Perspectives of the use of bacteriophages in agriculture, food and processing industries

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Abstract. The need to reduce excessive, and often not rational, use of antibiotics in bacteriosis treatment and prevention is acknowledged unanimously by representatives of medicine and veterinary medicine. One of the most important tasks for relevant research and productive organizations is finding the alternative treatments for bacterial infections in view of the growing trend of the increasing of antibiotic resistance. Against this background, limiting the use of antibiotics and developing new antibacterial agents is a priority. This review reflects the current view of specialized departments, government and public structures, business community on the field of application of antibacterial agents, including on the basis of bacteriophages. The work explains the feasibility of minimizing and rationalizing the use of antibiotics. There is also described the history of the researches about bacteriophages, their properties, peculiarities of application and examples of the use of bacteriophages in medicine and veterinary medicine, as well as in food and processing industries. Bacteriophages are a complete antibacterial agent which can be used not only in the direction of treatment and prevention of infectious diseases, but also in the field of biodesinfection, biodecontamination and bioconservation.

1. Introduction

According to Dr. Tedros Adhanom Ghebreyesus, the CEO of World Health Organization (WHO), “Antimicrobial resistance is a global crisis that threatens a century of progress in health and achievement of the Sustainable Development Goals.” Many states are already taking steps to reduce the amount of antibiotics used in medicine and livestock in order to prevent the development of such a scenario [1]. However, it is worth understanding that he maximum effect can be achieved only by joint efforts of the entire world community. It is assumed that more than 50% of bacteria have resistance to the bulk of antibiotics used in medicine, and in a quarter of a century this figure will be close to 100% [2]. In addition, international experts estimate that antimicrobial resistance causes more than 700,000 deaths in the world annually, and this figure could increase to 10 million people by the middle of the twenty-first century [3]. Given this situation, in 2017 WHO proposed the ranking of bacterial agents with the highest resistance trend, namely [4] Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacteriaceae, Enterococcus faecium, Helicobacter pylori, Salmonella species, Campylobacter species, Staphylococcus aureus, Neisseria gonorrhoeae, Streptococcus pneumonia, Haemophilus influenzae, Shigella species. All these species of microorganisms have a high degree of resistance to different groups of antibacterial preparations, and therefore are less susceptible to antibacterial therapy [5, 6, 7, 8]. The first solution to this problem is to minimize the use of antibacterial agents, which in turn will contain the development and spread of resistance. The second solution is to develop new antibacterial agents.
and put them into practice, but it should be taken into account that the effect of such implementation may be short-term, so it is worth using both options simultaneously [3].

2. Reduction of antibiotic use in livestock
The first European country which limited the use of antibiotics in the agricultural sector was the UK in 1971. For this purpose, antibiotics used in medicine were prohibited in livestock breeding. After 15 years, a similar ban was made in Sweden. Denmark banned avoparcin in pig breeding in 1996, and after four years refused to use all antibiotic growth stimulants voluntarily. The changes in pig growing technology did not lead to an increase in mortality among animals, and therefore the possibility of abandoning a number of antibacterial drugs was clearly demonstrated. Since 1999, the group of prohibited drugs in the EU has included virginamycin, carbachlo, olaquindox, spiramycin, tylosine and zincbacitracine. The remaining four types of feed antibiotics/coccidiostatics - avilamycin, monensin, salinomycin and flavomycin were allowed for use until 1 January 2006 - since that date the law banning them entered into force in the EU. Thus, a total ban on the use of feed antibiotics has entered into force in the EU since 2006. Unfortunately, these drugs are still used in some other States. For example, the United States is still the largest producer and consumer of feed antibiotics in livestock. The US use them in diets of about 80% of poultry, 75% of pigs and dairy cattle and 60% of beef cattle. High levels of feed antibiotic use also remain in China, India, Canada, South America and Africa [9].

It should be noted that the Russian Federation is implementing the Strategy on Prevention and Containment of Antimicrobial Resistance. The Government has submitted to the State Duma a draft federal law on biological safety of the Russian Federation [10], according to which, since 01.01.2021 it is supposed to ban the use of antimicrobial drugs in veterinary medicine for non-therapeutic purposes. A move to prescription release of antibiotics is also expected.

3. Bacteriophages as an alternative to antibiotics
Bacteriophages are viruses which are antagonists of bacterial cells that have the ability to selectively infect bacteria belonging to a single strain or antigen-homologous strains of a single species or genus [11]. There is a huge amount of phage particles both on skin, mucous membranes, external objects and in open cavities of people and animals [12]. The antimicrobial effect of bacteriophages is due to the incorporation of phages into bacterial cells, followed by their reproduction and lysis of infected cells. Bacteriophages released into the external environment as a result of the lysis re-infect and lyse with other bacterial cells and act until the target cells are completely killed at the inflamed area. Natural mechanisms of interaction of phages and bacteria allow to predict infinite diversity of both bacteriophages themselves (10^{30}, 10^{32}) and possible ways of their application [13]. With the advent of the antibiotic age in European countries despite the preliminary promising results of phagotherapy, research on bacteriophages has decreased, however, in the Soviet Union they started developments was continued. Research by G.A. Kokin and others led to the development of methods for parenteral phage use, which was necessary in the therapy of systemic infections developing against the background of cavity operations and combat wounds [14]. These methods were used by great surgeon N. N. Burdenko and were approved for use for therapy of military personnel during the Great Patriotic War. In the 1960s-1980s, research on bacteriophages has continued to develop actively in the USSR, despite a skeptical view about it among many Western scientists. Such famous researchers as Timakov, Zhukov-Verezhnikov, Goldfarb, Rautenstein, Tikhonenko, Revenko, Krylov and others worked in this direction [15].

At the present stage bacteriophages are used in medicine as antimicrobial drugs in generalized septic diseases, bacterial infections of gastrointestinal tract, nose and throat examination, organs of vision, respiratory tract, urogenital tract, burn wounds and surgical infections [16]. The results of researches on the effectiveness of phage lithium enzymes of bacteriophages as therapeutic drugs are promising. There is a number of researchers which shows that the peptidoglycan-lysing enzyme of bacteriophages (endolysin) causes accelerated lysis of cells of the relevant Gram-positive bacteria [17, 18]. One particularly promising area is exploring the possibility of bacteriophages functioning in biofilms.
Particularly effective for the destruction of microbial biofilms is the application of a dual enzymatic bacteriophage strategy that reduces the number of bacterial biofilm cells by about 4.5 orders of magnitude (removal of about 99.9%), which is nearly two orders of magnitude higher than non-enzymatic phage had [19].

There are two models of interaction of bacteriophage with sensitive cell - virulent and moderate types. Infection of bacteria with virulent phages always ends with a lytic effect: the release of mature phage particles which is ready to hit new bacterial cells. The genetic material of moderate phages integrates into the bacterial chromosome and multiplies with the host cell synchronously without causing lysis - such phages are called prophages and lead to lysogenic conversion. One way to reduce the risk of the spread of undesirable genes involving therapeutic bacteriophages is to use exclusively virulent phages for phagotherapy. The mechanism of action of phages consists in penetration through epithelial barriers of mucous membranes by receptor-dependent mechanism, actively carried out by beaker cells of intestinal epithelium and M-cells of immune system. The bacteriophage introduced by mouth reaches the hotbed of infection localization and after an hour enters the general circulation and is adsorbed by tissues, settling primarily in lymph nodes, liver and spleen, bursa fabricii (in birds) after 1-1.5 hours is fixed in bronchopulmonary exudate, and then after 2 hours in urine [20]. The blood-brain barrier is not a barrier to phage penetration into the central nervous system. The need to create and use bacteriophages in veterinary medicine as an alternative to antibiotics in the Russian Federation is provided for in the «Strategy for the Prevention of the Spread of Antimicrobial Resistance in the Russian Federation for the Period until 2030» (the Strategy). The «Action plan for 2019-2024 on implementation of the Strategy» of 30 March 2019 No. 604-R provides for the creation of banks of bacteriophages in order to research their genotypic features, development of genetically engineered strain-focused phages on the basis of reference centers of the Ministry of Health of the Russian Federation, Federal Service for Oversight of Consumer Protection and Welfare (Rospotrebnadzor) and Federal Service for Veterinarian and Vegetation Sanitary Supervision (Rosselkhoznadzor) [3]. The main directions for the design and introduction of bacteriophage-based drugs in veterinary medicine, manufacturing and processing industry are [21]:

- therapeutic and preventive use of bacteriophages in the fight against bacterial infections;
- bacteriophage-mediated biocontrol, which implies a set of measures to destroy bacteria that affect crops and production animals at a life-long stage, i.e. before they enter processing plants;
- phage bioprocessing which is used for the decontamination of food products of plant and animal origin (for example, from causative agent for toxicoinfections), as well as for the extension of their shelf life, directly at the processing plant;
- biodesinfection of objects of veterinary supervision, implying spraying of premises, equipment, litter, manure, etc.

3.1. Therapeutic bacteriophages in medicine and agriculture

The earliest records of phagotherapy in veterinary medicine are related to the name Felix d’Herelle, who in 1919 in France successfully tested bacteriophages with high lytic activity to Salmonella gallinarum in the outbreak of chicken salmonellosis [22, 23].

In 1983, Williams Smith and others discovered the preventive and therapeutic effectiveness of bacteriophages specific to the enterotoxigenic strain E. coli O9:K30.99.57, in case of calves, piglets and lambs diarrhoea [24]. In 2002, experiments to treat bacteremia caused by the vancomycin-resistant strain Enterococcus faecium were carried out [25]. The use of specific and lytically highly active bacteriophages has been successfully tested to combat salmonellosis among pigeons, which allow to sanitize poultry from carriage [26, 27]. Some encouraging progress is being made by the company RPC Micromir in preventing post-partum endometritis of cattle with phage products [28]. In 1995, employees of The Russian State Center for Quality and Standardization of Veterinary Drugs and Feed («VGNKI») developed a phage preparation with high therapeutic and preventive activity towards Salmonella enteritidis and Salmonella gallinarum-pullorum [29, 30]. Specialists of Center of food biotechnological
processing at the institute of applied microecology (CBO «Microecology») noted decisions on the use of bacteriophages for the control and prevention of salmonellosis on seropositive for Salmonella spp. enterprises, where due to an integrated approach at the experimental sites of poultry agricultural holding companies it is possible to stop the allocation of salmonella. A clear example of bacteriophage-based drugs for humans is the line of products of the «Scientific and Production Association for Immunological Preparations «Microgen» which includes E. Coli, Proteus, Streptococcus, Pseudomonas, Klebsiella, Staphylococcus, Salmonella, Shigella, etc. bacteriophages.

The innovative approach of bacteriophages use is their use as Biologically Active Additives [31]. In the United States, a probiotic food supplement based on Shigella bacteriophage is widely used to significantly reduce the risk of massive and sporadic cases of food toxicinfections [32]. The idea of taking the bacteriophages for preventive purposes is not original and was used in the armed forces of the Soviet Union [33]. In the Russian Federation, Institute of Epidemiology and Microbiology (Gabrichevsky MRIEM) Rospotrebnadzor, developed a specialized product of dietary preventive nutrition (Foodphag) based on bacteriophages for the prevention of alimentary transmitted infections, recommended to employees of food production, catering services, educational and pre-school institutions, medical facilities and other decreed groups of the population.

3.2. The bacteriophage-mediated biocontrol
A vivid example of phage-mediated biocontrol is a US EPA-registered biopesticide which contains bacteriophages lysing Xanthomonas campestris pv. Vesciectoria and Pseudomonas syringae pv. Tomato causing a «black» bacterial spot [34]. In 2006-2007 Omnilytex received USDA approval for use of bacteriophage-based composition against E. coli O 157: H 7 and Salmonella (BacWash) before slaughter of livestock [15]. In 1995, employees of «VGNKI» developed biological drugs against chicken salmonellosis. The drugs have high therapeutic and preventive activity towards Salmonella enteritidis and Salmonella gallinarum-pullorum. Its composition includes: vaccine genetically marked, phage-resistant strain Salmonella enteritidis and highly active to epizootic strains Salmonella enteritidis and Salmonella gallinarum-pullorum bacteriophages. The bacteriophage Phagum M. morganii M-20 UGSKHA was tested in pig breeding, which lysed 90.6% of the studied microorganisms, the remaining strains of bacteriophages were active against homologous bacteria in the range 79.3-89.3% [35].There is a positive experience in the use of specific and lithium-high-activity bacteriophages to combat salmonellosis among pigeons, which allows to sanitize poultry from salmonella carriage, provides protection against disease from the first days of life, while limiting the use of antibiotics [26, 27].

3.3. Phage bioprocessing
Illustration of bioprocessing of semi-finished products may be a processing aid registered by US Food and Drug Administration (FDA) which is created on the basis of a polyvalent listeria cocktail made up 6 different phages sensitive to 170 L. monocytogenes strains, allowing the treatment of raw meat and mince [36]. The effectiveness of this processing aid has also been experimentally proved in processed fruits [37]. In the same year, the US FDA classified a food additive (manufactured by Micreos, Netherlands) based on a strain of listeric bacteriophage P100 as a Generally Recognized as Safe food additive – «usually safe», i.e. confirming the safety of their use in the food industry, and allowed the use in the production of ready-to-eat food [38]. In 2011, Intralytics submitted to the US FDA a cocktail of 3 virulent bacteriophages belonging to the family Myoviridae, isolated from fresh and sea water, active against 111 E. coli O157:H7 strains, 76 E. coli strains of other serotypes and 20 strains of other types of bacteria, and received permission to use it in the processing of raw meat and mince [39]. The developers showed high efficiency (the number of E. coli bacteria decreased by 95% during 24 hours) of the developed agent while the decontamination of the permitted class of products - meat semi-finished products. The Dutch Medical Expert Council granted Micreos a temporary permit for using the product based on Salmonella bacteriophage in order to conduct full-scale tests based on one of Europe's largest poultry processing factories. In 2012, Intralytics submitted for consideration by the FDA to obtain approval for GRAS category cocktail of lithium bacteriophages specifically active against highly
pathogenic salmonella strains of Typhimurium serotypes, Enteritidis, Heidelberg, Newport, Hadar, Kent, Thompson, specially designed for raw meat decontamination and poultry before cutting the carcasses [40]. In experimental works when S. enteritidis infection of chicken skin is caused artificially, S. typhimurium of chicken legs and chicken meat sausage, independent researchers showed the effectiveness of phage cocktail, which allowed to reduce contamination of semi-finished products by 2 lg [41]. Cocktail treatment of bacteriophages (PC1) with a wide range of lytic activity allowed to achieve 99% decontamination of contaminated S. typhimurium pig skin [42].

Positive results in the development of preparations based on bacteriophages for decontamination, as well as for extension of shelf life of food products in the territory of the Russian Federation were obtained by Gabrichevsky Institute of Epidemiology and Microbiology. In 2017, on the basis of the Astrakhan State Medical University of the Ministry of Health of the Russian Federation the researches were carried out to identify leading pathogens which lead to cooled fish rotting. During the studies, on the basis of isolated from environmental objects and characterized from the phenotypic and molecular-genetic point of view of bacteriophages strains, a tool for decontamination of cooled fish was constructed. Pilot technology for production of polyvalent phage cocktails has been improved, which allows to increase by 5 days the shelf life of this category of semi-finished products and reduce the risk of becoming infected by alimentary infections [43].

Despite the development in America and western Europe of effective processing aids for food decontamination, as well as the decision taken by the Scientific Council of Rospotrebnadzor in 2011 to create new agents based on bacteriophages active for food pathogens, processing aids contained bacteriophages there is no mention of processing aids in Technical regulation of the Customs Union TR CU 029/2012 «Requirements for the safety of food additives, flavorings and technological aids». Thus, to date there is no one registered processing aid for decontamination and extension of shelf life of food semi-finished products, including chilled fish among the Member States of the Eurasian Economic Union (EAEU).

3.4. Biodesinfection of objects of veterinary supervision

Biodesinfection of objects of veterinary supervision is a new direction in Russian veterinary medicine. The basis for the use of bacteriophages in disinfectology was both experience of the use of biodesinfection in epidemiologically significant specialized departments of medical organizations (intensive care units, burn units) [44], and the formation of clones (strains) of microorganisms resistant to chemical disinfectants and antiseptics [45]. Scientists at the Union of the Ireland universities (the National University of Ireland) found that the introduction of increasing doses of disinfectants into laboratory Pseudomonas aeruginosa strains leads to addiction not only to the bacteria itself, but also to resistance to second-generation fluoroquinolones (ciprofloxacin), even without contact with an antibiotic. In 2009, it became known that in hospitals in Brazil, Mycobacterium massiliensis gained resistance to sterilizing fluid, and it was the first case of resistance to biocide. Due to the increasing introduction into the practice of surfactant-based products, in particular quaternary ammonium compounds (Q.A.C.), the problem of the possible formation of bacterial resistance to them is becoming more and more urgent. In this regard, in 2020, CBO «Microecology» specialists developed and declared a new veterinary product - a bacteriophage-based disinfectant designed to treat objects of veterinary supervision and prevent infectious diseases of domestic and farm animals and birds «Bio-Salm». The drug has the ability to specifically lyse the pathogenic serotypes Escherichia coli, as well as Salmonella enteritidis, Salmonella infantis, Salmonella typhimurium, Salmonella dublin, Salmonella gallinarum-pullorum, Salmonella choleraesuis, Salmonella bredeney, Salmonella reading, Salmonella virchow.

The E. Coli and Salmonella bacteriophages included in the agent have a high adsorption rate, reaching the target bacterial cells (via taxis), multiply in them, while the concentration of phage particles increases, which leads to an increase the efficiency of the drug. Coli bacteriophages are active only in regard to virulent cultures Escherichia coli, and do not cause lysis of non-pathogenic Escherichia, which are normal flora of the digestive system of animals and birds. Using bacteriophages as a method of
biological disinfection is sometimes the only possible measure for eliminating the pathogens of infections and allows to achieve a 15-fold reduction in incidence in a short time [44].

4. Conclusion

A modern view of the application of bacteriophages should be considered by veterinarians, doctors and specialists of the processing industry as an effective tool in the pro- and metaphylaxis of bacterial infections, an integral element of basic biosecurity programs in agricultural enterprises, an innovative tool for decontamination and extension of the shelf life of products of vegetable and animal origin.

The use of bacteriophage-based products in agriculture and the processing industry will allow us to reach a new technological level of production, ensuring:

- growth of the production segment based on natural and organic products;
- combating antibiotic resistance and limiting the use of antibiotics in agriculture;
- increased safety and functional efficiency of processing aids compared to foreign producers of similar products;
- creation new types and updating the range of available chilled products.

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