THE ROLE OF *MEF* AND *ERMB* DRUG RESISTANCE GENETIC MARKERS IN THE SELECTION OF FECAL MICROBIOTA DONORS

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Fecal microbiota transplantation (FMT) is prescribed to treat various gastrointestinal pathologies. One of the most important and significant stages of FMT is selection of the donor. In recent years, special attention has been paid to checking the biomaterial for genes marking resistance to various groups of antibiotics. This study aimed to analyze the occurrence of mef and *ermB* drug resistance genetic markers in population of various age groups, including breastfed infants, and to determine microbiological composition of the flora of distal part of the intestine of potentially healthy volunteering FMT donors. A total of 52 biological samples (46 stool samples and 6 breast milk samples) were analyzed by real-time polymerase chain reaction. The macrolides resistance gene (mef) was detected in 97.8% of stool samples (different age groups), the gene marking resistance to macrolides, lincosamides, streptogramin (*ermB*) — in 93.5%. In the isolated "mother-child" group, the mef gene was found in all samples of breast milk and feces. The *ermB* gene in this group was found in 3 out of 6 breast milk samples and 4 out of 6 infant stool samples. Since the *mef* and *ermB* genetic determinants were identified not only among in adults but also in infants, it was suggested that transplant material (feces) containing these genes can be used for FMT. The analysis of microbiological composition of stool samples from 23 healthy volunteers (potential FMT donors) revealed that it rarely (in 8.7% of cases only) corresponds to what is considered to be a normal microbiota of the intestine's distal part.

Keywords: FMT, antibiotic resistance gene, *mef*, *ermB*, fecal microbiota donor, PCR

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РОЛЬ ГЕНЕТИЧЕСКИХ МАРКЕРОВ ЛЕКАРСТВЕННОЙ УСТОЙЧИВОСТИ *MEF* И *ERMB* ПРИ ПОДБОРЕ ДОНОРОВ ФЕКАЛЬНОЙ МИКРОБИОТЫ

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Трансплантацию фекальной микробиоты (ТФМ) назначают в качестве терапии для лечения различных патологий желудочно-кишечного тракта. Подбор донора является одним из наиболее важных и значимых этапов для ТФМ. Особое внимание в последнее время уделяют проблеме наличия генов устойчивости к разным группам антибиотиков в биоматериале. Целью исследования было провести анализ встречаемости генетических маркеров лекарственной устойчивости *mef* и *ermB* среди разных возрастных групп населения, включая младенцев на грудном вскармливании, а также определить микробиологический состав дистальной части кишечника у потенциально здоровых добровольцев доноров ТФМ. Всего было проанализировано 52 образца биологического материала (46 образцов кала и шесть — грудного молока) методом полимеразной цепной реакции в режиме реального времени. Ген устойчивости к макролидам (*mef*) среди разных возрастных групп был выявлен в 97,8% образцах кала, ген устойчивости к макролидам, линкозамидам, стрептограмину (*ermB*) — в 93,5%. В отдельно выделенной группе «мать – дитя» ген *mef* обнаружен во всех образцах грудного молока и кала. Ген *ermB* в этой группе подтвержден в трех из шести образцов грудного молока и четырех из шести образцов кала младенцев. В результате детекции генетических детерминат *mef* и *ermB* не только среди взрослого населения, но и у младенцев, было выдвинуто предположение, что использование трансплантата (кал), содержащего данные гены допустимо для ТФМ. Анализ микробиологического состава кала 23 здоровых добровольцев — потенциальных доноров ТФМ, показал очень низкий процент соответствия (8,7%) нормам микробиоты дистальной части кишечника.

Ключевые слова: ТФМ, гены резистентности, *mef*, *ermB*, доноры фекальной микробиоты, ПЦР

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Presently, fecal microbiota transplantation (FMT) is becoming an increasingly popular approach to correcting microbiota dysbiosis caused by various pathologies. FMT is introduction of a suspension of feces from a healthy donor into the intestinal tract of a recipient; the procedure aims to treat or prevent a number of diseases by changing the recipient's microbiome [1–3]. Numerous randomized trials have shown the high efficiency of the method for treatment of refractory and recurrent forms of intestinal infections caused by *Clostridium difficile* [1, 4, 5]. Since 2013, FMT is a procedure officially approved by the US Food & Drug Administration (FDA) [6].

Donor screening and selection is one of the most important stages of FMT, since patient's safety depends thereon. Being the most time-consuming and resource-intensive stage part of the process, this stage consists of two steps: questionnaire filling (to collect the donor's medical history) and laboratory examination (to prevent possible transmission of pathogens to the recipient). Laboratory examination of the donor includes: basic hematological and biochemical blood tests, tests for hepatitis B and C, human immunodeficiency virus, syphilis, general urinalysis, coprogram, occult blood test, tests for protozoa and helminth eggs, fecal culture, PCR test for pathogenic intestinal flora and drug resistance genetic markers (presence of resistance genes) [7, 8].

The microorganisms carrying genes of resistance to antibiotics of various groups have been spreading actively recently [9]. One particular reason behind the spread is the generally more frequent use of broad-spectrum antimicrobial drugs. The pressure exerted by antibiotics on the microbial population translates into mutation or transformation of the genetic material, which leads to the development of new mechanisms enabling adaptation to the changing conditions [10]. In addition, bacteria can share the resistance genes with one another with the help of mobile elements (plasmids, transposons and integrons). The development of various sectors of industry drives outspread of the antibiotic resistance genes not only among microorganisms that directly interact with humans but also in the wild. Resistant bacteria have been found in deep underground trenches, in wastewater, surface and ground water, sediments and soil [11]. They are also present in organisms living in places relatively untouched by human civilization, such as Antarctica and the Arctic [12]. Antibiotic resistance genes are growing more and more common every year. One of the studies (lasted from 2011 through 2015) investigated the mechanisms of resistance to macrolides exhibited by *Streptococcus pyogenes* strains isolated from human microflora, as well as the frequency of occurrence of such resistance. The results of this study show that the pathogen has grown 6.8–12.6% more resistant to macrolides (among the total number of strains studied) [13].

In the context of screening microbiota transplantation donors, genes that determine the resistance of *Streptococcus* and *Staphylococcus* bacteria to macrolides, lincosamides, and streptogramins are of particular interest. The list of such genes includes mef (macrolide efflux), which encodes efflux proteins, and *ermB* (erytromimycin ribosome methylation), which encodes 23S rRNA methylase that modifies antibacterial drug (ABD) target molecules [14, 15]. Another study has registered prevalence of *mefA* and *ermB* genetic determinants, with both resistance genes found in all intestinal microbiota samples collected from patients with chronic obstructive pulmonary disease that abstained from taking antibiotics for at least three months; the said genes were detected by both metagenomic method and real-time PCR [16, 17]. The resistance genes have also been found in large numbers in stool samples and even meconium collected from newborns [18].

These genes are common in potential FMT donors, which complicates selection of those who can donate healthy microbiota.

The purpose of this study was to analyze the occurrence of *mef* and *ermB* drug resistance genetic markers in population belonging to different age groups, and to determine microbiological composition of the flora of distal part of the intestine of potential FMT donors.

METHODS

Collection of samples

The initial group of donors was selected with the help of questionnaires and an algorithm developed for the purpose [7]. The inclusion criteria were: any gender; age from 18 to 55 years; no history of ABD treatment for a year or more. Stool samples were collected in this group to determine the composition of microflora, detect pathogenic microorganisms and screen for the resistance genes. The exclusion criteria were: a history of diseases associated with intestinal microbiota composition upset, chronic diseases and/or AND courses. In addition, the results of laboratory tests (general blood count, biochemical tests) allowed excluding volunteers that had any deviations from the normal parameters. Ultimately, based on the data obtained, 23 healthy volunteers were selected from the initial group of 53 as potential fecal microbiota donors. Other volunteers were excluded from further analysis due to abnormal blood test results.

Additionally, the study included a "mother-child" group. After receiving informed consent from the participants selected through random sampling, we collected biological material from the following categories of study participants: a) breastfed infants under 1 year of age who have never taken ABD; b) formula-fed infants under 1 year of age who have never taken ABD; c) children aged 1–3 who have never taken ABD; d) children 3 to 7 years old who have not taken ABD for more than a year. The "mother-child" group consisted of six couples (nursing mother/breastfed child); stool (mother and infant) and breast milk samples were taken from all of them.

A total of 52 samples of biological material were included in the study, including 46 stool samples and 6 breast milk samples. Among the stool samples (n = 46), 29 were from adults, 9 were from infants (2–11 months; 6 breastfed and 3 formula-fed), 4 from children 1 to 3 years old, 4 more from children aged 3–7 years.

Feces and breast milk were collected into individual sterile plastic containers. The samples (stool sample — 10–20 g, breast milk sample — 10–20 ml) were not frozen but sent to the laboratory in a thermal container immediately or stored for no more than 8 hours at 4 °C before submission to the laboratory.

Stool culture test

The stool culture testing followed provisions of the applicable regulations [19]. One gram of the native stool sample was homogenized in 9 ml of saline (10^{-1}) and left at room temperature for 10–15 minutes. The resulting suspension was plated on the solid nutrient media to detect pathogenic enterobacteria (SS- and XLD-agar (HiMedia Laboratories; India)) and selenite broth (Biocompas-S; Russia) to isolate pathogenic *E. coli*. From the initial dilution (10^{-1}), we made a series of subsequent dilutions up to 10^{-8}.

The prepared dilutions were plated on nutrient media to cultivate various groups of microorganisms (1 ml of suspension

Table 1. Stool culture method

Dilution	Group microorganisms tested for	Nutrient medium	Amount of suspension plated, ml	Cultivation time, h	Results evaluation pattern
2	3	4	5	6	7
10^{-8}	*Bifidobacterium*	Blaurock	1	72	Gram staining, microscopy
10^{-7}	*Lactobacillus*	MRS-2			
10^{-5}	*Clostridium*	Iron sulfite agar	1	72	Emission of hydrogen sulfide (black color of the medium), gas formation
	Hemolytic species of bacteria	Blood agar	0.1	20–22	Type of hemolysis: α, β, γ
10^{-3}	*Enterobacteria*	Endo			Bacteria counting by lactose fermentation: + pink colonies and nutrient medium; — transparent colonies, no change in colony color
	Pathogenic fungi	Sabouraud		48	Chromogenic agar, determined by the color of the colonies
	Staphylococcus	Yolk-salt agar			Growth of colonies, yellow coloration of the medium — assimilation of mannitol, lecithinase activity
	Enterococcus	Milk inhibitor medium			Counting bacteria by esculin hydrolysis — colonies acquire dark brown or black color
10^{-1}	Pathogenic enterobacteria	XLD agar		20–22	*Shigella, Salmonella spp.* transparent, for salmonella — colorless with a black center, *E. coli* — opaque yellow with a yellow zone around, *Proteus mirabilis* — yellow with a black center
		SS agar			*Shigella, Salmonella spp.* transparent, for salmonella —- colorless with a black center, medium color — yellow; *E. coli* — raspberry, medium color — pink, *Proteus mirabilis* — brown with a dark center

plated on semi-liquid nutrient media, 0.1 ml on solid media, rubbed with a sterile spatula over the surface of the medium):

– 10^{-8} dilution — bifidobacteria on a semi-liquid nutrient medium Blaurock (FBUN GNC PMB Obolensk, Russia);

– 10^{-7} dilution — lactobacilli on semi-liquid nutrient medium MRS-2 (FBUN GNC PMB Obolensk, Russia) and bifidobacteria on Blaurock medium (FBUN GNC PMB Obolensk, Russia);

– 10^{-5} dilution — clostridia, 1 ml deep plated on iron sulfite agar (Biokompas-S; Russia); gram-negative enterobacteria — Endo medium (HiMedia Laboratories; India); hemolytic bacterial species - blood agar (HiMedia Laboratories; India).

– 10^{-3} dilution — plated on the Endo medium (Biokompas-S; Russia); plated on the Sabouraud medium (Biotechnovatsiya; Russia) to detect pathogenic fungi; plated yolk-salt agar (HiMedia Laboratories; India) to detect on staphylococci; and on milk-inhibitory medium ("Biokompas-S"; Russia) to detect enterococci (Table 1).

Culture test results were evaluated:

– after 20–22 hours for Endo, blood agar, SS- and XLD-agar;

– after 48 hours for Sabouraud, yolk-salt agar and milk inhibitor;

– after 72 hours for Blaurock, MRS-2, iron sulfite agar.

Where Endo media were used, we counted the number and the percentage of lactose-negative (colorless) colonies in relation to the total number of grown colonies. Colonies with mild enzymatic properties (weak decomposition of lactose — pink colonies) were counted against the total number of *E. coli* colonies. According to the available recommendations, the generic composition of lactose-negative enterobacteria not belonging to the intestinal pathogenic bacteria family can be left undetailed; it is sufficient to count the total amount of lactose-negative colonies on the Endo medium.

Extraction of DNA from biological material

We used the DNA-SORBENT kit (Litech; Russia) and followed the manufacturer's protocol to extract DNA from biological material (feces, breast milk). In case of breast milk, we extracted DNA from saliva, cerebrospinal fluid, and synovial fluid. Prior to submission for PCR testing, the isolated DNA was stored at –20 °C.

Analysis of genetic markers of drug resistance

We used the RESISTOM.mef monoplex kit to detect macrolides resistance mef-genes in Streptococcus spp., RESISTOM. *ermB* to detect macrolides, lincosamides and streptomycin B resistance erm-genes in *Streptococcus spp*. and *Staphylococcus spp*.; the format was FLUOROPOL-RV (Litech; Russia), method — real-time PCR in a CFX96 amplifier (Bio-Rad Laboratories; USA). A total of 52 DNA samples isolated from biological material (feces and milk) were analyzed. To control the quality of DNA extraction and to prevent the occurrence

Table 2. Bacteria culture test, stool samples from potentially healthy volunteers

№	Microflora	Normal, cfu/g	Number of volunteers, people		*p*
			Within normal limits	Deviation from the normal	
1	*Bifidobacterium spp*	10^8 AND ABOVE	13	10	0,678
2	*Lactobacillus spp*	10^6–10^7	12	11	0,835
3	Total number of enterobacteria	10^7–10^8	12	11	0,835
4	*Escherichia spp*	10^7–10^8	12	11	0,835
5	*Enterococcus spp*	10^6–10^7	4	19	0,004
6	Conditionally pathogenic enterobacteria: *Enterobacter cloacae*, *Enterobacter gergoviae*, *Citrobacter freundii*, *Citrobacter amalonaticus*. lactose-negative *Escherichia coli*	< 10^4	8	15	0,211
7	Intestinal pathogenic microorganism	Should be absent	23	0	< 0,001
8	*Staphylococcus*	≤ 10^4 / Should be absent	9	14	0,404
	S. aureus	≤ 10^4 / Should be absent	21	2	< 0,001
9	*Candida spp*	≤ 10^4	23	0	< 0,001
10	Non-fermenting gram-negative bacteria incl. *Pseudomonas aeroginosa*, *Pseudomonas putida*	≤ 10^4	19	4	0,004
11	Sulfite-reducing anaerobes of the *Clostridium* genus	≤ 10^6	12	11	0.835

of false negative results in the set we relied on internal exogenous control (detected via the HEX channel) introduced into the studied samples at the DNA extraction stage. The PCR pattern was as follows: 80 °C — 2 minutes, 95 °C — 1 minute 30 seconds, then 40 cycles: 95 °C — 15 seconds, 60 °C — 30 seconds, 72 °C — 40 seconds.

Statistical analysis

Statistical processing was performed with the help of Statistica 10.0 software (StatSoft Inc.; USA). The significance of differences in the registered prevalence of ABD resistance genes (in groups) was assessed by Pearson's χ^2 test with Yates's correction. The significance of differences in the registered prevalence among bacteriological parameters was assessed by McNemar's test. A difference was considered statistically significant at $p < 0.05$.

RESULTS

According to the questionnaire data and the results of clinical studies (complete blood count and blood chemistry tests), 23 volunteers out of 53 were included for further research as potential donors of fecal microbiota.

To determine the probable minimum age when a person becomes a carrier of the studied genes, as well as to identify the ways of transmission of macrolide resistance genes (mef and ermB), we formed a mother-child group (6 people). Twelve stool samples and six breast milk samples were included in the study from this group.

Bacteriological analysis

In the course of the work, we preliminarily assessed qualitative and quantitative bacteriological composition of the stool samples from potentially healthy volunteers ($n = 23$). The results of the bacteria culture test corresponded to the values given in regulatory documents [20]; they are presented in Table 2.

Composition of the obligate microflora species, such as *Bifidobacterium*, *Lactobacillus*, *Escherichia*, is normal in 52.2% of the participants, and that of *Enterococcus* — in 8.7%. In 34.8% of the participants the content of conditionally pathogenic bacteria of facultative microflora was normal or below normal; the species of that microflora are *Enterobacter cloacae*, *Staphilococcus aureus*, *Citrobacter freundii*, *Citrobacter amalonaticus*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Enterobacter gergoviae*, lactose-negative *Escherichia coli*.

Thus, the bacterial test has shown that only 8.7% of the volunteers met the standards of all indicators, which is only 4 people out of 23 examined.

Molecular genetic analysis

All 23 volunteering donors tested positive for genetic markers of drug resistance to macrolides, both genes (*mef* and *ermB*).

Therefore, in order to identify the age when these genes appear, find out if there is a relationship between their presence and intake of ABD and establish the transmission pathways we formed the "mother-child" sample and samples comprised of children of various ages. Both stool and breast milk samples were tested for *mef* and *ermB* resistance genes. Table 3 presents data on the presence of resistance genes in feces in all volunteers ($n = 46$; different age groups).

The stool sample analysis has revealed the mef macrolide resistance gene in 45 (97.8%) out of 46 samples (Tables 3 and 4). Only one sample, that collected from a 7-year old child, did not contain this gene. The presence of the *ermB* gene encoding resistance to macrolides, lincosamides, streptogramin was confirmed in 43 (93.5%) out of 46 cases (Tables 3 and 4). The *ermB* gene was not detected in stool samples from three breastfed and formula-fed infants.

Table 3. Prevalence of *mef* and *ermB* resistance genes in feces, different age groups

Genes	"Mother-child" group		Formula-fed children	Children 1-3 years old	Children 3-7 years old	Adults
	baby's feces	mother's feces				
mef	6/6	6/6	3/3	4/4	3/4	23/23
ermB	4/6	6/6	2/3	4/4	4/4	23/23

Note: * — ratio of the number of samples in which the gene was detected to the total number of samples in the respective group.

Table 4. Prevalence of *mefA* and *ermB* resistance genes in the "mother-child" group

Genes	"Mother-child"		
	Breast milk	Baby's feces	Mother's feces
mef	6/6	6/6	6/6
ermB	3/6	4/6	6/6

Note: * — ratio of the number of samples in which the gene was detected to the total number of samples in the respective group.

Forty-four (84.6%) out of 52 samples had both mef and ermB resistance genes simultaneously.

Statistical analysis of prevalence of *mef* and *ermB* genes did not reveal significant differences between the age groups ($p = 0.258$).

All breast milk samples collected from mothers breastfeeding their infants in the "mother-child" group (Table 4) contained the genetic determinants (mef gene). As for the ermB gene, it was found in 3 out of 6 breast milk samples and 4 out of 6 infant stool samples. A noteworthy case is that of a baby who was fed with breast milk that did not contain the *ermB* gene and whose feces proved to have it, nevertheless. There were no significant differences established between the subgroups in studying the joint prevalence of the mef and *ermB* resistance genes in the "nursing mother — breastfed child" group ($p = 0.423$).

DISCUSSION

Fecal microbiota transplantation donor screening is one of the most difficult and important stages of the therapy, since it is necessary to exclude or minimize the undesirable consequences of transplantation for the recipient. Special attention should be paid to resistant bacteria that, as a result of transplantation, can trigger emergence of resistant clones in the recipient's microflora [21]. In this connection, it should be remembered that a respective negative result of the bacterial culture test does not guarantee there are no resistance genes in the sample at all.

Bacterial culture allows identifying specifics of the fecal microflora, which reflects the microbial composition of the distal intestines, and to determine the qualitative and quantitative content of the microbiota. Changes in the ratio of certain types of microorganisms allows diagnosing dysbiotic disorders of the digestive tract.

In a healthy person, the colon typically hosts three groups of bacteria: 1) obligate microflora (more than 90%), which includes non-pathogenic types of bacteria (bifidobacteria, bacteroids, lactobacilli, *E. coli*, enterococci) and enables the main physiological functions of the body (digestion, absorption); 2) facultative microflora (less than 10%), which includes conditionally pathogenic microorganisms (clostridia, *Staphylococcus*, proteus, campylobacter, yeast-like fungi, etc.) and is involved in protective and digestive functions; 3) transient (random) microflora (not more than 1%), represented by Pseudomonas aeruginosa, fungi of the genus Candida, pathogenic enterobacteria, etc. Bacterial culture test has shown that in our sample, the healthy donors (4 out of 23) had a sufficient amount of bifidobacteria, lactobacilli, Escherichia and enterococci, and the number of opportunistic bacteria they hosted was within the normal range, which corresponds to regulatory documents [22]. The majority of the examined participants (17 out of 23) had dysbiotic disorders: quantitatively insufficient obligate microflora or overabundant facultative and transient microflora, which can result from an unbalanced diet and frequent stress.

Obviously, a bacteriological study of the feces cannot give a complete picture of the diversity of intestinal microflora, since microbiota may contain uncultivated bacteria and some bacteria present in small quantities only cannot be cultivated on artificial nutrient media. Currently, the two methods that yield a more accurate data on composition of the intestinal microbiota are whole genome sequencing and metagenomic analysis. However, a standard approach to medical examination of a person includes bacterial culture and biochemical markers tests only, the results thereof being the basis for conclusion about the state of health of the patient. The so-called omics analytical methods are not currently routine in the practice of a clinician. Undoubtedly, to understand the exact mechanism of action of FMT and, possibly, to isolate the most active components thereof, it is necessary to conduct multidisciplinary complex studies. At the same time, more and more medical institutions introduce FMT into their practice and can only rely on routine tests to screen donors. Thus, it is important to understand the significance of testing the potential FMT biomaterial for resistance genes.

In this work, we focused on the analysis of prevalence of mef and *ermB* genes, which determine resistance to macrolides, lincosamides, and streptomycin B. According to the data obtained, both of them were detected in stool samples from all 23 healthy volunteers.

In the additionally formed "mother-child" group, we identified the mef gene in all breast milk and stool samples of the following categories of participants who had never taken ABD: breastfed infants under 1 year of age; formula-fed babies; children 1–3 years old. This gene was also found in all adults and three children, aged 3 to 7 years, who had not taken ABD for more than a year. The results of our study are consistent with the report on another research effort that saw the mef gene in the feces of 100% of newborns participating therein [22].

The *ermB* gene was not detected in stool samples from three infants. Two of them were breastfed and their mothers' milk did not contain this gene, one baby was formula-fed. It should also be noted that feces from one baby has the *ermB* gene, although breast milk of this baby's mother does not have this gene. Two formula-fed children under one year of age have the *ermB* gene in their feces. The issue of the acquisition of genetic determinants in the feces of newborns in the prenatal period, during childbirth or thereafter is not fully understood. Previous studies have confirmed that resistance genes in the feces of newborns were acquired after birth (for example, through breast milk and/or air or drinking water, since resistance genes were not found in the amniotic fluid and meconium of newborns [23–25]).

CONCLUSIONS

The analysis of microbiological composition of feces from 23 potentially healthy volunteers showed that only 8.7% of them had the appropriate microbiota of the intestine's distal part and could be considered as potential FMT donors. This may indicate that population as a whole has a problem with composition of the microbiota. Additionally, the study analyzed the mef and ermB drug resistance genetic markers occurrence in different age groups, including infants. We have revealed a high prevalence of the macrolide resistance gene, mef, which

reached up to 97.8%, while that for ermB, gene of resistance to macrolides, lincosamides, streptogramin, was 93.5%. In the "mother-child" group, all stool and breast milk samples had the mef gene. Thus, in view of the fact that the mef and *ermB* genes can be found not only in the adult population but also in infants, we suggested that the use of a transplant (feces) containing these genes is acceptable for FMT. These data data can help clinicians introducing FMT into their practice in the context of independent search for donors and preparation of biomaterial.

References

1. Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, et al. European consensus conference on faecal microbiota transplantation in clinical practice. Gut. 2017; 66 (4): 569–80. DOI: 10.1136/gutjnl-2016-313017.
2. Baunwall SMD, Lee MM, Eriksen MK, Mullish BH, Marchesi JR, Dahlerup JF, et al. Faecal microbiota transplantation for recurrent Clostridioides difficile infection: An updated systematic review and meta-analysis. EClinicalMedicine. 2020; 23: 29–30. PMID: 33437951; PMCID: PMC7788438. DOI: 10.1016/j.eclinm.2020.100642.
3. Wang Y, Zhang S, Borody T, Zhang F. Encyclopedia of fecal microbiota transplantation: a review of effectiveness in the treatment of 85 diseases. Chinese Medical Journal. 2022; 10.1097/CM9.0000000000002339 DOI: 10.1097/CM9.0000000000002339.
4. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol. 2013; 108 (4): 478–98.
5. Debast SB, Bauer MP, Kuijper EJ, European Society of Clinical M, Infectious D: European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. Clin Microbiol Infect. 2014; 20 (Suppl 2): 1–26.
6. Enforcement Policy Regarding Investigational New Drug Requirements for Use of Fecal Microbiota for Transplantation to Treat Clostridium difficile Infection Not Responsive to Standard Therapies. 2013; www.fdagov.
7. Shherbakov PL, Belova ND, Generozov EhV, Zhgun ES, Ivanova OI, Il'ina EN, i dr. Primenenie fekal'noj transplantacii v lechenii zabolevanij pishhevaritel'nogo trakta (pervyj klinicheskij opyt). Doktor.Ru. 2019; 3 (158): 40–46. DOI: 10.31550/1727-2378-2019-158-3-40-46. Russian.
8. Yakupova AA, Abdulxakov SR, Safin AG, Alieva IM, Oslopova YuV, Abdulxakov RA. Transplantaciya fekal'noj mikrobioty: kriterii vybora donora, podgotovki i xraneniya biomateriala (obzor sovremennyx rekomendacij). Terapevticheskij arxiv. 2021; 93 (2): 215–21. DOI: 10.26442/00403660.2021.02.200615. Russian.
9. Ehjdelshtejn MV. Ehkstremal'no- i panrezistentnye klony bakterial'nyx vozbuditelej v klinike. Tezisy dokladov ezhegodnoj Vserossijskoj nauchno-prakticheskoj konferencii s mezhdunarodnym uchastiem «Kontrol' i profilaktika infekcij, svyazannyx s okazaniem medicinskoj pomoshhi (ISMP-2015)», 25 noyabrya 2015, Moskva Zhurnal MediAl'. 2015, 3 (17). Russian.
10. Turkutyukov VB. Molekulyarno-geneticheskij monitoring rezistentnosti mikroorganizmov k antibiotikam. Tixookeanskij medicinskij zhurnal. 2011; 2 (44): 28–31. Russian.
11. Zhuangab M, Achmonab Y, Cao Y, Liang X, Chen L, Wange H, et. al. Distribution of antibiotic resistance genes in the environment. Environmental Pollution. 2021; 285. Article 117402. DOI. org/10.1016/j.envpol.2021.117402.
12. Roberts MC. Environmental macrolide–lincosamide–streptogramin and tetracycline resistant bacteria Front. Microbiol. 2011. Available from: https://doi.org/10.3389/fmicb.2011.00040.
13. Katosova LK, Lazareva AV, Xoxlova TA., Ponomarenko OA, Alyabeva NM. Rasprostranenie i mexanizmy ustojchivosti k makrolidam Streptococcus pyogenes, vydelennyx u detej. Antibiotiki i ximioterapiya. 2016; 61 (3–4): 23–29. Russian.
14. Farrell DJ, Morrissey I, Bakker S, Morris L, Buckridge S, Felmingham D. Molecular epidemiology of multiresistant Streptococcus pneumoniae with both erm(B)- and mef(A)-mediated macrolide resistance. J Clin Microbiol. 2004; 42 (2): 764–8. DOI: 10.1128/JCM.42.2.764–768.2004. PMID: 14766850; PMCID: PMC344484.
15. Reinert RR, Ringelstein A, van der Linden M, Cil MY, Al-Lahham A, Schmitz FJ. Molecular epidemiology of macrolide-resistant Streptococcus pneumoniae isolates in Europe. J Clin Microbiol. 2005; 43 (3): 1294–300. DOI: 10.1128/JCM.43.3.1294-1300.2005. PMID: 15750098; PMCID: PMC1081259.
16. Ikryannikova LN, Senina ME, Lisicina ES, Ogorodova LM, Fedosenko SV, Karnaushkina MA, i dr. Vidovaya identifikaciya i analiz geneticheskix markerov lekarstvennoĭ ustoichivosti streptokokkov s pomoshh'yu kolichestvennoĭ mul'tipleksnoĭ polimeraznoĭ cepnoĭ reakcii u pacientov s xronicheskoĭ obstruktivnoĭ bolezn'yu legkix. Pul'monologiya. 2014; (6): 40–48. Dostupno po ssylke: https://doi.org/10.18093/0869-0189-2014-0-6-40-48. Russian.
17. Fedosenko SV, Ogorodova LM, Ilina EN, Senina ME, Lisicina ES, Karnaushkina MA, i dr. Geneticheskie determinanty ustojchivosti orofaringeal'nyx streptokokkov k antibakterial'nym sredstvam u pacientov s xronicheskoj obstruktivnoj bolezn'yu legkix i bol'nyx bronxial'noj astmoj. Terapevticheskij arxiv. 2015; 87 (8): 51–57. DOI: 10.17116/terarkh201587851-57. Russian.
18. Gosalbes MJ, Vallès Y, Jiménez-Hernández N, Balle C, Riva P, Miravet-Verde S, et al. High frequencies of antibiotic resistance genes in infants' meconium and early fecal samples. J Dev Orig Health Dis. 2016; 7: 35–44. DOI: 10.1017/S2040174415001506.
19. Ehpshtejn-Litvak RV, Vilshanskaya FL. Bakteriologicheskaya diagnostika disbakterioza kishechnika. Metodicheskie rekomendacii. M., 1977; 20 s. Russian.
20. Protokol vedeniya bol'nyx. Disbakterioz kishechnika: OST 91500.11.0004-2003. Prikaz Minzdrava Rossii # 231 ot 09.06.03. (Otraslevoj standart). Russian.
21. U.S. Food and Drug Administration. Information pertaining to additional safety protections regarding use of fecal microbiota for transplantation — screening and testing of stool donors for multi-drug resistant organisms. 2019. Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/information-pertaining-additional-safety-protections-regarding-use-fecal-microbiota-transplantation.
22. Klassert TE, Zubiria-Barrera C, Kankel S, Stock M, Neubert R, Lorenzo-Diaz F, et.al. Early Bacterial Colonization and Antibiotic Resistance Gene Acquisition in Newborns. Front Cell Infect Microbiol. 2020; 10: 332. DOI: 10.3389/fcimb.2020.00332. PMID: 32754449; PMCID: PMC7366792.
23. Zhang K, Jin M, Yang D, Shen Z, Liu W, Yin J, et al. Antibiotic resistance genes in gut of breast-fed neonates born by caesarean section originate from breast milk and hospital ward air. BMC Microbiol 22. 2022; 36. DOI: org/10.1186/s12866-022-02447-8.
24. Huang MS, Cheng CC, Tseng SY, Lin YL, Lo HM, Chen PW. Most commensally bacterial strains in human milk of healthy mothers display multiple antibiotic resistance. Microbiology Open. 2019; 8 (1): e00618 doi.org/10.1002/mbo3.618.
25. Carvalho MJ, Sands K, Thomson, Portal KE, Mathias J, Milton R, et al. Antibiotic resistance genes in the gut microbiota of mothers and linked neonates with or without sepsis from low- and middle-income countries. Nat Microbiol. 2022; 7: 1337–47. Available from: doi.org/10.1038/s41564-022-01184-y.

Литература

1. Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, et al. European consensus conference on faecal microbiota transplantation in clinical practice. Gut. 2017; 66 (4): 569–80. DOI: 10.1136/gutjnl-2016-313017.
2. Baunwall SMD, Lee MM, Eriksen MK, Mullish BH, Marchesi JR, Dahlerup JF, et al. Faecal microbiota transplantation for recurrent Clostridioides difficile infection: An updated systematic review and meta-analysis. EClinicalMedicine. 2020; 23: 29–30. PMID: 33437951; PMCID: PMC7788438. DOI: 10.1016/j.eclinm.2020.100642.
3. Wang Y, Zhang S, Borody T, Zhang F. Encyclopedia of fecal microbiota transplantation: a review of effectiveness in the treatment of 85 diseases. Chinese Medical Journal. 2022; 10.1097/CM9.0000000000002339 DOI: 10.1097/CM9.0000000000002339.
4. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol. 2013; 108 (4): 478–98.
5. Debast SB, Bauer MP, Kuijper EJ, European Society of Clinical M, Infectious D: European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. Clin Microbiol Infect. 2014; 20 (Suppl 2): 1–26.
6. Enforcement Policy Regarding Investigational New Drug Requirements for Use of Fecal Microbiota for Transplantation to Treat Clostridium difficile Infection Not Responsive to Standard Therapies. 2013; www.fdagov.
7. Щербаков П. Л., Белова Н. Д., Генерозов Э. В., Жгун Е. С., Иванова О. И., Ильина Е. Н. и др. Применение фекальной трансплантации в лечении заболеваний пищеварительного тракта (первый клинический опыт). Доктор.Ру. 2019; 3 (158): 40–46. DOI: 10.31550/1727-2378-2019-158-3-40-46.
8. Якупова А. А., Абдулхаков С. Р., Сафин А. Г., Алиева И. М., Ослопова Ю. В., Абдулхаков Р. А. Трансплантация фекальной микробиоты: критерии выбора донора, подготовки и хранения биоматериала (обзор современных рекомендаций). Терапевтический архив. 2021; 93 (2): 215–21. DOI: 10.26442/00403660.2021.02.200615.
9. Эйдельштейн М. В. Экстремально- и панрезистентные клоны бактериальных возбудителей в клинике. Тезисы докладов ежегодной Всероссийской научно-практической конференции с международным участием «Контроль и профилактика инфекций, связанных с оказанием медицинской помощи (ИСМП-2015)», 25 ноября 2015, Москва Журнал МедиАль. 2015; 3 (17).
10. Туркутюков В. Б. Молекулярно-генетический мониторинг резистентности микроорганизмов к антибиотикам. Тихоокеанский медицинский журнал. 2011; 2 (44): 28–31.
11. Zhuangab M, Achmonab Y, Cao Y, Liang X, Chen L, Wange H, et. al. Distribution of antibiotic resistance genes in the environment. Environmental Pollution. 2021; 285. Article 117402. DOI. org/10.1016/j.envpol.2021.117402.
12. Roberts MC. Environmental macrolide–lincosamide–streptogramin and tetracycline resistant bacteria Front. Microbiol. 2011. Available from: https://doi.org/10.3389/fmicb.2011.00040.
13. Катосова Л. К., Лазарева А. В., Хохлова Т. А., Пономаренко О. А., Алябьева Н. М. Распространение и механизмы устойчивости к макролидам Streptococcus pyogenes, выделенных у детей. Антибиотики и химиотерапия. 2016; 61 (3–4): 23–29.
14. Farrell DJ, Morrissey I, Bakker S, Morris L, Buckridge S, Felmingham D. Molecular epidemiology of multiresistant Streptococcus pneumoniae with both erm(B)- and mef(A)-mediated macrolide resistance. J Clin Microbiol. 2004; 42 (2): 764–8. DOI: 10.1128/JCM.42.2.764–768.2004. PMID: 14766850; PMCID: PMC344484.
15. Reinert RR, Ringelstein A, van der Linden M, Cil MY, Al-Lahham A, Schmitz FJ. Molecular epidemiology of macrolide-resistant Streptococcus pneumoniae isolates in Europe. J Clin Microbiol. 2005; 43 (3): 1294–300. DOI: 10.1128/JCM.43.3.1294-1300.2005. PMID: 15750098; PMCID: PMC1081259.
16. Икрянникова Л. Н., Сенина М. Е., Лисицина Е. С., Огородова Л. М., Федосенко С. В., Карнаушкина М. А. и др. Видовая идентификация и анализ генетических маркеров лекарственной устойчивости стрептококков с помощью количественной мультиплексной полимеразной цепной реакции у пациентов с хронической обструктивной болезнью легких. Пульмонология. 2014; (6): 40–48. Доступно по ссылке: https://doi.org/10.18093/0869-0189-2014-0-6-40-48.
17. Федосенко С. В., Огородова Л. М., Ильина Е. Н., Сенина М. Е., Лисицина Е. С., Карнаушкина М. А. и др. Генетические детерминанты устойчивости орофарингеальных стрептококков к антибактериальным средствам у пациентов с хронической обструктивной болезнью легких и больных бронхиальной астмой. Терапевтический архив. 2015; 87 (8): 51–57. DOI: 10.17116/terarkh201587851-57.
18. Gosalbes MJ, Vallès Y, Jiménez-Hernández N, Balle C, Riva P, Miravet-Verde S, et al. High frequencies of antibiotic resistance genes in infants' meconium and early fecal samples. J Dev Orig Health Dis. 2016; 7: 35–44. DOI: 10.1017/S2040174415001506.
19. Эпштейн-Литвак Р. В., Вильшанская Ф. Л. Бактериологическая диагностика дисбактериоза кишечника. Методические рекомендации. М., 1977; 20 с.
20. Протокол ведения больных. Дисбактериоз кишечника: ОСТ 91500.11.0004-2003. Приказ Минздрава России № 231 от 09.06.03. (Отраслевой стандарт).
21. U.S. Food and Drug Administration. Information pertaining to additional safety protections regarding use of fecal microbiota for transplantation — screening and testing of stool donors for multi-drug resistant organisms. 2019. Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/information-pertaining-additional-safety-protections-regarding-use-fecal-microbiota-transplantation.
22. Klassert TE, Zubiria-Barrera C, Kankel S, Stock M, Neubert R, Lorenzo-Diaz F, et.al. Early Bacterial Colonization and Antibiotic Resistance Gene Acquisition in Newborns. Front Cell Infect Microbiol. 2020; 10: 332. DOI: 10.3389/fcimb.2020.00332. PMID: 32754449; PMCID: PMC7366792.
23. Zhang K, Jin M, Yang D, Shen Z, Liu W, Yin J, et al. Antibiotic resistance genes in gut of breast-fed neonates born by caesarean section originate from breast milk and hospital ward air. BMC Microbiol 22. 2022; 36. DOI: org/10.1186/s12866-022-02447-8.
24. Huang MS, Cheng CC, Tseng SY, Lin YL, Lo HM, Chen PW. Most commensally bacterial strains in human milk of healthy mothers display multiple antibiotic resistance. Microbiology Open. 2019; 8 (1): e00618 doi.org/10.1002/mbo3.618.
25. Carvalho MJ, Sands K, Thomson, Portal KE, Mathias J, Milton R, et al. Antibiotic resistance genes in the gut microbiota of mothers and linked neonates with or without sepsis from low- and middle-income countries. Nat Microbiol. 2022; 7: 1337–47. Available from: doi.org/10.1038/s41564-022-01184-y.