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MICROECOLOGICAL APPROACH IN TAXONOMIC COMPOSITION STUDY AND MICROBIOTA POPULATION LEVEL OF LARGE BOWEL CONTENT IN PRACTICALLY HEALTHY PEOPLE OF CHERNIVTSI REGION

Abstract: Taxonomic composition study and microbiota population level of large bowel cavity in practically healthy people of Chernivtsi region has been conducted. It has been ascertained that taxonomic composition study and microbiota population level of large bowel cavity in practically healthy people of Chernivtsi region is represented by indigenous autochthonal obligative anaerobic bacteria of Bifidobacterium, Lactobacillus, Bacteroides, Peptostreptococcus, E.coli genera. Enterobacteria of Proteus genus and yeast-fungi of Candida genus contribute to additional microbiota. Initial bacteria are represented in the population level from $8,41\pm0,13$ to $9,12\pm0,10$ lg CFU/g, additional $4,29\pm0,16-5,53\pm0,09$ lg CFU/g.

Key words: a large bowel, cavity content, normal microbiota, microbial ecology.

INTRODUCTION

Extensive development of the microbial ecology of a human in the last decades has made dramatic changes in the viewpoints of reasons and consequences of the disturbance in the human natural symbiosis with indigenous obligative symbiotic microbial associations as well as has risen a question of a possibility of renewing disturbance of the human microecological system by the way of specific micropreparation application, that involve the most significant by representation in the biocenosis content in every biotope and by its multifunctional role in microecological homeostasis sustenance.

Human microecological problem has played one of the key roles in medicine and biology in the recent decades. The statistics of highly-developed countries of the world reports the growth of diarrhea diseases with the result in qualitative and quantitative microecological content of any biotope (dysbacteriosis/dysbiosis). Human microecological changes are apparently a logical consequence of radical lifestyle change, traditional stereotypes that have been formed. Rapid urbanization process, high industrial development and environmental pollution connected with it, wide spread stress frequency, global use of synthetic things in every day life and industrial production, wide effect medicine taking to constant-growing extent. These are not a full list of negative influence sources on human microecology and dysbacteriosis /dysbiosis formation that initially become the symptoms of a major disease and then at the therapeutical measure treatment absence they become leading in pathogenesis of a human disease and with difficulties are subjected to therapeutic tactics. Besides, infectious diseases, especially digestive tract illnesses (DT), are formed on the background of a certain microecological biotope disturbance. For establishing the microecological processes disturbance

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level the examination of qualitative and quantitative microbiotic content of any biotope including DT in healthy people of certain clinic-geographical, agricultural region.

Objectives:

to establish the main taxonomic composition, population level and microecological indexes of micro biota in large bowel cavity content of practically healthy people of Chernivtsi region that has its own climatic-geographical peculiarities and specific soil structure, water etc.

Resources and methods:

Taxonomic composition and microbiota population level of large bowel cavity in practically healthy people of Chernivtsi region has been examined for three years. The material under investigation was taken in people aged 10-30, who considered themselves to be absolutely healthy and hadn't suffered from any infectious or uninfectious diseases for the last 6 months (according to the anamnesis). Large bowel excrements were taken from patients and 2-3 grams of content from middle portions were selected. They were placed into sterile flasks (sterilization was done in an autoclave) and immediately delivered to the laboratory of the microbiology department of clinic immunology, allergology and endocrinology of the Bukovynian state medical university, where bacteriological and mycological examination of the large bowel cavity content was conducted. The material delivered later than two hours after the abstraction was not to be examined due to the possibility of the obligative anaerobic bacteria death, that's why a repeated abstraction of the material under investigation was done. Average annual microflora indexes, analytical coefficients and indexes were calculated according to the bacterial investigations data in practically healthy people throughout the year.

Investigation results and their discussion.

For the revealing of biotope microorganisms colonization mechanisms an ecological method has been used that allowed to make coexistence characteristics of ecological system representatives «microorganism – microbiota», to trace the direction of possible changes in microbial ecology content of a large bowel cavity – that is the main

human microbiota reservoir in general and the digestive tract in particular.

17 families, 45 genera and more than 400 microorganism species are identified in large bowel biotope of a human body that certifies the microbiota complexity of the biotope. However, the microbiota composition stability and its physiological functions are sustained by the complex megapolis of symbiosis with human body that has been formed through the lengthy adaptation process of coexistence in the form of a single ecological system. As in any biotope, especially in a large bowel cavity, «typical» microorganism groups with insignificant amount of species always dominate. However in a quantitative ratio they constitute the basis of microbiota.

The results of taxonomic composition study and microbiota population level of

large bowel cavity in practically healthy people are given in Table 1.

Table 1

Taxonomic composition study and microbiota population level of large bowel cavity in practically healthy people of Chernivtsi region.

Microorganisms	Number of peo-	Identi- fied strains	Index of con- stancy	Frequency	Marhaleph's generic abundance	Witteker's generic abun-	Simpson's ge- neric domina-	Berger-Par- ker's generic abundance	Biotope inhabitants relation ratio by
	amined		(%)		index	dance index	tion index	index	Zhakkard
	T			Autochthon	al obligative an	Autochthonal obligative anaerobic bacteria			
Bifidobacterium spp.	181	181	0,001	91'0	99.45	26,58	0,032	1,00	0'001
Lactobacillus spp.	181	181	100,0	91'0	99,45	26,58	0,032	1.00	100,0
Bacteroides spp.	181	181	0,001	91.0	99.45	26,58	0,032	1.00	0,001
Peptostreptococcus spp.	181	156	86.2	0,14	85.64	22,91	0,024	0.86	86,11
Peptococcus niger	181	37	20,4	0.03	19.89	5,43	10'0	0,20	20.00
			F	acultative ana	erobic and aero	Facultative anaerobic and aerobic microorganisms	ns		
E.coli	181	181	0,001	91'0	66,45	26,58	0,032	1.00	0'001
Proteus spp.	181	120	66,3	0,11	65,75	17,62	0,014	99'0	71,67
Klebsiella pneumonia	181	8	T.7	10'0	1.10	0,44	<0,001	0.02	1,10
Enterococcus spp.	181	7	3,9	10.0	3,31	1.03	100'0>	0.04	3,27
Staphylococcus spp.	181	29	0.01	0,03	15,47	4,26	100'0	0,16	16,02
Candida spp.	181	52	28.7	50'0	28,18	7,64	0,003	0.28	28,33
			1				-		

Typology of the dominant (resistant, obligative, indigenous, major) micro biota representatives was carried out on the basis of constancy and frequency indexes meaning. For characteristics of micro biocenosis variety of large bowel cavity indexes of generic abundance by Marhaleph and generic variety by Witekker have been calculated. These original «ratings» for the biotope stability protection and preservation that characterize spatial-nutritional resources and environmental conditions of microorganism existence in a human biotope.

For domination level identification of a certain microorganism species in a large bowel cavity of a practically healthy person the Simpson's and Berger-Parker's generic domination indexes have been calculated. The inhabitants' relationships in a large bowel cavity have been characterized with the help of Zhakkard's coefficient which is one of the most reliable indexes of microorganism relationships in association.

It has been shown that autochthonal obligative anaerobic bacteria of Bifidobacterium, Lactobacillus, Bacteroides, Peptostreptococcus and E.coli are the dominant microorganisms in a large bowel cavity of practically healthy people in Chernivtsi region. According to the constancy and frequency indexes, Marhaleph's generic abundance index, Simpson's and Berger-Parker's generic domination index, Witteker's generic abundance index and Zhakkard's coefficient we can regard Proteus and yeast-fungi of Candida genus as secondary microorganisms. Other microorganisms (P. niger, Enterococcus spp., K. pneumoniae) according to these indexes can also be referred to the secondary ones.

Normal micro biota of any biotope, especially a large bowel cavity of a practically healthy person is characterized by not only qualitative content, but also, which is more important by quantitative indexes that are more informative about micro biota state and its disturbance. It is known that cavitary micro biota is the most susceptible to various exogenic and endogenic factors. Nutritional changes, ecological environmental influences, short-termed medicamental treatment first of all have its impact on quantitative micro biota indexes. But this buffer stock of the micro biota population content in practically healthy people is rather high. That's why the next stage was the quantitative indexes study of a large bowel cavity in practically healthy people, as well as micro ecological indexes have been established that characterize micro biocenosis state of a large bowel cavity. The study results of the micro biota population level of a large bowel cavity in practically healthy people of Chernivtsi region are given in Table 2.

Table 2. The microbiota population level of a large bowel cavity in practically healthy people

Microorganisms	Population level (lg CFU/g) (M±m)	Quantitative domina- tion coefficient	Meaning coefficient
Obligative anaerobic bact	eria		
Bifidobacterium spp.	8,87±0,13	123.7	0,20
Lactobacillus spp.	7,38±0,11	102.9	0,16

Bacteroides spp.	9,12±0,10	127,2	0.20
Peptostreptococcus spp.	8,41±0,13	101,1	0,16
Peptococcus niger	8,23±0,10	23,4	0,03
Facultative anaerobic and	aerobie microorgai	nisms	
E.coli	9,13±0,10	127,3	0,02
Proteus spp.	5,53±0,09	32,6	0,05
Klebsiella pneumonia	6,99±0,16	1,7	0,01
Enterococcus spp.	3,89±0,05	4,3	0,01
Staphylococcus spp.	5,02±0,15	11,2	0,02
Candida spp.	4,29±0,16	17,2	0,03

According to the qualitative indexes (population level, quantitative domination coefficient and meaning coefficient) autochtonic obligative anaerobic bacteria of Bifidobacterium, Lactobacillus, Bacteroides, Peptostreptococcus genera refer to the main microbiota of a large bowel cavity in practically healthy people, and also facultative anaerobic and aerobic microorganisms of Escherichia genus. These data are coordinated by study results, conducted by V. M. Bondarenko, N.M. Hracheva and T.V. Matskulevych. Together with that, the latter authors refer Enterococci to the main microbiota. According to our data bacteria of Enterococcus genus as well as Staphylococcus and yeast-fungi of Candida genus with their microecological indexes refer to practically healthy people in Chernivtsi region and additional micro biota and they constitute the accompanying microflora.

Dominant biocenosis microbiota of the large bowel cavity of a practically healthy human is represented only by a few anaerobic bacteria groups (Bifidobacterium, Lactobacillus) that in quantitative ratio with bacteria of Bacteroides, Peptostreptococcus, constitute the micro biota basis of the large bowel cavity in a practically healthy human and fulfill the key role in supporting the optimal biocenosis state and its functions, nevertheless the huge taxonomic variety of this ecosystem as it is shown in tables 1, 2. High concentration of these anaerobic bacteria to some extent is connected with macroorganism immunologic protection tolerance to them. Physiological importance of the bacteria of Bifidobacterium, Lactobacillus genera for a human body and their exceptional significance in microbial ecosystem functioning has been proved by the numerous investigations and is out of any doubt.

Conclusions

1.Taxonomic composition of large bowel cavity in practically healthy people of Chernivtsi region represented by indigenous, autochtonic, obligative, anaerobic bacteria of Bifidobacterium, Lactobacillus, Bacteroides, Peptostreptococcus genera, by additional Enterobacteria of Proteus genus and yeast-fungi of Candida genus. Occasional micro biota is represented by P. niger, Enterococcus spp., K. pneumoniae.

2.According to the population level, quantitative domination and meaning indexes the leading role in microecological system formation «microbiota – macroorganism»

belongs to obligative anaerobic autochtonic bacteria of Bifidobacterium, Lactobacillus genera that have joint exceptional meaning in the microbial ecosystem functioning.

Perspectives of further investigations.

The given results are the reason for carrying out a comparative characteristics of microbiota taxonomic composition and population level of the large bowel cavity in practically healthy people who reside in Chernivtsi region and other regions of Ukraine.

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