



## Pro-Inflammatory Cytokine Levels and Their Association with the Emergence of Antibiotic Resistance in Children with Escherichiosis Before The

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**Abstract:** Currently, one of the important scientific directions in the treatment of acute intestinal diseases is the study of the immune system. It is known that with the development of infectious enterocolitis in children, changes in the number of T – lymphocytes, immunoglobulins and cytokines are noted. The study examined the indicators of cytokine levels in sick children and the associated antibiotic resistance of E.coli. The results obtained determine the features of the course of diarrhea developed as a result of hemolytic escherichia.

**Keywords:** children, E.coli, cytokines, interleukins, antibiotics, diarrhea, antigen.

**Introduction.** According to the World Health Organization, as many as 1 billion children a year develop acute intestinal infection (AEI) and diarrhoea, ranging from 3.1 million (8,400 per day) to 4 million children a year, most of them in developing countries. It is estimated that 1.87 million children under 5 die each year from diarrhoea. 8 out of 10 deaths occur in the first 2 years of a child's life. Acute intestinal infections account for the majority of hospital admissions (30-45 per cent).

In Uzbekistan, 30-32% of diarrhoeal diseases are caused by Escherichia coli. Common  $\beta$ -lactam antimicrobials, aminoglycosides, quinolones and many biologics are used in the treatment of diseases caused by Escherichia coli and in the correction of intestinal microflora. However, the increasing resistance of E. coli to most antibiotics used in recent years has resulted in ineffective treatment. In recent years, there has been an increased interest in studying the effects of cytokines on the development of infectious diseases. One should note the particular importance of interleukins in the general inflammatory process, immune response formation, T- and B-lymphocyte activation, chemotaxis, adhesion, prostaglandin synthesis, infantile immune system formation, and cell apoptosis. Determination of cytokines amount in peripheral blood of the children with escherichiosis allows controlling immunologic changes and pathologic process forming in the organism.

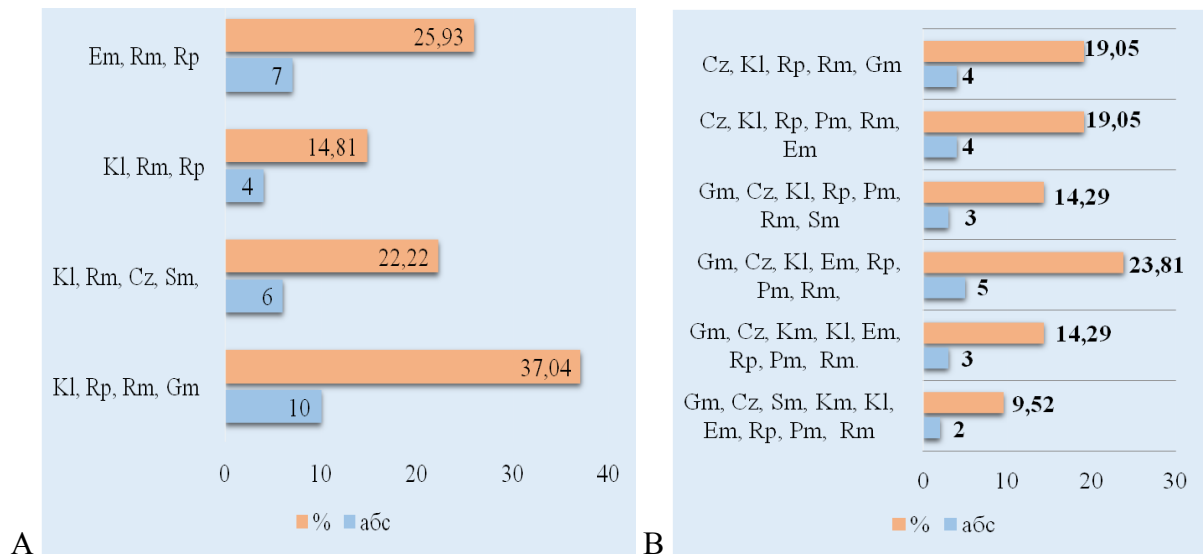
**Objective of the investigation:** investigation of proinflammatory cytokines amount and antibiotic resistance formation in children with hemolytic escherichiosis.

**Research Methods.** Solid-phase enzyme immunoassay was used to determine cytokines, Vector-Best (Russia) was used to detect interleukins IL-6 and IL-8. Bacteriological examination was performed using Endo medium and Müller-Hinton medium to determine antibiotic sensitivity.

Identification of E.coli strains was carried out by agglutination reaction on glass using polyvalent escherichiosis sera of OCA, OQA, OCS, OCD and OCE groups produced by I.I. Mechnikov Biomed JSC.

**Results and discussion:** During the study 232 strains of Hly+ escherichiae were isolated according to immune status of children with coliinfection and treated with common antibiotics (gentamicin (Gm), cefzol (Cz), streptomycin (Sm), kanamycin (Km), clofaran (Kl), erythromycin (Em), rifampicin (Rp), polymyxin (Pm), rovamycin (Rm) etc). The cytokines IL-6, IL-8 in peripheral blood

serum were also studied in the resistance of haemolytic escherichia isolated from these patients. From 21 seriously ill children 27 strains of haemolytic Escherichiae were isolated, and their resistance to antibiotics was determined. From 7 critically ill children 21 strains of haemolytic Escherichiae were isolated and antibiotic resistance was determined.



**Figure 1. A- Antibiotic resistance of haemolytic Escherichiae isolated from moderately ill children B- Antibiotic resistance of haemolytic Escherichiae isolated from severely ill children**

Figure 1 shows that strains of Escherichiae were not resistant to one or two antibiotics, 40.7% were resistant to three and 59.3% were resistant to four antibiotics.

In severe disease, with levels of IL-6 and IL-8 ranging from 10-18, strains of Escherichia coli were resistant to a number of antibiotics, including 38.1% to types 5-6, 38.1% to type 7, and 23.8% to types 8-9. Relative resistance was observed.

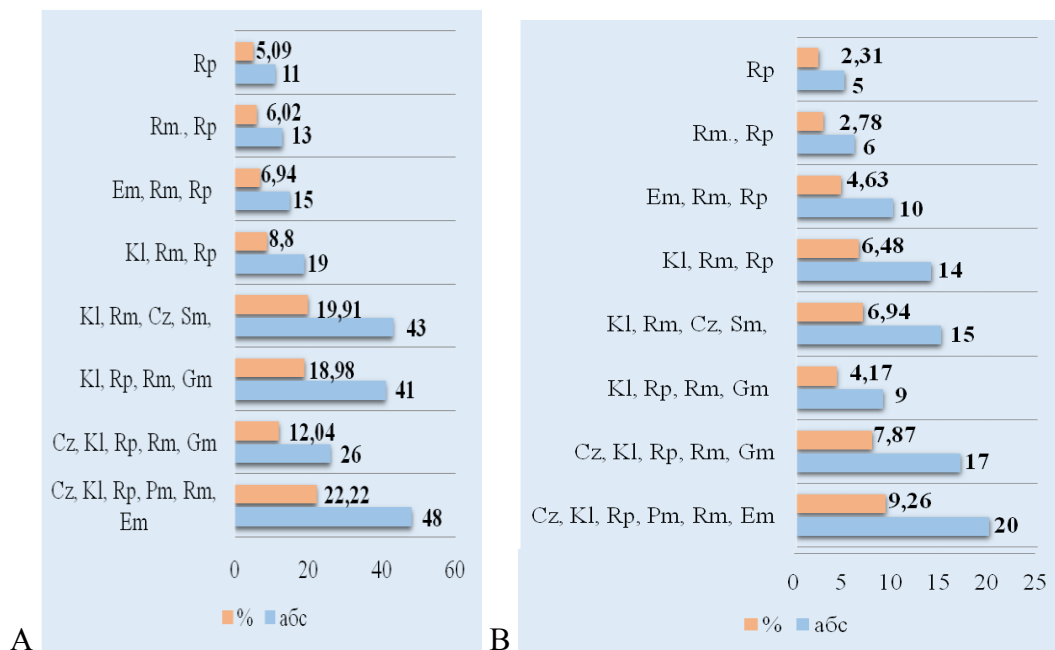
A change in the number of cytokines in relation to the severity of the disease resulted in an increase in antibiotic resistance of Escherichia coli.

Thus, in patients with OCI, caused by hemolytic Escherichiae, stabilization of IL-8 to a normal level during the period of recuperation shows the recovery without development of special complications. A correlation between cytokine concentrations and clinical symptoms showed that elevated serum IL-6 concentrations in the acute period of the disease ( $r_1$ ) and at the onset of clinical signs of UCI caused by hemolytic escherichia ( $r_2$ ) correlated with the following symptoms pain ( $r_1 = 0.31$ ,  $r_2 = 0.35$  respectively), liquid stool ( $r_1 = 0.30$ ,  $r_2 = 0.32$ ), vomiting ( $r_1 = 0.31$ ,  $r_2 = 0.33$ ). Pro-inflammatory cytokine production is associated with the development of symptoms of gastroenteritis and enterocolitis. In UCI caused by Haemolytic Escherichia coli, a direct link was established between high levels of inflammatory mediators in the acute period of the disease and changes such as increased body temperature, the appearance of symptoms of gastroenteritis and enterocolitis. Increased amount of anti-inflammatory cytokines indicated that inflammatory processes in patients were not over. Preservation of IL-6 at high levels in blood serum indicated the development of complications caused by hemolytic escherichia in UCI patients. Relationship between the amount of inflammatory and anti-inflammatory cytokines in blood serum, inflammatory mediators and clinical and laboratory symptoms, appearing in patients with UCI, determined the development of severe forms of the disease and its course. In UCI caused by Escherichia haemolytica, the disease mainly manifested as gastroenteritis (92.9%) and enterocolitis (7.1%). In patients with UCI caused by hemolytic escherichia, increased levels of inflammatory cytokines in blood serum were observed during exacerbation of the disease. In the recuperation phase, the concentration of inflammatory cytokines decreased sharply. The highest stage of IL-8 production coincided with a reduction in clinical symptoms and the period of recuperation. A direct correlation between IL-6 and other interleukins was observed during exacerbation and recurrence of clinical symptoms, and this was observed together with the development of gastroenteritis symptoms. At disease severity levels (IL-6

and IL-8:10-18), resistance of strains of *Escherichia coli* to a number of antibiotics was observed, including 38.1% to type 5-6, 38.1% to type 7, and 23.8% to types 8-9. Haemolytic *Escherichia coli* strains isolated from children with moderate disease severity were not resistant to one or two antibiotics, 40.7% were resistant to three and 59.3% were resistant to four antibiotics.

A total of 492 strains of *Escherichia coli* isolated from children with UCI caused by haemolytic *Escherichia coli* did not show resistance to one or two antibiotics. Most Hly+ *E.coli* strains were isolated from sick children, i.e. 159 (73.6%) strains were resistant to 5-9 antibiotics, 57 strains (26.4%) were resistant to 3-4 antibiotics.

Out of 260 Hly-*E.coli* strains isolated from sick children, 74 strains (34.2%) were resistant to 5-6 antibiotics, 62 strains (54.6%) were resistant to 3-4 antibiotics and 24 strains (18.2%) were determined to be resistant to 1-2 antibiotics.



**Figure 2. Antibiotic resistance of A-Hly+ *E.coli* and B-Hly-*E.coli* strains isolated from sick children caused by A-haemolytic *Escherichiae***

Of the 96 strains of Hly- *Escherichia coli* isolated from healthy children, 37 (38.5±1.5%) were resistant to 5-6 antibiotics, 48 strains (50.0±1.7%) to 3-4 antibiotics and 11 strains (11.5±2.5%) were resistant to 1-2 antibiotics.

Polyresistance to 7-9 types of antibiotics was not detected in Hly-*E.coli* strains isolated from children with UCI caused by hemolytic *Escherichia spp.* *Escherichiae* Hly+ and Hly-, isolated from ill children, developed resistance to erythromycin and polymyxin in nearly 90% of bacteria studied for primary selection, and to streptomycin and kanamycin in 65-80%.

A statistical study by region found that strains of *Escherichia coli* are highly resistant to antibiotics in Samarkand city and Samarkand district, while antibiotic resistance is low in neighbouring districts.

The unwarranted use of various antibiotics on a large scale has caused the emergence and spread of multidrug-resistant strains of *E.coli*. Haemolytic *Escherichia coli* which developed coli-infection in children showed resistance to 6-9 types of antibiotics, while in healthy children this figure was 2-4 antibiotics.

The sensitivity of Hly+ and Hly- *Escherichia coli* to antibiotics has been found to vary greatly. This requires that the sensitivity of the isolated culture to antibiotics be determined before treating children with coli infection caused by Haemolytic *Escherichia coli*. The diagnostic significance of proinflammatory cytokines (IL-6 and IL-8) in diseases caused by haemolytic *E. coli* lies in the fact that changes in cytokines, depending on the severity of the disease, resulted in increased resistance of *E. coli* to antibiotics.

**Table 1. Antibiotic resistance of Escherichia strains in Samarkand city and Samarkand region. Samarkand province.**

Antimicrobials	Pastdargom	Jomboy	Okdaryo	Toylack	Urgut	Samarkand region	Samarkand
Amoxicillin	36,2	49,7	42,6	47,9	51,1	56,3	58,8
Co-trimoxazole	19,3	33,3	32,1	30,5	38,5	39,7	44,3
Nalidix acid	16,9	12,5	8,7	9,6	9,9	11,5	21,9
Ciprofloxacin	5,5	3,7	4,9	3,8	3,8	4,4	6,7
Gentamicin	3,7	6,4	12,5	11,8	12,4	13,1	17,5
Netilmicin	2,4	2,5	5,1	3,3	3,8	4,1	6,4
Cefoperazone	4,2	7,6	9,3	4,6	4,7	5,4	12,1
Cefuroxime	5,8	8,2	10,1	7,4	7,1	7,6	14,8
Amoxicillin/clavulanate	5,9	8,7	6,5	7,2	6,8	7,5	11,1
Cefepim	1,9	2,1	2,6	1,7	0,9	3,6	5,2
Nitrofurantoin	0	0	1,8	1,1	0,7	1,3	3,6
Ceftriaxone	1,1	2,4	1,9	2	1,1	1,5	4,8
Cefotaxime	1,3	2,7	4,4	3,4	2,7	4,7	8,9
Ceftazidime	1,2	0	0,9	0	1,3	1,1	2,6
Amikacin	0,5	0	0,3	0	0,8	1,7	2,1
Fosfomycin	0,2	0,8	0	0,7	0	1,1	1,9

Determination of the antibiotic sensitivity of Hli+ and Hli - escherichiae has shown that the sensitivity of the pathogens to antibiotics is highly variable. Antibiotic sensitivity testing of isolated cultures is recommended prior to the treatment of children with coli infection resulting from Haemolytic Escherichia coli. A study of resistance of haemolytic Escherichia coli to antibiotics commonly used in clinical settings showed high resistance to amoxicillin and co-trimoxazole.

### Conclusions:

1. From the above, it can be concluded that the widespread unwarranted use of various antibiotics is responsible for the emergence and spread of multidrug-resistant strains of E.coli. Hlu+ and Hly - Escherichia coli, isolated from ill children, were found to be 90% resistant to erythromycin and polymyxin, and 65-80% resistant to streptomycin and kanamycin.
2. The study found that pathogens in children with coli infection developed as a result of haemolytic Escherichia coli showed resistance to 6-9 types of antibiotics, whereas in healthy children this figure was 2-4 antibiotics.
3. The diagnostic significance of proinflammatory cytokines (IL 6 and IL 8) in E. coli-induced disease is that changes in the number of cytokines, depending on the severity of the disease, also lead to increased antibiotic resistance of E. coli.

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