

**THE NORMAL INTESTINAL MICROFLORA
AND THE ANTIBODIES (THE ANTIBODIES STUDY
TO THE OPPORTUNISTIC INTESTINAL ENTEROBACTERIA)**

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The specific antibodies to the CPE antigens have been discovered at the 80,3% examined and the studied children. So, the antibodies revealed titres to the CPE antigens at the adult persons have had the spread wide range, in average, from 13 up to 29%. Then, it had also been revealed, that, at the early age children, the seronegative indices were in 2,5–3 times more, than the seropositive sera with all the examined and the studied CPE antigens. The specific immune level, in the form of the antimicrobial and the antibacterial antibodies is being reliably raised, with the examined children age, at their early age, following increase.

Keywords: microflora, bowels or intestines, antigens, antibodies, immunity, enterobacteria, opportunistic microflora

The significant role is being belonged to the intestine microflora in the immunological responsiveness and the human body tolerance maintaining [8, 9, 10]. The allogenic substances to the human body infinite number confrontation is constantly being taken its place approximately on the 200 square meters of the gastrointestinal tract surface. The scholars and the scientists are still trying to be comprehended the gastrointestinal tract immune system peculiarities and the special features and to be based the quite new medical treatment methods without the harmful and the unhealthy side effects [1, 4, 7].

The level peculiarities and the specific features and the specific antibodies spectrum are being presented the essential and the considerable interest, they have genetically been programmed, and they have been defined the macroorganism immune response force and also its susceptibility to the infectious diseases and the other illnesses [3, 9].

So, the antibodies formation reinforcement to their proper and their own tissues and to the human body microflora antigens is being taken its place against the background of the cell immunity and the humoral immunity moderate lowering inhibition [2; 6]. Just between the bacteria and the proper and their own tissues antigens, it is quite possible the autoimmune reactions development, and also the immunopathological processes development, because of having had the common antigenic determinant. The antibodies titer to the human organism bacteria is one from the indicated signs.

Thus, the present research and the study target has been the definition and the circulated antibodies level valuation to the opportunistic enterobacteria at the practically healthy adult people and the children, having taken into consideration the above – mentioned material.

The Materials and the methods

The children at the age from 4 months up to 14 years (e.g. 168 months) have already been examined by us for

the set goals achievement. The III, IV – degree bowels dysbiosis have been found at the 66 children among the examined children. After this, they have been included into the main group. The 30 practically healthy children of the same specific age – related composition have been consisted in the control group. Thus, the sera have been preserved in the soldered glass ampullae, in the frozen state in the low – temperature refrigerator car at – 45°C up to the research and the study moment.

The conditionally pathogenic enterobacteria (CPE) antigens have been received by Boivin – the complex microbial antigen (e.g. Boivin A., Mesrobian I., 1935), by means of the trichloroacetic acid (TCA) microorganisms diurnal culture extraction. Thus, this kind of approach is being related to the antigen extraction chemical methods group, and that is why, it is widely being used in the laboratory practice.

So, the microorganisms cultures have already been received from «The Human Infections Microorganisms National Collection» of the MPH RUz Epidemiology, the Microbiology and the Infectious Diseases the Research Institute. The passports for the bacteria strains, having contained their main characteristics, have been received together with the cultures. The 7 types of the Gram – negative bacteria: E.coli – 004136, ATCC 25922; Proteus vulgaris – 003341, 7; Citrobacter freundii – 0028011, 27; Klebsiella pneumoniae – 000691, 691; Enterobacter aerogenes – 003696, 27-C; Enterobacter cloacae – 004339, B-048; Pseudomonas aeruginosae – 004135, ATCC 27853 have already been used in the work. The Paeruginosae have been used side by side with the TCA for the reason, that this Gram – negative microorganism sufficiently is often colonized the bowels and the mucous membranes at the early age children.

The antibodies serum indication has been made in the immunofluorescence analysis (IFA) with the systems – test experimental series application, on the basis of the reagents from the commercial sets. The antigen sorption on the solid phase polystyrene, the flat – bottomed immunological plane tables have been made under the laboratory conditions in the optimal regime, having selected in the preliminary experiments. The IFA has been made, in accordance with the generally accepted methods and the generally used primary standards. The systems – test experimental series with the commercial sets components application for the IFA has already been prepared by us [7].

The already prepared complex bacterial antigens have been reduced to the 40 mkg/ml concentration, at the 9,6–9,8 medium pH. This kind of concentration has

been used for the solid phase – polystyrene plane tables sensitizing, by the «Medpolymer» production, RF. The studied blood sera, preliminarily, having titrated in the beyond buffer physiological solution from 1:25 up to 1:6,400 have been carried into the small holes after the immunological plane tables sensitized antigens washing by the washing solution. They have been incubated 1 hour, they have been thoroughly washed, and the antibody commercial reagent has been carried into against the IgG human, having labelled by the horseradish peroxidase (HRP) (e.g. the conjugate), it has been washed in the thorough way, and the developing solutions – the commercial OFD and the perhydrol have been carried into the small holes. Then, the recording has been carried out visually by the solution staining in the small holes. Thus, the last serum dilution has been taken for the titre, having given the solution staining in the hole much more intensive, than in the control holes (e.g. the negative control).

Thus, the received data have statistically been processed with the calculation of the mean one – M , the error of the mean – m , the roof – mean – square deviation – δ , the Student criterion – t by Fisher – Student.

The Results

The received data had been given in the form of the Table, where the data by the anti-

bodies frequency rate in the human blood serum to the 7 above – indicated CPE representatives were presented. The antibodies titres spread has been in the quantities wide range (see, Table 1). The sharply positive results (e.g. the antibodies titre 1:1,600 and more) have been, in average, within the limits from $13,0 \pm 2,4$ up to $18,0 \pm 4,3$ %. The sharply positive titres have not been determined for the *Citrobacter freundii* antigen. The positive titres have already been registered, in average, within the limits of the $18,0 \pm 4,3$ % – $24,0 \pm 5,9$ % observed ones. The weakly positive results by the antibodies titres to the 7 CPM studies have been noted, in average, at the $22,0 \pm 5,5$ % – $29,0 \pm 6,6$ % persons. The doubtful results (e.g. the 1:25–1:50 titre) have already been registered, in average, at the $13,0 \pm 2,4$ % – $29,0 \pm 6,6$ % observed ones. The antibodies to the CPM antigens have not been revealed (e.g. the negative result), in average, at the $13,0 \pm 2,4$ % – $24,0 \pm 5,9$ % blood sera observed ones.

Table 1

The IFA Results by the Antibodies Definition in the Blood Serum of the Practically Healthy Children against CPE Antigens and *P.aeruginosae*, in %

The Complex Microbial Antigen	The Blood Serum Titre				
	The Sharply Positive Ones	The Positive Ones	The Weakly Positive Ones	The Doubtful Ones	The Negative Ones
<i>Escherichia coli</i>	$18,0 \pm 4,3$	$24,0 \pm 5,9$	$29,0 \pm 6,6$	$13,0 \pm 2,4$	$16,0 \pm 3,5$
<i>Enterobacter aerogenes</i>	$15,5 \pm 3,5$	$18,0 \pm 4,3$	$27,0 \pm 6,3$	$15,5 \pm 3,5$	$24,0 \pm 5,9$
<i>Enterobacter cloacae</i>	$15,5 \pm 3,5$	$20,0 \pm 5,0$	$24,0 \pm 5,9$	$20,0 \pm 5,0$	$20,0 \pm 5,0$
<i>Citrobacter freundii</i>	0	$22,0 \pm 5,5$	$22,0 \pm 5,5$	$29,0 \pm 6,6$	$18,0 \pm 4,3$
<i>Klebsiella pneumoniae</i>	$15,5 \pm 3,5$	$24,0 \pm 5,9$	$27,0 \pm 6,3$	$20,0 \pm 5,0$	$15,5 \pm 3,5$
<i>Proteus vulgaris</i>	$18,0 \pm 4,3$	$20,0 \pm 5,0$	$27,0 \pm 6,3$	$22,0 \pm 5,5$	$13,0 \pm 2,4$
<i>Pseudomonas aeruginosae</i>	$13,0 \pm 2,4$	$24,0 \pm 5,9$	$29,0 \pm 6,6$	$20,0 \pm 5,0$	$13,0 \pm 2,4$

Note: the sharply positive – titre from 1: 1,600 and more;
 the positive – titre from 1:400 up to 1:800;
 the weakly positive – titre from 1:100 up to 1:200;
 the doubtful – titre from 1:25 up to 1:50;
 the negative – titre 0.

Thus, the differences by the serum antibodies the frequency rate in the human blood at the examined practically healthy children have been determined by the carried out studies and the researches. So, the antibodies revealed titres to the CPE antigens (e.g. *Escherichia coli*, *Proteus vulgaris*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Pseudomonas aeruginosae*) have had the spread wide range, in average, from 13 up to 29%. The examined and the studied groups carried out separation up to the 5 indices (e.g.

the sharply positive, the positive, the weakly positive, the doubtful, and the negative ones), depending on the antibodies titres quantities of the healthy persons blood is being permitted to be carried out the relative rating.

It should be considered the indices complex by the CPE aetiological significance criterion, among which one from the most important – is the specific serum antibodies revealing to the assumed causative agent antigens. The false – positive results, owing to the crossed reactive antibodies in the agglutination reaction with

the autostrain are being observed not more, than at 5–10% healthy persons and the bearers, and, by the data of some authors, they are not exceeded 1: 10 – 1: 20 for the H – antigen, and 1:40 of the other authors. Then, it, moreover, should be especially emphasized that fact, that as the positive and the false – positive reactions are not quite observed at the early age healthy children, owing to the immune system imperfection and also the antigenic stimulation short – term period.

Having proceeded from the above – stated material, the circulated antibodies to the CPE level study and the examination and also the valuation at the children with the bowels disbioz has been our study and the researches next stage.

The antibodies medial geometrical titres, having expressed in the form of the negative logarithms with the 2 ($-\log_2$) basis have been calculated for the antibody formation intensity definition for the quite different and the various CPE antigens. The relative indices difference error in the percent had been calculated by the formula for the non – equivalent samplings at the difference reliability valuation between the indices, when the one comparative group quantity was exceeded the

other quantity not less, than for 25 % (e.g. Lakin M.F. 1980).

So, the specific and the peculiar antibodies to the CPE antigens have already been discovered at the human blood serum of the examined children overwhelming majority. Then, the positive result has been received at the 80,0 \pm 2,6 %, though the human organism response immune reaction with the high level frequency has been discovered at the children with the CPE association – mixstautostrains of all the listed large intestines microflora representatives, than at the children, at whom mainly the monocultures have been isolated and have been taken.

The antibodies definition results to the 7 CPE representatives has been shown the comparable results for the Enterobacteriaceae family 6 representatives – the seronegative results have been in the range from 19,7 up to 30,3 % (e.g. see, Table 2), the seropositive results have been from 69,7 up to 80,3 %. So, the seropositive serahas been somewhat higher for the P.aeruginosa, but it is not significantly statistically, in comparison with the other antigens (e.g. the seronegative ones 15,2 %, the seropositive ones 84,8 %).

Table 2

The Blood Sera IFA Results with the CPE Antigens

The Microbial Antigen	The Seronegative Ones	The Seropositive Ones
Escherichia coli	19,7 \pm 8,9	80,3 \pm 8,9*
Enterobacter aerogenes	24,2 \pm 9,7	75,8 \pm 9,7*
Enterobacter cloacae	30,3 \pm 10,2	69,7 \pm 10,2*
Citrobacter freundii	19,7 \pm 8,9	80,3 \pm 8,9*
Klebsiella pneumoniae	30,3 \pm 10,2	69,7 \pm 10,2*
Proteus vulgaris	30,3 \pm 10,2	69,7 \pm 10,2*
Pseudomonas aeruginosae	15,2 \pm 7,9	84,8 \pm 7,9*

Note: * – the differences reliability.

However, the seropositive sera frequency rate has been reliably higher, than the seronegative ones (e.g. $p < 0,001$) by the all 7 antigens. Especially, it is concerned the E.coli (e.g. 80,0 \pm 8,9%), the C.freindii (e.g. 80,0 \pm 8,9%) and the P.aeruginosae (e.g. 85,0 \pm 7,9%), the detectability percent of which has been higher, than the other microorganisms. Apparently, all these causative agents have already colonized the whole bowels, and they, moreover, have aggravated the large intestines disbioz at the children.

The antibody formation intensity study to the already studied CPE and the P.aeruginosae – all the examined children up to 2 years have already been divided into the following groups: the 1-st group: the children up to the 6 months;

the 2-nd group: the 7–9 months children, the 3-rd group: at the age of the 10–13 months and, at last, the 4-th group: the 13–24 months old. So, the received results have been shown (e.g. Table 3), that specific immunity level in the form of the antimicrobial and the antibacterial antibodies is appropriately being increased from the 1-st group up to the 4-th group. The fact is being drawn its attention, that the higher antibodies titre has already been fixed in the 3-rd and the 4-th groups (e.g. $-\log_2$ 7,5 up to $-\log_2$ 4,3), than in the 1-st and the 2-nd groups – 4,0–4,2 (in $-\log_2$) – $p < 0,05$.

At the same time, we make the special emphasis, that the antibodies formation has been taken its place only against antigens, against the E.coli and the P.vulgaris. If to be taken into

consideration that moment, that the newborn immune system is not only completely developed, and the antibodies formation is not taken its place in the complete volume, then, apparently, all these antibodies are maternal, we are quite to be assumed, that the bowels disbioz of the Escherichia and the protein aetiology has been revealed also at the mother.

So, the very near to them results have been received and at the 2-nd group children. So, the whole antibody formation intensity has been in the complete volume and in the large quantities from the 10-th months old (e.g. the 3-rd and the 4-th groups), than at the 1-st and the 2-nd groups children (e.g. $p < 0,05$). It makes no difference, the antibodies titre has been left the high one to the Escherichia and the protein antigens at all these children, and apparently, they are the disbiozes reason at the children up to the 2 years old.

Thus, the specific antibodies to the CPE antigens have been discovered at the 80,3% examined and the studied children with the III-IV degree bowels disbioz, the human organism response immune reaction with the high level frequency has been discovered at the children with the CPE association. So, it had also been revealed, that the seronegative indices were

in 2,5–3 times less, than the seropositive sera with all the examined and the studied CPE antigens, though the seropositive sera have been in somewhat higher for the P.aeruginosa, with respect to the CPE antigens (e.g. $p < 0,05$). The specific immune level, in the form of the antimicrobial and the antibacterial antibodies is being reliably raised, with the examined children age following increase (e.g. $p < 0,05$).

The Results' Discussion

The human digestive tract is being presented itself the opened system, by means of which is being carried out its contact with the outdoor and the external environment and the microbes' world, which is being populated it. At present, it is quite considered to be proved, that the qualitative and the quantitative composition of the colonized microflora are being controlled the natural resistance and the immunological protection factors. The existing contacts with the bacterial and the microbial antigens are being defined the immune system maturing, and the intestines microflora composition will have to be reflected indirectly the peculiarities and the special features of the physiological condition, as the protective, well as the human organism other systems in the quite different and the various its life period [9].

Table 3

The Antibody Formation Intensity against the CPE Various Antigens

The Groups	The Specific Immunity Level to the Antigens from													
	E.coli		E.aerogenes		E.cloacae		C.freindii		P.vulgaris		K.pneumoniae		P.aeruginosae	
	max min	log2 m.g.t.	max min	log2 m.g.t.	max min	log2 m.g.t.	max min	log2 m.g.t.	max min	log2 m.g.t.	max min	log2 m.g.t.	max min	log2 m.g.t.
1	25	4,2	0	0	0	0	0	0	25	4,2	0	0	0	0
	25	25,0	0	0	0	0	0	0	25	25,0	0	0	0	0
2	25	4,2	0	0	0	0	0	0	25	4,0	0	0	25	4,2
	25	25,0	0	0	0	0	0	0	50	32,3	0	0	50	32,6
3	25	5,3	25	4,4	25	4,3	25	4,7	25	6,1	0	4,5	25	5,3
	50	36,3	25	25,0	25	25,0	50	30,4	100	68,8	25	20,0	50	37,5
4	25	7,5	25	4,0	25	4,0	25	7,2	25	7,0	25	7,3	25	6,1
	200	150,4	50	35,6	25	25,0	200	132,6	200	128,6	200	136,0	100	65,3

Note : in the numerator – (max) the antibodies maximum titre (e.g. the reverse titre);
 in the denominator – (min) the antibodies minimum titre (the reverse titre);
 in the numerator – (the log₂ m.geom. titre) the negative log by the medial geometrical titre;
 in the denominator – (m.geom. titre) the medial geometrical titre.

The normal microflora, the lymphoid tissue, having associated with the bowels mucous membrane, and also the cytokines, as the intercellular interaction factor, take their part in the immune mechanisms realization at the GIT level [10]. The circulating antibodies, the com-

plement level, and also the white blood cells functional state have the special significance for the antibacterial and the antimicrobial significance immune.

So, the main and the basic elements, having formed the protection lines, are being singled

out in the bowels mucous membrane immunological protection. The immune exclusion is being restricted the epithelium colonization by the microorganisms and, it is also being controlled and restrained the allogenic soluble antigens further penetration.

The mucous membrane is being presented itself the human organism protection first line against the different and the various environmental pathogenic factors, such as the following: the bacterial, the microbial, the viral, the industrial and the chemical irritants and the pollution stimuli, and the contamination in the «external barriers» system [8].

The fixed and the established differences and the distinctions by the frequency rate of the serum antibodies in the blood of the examined and the studied healthy children have been shown the whole population heterogeneity, in the plan of the antibodies production to the bowels micrifiore. Thus, the revealed and the exposed antibodies titres to the CPE antigens (e.g. the *Escherichia coli*, *Proteus vulgaris*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*) have had the spread wide range at the practically healthy adult people (e.g. in average, from 13 up to 29%).

The all received results, owing to the spread wide range, have been permitted to be conducted these indices conditional rating. So, the observed and the examined groups' separation, depending on the results quantities upon 5 indices (e.g. the sharply positive, the positive, the weakly positive, the doubtful, and also the negative ones), depending on the blood antibodies titre quantities of the healthy adult people is being permitted to be carried out the relative rating. So, the specific antibodies to the CPE antigens have been discovered at the 80,3% examined and the studied children. The metabolic products and the microorganisms' cellular elements, which are being lived in the human intestines, are quite able to be caused harm to the quite different and the various macroorganism systems.

The Conclusions

1. The differences have already been determined by the frequency rate of the serum antibodies in the blood at the examined and the studied practically healthy children. So, the antibodies revealed titres to the CPE antigens at the adult persons have had the spread wide range, in average, from 13 up to 29%. The examined and the studied groups carried

out separation up to the 5 indices, depending on the antibodies titres quantities of the healthy persons blood is being permitted to be carried out the relative rating.

2. The specific antibodies to the CPE antigens have been discovered at the 80,3% examined and the studied children with the III-IV degree bowels disbioz, the human organism response immune reaction with the high level frequency has been discovered at the children with the CPE association. So, it had also been revealed, that, at the early age children, the seronegative indices were in 2,5–3 times less, than the seropositive sera with all the examined and the studied CPE antigens, though the seropositive sera have been in somewhat higher for the *P.aeruginosa*, with respect to the CPE antigens (e.g. $p < 0,05$).

3. The specific immune level, in the form of the antimicrobial and the antibacterial antibodies is being reliably raised, with the examined children age following increase (e.g. $p < 0,05$).

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