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Indicators of the Intestinal Microflora of Laboratory Animals (Rats, Rabbits and Guinea Pigs) With Dysbacteriosis in the Dynamics of Age

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ARTICLE INFO	ABSTRACT
Published Online: 31 May 2022 Corresponding Author: **Abdukarimov D.I.**	Dysbiotic disturbances in the qualitative and quantitative composition of the microflora of the body and its functions, caused by various reasons, still remain one of the leading and most difficult problems to solve in modern medicine. In experimental modeling of dysbiosis in laboratory animals, increased adhesion of opportunistic microorganisms to intestinal epithelial cells was revealed. The mechanisms of development of dysbacteriosis and its consequences are very complex and multifaceted, so the need to find tools that can cover as many links of this pathogenesis as possible is still relevant for medicine.

KEYWORDS: Dysbiosis, oxacillin, bifidobacteria, lactobacilli, bacteroids, peptostreptococci, Mushrooms of the genus Candida, Escherichia, Staphylococci, pseudomonas.

INTRODUCTION

An important role in solving many problems in biology and medicine is played by experimental studies on animals, which make it possible to model and study the dynamics of the pathological process under various metabolic conditions. The literature describes various approaches to modeling dysbacteriosis in animals. Known models of dysbacteriosis caused by starvation, total blood loss, radiation exposure, administration of antibiotics [1]. It has been proven that from 300 to 500 species of various microorganisms live in the large intestine, 90% of them are mutualist microbes (bifido-, lactobacilli, bacteroids, peptostreptococci), only a small group is represented by conditionally pathogenic microorganisms. The mass of the normal intestinal microflora of an adult is more than 2.5 kg, and its number is 1014 [2,3]. The objective of this study was to study the quantitative and qualitative composition of the intestinal microbiocenosis of rats with experimental dysbacteriosis induced by prolonged administration of oxacillin, as well as the effect of various groups of probiotic preparations on the microflora of animals in the experiment. Bacteria of normal microflora represent an evolutionarily created "biological barrier" of a macroorganism, allowing it to exist in the biosphere [4,5,6]. The symbiotic intestinal microflora, primarily bifidobacteria and lactobacilli, through antigenic stimulation enhances the formation of complement, lysozyme, immunoglobulins, induces the synthesis of interferon, stimulates the lymphoid apparatus of the intestine, having a direct effect on the differentiation of T- and B-lymphocytes in Peyer's patches, inducing functional activity phagocytes [7,8]. However, under the influence of harmful exogenous and endogenous factors, this microbiocenosis, powerful in composition and quantity, is disturbed, towards an increase in unfavorable microflora and contributes to the development of dysbacteriosis [9,10,11]. As a result, microbial associations cannot perform the protective and physiological functions that they perform under conditions of normocenosis. An imbalance in the microbial ecosystem of the intestines of animals entails a decrease in the antagonistic and metabolic activity of microorganisms, which is reflected in a number of morphological and functional features of various organs and systems, manifested in a violation of the digestion and absorption of food, the synthesis of vitamins, enzymes, amino acids, a decrease in overall resistance and the development of inflammatory processes.

MAIN PART

Intestinal dysbacteriosis is a pathological condition characterized by a violation of the qualitative and quantitative composition of the intestinal microflora. With dysbiosis, there is an increase in pathogenic microorganisms and a decrease in the concentration of beneficial lacto- and bifidobacteria. Dysbacteriosis is not an independent disease. Dysbacteriosis is a condition in which the intestinal microflora is disturbed due to a change in the composition of the bacteria that inhabit it. At the same time, both the quantitative and qualitative composition of the microflora changes: the number of beneficial bifidus and lactobacilli decreases, and the number of pathogenic bacteria increases.

Violation of the composition and quantity of microorganisms (bifidobacteria and many others) that inhabit the digestive tract is most often the result of the uncontrolled use of various drugs, especially antibiotics. Of great importance is the composition of the diet, as well as the presence of various chronic and functional diseases of organs and systems, and, above all, the organs of the gastrointestinal tract. An unfavorable psycho-emotional background contributes to the development of this condition. If dysbiotic changes were caused by the use of antibiotics or an acute intestinal infection, then with timely diagnosis and correct correction of this condition, it is possible to achieve a complete restoration of the balance of the intestinal microflora, not only bifidobacteria, but also other microbes. If dysbacteriosis is the result of a chronic disease, such as chronic gastritis, duodenitis, colitis, etc., then it is impossible to cure dysbacteriosis once and for all, but it is simply necessary to periodically carry out therapy aimed at normalizing the microbial landscape of the intestine, since quantitative and qualitative changes in the microflora can lead and maintain exacerbations of the underlying disease. Self-treatment of "dysbacteriosis" with bioadditives, kefir diet and "probiotics" does not always give any beneficial effect. Equally important for the prevention of dysbacteriosis is a rational diet and a balanced composition of food. When prescribing antibiotics in parallel, it is recommended to use drugs that support the growth and development of normal microflora. The choice of support drugs is the prerogative of the doctor, since only the doctor knows all the features of the course of the disease in the patient and can choose the right individual treatment. In the presence of chronic digestive diseases, it is necessary to strictly follow all the doctor's instructions for the treatment of the underlying disease. If necessary, after an additional examination, the doctor may, in parallel or after the main course of therapy, prescribe a regimen aimed at correcting dysbacteriosis.

MATERIALS AND METHODS

The experiment was carried out on laboratory animals at the age of 1, 2 and 3 weeks, such as white rats, guinea pigs and rabbits. Animals were kept under standard conditions: 12-hour light period, temperature 20°C. To simulate dysbacteriosis, laboratory animals were orally administered oxacillin dissolved in physiological saline through a rigid probe. The introduction of the drug was started from the 2nd day after birth, daily, 2 mg of the drug twice a week. The sampling of material for bacteriological examination was carried out on the 7th, 14th and 21st days. Determination of the intestinal microflora of rats, guinea pigs and rabbits after dysbacteriosis was carried out by inoculation of feces on selective media for bifidobacteria, lactobacilli, bacteroids and peptostreptococci by the method of serial serial dilutions.

RESULTS AND ITS DISCUSSION

When studying the effect of oxacillin on the composition of the microflora of mice, a sharp change in the composition of the microflora was shown (data are presented in table No. 1).

Table 1. The effect of oxacillin on the natural microflora in the large intestine of rats

№	Name of microorganisms	7 days		14 days		21 days	
		The number of microorganisms. In 1 gr. f.		The number of microorganisms. In 1 gr. f.		The number of microorganisms. In 1 gr. f.	
		Norm	experiment	Norm	experiment	Norm	experiment
1.	Total number of anaerobes	10^{10} 5%	8×10^8	10^{10} 5%	6×10^6	10^{10} 5%	8×10^8
2.	of them with gem. St. you	10^9	6×10^5↓	10^9	6×10^4↓	10^9	6×10^5↓
3.	bifidobacteria	10^8	4×10^4↓	10^8	6×10^5↓	10^8	6×10^5↓
4.	lactobacilli						
5.	Bacteroids						
6.	Total number of aerobes of which with hemolytic properties	10^8 5%	$2,8\times10^8$	10^8 5%	$3,2\times10^6$	10^8 5%	$2,8\times10^8$
7.	Escherichia:						
	Lactose positive:	10^8	6×10^4↓	10^8	6×10^5↓	10^8	6×10^5↓
	Lactose negative:	10^2	--	10^2	--	10^2	--
	Hemolytic:	10^1	--	10^1	--	10^1	8×10^6↑
8.	Enterococci	10^6	10^4	10^6	10^6	10^6	10^6
9.	Streptococcus gr. BUT	10^1	--	10^1	--	10^1	--
10.	Staphylococci:	10^2	--	10^2	--	10^2	--
		10^4	10^4	10^4	10^6	10^4	10^6
		10^2	10^4	10^2	10^4	10^2	10^6

11.	Golden:	10^{2}	--	10^{2}	8×10^{6}↑	10^{2}	--
12.	Saprophytic:	10^{2}	--	10^{2}	--	10^{2}	--
13.	Klebsiella	10^{2}	--	10^{2}	--	10^{2}	--
14.	Mycoplasmas	0	--	0	--	0	--
15.	Clostridia	0	--	0	--	0	--
16.	pseudomonas	0	--	0	--	0	--

The obtained results indicate the development of dysbacteriosis and confirm the success of the chosen model of experimental dysbacteriosis. Observation of the appearance, activity, appetite and nature of the feces of experimental animals revealed that after the use of oxacillin, the vast majority of animals of all experimental groups showed characteristic signs of a digestive tract disorder: anxiety or lethargy, a decrease in the amount of food consumed, flatulence, feces had an oily or liquid consistency sometimes with an admixture of mucus, greenish-brown. In the course of the studies, it was found that after a weekly administration of oxacillin to rats, there was a decrease in the number of anaerobic bacteria in the large intestine of rats: bifidobacteria and lactobacilli on. It should be noted that on the 14th day of the experiment, the number of fungi from the genus Candida increased against the background of a decrease in the number of bifidobacteria and lactobacilli populations compared to the 7th day of the experiment, on the 21st day the number of hemolytic Escherichia increased against the background of a significant decrease in the populations of bifidobacteria and lactobacilli.

Table 2. The effect of oxacillin on the natural microflora in the large intestine of guinea pigs

№	Name of microorganisms	7 days		14 days		21 days	
		The number of microorganisms. In 1 gr. f.		The number of microorganisms. In 1 gr. f.		The number of microorganisms. In 1 gr. f.	
		Norm	experiment	Norm	experiment	Norm	experiment
1.	Total number of anaerobes	10^{10}	8×10^{8}	10^{10}	8×10^{8}	10^{10}	8×10^{8}
		5%		5%		5%	
2.	of them with gem. St. you	10^{9}	6×10^{5}↓	10^{9}	6×10^{4}↓	10^{9}	6×10^{4}↓
3.	bifidobacteria	10^{8}	4×10^{4}↓	10^{8}	4×10^{4}↓	10^{8}	6×10^{5}↓
4.	lactobacilli						
5.	Bacteroids						
6.	Total number of aerobes	10^{8}	$2,8\times10^{8}$	10^{8}	$3,2\times10^{8}$	10^{8}	$3,4\times10^{8}$
	of which with hemolytic properties	5%		5%		5%	
7.	Escherichia:						
	Lactose positive:	10^{8}	6×10^{4}↓	10^{8}	6×10^{4}↓	10^{8}	6×10^{4}↓
	Lactose negative:	10^{2}	--	10^{2}	6×10^{5} ↑	10^{2}	6×10^{6} ↑
	Hemolytic:	10^{1}	--	10^{1}	--	10^{1}	--
8.	Enterococci	10^{6}	10^{4}	10^{6}	10^{6}	10^{6}	10^{6}
9.	Streptococcus gr. BUT	10^{1}	--	10^{1}	--	10^{1}	--
10.	Staphylococci:	10^{2}	--	10^{2}	--	10^{2}	--
		10^{4}	10^{4}	10^{4}	10^{6}	10^{4}	10^{6}
		10^{2}	10^{4}	10^{2}	10^{6}	10^{2}	10^{4}
11.	Golden:	10^{2}	--	10^{2}	--	10^{2}	--
12.	Saprophytic:	10^{2}	--	10^{2}	--	10^{2}	--
13.	Klebsiella	10^{2}	--	10^{2}	--	10^{2}	--
14.	Mycoplasmas	0	--	0	--	0	--
15.	Clostridia	0	--	0	--	0	--
16.	pseudomonas	0	--	0	--	0	--

It should be noted that on the 7th day of the experiment, the same changes were observed in guinea pigs as in rats. the results of the study showed 2 degrees of dysbacteriosis in the intestines of guinea pigs on the 14th day. Day 14 - in the body of guinea pigs The number of lactose-negative Escherichia increased against the background of a decrease in bifidus and lactobacilli. Day 21 The number of lactose-negative Escherichia increased against the background of a decrease in bifidus and lactobacilli.

Table 3. The effect of oxacillin on the natural microflora in the large intestine of rabbits

№	Name of microorganisms	7 days		14 days		21 days	
		The number of microorganisms. In 1 gr. f.		The number of microorganisms. In 1 gr. f.		The number of microorganisms. In 1 gr. f.	
		Norm	experiment	Norm	experiment	Norm	experiment
1.	Total number of anaerobes	10^{10}	8×10^{8}	10^{10}	6×10^{6}	10^{10}	8×10^{8}
		5%		5%		5%	
2.	of them with gem. St. you	10^{9}	$6\times10^{5}\downarrow$	10^{9}	$4\times10^{4}\downarrow$	10^{9}	$6\times10^{4}\downarrow$
3.	bifidobacteria	10^{8}	$4\times10^{4}\downarrow$	10^{8}	$4\times10^{4}\downarrow$	10^{8}	$6\times10^{4}\downarrow$
4.	lactobacilli						
5.	Bacteroids						
6.	Total number of aerobes	10^{8}	$2,8\times10^{8}$	10^{8}	$3,4\times10^{6}$	10^{8}	$3,4\times10^{8}$
	of which with hemolytic properties	5%		5%		5%	
7.	Escherichia:						
	Lactose positive:	10^{8}	$6\times10^{4}\downarrow$	10^{8}	$4\times10^{4}\downarrow$	10^{8}	$6\times10^{4}\downarrow$
	Lactose negative:	10^{2}	--	10^{2}	--	10^{2}	--
	Hemolytic:	10^{1}	--	10^{1}	$6\times10^{6}\uparrow$	10^{1}	$6\times10^{6}\uparrow$
8.	Enterococci	10^{6}	10^{4}	10^{6}	10^{6}	10^{6}	10^{6}
9.	Streptococcus gr. BUT	10^{1}	--	10^{1}	--	10^{1}	--
10.	Staphylococci:	10^{2}	--	10^{2}	--	10^{2}	--
		10^{4}	10^{4}	10^{4}	10^{6}	10^{4}	10^{6}
		10^{2}	10^{4}	10^{2}	10^{4}	10^{2}	10^{4}
11.	Golden:	10^{2}	--	10^{2}	--	10^{2}	--
12.	Saprophytic:	10^{2}	--	10^{2}	--	10^{2}	--
13.	Klebsiella	10^{2}	--	10^{2}	--	10^{2}	--
14.	Mycoplasmas	0	--	0	--	0	--
15.	Clostridia	0	--	0	--	0	--
16.	pseudomonas	0	--	0	--	0	--

On the 7th day, the same results were observed in rabbits as in rats and guinea pigs. Day 14 Increased the amount of hemolytic Escherichia against the background of a decrease in bifidus and lactobacilli. Day 21 - The amount of hemolytic Escherichia increased against the background of a decrease in bifidus and lactobacilli. A method for modeling intestinal dysbacteriosis in laboratory animals, accompanied by toxic damage to many organs, by introducing a drug into the stomach through a tube, characterized in that oxacillin is administered as a drug at a dose of 2 mg/kg of body weight with an interval of 24 hours for 7 days. The invention relates to the field of experimental medicine, namely to experimental microbiology, and can be used to study the effectiveness of probiotics in their prophylactic or therapeutic use. Violation balance of the microbial ecosystem of the intestines of animals entails a decrease antagonistic and metabolic activity of microorganisms, which is reflected in a number of morphofunctional features of various organs and systems, manifested in violation of digestion and absorption food, the synthesis of vitamins, enzymes, amino acids, as well as in reducing researches of the general resistance and development of inflammatory processes. The mechanisms of development of dysbacteriosis and its consequences are very complex and multifaceted, so the need to find funds that can can cover as many links of this pathogenesis as possible, for veterinary science is still relevant.The modern view on the correction of pathological processes associated with known with a violation of microflora, involves the use of complex approach to the improvement of intestinal ecology.

CONCLUSION

The results of the study showed that as a result of the use of oxacillin, there was an oppression of representatives of the traditional normal intestinal microflora in 100% of animals of all experiments tal groups equally, which was expressed in a decrease in the number bifidobacteria by 2.5 times, lactobacilli - by 2.3 times, Escherichia coli with normal enzymatic properties - 3 times. Significantly the percentage of excretion and the number of enterococci decreased. In 100% of living In some cases, after the use of an antibiotic, the appearance of intestinal sticks with reduced enzymatic properties, as well as increased decrease in the number of fungi of the genus Candida by 2.6 times.

Thus, the obtained results allow us to conclude that the experimental use of anabotics significantly affects the state of the intestinal microflora, manifested by a significant decrease in the populations of bifidobacteria and lactobacilli against the background of a sharp increase in strains of pathogenic anaerobes with hemolytic properties and fungi of the genus Candida. Ultimately, this leads to inhibition of the functionality of the intestine.

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