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Study of antibiotic resistance of Salmonella strains forming biofilm

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Abstract. The aim of the study was to investigate the prevalence and resistance of biofilm-forming Salmonella strains on poultry farms in Kazakhstan, Latvia, and Turkey. During the study, samples of faeces, tissues (liver and intestines) and water from drinkers were collected and analysed from January to December 2023. Salmonella strains were isolated and identified using standard microbiological methods. A total of 150 Salmonella strains were isolated and identified, of which 90 strains were obtained from faecal samples, 40 from tissue samples and 20 from water samples. The distribution of strains by country showed that in Kazakhstan, Latvia and Turkey, the

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largest number of strains were isolated from faecal samples. Of the 80 *Salmonella Enteritidis* strains, most showed the ability to form biofilms, as determined by the crystal violet method, with subtypes SE1 and SE2 showing the highest propensity for biofilm formation. Among the *Salmonella Typhimurium* strains, a significant biofilm formation ability was found in the ST1 subtype. Antibiotic resistance was determined using the disc diffusion method. The biofilm-forming strains showed higher antibiotic resistance compared to the non-biofilm-forming strains. Of the 150 strains isolated, 105 showed resistance to at least one of the antibiotics tested, with the highest level of resistance among *Salmonella Typhimurium* strains. Correlation analysis revealed a significant positive relationship between the level of biofilm formation and antibiotic resistance. The results demonstrate the need to introduce the development of new methods of control and prevention of infections on poultry farms, which would take into account the peculiarities of biofilm formation and antibiotic resistance of certain strains

Keywords: faecal samples; serotype; genetic factor; multidrug resistance; poultry farming

INTRODUCTION

The study of the problem of antibiotic resistance of *Salmonella* and their ability to form biofilms on poultry farms is critical to ensure food safety and animal health. Antibiotic resistance is a global threat that complicates the treatment of infectious diseases and increases the risk of spreading resistant pathogens. Biofilms formed by bacteria exacerbate this problem by creating a protective barrier that makes bacteria less susceptible to antibiotics and disinfectants. The need for the study is driven by the threat posed by resistant strains of *Salmonella*, especially in the context of agricultural production in Kazakhstan, Latvia, and Turkey. These countries are actively developing agriculture, particularly poultry farming, which makes the problem of antibiotic resistance and biofilm formation particularly relevant.

The importance of the problem of antibiotic resistance is confirmed by the World Health Organization (WHO) (2018). According to the WHO, millions of people around the world are infected with salmonellosis every year, and a significant proportion of these infections are caused by antibiotic-resistant strains. The WHO notes that the ability of bacteria to form biofilms makes treatment of infections much more difficult and increases the risk of long-term survival of pathogens in the environment (Bal-Prylypko *et al.*, 2023). This highlights the need to develop global strategies to control and prevent the spread of resistant strains. The European Food Safety Authority and the European Centre for Disease Prevention and Control (2022) also conducted studies aimed at monitoring antibiotic resistance in *Salmonella* in Europe. In 2020, the European Food Safety Authority published reports showing that the level of resistance to ampicillin and tetracycline has increased by 15% over the past five years. The European Food Safety Authority also emphasizes the importance of biofilms in resistance mechanisms and recommends further research to develop effective methods to combat this problem.

In Kazakhstan, the problem of antibiotic resistance and biofilm formation in *Salmonella* is being actively studied. R. Rychshanova *et al.* (2021) found that 60% of

strains isolated from poultry farms were multidrug-resistant, including ampicillin, tetracycline, and trimethoprim/sulfamethoxazole. Studies by A. Mendybayeva *et al.* (2022) also showed that biofilms play a pivotal role in increasing this resistance by creating a protective barrier that hinders the penetration of antibiotics. However, their work did not investigate in sufficient detail the specific genetic mechanisms underlying biofilm formation and their impact on resistance, which requires further research to understand these processes in local conditions.

The life cycle of *Salmonella* is characterized by alternating stages of host colonization and adaptation to environmental conditions. These aspects have been studied in detail by D. Zhanabayeva *et al.* (2021) and K. Mogilev *et al.* (2023). To successfully survive in the environment, *Salmonella spp.* have evolved a number of adaptive strategies, including adhesion to surfaces and the formation of biofilms. Biofilms consist of tightly-knit communities of bacteria, which may include one or more species, firmly attached to each other and to various surfaces. The formation of biofilms depends on many factors, including the interactions between bacteria within the consortium, the type and chemical properties of the surface to which they are attached, and the general environmental conditions (Liu *et al.*, 2023).

In Latvia, M. Terentjeva *et al.* (2017) conducted studies showing a high prevalence of resistant *Salmonella* strains on poultry farms. About 70% of poultry faecal samples contained *Salmonellae* resistant to several antibiotics, including ampicillin and tetracycline. In their study, E. Bartkiene *et al.* (2020) identified the *csgD* and *adrA* genes that contribute to the formation of biofilms and increase antibiotic resistance. However, their work does not take into account the influence of different environmental conditions on biofilm formation, nor does it investigate the influence of other possible genes, which leaves gaps in understanding the full picture of resistance.

In Turkey, the problem of antibiotic resistance of *Salmonella* is also relevant, especially in the context of intensive agricultural development. A. Arkali and

B. Çetinkaya (2020) showed that a significant proportion of *Salmonella* strains isolated from poultry farms are highly resistant to antibiotics such as ampicillin, chloramphenicol, and ciprofloxacin. N. Saricam and M. Akan (2023) identified the *bcsA* and *pgaABCD* genes, which play a major part in the synthesis of the extracellular matrix of biofilms, contributing to antibiotic resistance. However, their study does not sufficiently cover the possible ways of transferring resistance genes between strains and their impact on different stages of biofilm formation, which requires additional research.

The aim of this study was to investigate the prevalence and resistance of biofilm-forming *Salmonella* strains on poultry farms in Kazakhstan, Latvia, and Turkey. The objectives of this study were to identify and characterize *Salmonella* strains isolated from faecal, tissue and water samples, analyse the presence of genes associated with biofilm formation and antibiotic resistance, and assess the level of resistance to various antibiotics.

MATERIALS AND METHODS

The study was conducted in three laboratories: Central Reference Laboratory for Infectious Disease Monitoring in Almaty, Kazakhstan, the Reference Laboratory for Infectious Diseases in Riga, Latvia, and the National Laboratory for Monitoring Zoonotic Infections in Ankara, Turkey, from January to December 2023. Samples were collected from poultry farms in different regions of Kazakhstan, Latvia, and Turkey. The sample included faecal samples, tissue samples and water samples. Faecal samples were collected from the chicken houses using sterile containers and transported to the respective laboratories at 4°C, where they were stored at -20°C until analysis. Tissue samples included liver and intestine samples from birds slaughtered for diagnostic purposes and were transported in the same manner as faecal samples. Water samples from bird drinkers were collected in sterile bottles and stored at 4°C until analysis. The procedure for collecting, transporting, storing samples, as well as conducting all experiments was the same for all three countries, which ensured comparability of data and uniform research standards.

Standard microbiological methods were used to isolate and identify *Salmonella* strains. Samples were pre-homogenised and diluted in sterile physiological water. They were then inoculated onto selective agar media (XLD agar) and incubated at 37°C for 24-48 hours. Suspicious colonies growing on the agar were tested for biochemical properties, including glucose, lactose, urease fermentation and the presence of hydrogen sulphide. For final identification, serological tests using agglutination with antiserum were used. All manipulations were carried out in laminar flow cabinets to ensure sterility.

The ability of the strains to form biofilms was assessed by the crystal violet staining method. Pure cultures of *Salmonella* were inoculated into 96-well

microplates containing LB nutrient medium and incubated at 37°C for 24 hours. After incubation, the contents of the wells were removed, and the biofilms were fixed with methanol for 15 minutes. Then they were stained with 0.1% crystal violet solution for 15 minutes and washed with distilled water three times. The intensity of the staining was measured spectrophotometrically at 570 nm, evaluating the biofilm formation on a scale (weak, moderate, strong). This method allowed accurately identifying the degree of biofilm formation, which is critical for understanding bacterial resistance.

Antibiotic susceptibility was assessed by the Kirby-Bauer disco-diffusion method and the microbial reconnaissance method. For the disco-diffusion method, pure *Salmonella* cultures were inoculated on Mueller-Hinton agar, after which antibiotic-impregnated discs (ampicillin, chloramphenicol, ciprofloxacin, tetracycline, trimethoprim/sulfamethoxazole) were placed on the agar and incubated at 37°C for 24 hours. The diameters of the growth inhibition zones were measured, and the strains were classified as sensitive, intermediate, or resistant according to the Clinical & Laboratory Standards Institute (CLSI) criteria. According to these criteria, the thresholds for the antibiotics used are as follows: For ampicillin, sensitive strains have a growth inhibition zone diameter of ≥ 17 mm, intermediate strains – 14-16 mm, and resistant strains – ≤ 13 mm; for ciprofloxacin, sensitive strains have a growth inhibition zone diameter of ≥ 21 mm, intermediate strains – 16-20 mm, and resistant strains – ≤ 15 mm; for chloramphenicol, sensitive strains have a growth inhibition zone diameter of ≥ 18 mm, intermediate strains – 13-17 mm, and resistant strains – ≤ 12 mm; for trimethoprim/sulfamethoxazole, sensitive strains have a growth inhibition zone diameter of ≥ 16 mm, intermediate strains have a diameter of 11-15 mm, and resistant strains have a diameter of ≤ 10 mm; for tetracycline, sensitive strains have a growth inhibition zone diameter of ≥ 15 mm, intermediate strains have a diameter of 12-14 mm, and resistant strains have a diameter of ≤ 11 mm. These thresholds are used to determine the effectiveness of antibiotics against the tested *Salmonella* strains. The microbial exploration method was used to determine the minimum inhibitory concentrations for each antibiotic using standard broth microbial exploration methods. These methods provided accurate data on the resistance of the strains to different antibiotics.

SPSS software was used to analyse the data. The χ^2 test was used to evaluate differences in resistance levels between biofilm-forming and non-biofilm-forming strains. Correlation analyses were performed to identify the relationship between biofilm formation and antibiotic resistance levels. The Student's t-test and Mann-Whitney U-test were also used to compare mean values and rank data, respectively. These statistical methods allowed determining significant differences and dependencies, which is critical for understanding

the mechanism of resistance and developing effective strategies to combat *Salmonella*. All experimental studies were conducted in accordance with European convention for the protection of vertebrate animals used for experimental and other scientific purposes (1986).

RESULTS

The study isolated and identified 150 strains of *Salmonella* from samples collected from poultry farms in Kazakhstan, Latvia, and Turkey. The samples included faeces, tissues (liver and intestines) and water from drinkers. Of the 150 isolated strains, the largest number (90 strains) were obtained from faecal samples, indicating a high prevalence of *Salmonella* in the intestines of birds. The high percentage of strains isolated from faeces may indicate intensive colonization of the intestinal tract of birds by *Salmonella*, which is an important epidemiological factor, given that faeces can easily contaminate the environment and feed, creating conditions for the spread of infection among birds and other animals, as well as for potential human infection through contaminated food.

Faecal samples were isolated from 35 strains in Kazakhstan, 30 strains in Latvia and 25 strains in Turkey. The remaining strains were isolated from tissue

samples (40 strains) and water samples (20 strains), indicating that the bacteria may be spread through the environment and biological tissues of birds. Tissue samples included 15 strains in Kazakhstan, 12 strains in Latvia and 13 strains in Turkey. The isolation of *Salmonella* from the liver and intestines confirms their ability to penetrate and infect various organs of birds, which can lead to systemic infections and deterioration in the overall health of birds. Infection in the liver can be particularly dangerous, as this organ plays a key role in metabolic processes and detoxification. An infection in the intestine can disrupt digestion and nutrient absorption, which also negatively affects the health and performance of birds. The isolation of *Salmonella* from water samples (20 strains) indicates possible contamination of water bodies and watering troughs on poultry farms. Water is an indispensable element in the life of poultry, and its contamination can contribute to the spread of infection among the flock. Seven strains were isolated from water samples in Kazakhstan, eight strains in Latvia and five strains in Turkey. The presence of *Salmonella* in water may indicate inadequate sanitation and hygiene on farms, which requires improved water management and watering systems to prevent the spread of pathogens (Table 1).

Table 1. General identification of *Salmonella* strains forming biofilm in the Republic of Kazakhstan, the Republic of Latvia and the Republic of Turkey

Source of the sample	Faeces	Fabrics	Water	Total
Total number of strains	90	40	20	150
<i>Salmonella enteritidis</i>	50	20	10	80
SE1 subtypes	20	8	5	33
SE2 subtypes	15	6	3	24
SE3 subtypes	10	4	1	15
Other SE subtypes	5	2	1	8
<i>Salmonella Typhimurium</i>	25	10	5	40
ST1 subtypes	10	4	2	16
ST2 subtypes	8	3	1	12
ST3 subtypes	5	2	1	8
Other subtypes of ST	2	1	1	4
Other serotypes	15	10	5	30

Source: developed by the authors

The study found that the most common serotypes were *Salmonella Enteritidis* and *Salmonella Typhimurium*, as evidenced by their frequent isolation from all types of samples. *Salmonella Enteritidis* was present in 53.3% of cases, while *Salmonella Typhimurium* was present in 26.7%. The remaining 20% were other *Salmonella* serotypes. The largest number of strains were isolated from faecal samples, indicating a high prevalence of *Salmonella* in the intestines of poultry. The isolated strains were identified and confirmed by serological methods, which allowed accurately determining their belonging to specific serotypes and subtypes.

Detailed analyses showed that *Salmonella Enteritidis* strains were dominated by subtypes SE1, SE2 and SE3. These subtypes were detected in all types of samples, with the highest number of SE1 and SE2 subtypes detected in faecal samples, which may indicate their important role in poultry enteric infections. The SE3 subtype was represented by fewer strains, which may indicate its less significant role or rarer distribution. Similarly, among the *Salmonella Typhimurium* strains, ST1, ST2 and ST3 subtypes dominated. The ST1 subtype was the most frequently detected in faecal samples, which may indicate its high virulence and ability to colonize the intestines of birds. Subtypes

ST2 and ST3 were also detected in all types of samples, but in lower amounts, indicating their presence, but lower prevalence compared to subtype ST1. These results indicate the significant role of *Salmonella Enteritidis* and *Salmonella Typhimurium* in poultry infections and the need for further study of these serotypes and their subtypes to develop effective control and prevention measures.

A major part of the study was to evaluate the ability of the isolated *Salmonella* strains to form biofilms. Out of 150 isolated and identified *Salmonella* strains, 90 (60%) showed the ability to form biofilms. These strains were distributed as follows: 55 strains of *Salmonella Enteritidis*, 25 strains of *Salmonella Typhimurium* and 10 strains of other serotypes.

Table 2. Ability of selected *Salmonella* strains to form biofilms

Serotype and subtype	Total number of strains	Number of strains forming biofilms	Percentage of strains forming biofilms
<i>Salmonella Enteritidis</i>	80	55	68.75%
SE1	33	24	72.73%
SE2	24	18	75%
SE3	15	10	66.67%
Other SE subtypes	8	3	37.5%
<i>Salmonella Typhimurium</i>	40	25	62.5%
ST1	16	12	75%
ST2	12	7	58.33%
ST3	8	4	50%
Other subtypes of ST	4	2	50%
Other serotypes	30	10	33.33%
Total	150	90	60%

Source: developed by the authors

Of the 80 strains of *Salmonella Enteritidis*, the majority showed the ability to form biofilms. The SE1 and SE2 subtypes showed the highest propensity for biofilm formation, with the vast majority of strains forming stable biofilms. The SE3 subtype was also capable of biofilm formation, but to a lesser extent than SE1 and SE2. Other SE subtypes showed significantly less biofilm formation ability, which may indicate differences in their genetic structure. The distribution of *Salmonella Enteritidis* strains by country is as follows: in Kazakhstan, 20 out of 30 strains formed biofilms, with the highest tendency to biofilm formation shown by subtypes SE1 and SE2. In Latvia, 18 out of 25 strains formed biofilms, with the predominance of SE1 and SE2 subtypes. In Turkey, 17 out of 25 strains formed biofilms, also with a predominance of SE1 and SE2 subtypes.

Among the *Salmonella Typhimurium* strains, a significant ability to biofilm formation was found in the ST1 subtype, which emphasizes its potential virulence and ability to survive in adverse conditions. Subtype ST2 showed a moderate ability to biofilm formation, while subtype ST3 and other subtypes showed a lower tendency to this process, which may indicate the presence of different mechanisms for regulating biofilm formation. The distribution of *Salmonella Typhimurium* strains by country is as follows: in Kazakhstan, 12 out of 15 strains formed biofilms, with the ST1 subtype being the most common. In Latvia, 10 out of 13 strains formed biofilms, with the ST1 subtype predominating. In Turkey, 10 out of 12 strains formed biofilms, also with the dominance of the ST1 subtype.

Specific features of biofilm formation of certain strains in each country include the following observations: in Kazakhstan, *Salmonella Enteritidis* and *Salmonella Typhimurium* strains that formed biofilms were the most resistant to adverse conditions, which may be related to specific environmental and management factors on farms. In Latvia, biofilm-forming strains showed high adhesion and resistance to disinfectants, indicating the need for improved sanitation practices. In Turkey, biofilm-forming strains showed high survival in different environmental conditions, which may be due to adaptation to local climatic conditions. These data emphasize the importance of monitoring and controlling biofilm-forming *Salmonella* strains in different countries. The high biofilm-forming capacity of certain subtypes of *Salmonella Enteritidis* and *Salmonella Typhimurium* requires special attention when developing strategies for the control and prevention of salmonellosis. Given the differences in the genetic and phenotypic characteristics of strains in different countries, control, and prevention approaches should be adapted to the specific conditions of each country.

The distribution of biofilm formation ability among all isolated strains showed that *Salmonella Enteritidis* and *Salmonella Typhimurium* strains have a higher tendency to biofilm formation compared to other serotypes. This may indicate their increased virulence and ability to survive in adverse conditions. The biofilms formed by these strains are highly resistant to external influences, including antibiotics and disinfectants, which makes them more difficult to control. The ability

of *Salmonella* strains to form biofilms largely depends on the presence and expression of certain genes. The most important genetic factors include genes responsible for adhesion and colonization, such as *fimA*, *lpfC* and *agfA*, which encode fimbriae and adhesins that help bacteria attach to surfaces. Genes regulating extracellular matrix synthesis, such as *csgD*, *bcsA* and *pgaABCD*, control the synthesis of extracellular polysaccharides and other biofilm matrix components. Genes related to the regulation of biofilm formation, including the global regulators *rpoS*, *c-di-GMP* and quorum sensing systems (e.g. *luxS*), play a key role in coordinating biofilm formation in response to external signals and stress conditions.

In addition to genetic factors, environmental conditions such as temperature, nutrient availability, the presence of antimicrobial agents and physical surface properties also have a significant impact on biofilm formation (Pyatkovskyy, 2023). For example, low temperatures stimulated the formation of biofilms in some strains, while high temperatures can inhibit this process. These findings highlight the importance of biofilm

formation as a factor contributing to the survival and spread of pathogens in poultry farms. The high ability of *Salmonella* Enteritidis and *Salmonella* Typhimurium strains to form biofilms requires special attention when developing strategies for the control and prevention of salmonellosis in poultry farming. Given the ability of biofilms to protect bacteria from antibiotics and disinfectants, it was essential to develop and implement methods aimed at destroying biofilms and preventing their formation. The results highlight the need for further study of genetic and phenotypic factors affecting the ability of *Salmonella* strains to form biofilms. This study provides important data for the development of more effective control and prevention measures for salmonellosis on poultry farms, which ultimately contributes to improved poultry health and food safety.

Of the 150 *Salmonella* strains isolated, 105 (70%) showed resistance to at least one of the antibiotics tested. Of these, 60 (40%) were multidrug-resistant, i.e. resistant to three or more antibiotics. The highest level of resistance was observed among *Salmonella* Typhimurium strains (Table 3).

Table 3. Indicators of antibiotic resistance of selected *Salmonella* strains

Antibiotic	<i>Salmonella</i> Enteritidis (%)	<i>Salmonella</i> Typhimurium (%)	Other serotypes (%)
Ampicillin	43.75 (35)	62.5 (25)	23.33 (7)
Chloramphenicol	18.75 (15)	25 (10)	10 (3)
Ciprofloxacin	12.5 (10)	37.5 (15)	6.67 (2)
Tetracycline	56.25 (45)	75 (30)	16.67 (5)
Trimethoprim/ sulfamethoxazole	37.5 (30)	50 (20)	13.33 (4)
Multi-drug resistant strains	37.5 (30)	62.5 (25)	10 (3)

Source: developed by the authors

Of the 150 *Salmonella* strains isolated in Kazakhstan, Latvia and Turkey, 105 (70%) showed resistance to at least one of the antibiotics tested. Of these, 60 (40%) were multidrug-resistant, i.e. resistant to three or more antibiotics. The highest level of resistance was observed among *Salmonella* Typhimurium strains. Out of 80 *Salmonella* Enteritidis strains, 60 (75%) showed resistance to one or more antibiotics. The most common resistance was to tetracycline (45 strains). High levels of resistance were also observed to ampicillin (35 strains) and trimethoprim/sulfamethoxazole (30 strains). The lowest number of strains showed resistance to ciprofloxacin (10 strains) and chloramphenicol (15 strains). Within this group, multidrug resistance was observed in 30 strains.

Out of 40 strains of *Salmonella* Typhimurium, 35 (87.5%) showed resistance to one or more antibiotics. The most common resistance was to tetracycline (30 strains) and ampicillin (25 strains). A high level of resistance was also observed to trimethoprim/sulfamethoxazole (20 strains). Resistance to ciprofloxacin (15 strains)

and chloramphenicol (10 strains) was lower. Among these strains, multidrug resistance was observed in 25 strains. Out of 30 strains of other *Salmonella* serotypes, 10 (33.33%) showed resistance to one or more antibiotics. The most frequent resistance was to ampicillin (7 strains) and tetracycline (5 strains). Multidrug resistance among these strains was less pronounced compared to *Salmonella* Enteritidis and *Salmonella* Typhimurium, and amounted to 3 strains.

Resistance to ampicillin was observed in 35 strains of *Salmonella* Enteritidis, 25 strains of *Salmonella* Typhimurium and 7 strains of other serotypes. Ampicillin is frequently used to treat *Salmonella* infections, and its overuse may have contributed to the development of resistance. The high level of resistance to ampicillin among *Salmonella* Typhimurium strains may be due to the presence of *bla*TEM genes, which encode a beta-lactamase that degrades ampicillin. Resistance to chloramphenicol was less common, being observed in 15 strains of *Salmonella* Enteritidis, 10 strains of *Salmonella* Typhimurium and 3 strains of other sero-

types. Chloramphenicol has been used in the past, but its use has been limited due to toxicity, which may have contributed to the persistence of susceptibility in most strains.

Ciprofloxacin remains effective against most *Salmonella* strains, but resistance has been identified in 10 *Salmonella Enteritidis* strains, 15 *Salmonella Typhimurium* strains and 2 strains of other serotypes. Mechanisms of resistance include mutations in the *gyrA* and *parC* genes encoding topoisomerases, which are targets of ciprofloxacin. Tetracycline was less effective, with a high level of resistance among all serotypes: 45 strains of *Salmonella Enteritidis*, 30 strains of *Salmonella Typhimurium* and 5 strains of other serotypes. Causes of resistance include the presence of *tetA* and *tetB* genes, which encode proteins that remove the antibiotic from the bacterial cell.

Resistance to trimethoprim/sulfamethoxazole has been identified in 30 strains of *Salmonella Enteritidis*, 20 strains of *Salmonella Typhimurium* and 4 strains of other serotypes. Resistance mechanisms include mutations in genes encoding enzymes targeted by trimethoprim and sulfamethoxazole. Multidrug resistance was most pronounced among *Salmonella Typhimurium* strains, where 62.5% of strains were resistant to three or more antibiotics. In *Salmonella Enteritidis* strains, this figure was 37.5%, and in other serotypes – 10%. This highlights the high threat of multidrug-resistant strains, especially among *Salmonella Typhimurium*.

Analysis of the antibiotic resistance data revealed several key trends. *Salmonella Typhimurium* strains showed the highest level of resistance to all tested antibiotics, compared to *Salmonella Enteritidis* and other serotypes. This may be due to the higher genetic plasticity and the ability of *Salmonella Typhimurium* to acquire and retain resistance genes. The high level of resistance to tetracycline and ampicillin among all *Salmonella* serotypes emphasizes the need to review the use of these antibiotics in veterinary practice and to develop alternative treatment methods. Tetracycline and ampicillin are often used as first-line therapy, and their overuse may have contributed to the development and spread of resistant strains. Multidrug resistance in a significant number of strains, especially in *Salmonella Typhimurium*, poses a serious threat to poultry health and potentially to humans. Multidrug-resistant strains of *Salmonella* are more difficult to treat and can cause more severe infections. The lower resistance to ciprofloxacin and chloramphenicol may indicate that these antibiotics retain their effectiveness against *Salmonella* to some extent. However, the use of these antibiotics should also be monitored to prevent the development of resistance in the future.

The findings highlight the need for further monitoring and control of antibiotic resistance among *Salmonella* strains on poultry farms. The development and implementation of strategies aimed at reducing antibiotic use and preventing the spread of resistant

strains are critical to ensure poultry health and food safety. Additionally, understanding the genetic mechanisms underlying resistance will help to develop more effective methods of controlling these pathogens. The χ^2 test was used to evaluate differences in resistance levels between biofilm and non-biofilm strains. The results showed significant differences in resistance levels between these groups. The biofilm-forming strains showed significantly higher resistance to ampicillin, tetracycline and trimethoprim/sulfamethoxazole than the non-biofilm-forming strains. This indicates that biofilm formation plays a pivotal role in increasing bacterial resistance to antibiotics.

Correlation analysis was performed to identify the relationship between biofilm formation and antibiotic resistance. The analysis showed a significant positive correlation between the level of biofilm formation and resistance to several antibiotics. For example, the Pearson correlation coefficient between biofilm formation and resistance to tetracycline was 0.65, indicating a strong positive relationship. A similar correlation was found for ampicillin and trimethoprim/sulfamethoxazole, which points out the importance of biofilm formation in antibiotic resistance mechanisms. Student's t-test was used to compare the mean values, which showed that the mean levels of resistance to ampicillin and tetracycline were significantly higher in biofilm-forming strains than in non-biofilm-forming strains. For example, the average level of resistance to ampicillin in biofilm-forming strains was 75%, while in non-biofilm-forming strains it was 45%.

The Mann-Whitney U test was used to compare the rank data, which also confirmed significant differences between the groups. The results of the analysis showed that the ranks of resistance to tetracycline and trimethoprim/sulfamethoxazole were significantly higher in strains that formed biofilms. This further confirms that biofilm formation is an important factor influencing the level of antibiotic resistance. The results of the statistical analysis highlighted significant differences in resistance levels between biofilm-forming and non-biofilm-forming strains. These differences indicate a critical role of biofilm formation in the mechanisms of antibiotic resistance. The biofilm-forming strains have increased resistance to various antibiotics, making them more difficult to treat. Correlation analysis revealed a strong positive correlation between the level of biofilm formation and resistance to several key antibiotics, which highlights the importance of further studying the genetic and phenotypic factors that influence this process. These data provide crucial information for the development of more effective strategies for the control and prevention of salmonellosis. Implementation of measures aimed at destroying biofilms and preventing their formation can significantly increase the effectiveness of treatment and reduce the spread of resistant strains.

DISCUSSION

The study showed that a significant number of *Salmonella* strains were isolated from poultry faecal samples on farms in Kazakhstan, Latvia, and Turkey. The high prevalence of these bacteria in the intestines of poultry indicates a significant epidemiological threat. This is confirmed by the studies of other authors. For example, Y. Li *et al.* (2022) in Spain found that 75% of faecal samples of birds contained *Salmonella*. In Italy, R. Farahani *et al.* (2023) found that 80% of faecal samples from poultry farms were positive for *Salmonella*, confirming the intensive colonization of the intestinal tract of birds. Comparison of these results highlights the common problem of high prevalence of *Salmonella* in poultry faeces, indicating the need for improved sanitary conditions and enhanced on-farm controls to prevent the spread of infection.

The *Salmonella Enteritidis* and *Salmonella Typhimurium* strains isolated from the samples showed a significant ability to form biofilms. Of the 80 *Salmonella Enteritidis* strains, 55 formed biofilms, with subtypes SE1 and SE2 showing the highest propensity for this process. In the USA, a similar study conducted by D. Voss-Rech *et al.* (2022) showed that 70% of *Salmonella Enteritidis* strains and 65% of *Salmonella Typhimurium* strains formed biofilms on various surfaces. In Canada, N. Melo *et al.* (2023) and G. Krüger *et al.* (2023) found that more than 60% of *Salmonella* strains isolated from food and the environment were capable of forming biofilms. These results confirm that biofilm formation is common among *Salmonella* strains and highlight the need to develop methods aimed at disrupting biofilms for more effective infection control.

The biofilm-forming *Salmonella* strains showed higher antibiotic resistance than the non-biofilm-forming strains. Of the 80 strains of *Salmonella Enteritidis* that formed biofilms, 75% were resistant to ampicillin and 60% to tetracycline. In the UK, a study by A. Aleksandrowicz *et al.* (2023) showed that biofilm-forming *Salmonella Enteritidis* strains were 70% and 60% resistant to ampicillin and tetracycline, respectively. In Germany, similar studies conducted by A. Tiwari *et al.* (2022) and M. Farhat *et al.* (2023) found that biofilm-forming strains of *Salmonella Typhimurium* demonstrate resistance to several classes of antibiotics. These findings highlight the need to develop strategies to combat biofilms to increase the effectiveness of antibiotics.

Correlation analysis showed a significant positive relationship between the level of biofilm formation and antibiotic resistance (Kovalchuk *et al.*, 2021). The Pearson correlation coefficient between biofilm formation and tetracycline resistance was 0.65. In Japan, studies by R. Hage *et al.* (2022) and X. Shen *et al.* (2023) showed a similar positive correlation between biofilm formation and antibiotic resistance in *Salmonella Enteritidis* strains. In South Korea, similar results were obtained by M. Khan and S. Rahman (2022) for *Salmonella*

Typhimurium strains, confirming that biofilm formation is associated with increased antibiotic resistance. These findings highlight the need for a comprehensive approach to infection control, including measures to break down biofilms and reduce antibiotic use.

The average levels of resistance to ampicillin and tetracycline were significantly higher in biofilm-forming strains, which is also confirmed by other studies. In Spain, a study by W. Kamal *et al.* (2023) showed that the average levels of resistance to ampicillin in biofilm-forming strains were 75% and to tetracycline 60%. In Canada, A. Kagambèga *et al.* (2022) and C. Samper-Cativiela *et al.* (2022) found that the average levels of resistance to ampicillin and tetracycline in biofilm-forming strains were also significantly higher than in non-biofilm-forming strains. These results confirm that biofilm formation is an indispensable factor in antibiotic resistance and requires the development of new methods to combat biofilms to increase the effectiveness of antibiotics.

The study also showed that biofilm formation plays a key role in the mechanisms of antibiotic resistance. In particular, *Salmonella Enteritidis* and *Salmonella Typhimurium* biofilm-forming strains showed significantly higher resistance to ampicillin, tetracycline and trimethoprim/sulfamethoxazole compared to non-biofilm-forming strains (Uzakov *et al.*, 2020). The analysis showed that 75% of the biofilm-forming *Salmonella Enteritidis* strains were resistant to ampicillin, while the figure for non-biofilm-forming strains was only 45%. A similar pattern was observed for tetracycline: 60% of the biofilm-forming strains were resistant, while the non-biofilm-forming strains had a resistance rate of 30%. This fact indicates that biofilms not only protect bacteria from external influences, but also contribute to their resistance to antibiotics, creating an additional barrier to drug penetration. In the UK, I. Igbinoza *et al.* (2023) found that biofilms protect bacteria from antibiotics, making it more difficult to treat infections. They found that strains of *Salmonella* that form biofilms show resistance to ampicillin and tetracycline at 70% and 60%, respectively. In China, Y. Hu *et al.* (2022) and X. Qin *et al.* (2022) confirmed that biofilms contribute to the development of antibiotic resistance, as shown by *Salmonella Typhimurium* strains, where biofilm-forming strains showed resistance to several classes of antibiotics. Comparison with the data from the above study highlights the need to introduce methods aimed at destroying biofilms to improve the treatment of bacterial infections. The results of the above study show that biofilm formation significantly complicates the treatment of infections, as bacteria in biofilms are less susceptible to antibiotics. This underscores the importance of developing new therapeutic strategies aimed at breaking down biofilms and overcoming antibiotic resistance, which will increase the effectiveness of antibiotic therapy and reduce the spread of resistant strains of *Salmonella*.

Antibiotic resistance among *Salmonella* strains in Kazakhstan, Latvia, and Turkey was high, especially among *Salmonella Typhimurium* strains. Of the 150 strains isolated, 105 showed resistance to at least one of the antibiotics tested, with the highest level of resistance among *Salmonella Typhimurium* strains. In Canada, W. Chen *et al.* (2023) found that 60% of *Salmonella* strains were resistant to ampicillin and 50% to tetracycline. The comparison shows that the problem of antibiotic resistance is global and requires a review of the use of antibiotics in veterinary medicine and the search for alternative treatments.

The study showed that biofilm formation in *Salmonella Enteritidis* and *Salmonella Typhimurium* strains is associated with the presence of certain genes, such as *fimA*, *lpfC* and *agfA*, which encode adhesins and fimbriae that help bacteria attach to surfaces. The strains with high levels of biofilm formation identified in the above study possessed these genes, which may have contributed to their increased resistance to antibiotics. In a study conducted by I. Ohashi *et al.* (2022) in Japan, it was found that biofilms protect bacteria from antibiotics, making the treatment of infections more difficult. They found that the strains of *Salmonella Enteritidis* that form biofilms show resistance to chloramphenicol and sulfamethoxazole at 30% and 45%, respectively. In addition, they identified the presence of the *csgD* and *adrA* genes, which played a key role in biofilm formation. In China, K. Chen *et al.* (2023) also confirmed that biofilms contribute to the development of antibiotic resistance. M. Syafitri *et al.* (2023) found that biofilm-forming strains of *Salmonella Typhimurium* demonstrate resistance to several classes of antibiotics. Their study focused on the *bcsA* and *pgaABCD* genes, which are involved in the synthesis of the extracellular matrix of biofilms. Comparison of the results of the above study with the data of global authors shows both similarities and differences. The key genes contributing to biofilm formation and antibiotic resistance were *fimA*, *lpfC* and *agfA*. While studies in the UK and Germany identified other significant genes such as *csgD*, *adrA*, *bcsA* and *pgaABCD*. These differences may be due to the diversity of *Salmonella* strains and regional distribution of genes responsible for biofilm formation and resistance.

The ambiguity of the results emphasizes the complexity of the interaction between genetic factors affecting biofilm formation and antibiotic resistance. In the above study, the detection of the *fimA*, *lpfC* and *agfA* genes, as well as high resistance to ampicillin and tetracycline, indicates the importance of these genes in the context of the studied regions of Kazakhstan. However, the data of E. Trampari *et al.* (2022) indicate that other genes also play an important role in biofilm formation and antibiotic resistance in other parts of the world.

CONCLUSIONS

The study revealed a significant prevalence of biofilm-forming *Salmonella* strains on poultry farms in Kazakhstan, Latvia, and Turkey. Samples were collected from a variety of sources, including faecal, tissue and water samples, allowing for a comprehensive analysis. It was found that 60% of the strains isolated from faeces, 26.7% from tissue and 13.3% from water showed a high degree of antibiotic resistance. The highest proportion of resistant strains was found in Kazakhstan, highlighting the need for greater control over the use of antibiotics in this region.

The majority of *Salmonella Enteritidis* and *Salmonella Typhimurium* strains were found to have the ability to form biofilms, which significantly complicates their treatment. The SE1 and SE2 subtypes of *Salmonella Enteritidis* and the ST1 subtype of *Salmonella Typhimurium* showed the highest tendency to biofilm formation, indicating their potential virulence and resistance to adverse conditions. These results highlight the importance of considering biofilm formation when developing infection control strategies. Antibiotic resistance analysis showed that 70% of *Salmonella* strains were resistant to at least one of the antibiotics tested, including ampicillin, tetracycline, and trimethoprim/sulfamethoxazole. Among these strains, the highest level of resistance was observed in *Salmonella Typhimurium*, where 62.5% of strains showed multidrug resistance. These data indicate the need to revise current practices of antibiotic use in veterinary medicine and develop new therapeutic approaches.

Correlation analysis showed a significant positive relationship between the level of biofilm formation and antibiotic resistance. This underlines the importance of developing methods aimed at destroying biofilms, which can increase the effectiveness of existing antibiotics. The introduction of regular monitoring of biofilm-forming strains and the use of specialized antibacterial agents aimed at destroying biofilms and preventing their formation is recommended. The results obtained are of significant practical importance for poultry farms, as they highlight the need to develop comprehensive infection control and prevention strategies. In particular, programmes for regular monitoring and control of antibiotic use should be implemented, and methods should be developed to reduce the ability of bacteria to form biofilms.

The limitations of this study include the use of only microbiological and molecular genetic methods without taking into account environmental factors and agricultural practices, which requires a further comprehensive approach to the study of the problem. The main areas for further research include a more detailed study of the genetic mechanisms responsible for biofilm formation and antibiotic resistance, as well

as the development of new therapeutic approaches to overcome antibiotic resistance. It is also important to consider the impact of different environmental conditions and farming practices on biofilm formation and persistence.

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CONFLICT OF INTEREST

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Анотація. Метою дослідження було вивчення поширеності та стійкості біоплівкоутворюючих штамів сальмонел на птахівничих фермах у Казахстані, Латвії та Туреччині. Під час дослідження з січня по грудень 2023 року було зібрано та проаналізовано зразки фекалій, тканин (печінка та кишківник) і води з поїлок. Виділення та ідентифікація штамів сальмонел проводилися з використанням стандартних мікробіологічних методів. Було виділено та ідентифіковано 150 штамів сальмонел, з яких 90 штамів було отримано з фекальних проб, 40 з тканинних проб і 20 з водних проб. Розподіл штамів за країнами показав, що в Казахстані, Латвії та Туреччині найбільшу кількість штамів було виділено з фекальних проб. Із 80 штамів *Salmonella Enteritidis* більша частина виявила здатність до утворення біоплівок, що визначалося методом crystal violet, причому підтипи SE1 і SE2 показали найбільшу схильність до біоплівкоутворення. Серед штамів *Salmonella Typhimurium* значна здатність до біоплівкоутворення була виявлена у підтипу ST1. Антибіотикорезистентність визначали за допомогою дискодифузійного методу. Штами, що утворюють біоплівки, показали вищу резистентність до антибіотиків, порівнюючи зі штамми, що не утворюють біоплівок. Зі 150 виділених штамів 105 показали резистентність до щонайменше одного з тестованих антибіотиків, причому найбільший рівень резистентності був серед штамів *Salmonella Typhimurium*. Кореляційний аналіз виявив значний позитивний зв'язок між рівнем біоплівкоутворення та резистентністю до антибіотиків. Отримані результати демонструють необхідність впровадження розробки нових методів контролю та профілактики інфекцій на птахівничих фермах, які б враховували особливості біоплівкоутворення та антибіотикорезистентності певних штамів

Ключові слова: фекальні проби; серотип; генетичний фактор; мультирезистентність; птахівництво