

STUDY OF ANTIBODIES TO OPPORTUNISTIC ENTEROBACTERIA IN THE BLOOD OF ADULTS

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Received: Jun 22, 2024; Accepted: Jul 29, 2024; Published: Aug 18, 2024;

Abstract: Background: Opportunistic enterobacteria (OE), including various Gram-negative bacteria, are known to cause infections, particularly in immunocompromised individuals. **Specific Background:** Despite their clinical relevance, there is limited data on the prevalence of antibodies against these bacteria in the general adult population. **Knowledge Gap:** The extent to which antibodies to opportunistic enterobacteria are present in serum samples from healthy adults has not been thoroughly investigated. **Aims:** This study aimed to evaluate the presence and frequency of antibodies to seven types of Gram-negative opportunistic enterobacteria in adult serum samples using enzyme-linked immunosorbent assay (ELISA). **Results:** The analysis revealed a significant prevalence of antibodies against the bacteria *E. coli*, *Proteus vulgaris*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* among the subjects. **Novelty:** This research provides new insights into the widespread presence of antibodies to OE in a healthy adult population, highlighting previously underexplored immunological responses. **Implications:** These findings could enhance diagnostic approaches and inform treatment strategies for infections caused by opportunistic enterobacteria, potentially leading to improved management of such infections in clinical settings.

Keywords: Antibodies, Opportunistic enterobacteria, Blood, Enzyme-linked immunosorbent assay, *E. coli*



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Introduction

Recently, there has been an increase in the activity of opportunistic microorganisms worldwide. These bacteria exhibit diverse characteristics: on one hand, they are part of the human body's natural microflora, and on the other, they can cause infectious diseases. The problem of infections caused by these microorganisms is particularly significant when considering the Enterobacteriaceae family. Acute intestinal infections (AIs) are one of the most common infectious diseases, especially among children.

Purpose of the study:

The aim of this study was to identify and evaluate the level of antibodies to opportunistic enterobacteria (OE) in practically healthy adults.

Methods

A total of 45 serum samples were collected from adults who did not complain of gastrointestinal problems and had no history of acute intestinal infections. The age of the subjects ranged from 19 to 37. Among them, 21 (47%) were men and 24 (53%) were women. To investigate the serum for the presence of antibodies to opportunistic enterobacteria (OE), 0.2-0.3 ml of serum was sufficient. Cultures of microorganisms were obtained from the "National Collection of Human Infection Microorganisms" of the Research Institute of Epidemiology, Microbiology, and Infectious Diseases of the Ministry of Health of the Republic of Uzbekistan. Along with the cultures, passports for bacterial strains containing their main characteristics were provided. The study used seven types of 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 aeruginosa* - 004135, ATCC 27853. Complex bacterial antigens were prepared to a concentration of 40 µg/ml at a pH of 9.6-9.8. This concentration was used for the sensitization of the solid phase - polystyrene plates manufactured by "Medpolymer" RF. After washing the antigen-sensitized immunological plates with a washing solution and drying, the test sera were added to the wells, previously diluted in phosphate-buffered saline in a ratio of 1:25 to 1:6400. Incubation was carried out for 1 hour, then the plates were washed and a commercial reagent of antibodies against human IgG labeled with horseradish peroxidase (conjugate) was added. After thorough washing, developing solutions were added to the wells - commercial OFD and hydrogen peroxide. The results were evaluated visually by the staining of the solution in the wells. The obtained data were statistically processed with the calculation of the mean (M), standard error of the mean (m), standard deviation (δ) and Student's t-test (t) according to Fisher-Student.

Results and Discussion

The results of the study are presented in Table 1, which shows the frequency of antibodies to the 7 mentioned representatives of opportunistic enterobacteria (OE) in the serum of the subjects. The reactions were evaluated, conditionally dividing them into the following categories: strongly positive - titer from 1:1600 and above; positive - titer from 1:400 to 1:800; weakly positive - titer from 1:100 to 1:200; doubtful - titer from 1:25 to 1:50; negative - titer 0. The determination of antibodies to 7 representatives of OE showed comparable results for 6 representatives of the Enterobacteriaceae family - seronegative results were from 20 to 30% of sera (Table 2), and seropositive - from 70 to 80%. For *P. aeruginosa*, the number of seropositive sera was slightly higher, but not statistically significant compared to other antigens. Seronegative sera for *P. aeruginosa* - 15%, seropositive - 85%.

Table 1. Results of ELISA for the determination of antibodies in serum against OE antigens, in %.

Complex Microbial antigen	Blood serum titer				
	Strongly positive	Positive	Weakly positive	Doubtful	Negative
E.coli	18±4,3	24±5,9	29±6,6	13±2,4	15,5±3,5
Enterobacter aerogenes	15,5±3,5	18±4,3	27±6,3	15,5±3,5	24±5,9
Enterobacter cloacae	15,5±3,5	20±5	24±5,9	20±5	20±5

Citrobacter freindii	0	22±5,5	22±5,5	29±6,6	18±4,3
Klebsiella pneumoniae	15,5±3,5	24±5,9	27±6,3	20±5	15,5±3,5
Proteus vulgaris	18±4,3	20±5	27±6,3	22±5,5	13±2,4
Pseudomonas aeruginosae	13±2,4	24±5,9	29±6,6	20±5	13±2,4

"For all 7 antigens, the frequency of seropositive sera was significantly higher than seronegative ($P<0.001$)."

Table 2. Results of ELISA for serum with OE antigens, in %.

Complex antigen from	Seronegative sera	Seropositive sera
E.coli	20±8,9	80±8,9*
Enterobacter aerogenes	25±9,7	75±9,7*
Enterobacter cloacae	30±10,2	70±10,2*
Citrobacter freindii	20 ±8,9	80±8,9*
Klebsiella pneumonia	30±10,2	70±10,2*
Proteus vulgaris	30 ±10,2	70±10,2*
Pseudomonas aeruginosae	15±7,9	85±7,9*

Note: * - significance level ($P<0.001$).

Conclusion

- Differences in the frequency of serum antibodies were found among the examined healthy adults.** This indicates that there is a variation in the levels of antibodies against these bacteria among individuals.
- The detected antibody titers against OE antigens (E.coli, Proteus vulgaris, Citrobacter freundii, Klebsiella pneumoniae, Enterobacter aerogenes, Enterobacter cloacae, Pseudomonas aeruginosa) in adults showed a wide range, averaging from 13 to 29%.** This means that the levels of antibodies varied greatly from person to person, even within the healthy adult population.
- The division of the examined groups into 5 categories (strongly positive, positive, weakly positive, doubtful, negative), based on antibody titer values in healthy adults, allows for relative standardization.** This suggests that by categorizing the antibody levels into these groups, it becomes easier to compare results and establish a reference range for healthy individuals.
- The study demonstrated the presence of antibodies to enterobacteria in blood samples of the examined individuals. This indicates contact with these bacteria and a response from the immune system.** This finding suggests that most people have been exposed to these bacteria at some point in their lives, and their bodies have produced antibodies as a defense

mechanism. **Further research may help to better understand the significance of these antibodies for the body's defense and develop methods for preventing and treating related diseases.** This implies that there is still much to learn about the role of these antibodies in protecting against infections and that future studies could lead to new treatments or preventive measures.

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