

Study of *E. coli* Pathogens Causing Intestinal Infections and Use of Bacteriophage Preparation-Coli Phage

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Abstract

According to the data of World Health Organization (WHO) about 2 billion of people get sick with intestinal infections annually. The pathogens which spread through food and water are the main causes of the sickness and mortality; they lead to death of about 1.8 million each year. Resulting from the mentioned problem, it carries particular importance to conduct microbiological and molecular research of bacterial pathogens causing intestinal infections. One of the main pathogens, which spread through waters, is variety of Salmonella, pathogenically *Escherichia coli* and Shigella. Monitoring the food and water related pathogens would facilitate the process of downsizing their frequency in the environment, which on its own would reduce the risk of spreading diseases caused by the mentioned pathogen amongst humans. Early identification of these pathogens has the vital importance for quick and effective treatment.

Keywords: Pathogens; *E. coli*

Introduction

Given the specific resistance of bacterial strains to antibiotics, the determination of sensibility to antimicrobial drugs is very important for the monitoring of pathogens and may be used as a necessary epidemiological marker for mentioned bacteria [1-4]. Medications, treatment of the infectious diseases gains more and more problematic characteristics. The reason for this is the fact that throughout the time there have been produced strains of bacteria, which are stable, selective and resistant towards various medication, in the nature. The latter fact has significantly reduced effects of many antibiotics, Sulfanilamide and of other medicines used for treatment, in some cases they even got useless. Resistance towards antibiotics, obtained by microorganisms, remains to

be an important problem until today, because of the wide distribution of the resistant forms of bacteria and reduced effectiveness of existing and widely utilized medicament in the sphere of medicine. Poly-resistant strain that cause group infection diseases [5-7]. Treatment of the intestinal infections represents a unification of the complex measures and includes therapy-first of all detoxication. Treatment is focused on the inactivation of the cause of the disease, besides, throughout the treatment the microbial balance, which later on determines normal viability of the organism, should not be disturbed. While selecting the medication, firstly, the sensibility of the cause towards chosen form of treatment should be taken into consideration. According to the standards of the National Committee for Clinical Laboratory Standards (NCCLS), antimicrobial medication may be utilized for the treatments, whenever 80% of the strains demonstrate sensibility towards them [8-10].

The alternative for the antibiotics are commercial phage medicines, among which is Coli Bacteriophage, which contains specific bacteriophages, which has specific bacteriophages against pathogenic *E. coli*. Using phage-based medications are harmless for human body, since phages have only lysis activity toward pathogenic bacteria [11, 12].

The represented work here aims to identify pathogenic *E. coli* causing intestinal infections in the clinical samples as well as in the food (ground-beef). To define the sensibility of the antibiotics towards the mentioned strains also along with commercial phage medicine-Coli Phage, the structure of which contains bacteriophages against pathogenic *E. coli*. In addition, to determine sensibility of pathogenic strains towards this medicine.

Materials and Methods

Isolation of *E. coli* strains

For isolation of *E. coli* strains, a loopful from the diluted specimens was inoculated into MacConkey's agar and

incubated at 37°C for 18-24 hours. Bacterial colonies were identified based on gross morphology, a number of colonies and the hemolytic pattern. Appropriate biochemical and serological tests were performed on the colonies isolated to identify the pathogens.

Serogrouping of *E. coli* isolates

E. coli serogroups were identified serologically by slide agglutination test using standard polyvalent and monovalent *E. coli* antisera (O114, O86, O26, O55, O36, O111, O119, O125, O126, O142, O157) according to Edwards and Ewing (1972) [13].

Antimicrobial susceptibility testing

To analyze the anti-biogram profile of the isolates, all isolated *E. coli* strains were tested for susceptibility to 15 different antimicrobial agents that are most widely used in clinics: penicilin (P), ampicillin (A), carbenicilin (Cb), ampiclox (Ap) chloramphenicol (C), streptomycin (S), tetracycline (T), gentamicin (G), kanamycin (K), erythromycin (E), metilicin (M), fortum (F), Cephamezin (Cf), ketotefen (Kt), claforan (Cl) Azythromicin (Az), Ciprofloxacin (Cip), Imipenem (Im), Cefazoline (Cef), Polymoxin (Pm). The susceptibility of these isolates to antimicrobial agents was performed by disk-diffusion method in accordance with the standards of the National Committee for Clinical Laboratory Standards [14].

Identification system of the bacteria of the intestinal group

Represents a standardized system, which contains 21 mini biochemical tests and is applied to identify the bacteria of the gastrointestinal group and other gram-negative bacteria.

Principle

Api 20 E consists of 20 micro test-tubes, which contain dehydrated substrates. Bacterial suspensions are put into reaction with a given substrate. While incubation the color changes, which might be defined directly or through adding the reagents.

The process of reading the reactions is carried out through utilizing respective interpretative table.

While cumulating positive results digital profile is produced and through respective analytical catalogue the name and the variety of cause is defined.

Bacteriophage isolation

The bacteriophages were isolated from wastewater by filtration, followed by the addition of broth concentrate and 18 h cultures of different strains (test strains) to the filtrate. After 24 h incubation in a thermostat, the mixture was filtered through filters with a pore diameter of 0.45 µm (Millipore, United States), and the filtrates were spot-tested for the presence of phages by application of the filtrate (0.1 mL) on the lawn of the test strain on a solid nutrient medium. The

result was considered positive if there was a lysis zone on the lawn in 18-24 h of cultivation at 37°C [15]. The highly specific bacteriophage strains were screened and selected by the plate method according to Gracia [16].

Bacteriophage cloning by repetitive single plaque isolation

The phages isolated from the initial material are not uniform. Mixtures of various phage types are frequent, as judged by plaque morphology. Plaques are of various sizes determined by virion size, speed of phage reproduction, etc. Plaques have other characteristic features, such as the sharpness of their edges, the presence of a halo and the completeness of the clearing. A single plaque may contain 10^7 - 10^9 virions. A number of theoretical and practical considerations require the use of "pure" phage lines (clones) during therapy. For this purpose an isolated phage plaque is touched with the fine end of a Pasteur pipette and small specimen is collected, subjected to serial dilution, mixed with plating bacteria and molten soft agar and spread on a plate. Isolation of a reliably pure bacteriophage clone requires about 3-5 such steps.

Electron microscopy: The morphology group membership of bacteriophages was investigated by the method of electron microscopy. Phages with a high titer of $>10^{10}$ CFU/mL were negatively stained with 1% uranyl acetate. Pictures were obtained by transmission electron microscopy (JEM 100 SX, JEOL, Japan) with negative contrasting of the preparations by uranyl acetate.

Results and Discussion

To reach out the defined objectives and aims, in frames of the project we have communicated with clinical laboratory, from there we received strains of the *E. coli* derived from the patients. From the mentioned laboratory we got the various *E. coli* derived from the patients for the further research.

From the Bacteriological Laboratory of the clinical we were given 56 pathogenic strain of *E. coli*. The mentioned pathogens were derived from the various organs of the patients, as follows: Ear, dis-bacteriosis, pus, wound, skin.

Herewith, from the second Bacteriological Laboratory we were given strains of the pathogenic *E. coli*. The mentioned pathogens were derived from the various organs of the patients, as follows: Ear, dis-bacteriosis, pus, wound, and skin. From the second bacterial laboratory, we were handed over with 33 strains of the pathogenic *E. coli*.

In April-May, 2019, the number of various strains given, in accordance with the biosafety procedures, from the clinical microbiological laboratories had been totaled 89.

The above mentioned strains were researched according to the morphological, cultural, biochemical, serological and pathogenic characteristics (**Figure 1**).

Based on the specifics of the work we have as well started conducting analysis on microbiological pollution of foods, namely of ground- beef and pork (**Figure 2**).

In total 20 samples were studied, out of which in 16 cases *E. coli* were identified.

Investigation of morphological, cultural properties and their antigenic structure of the isolated bacteria indicated that the isolated strains of *E. coli* belong to the serotypes, such as O114 (30%), O26 (20%), O36 (25%), O125 (15%), O126 (10%).



Figure 1 Api systems in *E. coli* diagnostics.



Figure 2 Hemolytic activity of *E. coli*.

Pathological strains of *E. coli* derived from the patients were assessed on the antibiotic resistancy. Some of the strains were resistant towards: Azythromicin, Ciprofloxacin, Imipenem, Cefazolin, Polymoxin and chloramphenicol (**Figures 3 and 4**).

Majority of the strains were resistant towards two and more antibiotics.

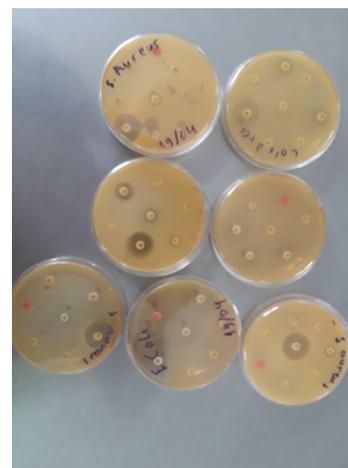


Figure 3 Antibioticograms of the various bacterial strains.

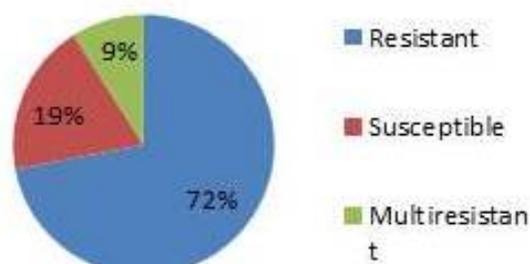


Figure 4 Antibiotic resistance of *E. coli* strains.

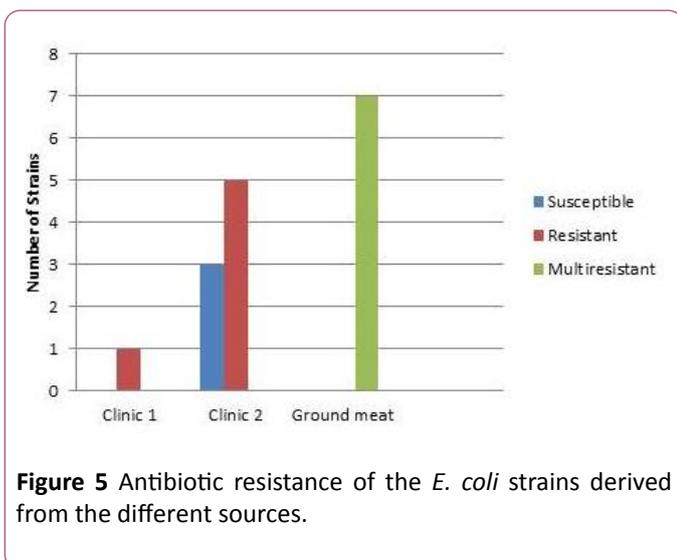
Ground meat bacteriological tests were performed to detect contamination of the above mentioned products.

Overall 20 samples were assessed, out of which 16 samples were contaminated by *E. coli*. This strains were characterized by resistance towards two or more antibiotics, in some cases-towards seven antibiotics.

E. coli strains derived from ground meat were resistant against: Amicacin, Azythromicin, Polymixin, Imipenem.

Interesting fact to mention is that strains of *E. coli* derived from the ground meat are resistant towards the same antibiotics (Amicacin, Azythromicin, Polymixin, Imipenem) as the strains of *E. coli* derived from the patients in the lab (**Figure 5**). Antibiotic susceptibility test revealed high resistance (**Figure 5**).

22 strains out of 56 of *E. coli* obtained from clinic I showed resistance toward antibiotics. From 33 *E. coli* strains obtained from clinic II only 9 isolates revealed resistance. High resistance was shown from strains isolated from ground meat-32 resistant and 7 multi-resistant strains (resistant to four or more antibiotics).



To isolate bacteriophages effective against new isolated pathogenic *E. coli* strains 40 samples of urban sewage water, river water and other environmental sources were investigated. From them 16 samples contained phages lysed strains of pathogenic *E. coli*. For formulating polyvalent phage preparation with widest spectrum of action and high lytic activity all 16 phages were characterized according to host range. These phages were tested against all 89 new isolated pathogenic *E. coli* clinical strains and 16 *E. coli* strains isolated from food. 6 phages conditionally named vB-Eco1, vB-Eco2, vB-Eco3, vB-Eco4, vB-Eco5 and vB-Eco6, with wide, complementary, non-overlapping host ranges were selected for further characterization (**Figures 6-8**).

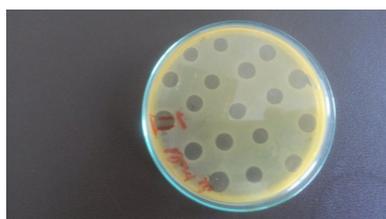


Figure 6 vB-Eco1 effectiveness against *E. coli* strains.

A phage preparation on the base of 6 newly isolated and characterized bacteriophages with a high range of activity against different strains of pathogenic *E. coli* was created. This preparation was effective against 89.9% of investigated strains.

The study revealed that 80 out of 89 strains of *E. coli* were sensitive towards *Coli* phage cocktail. Also 14 out of 16 strains of *E. coli* derived from the ground meat were 87.5% sensitive towards *Coli* phage cocktail (**Figure 9**).

Considering high lytic activity and broad spectrum of this polyvalent phage preparation, it will be significant alternative for prevention and treatment of colibacillosis caused by multidrug resistant *E. coli* pathogens.



Figure 7 *E. coli* phage vB-Eco1 electron micrograph.

Description: Siphoviridae. Size: head: 50 nm×50 nm tail: 125 nm × 10 nm.



Figure 8 *E. coli* phage vB-Eco1.

Description: Myoviridae. Size: head: 50 nm×50 nm tail: 120 nm × 15 nm.

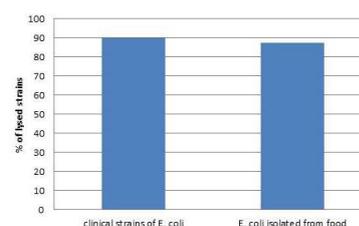


Figure 9 Sensitivity of the *E. coli* strains towards *Coli* phage cocktail.

Conclusion

- The bacteriological laboratory of the first clinic tested 56 pathogenic strains that were resistant towards: Azithromycin, Ciprofloxacin, Imipenem, Cefazolin, Polymixin and Chloramphenicol.

- The bacteriological laboratory of the second clinic tested 33 pathogenic strains that were resistant towards: Amicacin, Azythromycin, Ciprofloxacin, Imipenem, Polymixin.
- 20 samples of ground meat were studied in April-May, 2019. In 16 samples was detected. The strains were resistant towards two or more, in several cases as much as seven antibiotics. Strains derived from ground meat were resistant towards: Amicacin, Azythromycin, Imipenem, Polymixin.
- We have produced the phages from the 89 strains derived from the patients and 16 strains derived from the ground meat and created the cocktail with the 6 most effective phages. 89.9% of patient derived strains and 87.5% of meat derived strains were sensitive towards this cocktail.

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Declarations

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