ISSN NO: 2770-2936
Date of Publication:10-10-2023

# In vitro antibacterial efficacy of bacteriophages against intra-abdominal infection

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**Abstract.** To study the structure and antibiotic resistance of pathogens of intraabdominal infections and to evaluate in vitro antibacterial efficacy of polyvalent pyobacteriophage "Sextaphag" on the biomaterial obtained from patients with peritonitis of different etiology.

**Keywords:** peritonitis, antibiotic therapy, resistance, bacteriophage.

## Introduction

Peritonitis as a complication of a limited (organ) focus of infection or abdominal intervention refers to severe infections with a high mortality rate [1].

The issue of the rational use of antibiotics in recent years has become increasingly important due to the increase in the number of microorganisms resistant to antibiotics. Insufficient development of antibacterial therapy, taking into account the structure of microflora and its antibiotic resistance, is one of the reasons for the unsatisfactory results of treatment of patients with diffuse and diffuse peritonitis.

Antibiotic resistance is currently becoming a problem not only in hospitals, but also for community-acquired infections, especially in their severe course [2].

In a study conducted in 24 hospitals in Spain, the result of inadequate initial therapy compared with adequate, in patients with complicated intraabdominal infection, was a significant deterioration in outcomes (79% vs. 26%, $p<0.001$), which was expressed in more frequent additional administration of antimicrobial drugs, lengthening the duration of hospitalization and an increase in the number of cases of readmission [3].

Also, in the course of another study conducted by J.E. Solomokhin and co-authors, it was revealed that delayed adequate therapy no longer affects the outcome of the disease. In relation
at the same time, the choice of an adequate starting therapy, and most importantly, its timeliness (before receiving the results of a microbiological study) significantly affects survival [4].

To date, the multi-resistance of bacteria against antibiotic therapy is a serious obstacle in the treatment of infectious diseases, and therefore one of the promising directions for solving this problem is the use of bacteriophages for therapeutic purposes.

An example of the use of bacteriophages is a multicenter study conducted by D.S.Parshin and co-authors (2019-2022) in the field of general and emergency surgery, where there was a decrease in the duration of inpatient treatment and mortality by more than 2 times [5]. Also, many other studies conducted in the field of surgery describe the positive effect of the use of bacteriophages [6,7,8].

The above studies prompted the authors of this article to the experimental use of bacteriophages in vitro, in order to substantiate the effectiveness in patients with peritonitis in our region.

## Material and methods

This study was conducted on the basis of the Republican Scientific Center for Emergency Medical Care (RNCEMP) in the period from April to June 2023, where 94 patients were examined who were treated in the emergency surgery department for acute destructive diseases that led to the formation of peritonitis (Table No. 1). The average age of patients was 48.1±15.2 (21-77) years. All 94 (100%) patients underwent surgical treatment.

| Diagnosis | Quantity (n) | % |
|---|---|---|
| Acute gangrenous (gangrenous-perforated) appendicitis | 32 | 34,04 |
| Perforated ulcer of the stomach and duodenum | 13 | 13,83 |
| Pyosalpingitis | 5 | 5,32 |
| Housing and communal services. Acute destructive | 21 | 22,34 |

ISSN NO: 2770-2936
Date of Publication:10-10-2023

| | | |
|---|---|---|
| cholecystitis | | |
| Acute adhesive intestinal obstruction | 10 | 10,63 |
| Sigmoid colon tumour | 8 | 8,51 |
| Acute pancreatitis, pancreonecrosis | 5 | 5,32 |

Table No. 1. Selected destructive diseases that caused purulent peritonitis.

The studied material was the contents of the abdominal cavity obtained directly at the time of surgery, and biomaterial obtained from the drainage tube in the postoperative period. This material in transport media was delivered to the microbiological laboratory within one hour from the moment of sampling.

When performing bacteriological studies, nutrient media produced by HiMedia (India), certified in the Republic of Uzbekistan, were used. HiMedia's products have an International Quality Certificate IS/ISO 9001:2000 and DNV 2005-OSL-AQ-0356.

For laboratory studies, an agar culture of microorganisms was used, diluted in sterile saline solution and standardized according to the turbidity standard 0.5 according to McFarland. Seeding of isolated strains of microorganisms was carried out on Muller Hinton plate nutrient agar (Muller Hinton M173, HiMedia, India) by lawn method. After drying the surface of the agar, standard disks with antibiotics (HiMedia, India) were laid out along the perimeter of the cup, a drop of commercial bacteriophage "Sextaphage" (NPO "Microgen" Russia) was placed in the central part. The results were taken into account after 18-24 hours of incubation in a thermostat at 370 C by the presence of zones of inhibition of microbial growth around the disk with an antibiotic (disco-diffusion method) (Fig. No. 1). The sensitivity of the obtained isolates of microorganisms was determined to 8 antibacterial drugs (amikacin, ceftriaxone, cefazolin, cefoperazone + sulbactam, levofloxacin, ciprofloxacin, meropinem, polymyxin). When performing the study, taking into account and interpreting the results of the sensitivity of antibacterial drugs, they were guided by the EUCAST standard[9].

Fig. No. 1 Decomposed disks with antibacterial drugs (along the perimeter), a spot of bacteriophages "Sextaphage" (in the center).

The assessment of the lysing ability of preparations with bacteriophages was taken into account according to a four-point (four-cross) system proposed by federal clinical guidelines [10]. The results from 3+ to 4+ were taken into account as positive reactions (Fig. No. 2).


Fig. Assessment of the degree of lysis of bacterial isolates by bacteriophage preparations:
"-" lack of lytic activity;
"+" low activity;

ISSN NO: 2770-2936
Date of Publication:10-10-2023

"++" formation of a lysis zone with a large number of colonies of secondary bacterial growth;
"+++" lysis zone with single colonies of secondary growth;
"++++" transparent lysis zone without secondary growth colonies.

**Results**

In biomaterials obtained intraoperatively from patients with peritonitis, etiologically significant microorganisms were isolated (etiologically significant microorganisms were found in concentrations of 104 microbial bodies in 1 ml and above) in 93.6% (88/94) of cases, including 69 aerobic and 12 anaerobic microorganisms, in monoculture – in 26 (27.66%), in microbial associations – in 68 (72.34%) patients.

A total of 23 strains of the Enterobacteriaceae family were isolated, of which 15 were producers of extended-spectrum beta-lactamase (BLRS). Isolation of E.coli was noted in 47.87%, Klebsiella spp. – 18.08%, Enterobacter spp. – 3.19%, Proteus spp. – 2.12%, Acinetobacter spp. – 4.25%, P.aeruginosa – 6.38%, Staphylococcus spp. – 12.76%, S.aureus – 4.25%, Streptococcus spp – 2.12%, Enterococcus spp. – 6.38% (tab No. 2).

| **Isolated pathogen** | **Quantity (n)** | **%** |
|---|---|---|
| E.Coli | 45 | 47,87 |
| Klebsiella spp. | 17 | 18,08 |
| Enterobacter spp. | 3 | 3,19 |
| Proteus spp. | 2 | 2,12 |
| Acinobacter spp. | 4 | 4,25 |
| P. aeruginosa | 6 | 6,38 |
| Staphylococcus spp. | 12 | 12,76 |
| S. aureus | 4 | 4,25 |
| Streptococcus spp. | 2 | 2,12 |
| Enterococcus spp. | 6 | 6,38 |

Table No.2. Pathogenic microflora obtained from biomaterial from patients with peritonitis.

The sensitivity of E.coli strains to the antibiotic drugs ciprofloxacin and ceftriaxone, most often used as starting therapy, turned out to be effective in 31.11% and 6.66%, respectively. According to the results of the disco-diffusion method of sensitivity to antibiotic drugs of other groups, the following results were revealed:

To amikacin was 62.22% of cases, cefazolin - 2.22%, meropenem - 51.11% cefoperazone/sulbactam - 28.88%, levofloxacin - 28.88%, polymyxin B in 37.77%.

According to the evaluation of the effectiveness of the lysing activity of bacteriophages, the following results were obtained: "4+" - in 22 (48.88%) cases, "3+" - in 13 (28.88%) cases; "2+" - in 8 (17,77%); "1+" - in 2 (4.44%) cases, respectively.

| **Antibacterial drug** | **Количество (n)** | **Чувствительность в %** |
|---|---|---|
| Ciprofloxacin | 14 | 31,11 |
| Ceftriaxone | 3 | 6,66 |
| Cefazolin | 1 | 2,22 |
| **Amikacin** | **28** | **62,22** |
| **Meropenem** | **23** | **51,11** |
| Cefoperazone + sulbactam | 13 | 28,88 |
| Levofloxacin | 13 | 28,88 |
| **Polymyxin B** | **17** | **37,77** |

Table No. 3. E.Coli sensitivity (n 100% = 45) to antibacterial drugs.

**Conclusion.**

According to the results of the study, the use of ceftriaxone and cefazolin with an efficiency of 6.66% and 2.22% in peritonitis is not rational. The use of bacteriophages with a sensitivity of 77.76%, in combination

ISSN NO: 2770-2936
Date of Publication:10-10-2023

with the antibacterial drug amikacin, the effectiveness of which was 62.22% as a starting therapy for peritonitis, seems more appropriate in our region.

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