ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8

International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies

http://TuEngr.com

Biological Properties and Genetic Characteristics of Collection and Epizootic Strains of Salmonella

Sergey Lenev[1*], Ekaterina Krylova[1], Veronika Gordeeva[1,2], Olga Prasolova[1], Irina Soltynskaya[1], Nadezhda Abrosimova[1]

[1] *The Russian State Center For Animal Feed And Drug Standardization And Quality, RUSSIA.*
[2] *Federal State Budgetary Institution Federal Scientific and Clinical Center for Physical and Chemical Medicine of the Federal Medical and Biological Agency of Russia, RUSSIA.*
**Corresponding Author (Email: slenev@yandex.ru)*

Paper ID: 13A12L

Volume 13 Issue 12

Received 10 June 2021
Received in revised form 10 September 2022
Accepted 17 September 2022
Available online 24 September 2022

Keywords:
Salmonella; Strains; Virulence; Immunogenicity; Sequencing.

Abstract

The paper presents the results of studies of the biological properties of 54 collection and epizootic strains of Salmonella, performed in order to select promising strains for the manufacture of vaccines against salmonellosis in animals. It was established that the industrial strains of Salmonella Typhimurium No. 371, Salmonella Dublin No.373 and Salmonella Choleraesuis No.370, currently used for the manufacture of vaccines, had the highest immunogenicity among the studied strains. Bioinformatics analysis of the data of whole genome sequencing of these strains was carried out, which makes it possible to identify them by molecular genetic methods.

Discipline: Microbiology and Veterinary.

Cite This Article:

Lenev, S., Krylova, E, Gordeeva, V., Prasolova, O., Soltynskaya, I., Abrosimova, N. (2022). Biological Properties and Genetic Characteristics of Collection and Epizootic Strains of Salmonella. *International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies, 13*(12), 13A12L, 1-11. http://TUENGR.COM/V13/13A12L.pdf DOI: 10.14456/ITJEMAST.2022.243

1 Introduction

In recent decades, salmonellosis has been regarded as one of the most common zoonoses in the world. According to the conclusion of WHO experts, salmonellosis, as a zoonotic infection, is unparalleled in the complexity of the development of both epizootic and epidemic processes, as well as in the difficulties of combating it [23].

In Russia, salmonellosis is the main cause of acute intestinal infections in people with bacterial etiology. Salmonellosis accounts for about 20% of cases of intestinal infections with an established etiology.

Human incidence of salmonellosis is the result of a combination of risk factors that affect the final level of contamination of food products and compliance with the rules of sanitary legislation during their storage, transportation, sale and preparation. The final level of contamination of products intended for sale in the retail network reflects the final effectiveness of measures to prevent salmonellosis in birds and other types of productive animals.

According to many researchers and the WHO expert committee on salmonellosis, the problem cannot be solved by using antibiotics and chemotherapy drugs [3, 23], while specific immunoprophylaxis of salmonellosis in animals is the most effective way to prevent the vertical spread of the pathogen and largely protects animals from Salmonella infection. [2–5, 7–9, 23].

In Russia, 15 vaccines against salmonellosis in animals are currently being produced, based on industrial strains of Salmonella serovars S. Choleraesuis, S. Typhimurium, S. Dublin, S. Enteritidis and S. Infantis.

The purpose of this study was to study the biological properties, virulence, immunogenicity and individual genetic characteristics of the collection and epizootic strains of Salmonella, in comparison with the properties of industrial strains.

2 Method

2.1 Strains

We used strains of serovars S. Choleraesuis, S. Typhimurium, S. Dublin, S. Enteritidis and S. Infantis of the genus Salmonella, received in the collection of the Museum of Microorganisms of the Federal State Budgetary Institution "VGNKI" in 1939 - 1980, as well as epizootic strains of these Salmonella serovars isolated in 2010-2021. in various regions of the Russian Federation.

2.2 Animals

White outbred mice weighing (14–16) g.

Nutrient media, reagents and diagnostics:

Meat peptone broth; meat-peptone agar with agar concentrations of 0.25% and 1.2%; Wednesday Endo; chromogenic salmonella agar (Oxoid, UK); Rappaport-Vassiliadis medium; a set of reagents for Gram stain; test system for the identification of Enterobacteriaceae and other unpretentious gram-negative rods api20E ("bioMerieux, SA", France); sera diagnostic salmonella "PETSAL" (SPbNIIVS FMBA, RF).

2.3 Microbiological Methods

2.3.1 Methods for Isolation and Identification of Salmonella spp.

The studies were carried out in accordance with MU 4.2.2723-10 “Laboratory diagnostics of salmonellosis, detection of salmonella in food products and environmental objects”, GOST R 50455-92 (ISO 3565-75), “Meat and meat products. Salmonella detection (arbitration method)”, GOST 31659-2012 (ISO 6579:2002) “Food products. Method for the detection of bacteria of the genus Salmonella.

To classify the isolated isolates of microorganisms to the genus Salmonella, their enzymatic properties were studied using the test system for the identification of Enterobacteriaceae and other unpretentious gram-negative rods api20E (bioMerieux, SA, France).

Salmonella isolates were also examined on a Sensititre bacteriological analyzer using the GNID test system, which determines 32 enzymatic reactions to identify the strain. Serological identification of the serovar was carried out using Salmonella agglutinating sera "PETSAL".

2.3.2 Cultural Methods for Studying Salmonella Strains

The morphology of colonies of cultures of Salmonella strains was studied on Petri dishes with meat-peptone agar (MPA), after sieving suspensions of cultures grown for 18-20 hours on them at a temperature of 37 ° C in meat-peptone broth (MPB). Attention was paid to the homogeneity of the composition of the population of colonies, their color, transparency, size, and shape of the edge of the colony. For a comparative study of cultural properties, one batch of the nutrient medium was used.

2.3.3 Determination of the Virulence of Salmonella Strains

The virulence of cultures of the studied strains of Salmonella was determined on white mice weighing (14–16) g. Dilutions of cultures of strains with a tenfold step were administered subcutaneously to animals, using 5 mice per dose. The LD50 value was calculated from the death of white mice on the 10th day after infection according to the Kerber method, modified by Ashmarin [1].

2.3.4 Method for Obtaining Vaccines

Cultures of selected strains of Salmonella grown during (18–20) hours of cultivation on MPA at a temperature of 37°C were washed off with sterile saline and the concentration of 1 billion microbial cells was determined in them according to the optical turbidity standard. A microbial suspension of cultures was heated in a water bath at a temperature of 58°C for 60 minutes. The obtained heated monovaccines, after establishing their sterility, were used to determine the immunogenicity of Salmonella strains.

2.3.5 Determination of the Immunogenicity of Salmonella Strains

The immunogenicity of Salmonella strains was studied by subcutaneously immunizing white mice with heated vaccines derived from them. Monovaccines were administered to animals using 5-fold dilutions of vaccines at doses of 40 million, 8 million, 1.6 million and 0.32 million microbial cells. 14 days after immunization, the animals were infected with 5 LD50 of the virulent strain of the corresponding Salmonella serovar. The ED50 value was calculated from the survival of white mice on day 10 after infection according to the Kerber method, modified by Ashmarin [1].

2.3.6 Molecular Genetic Methods, Software and Resources Used

DNA isolation was carried out using the DNA-sorb-V reagent kits (Federal Scientific Research Institute of Experimental Engineering, Russia) in accordance with the manufacturer's instructions. The DNA library was prepared using the Nextera XT DNA Sample Preparation Kit according to the manufacturer's instructions. Whole genome sequencing was performed on a MiSeq system (Illumina) according to standard operating procedure.

For bioinformatics analysis of whole genome sequencing data and de novo genome assembly, the following programs were used: FastQC 0.11.17, Trimmomatic v.0.36, SPAdes 2.11.1, QUAST 4.6.3, MAUVE v.20150226. To determine the species of bacteria using the collected contigs, we used the search for common k-mers implemented in the KmerFinder program (version 3.0.2), as well as the multilocus typing method (MLST, version 2.0.4) on the online service of the Center for Genomic Epidemiology of the Danish Technical University (CGE) [10, 11]. Annotation of bacterial genomes was performed using the RAST server [6, 16].

The search for genetic factors providing bacterial resistance to various antibiotics was carried out using the ResFinder online service on the server of the Center for Genomic Epidemiology of the Technical University of Denmark [13, 24] and the NCBI's Bacterial Antimicrobial Reference Resistance Gene Database (NCBI BARRGD) [14]. The Virulence Factor Database (VFDB) was used to search for the main virulence factors in bacterial genomes [12, 20]. The search for Salmonella pathogenicity islands was carried out using the online service SPIFinder (version 2.0) on the server of the Center for Genomic Epidemiology of the Technical University of Denmark [17].

3 Result and Discussion

The results of determining the cultural and serological properties of Salmonella strains that entered the collection of the Museum of Microorganisms of the Federal State Budgetary Institution "VGNKI" in 1939–1980 are presented in Table 1.

Table 1: The results of determining the cultural and serological properties of Salmonella strains

№	Name of the strain	Strain number	Dissociation of colonies	Agglutinability
1	*S. Choleraesuis*	4091	S-shape	#
2	*S. Choleraesuis*	1045	S-shape	#
3	*S. Choleraesuis*	371	R-shape-30%	++
4	*S. Choleraesuis*	370*	S-shape	#
5	*S. Choleraesuis*	3089/15	R-shape-30%	++
6	*S. Choleraesuis*	7035	S-shape	#
7	*S. Choleraesuis*	127/41	R-shape-30%	++
8	*S. Dublin*	89	R-shape-50%	++
9	*S. Dublin*	42	S-shape	#
10	*S. Dublin*	1449	R-shape-50%	++
11	*S. Dublin*	730	R-shape-50%	++
12	*S. Dublin*	780	S-shape	#
13	*S. Dublin*	373*	S-shape	#
13	*S. Infantis*	158-51	S-shape	+++
14	*S. Infantis*	12454	S-shape	#
19	*S. Enteritidis*	269/69	R-shape	++
20	*S. Enteritidis*	64	S-shape	+++
21	*S. Enteritidis*	918-L	S-shape	+++
22	*S. Enteritidis*	3131	S-shape	+++
23	*S. Enteritidis*	7	S-shape	#
24	*S. Enteritidis*	5785	R-shape	++
25	*S. Enteritidis*	11272	S-shape	+++
26	*S. Enteritidis*	1226	S-shape	#
27	*S. Enteritidis*	1282	S-shape	+++
28	*S. Enteritidis*	1330	S-shape	+++
29	*S. Enteritidis*	1383	S-shape	#
30	*S. Enteritidis*	1349	S-shape	#
31	*S. Typhimurium*	371*	S-shape	#
32	*S. Typhimurium*	159	R-shape	++
33	*S. Typhimurium*	1282	S-shape	#
34	*S. Typhimurium*	1279	S-shape	#

*marked strains are industrial strains for the manufacture of vaccines against salmonellosis in animals

The results of determining the cultural and serological properties of epizootic strains of Salmonella are presented in table 2.

3.1 The Results of the Study of the Cultural Properties of Strains

As a result of studying the cultural properties of Salmonella strains, it was found that:

1. among the studied strains of Salmonella Enteritidis, most (9 out of 10) epizootic strains of Salmonella Enteritidis formed on MPA from 30% to 50% of colonies in the R-form, in addition, strains of Salmonella Enteritidis "Lipetsk" and Salmonella Enteritidis "4B" tend to dissociation with the formation of more than 30% of colonies that differ in color and size;

2. among strains of Salmonella Infantis, dissociation of colonies from 30% to 50% of the population size was revealed, among strains of Salmonella Infantis S-12, Salmonella Infantis "Kuznetsov" and "758";

3. among strains of S. Typhimurium, dissociation of colonies of strains of S. Typhimurium 159, S. Typhimurium "Severin" and S. Typhimurium b/n isolated from chickens in the Tula region was revealed;

4. strains of Salmonella Dublin 89, 730, 1449 and Immonoilin 2 formed more than 50% in the R-form;

5. Among strains of Salmonella Choleraesuis, a significant dissociation of colonies (more than 30%) was found in strains of Salmonella Choleraesuis 127/41, 371 and 3089/15.

Strains prone to dissociation, forming colonies in the R-form, were not used in subsequent studies, as they did not meet the criteria for selecting promising strains for vaccine production

Table 2: The results of determining the cultural and serological properties of epizootic strains of Salmonella

№	Name of the strain	From which animal species is isolated	Region	Year of allocation	Dissociation of colonies	Agglutinability
1	S. infantis "Kuznetsov"	chickens	Tomsk region	2017	R-shape (30–50)%	+
2	S. infantis 4632	chickens	Stavropol region	2014	S-shape	+++
3	S. Infantis 758	chickens	Yaroslavl region	2014	R-shape (30–50)%	++
4	S. infantis 663	chickens	Kaluga region	2021	S-shape	+++
5	S. infantis 2108	chicken feces	Moscow region	2021	S-shape	#
6	S. Infantis S-11	chicken skin	Novosibirsk region	2019	S-shape	#
7	S. Infantis S-12	minced chicken	Novosibirsk region	2019	R-shape (30–50)%	+
8	S. Infantis S-13	chicken bones	Novosibirsk region	2019	S-shape	#
5	S. Enteritidis Kursk	chickens	Kursk region	2021	S-shape	+++
6	S. Enteritidis 25	chickens	Ryazan region	2015	R-shape-30%	++
7	S. Enteritidis "Lipetsk"	chickens	Lipetsk region	2015	R-shape-30%	+
8	S. Enteritidis "4B"	chickens	Belgorod region	2010	R-shape-30%	+
9	S. Enteritidis M-6	chickens	Kursk region	2021	R-shape-30%	+++
10	S. Enteritidis M-5	chickens	Kursk region	2021	R-shape-30%	+++
11	S. Enteritidis M-4	chickens	Kursk region	2021	R-shape-30%	+++
12	S. Enteritidis M-3	chickens	Moscow region	2020	R-shape-30%	+++
13	S. Enteritidis M-	chickens	Moscow region	2020	R-shape-30%	+++
14	S. Enteritidis M-1	chickens	Moscow region	2020	R-shape-30%	+++
15	S. Dublin Immonoilin 2	calves	Moscow region	2017	R-shape-50%	++
16	S. Dublin "Maxim"	calves	Chelyabinsk region	2016	S-shape	#
17	S. Typhimurium "Dove"	pigeons	Moscow region	2017	S-shape	#
18	S. Typhimurium no.	ducks	Ufa region	2017	S-shape	#
19	S. Typhimurium 'Severin'	pigeons	Moscow region	2017	R-shape	++
20	S. Typhimurium no.	chickens	Tula region	2015	R-shape	++

3.2 The Results of the Study of Serological (antigenic) Properties of Strains

Serological properties of Salmonella strains, with the exception of strains culled according to the results of the study of cultural properties, were studied using Salmonella O and H agglutinating sera "PETSAL", assessing their agglutenability in crosses, in accordance with the instructions for their use.

Due to the fact that the agglutenability of enterobacteria strains is associated with their potential immunogenicity, based on the results of these studies, the following strains were selected for further work:

1. Salmonella Enteritidis strains 1226, 1383,1349 and 7 among the studied Salmonella Enteritidis strains;

2. from strains of Salmonella Infantis - strains of Salmonella Infantis S-11, 12454, S-13, 2108;

3. from strains of Salmonella Typhimurium - strains of Salmonella Typhimurium 1282, 1279, "Dove", no. isolated from ducks;

4. among strains of Salmonella Dublin - strains of Salmonella Dublin "Maxim", 42, 780;

5. among strains of Salmonella Choleraesuis - strains of Salmonella Choleraesuis 4091, 1045 and 7035.

3.3 Virulence of Salmonella strains

The virulence of Salmonella strains was determined on white mice weighing (14–16) g according to the LD50 value calculated from the death of animals on the 10th day after infection. Cultures of the studied strains were administered to mice subcutaneously at doses of 103, 104, 105, 106, and 107 microbial cells, using 5 animals per dose. The results of the determination of virulence are presented in Table 3.

Table 3: The results of the determination of virulence

№	Serovar strain	No., strain name	LD_{50} value
1	*Salmonella Enteritidis*	1226	$1,3\times10^3$
2	*Salmonella Enteritidis*	1383	$3,2\times10^3$
3	*Salmonella Enteritidis*	1349	$6,3\times10^3$
4	*Salmonella Enteritidis*	7	$1,3\times10^3$
5	*Salmonella Infantis*	S-11	$4,5\times10^6$
6	*Salmonella Infantis*	12454	$2,2\times10^6$
7	*Salmonella Infantis*	S-12	$1,3\times10^4$
8	*Salmonella Infantis*	2108	$>1,0\times10^7$
9	*Salmonella Typhimurium*	1282	$3,5\times10^5$
10	*Salmonella Typhimurium*	1289	$3,2\times10^5$
11	*Salmonella Typhimurium*	"Pigeon"	$2,2\times10^6$
12	*Salmonella Typhimurium*	b / no. from ducks	$4,2\times10^6$
13	*Salmonella Dublin*	"Maksim"	$2,5\times10^6$
14	*Salmonella Dublin*	42	$5,4\times10^5$
15	*Salmonella Dublin*	780	$3,2\times10^5$
16	*Salmonella Choleraesuis*	1035	$5,6\times10^4$
17	*Salmonella Choleraesuis*	4091	$1,2\times10^5$
18	*Salmonella Choleraesuis*	7035	$5,0\times10^5$
19	*Salmonella Choleraesuis*	370*	$1,1\times10^3$
20	*Salmonella Typhimurium*	371*	$1,3\times10^3$
21	*Salmonella Dublin*	373*	$2,5\times10^3$

* strains marked with an asterisk are production strains for the manufacture of vaccines against salmonellosis in animals

The highest virulence was found in industrial strains of Salmonella and in strains belonging to the serovar Salmonella Enteritidis. Collection and epizootic strains of Salmonella Infantis serovar had the lowest virulence.

3.4 Immunogenicity of Salmonella strains

The immunogenicity of Salmonella strains was determined by immunizing white mice with heated vaccines obtained from cultures of the corresponding strain. Heat-inactivated microbial cells were administered to animals subcutaneously at doses of 40 million, 8 million, 1.6 million and 0.32 million microbial cells. 14–16 days after immunization, animals were challenged with 5 LD50 of the virulent strain corresponding to Salmonella serovar. The value of ED50 was calculated from the survival of white mice on the 10th day after infection by the method of Kerber, modified by Ashmarin.

The potential immunogenicity of the studied strains was compared with the immunogenicity of industrial strains of Salmonella Typhimurium No. 371, Salmonella Dublin No. 373 and Salmonella Choleraesuis No. 370, selected in 1970-1980. and currently used for the manufacture of animal salmonellosis vaccines. The results of determining the immunogenicity of cultures of Salmonella strains are presented in table 4.

As follows from the data presented in the table, the production strains of Salmonella Typhimurium No. 371, Salmonella Dublin No. 373 and Salmonella Choleraesuis No. 370 had the highest immunogenicity among the studied strains. Their immunogenicity was 10–20 times higher than that of museum and epizootic strains of the corresponding Salmonella serovars. Among Salmonella serovars Salmonella Enteritidis and Salmonella Infantis, no highly immunogenic strains promising for use as industrial ones were found. In our opinion, this is due to the high instability and dissociation of cultures of the studied strains of these serovars.

Table 4: The results of determining the immunogenicity of cultures of Salmonella strains

№	Serovar strain	No., strain name	LD_{50} value
1	*Salmonella Enteritidis*	1226	$9,2\times10^6$
2	*Salmonella Enteritidis*	1383	$7,5\times10^6$
3	*Salmonella Enteritidis*	1349	$9,0\times10^6$
4	*Salmonella Enteritidis*	S-7	$5,6\times10^6$
5	*Salmonella Infantis*	S-11	$1,6\times10^7$
6	*Salmonella Infantis*	12454	$2,8\times10^7$
7	*Salmonella Infantis*	S-13	$2,2\times10^7$
8	*Salmonella Infantis*	2108	$7,4\times10^6$
9	*Salmonella Typhimurium*	1282	$2,5\times10^7$
10	*Salmonella Typhimurium*	1289	$2,6\times10^7$
11	*Salmonella Typhimurium*	"Pigeon"	$9,7\times10^6$
12	*Salmonella Typhimurium*	No. 371 *	$1,5\times10^6$
13	*Salmonella Dublin*	"Maksim"	$2,5\times10^7$
14	*Salmonella Dublin*	42	$3,1\times10^7$
15	*Salmonella Dublin*	780	$2,7\times10^7$
16	*Salmonella Dublin*	No. 373 *	$1,6\times10^6$
17	*Salmonella Choleraesuis*	1035	$2,5\times10^7$
18	*Salmonella Choleraesuis*	4091	$3,2\times10^7$
19	*Salmonella Choleraesuis*	7035	$2,8\times10^7$
20	*Salmonella Choleraesuis*	No. 370 *	$2,0\times10^6$

*marked strains are industrial strains for the manufacture of vaccines against salmonellosis in animals

3.5 Results of Whole Genome Sequencing of Industrial Salmonella Strains with High Immunogenicity

For the purpose of genetic identification and certification, whole genome sequencing of industrial strains of Salmonella Typhimurium No. 371, Salmonella Dublin No. 373, and Salmonella Choleraesuis No. 370 was carried out, and bioinformatics analysis of the obtained data was made.

The quality of sequencing data (FASTQ files) was assessed using the FastQC_0.11.17 program. Removal of technical sequences and low-quality nucleotides was performed in the Trimmomatic v.0.36 program with the following ILLUMINACLIP parameters: NexteraPE-PE.fa: 2:30:10, SLIDINGWINDOW: 4:15. De novo assembly of bacterial genomes was performed using the SPAdes 2.11.1 assembler with sequencing error correction and automatic selection of the k-mer length (21, 33, 55, 77, 99). Contigs less than 500 bp were excluded from further analysis. The assembly with the smallest number of contigs and the largest N50 value was chosen as the best one. The main characteristics of the assembly were obtained using the QUAST 4.6.3 program and are presented in Table 5.

Table 5: Characteristics of S. enterica genome assembly

Sample	Number of contigs > 500 b.p.	Maximum contig length	Total length of contigs	N50
Salmonella Typhimurium №371	128	287840	4963821	87316
Salmonella Dublin №373	39	419864	4878033	248655
Salmonella Choleraesuis №370	131	223605	4732069	90144

The classification of contigs into chromosomal and plasmid ones was carried out using our own algorithm implemented in Python 2.7. Subsequent scaffolding was performed using the Ragout program (version 2.3) [22], using the genomes of the corresponding model samples listed in Table 6 (KmerFinder column) as a reference. The result was:

• for Salmonella Typhimurium №371 - 2 chromosome scaffolds with a total length of 4902530 bp, as well as the sequence of one of the plasmids with a length of 94045 bp;

• for Salmonella Dublin No. 373 - 6 scaffolds, including the chromosome sequence of 5092130 bp, as well as the sequence of the plasmid contig of 74698 bp;

• for Salmonella Choleraesuis No. 370 - 8 scaffolds, including the chromosome sequence of 4753177 bp in length, as well as the sequence of the plasmid contig of 49547 bp in length.

Genotyping of S.enterica samples was carried out at the aroC, dnaN, hemD, hisD, purE, sucA, and thrA loci. The results are shown in Table 6.

Table 6: Results of genotyping of S.enterica samples

Sample	MLST	KmerFinder
Salmonella Typhimurium №371	ST-19	NZ_CP035301.1 *Salmonella enterica* subsp. enterica strain ST1539
Salmonella Dublin №373	ST-10	NZ_CP032390.1 *Salmonella enterica* subsp. enterica serovar Dublin strain CVM 34981
Salmonella Choleraesuis №370	ST-68	NZ_CP007639.1 *Salmonella enterica* subsp. enterica serovar Choleraesuis strain C500

The annotation was performed using the RAST server on the open platform for comparative analysis of SEED genomes. The preliminarily obtained contigs were ordered using the MAUVE v.20150226 programs relative to the genomes of the corresponding model samples (NZ_CP035301.1, NZ_CP032390.1, NZ_CP007639.1). The result of genome annotation is presented in Table 7.

Table 7: Annotation of S. enterica genomes

Sample	Number of subsystems	CDS	RNA
Salmonella Typhimurium №371	373	5086	87
Salmonella Dublin №373	369	5009	83
Salmonella Choleraesuis №370	368	4904	88

The identification of genes providing resistance to various antibiotics was carried out by the ABRicate program [18] using BLASTN and BLASTX against nucleotide and amino acid sequences from various databases (ResFinder, NCBI BARRGD). The following criteria were used to analyze contig sequences for the presence of resistance genes: >95% identity, >80% minimum crossing length. In all samples (Salmonella Typhimurium No. 371, Salmonella Dublin No. 373, Salmonella Choleraesuis No. 370), the aac(6')-Iaa gene was found, which provides resistance to aminoglycosides due to enzymatic inactivation of the antibiotic. Encodes chromosomal aminoglycoside acetyl transferase. In Salmonella, this gene can be “silent” and does not always contribute to the development of resistance to aminoglycosides; aac(6')-Iaa can be involved in pentose catabolism [15].

To search the VFDB database for the main Salmonella virulence factors, the following criteria were used: >95% identity, >80% minimum intersection length. The results are presented in table 8.

Table 8: S. enterica virulence factors

Sample	Virulence factors
Salmonella Typhimurium №371	avrA, csgA/B/C/D/E/F/G, fimC/D/F/H/I, gogB, grvA, invA/B/C/E/F/G/H/I/J, lpfA/B/C/D/E, mgtB/C, mig-14, misL, orgB/C, pefA/B/C/D, pipB2/B, prgH/I/J/K, ratB, rck, sicA/P, sifA/B, sinH, sipA/sspA, sipB/sspB, sipC/sspC, sipD, slrP, sodCI, sopB/sigD, sopA/D2/D/E2, spaO/P/Q/R/S, spiC/ssaB, sptP, spvB/C/R, ssaC/D/E/G/H/I/J/L/M/N/O/P/Q/R/S/T/U/V, sscA/B, sseA/B/C/D/E/F/G/J/K2/L, sseI/srfH, sspH2, steA/B/C
Salmonella Dublin №373	avrA, csgA/B/C/D/E/F/G, fimC/D/F/H/I, grvA, invA/B/C/E/F/G/H/I/J, lpfA/B/C/D/E, mgtB/C, mig-14, misL, orgB/C, pipB/B2, prgH/I/J/K, ratB, sicA/P, sifA/B, sinH, sipA/sspA, sipB/sspB, sipC/sspC, sipD, slrP, sodCI, sopB/sigD, sopA/D2/E2, spaO/P/Q/R/S, spiC/ssaB, sptP, spvB/C/R, ssaC/D/E/G/H/I/J/L/M/N/O/P/Q/R/S/T/U/V, sscA/sscB, sseA/B/C/E/F/G/J/K1, sseI/srfH, sspH2, steB/C
Salmonella Choleraesuis №370	csgA, csgB/C/D/E/F/G, fimC/D/F/I, gogB, grvA, invA/B/C/E/F/G/H/I/J, isdE, lpfA/B/C/D/E, mgtB/C, mig-14, orgA/C, pefB/D, pipB/B2, prgH/I/J/K, ratB, sicA/P, sifA/B, sinH, sipA/sspA, sipB/sspB, sipC/sspC, sipD, sodCI, sopB/sigD, sopD/D2/E2, spaO/P/Q/R/S, spiC/ssaB, sptP, spvB/C/R, ssaC/D/E/G/H/I/J/L/N/O/P/Q/R/S/T/U/V, sscA/B, sseA/E/G/J/K1, sseI/srfH, sspH2, steB/C

The following criteria were used to search for Salmonella pathogenicity islands using the SPIFinder service: >95% identity, >60% minimum intersection length. The results are presented in table 9.

Table 9: Islets of pathogenicity S. enterica

Sample	Islets of pathogenicity
Salmonella Typhimurium №371	SPI 1, SPI 4, SPI 5, SPI 9
Salmonella Dublin №373	SPI 1, SPI 2, SPI 4, SPI 5, SPI 9
Salmonella Choleraesuis №370	SPI 2, SPI 4, SPI 5, SPI 9

In Salmonella, pathogenicity islands (SPIs) include a number of genes containing signs of virulence. Twenty-one SPIs are currently defined. Pathogenicity island-1 (SPI-1) is a 40 kb DNA region. This module encodes 33 proteins, incl. components of the type III secretion system (T3SS), regulatory and secretory effector proteins. Pathogenicity island 2 (SPI-2) has a size of 40 kb. It codes for the second kind of type III secretion system that is involved in intracellular survival and the flagellum assembly system. SPI-4 is 24 kb. and participates in adhesion to epithelial cells. SPI-5 is a small pathogenicity island less than 8 kb in size, its main role is to ensure penetration into the cell of the intestinal epithelium. SPI-9 is a 16 kb locus. and contains three genes encoding components of the type I secretion system (T1SS) [19, 21].

4 Conclusion

The biological properties, virulence, and immunogenicity of 54 strains of Salmonella serovars S. Choleraesuis, S. Typhimurium, S. Dublin, S. Enteritidis, and S. Infantis, received in the collection of the Museum of Microorganisms of the Federal State Budgetary Institution "VGNKI" in 1939-1980, were studied. as well as epizootic strains of these Salmonella serovars isolated in 2010-2021. in various regions of Russia. It has been established that the production strains of S. Typhimurium No. 371, S. Dublin No. 373 and S. Choleraesuis No. 370, selected in 1970 - 1980, are currently the most immunogenic and suitable for the production of vaccines against salmonellosis in terms of their combination of properties. animals.

Bioinformatics analysis of whole genome sequencing data of these strains will allow their genetic identification.

Among the Salmonella serovars S. Enteritidis and S. Infantis, no highly immunogenic strains promising for their use as industrial ones were found.

5 Availability of Data and Material

Data can be made available by contacting the corresponding authors.

6 Acknowledgement

This work was carried out within the framework of the research program "Creation of a complex of means of protection against economically and socially significant animal diseases, based on the production strains of microorganisms selected by genomic sequencing methods" (Agreement No. 075-15-2021-1054 of 29.09.2021).

7 References

[1] Ashmarin, I.A. Vorobyov A.A. (1962). Statistical methods in microbiology. Leningrad. Medicine, 1962. 180 p.

[2] Volozhantsev, N.V., et al. (1997). Obtaining vaccine strains of Salmonella using insertional mutagenesis. Veterinary. 9, 20-24.

[3] Panin, A.N., et al. (2017). The problem of resistance to antibiotics of pathogens common to humans and animals. Veterinary medicine, zootechnics and biotechnology. 5, 18-24.

[4] Panin, A.N., Kulikovskii, A.V., Davleev, A.D. (2010). Prevention of salmonellosis in the cultivation and processing of poultry. Available at: webpticeprom.ru.

[5] Barrow, P. A., Lovell, M. A., Berchieri, A. (1991). The use of two live attenuated vaccines to immunize egg-laying hens against Salmonella enteritidis phage type 4. Avian Pathology. 20(4), 681-692.

[6] Brettin, T., et al. (2015). RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Scientific reports. 5, 8365.

[7] Charles,,S. D., et al. (1994). Adjuvanted subunit vaccines for the control of Salmonella enteritidis infection in turkeys. American journal of veterinary research. 55(5), 636-642.

[8] Ghosh, S. S. (1989). Comparative efficacy of four vaccines against Salmonella virchow in chicks in India. Research in Veterinary Science. 47(2), 280-282.

[9] Gooderham, K. (1998). Biosecurity and vaccination in eradicating Salmonella enteritidis [chickens-United Kingdom]. Selezione Veterinaria (Italy). 8/9, 561-571.

[10] Hasman, H. et al. (2014). Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. Journal of clinical microbiology. 52(1), 139-146.

[11] Larsen, M. V. et al. (2012). Multilocus sequence typing of total-genome-sequenced bacteria. Journal of clinical microbiology. 50(4), 1355-1361.

[12] Liu, B. et al. (2018). VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic acids research. 47(D1), 687-692.

[13] Identification of acquired antibiotic resistance genes. ResFinder. Available at: https://www.cge.cbs.dtu.dk/services/ResFinder.

[14] NCBI's Bacterial Antimicrobial Resistance Reference Gene Database. Available at: https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder.

[15] Magnet, S., Courvalin, P., Lambert, T. (1999). Activation of the cryptic aac (6′)-Iy aminoglycoside resistance gene of Salmonella by a chromosomal deletion generating a transcriptional fusion. Journal of Bacteriology. 181(21) 6650-6655.

[16] Rapid Annotation using Subsystem Technology (RAST). Available at: http://wwww.rast.theseed.org/FIG/rast.cgi.

[17] Roer L. et al. (2016). Is the evolution of Salmonella enterica subsp. enterica linked to restriction-modification systems?. Msystems. 1(3) e00009-16.

[18] ABRicate program. Avalable at: https://github.com/tseemann/abricate

[19] Chugunova, E. O., Tatarnikova, N. A. (2014). Salmonella genome (review). Modern problems of science and education. 6, 1828-1828.

[20] The Virulence Factor DataBase (VFDB). Available at: http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi.

[21] Semina, A. N., Novikova, O. B., Abgaryan, S. R. (2018). Study of the Salmonella genome for the specific determination of s. Enteridis, s. infantis and s. Typhimurium. Effective animal husbandry. 9(148), 6-6.

[22] Kolmogorov, M. et al. (2014). Ragout – a reference-assisted assembly tool for bacterial genomes. Bioinformatics. 30(12), i302-i309.

[23] World Health Organization. (1991). Control of Salmonellosis: Part Played by Veterinary Science and Nutrition Hygiene. 11-12 June 1991. Geneva, Switzerland: WHO/CDD/SER/91.14, 436-437.

[24] Zankari, E. et al. (2012). Identification of acquired antimicrobial resistance genes. Journal of antimicrobial chemotherapy. 67(11) 2640-2644.

Lenev Sergey is a Candidate in Biological Sciences, Leading Researcher at the Scientific and Technological Laboratory of The Russian State Center For Animal Feed And Drug Standardization And Quality. His research focuses on the use of bacteriophages as biological preparations in the fight against salmonellosis and colibacillosis, as well as reducing the likelihood of human disease through slaughter products and animal and poultry meat contaminated with pathogens of these infections.

Krylova Ekaterina is a Candidate in Biological Sciences, Leading Researcher, the Department of Molecular Biology of The Russian State Center For Animal Feed And Drug Standardization And Quality. Her research focuses on medicines for animals and feed.

Gordeeva Veronika is a Leading Specialist in the Department of Molecular Biology of The Russian State Center For Animal Feed And Drug Standardization And Quality. Her research focuses on molecular genetics, cell biology and bioinformatics.

Prasolova Olga is a Candidate in Veterinary Sciences, Leading Researcher, at the Department of Molecular Biology of The Russian State Center For Animal Feed And Drug Standardization And Quality. Her research focuses on molecular genetics and cell biology.

Soltynskaya Irina is a Head of the Department of Molecular Biology of The Russian State Center For Animal Feed And Drug Standardization And Quality. Scientific interests: veterinary monitoring of bacterial resistance to antimicrobial agents.

Abrosimova Nadezhda is a Researcher at the Scientific and Technological Laboratory of The Russian State Center For Animal Feed And Drug Standardization And Quality. Her research focuses on carrier testing hereditary diseases.