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The issue of the correct use of probiotics in the absence of recommendations

Problemy prawidłowego stosowania probiotyków w związku z brakiem rekomendacji

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Summary: Antibiotics are important for saving both human health and life. Antibiotics destroy all bacteria within their spectrum, because they do not distinguish between good and bad bacteria. Even if an antibiotic therapy lasts only a few days, it may cause diarrhoea and mycosis. Antibiotics destroy most bacterial species in the intestines. These changes may affect one's whole life. Today it is a challenge for medicine to be able to manipulate the microbiome so as to restore normal relations between microorganisms. At present, when antibiotics are abused, probiotics are very often applied. However, as there are no recommendations, a lot of mistakes can be made when using them. Both drugs and dietary supplements can be classified as probiotics. Medicinal probiotics are subject to very strict registration requirements and their use is associated with a specific disease or ailment. Probiotic microorganisms must be classified according to their genus, species and strain. These preparations may contain one or more probiotic strains depending on its application. At present there are no established schemes or rules concerning the dosage of probiotic preparations. This issue arouses numerous controversies. It is assumed that the probiotic should be applied at a dose which proved to have a beneficial effect in tests conducted on humans. Patients usually make decisions on the choice and dosage of preparations themselves. Individualised probiotic therapy is the key to success. There is no universal preparation – a specific probiotic should be used in a particular clinical case.

Keywords: **probiotics, dietary supplements, antibiotics, gut microbiome, recommendations**

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INTRODUCTION

Antibiotics are important for saving both human health and life. In some situations it is necessary to apply a broad-spectrum therapy. Antibiotics destroy all bacteria within their spectrum, because they do not distinguish between good and bad bacteria. It is always necessary to remember this before beginning an antibiotic therapy. Both the bacteria causing an infection and the ones which are part of the human physiological microflora will be eradicated. Even if an antibiotic therapy lasts only a few days, it may cause diarrhoea and mycosis. In some cases it may cause pseudomembranous colitis due to the infection with *Clostridioides difficile*. The Summary of Product Characteristics (SmPC) for antibiotics does not provide precise information about their harmful effect on human microflora. Many antimicrobial drugs were registered long time ago, when the knowledge about the microbiome (formerly known as physiological flora) was very poor. The use of sequencing methods to assess the qualitative composition of the intestinal microbiome turned out to be a breakthrough as it provided an opportunity to see a full spectrum of microorganisms living in the human intestines [5, 42, 54]. Today, it is known that each antibiotic therapy is harmful to the microbiome, especially in the intestines. The intestinal microbiota of a healthy human is an ecosystem composed of about 1,000 microbial species [38, 57, 80]. Thanks to this diversity the homeostasis of the body can be preserved [26].Antibiotics destroy many bacterial species in the intestines. However, those bacteria which are not covered by the spectrum of used antibiotics will survive. In consequence, the qualitative composition of the intestinal microbiota is disordered [17, 84]. These changes may persist in the human body even as long as several months, and the intestinal microbiome will be normalised only after two years [29, 39]. The microbiome will never regain the original species diversity it had before the implementation of the antibiotic therapy. Changes in the intestinal microbiome may indirectly affect the occurrence of many diseases. Studies have shown that there is a relation between the composition of the intestinal microflora and systemic diseases such as cancer, diabetes, obesity, autism, depression, and above all – intestinal diseases. The inheritance of diseases may be related with the inheriting of bacterial microflora. The abuse of antibiotics in early childhood is the biggest problem, because it causes irreversible changes during the formation of the microbiome. These changes may affect one's whole life. Today it is a challenge for medicine to be able to manipulate the microbiome so as to restore normal relations between microorganisms. The intestinal microbiome can be repaired with a proper diet, which has high content of fibre and low content of sugar. It is also recommended to enrich the diet with lactic acid bacteria contained in silages, fermented dairy products or probiotics. Ilja Miecznikow was the first to notice the enormous role of these bacteria and their beneficial effect on the intestines. He noticed the relation between the longevity and health of rural inhabitants in Bulgaria and their consumption of sour milk containing *Lactobacillus* spp. [9, 46, 53, 60]. Preparations containing lactic acid bacteria were named probiotics due to their positive effect on the human body (pro-bios) [31, 40, 50, 64]. Probiotics may not be fully effective in some clinical situations. This concerns the condition of severe dysbiosis, in which there is an imbalance between individual components of the microbiota and the host organism and its microbiota [57, 79]. Dysbiosis mainly concerns patients who received long-term, multiple, and broad-spectrum antibiotic therapy (intensive care patients) [45] and patients with severe intestinal diseases as well as patients after chemotherapy. In some clinical situations in severe dysbiosis, the only way to restore the microbiome is to transplant the intestinal microbiota from a healthy donor. Thus, intestinal bacteria are provided together with their environment [7, 65]. At present, when antibiotics are abused, probiotics are very often applied. However, as there are no recommendations, a lot of mistakes can be made when using them.

PROBIOTICS – DRUGS OR DIETARY SUPPLEMENTS?

Both drugs and dietary supplements can be classified as probiotics [30]. If they are applied at a right dose, they may have a beneficial effect on the human body [28, 48]. The properties of probiotics depend on a particular bacterial strain, which has specific biochemical and microbiological parameters. Probiotics mainly contain lactic acid bacteria (LAB) of the *Lactobacillus* and *Bifidobacterium* genera as well as *Saccharomyces cerevisiae* var. *boulardiiyeasts* [35]. They are referred to as 'generally recognised as safe' (GRAS) due to the low level of adverse effects they cause [41]. Due to the lack of specific recommendations concerning the administration of probiotics, they are very often used mistakenly. Currently the market offers a wide range of preparations, but only some of them are medicines, while most of them are dietary supplements. Different methods of production, quality control procedures and registration principles are applied before they are available on the market [41]. The information on the species and count of microorganisms contained in a dose of a dietary supplement can be found on its label. However, it is usually not true [24, 25, 63]. There is no specific information how dietary supplements should be used. It is obligatory to provide this information only for medicines. The interest in the market of probiotics and the number of available preparations are increasing [51]. Most of them are dietary supplements. By definition, these are not medicinal products but foodstuffs supplementing a normal diet [76]. Medicinal probiotics are subject to very strict registration requirements and their use is associated with a specific disease or ailment [34].

CHARACTERISTICS OF PROBIOTIC STRAINS

Good probiotics should be easy to produce. The microbial strains they contain should resist and survive fixation processes and they should be stable during storage. It is also important that the organoleptic characteristics of a finished product should not be worse. The criteria defining the properties of probiotic strains and describing the conditions of the production, storage and distribution of probiotic preparations were described in the FAO/WHO

recommendations [23]. Probiotic microorganisms must be classified according to their genus, species and strain [69] and the information on the package of a probiotic preparation must be provided only in this form. It is recommended that probiotic strains should come from the population in which they will be used due to age-, region- and living environment-dependent differences in the intestinal microbiome [2, 28]. Some probiotics are composed of one strain, others a few. All these preparations have a different purpose and are used in specific ailments. One-component preparations are used e.g. in the reduction of irritable bowel symptoms, in the treatment of constipation [21] or diarrhea, thus multi-component preparations in the treatment of obesity or mood disorders. It is important that the multi-component preparations are composed so that the strains composed of them do not act antagonistically [28]. It is required to exclude pathogenic and carcinogenic effects of strains, check the degree of adherence to epithelial cells and determine their metabolic activity. The stability of microorganisms should also be controlled after the technological process and at various stages of the shelf life of the preparation. The resistance of probiotic bacteria to antibiotics should also be tested. It is a key element to prove the genetic stability of microbial strains in tests, i.e. to determine the presence of resistance genes on mobile genetic elements (plasmids, transposons) [13, 23]. The final stage is to test probiotics in double-blind and randomised analyses with a placebo group [67].

MECHANISM OF ACTION OF PROBIOTIC STRAINS

There are various mechanisms of interaction between probiotic bacteria and microorganisms belonging to the natural microflora or pathogens. Probiotics can interact directly in the intestines, or they can also act indirectly, by modulating the immune response and epithelial mechanisms [18, 37, 69]. One of the mechanisms of action can be observed directly in the intestinal lumen, where probiotic strains limit the possibility of adhesion of pathogens to enterocytes by competing for nutrients and receptor sites on intestinal epithelial cells [30, 48, 49]. The adhesion strength is an individual characteristic of a specific probiotic strain. As chyme passes in the intestinal lumen, weakly attached microbial cells are removed, making new space for stronger microorganisms. The probiotic strains which adhere to enterocytes more strongly are more difficult to remove from the surface of the intestine. In consequence, this reduces the formation of new surfaces, where pathogenic bacteria may appear [28]. Thanks to the production of bacteriostatic substances, probiotic bacteria inhibit the growth of pathogenic microorganisms in the human body. In addition, the production of such metabolites as lactic acid and short-chain fatty acids lowers the pH in the intestines and creates an adverse environment for the development of pathogens [67]. Probiotics are also capable of stimulating cellular and humoral immune responses [48]. The immunological interactions of probiotic microorganisms inhibit the production of pro-inflammatory cytokines, e.g. IL-8, TNF-alpha, and stimulate phagocytosis [69]. *L. acidophilus*, *L. caseiand Bifidobacterium* spp. affect the proliferation and activity of B and T lymphocytes and interferon gamma [48]. Probiotic strains are capable of inducing anti-inflammatory factors, which stimulate the maturation of dendritic cells [82].

The microorganisms contained in probiotics can also use the epithelial mechanism, for example, through the enzymatic modification of receptors of bacterial toxins, the binding and activation of toll-like receptors and stabilisation of the intestinal barrier [68].

THE ROLE OF SELECTED PROBIOTIC STRAINS IN THE HUMAN BODY

The intestines are part of the immune system. The intestinal microflora affects human health both directly and indirectly. In some situations probiotics modulate the immune response in immunodeficient patients. Even if the bacteria contained in the probiotic do not survive, they may also have immunological significance and act as an oral vaccine. Vitamin D is the main precursor of the formation of antibacterial peptides which control the intestinal bacterial microflora. Its deficiency may disorder the synthesis of these peptides and thus, disorder the bacterial microflora. In consequence, the susceptibility to infection will increase. Vitamin D deficiency may result in lower immunity of the human body. The intestinal microflora is a protective barrier against pathogens and infections. When good bacteria colonise the intestines, they do not allow pathogenic microorganisms to replace them. They provide protection by competing with pathogenic bacteria for nutrients[21, 44, 47, 83]. Weaker bacteria do not survive because they have no nutrients. The microorganisms contained in probiotics produce short chain fatty acids, which have an anti-inflammatory effect. *Lactobacillus*, *Bifidobacterium* and *S. cerevisiae* var. boulardii are the most common strains used in probiotics. *Faecali bacterium prausnitzii*, *Akkermansia muciniphila*, *Butyricococcus pullizaecorum*, *Eubacterium hallii* and *Roseburia* spp. also have the characteristics of good bacteria. Currently there are no probiotics containing these species. These are probiotics of the future, which have not been thoroughly tested yet [10, 60]. Only probiotic preparations tested on humans have proven effects for particular indications. As each probiotic is responsible for something different, it is important to use specific probiotic preparations containing appropriate strains in specific clinical situations (Table 1). The effect on the human body depends on a specific strain rather than species [39, 68]. *Lactobacillus acidophilus* is one of the most commonly used bacterial strains of the *Lactobacillus* genus. It is used to treat ulcerative colitis. *In vitro* studies have shown that it inhibits the growth of *Helicobacter pylori* (L. acidophilus CRL639). When *L. acidophilus* is combined with *L. bulgaricus* or *B. longum*, it has beneficial health effect on patients suffering from post-antibiotic diarrhoea [31, 68]. The combination of *L. acidophilus* and *B. bifidum* is the best option in terms of the time of recolonisation of the intestine with aerobic and anaerobic bacteria [23]. Studies have shown that *L. rhamnosus* GG was highly efficacious in the treatment of children in Europe, who suffered from acute infec-

Table 1. Evidence-based adult indications for probiotics, prebiotics, and synbiotics in gastroenterology

Clinical Condition	Probiotic strain	Recommended dose	References
acute diarrhea in adults	– *Lactobacillus paracasei* B 21060 – *Lactobacillus rhamnosus* GG – *Saccharomyces cerevisiae* var. *boulardii* CNCM I-745	10^9 CFU twice daily 10^9 CFU twice daily 5x 10^9 CFU/capsule	[16, 66]
acute gastroenteritis in children	– *L. rhamnosus* GG – *S. boulardii* CNCM I745 – *Lactobacillus reuteri* DSM 17938	≥ 10^{10} CFU/day 250–750 mg/day 10^8 to 4 x 10^8 CFU	[16, 22, 66]
antibiotic-associated diarrhea	– *L. rhamnosus* GG – *S. boulardii* CNCM I-745 – *L. reuteri* DSM 17938 – Yogurt with *Lactobacillus casei* DN114, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* – *Lactobacillus acidophilus* and *L. bulgaricus* or *Bifidobacterium longum*	10^{10} CFU/capsule twice daily 5 x 10^9 CFU/capsule 1 x 10^8 CFU twice daily ≥ 10^{10} CFU daily	[28, 66, 68, 69, 71]
Clostridium *difficile*-associated diarrhea	– *L. acidophilus* CL1285 and *L. casei* LBC80R – *S. boulardii*	5 x 10^{10} CFU daily and 4–10 x 10^{10} CFU daily 5 x 10^9 CFU/capsule	[58, 66, 68]
Helicobacter pylori infection	– *S. boulardii* – *L. rhamnosus* GG – *L. casei* DN-114 001 (in children)	5 x 10^9 CFU/capsule 6 x 10^9 twice daily	[66, 68, 70]
Irritable Bowel Syndrome	– *Bifidobacterium infantis* 35624 – *Bifidobacterium animalis* DN-173 010 – *L. plantarum* DSM 9843 (299v) – VSL#3*	1 x 10^8 CFU/capsule 10^{10} CFU, twice daily 10 billion CFU once daily	[16, 32, 66, 68, 81]
ulcerative colitis (mild to moderate CU for the induction of remission)	– *Escherichia coli* Nissle 1917 – VSL#3	5 x 10^{10} viable bacteria twice daily	[6, 47, 66, 72]
infantile colic	– *L. reuteri* DSM 17938 – *L. rhamnosus* GG	10^8 CFU, once daily 10^{10}–10^{11} CFU, twice daily	[66, 68, 73]
hepatic encephalopathy	– VSL#3	10^8 CFU three times daily	[16, 66]
traveler`s diarrhea	– *S. boulardii* CNCM I-745	250–1000 mg, once daily	[12]

* VSL#3 – *Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus helveticus*

tious diarrhoea. The strain significantly shortened the duration of diarrhoea and increased the patients' immune response to rotaviruses [53, 80]. *S. cerevisiae* var. *boulardii* is another microorganism, whose positive therapeutic effect has been documented. It produces protease, which inactivates *Clostridium difficile* and *Vibrio cholerae* toxins. It is also used to treat patients with post-antibiotic diarrhoea [31, 33, 82]. The Summary of Product Characteristics containing the *S. cerevisiae* var.*boulardii* strain indicates that it is efficacious against diarrhoea caused by enterohemorrhagic strains and that it prevents travellers' diarrhoea [82].

DOSAGE AND FORM OF PROBIOTICS, DURATION OF THERAPY

At present there are no established schemes or rules concerning the dosage of probiotic preparations. This issue arouses numerous controversies. It is assumed that the probiotic should be applied at a dose which proved to have a beneficial effect in tests conducted on humans [15]. Patients usually make decisions on the choice and dosage of preparations themselves. They often assume that if the antibiotic has caused so many side effects, it is best to choose a preparation containing several bacterial strains and to take a larger dose of it. However, more does not always mean better. This applies both to the number of strains in the preparation and the content of living cells. If a preparation contains several strains, it is necessary to exclude their antagonistic effect. There is a lot of very divergent information on the dosage of probiotics in reference publications. Some authors recommend a minimum therapeutic dose of 106 CFU (colony forming units), whereas others say that it should be even as high as 10^{11}CFU per day [43, 48, 69, 77]. For example, the administration of *L. rhamnosus* GG at a dose of 10^{10}-10^{11} CFU shortened the duration of acute diarrhoea caused by a rotavirus infection [77]. According to the regulations of the Canadian Natural Health Products, the minimum therapeutic dose of *L. rhamnosus* GG should be about 6 x 10^9

CFU per day. A minimum dose of 10^{10} CFU per day is recommended for *S. cerevisiae* var. *Boulardii* to reduce the risk of post-antibiotic diarrhoea [52]. Numerous studies have also shown that when a probiotic preparation is applied at a smaller dose than 109 CFU per day, it treats antibiotic diarrhoea and C. *difficile* infections less effectively than a dose exceeding 10^{10} CFU per day [56].

The form of the probiotic (capsules, tablets, powder, drops) considerably affects its efficacy. Capsules may seem to provide the best protection from an acidic environment. The substance should not be removed from the capsule, but manufacturers do not always provide this information in the leaflet. Also, there is no precise data on the count of microorganisms that can be encapsulated. Powder probiotic preparations usually contain more bacteria, probably because at least some of them may survive if others die in a low-pH environment.

The moment when a probiotic therapy should be included and its duration are also controversial issues. As most preparations are registered as dietary supplements rather than medicines, it is not obligatory to provide specific recommendations concerning their use and dosage. There is no information when and which preparation should be included, and whether it should be done during or after an antibiotic therapy. When analysing the problem, it is worth considering what happens to probiotic bacteria during an antibiotic therapy. The probiotic contains the same species that can be found in the intestinal microbiome. Therefore, it is likely that probiotic strains will also be destroyed during an antibiotic therapy if the given species in the probiotic is in the spectrum of the antibiotic used. Therefore, it is worth choosing a probiotic which contains strains not found in the spectrum of the antibiotic that is planned to be included in the therapy. There is still no specific full answer to the question of how long probiotic strains colonize the intestine, and how long they should be used [53]. It is important to administer the probiotic when finished antibiotic therapy, to replenish the lost microbiota.

Can these bacteria survive in an antibiotic environment?

Another problem related with the dosage of probiotics is the time interval between the administration of the antibiotic and probiotic. According to the recommendations of the Canadian Natural Health Product, the probiotic should be taken 2-3 hours before or after the administration of the antibiotic [52]. It is likely that this time is given to limit the contact of probiotic microorganisms with the antibiotic in the gastrointestinal tract. According to other publications, the probiotic should be taken two hours before or after the antibiotic [61]. Probably this theory is related to the fact that there should be no direct contact between the probiotic and the orally administered antibiotic. However, this theory is wrong because orally administered antibiotics have systemic rather than local effect, which is limited to the intestine. This effect lasts long after the end of the antibiotic therapy.

The type of food consumed while using probiotics also affects the absorption and viability of probiotic microorganisms. An in vitro study with an artificial gastrointestinal tract conducted two experiments. The first study showed that taking the Protec Flor probiotic preparation (*Lactobacillus helveticus* R0052, *L. rhamnosus* R0011, *Bifidobacterium longum* R0175, *S. cerevisiae* var. *boulardii*) together with 1% milk fat or with oatmeal and 1% milk fat provides the higher survival of lactobacilli and B. longum strains than apple juice or spring water. This may suggest that proteins and fats present in milk and oatmeal have a positive effect on the viability of some probiotic microorganisms. There was no relation between the meal type and the survival of *S. cerevisiae* var. *boulardii*. The second study assessed the effect on the viability of probiotic organisms consumed before, during or after a meal. It showed that the probiotic bacteria (L. helveticus R0052, *L. rhamnosus* R0011, and B. longum R0175) were most likely to survive if they were consumed 30 minutes before or during a meal of oatmeal with 1% milk fat. When a dose of the ProtecFlor probiotic preparation was taken 30 minutes after the meal, a significant amount of the lactobacilli and B. longum strains were reduce compared to dosing before and during a meal. The probiotic yeast, *S. cerevisiae* var. *boulardii* showed no significant differences in levels attained before, during or after the meal [78].

The viability of the bacteria contained in the probiotic is also important. In most cases the probiotic microorganisms can be found in stool only during the supplementation with the preparation. Probiotic strains do not have to colonize the intestine permanently [3, 57]. After supplementation with the preparation probiotic microorganisms can no longer be isolated from stool. It depends on the survival of microorganisms contained in the probiotic during passage through the gastrointestinal tract [61].

In almost 90% of the tested probiotic preparations the count of living bacteria decreased as the time after their production passed, although they were still before the expiry date [61, 66]. Another question arises: How long should probiotics be taken? Even if they are taken for a long time, they cannot change and restore the microflora after the destruction caused by the antibiotic.

Can each microorganism contained in the probiotic survive the antibiotic therapy?

The bacterial microflora of the intestines cannot be restored during the antibiotic therapy. The restoration process can only take place after the therapy has been completed. Many doctors recommend the use of probiotics during the antibiotic therapy. However, only some of them recommend taking probiotics also after the antibiotic therapy. This period is the most important. Some doctors rationally recommend that patients should take care of their microbiome after the antibiotic therapy. However, it may be too late, especially if a patient underwent a long therapy with several broad-spectrum antibiotics. Enterol is the only preparation that is effective during an antibiotic

therapy, because it contains the fungi which are by nature resistant to antibiotics. The task of S. cerevisiae var. *boulardii* is to adhere to the intestinal wall and 'hold' places for bacteria from the probiotic, which will be taken later, i.e. after the antibiotic therapy. This will protect the intestines from colonisation by pathogenic bacteria. The task of the fungi is only to adhere to the intestinal wall, without restoration of the population of lost microbes. The most important thing is to use bacterial probiotics long after the end of the antibiotic therapy. There is no point in simultaneous administration of the antibiotic and bacterial probiotic.

Information on the best time of the day to take the probiotic is also very controversial

Probiotics should be treated individually because each of them has a different task. They should be applied at the right time, depending on the antibiotic administered and the underlying disease.

SAFE USE OF PROBIOTICS AND POTENTIAL DANGERS

Probiotics are generally considered to be safe. However, sometimes they may have side effects. They cannot be administered to all patients in all clinical situations. It is necessary to remember that probiotic microorganisms may cause infections. There are groups of patients who should be careful when using probiotics, i.e. people with immunodeficiency, pregnant women, premature babies, critically ill patients and patients with venous catheters [13, 30, 35, 67, 69].

Bloodstream infections are some of the most common infections caused by probiotic strains [13, 35, 41]. Only until 1999 89 cases of bacteraemia with the *L. rhamnosus* aetiology were described, 11 of which were with a GRAS strain [41]. It is estimated that 0.1-0.2% of all cases of bacteraemia are caused by Lactobacillus spp., whereas there is a minimal percentage of cases of bacteraemia with the *Bifidobacterium* spp. aetiology [35]. Vascular bed infections caused by Lactobacillus spp. strains are regarded as opportunistic infections [35, 67]. The mortality rate among patients from risk groups who developed bacteraemia induced by LAB strains is up to 70% within one year after the first symptoms of infection [35]. There are also documented reports on fungaemia caused by the probiotic strains of *S. Cerevisiae* var. *boulardii*. The infections are usually caused by vascular catheter contaminations [13, 67].

Probiotics are not recommended to patients with acute pancreatitis as probiotic prophylaxis resulted in a double increase in their mortality. Death may have been caused by an increased demand for oxygen in the intestinal lumen, which was hypoxic due to the disease [4, 30, 35, 69].

Manufacturers issue warnings about dangers only for preparations registered as medicines. There is no such obligation for dietary supplements. A warning might discourage customers from buying a supplement and have a counter-marketing effect. Unfortunately, leaflets attached to various supplements provide information on the count and species of microorganisms contained in these products that is divergent from tests [24, 25, 27, 41, 59, 63]

As probiotics contain strains that are resistant to most antibiotics, they contain resistance genes. Is it safe to use such preparations? Resistance genes may be passed on to microorganisms belonging to the intestinal microbiome. Each probiotic should be tested for the presence of mobile genetic elements, which determine resistance to antibiotics. Plasmid location of resistance determinants should be excluded [1, 8, 11, 62, 67]. In the future tests on genes located on mobile plasmids will enable the withdrawal of dangerous preparations from the market. Thus, it will possible to minimise the risk of spreading resistance from probiotic strains to the microorganisms which are part of the intestinal microflora [41].

CONCLUSION

There are 1000 species of microorganisms in the intestinal microbiome of a healthy adult human. Many of them die during an antibiotic therapy. Can a probiotic that contains several strains of microorganisms supplement the lost bacteria? Over 98% of all species belong to one of the four dominant phyla: *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%), *Actinobacteria* (3%). The share of other microorganisms amounts to 2% and it is variable. The probiotic can supplement only the percentage content in individual phyla, but it cannot restore the species diversity. After a debilitating antibiotic therapy in severe clinical conditions the situation can be changed only by transplantation of the intestinal microbiota with various intestinal bacteria and their entire environment.

Probiotics are recommended both by doctors and nutritionists. Some of them speak of targeted probiotics and recommend that patients should have a stool culture test after an antibiotic therapy to check whether the situation is normal or a probiotic should be administered. If it is necessary to apply a probiotic, they choose one based on the results of a stool culture test. This test is based on classical microbiology, and only 20% of microorganisms can be cultured. Most of the other microbes colonising the intestines are absolute anaerobes, which cannot be cultured. Therefore, these tests are not objective and they are misleading both for the doctor and patient. Routine microbiome tests by means of sequencing is a method of the future. At present these tests are conducted mainly for scientific purposes. They are not widely available and they are very expensive. But only these methods will enable assessment of the real situation in the intestinal microbiome.

Many probiotics are dietary supplements [20]. Only products registered as medicines need to undergo numerous tests. The administration of a probiotic containing several strains will not restore the microbiome, which includes several hundred species. A microbiologist should be consulted in difficult clinical situations related with the intestinal microbiome. Taking probiotics makes sense if patients

take a preparation containing antibiotic-resistant fungi during an antibiotic therapy, and they start taking a bacterial probiotic only after the end of the antibiotic therapy. It is best to use preparations containing several different strains to ensure the biodiversity of the microflora while it is being restored. It is also important that the patients who received antibiotics in the past and who often have infections should take probiotics to increase their immunity (immunomodulation).

Healthy people should not take probiotics because they may make unnecessary and irreversible changes to their normal microbiome. The administration of probiotic preparations to healthy children to strengthen their immunity is also controversial. If a child is not ill, has no infection and has not taken an antibiotic before, the probiotic will disturb the child's natural microbiome.

Untested preparations should not be used because they are only dietary supplements rather than medicines. If the exact species composition of the preparation is unknown, it is also risky to take it, because it may be contaminated with other pathogenic species, which are not listed in the composition of the probiotic that is provided in the information leaflet [61].

Patients taking probiotics should have an adequate diet of vegetables with high content of fibre. They should not consume products containing sugar, because it promotes the growth of pathogenic bacteria and Escherichia coli. Even the most expensive probiotics will not cause microbial strains to survive and proliferate if the patient's diet is inadequate. The host's diet has enormous influence on the intestinal microbiome. Changes in the microflora might be related with the consumption of specific foods. The influence of various diets on the inhibition of intestinal diseases has been tested. If the diet is appropriate, the intestinal microflora is stable and does not change much. However, long-term inappropriate nutrition may cause irreversible changes in the intestinal microflora.

When doctors prescribe antibiotics, they often recommend patients to eat yoghurts regularly during the therapy. However, it is not the right solution in every situation. For example, calcium, which is contained in dairy products, may inactivate tetracyclines in the drug.

Probiotics only supplement a therapy but they cannot replace the treatment of an underlying disease. Individualised probiotic therapy is the key to success. There is no universal preparation – a specific probiotic should be used in a particular clinical case [33]. Recommendations for the use of probiotics are not consistent [61]. When it is decided to administer probiotic to the patient, the current guidelines and recommendations of major organizations and companies should be considered, depending on the indication: European Society for Paediatric Gastroenterology, Hepatology and Nutrition, European Society of Clinical Microbiology and Infectious Diseases, European Crohn's and Colitis Organisation, European Society for Clinical Nutritionand Metabolism, World Allergy Organization and European Society for Paediatric Infectious Diseases.

Due to the widespread consumption of probiotics around the world it is necessary to monitor the safety of these preparations [14, 35]. The right probiotics can be applied if the state of intestinal bacteria is known. This can be assessed only in genetic sequencing tests.

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