

Herald of Science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences.
– Astana: S. Seifullin Kazakh Agrotechnical Research University, 2025. – № 4 (012). – P.4-18.
- ISSN 2958-5430, ISSN 2958-5449

VETERINARY SCIENCES

doi.org/10.51452/kazatuvc.2025.4(012).2063

UDC 619:616.98:636.5

Research article

Study of the Distribution of *Salmonella* Infection in Poultry Farms in the Northern Region of Kazakhstan

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Received: 18.10.2025 **Accepted:** 22.12.2025 **Published:** 30.12.2025

Abstract

Background and Aim. Avian salmonellosis represents a serious public health concern, as infected birds and contaminated poultry products serve as major sources of transmission. This study aimed to perform serological analyses and isolate *Salmonella enterica* from samples collected from poultry farms in northern Kazakhstan.

Materials and Methods. A total of 334 chicken serum samples and 285 biological and pathological samples were collected from poultry farms located in the Akmola, Kostanay, and Karaganda regions. The serum samples were analyzed using the indirect enzyme-linked immunosorbent assay (ELISA). To isolate *Salmonella* strains, the samples were cultured on differential diagnostic media, and resulting isolates were identified using biochemical and molecular genetic methods. The antimicrobial resistance of the isolated strains was determined by the disk diffusion method.

Results. Antibodies specific to *Salmonella* antigens were detected in serum samples from chickens at one poultry farm. Nine *Salmonella* isolates were recovered from pathological material. The isolates were identified as *Salmonella enterica* subsp. *enterica*, and the following serotypes were determined: *S. enteritidis*, *S. paratyphi*, *S. moscow*, *S. infantis*, and *S. mbandaka*. Antimicrobial susceptibility testing revealed sensitivity to amikacin, ceftriaxone, gentamicin, amoxicillin, and ciprofloxacin. However, the isolates exhibited multidrug resistance to several antibiotic classes, including rifamycins, macrolides, glycopeptides, cephalosporins, tetracyclines, lincosamides, aminoglycosides, nitrofurans, and penicillins.

Conclusion. The results of this study confirm the circulation of *Salmonella* at certain poultry farms in the northern region of Kazakhstan and provide insight into the serotypes of the strains and their antimicrobial resistance profiles. These findings may be used to support the development of effective antimicrobial therapy strategies in poultry farming.

Keywords: avian salmonellosis; antibody titers; isolates; identification; antimicrobial resistance.

Introduction

Poultry farming is currently one of the most economically profitable sectors of animal husbandry in the Republic of Kazakhstan, providing the country with valuable poultry products. However, infectious diseases, particularly salmonellosis, can hinder the development of this industry. Salmonellosis causes

significant damage to poultry production and is one of the most common causes of foodborne diseases in humans [1]. A high level of *Salmonella* infection in poultry has been reported in many countries around the world, including Kazakhstan [2, 3, 4, 5]. The main source of infection is infected birds, which excrete large quantities of the pathogen via feces and eggs. Transmission can occur via the digestive tract (feed, water), transovarially (to embryos), as well as by airborne and ocular routes. Adult birds often experience asymptomatic infection and act as carriers, with the pathogen primarily localized in the ovaries [6, 7]. The principal causative agent of chicken salmonellosis is *Salmonella pullorum/gallinarum*; however, chickens are frequently infected with *Salmonella enteritidis* and other serovars that do not cause clinical signs or mortality, complicating the assessment of the infection status of a poultry farm [8]. Reports suggest that salmonellosis outbreaks have demonstrated an increased frequency of *Salmonella* isolation from domestic poultry, including chickens [9]. According to WHO experts, the absence of clinical signs in birds and the difficulty in identifying the carrier birds pose a constant risk of environmental and food contamination [10]. The maintenance and spread of infection are also facilitated by birds from private household farms [11] and wild migratory birds. Changes in feeding behavior and migration patterns associated with climate change have increased interactions between wild bird and urban environments. *Salmonella* infection has been documented in approximately 140 wild bird species, highlighting their role in the long-distance spread of the pathogen [12].

According to statistical data, there has been a decline in the number of reported cases of salmonellosis in Kazakhstan [13]. However, studies conducted at individual poultry farms confirm the continued presence of *Salmonella* [5, 14, 15]. Additionally, research into antimicrobial resistance of *Salmonella* bacteria is currently highly relevant due to several factors. Firstly, *Salmonella* remains one of the major pathogens causing foodborne poisoning and infectious diseases in humans. Secondly, the continuous emergence of new *Salmonella* strains that are resistant to antimicrobial agents poses serious challenges for both veterinary medicine and public health, as standard treatment regimens may prove ineffective [16].

This study aimed to investigate samples collected from poultry farms in the Akmola, Kostanay, and Karaganda regions of the Republic of Kazakhstan, through isolation and identification of *Salmonella* strains and evaluating their microbial resistance profiles.

Materials and Methods

Ethical approvals

All research procedures were approved by the Ethics Committee of S. Seifullin Kazakh Agrotechnical Research University (Protocol No. 2, dated November 1, 2023) and were conducted in accordance with biosafety regulations and ethical standards for animal care and use.

The study material included blood serum collected from chickens of various ages, as well as other biological and pathological samples (feed, surface swabs from equipment and tools, as well as the organs of deceased birds and gastrointestinal contents). Samples were collected from poultry farms located in the Kostanay and Akmola regions of Kazakhstan. Sampling was conducted in accordance with the "Rules for Sampling and Biological Material Collection" (Order of the Ministry of Agriculture of the Republic of Kazakhstan, dated April 30, 2015, No. 7-1/393).

Biological material samples were placed in transport medium tubes (Swab, Tokyo, Japan). Blood samples from chickens and chicks were collected from the subclavian vein into Vacutainers and processed to obtain serum. Pathological material samples (parenchymal organs from deceased birds) were collected in sterile disposable containers and kept in an icebox. Sampling was supervised by the poultry farm veterinarians, and all the samples were delivered to the Kazakhstan-China Laboratory for Biosafety at Saken Seifullin Kazakh Agrotechnical Research University in strict compliance with the "cold chain" protocol.

The research protocols were approved by the Ethics Committee of Saken Seifullin Kazakh Agrotechnical Research University (Protocol No. 1, dated November 15, 2023). All procedures were conducted in accordance with biosafety regulations and the ethical standards for animal care and use.

For the serological examination, a commercial enzyme-linked immunosorbent assay (ELISA) kit (ID Screen Avian Salmonella Indirect, Montpellier, France) was used to quantitatively assess the presence of antibodies against *Salmonella* (groups B and D) in chicken and turkey sera.

Microbiological and bacteriological studies were performed in accordance with GOST 31659-2012: "Food Products. Methods for the Detection of *Salmonella* spp." [17]. The following culture media were used for pathogen isolation: buffered peptone water (LLC "Scientific and Production Center Biokompas-S," Russia), Rappaport-Vassiliadis soybean broth (LLC "Scientific and Production Center Biokompas-S," Russia), bismuth sulfite agar, Ploskirev medium, and Endo medium (Federal State Scientific Center for Applied Microbiology and Biotechnology, Russia).

Colony morphology was documented after incubation in a thermostat for 24-48 h. It is well known that on Ploskirev medium, *Salmonella* colonies appear as colorless, round colonies with black centers. On Endo medium, *Salmonella* form round, colorless or slightly pink colonies. On bismuth sulfite agar, *Salmonella* produces black colonies with a characteristic metallic sheen or greenish colonies encircled by a dark green border.

For rapid detection of *Salmonella*, Compact Dry SL indicator tests (Tokyo, Japan) were employed with a ready-to-use selective dry medium for *Salmonella*. Solid samples were pretreated by adding sterile peptone water in a 1:9 ratio and then homogenized for 1 min. For liquid samples, peptone water was added in the same 1:9 ratios. The liquid sample was filtered through a membrane filter and incubated in a thermostat for 20-24 h at 35-37 °C. Using a sterile pipette, 0.1 mL of the enriched sample was applied to the surface of the Compact Dry SL plate and incubated at 41-43 °C for 20-24 h. The results were evaluated visually according to the manufacturer's instructions.

The following materials were used for biochemical identification: Hiss medium with sucrose and mannitol (LLC "Scientific and Production Center Biokompas-S," Russia), Mueller-Hinton agar (HiMedia Laboratories, India), lead paper, oxalate paper, OF-test (Erba Lachema, Czech Republic), a reagent kit for the Voges-Proskauer reaction (Micro-VOGES-PROSKAUER-NICF, Russia), and reagents for the catalase and oxidase activity tests (Erba Lachema, Czech Republic). Biochemical identification included determining the ability to ferment glucose, lactose, mannitol, and sucrose; to produce hydrogen sulfide and indole; and the assessment of catalase and oxidase activity.

Isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with Bruker Realtime Classification software (Bruker Daltonics, Billerica, Massachusetts, USA), where a score of ≥ 2.0 was considered reliable.

Bacterial cultures were identified at species level by analyzing the nucleotide sequence of the 16S rRNA fragment and by intact cell mass spectrometry. The 16S rRNA fragment was amplified and further sequenced according to a previously described protocol [18].

Antibiotic susceptibility was determined using the disk diffusion method in accordance with MUK 4.2.1890-04: "Determination of Microorganism Sensitivity to Antibacterial Drugs". The method was conducted on Mueller-Hinton agar, and 33 antibiotic disks were used for the testing (NICF, Russia) [19]. Interpretation of the results was based on EUCAST criteria (versions 8.0, 2018 and 9.0, 2019). Statistical data analysis was performed using Microsoft Excel 2010, applying the student's t-test at a significance level of $\alpha < 0.05$.

Results and Discussion

The samples selected for the study included feces, surface swabs from equipment and tools, blood, and parenchymal organs from deceased birds (liver, kidneys, pancreas) (Figure 1).

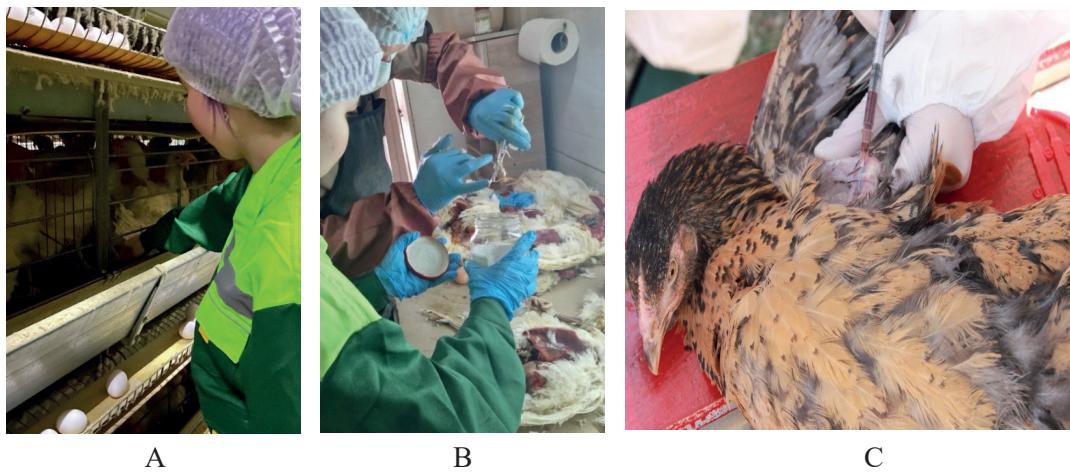


Figure 1 – Sampling of material from the poultry farm
(A – surface swabs from equipment, B – organ sampling, C – blood collection)

Screening for salmonellosis in chickens at a poultry farm in the Kostanay region using the ELISA revealed that among 100 birds of different ages, young chickens (56 and 153 days old) tested negative. Specific antibodies against *Salmonella* antigens were detected in 33 samples from adult birds (340 and 431 days old) (Figure 2).

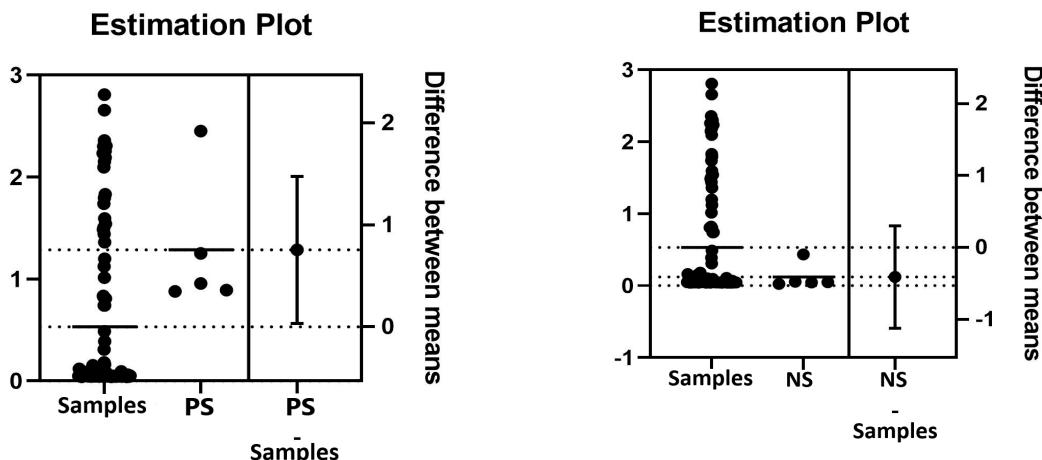


Figure 2 – Comparative analysis of tested chicken serum samples with sera from healthy and infected birds
(PS – positive samples, NS – negative samples)

As depicted in Figure 2, the optical density (OD) values of the ELISA results comprising sera from birds, as well as from positive and negative controls, displayed a fairly normal distribution. Consequently, it confirmed the homogeneity of the samples and allowed for the application of parametric statistical methods. Comparative analysis of the OD values between the studied groups was performed using the student's t-test. The use of this test allowed for an objective determination of the significance of the observed differences and confirmed that the experimental data accurately reflected the actual relationship between the clinical condition of the birds and the level of specific antibodies detected by the ELISA method.

Similarly, serum samples collected from two different poultry houses at poultry farms "M" and "A" in the Akmola region, as well as serum samples from poultry farm "K" in the Karaganda region, were examined. No specific antibodies against the *Salmonella* genus were detected in the bird serum samples. The analysis of the ELISA results for the blood serum samples from various poultry farms is presented in Table 1.

Table 1 – Results of blood serum sample testing from different poultry farms

| No. | Poultry farm | Number of samples | Bird age (days) | Positive | Negative |
|-------|-----------------------------------|-------------------|--------------------|----------|----------|
| 1 | Kostanay region. Poultry farm "A" | 12 | 56 | 0 | 12 |
| | | 30 | 153 | 0 | 30 |
| | | 28 | 340 | 14 | 14 |
| | | 30 | 431 | 19 | 11 |
| 2 | Akmola region. Poultry farm "M" | 72 | Adult bird | 0 | 72 |
| 3 | Akmola region. Poultry farm "A" | 126 | (broilers) 50 days | 0 | 126 |
| 4 | Karaganda region Poultry farm "K" | 36 | Adult bird | 0 | 36 |
| Total | | 334 | | 33 | 301 |

Investigation of the serum samples from chickens at several poultry farms revealed a high seroprevalence rate in adult birds at a poultry farm in the Kostanay region. Specific antibodies to *Salmonella* antigens were detected in 17.55% of the tested samples. No antibodies specific to *Salmonella* bacteria were detected at any of the other poultry farms.

Simultaneously, microbiological studies were conducted to detect *Salmonella* bacteria in the samples collected from the poultry farms (Figure 3). For rapid detection, the material samples were applied to the chromogenic test substrates in a Compact Dry SL *Salmonella* kit (Tokyo, Japan).



Figure 3 – Results of sample testing for the presence of *Salmonella* on Compact Dry plates
(1, 2 – negative results, 3 – positive result, 4 – control)

The analysis results showed that the presumptive presence of *Salmonella* was detected in 12 samples collected from a poultry farm in the Kostanay region. After inoculation onto differential diagnostic media and incubation for 24 and 48 h, the colonies were examined, and bacterial colonies typical of the genus *Salmonella* were selected (Figure 4).

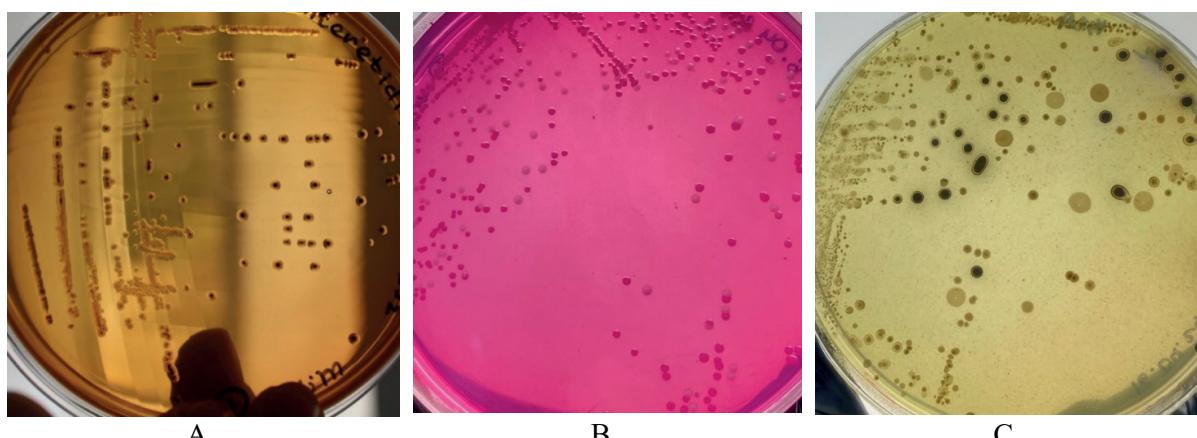


Figure 4 – Growth characteristics of *Salmonella* bacteria on Ploskirev medium (A), Endo medium (B), and bismuth sulfite agar (C)

The samples from poultry farm “A” in the Kostanay region and poultry farm “M” in the Akmola region showed growth of typical *Salmonella* colonies, which were further confirmed using Gram staining and microscopic examination. The microscopic examination confirmed Gram-negative rod-shaped bacteria measuring 3-7 μm in length and 0.30.7 μm in width.

Because birds serve as natural reservoirs for *Salmonella* and *E. coli*, they are commonly found on poultry farms, and production conditions may facilitate their spread, leading to the contamination of poultry products. On simple media such as nutrient agar and nutrient broth, *Salmonella* and *E. coli* can exhibit similar growth patterns, making it difficult to distinguish them based solely on colony morphology. To differentiate these microorganisms, biochemical tests are warranted. Table 2 presents the comparative analysis of the biochemical properties of *Salmonella* and *Escherichia* bacteria.

Table 2 – Comparison of the biochemical properties of bacteria of the genera *Salmonella* and *Escherichia*

| A genus of bacteria belonging to the family Enterobacteriaceae | Products | | Fermentation of sugars | | | | catalase | oxidase |
|--|------------------|--------|------------------------|---------|---------|----------|----------|---------|
| | Hydrogen sulfide | Indole | lactose | glucose | Sucrose | mannitol | | |
| <i>Salmonella</i> | + | - | - | + | - | + | + | - |
| <i>Escherichia</i> | - | + | + | + | - | + | + | - |

Biochemical identification revealed that the isolated cultures could produce hydrogen sulfide, ferment glucose and mannitol, and exhibit a positive catalase reaction: indicating characteristic features of the genus *Salmonella*.

To confirm the affiliation of the isolates with *Salmonella* bacteria, identification was performed using MALDI-TOF MS (Figure 5).

Organism names which are in blue and underlined are linked to the matching nine table below.

| Analyte Name | Analyte ID | Organism (best match) | Score Value | Organism (second best match) | Score Value |
|-----------------------|------------|-----------------------------|--------------|------------------------------|--------------|
| <u>A8</u> (+) (B) | A8 | <u>Salmonella sp</u> | <u>1.96</u> | <u>Salmonella sp</u> | <u>1.941</u> |
| <u>A9</u> (++) (C) | A9 | <u>Salmonella sp</u> | <u>2.143</u> | <u>Salmonella sp</u> | <u>2.141</u> |
| <u>B8</u> (-) (C) | B8 | no peaks found | <u>≤ 0</u> | no peaks found | <u>≤ 0</u> |
| <u>B9</u> (++) (A) | B9 | <u>Niallia circulans</u> | <u>2.255</u> | <u>Niallia circulans</u> | <u>2.037</u> |
| <u>C8</u> (-) (C) | C8 | not reliable identification | <u>1.697</u> | not reliable identification | <u>1.681</u> |
| <u>C9</u> (+) (B) | C9 | <u>Niallia circulans</u> | <u>1.889</u> | <u>Niallia circulans</u> | <u>1.826</u> |
| <u>D8</u> (++) (A) | D8 | <u>Salmonella sp</u> | <u>2.152</u> | <u>Salmonella sp</u> | <u>2.14</u> |
| <u>D9</u> (+) (B) | D9 | <u>Niallia circulans</u> | <u>1.84</u> | <u>Niallia circulans</u> | <u>1.727</u> |
| <u>E8</u> (++) (A) | E8 | <u>Salmonella sp</u> | <u>2.272</u> | <u>Salmonella sp</u> | <u>2.264</u> |
| <u>E9</u> (+) (B) | E9 | <u>Niallia circulans</u> | <u>1.822</u> | <u>Niallia circulans</u> | <u>1.76</u> |
| <u>F8</u> (-) (C) | F8 | not reliable identification | <u>1.543</u> | not reliable identification | <u>1.542</u> |
| <u>F9</u> (++) (A) | F9 | <u>Niallia circulans</u> | <u>2.051</u> | <u>Niallia circulans</u> | <u>2.034</u> |
| <u>G8</u> (++) (A) | G8 | <u>Salmonella sp</u> | <u>2.265</u> | <u>Salmonella sp</u> | <u>2.225</u> |
| <u>G9</u> (+) (B) | G9 | <u>Niallia circulans</u> | <u>1.926</u> | <u>Niallia circulans</u> | <u>1.766</u> |
| <u>H8</u> (++) (C) | H8 | <u>Salmonella sp</u> | <u>2.237</u> | <u>Salmonella sp</u> | <u>2.225</u> |
| <u>H9</u> (++) (A) | H9 | <u>Niallia circulans</u> | <u>2.004</u> | <u>Niallia circulans</u> | <u>1.927</u> |

Figure 5 – Results of bacterial isolate identification using mass spectrometry

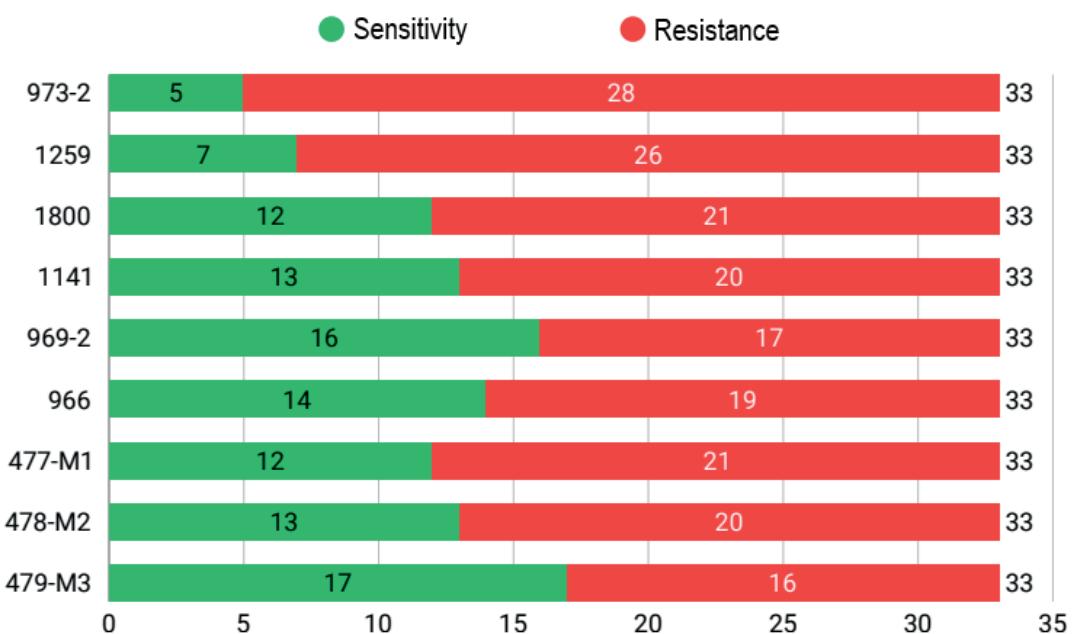
As a result of ion detection and comparison of their mass, structure, and abundance with the reference database integrated into the mass spectrometer, nine cases demonstrated their affiliation with the bacteria of genus *Salmonella*. These data are consistent with the results obtained using Compact Dry SL *Salmonella* kit. Genotyping of the isolated strains determined their taxonomy as *Salmonella enterica*, subspecies *enterica* (subspecies I) and identification of the serotype of each isolate (Table 3).

Table 3 – Characteristics of the isolated bacterial strains

| Isolate number | № 973-2 | № 1259 | № 1800 | № 1141 | № 969-2 | № 966 | № 477-M1 | № 478-M2 | № 479-M3 |
|--------------------------|--|--|--|--|--|--|--|--|--|
| Predicted identification | <i>Salmo-nella enterica sub-species enterica (sub-species I)</i> |
| Serotype | <i>S. enteritidis</i> | <i>S. paratyphi C</i> | <i>S. enteritidis</i> | <i>S. moscow</i> | <i>S. enteritidis</i> | <i>S. enteritidis</i> | <i>S. infantis</i> | <i>S. infantis</i> | <i>S. mbandaka</i> |

As shown in Table 3, all isolates belonged to *Salmonella enterica* subspecies enterica (subspecies I) but represented different serotypes.

After studying the biochemical properties of the bacterial isolates and confirming their taxonomic classification as *Salmonella*, antibiotic susceptibility was tested via the disk diffusion method. The results were evaluated after 24 h and found antimicrobial resistance of the nine *Salmonella* isolates obtained from poultry farm samples in the Republic of Kazakhstan (Figure 6).

Figure 6 – Determination of the antimicrobial susceptibility of isolated *Salmonella* strains

The figure illustrates the sensitivity and resistance profiles of nine *Salmonella* strains to 33 tested antimicrobial agents. The Y-axis represents the strain identifiers, while the X-axis indicates the number of antimicrobial agents for which efficacy was assessed. Green bars indicate the number of drugs to which each strain was sensitive, whereas the red bars represent the number of drugs to which resistance was detected. As shown in the chart, the highest level of susceptibility was observed in strain 479-M3 (17 out of 33 agents), whereas the lowest susceptibility was recorded in strain 973-2 (5 out of 33 agents).

Analysis of the antimicrobial susceptibility results revealed cases of multidrug resistance among the isolated *Salmonella* strains. The isolates demonstrated sensitivity to several antibiotics, including amikacin, ceftriaxone, gentamicin, amoxicillin, and ciprofloxacin. However, resistance was recorded to multiple classes of antibiotics, including rifamycins (rifampicin), macrolides (azithromycin, erythromycin), glycopeptides (vancomycin), cephalosporins (cephadroxil, cefuroxime, cefaclor, cephalothin, cefazolin), tetracyclines (doxycycline), lincosamides (clindamycin), aminoglycosides (kanamycin, streptomycin), nitrofurans (nitrofurantoin), and penicillins (piperacillin).

Differences in susceptibility among isolates of different serotypes were noteworthy. *Salmonella infantis* exhibited a particularly high level of resistance, including resistance to antibiotics that were effective against most other serovars. In contrast, *Salmonella mbandaka* showed sensitivity to a wide range of antimicrobial agents, including amoxicillin/clavulanic acid and co-trimoxazole.

The increasing resistance of bacterial strains to antimicrobial agents often necessitates the use of higher drug dosages. Therefore, based on the results of antimicrobial susceptibility testing, it is recommended that antibiotics to which the studied strains demonstrated the highest sensitivity be preferentially used in practical applications.

Salmonella is a major cause of foodborne disease outbreaks in many countries. In the European Union, the number of reported cases of salmonellosis reached nearly 88,000 in 2019, but decreased to 57,000 in 2020, representing the lowest level recorded since 2007 [20]. In Asian countries, as well as in North and South America and Africa, non-typhoidal salmonellosis remains one of the leading causes of foodborne zoonoses [21, 22, 23].

In veterinary laboratories, the diagnosis of salmonellosis is performed using bacteriological methods along with modern tests, such as ELISA and PCR [24]. Although ELISA has long been used to detect specific antibodies in poultry serum, the method continues to be refined. For example, Ma et al. (2018) demonstrated that the outer membrane protein, PagC, is present in all common *Salmonella* serovars, with a sequence similarity of 98%, and used purified recombinant PagC protein to test chicken serum samples. The ELISA results using rPagC, compared with agglutination tests, showed 80.6% agreement with agglutination tests when analyzing 252 clinical chicken serum samples, suggesting that indirect ELISA based on PagC antibodies may serve as a convenient and novel diagnostic method for salmonellosis [25].

Other researchers have also employed recombinant proteins to develop new variants of ELISA. For example, Gao et al. (2023) utilized recombinant SifA protein for the early diagnosis of salmonellosis in poultry [26]. Additionally, an indirect ELISA based on recombinant IpaJ protein has been proposed as a novel method for the specific detection of *S. pullorum* infection, which may facilitate the eradication of this pathogen in poultry farming [27]. In another study, Yeh et al. used an automated capillary ELISA to quantitatively determine antibodies in chicken serum against recombinant proteins of *Salmonella enterica* serotype Heidelberg [28].

Nevertheless, bacteriological methods remain the primary approach for isolating and characterizing the pathogen. For instance, in Romania, 112 isolates were obtained from raw poultry meat between 2011 and 2021 [29]. That study determined the serovar characteristics of *Salmonella*, their susceptibility to antimicrobial agents, and presence of antimicrobial resistance genes. The most common serotypes were *Salmonella enterica* serovars Enteritidis and Typhimurium (56% and 25%, respectively), and most isolates were resistant to at least three antimicrobial agents, indicating the presence of multidrug-resistant *Salmonella* serovars in poultry meat products [29].

According to Drauch et al. (2022), *Salmonella infantis* is currently the most prevalent serovar in broilers within the European Union. Previous studies have shown that *Salmonella* is detected less frequently in the feces of laying hens than in that of broilers; therefore, fast-growing broilers pose the greatest risk of transmitting the infection to humans [30].

In Iraq, 300 samples of poultry products and human feces were examined for the presence of *Salmonella enterica*. The pathogen was detected in 8.66% of poultry samples and in 4.6% of human samples. Genetic mutations associated with alterations in molecular characteristics and development of multidrug resistance were identified in *S. enterica* isolates [31].

The resistance of *Salmonella* strains represents a serious challenge in controlling avian salmonellosis, and numerous studies have reported multidrug resistance (MDR) among *Salmonella* isolates. For example, in Ethiopia, 32.7% of strains tested in one study showed resistance to streptomycin (75%) and ampicillin (59.4%) [32]. In Sudan, an antimicrobial resistance study of 64 *Salmonella* isolates revealed frequent resistance to ampicillin and cephalexin [33]. In Vietnam, a meta-analysis of publications from 2013 to 2020 showed that bacterial isolates, including *Salmonella*, obtained from pigs and poultry exhibited multidrug resistance, emphasizing the need to restrict antibiotic use in farm animals [34].

Our findings are consistent with previously published studies on avian salmonellosis in Kazakhstan, where *Salmonella* spp. isolates were recovered from retail outlets and poultry farms in the northern regions of the country. Most of the isolates were identified as *S. enteritidis*, with 64.3% demonstrating resistance to three or more classes of antimicrobial agents, indicating the widespread occurrence of multidrug resistance among poultry-associated *Salmonella* strains [35, 36].

Taken together, these results indicate the widespread distribution of *Salmonella enterica* and the presence of multidrug resistance, which is likely attributable to the indiscriminate use of antimicrobial agents in farm animals [37, 33].

Conclusion

Examination of chicken serum samples from several poultry farms in northern Kazakhstan using the ELISA method revealed the presence of antibodies specific to the *Salmonella* pathogen. Subsequently, nine bacterial isolates, presumptively identified as *Salmonella* were recovered, from samples collected at these farms, primarily from parenchymatous organs of deceased birds. Biochemical identification demonstrated that the isolates were capable of producing hydrogen sulfide, fermenting glucose and mannitol, and exhibiting a positive catalase reaction, features characteristic of bacteria belonging to the genus *Salmonella*.

Identification using MALDI-MS and genotyping confirmed that the bacteria belonged to *Salmonella enterica* subsp. *enterica* and revealed the following serotypes: *S. enteritidis*, *S. paratyphi*, *S. moscow*, *S. infantis*, and *S. mbandaka*. Investigation of antimicrobial resistance in the isolated strains revealed their sensitivity to several antibiotics (amikacin, ceftriaxone, gentamicin, amoxicillin, ciprofloxacin), but their resistance to multiple classes of antibiotics, including rifamycins, macrolides, glycopeptides, cephalosporins, tetracyclines, lincosamides, aminoglycosides, nitrofurans, and penicillins, confirming multidrug resistance among the isolates.

These findings are of concern, as the isolates were recovered from poultry farm environments that may serve as sources of contamination for poultry products. Therefore, careful selection of effective antimicrobial agents by veterinary specialists is essential. Differences in antimicrobial susceptibility among *Salmonella* strains at the serotype level should also be considered when assessing the effectiveness of antibiotic therapy. Furthermore, comprehensive surveillance studies across all regions of the country are needed to identify circulating *Salmonella* strains and characterize their resistance profiles.

Authors' Contribution

SB, ZhA and GD: designed and supervised the study, conducted a comprehensive literature search, analyzed the collected data and drafted the manuscript. DSh and EB: performed statistical analysis and contributed to drafting the manuscript. AS: conducted the final revision and proofreading. All authors have read, reviewed, and approved the final manuscript.

Acknowledgements

This study was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan AP23490406.

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