ANNOTATION

of the dissertation work for the degree of Doctor of Philosophy (Ph.D.) in the specialty 6D070100 – Biotechnology

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Development of PCR test systems for the typing of *Salmonella* in clinical material, food raw materials and foodstuffs

General characteristics of the dissertation research. The dissertation work relates to the field of biotechnology and is devoted to the development of rapid tests suitable for identifying and typing salmonella in various samples. The paper presents the results of collecting samples of clinical materials, food raw materials and foodstuffs sold on markets in various regions, data on the selection of specific oligonucleotides, testing the conditions for PCR, DNA extraction, determining the specificity, sensitivity and diagnostic efficiency of the developed test systems. Using the developed test systems, the prevalence and genetic heterogeneity of Salmonella in Kazakhstan was shown. The data obtained formed the basis for recommendations and regulatory and technical documentation for the test system.

The relevance of research.

In the Republic of Kazakhstan, salmonellosis remains one of the most pressing health problems. An analysis of statistical data from the Ministry of Health shows that the incidence rate of salmonellosis per 100,000 population in 2019-2021 was 2.59-6.01. In 2017-2021, the microbial landscape of *Salmonella* cultures isolated from patients was dominated by *S. Enteritidis* and *S. Typhimurium*. According to the sanitary and epidemiological service, from 70 to 90% of salmonellosis cases occur as a result of consumption of low-quality products.

Detection and identification of bacterial pathogens in the food industry are essential in preventing foodborne outbreaks. In the Republic of Kazakhstan, the most commonly used method for detecting *Salmonella* is the traditional microbiological method. These methods are typically labor intensive and require a minimum of 4–6 days, increasing the risk of contamination or transmitting pathogens. The need to obtain timely results has led to the development of many rapid detection methods with high specificity and sensitivity, among which PCR is a powerful method that provides rapid, sensitive and specific detection of pathogens. In the available literature, there is only one report by Kazakh researchers on developing PCR for detecting *Salmonella* using the fimA virulence gene (Daugalieva A.T. et al., 2015). The authors showed the possibility of detecting *Salmonella* in sick animals but did not report the capabilities of this method for detecting *Salmonella* in food. Recently, based on an analysis of the complete genome of 145 strains of various *Salmonella* species, Hu, L. et al. (2021) showed that the fimA gene has questionable sites for use as a target when developing a method for detecting *Salmonella*.

Thus, the development of PCR for the detection and typing of *Salmonella* in clinical material, food raw materials and food products in the Republic of Kazakhstan is relevant.

Objects of study: Serotypes of *Salmonella enterica* subsp. Samples from clinical material of patients suffering from acute intestinal infection. Samples from food raw materials and semi-finished products used in preparation, daily samples of prepared food and other suspected factors in transmitting the infectious agent.

Subject of study. Classic PCR and real-time PCR (RT) test systems for detecting *Salmonella enterica* subsp. *Enterica* (*S. enterica*) and typing of *Salmonella enterica* subsp. *Enterica* serovar *Enteritidis* (*S. Enteritidis*), *Salmonella enterica* subsp. *Enterica* serovar *Typhimurium* (*S. Typhimurium), Salmonella enterica* subsp. E*nterica* serovar *Virchow* (*S. Virchow*) in clinical material, food raw materials and food products. RAPD-PCR for *S. enterica* serotyping.

Purpose of the study. Creation of PCR test systems for typing Salmonella in clinical material, as well as in food raw materials and food products sold in the Republic of Kazakhstan.

Research objectives:

1. Development of PCR test systems for the detection and typing of Salmonella in clinical material, food raw materials and foodstuffs.

2. Determination of the specificity, sensitivity and diagnostic efficiency of PCR test systems for the detection and typing of Salmonella in clinical material, food raw materials and food products.

3. Determination of the prevalence and genetic diversity of Salmonella isolated from clinical material in food raw materials and foodstuffs.

4. Development of recommendations for the use of PCR for typing Salmonella in clinical material, food raw materials and foodstuffs.

Research methods. The work used modern research methods of molecular biology and microbiology: collection of biological samples from sick children in medical institutions, collection of food raw materials and food products in their distribution network, selection of primers and probes using the NCBI bioinformation database, synthesis of primers and probes, DNA extraction isolates of *Salmonella* bacteria, PCR analysis to identify *Salmonella* bacteria, determination of the specificity and sensitivity of classical PCR and RT PCR, RAPD-PCR, determination of the nucleotide sequence using the method using NGS technology (MiSeq, Illumina), analysis of oligonucleotide sequences using the Basic Local Alignment Search Tool (BLAST) bioinformatics program from the NCBI database (https://blast.ncbi.nlm.nih.gov).

The scientific novelty of the study lies in the fact that for the first time in Kazakhstan, test systems based on classical PCR and RT PCR have been developed

for the molecular diagnosis of salmonellosis to identify *S. enterica* and its common serotypes *S. Enteritidis*, *S. Typhimurium* and *S. Virchow*.

For the first time, using developed modern methods, it was shown that the prevalence of *S. enterica* in various food products in 2018 was 5%, and in 2019 it was 2.34%. 65 clinical cases of *Salmonella* infection have been confirmed. A total of 99 samples were isolated and confirmed by PCR, of which 21 (21.2%) isolates were assigned to *S. Enteritidis*, 43 (43.4%) isolates to *S. Typhimurium*, and 26 (26.3%) isolates to *S. Virchow*.

2 patents of the Republic of Kazakhstan were received for a set of new oligonucleotides for identifying the DNA of *S. enterica* and its types, as well as for the PCR method for identifying *S. enterica*, which confirms the scientific novelty of the dissertation work.

For the first time, using the developed RAPD PCR, the genetic diversity of salmonella in food and clinical material isolated on the territory of Kazakhstan was shown. PCR analysis of the genomic DNA of *S. enterica* bacteria obtained from the food chain and clinical materials with the RAPD primer showed the genetic relatedness of the *S. Enteritidis* isolates, and the genetic heterogeneity of the *S. Typhimurium*, *S. Virchow* isolates.

For the first time, the nucleotide sequence of the complete genome of the Kazakh strain QazSL-4 of *S. enterica* was determined and deposited in the international GenBank database.

Practical significance of the study

The possibility of using the developed PCR test systems for the analysis of clinical materials isolated from sick children and food raw materials, food products contaminated with *S. enterica* and the types *S. Enteritidis*, *S. Typhimurium* and *S. Virchow* has been shown.

A package of normative and technical documentation (NTD) has been developed: Organizational standards, instructions for production and control, and instructions for the use “Test system for detecting *S. enterica* by real-time PCR method.”

Based on the materials of the dissertation work, a “Recommendation on the use of PCR for typing *Salmonella* in clinical material, food raw materials and food products” was compiled. The collected research data will allow us to develop measures to reduce the prevalence of salmonellosis.

The established complete genome of the Kazakh strain QazSL-4 of *S. enterica*, isolated from chicken fillet, is an essential addition to the existing genome database of the *Salmonella* bacterium, which makes it possible to use them in determining the variability of the *Salmonella* genome circulating in Kazakhstan.

The theoretical significance lies in assessing the prevalence and diversity of *Salmonella* isolates on the territory of the Republic of Kazakhstan. As a result of the

work, the complete genome sequence of the Kazakhstan isolate QazSL-4 of *S. enterica* was deposited in the NCBI GenBank database.

The main provisions of the dissertation submitted for defense:

1. PCR test systems have been created to quickly detect S. enterica and identify serotypes S. Enteritidis, S. Typhimurium and S. Virchow in clinical material, food raw materials and foodstuffs.

2. High specificity, sensitivity and diagnostic efficiency of PCR test systems for the detection and typing of Salmonella have been demonstrated

3. Prevalence and genetic heterogeneity of Salmonella serotypes in clinical material, food raw materials and food products in Kazakhstan.

4. The high diagnostic efficiency of the developed PCR test systems made it possible to formulate recommendations for the early detection of salmonella. The developed set of regulatory and technical documentation opens the way to the introduction of the drug into production.

Main results and conclusions

1. Specific primers were designed and a PCR test system was developed to detect the genomic DNA of *S. enterica* in food and clinical material.

2. Specific primers were designed and PCR test systems were developed to identify the serotypes of *S. Enteritidis*, *S. Typhimurium*, *S. Virchow*.

3. Specific primers and probes were designed, and a RT-PCR test system was developed to detect *S. enterica* genomic DNA in food and clinical material.

4. Specific primers and probes were designed, RT PCR test systems were developed to identify *S. Enteritidis, S. Typhimurium*, *S. Virchow* serotypes.

5. The high diagnostic efficiency of the developed test systems has been shown. As a result of the study of 1020 biological samples collected in 2018-2019. in Almaty, 99 isolates of *S. enterica* were identified using developed PCR methods and isolated by microbiological cultivation.

6. The prevalence of *S. enterica* in various food products in 2018 was 5%, and in 2019 it was 2.34%. 65 clinical cases of *Salmonella* infection have been confirmed. A total of 99 samples were isolated and confirmed by PCR, of which 21 (21.2%) isolates were assigned to *S. Enteritidis*, 43 (43.4%) isolates to *S. Typhimurium*, and 26 (26.3%) isolates to *S. Virchow*.

7. RAPD-PCR was developed to determine the genetic polymorphism of various *S. enterica* species. PCR analysis of the genomic DNA of *S. enterica* bacteria obtained from the food chain and clinical materials with the RAPD primer showed the genetic relatedness of the *S. Enteritidis* isolates, and the genetic heterogeneity of the *S. Typhimurium*, *S. Virchow* isolates.

8. Whole genome sequencing of the QazSL-4 strain of serovar *S.enterica* subsp. *Enterica*, isolated from chicken fillet purchased at the Almaty market in 2018, allowed us to obtain a genome sequence with a length of 4,711,816 bp. spanning 49 contigs, with an N50 value of 491,954 bp and an L50 value of three contigs. The genome of strain QazSL-4 contains one homologue of the resistance gene aac(6')-Iaa. The PHASTER program identified one prophage region in the genome sequence; no intact regions were found. The presence of widespread *Salmonella* pathogenicity islands SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-10, SPI-13 and SPI-14 was revealed.

Connection with the plan of essential scientific work. The dissertation work was carried out within the framework of project No. AP05131147 "Genotyping of pathogenic microorganisms in food raw materials and food products sold in markets and supermarkets of the Republic of Kazakhstan, development of recommendations for reducing the risk of morbidity in children of preschool and school age" (2018-2020), as well as in within the framework of the project "Zhas Galym", No. AP15473285 "Study of the prevalence and genetic diversity of *Salmonella* in the southern regions of Kazakhstan" (2022-2024)

Approbation of work. The research results were published at international scientific and practical conferences: VI International Conference of Young Scientists (Novosibirsk, 2019), "VI International Farabian Readings" (Almaty, 2019), International Scientific and Practical Conference "Current Problems of Biodiversity and Biotechnology" (Astana, 2019), 2021: Proceedings of World Congress on Food Science & Food Safety and for Proceedings of 2nd World congress on Diabetes & Metabolism (2021), international scientific conference of students and young scientists "Farabi Әlemi" (Almaty, 2022), international scientific and practical conference "Biotechnology and biological safety: achievements and development prospects" (Almaty, 2023).

Publications. The main content of the dissertation is reflected in 16 published works, including 2 article in a publication indexed in the Web of Science or Scopus database with a non-zero impact factor; 4 articles in republican scientific journals included in the list of Committee for Quality Assurance in the Field of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, 1 article in a publication indexed in the RSCI database, 7 theses in materials of international conferences held in Switzerland, Russia and the Republic of Kazakhstan. Based on the work results, 2 patents for utility models were received.

Personal contribution of a doctoral student. All sections of the dissertation work were completed with the personal participation of the author. The dissertation work was completed by the author independently, under the guidance of scientific consultants. An analysis of literary sources was carried out, laboratory experiments were performed, methods for performing molecular genetic analyzes were optimized, the results were independently analyzed and processed, conclusions were drawn and proposals were formed.

Scope and structure of the dissertation. The dissertation is 123 pages and consists of symbols and abbreviations, an introduction, a literature review, materials and methods, results and conclusion, and a list of sources used.