Bondareva Anastasia Valeryevna

Clinical and epidemiological features and therapy of colibacillosis in children in the modern stage

14.01.09 - Infectious diseases

14.02.02 – Epidemiology

Synopsis of thesis for the degree of

Candidate of Medical Sciences

Moscow-2013

The work has been carried out in the Federal Budget Scientific Institution "Central Scientific - Research Institute of Epidemiology", Russian Agency for Health and Consumer Rights.

Supervisor:

Doctor of Medical Sciences,

Professor Alexander Gorelov

PhD Alexander Podkolzin Tihonovich

Official opponents:

Novokshonov Alex Amosovich- PhD

Medical University "Russian National Research Medical

University of Pirogov "Ministry of Health and

Social Development, Department of Infectious Diseases in Children №1,

Professor

Elena Simonova G. - MD

Medical University "First Moscow State Medical

University IM Sechenov ", Department of Epidemiology health

prophylactic faculty, Professor

Leading organization - State educational institution

Higher Professional Education "Russian University of Friendship

peoples. "

Thesis held on 19 April 2013 in ____ hours

Dissertation Council meeting D208.114.01 ederalnogo budget

institutions of science, "Central Research Institute

Epidemiology "Rospotrebnadzor at: 111123, Moscow, st.

Novogireevskaya, d. 3a.

With the thesis is available in the library BUN "CRI

Epidemiology "Rospotrebnadzor.

Abstract of distributed "__" marta2013g.

Scientific Secretary

Dissertation Council

Doctor of Medical Sciences,

Professor Alexander Gorelov

General work characteristics

Problem relevance

Acute intestinal infections (AII) still represent one of the most urgent problems of infectious pathology. According to WHO, more than four billion cases of acute intestinal infections is annually registered in the world, of them 60 % in children under three years. Among the causes of death in children acute intestinal infection always take 2-3 place after infectious diseases of upper respiratory tract and HIV (Gorelov A.V., 2006; Lobzin Yu.V., 2009). AII problem is as well important for Russian healthcare. For example, in 2011 more than 776,000 cases of acute intestinal infections of established and unknown etiology (Onishchenko G.G., 2012) was registered in the Russian Federation.

However, during the last two decades there has been a clear reduction in colibacillosis cases in our country, while they are widespread throughout the world and their accidence does not decrease, especially in the etiological structure of acute intestinal infections in children (Prère MF., 2006; Jensen C., 2007). In Russia every year officially only from 17 to 20,000 cases of acute intestinal infections caused by Escherichia are registered (Onishchenko G.G., 2012). The vast majority of colibacillosis cases (75%) concern children in the age of 14. The reduction in the number of detected colibacillosis cases is due to primary diagnostic difficulties.

Some difficulties have recently arisen in differentiation of various groups of diarrheal *Escherichia coli* using classical microbiological studies in clinical practice. The most common identification method for the groups of diarrheal *E. coli* in the conventional healthcare is to define their serogroup / serotype followed by an indirect determination of the isolate belonging to one of the known groups of diarrheal *E. coli*.

Direct detection methods of specific virulence factors are carried out using biopsy methods for identifying specific toxins capable of adhesion to cell cultures, as well as less-widespread identification of genes encoding virulence factors by PCR (Griffin P.M., 1991; Nataro J.P., 1998; Wanke C.A., 1998). Serological tests in children with colibacillosis have no diagnostic value (Gorelov AV, 2003). Several colibacillosis types, such as enteroaggregative or diffuse adhesive colibacillosis cannot so far be diagnosed at all in the Russian Federation.

The greatest difficulty for the doctor is appointment of an optimal causal treatment, one of the important points of which is adequate choice of antibiotic connected with constant variability in sensitivity of pathogens to commonly used antibiotics and the increasing number of antibiotic-resistant strains (Milutin L.N., 2005; Kadzhaeva E.P., 2006). Among *Escherichia* types antibiotic resistant strains are up to 60% (Melnikova I.Y., 2009).

In our country, clinical and epidemiological features and issues of differential diagnosis of AII caused by different types of *Escherichia*, especially enteroaggregative, enterohaemorrhagic and diffuse adhesive types are studied insufficiently, which requires further research on this issue and determination of the role of diarrheal *Escherichia coli* in the etiology of acute intestinal infections in children today. All the above has defined purpose of the work and allowed to formulate the study objectives.

Study purpose

The purpose of this work is optimization of colibacillosis diagnosis and therapy in children based on a study of clinical and epidemiological features on the modern stage.

Research objectives

1. To determine current epidemiological features of colibacillosis in children.

2. To evaluate the clinical significance of identifying the most common groups of diarrheal *Escherichia*.

3. To define clinical features of AII caused by enteroaggregative *Escherichia* in children, a comparative analysis of clinical features of enteropathogenic and enteroaggregative forms.

4. To assess the effectiveness of different therapeutic methods in the initial therapy of colibacillosis in children.

Scientific novelty of the research

Inclusion of the original test - systems for PCR diagnostics in the complex examination of AII patients expands the understanding of colibacillosis prevalence in children and makes it possible to identify true role of colibacillosis infection in etiological structure of acute intestinal infections in children of different ages in different seasons.

High clinical significance of and enteropathogenic and enteroaggregative *Escherichia* in children in the first six months of age has been shown within etiological structure of acute intestinal infections.

For the first time clinical and epidemiological features of enteroaggregative colibacillosis in children has been defined. Comparative characteristics of diseases associated with the well-studied enteropathogenic *E.coli* and less studied enteroaggregative *E.coli* has been performed.

Different approaches to the starting points of mono- and combined therapy of colibacillosis in children have been defined.

Practical significance

Evaluation of clinical and epidemiological significance of the results of use of direct methods for detection of various groups of diarrheal *Escherichia* provides higher diagnostic results in the clinical practice. The identified clinical and epidemiological peculiarities of colibacillosis allow to quickly solve problems of diagnosis and therapy of these infections, which provide proper and timely treatment, thereby ensuring more favorable disease course and outcome, reducing material costs for the treatment.

Implementation of the results

The results of the work have been put into practice on the State healthcare institution "Pediatric Hospital for Infectious Diseases No. 5 », Moscow.

Personal contribution

The author has personally carried out a clinical observation and analysis of the results of laboratory and instrumental methods of research in patients hospitalized with the diagnosis of acute intestinal infection. The following examinations were carried out in all patients: medical history taking, medical examination, sampling for additional laboratory tests; the development and filling of individual case cards card was conducted, as well as creating and filling in of databases, analysis of computer data base, statistical processing of the results, literature analysis as well as preparation of publications on the work performed.

Work approbation

The main provisions of the thesis were presented and discussed in the X Congress of Russian children's infectious diseases, "Topical Issues of infectious diseases and vaccine prevention ", Moscow, 2011; IX Scientific and practical conference "Infectious diseases and antimicrobial agents ", Moscow, 2011; IV Annual All-Russia Congress on Infectious Diseases, Moscow, 2012.

Approbation of the dissertation took place at a meeting of Department of clinical infectious diseases, Budgetary institutution "CSRI of Epidemiology", Russian Agency for Health and Consumer Rights, June 26, 2012.

Publishing

Eight works have been published on the topic of the dissertation, of them three – in the publications named in the list of Russian HAC.

Structure and scope of the thesis

Dissertation consists from 174 pages of typewritten text and includes introduction, literature review, Materials and Methods chapter, 3 chapters of the research, discussion, conclusions, practical recommendations and list of references containing 119 literature sources (62 Russian and 57 foreign sources). Thesis is illustrated with 56 tables, 23 figures and two clinical cases.

In-house studies

Materials and methods

The present study includes data on 789 patients aged from one month to 5 years treated in the Department of acute intestinal infections of children's infectious diseases hospital No. 5, Moscow (head physician Vlasov E.V.), from November 2009 to June 2011.

The patients were chosen by random sampling during all seasons of the year. The comparison group included 147 clinically healthy children from the Central Children clinical health resort "Malakhovka" (42 children), and children surveyed prior to vaccination in children's polyclinics of Moscow region (105 children).

General characteristics of the examined patients and the comparison group are presented in table 1.

Table 1. Characteristics of examined AII patients and the comparison group

Comparison parameters	Study group, n=789		Comparison group, n=147	
	absolute	%	absolute	%
Aged 1 month – 1 year	395	50.1	62	42.2
1 year – 3 years	296	37.5	60	40.8
3-5 years	98	12.4	25	17
Gender: boys	426	54	75	51
Girls	363	46	72	49
Burdened premorbid background	550	69.7	92	62.6

The difference is significant: $p < 0.05$

The groups were matched in all comparison parameters (age, gender, premorbid background). For each patient a specially developed account card was filled in containing daily data on the disease course, clinical and laboratory examinations as well as recorded data history.

To study colibacillosis in children under 5 years epidemiological approach was used including descriptive and evaluative methods and elements of analytical epidemiological studies. Etiological structure of colibacillosis in children was described, as well as accidence of different types of diarrheal colibacillosis depending on the seasons, age and gender. To collect epidemiologically important information on possible routes of transmission of pathogens questioning the parents of the patients was carried out.

Assessment of AII severity and clinical form was conducted based on doctors' textbook “Clinical recommendations on diagnosis and treatment of acute intestinal infections in children "(Gorelov A.V., Milutin L.N., Usenko D.V., 2005) approved by Ministry of Health and Social Development of the Russian Federation.

To identify AII causative agents, all the patients were examined by routine methods on day 1 of hospitalization (bacteriologic culture, ELISA for detection of rotavirus antigen) and by PCR. Standard comprehensive laboratory examination was performed as well (CBC and urinalysis, blood chemistry, determination of acid-base blood composition, coprological study). In the second week of the disease serological blood tests done by passive hemagglutination test for salmonellosis, colibacillosis and yersiniosis diagnosticums. Status of abdominal cavity and heart was evaluated using ultrasound method. For objective assessment of intoxication degree leukocyte index was calculated (leukocyte intoxication index (LII) on Kalf-Caliph, Rice, Ostrovsky, Dashtayanz endotoxemia index, sensitization index as well as shift index).

Colibacillosis was diagnosed on AII clinical performance and coprological identification of diarrheal *Escherichia* in the analysis by PCR. Test systems "AmpliSense Colibacillosis- FL» registration No. - ФСР 2010/07977 from June 11, 2010) Bacteriological studies of colibacillosis were not performed due to lack of diagnostic serum in bacteriological laboratory of the hospital. In 453 patients PCR coprological study was performed over time on days 1 and 5 of inpatient stay to assess elimination of diarrheal *E. coli*.

To evaluate the effectiveness of initial therapy, patients with colibacillosis were divided into groups. Group I included 40 children treated with antibiotics in combination with oral rehydration, in Group II - 36 children treated with enterosorbents combined with oral rehydration. Treatment effectiveness was evaluated by duration of symptoms: fever, intoxication, vomiting, abnormal stool or abnormal CBC leukocytes in the period of early convalescence.

To compare the efficiency of different classes of antibacterial drugs, group I was divided into four subgroups of patients. First subgroup included 11 patients treated with aminoglycosides; second subgroup - 8 children with antibiotic AII therapy including nifuroxazide; third subgroup - 11 children receiving cephalosporins generation III; the fourth subgroup - 10 children receiving nalidixic acid. To compare the efficiency of different classes of enterosorbents, group II was divided into two subgroups: I subgroup - 18 children receiving dioctahedral smectite (Neosmectin), II subgroup - 18 children treated with polymethylsiloxane polyhydrate (Enterosgel). To evaluate the effectiveness of initial therapy of patients with uncomplicated combined colibacillosis – rotavirus, moderate to severe form, analyses were performed in two groups of patients. Group I included 26 children treated with immune preparations (Genferon Light, Kipferon) in combination with antibacterial drugs, group II – 14 children treated with immune preparations (Genferon Light, Kipferon) in combination with enterosorbents. To compare the efficeincy of immune preparations two subgroups of patients were identified: I subgroup - 13 children receiving Genferon Light, II subgroup - 13 children receiving Kipferon.

Table 2 summarizes the volume of performed studies.

Table 2. The performed analyses

Studies performed	Material	Number of studied persons	Number of studies

CBC	Blood	789	1190
Urinalysis	Urine	789	1002
Blood chemistry	Blood	326	329
Acid-base blood count	Blood	240	284
Coprological study	Feces	339	345
Opportunistic pathogenic microflora	Feces	55	55
Bacteriologic culture for pathogenic microflora	Feces	789	13-6
Stomach washings for pathogenic microflora	Washings	15	15
ELISA for rotavirus antigen	Feces	410	445
Passive hemagglutination test with *Shigella, Salmonella, Yersinia*	Blood	114	128
Coprological PCR studies of children with AII and healthy children	Feces	936	1189
Ultrasound of abdominal cavity and heart	---	512	512
ECG	---	350	350
Questioning	Questionnaires	450	450

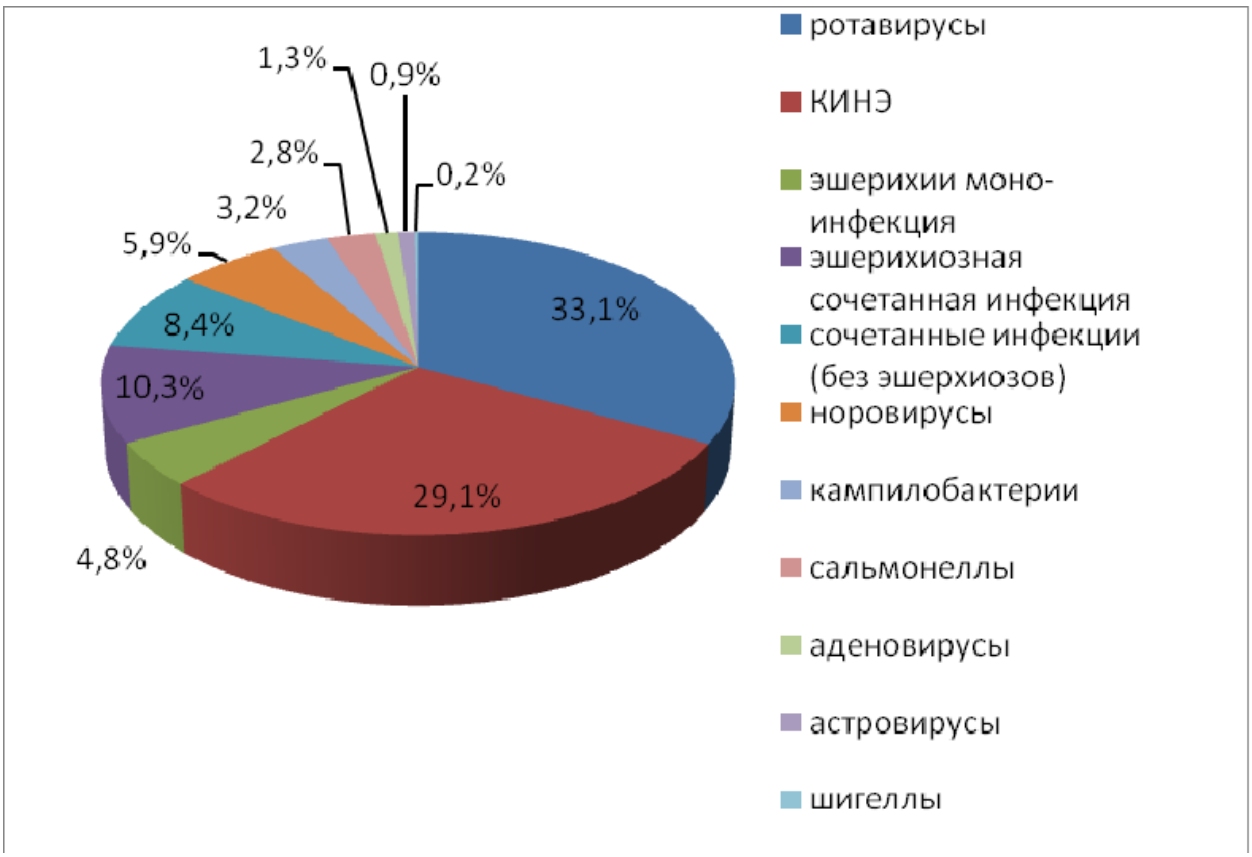
Statistical data processing was carried out using methods of variation statistics on a computer with licensed software tools Microsoft Excel, as well as Statistical program STATISTICA-6. Percentage of some data (%), the arithmetic mean (M) and standard error (m) were determined. To evaluate the differences between the values, Student's t-test and χ^2 test were used. Differences were considered significant at $p < 0.05$, highly significant - at $p < 0.01$ and $p < 0.001$, insignificant at $p > 0.05$. To compare the degree of homogeneity of statistical groups, dispersion comparison was applied using Fisher's test.

Results of the author's research and their discussion

Epidemiological features of colibacillosis in children

In general, among the surveyed 789 patients A rotaviruses predominated - in 261 cases (33.1%), and co-infection; several pathogens at the same time (without *Escherichia*) – in 66 (8.4%) patients; noroviruses G II - in 47 (5.9%) patients; other AII pathogens were determined more seldom – less than 5%. In 29.1% cases AII remained undeciphered, even with the use of modern molecular and genetic methods (Figure1).

Fig. 1. Distribution of AII patients by etiological factor



Escherichia was found in 119 children (15.1%), of them in 38 (4.8%) as a mono-pathogen and in 81 (10.3%) - in combination with other AII agents.

Structure of colinacillosis mono-infection was as follows: in 20 children (52.6%) EPEC was identified; in 15 (39.5%) - EAgEC; in 2 (5.3%) – EHEC and in 1 (2.6%) - ETEC. Among the combined colibacillosis forms in 57 (70.4%) patients diarrheal *Escherichia* was determined simultaneously with the viruses (often with rotaviruses - 59.3%), in 14 (17.3%) - with bacteria, in 10 (12.3%) children multiple combinations were found (viruses and bacteria simultaneously).

The most widespread among children with AII and healthy children were enteropathogenic and enteroaggregative groups of *Escherichia* (9.1% and 4.9% in patients and 12.9% and 8.2% - in healthy children, respectively). The frequency of diarrheal *E coli* in the main group and the comparison group is presented in Table 3. The fact is noteworthy that many of "healthy" patients carried *Escherichia* pathogens.

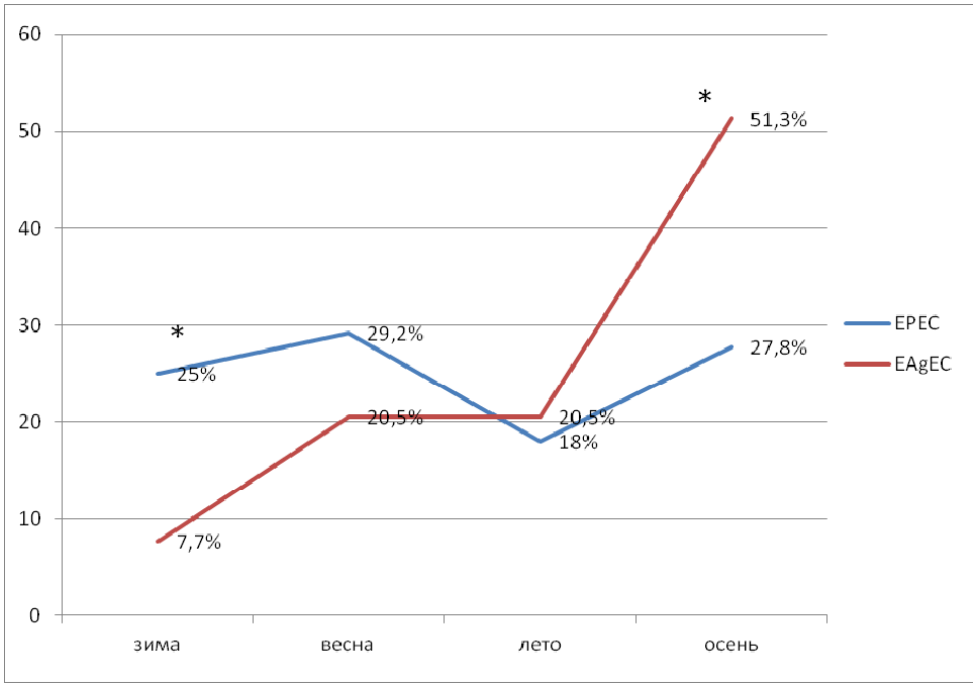
Table 3. Diarrheal *E. coli* detection rate in the study group and the comparison group

Diarrheal Escherichia	Main group, n=789	Comparison group, n=147
	%	%
EPEC	72 (9.1%)	19 (12.9%)
EAgEC	39 (4.9%)	12 (8.2%)
ETEC	6 (0.8%)	6 (4%)*
EHEC	4 (0.5%)	4 (2.7%)
EIEC+*Shigella spp.*	8 (1.0%)*	0

Dignificant difference: $p < 0.05$

Enterotoxigenic *Escherichia* was determined significantly more frequent in healthy children compared with those with AII (p <0.05). Enteroinvasive *Escherichia* combined with *Shigella* was detected onlyin patients with AII (p <0.05).

Figure 2 shows seasonality of enteropathogen and enteroaggregative colibacillosis.



The significant difference: * p <0.05

Figure 2. Seasonality of enteropathogenic and enteroaggregative colibacillosis

In our observation enteropathogenic colibacillosis was recorded evenly throughout the year. Enteroaggregative colibacillosis was characterized mainly by autumn season - in 20 (51.3%) patients (p <0.05). In winter enteropathogenic colibacillosis was significantly more frequent than enteroaggregative form (25% vs 7.7%), while in autumn, on the contrary, enteroaggregative colibacillosis was recorded significantly more than enteropathogenic form (51.3% vs 27.8%). Colibacillosis was more often diagnosed in children from 1 year to 3 years than in children of the first year of life (19.9 vs 10.9%) (p <0.05). Combined colibacillosis infection was recorded significantly more frequently in patients of age group 1-3 years or 3-5 years than those of the first year of life (Table 4). No gender differences were found.

Table 4. Age distribution of children with colibacillosis infection

AII children, n=789	1 month – 1 year (n=395)		1-3 years (n=296)		3-5 years (n=98)	
Colibacillosis, n=119	Abs.	%	Abs.	%	Abs.	%
	43	10.9	59	19.9*	17	17.3

Monoinfection, n=38	19	4.8	15	5	4	4
EPEC	11	2.8	7	2.4	2	2
EAgEC	7	1.8	6	2	2	2
ETEC	1	0.2	0	0	0	0
EHEC	0	0	2	0.6	0	0
Combined infection, n=81	24	6.1	44	14.9*	13	13.3*

The significant difference: * $p < 0.05$

Due to literature data on significant association of EPEC with clinical AII symptoms only in children of first months of life, we have evaluated distribution of cases and identification of EPEC/EAgEC by age in the main and control groups (Table 5).

Table 5. Distribution of identified EPEC/EAgEC cases by age in the main and control groups

Main group, n=789						Comparison group, n=147					
Age, months	Number of children	EPEC, abs.	EPEC, %	EAgEC, abs.	EAgEC, %	Age, months	Number of children	EPEC, abs.	EPEC, %	EAgEC, abs.	EAgEC, %
1-6	195	4	2.05*	6	3.08*	1-6	25	0	0	0	0
7-12	243	28	11.52	13	5.35	7-12	37	5	13.51	3	8.1
13-18	121	20	16.52	6	4.96	13-18	24	7	29.16	4	16.66
19-24	63	8	12.7	2	3.17	19-24	13	2	15.38	1	7.69
25-36	99	6	6.06	8	8.08	25-36	22	6	27.27	2	8.33
37-18	43	6	13.95 *	4	9.3	37-18	9	0	0	1	11.1
49-60	25	0	0	0	0	49-60	17	2	11.76	1	5.88

Significant difference: * $p < 0.05$

* Percentage of the pathogen in this age group

n - combined detection

Considering the fact that no diarrheal *Escherichia* were found in a group of healthy children from 1 to 6 months, we have supposed it to be a valid argument for association of EPEC and diarrheal symptoms in children during the first months of life. A similar observation is relevant for EAgEC group ($p < 0.05$).

To clarify the epidemiological history, we have conducted a survey of parents of patients with laboratory-confirmed colibacillosis infection (Table 6).

Table 6. Supposed ways and factors of contamination with enteropathogenic and enteroaggregative colibacillosis according to survey

Supposed ways and factors of contamination	EPEC, n=72		EAgEC, n=39	
	Abs.	%	Abs.	%
Alimentary, including	9	12.5	10	4.3

products: - Milk and dairy products				
- Vegetables and herbs	12	16.7	5	12.8
- Fruit and berries	14	19.4	6	15.4
- Water, esp. of low quality	8	11.1	5	12.8
- Contacts: with a sick family member, in the kindergartens, playgrounds etc.	19	26.4	7	17.8
Not established	17	23.6	10	25.6

Significant difference: $p < 0.05$

Parents of patients with enteroaggregative colibacillosis associated infections with medical assistance (previous hospitalization in other hospitals) significantly more often than parents of children with enteropathogenic colibacillosis (25.6% and 9.7%, respectively).

Analyzing premorbid background of children with colibacillosis and children in the comparison group, we have found that most colibacillosis cases were recorded in children with family history – in 37 patients (31%), nervous system pathology - in 12 (10%), pathology of pregnancy and childbirth – in 48 (43%).

Analysis of feeding of children with colibacillosis at the age of 1-1.5 years showed that 14 of them (36.8%) received breastfeeding up to one year of age, 4 (10.5%) children received breast milk until 2 and 6 months, and only 4 (10.5%) patients received bottle-feeding from the very birth. Thus we have concluded that preservation of breastfeeding in the first year of life does not prevent contamination.

Clinical features of enteroaggregative and enteropathogenic colibacillosis in children

Given the lack of data on clinical manifestation EAgEC in the Russian literature, the description of clinical manifestations of the disease was of interest. Enteroaggregative colibacillosis was identified in 39 patients with acute intestinal infections, of them 15 patients were diagnosed with mono-infection, among them 7 (46.7 %) in the first year of life, from 1-3 years - 6 children (40%), 3-5 years- 2 children (13.3%), and 24 patients – combined form.

Detailed analysis of enteroaggregative colibacillosis mono-infection has shown that acute onset of the disease was observed in 8 (53.3%) patients, subacute - in 7 (46.7%). Fever was the first monosymptom in 5 (33.3%) patients. In 9 (60%) patients the disease began with a combination of several disease symptoms, of them fever and liquid stool was reported more frequently – in 6 (40%) patients. Manifestation of the disease with fever and vomiting was recorded in 1 (6.7%) patient, with vomiting and loose stool - in 1 (6.7%) patient, with fever, vomiting and liquid stool at the same time – in 1 (6.7%) patient.

Fever was recorded in all patients on the first day of the disease, the average value of fever was – $39.03 \pm 0.01^{\circ}C$. Hyperthermia (body temperature more than $39.5^{\circ}C$) was noted in 5 (33.3%) patients, febrile (body temperature up to 38 – $39.5^{\circ}C$) – in 9 (60%) patients, subfebrile (fever up to 38° C) – in 1 (6.7%) child. Middle duration of fever was 3.7 ± 0.1 days, lytic temperature decrease was most frequent - in 12 (80%) patients, intermittent fever was recorded in 3 (20%) patients.

Vomiting was detected in 10 patients (66.7%). Average day of this symptom was 2.5 ± 0.6 days, average frequency – 3.4 ± 0.9 times / day, average duration – 2.2 ± 0.4 days.

Abnormal stool was recorded in all patients with enteroaggregative colibacillosis. Average daily occurrence of diarrhea was 1.4 ± 0.2 days, average rate - 4 ± 0.5 times / day, mean duration of diarrhea – 2.8 ± 0.7 days. In 10 (66.7%) patients liquid stool was reported, watery - in 5 (33.3%) patients. A number of patients revealed pathological impurities in the stool: in 7 (46.7%) patients- mucus, in 1 (6.7%) - blood.

Enteroaggregative colibacillosis was often of gastroenteritis type - 6 (40%), and enteritis type - 6 (40%) patients, less by gastroenterocolitis type – in 3 (20%) patients; no other forms of the disease were registered. Complaints of epigastric abdominal pain was recorded in 1 (6.7%) patient. Bloating was noted in 2 (13.3%) patients, urchanie- in 1 (6.7%) patient. Hepatomegaly was detected in 4 (26.7%) patients, splenomegaly- in 2 (13.3%) patients.

Enteroaggregative colibacillosis was in mainly moderate form - in 10 (66.7%) patients, in the mild form - in 5 (33.3%) patients, no severe forms of the disease have been recorded. The severity of the condition was determined mainly by intoxication symptoms - in 12 (80%) patients, of them in 11 (73.3%) patients symptoms were moderate, expressed - in 1 (6.7%) patient. Loss of appetite was noted in 50% of affected children - in 8 (53.3%) patients. The average duration of intoxication syndrome was 2.33 ± 0.3 days. Exsicosis, grade 1-2 was recorded in 1 (6.7%) patient. In general, CBC in the acute phase of the disease revealed leukocytosis (> 11 x 10^9/ L) in 7 (46.7%) patients, leukopenia- in 4 (26.7%) patients, increased segmented leukocytes – in 9 (60%) patietns, increased rod nuclear leukocytes - in 2 (13.3%), eosinopenia - in 2 (13.3%), lymphocytopenia - in 10 cases (66.7%), monocytopenia- in 4 (26.7%) patietns, ESR acceleration – in 4 (26.7%) cases.

It is important to emphasize that enteroaggregative colibacillosis was complicated with the urinary system pathology in 8 (53.3%) patients, which was manifested by urinalysis changes as a moderate leukocyturia, proteinuria, acetonuria, induration of pelvicalyceal system in ultrasound. Among patients with complicated course of enteroaggregative colibacillosis boys were 3 (37.5%), girls – 5 (62.5%). Of them, 4 (50%) children in the first year of life, 3 (37.5%) patients 1-3 years, 3-5 years - 1 (12.5%) patient. Complications concerning urinary tract were noted in 3 patients (37.5%) in summer, in 4 (50%) patients in the autumn, in 1 (12.5%) patient in winter. In the first three days of the disease this complication was diagnosed in 2 (25%) children, by day 4-5 – in 4 (50%), after 5 days of the diesease - in 2 (25%) children. A repeated urinalysis on day 5 of therapy showed that lab parameters came to the age norm. In children with urinary tract infection complications neither burdened history, nor hereditary urinary system pathology had previously been mentioned. Enteroaggregative colibacillosis was as well complicated with infectious cardiomiopathy in a small number of patients – in 3 (20%), which was confirmed by auscultation, electrocardiogram, echocardiogram, changes in the level of cardioenzymes (CPK, CPK-MB, LDH, LDH-1.2, troponin) in the blood chemistry.

Since in our study enteropathogenic and enteroaggregative *Escherichia* was the most common, we have conducted a differential search - diagnostic hallmarks of enteroaggregative and enteropathogenic mono-infection (Table 7).

Comparison groups were matched in age, premorbid background, terms of hospitalization.

Table 7. Differential-diagnostic features of enteroaggregative and enteropathogenic mono-infections

Differential diagnosis signs	EPEC, n=20		EAgEC, n=15	
	Abs.	%	Abs.	%
Onset, with monosymptome (liquid stool)	4	20*	0	0

Fever on day 1	12	60	15	100*
Fever by day 2-3	4	20*	0	0
Pathological green feces impurities	5	25*	0	0
Frequency of exsicosis, grade 1	3	15*	0	0
GIT disorder, enterocolitis type	4	20*	0	0
Frequency of leukocytosis in the acute period	4	20	7	46.7*
Average duration of fever, days	5.2±0.6*		3.73±0.1	
Average duration of pathological stool, days	6.94±0.2**		2.8±0.7	
Average frequency of pathological stool, times/day	5.6±0.2**		4±0.5	

Significant difference: * $p < 0.05$; ** $p < 0.01$

Unlike enteroaggregative colibacillosis, enteropathogenic colibacillosis often began with a monosymptome - loose stool and was characterized by a longer fever that often began on days 2-3 from the onset of the disease, as well as a longer duration and severity of intestinal dysfunction, presence of abnormal stool with green impurities, gastrointestinal lesions by enterocolitis type and development of dehydration. At the same time enteroaggregative colibacillosis was characterized by fever, which appeared on day 1 of the disease, as well as higher frequency of leukocytosis and UTI in the acute phase.

Analysis of the effectiveness of complex initial colibacillosis therapy in children

Given absence of unified approaches to colibacillosuis therapy in children to date, we have retrospectively evaluated "initial" treatment of these diseases.

Table 8 presents data on the duration of the main colibacillosis symptoms and deviation from the age norm of leukocytes in CBC during the early recovery in patients treated with different initial therapies, including oral rehydration and antibacterial agents, or entersorbents. The compared groups were matched by age, premorbid background and hospitalization terms.

Table 8.

Duration of the main symptoms of colibacillosis and deviation of leukocytes from the age norm in the CBC during early convalescence in patients treated with different initial therapies

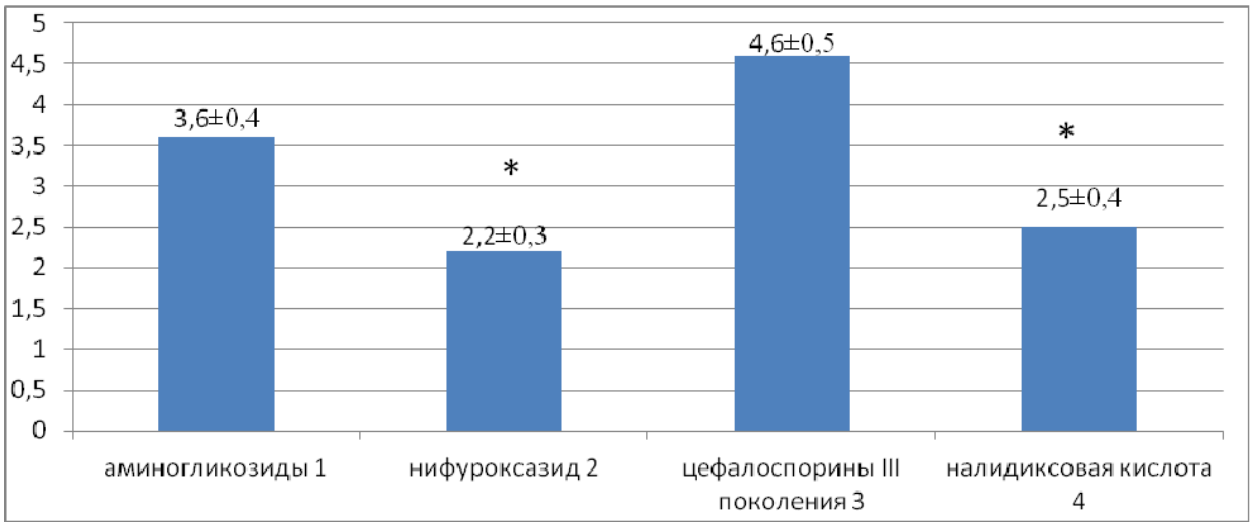
Symptom duration, days	Antibacterial therapy (n=40)	Enterosorbents (n=36)
Fever	4.36±0.4*	2.86±0.3
Intoxication	2.45±0.2	2.37±0.2
Vomiting	2.36±0.4	2.58±0.3
Pathological stool	5.9±0.8	4.29±0.5

Leukocytosis	16±5.8	5.9±3.9
Leukopenia	40±7.7	23.5±7.1
Normocytosis	44±7.8	70.6±7.6*

Significant difference: * p <0.05

We have not found any essential differences in the dynamics of the clinical relief symptoms in the two groups, but the fever against the background of antibiotic therapy was longer, moreover, during the treatment with enterosorbents level of white blood cells did not change significantly and remained within the age norm.

Assessing the clinical efficacy of different antibiotic groups, we have noted that nifuroxazide and nalidixic acid had the greatest efficacy: in patients receiving these preparations, fever duration was significantly less compared with patients receiving aminoglycosides and cephalosporins of III generation (2.2 ± 0.3 days in patients treated with nifuroxazide, 2.5 ± 0.4 days in patients receiving nalidixic acid vs 3.6 ± 0.4 and 4.6 ± 0.5 days, respectively) (Figure 3).

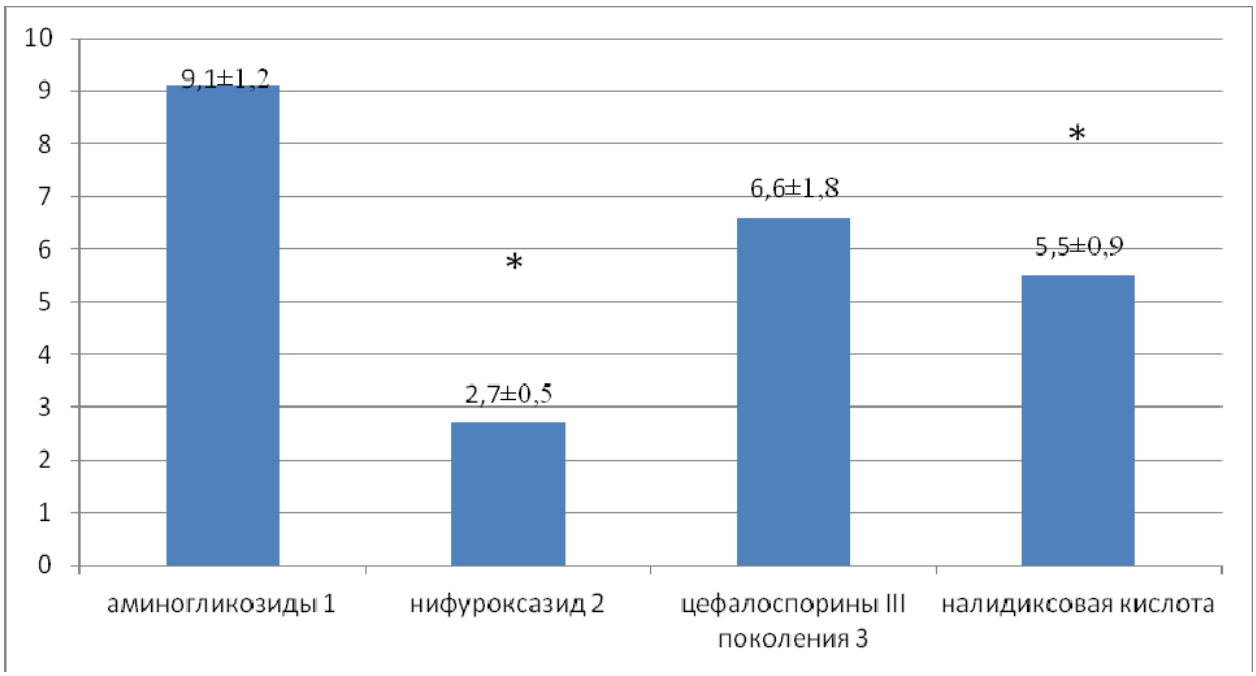


Significant difference: p <0.05: * between 2 and 1; between 2 and 3 ** between 4 and 1; between 4 and 3

Figure 3. Duration of fever in patients receiving initial treatment with various antibacterial drugs

Duration of vomiting in patients treated with aminoglycosides (1.8 ± 0.3 days) and nalidixic acid (2 ± 0.3 days) was significantly lower compared with that of patients receiving nifuroxazide (3± 0,7 days).

Duration of pathological stool in patients receiving nifuroxazide was 2.7 ± 0.5 days, which was significantly less compared with patients receiving aminoglycosides – 9.1 ± 1.2 days. In patients treated with nalidixic acid duration of diarrhea was 5.5 ± 0.9 days, which was significantly less than in patients receiving cephalosporines of III generation- 6.6 ± 1.8 days (Figure 4).



Significant difference: p <0.05 * between 2 and 1

** Between 4 and 3

Figure 4. Duration of abnormal stool in children receiving initial treatment with various antibacterial drugs

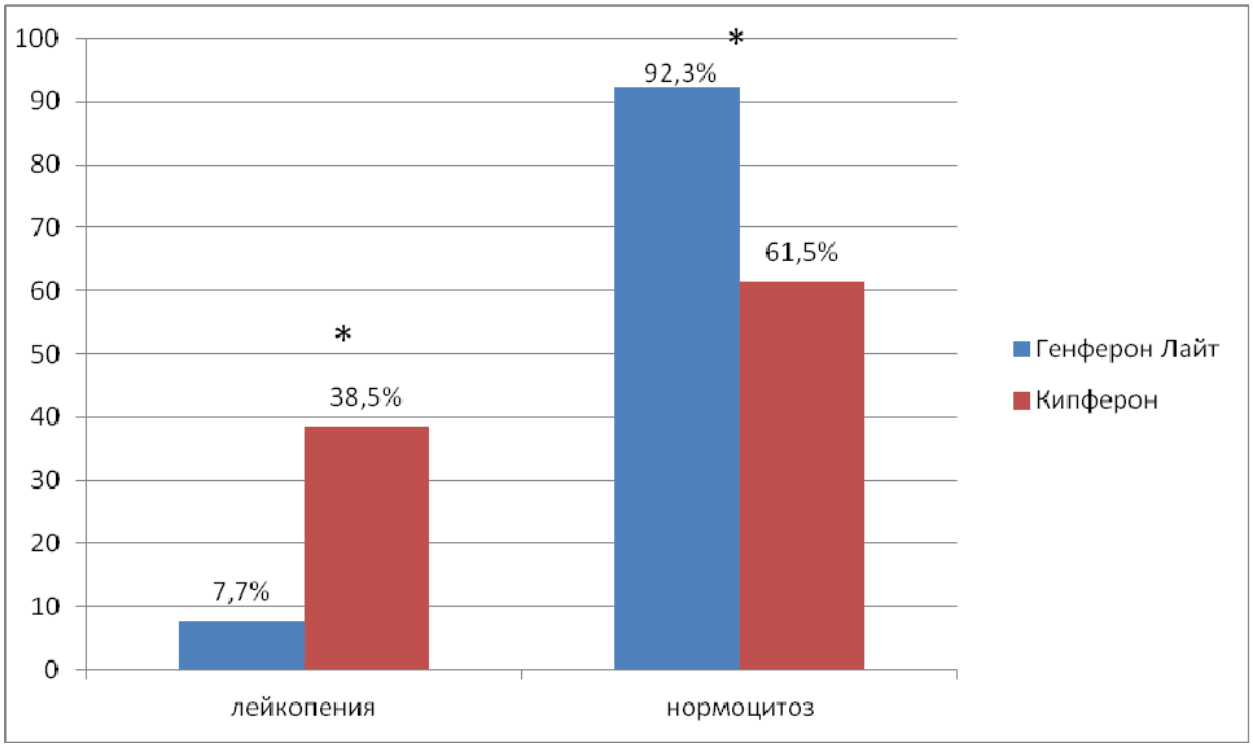
When comparing the effectiveness of different enterosorbents no significant difference has been found in terms of disappearance of the disease symptoms, except that the duration of pathological stool in patients treated with dioctahedral smectite was significantly higher than in patients taking polymethylsiloxane polyhydrate (5.8 ± 0.7 and 4± 0.4 days).

Given the fact that the majority of colibacillosis cases was combined (mostly with viruses, of them - with rotavirus), we have for the first time tested a new algorithm of combined therapy of colibacillosis-rotavirus, which would incorporate "Initial therapy "immune preparations (Genferon Light, Kipferon). The patients were divided into two groups: the first group included 26 patients receiving immunotropic drugs and antibiotics, the second group included 14 children treated with immunotropic tools and enterosorbents.

Comparison groups were matched for age, gender, premorbid background, terms of hospitalization (the sample included children with colibacillosis and rotavirus of moderate forms).

It has been found that in patients receiving immunotropic and antibacterial agents as initial therapy, intoxication persisted for a longer period compared to those treated with enterosorbents and immunotropic drugs (3.3 ± 0.2 and 2.3± 0.3 days, respectively, p <0.05).

In a comparison between the effectiveness of various immune preparations (Genferon Light and Kipferon) it has been revealed that in patients receiving Genferon Light, fever duration was significantly lower compared with that of patients receiving Kipferon (2.5 ± 0.3 and 3.6± 0,3 days, respectively, p <0.05), intoxication duration was as well significantly less (2.3 ± 0.3 and 4.3 ± 0.8 days, respectively, p <0.05). During the convalescence period in patients treated with Genferon Light CBC level of leukocytes was often within the age norm, whereas patients receiving Kipferon had more frequent leukopenia (Fig. 5).



Significant difference: p <0.05

Figure 5. Deviations from the age norm of CBC leukocytes in the early convalescence in patients treated with various immune preparations, %

Thus, our analysis has allowed to develop and implement the new algorithm of combined colibacillosis therapy providing a combination of oral rehydration and enterosorbents, when necessary - antibacterial drugs (nifuroxazide or nalidixic acid). In combination of colibacillosis with rotavirus, combination of initial therapy with Genferon Light with enterosorbents and oral rehydration has been optimal.

Conclusions

1. Frequency of diarrheal *E. coli* among children admitted to hospital with acute intestinal infections, was 15.1% (mono-infection - 4.8%, combination - 10.3%). In colibacillosis monoinfections structure enterotoxigenic E. coli had 52.6%, enteroaggregative - 39.5%, enterohaemorrhagic -5.3 %, enterotoxigenic - 2.6. Combined infection was in 70.4% an association of *Escherichia* with viruses (often with rotavirus), 17.3% - *Escherichia* with bacteria and in 12.3% - multiple pathogens (*Escherichia* in combination with pathogenic viruses and bacteria simultaneously).

2. Colibacillosis infection is recorded in all the seasons, while for enteroaggregative colibacillosis autumn is more characteristic.

3. Colibacillosis is more often detected in young children (1-3 years) with a premorbid background (pregnancy and birth pathology, family history, nervous system disorders) regardless of gender. Continuation of breastfeeding in the first year of life does not prevent the contamination.

4. A direct association is in the presence of the most common *Escherichia* groups (EPEC and EAgEC) and diarrheal symptoms in children during the first six months of life.

5. The clinical picture of enteroaggregative colibacillosis is characterized by the acute onset of the disease with a combination of several symptoms at the same time (usually fever and watery stool). Characteristic

symptoms of the disease are fever (appears on the first day of the disease, often has febrile nature and is characterized by a lytic decrease) and pathological stool (usually liquid fecal stool with pathological mucus impurities). Enteroaggregative colibacillosis occurs mainly by enteritis and gastroenteritis type, in the moderate form and with moderate intoxication. In a small group of patients abdominal pain, bloating, rumbling along the large intestine, hepatosplenomegaly and exsicosis are recorded. In half of the patients the disease pathology is complicated by urinary tracts infections, less frequent - with infectious cardiomyopathy.

6. Differential diagnostic differences between enteropathogenic and enteroaggregative colibacillosis includes the nature of manifestations - primarily with monosymptomes (liquid stools), longer duration and frequency of diarrhea, longer fever (usually appears by day 2-3), as well as frequent development of exsicosis and the digestive tract disorder by enterocolitis type.

7. High clinical effect has been proved for enterosorbents combined with oral rehydration in the initial treatment of uncomplicated colibacillosis. Antibiotics (nifuroxazide and nalidixic acid) are the drugs of choice in complicated colibacillosis in children.

8. Appointment of immune preparations (Genferon Light, Kipferon) in the acute phase of the combined colibacillosis and rotavirus contributes to an earlier clinical reduction of the disease manifestations.

Practical recommendations

1. To improve the diagnostic quality of colibacillosis in the hospital, it may be recommended to use molecular biological methods more widely to identify differentiated groups of diarrheal *E. coli*.

2. In uncomplicated colibacillosis, application of enterosorbents (dioctahedral smectite and polymethyloxane polyhydrate) in combination with oral rehydration dose is recommended in the age doses during courses of 3-5 days.

3. In the treatment of complicated colibacillosis nifuroxazide is recommended (in children under two years the drug is prescribed only in the form of suspension: 1-6 months- 100 mg (2.5 ml or1 / 2 measuring spoon) 2-3 times / day; from 7 months to 2 years of age the recommended dose is 100 mg (2.5 ml or 1/2 measuring spoon) 4 times / day.; in children from 2 to 7 years - 200 mg 3 times / day, daily dose - 600 mg) and nalidixic acid (60 mg / kg distributing the daily dose into 4 equal parts, for at least 5 days).

4. In combination of colibacillosis with rotavirus infection, initial therapy is indicated including immune preparations, especially Genferon Light (children under 7 years - 125 000 IU of interferon alpha-2b *per rectum* twice a day for 5-7 days).

5. Given the fact that there are often "healthy" carriers of pathogenic *Escherichia*, it is recommended to interpret detection of enteroaggregative and enteropathogenic *Escherichia* as clinically significant in children under six months.

List of papers published on the topic

1. Role of pathogenic Escherichia in the structure of acute intestinal infections in children / Bondarev A.V., Gorelov A.V., Podkolzina A.T., Nikolaeva T.A. // Topical issues of infectious diseases in the clinic. Standards of diagnosis and treatment: Proceedings of XV Russian scientific-practical conference dedicated to 75th anniversary of the Department of Infectious Diseases. Academician Rudnev G.P. - Makhachkala. - 2010. - P. 72-73.

2. The effectiveness of causal treatment of acute bacterial intestinal infections in children at the present stage / Ploskireva A.A., Gorelov A.V., Usenko D.V., Bondarev A.V. et al // Infectious Diseases. -2011. – Vol. 9. – No. 4. - P. 79-83.

3. Pathogenic *Escherichia* in the etiological structure of acute intestinal infections in children at the present stage / Bondarev A.V., Gorelov A.V., Podkolzina A.T., Nikolaev T.A. // Topical Issues of infectious diseases and vaccine prophylaxis: Materials of X Congress of Russian children's infectious diseases. - Moscow. - 2011. - P. 17-18.

4. Modern approaches to the treatment of acute viral diarrhea in children / Gorelov A.V., Ploskireva A.A., Bondarev A.V., Lytvynchuk O.A., Zhuchkova S.N. // Infectious diseases and antimicrobial agents: Materials of IX scientific conference. - Moscow. - 2011. - P. 26-28.

5. New possibilities in the treatment of acute intestinal infections in children / Gorelov A.V., Feklisova L.V., Ploskireva A.A., Meskin E.R., Bondarev A.V. et al. // Infectious Diseases. - 2012. - Vol.10. – No. 1. - P. 42-49.

6. Modern approaches to intensive therapy of acute intestinal infections in children / Ploskireva A.A., Gorelov A.V., Zhuchkova S.N., Bondarev A.V. et al. // Infectious Diseases. - 2012. - Vol.10. – No. 1. - P. 50-55.

7. The role of pathogenic *Escherichia* in the seasonal incidence of acute intestinal infections in children at the present stage / Bondarev A.V., Gorelov A.V., Podkolzin A.T., Nikolaeva T.A. // Infectious Diseases. - 2012. - Vol.10. - Appendix No. 1. Proceedings of IV annual Russian Congress on Infectious Diseases. - Moscow. - 2012. - P. -60-61.

8. The role of pathogenic *Escherichia* in the etiological structure of acute intestinal infections in children at the present stage / Bondarev A.V., Gorelov A.V., Podkolzin A.T., Nikolaeva T.A. // Infectious Diseases. - 2012. - Vol.10. - Appendix No. 1. Proceedings of IV annual Russian Congress on Infectious Diseases. - Moscow. - 2012. – P. 61.

Abbreviations

GIT- gastrointestinal tract

UTI- urinary tract infection

IIUE- intestinal infection of unknown etiology

UT- urinary tract

CBC- complete blood count

AII - acute intestinal infection

PCR - polymerase chain reaction

EPEC- enteropathogenic *Escherichia*

EAgEC- enteroaggregative *Escherichia*

EHES - enterohaemorrhagic *Escherichia*

ETES - Enterotoxigenic *Escherichia*

EIEC + *Shigella spp* – complex of enteroinvasive *Escherichia* with *Shigella*