

## ANNOTATION

**of the dissertation work of Mendybayeva Anara Muratovna  
on the topic «Investigations of phenotypic and genotypic resistance of  
*Salmonella enterica* strains circulating in the Northern region of Kazakhstan»  
submitted for the degree of Doctor of Philosophy (PhD) in the specialty  
6D120200 - Veterinary Sanitation**

**Relevance of the research topic.** The development and spread of antibiotic resistance is one of the most important evolutionary changes in the modern era. Antimicrobial resistance is a public health issue of concern worldwide. Resistance has increased among bacteria of clinical importance. According to Craig Baker-Austin et al., the root cause is selective pressure caused by the widespread use of commercial antibiotics in human and veterinary medicine.

In 2015, the 68th session of the World Health Organization adopted a global action plan on antimicrobial resistance. Recognizing the urgent need for a more coordinated and harmonized surveillance system for monitoring antimicrobial resistance at the national, regional and global levels, WHO invited international, regional and national partners to take the necessary actions to contribute to the goals of the ambitious action plan on antimicrobial resistance.

At the end of 2016, UN member countries adopted a joint statement on the need to take action to combat drug-resistant microorganisms and ensure control of the use of antimicrobials. In the history of the organization, the problem of antibiotic resistance of microorganisms has become the fourth public health problem to be discussed by the General Assembly after HIV infection, Ebola and non-communicable diseases (such as heart disease, diabetes mellitus and others).

*Salmonella* is among the microorganisms that have developed a number of resistant serotypes found in the food chain. Resistance to antibacterial drugs in *Salmonella* strains that can cause disease or carriage in humans and animals is an undesirable but almost inevitable consequence of their widespread use in animal husbandry, poultry farming and medicine. The intensive use of antibacterial drugs enhances the selection of resistant strains of *Salmonella*, the reservoir of which are animals. Through the food chain, such strains are often transmitted to humans. *Salmonella* resistance to drugs used for treatment is a major public health problem.

The use of antibiotics, combined with the horizontal transfer of resistance genes between bacteria, has led to the emergence of multidrug resistance. Multidrug resistance is resistance to two or more antibacterial drugs, which greatly limits the therapeutic options for treating diseases in both humans and animals.

A huge number of different genes may be responsible for antimicrobial resistance. The identification of such genes is important for understanding the epidemiology of resistance, for testing for insensitive phenotypes, and for identifying resistant strains when the genes are weakly expressed *in vitro*.

**The purpose of the dissertation research:** features of phenotypic and genotypic resistance of *Salmonella enterica* strains circulating in the northern region of Kazakhstan.

**Object of study:** bacterial isolates of *Salmonella* isolated from various sources on the territory of Kostanay and North Kazakhstan regions.

**Subject of study:** antibiotic resistance and molecular genetic mechanisms of resistance of *Salmonella* strains.

**Research objectives:**

- 1) Isolate and identify bacterial cultures of *Salmonella* spp. from various sources, to study their biological properties.
- 2) Carry out molecular genetic typing of *Salmonella* strains.
- 3) To test and determine the spectrum of drug resistance of isolated *S. enterica* strains to antibacterial drugs of various pharmacological groups.
- 4) Determine the genetic profile of antibiotic-resistant bacterial strains.
- 5) Investigate the ability of *S. enterica* strains to biofilm formation.

**Research methods.** The work was carried out in the laboratories of microbiology and molecular genetic analysis on the basis of the Research Institute of Applied Biotechnology NLC "KRU named after A. Baitursynov", the laboratory of microbiology of the Institute of Microbiology and Virology of the Lithuanian University of Health Sciences (Kaunas), the laboratory of chemical and molecular genetic methods of research and analysis TC LLP "SPC Microbiology and Virology" (Almaty).

Isolation and identification of *Salmonella* strains was carried out according to the guidelines "Laboratory diagnosis of salmonellosis, detection of *Salmonella* in food and environmental objects." Serotyping was carried out in the agglutination reaction with diagnostic salmonella sera.

Molecular genetic methods. Typing of *Salmonella* strains was carried out according to the Sanger method. Resistance genes were detected by PCR with visualization of amplification products in agarose gel. Oligonucleotide sequences from the literature data were used as primers.

Antibiotic resistance testing. The sensitivity of strains to antibacterial drugs was carried out by the method of disk diffusion, according to the methodological recommendations "Determination of the sensitivity of microorganisms to antibacterial drugs."

Spectrophotometric methods. Determination of the ability of *Salmonella* strains to form biofilms on the surface of a polystyrene plate was carried out by staining with crystal violet. The optical density was measured on a Multiskan multichannel microbiological spectrophotometer at a wavelength of 620 nm.

**The main provisions submitted for protection**

1. Serotypes of salmonella circulating in the territory of Kostanay and North Kazakhstan regions.
2. The prevalence of salmonella resistant to antibacterial drugs isolated from various sources.
3. Profile of phenotypic and genotypic resistance of *Salmonella* strains to antibacterial drugs of various pharmacological groups.

**Scientific novelty.** For the first time in the territory of northern Kazakhstan, studies have been conducted to study the antibiotic resistance of salmonella strains isolated from various sources to a wide range of antibacterial drugs. The new data

obtained made it possible to assess the existing prevalence of resistant forms of salmonella isolated from various sources to groups of antibacterial drugs, including those critically important for human therapy.

It has been established that more than 90% of salmonella strains isolated from various sources are resistant to at least one group of antibacterial drugs used to treat enterobacterial infections.

A high frequency of isolation of *Salmonella* strains resistant to tetracycline, nitrofurans, beta-lactams and quinolones was revealed.

The genotypic resistance of salmonella strains was determined, the presence of 20 genes encoding resistance to antibacterial drugs of 6 pharmacological groups was detected. The presence of integrons of classes 1 and 2 (*teg1* and *teg2*) was detected in DNA samples from biological material from animals and animal products.

#### **Theoretical and practical significance**

Based on the conducted research, developed and proposed:

- Practical recommendation "Laboratory diagnostics and identification of pathogens of staphylococcosis, salmonellosis and escherichiosis" for specialists of diagnostic laboratories, teachers, students, undergraduates and doctoral students of veterinary specialties;

- Methodical manual "Determination of the sensitivity of microorganisms to antibacterial drugs" for specialists of diagnostic laboratories, teachers, students, undergraduates and doctoral students of veterinary specialties;

- The textbook "Diagnostics of pathogens of enteropathogenic zoonanthroponotic diseases" has been introduced into the educational process of the Department of Veterinary Sanitation and is used as an educational material when lecturing and conducting laboratory and practical classes.

The results of research have been put into production, and practical recommendations have been given for the prevention and monitoring of the spread of antibiotic resistance.

**Approbation of work.** The main provisions submitted for defense were reported at international scientific and practical conferences:

- in the materials of the international scientific and practical conference "Baitursynov Readings - 2019" April 26, 2019 (Kostanay);

- in the materials of the international scientific and practical conference "Prospects for the development of breeding livestock", dedicated to the day of honoring the 80th anniversary of the Doctor of Agricultural Sciences, Professor Naimanov D.K., October 9, 2020 (Kostanay).

**Publications.** The results of dissertation research and the main provisions are reflected in 9 publications, including 3 - in journals included in the international databases Scopus and Web of Science (86%, 83% and 24% percentile), 2 - in publications recommended by CQAFSHE MSHE RK, 2 - in journals peer-reviewed in the Russian Science Citation Index. 1 utility model patent received. Published: 1 - practical recommendation, 1 - study guide, 1 - methodological guide.

#### **Relationship of work with research programs**

The work was carried out within the framework of 2 scientific projects:

- grant funding project of the Ministry of Education and Science of the Republic of Kazakhstan No. AP05131447 "Monitoring of antibiotic resistance of pathogens of enteropathogenic zoonotic diseases of the Northern region of Kazakhstan" under the budget program 217 "Development of science", subprogram 102 "Grant funding of scientific research";

- project "Analysis of the risks of the emergence of resistance to antibiotics in pathogenic microflora isolated from animals and from raw materials and products of animal origin" within the framework of the scientific and technical program BR10764944 "Development of methods for analytical control and monitoring of food safety" funded by the Ministry of Agriculture within the framework of the budget program 267 "Improving the availability of knowledge and scientific research", subprogram 101 "Program-targeted financing of scientific research and activities".

**Degree of reliability of results.** The reliability of the data obtained is determined by a sufficient amount of research, the use of modern methods. The results of the research are reflected in the final report on the project AP05131447 "Monitoring of antibiotic resistance of pathogens of enteropathogenic zoonotic diseases in the Northern region of Kazakhstan" (reg. No. 0118RK00397, inventory No. 0220RK00538).

#### **Personal contribution**

The author carried out theoretical and practical research at all stages of the dissertation work. Literature data were collected and summarized. Experimental studies have been carried out. The author generalized and analyzed the obtained data, formulated conclusions and practical recommendations. Articles have been published, a patent for a utility model has been obtained, and relevant sections of educational and methodological works have been prepared.

#### **Description of the main results of the study:**

1. The results of microbiological studies (2018-2020) on the territory of Northern Kazakhstan showed that out of 2010 samples of biomaterial from animals, birds and animal products, 90 (4.5%) *Salmonella* strains were isolated and identified. On the territory of Kostanay region, out of 1035 samples, 74 strains of *Salmonella* (7.1%) were isolated, in the North Kazakhstan region, 16 strains of *Salmonella* (1.6%) were isolated from 975 sources. The presence of *Salmonella* prevailed in samples from animals, in particular from cattle (37.7%). Studies of products of animal origin showed that 35 strains of *Salmonella* were isolated from 429 product samples, which accounted for 8.1% of the total number of food isolates. In products and raw materials of animal origin, the predominant number of *Salmonella* strains was isolated on the territory of the Kostanay region, 30 strains of *Salmonella* were isolated from 223 samples, which amounted to 13.4%. In the North Kazakhstan region, salmonella was isolated in 5 (2.4%) out of 206 product samples.

2. A wide variety of *Salmonella* serotypes circulate on the territory of Kostanay and North Kazakhstan regions: *S. Enteritidis*, *S. Typhimurium*, *S. Paratyphi C*, *S. Typhi*, *S. Abortus equi*, *S. Derby*, *S. Blegdam*, *S. Tshiongwé*, *S. Cholerae suis*, *S. Dublin*, *S. Tennessee*, *S. Moscow*, *S. Virchow*. A significant part, 41.1% of the isolated strains of *Salmonella* belongs to the serotype *S. Enteritidis* and 15.5% - *S. Typhimurium*. Moreover, the *S. Enteritidis* serotype prevailed among the strains

isolated from animal products and amounted to 68.6%, while in the biomaterial from animals and birds this serotype was found in 23.6% of cases.

3. The problem of salmonellosis is complicated by the progressive spread of antibiotic resistance in strains of *S. enterica*. It was found that 93.3% of *Salmonella* strains were resistant to at least one group of antibacterial drugs and only 6.7% were sensitive to all antibacterial drugs. *Salmonella* strains with resistance were mostly resistant to antibiotics of the tetracycline group (64%). The second most common group is nitrofurans (61%), followed by quinolones (51%), fluoroquinolones (46%) and beta-lactams (41%). The least resistant strains were found to the groups of aminoglycosides (24%), sulfonamides (19%) and amphenicols (11%).

4. The highest level of multiresistant strains was found in isolates of livestock products and amounted to 71.4%, while isolates isolated from animals were multiresistant in 63.6% of cases. Eight cases of extremely resistant *Salmonella* strains were identified, where the *S. Enteritidis* serotype is most common (n=3). Two strains (*S. Virchow* and *S. Tennessee*) were also found to be resistant to all eight tested groups of antibacterial drugs.

5. A total of 44 antibiotic resistance profiles were identified with different combinations of resistance to antibacterial drug groups. The predominant resistance profile of the isolated strains of *Salmonella* was the profile "tetracyclines + fluoroquinolones + quinolones + nitrofurans".

6. The study of genotypic resistance of *Salmonella* strains showed the presence of 20 genes encoding resistance to antibacterial drugs of six pharmacological groups, such as:

- beta-lactams - genes BlaTEM, BlaSHV, OXA1 and ctxM;
- aminoglycosides - genes aacA4, aadA, aadB, aphA1, strA, strB;
- tetracyclines - tetA and tetB genes;
- sulfonamides - genes Sul1, Sul2, Sul3 and dfr1;
- amphenicols - genes cmlA, catII;
- quinolones - genes qnrA, qnrB.

7. In DNA samples of strains *S. Enteritidis*, *S. Paratyphi C*, *S. Virchow*, *S. Tennessee*, characterized by multiple resistance, the presence of integrons of classes 1 and 2 (teg1 and teg2) was found.

8. Studies of the ability of *Salmonella* strains to form biofilms showed that the studied strains in most cases did not have the ability to form biofilms, which indicates that *Salmonella* is more adaptable to circulation inside organisms than in the environment.

### **Practical Suggestions**

1. The conducted studies and the results obtained have shown the pattern of a steady increase in the resistance of *Salmonella* strains to most drugs used to treat infectious animal diseases, in this regard, in order to prescribe adequate etiologic therapy and in order to monitor sensitivity to various antimicrobial drugs, in isolated pathogens, it is necessary to mandatory to determine the sensitivity to antimicrobial drugs.

2. For the treatment of salmonellosis infection in animals, antimicrobials critical for human medicine should not be used: fluoroquinolones and cephalosporins of the third and fourth generations.

**Scope and structure of dissertation research**

The dissertation work is presented on 173 sheets of typewritten text and includes sections: introduction, literature review, own research, research results, generalization and evaluation of research results, list of references, applications.

The work contains 24 figures, 20 tables, 20 appendices, 269 literature sources.