacteria of great diversity. They include absolutely anaerobic, aerobic and spore-producing species [5].

Bacteroides, Prevotella and Ruminococcus are the most abundant. It has been observed that in people with poor microbiota, Bacteroides are dominant, and Prevotella and Ruminococcus are negligible. With the increase in the diversity of the microbiota, the share of Bacteroides decreases, and the share of Prevotella bacteria, and in particular Ruminococcus, increases [53].

In people considered healthy, occasional bacteria have also been observed, i.e. bacteria that are not present in every person and their number varies. In addition, it has been noticed that the distribution of bacteria in the stool is not even. This is because some bacteria (such as Enterobacteriaceae) exist at the interface between the mucus layer and the faeces. In turn, Bifidobacterium bacteria are found mainly outside the mucus layer. Some bacteria, such as Eubacterium rectale, Faecalibacterium prausnitzii, Bacteroides, Eubacterium cylindroides, Clostridium histolyticum and Clostridium lituseburense, can be found in any part of the stool - regardless of the presence of mucus [54]. Such uneven distribution of individual bacteria may have an impact on the results obtained in the research and is information that should be taken into account when planning tests, which also include analysis of stool samples. Regardless of the test, it is worth using the same procedures for collecting, storing and preparing test samples. It is worth using the procedures prepared as part of the International Human Microbiome Standards (IHMS) project.

The intestinal microbiota consists not only of bacteria. It is estimated that apart from bacteria, there are about 2.5% of other microorganisms: archaea (2.2%), viruses (0.2%), fungi (1%), eukaryotic organisms (<0.01%) [55] .

The epithelial cells of the intestinal mucosa are the first line of contact with the microbiota. They also protect against the translocation of microorganisms and their metabolites into the body. Enterocytes also constitute the boundary between the external environment and the most active gut-associated lymphoid tissue (GALT), which is a rich cluster of cells that make up the innate and acquired human immunity. GALT cells process and transmit information to the body from contact with intestinal bacteria. This communication is possible thanks to dendritic cells, TLRs receptors and the NOD domain (nucleotide-binding oligomerization domain) [5]. The intestinal microbiota performs the following functions, important from the point of view of the host organism [2, 5]:

- Production of short-chain fatty acids
- Production of vitamins and metabolites
- To break down nutrients that the host organism cannot break down on its own

• Decomposition of ingredients of plant origin
• Neutralization of potential mutagens and carcinogens
• Influence on the development of enterocytes and intestinal epithelium
• Development and functioning of the immune system
• Protection of the host organism against pathogens
• Influencing the proper development of organs (except the intestines), for example by regulating angiogenesis or developing the behavior of the host organism.
• Effects, including adverse host effects, on drug metabolism.

5. NEXT-GENERATION SEQUENCING-BASED TECHNIQUES FOR THE STUDY OF THE HUMAN MICROBIOME

5.1. MICROBIOME STUDIES BACKGROUND

The first microbiological studies to determine the composition of the human microbiota were based on traditional microbial breeding and isolation. These methods are still used today, but their use is limited as growing conditions may favor the growth of one or more species of bacteria over others. Additionally, it is estimated that about 99% of human microbiota's microorganisms belong to non-cultivated microorganisms [21]. Methods such as quantification by PCR or gel electrophoretic separation use specific probes to detect specific bacteria, and therefore they are not currently used to study the composition of the microbiome

In the last several decades, next-generation sequencing (NGS) technology has been developed and developed, which significantly increases the throughput of sequenced fragments of genetic material and allows to reduce costs, which significantly allowed the development of metagenomics. Based on NGS methods, a specific microbiome can be quantitatively and qualitatively very accurately quantified without selection errors and without the constraints associated with culture and isolation methods. These technologies are also used in the Human Microbiome project (HMP), the aim of which is to classify all microbes that make up the different microbiome of the human body and to understand their functions in detail [15, 22, 23]. The NGS-based methods currently in use for metagenomic purposes are briefly characterized below.

5.2. SHOTGUN SEQUENCING

Shotgun sequencing is an analysis that extracts genomic DNA directly from a patient sample. This DNA is then used to prepare the NGS library for high-throughput sequencing. Subsequent data analysis, performed with the use of specific bioinformatics tools, enables the attribution of the obtained readings to the host and its microbial organisms and to the execution of the genome pattern. The great advantage of this method is that the culture and PCR steps are avoided due to the direct DNA analysis. In this way, bacteria can be identified down to the species level and assembled a complete or nearly complete genome. Shotgun sequencing is also used for virus analysis (there is no universal tag for virus analysis). The limitations of this method include: defective mapping of the genome in the case of less abundant and / or closely related species, ambiguous assignment of functions, systematic errors that may be related to the method used for DNA extraction [24].

5.3. 16S rRNA SEQUENCING

Targeting sequencing of specific genes makes it possible to study the microbiome in all its complexity in an easy and cost-effective way. All bacteria contain the 16S rRNA gene which is commonly used for phylogenetic purposes. The 16S rRNA gene has a peculiar structure characterized by hypervariable regions, separated by ultra-conserved regions [25]. By amplifying conserved regions of the 16S rRNA gene, universal primers are created, which can then be used to amplify virtually all bacteria present in the target environment in a single PCR reaction and to uniquely identify them at the end of sequencing [26]. Although 16S rRNA sequencing is easy to perform, fast and relatively inexpensive, DNA extraction can be biased and PCR methodology can confuse the analysis and give inconclusive results [24, 27]. Again, the assignment of the readings depends on the accuracy and timeliness of the reference databases used, and the procedure does not provide data on the bacterial functions.

5.4. METATRANSCRIPTOMICS

Metatranscriptomics is used to analyze the entire genome of the microbiome to obtain a complete picture of gene expression profiles and functional data. Several factors influence the large-scale application of metatranscriptomics, namely technical issues related to RNA extraction and storage procedures, RNA quality and quantity, and the enrichment procedure used to remove rRNA [28]. Bioinformatics tools for metatrancriptomic data analysis are still being developed.

5.5. BIOINFORMATIC TOOLS

The increasing use of the above-mentioned metagenomic strategies results in a need for accurate databases capable of interpreting complex NGS data, as described elsewhere [29, 30].

In recent years, various tools have been developed to analyze 16S rRNA readings. Most of these methods include both taxonomic identification and diversity analysis [31, 32, 33]. The accuracy of the

taxonomic identification is variable and depends not only on the linear sequence used, but also on the portion of the sequenced 16S rRNA, PCR systematic errors and the availability of updated databases. In general, taxonomic assignment is type-to-genus, while species identification is more difficult [34]. 16S rRNA sequencing shows the qualitative and quantitative composition of the microbiome, but does not provide information about the functions of the bacteria.

Shotgun sequencing, which is NGS sequencing of all DNA isolated from the sample, shows the host microbiome and all microorganisms present in the environment (bacteria, archaea, fungi and viruses), excluding errors due to culture or PCR. Specific tools are available to obtain taxonomic identification to the species and strain level [35, 36, 37]. In addition to providing more accurate identification, these tools can predict the functional properties of microorganisms in the human microbiota.

Metatranscriptomics is a relatively new tool that seems to be able to overcome the existing limitations. Several tools for this type of analysis are currently available, although most depend on the availability of genome reference sequences [38, 39, 40].

6. THE SIGNIFICANCE OF THE GASTROINTESTINAL TRACT MICROBIOTA

The evidence presented in this publication on the importance of the correct composition of the intestinal microbiota for the proper functioning of the gastrointestinal tract and the entire host organism allows us to state that the relationship between the microbiota and the human organism is an example of symbiosis. One should look at the microbiota in a broader context, not only focus on a closed set of microorganisms, but also consider the host organism and the network of interactions between the microorganisms and the host. Disruption of the relationship between the microbiota and the host organism is called dysbiosis. Microbiota, in the context of the cited scientific evidence, is a candidate to be seen in the future as a marker or diagnostic tool, as well as a therapeutic target. As knowledge about the functions of the gut microbiome grows, it becomes more and more possible to develop new diagnostic, prognostic and therapeutic strategies based on the diagnosis and modification of changes in the composition of the microbiota.

7. SUMMARY

This publication attempts to summarize the current state of knowledge on the microbiota of various parts of the human gastrointestinal tract in the state of health. In addition, modern techniques for sequencing the composition of the microbiota are also briefly described.

Metagenomics has shed much light on microbiomes, including the human microbiome, and on the complex relationships between microbes and their hosts. Today we know that the gut microbiome plays a role in the functions necessary for the physiology and proper development of our organs, and its composition is related to aging, environmental factors (diet, exercise, etc.) and pathological conditions.

The organization and systematization of recently known physiological phenomena affecting the diversity of microbiota in various organs and systems of the human body is an excellent basis for further research on the impact of disturbances of these physiological phenomena on the pathogenesis of various disease phenomena. Understanding the principles determining the interaction of the human body with microorganisms in the gastrointestinal tract may be of great importance in the prevention and treatment of many diseases.

In many areas of microbiota, knowledge is still incomplete and many questions remain unanswered. It is an incentive to conduct further research and expand current knowledge on this topic. Understanding the function of specific microbes in the human body can open the way to the development of new strategies for the diagnosis, monitoring and treatment of disease. Modulating the gut microflora to restore homeostasis and health is a challenge metagenomic research will face in the years to come.

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