Original Article

Genetic-molecular Sources of Multidrug Resistance of Salmonella Isolated from Eggs

Mohammad Saram [1], Arash Chaichi Nosrati [1*], Leila Modiri [1], Khossrow Issazadeh [1]
[1] Division Microbiology, Faculty of Basic Sciences, Department of Molecular & Cell Biology, Lahijan Branch, Islamic Azad University, Lahijan, Iran.
***Corresponding author and reprints: Arash Chaichi Nosrati,** Division Microbiology, Faculty of Basic Sciences, Department of Molecular & Cell Biology, Lahijan Branch, Islamic Azad University, Lahijan, Iran.
Email: Leim_clinpathem@yahoo.ir
Received: 04 Sep 2022 **Accepted:** 05 Oct 2022 **Published:** 08 Nov 2022

Abstract

Background: Bacteria can be transmitted to consumers around the world due to the movement of animals, and food products. The current study's goal is to look into the genetic and molecular causes of Salmonella isolated from eggs' multidrug resistance.

Methods: 500 egg samples from 37 Iranian brands were extracted from Salmonella yolk and shell source and after confirmation by differential phenotypic methods and resistance test for tetracycline, cellophanamide and nitrofurantoin-furazolidone antibiotics were performed. DNA of strains was extracted and using PCR with specific primers, the presence of tetA, tetB, tetC, tetD, tetG, tetE, tetE, tetH, sul (I), sul (II), sul (III), nfsA, nfsB were examined according to control DNA (ATCC14028).

Results: Among the genes of teta, tetb.tetc, tetd, tete, tetf, tetg and teth, the presence of tetc was seen in 81% of the samples and not in 19% of them. Other tetracycline resistance genes were not found in the samples. Tracing of genes related to sulfonamide resistance by PCR showed that among the Sul1, Sul2, and Sul3 genes, sulfonamide resistance of Sul2 was observed in 95.5% of the samples. Nitrofurantoin resistance genes in the samples showed that 9.1% of the samples contained nfsa gene and 4.5% contained nfsb gene. Other samples did not have any nitrofurantoin resistance gene.

Conclusion: Improper use of antibiotics has caused multidrug resistance to Salmonella, so the study of genes and resistance mechanisms in Salmonella strains isolated from produced eggs in Iran is vital.

Keywords: **Drug Resistance, Multiple; genes, MDR; Nitrofurantoin; Salmonella; Sulfonamides; Tetracycline.**

Cite this article as: Saram M, Chaichi Nosrati A, Modiri L, Issazadeh K. Genetic-molecular Sources of Multidrug Resistance of Salmonella Isolated from Eggs. *Soc Determinants Health*. 2022;8(1):1-11. DOI: http://dx.doi.org/10.22037/sdh.v8i1.39325

Introduction

In many nations, particularly underdeveloped nations, the infection caused by a microbe resistant to antibiotics is the main cause of death. Currently, the aim of food safety in industrialized countries is to shift the focus of research on foodborne illness causes to active food, contamination prevention, and changed coping mechanisms (1). With more than 2600 serotypes, Salmonella is one of the main and most prevalent infections in humans and animals and has been a common public health concern for more than a century (2, 3). Salmonella in eggs can be identified and characterized to offer information that can be used to lessen the financial losses associated with Salmonella infections (4). Concern has

been raised in Iran by the rising rate of antibiotic resistance among Salmonella isolates from children under the age of 15 (5). According to reports, the majority of countries in the world rank poultry and poultry products first and second in terms of the prevalence of foodborne disease, while the United States ranks third (6). According to a study conducted in the United States, in 2020, 1.2 million people will be projected to contract salmonellosis annually, resulting in 23,000 hospitalizations and 450 fatalities. Each year, more than half of the 155,000 deaths caused by salmonellosis, which affects 94 million people globally, are attributable to tainted food (7). This increase is concerning. Salmonella outbreaks in meat or eggs have been documented in numerous investigations. The vertical-horizontal transmission of infection from poultry-to-poultry meat to poultry eggs, as well as the epidemiology of Salmonella species infections, is one of the significant issues in the poultry sector (8). In terms of Salmonella species isolated from domestic and farm poultry, poultry farms were home to five different species (S. Typhi, S. enteritids, S. paratyphi, S. pullorum, and S. gallinarum), while local chickens were home to four different species (S. Typhi, S. enteritids, S. pullorum, and S. gallinarum). But over 95% of Salmonella cases have been linked to food-borne illnesses (9). The well-known pathogen Salmonella can cause diarrhea in both people and animals (10).

Studying the genes and resistance mechanisms in Salmonella strains isolated from eggs produced in Iran that have been exposed to various antibiotics is crucial since eggs are one of the sources of Salmonella transmission to people and because this bacterium has developed multidrug resistance. This study sought to determine whether Salmonella isolates from several brands of eggs produced in Iran had various genes for tetracycline and sulfonamide resistance.

Methods

The way of isolating and laboratory diagnosis of Salmonella isolates:

A sampling of 835 eggs from 36 brands with a consumption date of 1-31 days with an average of 11 days was performed from the northern regions of Iran and the yolks were separated from albumin, stirred, and stored in the refrigerator before analysis. Tetracyclines and sulfonamides were sampled by ELISA using the Ridascreen-RbioPharm kit. Used to prove laboratory and differential diagnostic tests of hot staining, production of hydrogen sulfide in TSI medium, ability to move in SIM culture medium, growth in McConkey agar medium, urease test, fermentation of sugars. Salmonella samples were stored in LB Broth medium containing 30% glycerol at -70 ° C until further processing.

Determination of antibiotic resistance pattern using disk diffusion method

According to the disk diffusion method and the 2019 CLSI guidelines, sensitivity testing on 12 Salmonella isolates was done. First, 24-hour culture of bacteria on solid medium was prepared. Then, using a loop, a single colony of pure culture was dissolved in sterile physiological saline. The turbidity of the suspension obtained was evaluated with half McFarland turbidity. In the next step, using a sterile swab, bacterial grass culture was performed on Müller-Hinton agar medium. Then, the desired antibiotic discs, prepared by Padtan Teb Company, were placed on the culture medium. The results of bacterial susceptibility or resistance were assessed after 24 hours of incubation at 37 °C in accordance with the growth inhibition halo values listed in the 2019CLSI tables. Discs used in this study included Chlortetracycline, Oxytetracycline, Doxycycline, Tetracycline Sulfonamide, nitrofurantoin.

Determining MIC / MBC by microplate dilution?

Table 1. Temperatures used for PCR

Factor	Temperature (ºC)			Time		
Gene Step	tetA,B,C,D,G	tetE,H	nfsA,B	tetA,B,C,D,G	tetE,H	nfsA,B
Initial denaturation	95	95	95	5 Min	5 Min	5 Min
Denaturation	95	95	95	20 Secs	20 Secs	20 Secs
Annealing	55	53	58	20 Secs	20 Secs	20 Secs
Extension	72	72	72	20 Secs	20 Secs	20 Secs
Final extension	72	72	72	5 Min	5 Min	5 Min
Cycle	40	40	40			
Factor	Temperature (ºC)			Time		
Gene Step	sulI	sulII	sulIII	sulI	sulII	sulIII
Initial denaturation	95	95	95	5 Min	5 Min	5 Min
Denaturation	95	95	95	20 Secs	20 Secs	20 Secs
Anealing	56	62	51	20 Secs	20 Secs	20 Secs
Extension	72	72	72	20 Secs	20 Secs	20 Secs
Final extension	72	72	72	5 Min	5 Min	5 Min
Cycle	40	40	40			

Sample preparation for molecular analysis

To extract Salmonella 500 Microlanda bacterial DNA from the LB broth medium cultured with the bacterium, it was removed and poured into 1.5 ml microtubes. The microtubes were transferred to Ben Marie for heating at 96 ° C. The degrees were placed in a pan for 15 minutes. After leaving the pan, it was immediately transferred to ice for a cold shock, then placed in the refrigerator freezer for three minutes. To separate the slippery wall and free contents Bacterial microfusion was performed at 13,000 rpm for two minutes. ThermoNanoDrop 2000c nanodrop device was used to measure DNA concentration. After isolation of DNA from bacteria by the above method, identification of tetA, tetB, tetC, tetD, tetG, tetE, tetH, sul I, sul II, sul III, nfsA, nfsB genes by PCR using Sina gene kit according to the kit protocol was performed PCR for unknown DNA and control DNA samples (ATCC14028) according to the temperature Table 1, and with specific primers as in Table 2.

The PCR product was run on 0.5% agarose gel by electrophoresis.

Table 2. Sequence of primers used for PCR

Primers	Primer sequence
F-tetA	GCGCCGATCTGGTTCACTCG
R-tetA	AGTCGACAGTAGCGCCCGC
F-tetB	۴TACGTGAATTTATTGCTTCGG
R-tetB	ATACAGCATCCAAAGCGCAC
F-tetC	۴GCGGGATATCGTCCATTCCG
R-tetC	GCGTAGAGGATCCACAGGACG
F-tetD	GGAATATGTCCCGGAAGCGG
R-tetD	CACATTGGACAGTGCCAGCAG
F-tetG	GCAGAGCAGGTCGCTGG
R-tetG	CCYGCAAGAGAAGCCAGAAG
F-tetH	CAGTGAAAATTCACTGGCAAC
R-tetH	ATCCAAAGTGTGGTTGAGAAT
F-tetE	GTTATTACGGGAGTTTGTTGG
R-tetE	AATACAACACCCACACTACGC
F-sulI	5'-TGA GAT CAG ACG TAT TGC GC-3'
R-sulI	5'-TTG AAG GTT CGA CAG CAC GT-3'
F-sulII	5'-GCG CTC AAG GCA GAT GGC ATT-3'
R-sulII	5'-GCG TTT GAT ACC GGC ACC CGT-3'
F-sulIII	5'-GAGCAAGATTTTTGGAATCG-3'
R-sulIII	5'-CATCTGCAGCTAACCTAGGGCTTTGGA-3'

Results

Phenotypic tests to identify the genus and species of Salmonella

Isolated bacteria were observed as gram-negative bacilli in hot staining Figure 1A. As shown in Table 3, Salmonella isolates are motile bacteria. Colony most isolates were found on the XLD medium due to the presence of dark iron sulfite Figure 1B. In the TSI environment, Salmonella uses only glucose and is not able to use lactose. Therefore, as can be seen in the figure, it causes the depth of the tube to turn yellow and blackens the culture medium due to the production of hydrogen sulfide Figure 1C. Additionally, all isolates could develop on Simon citrate agar medium and cause the medium's color to switch from green to blue (Figure 1D). Salmonella lysine is a decarboxylase positive and causes the culture medium to blacken Figure 1E. Salmonella urease is negative, as shown in Figure 1F. Salmonella methyl red is positive, which becomes a sour cherry dye by adding the reagent to the culture medium Figure 1G. For the VP test, three drops of alpha-naphthol and one drop of potash were added. Since Salmonella VP is negative, the environment did not change color Figure 1H. Salmonella bacteria grow in phenol-red medium and produce glucose with acid fermentation. Because of the glucose fermentation caused by the strains isolated in this medium, the culture media turns yellow instead of red. Figure 1I.

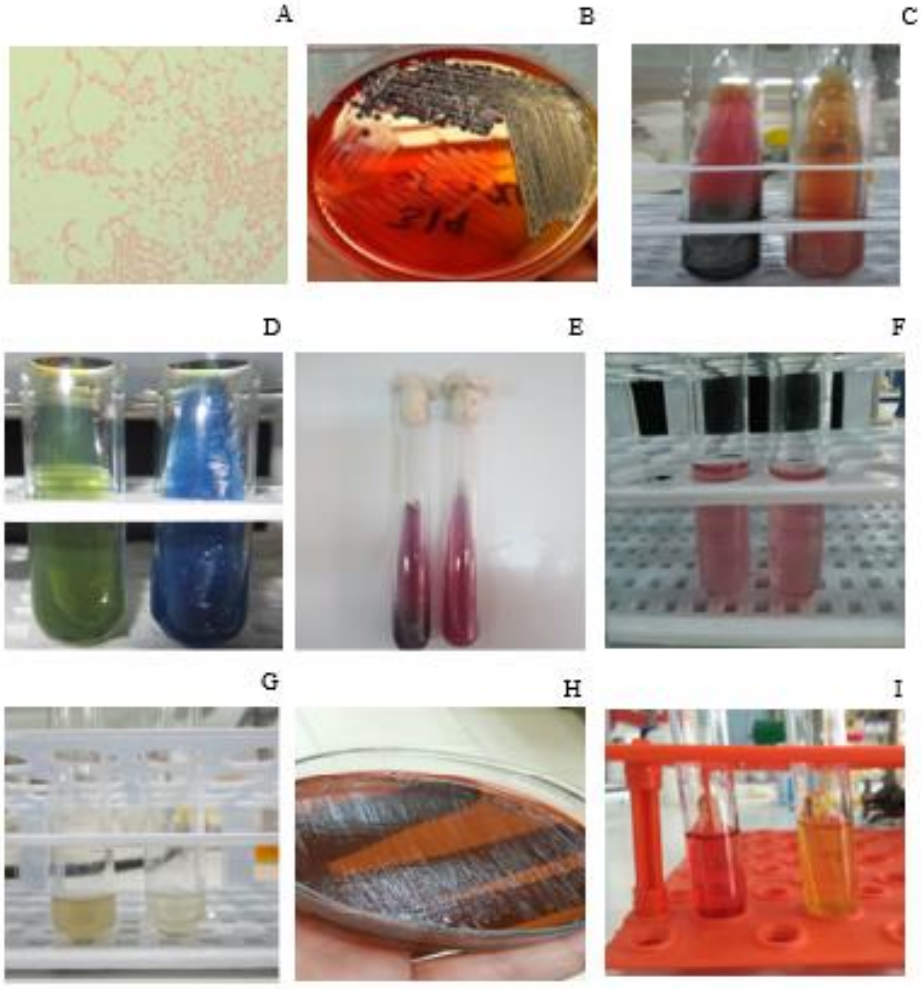

Figure 1. Phenotypic tests to identify the genus and species of Salmonella A-- Gram-negative Salmonella bacilli resulting from gram / B staining Salmonella cultured on XLD medium C: Salmonella bacteria cultured in TSI / D medium: Culture of Salmonella bacteria in Simon Citrate Environment E /: Positive Lysine Decarboxylase F Test Urease Test / G: SIM / H Test Salmonella Bacteria Growth on EMB / I Sugar Fermentation Environment

Table 3. Differential tests to identify Salmonella

Diagnostic tests	Results
Warm coloring	In the form of gram-negative
TSI	+
Ability to move in SIM culture medium	+
Growing in McConkie agar medium	+
Urease test	-
Fermentation of sugars	+
VP	+
Growth in XLD environment	+
Lysine decarboxylase	+
Hydrogen sulfide Production	+
MR	+
Simon Citrate	+
Fermentation of glucose	+
Fermentation of lactose	-

Determination of antibiogram results using disk diffusion method:

Figure 2 shows the findings of the disk diffusion method, which revealed that 18 of the 20 bacterial samples tested positive for antibiotic resistance.

Figure 2 shows the findings of the disk diffusion method, which revealed that 18 of the 20 bacterial samples tested positive for antibiotic resistance. The antibiotic resistance of Salmonella strains shown by disk diffusion revealed that Oxytetracycline (90%), Tetracycline (90%), Doxycycline (90%), and Chlortetracycline (90%) were detected.

The number of results obtained from PCR of antibiotic residues resulting from ELISA

The presence of resistance genes in multidrug-resistant organisms was determined by electrophoresis of PCR products. The presence of each gene in the strain produced the following results:

Gene analysis: tetA

The tetA gene was not discovered in any of the samples, as shown in Figure 3, according to the PCR and electrophoresis results of the samples. Examination of the frequency of tetracycline resistance genes by PCR showed that among the teta, tetb, tetc, tetd, tete, tetf, tetg and teth genes, the presence of tetc was seen in 81% of the samples and in 19% of the samples. Failed Figure 4A. Other tetracycline resistance genes were not found in the samples.

Sul2 was present in 95.5% of the samples and not in 4.5% of the samples, according to a frequency analysis of the presence of genes relevant to sulfonamide resistance conducted using the PCR method. Image 4B. There were no further sulfonamide resistance genes in the samples.

Examination of the frequency of nitrofurantoin resistance genes in the samples showed that 9.1% of the samples contained the nfsa gene and 4.5% contained the nfsb gene Figure 4C-D. The rest of the samples did not have any of the nitrofurantoin resistance genes.

Figure 2. Antibiogram test by disk diffusion method

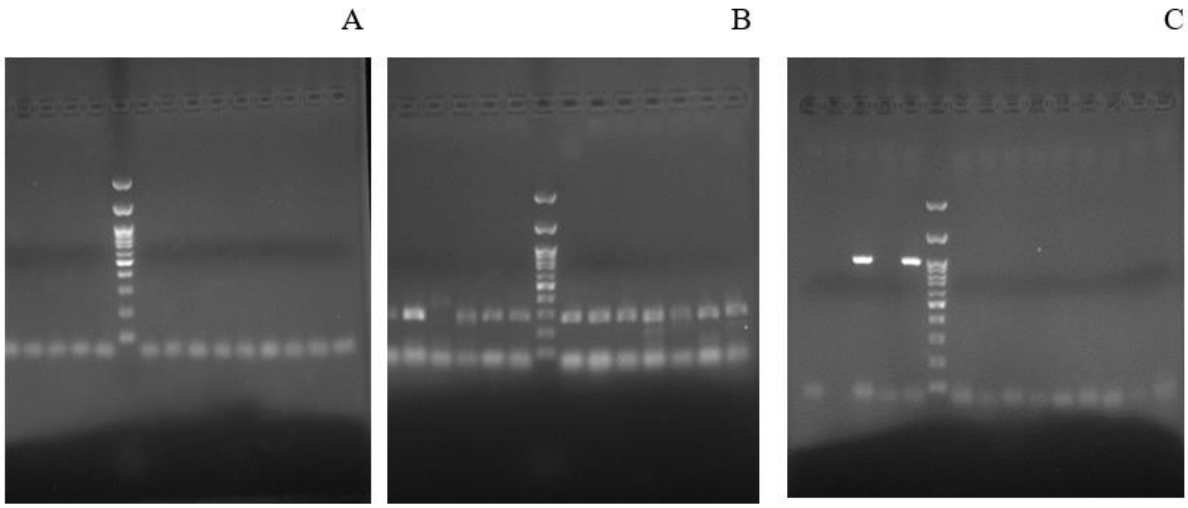

Figure 3. Results of PCR product electrophoresis for the studied genes. A: gel related to the presence or absence of genes. B: tetD gel related to the presence or absence of sulII gene. C: Gel related to the presence or absence of nfsA gene

Discussion

Based on the present study, in which 22 salmonella-resistant bacteria were isolated from 500 egg samples of different Iranian brands. Confirmation of Salmonella through phenotypic tests of Gram-negative Salmonella bacilli from gram staining / Salmonella cultured in XLD medium: Salmonella cultured in TSI medium / and Salmonella cultured in Simon Citrate medium, positive lysine decarboxylase test and urease test, SIM / H Salmonella bacterial growth test was performed on EMB / culture medium and sugar paste. Figure 1. By employing PCR to look for tetracycline-related genes, the mechanism of antibiotic resistance was examined. The findings demonstrated that the presence of the TetC gene, which was found in 81%

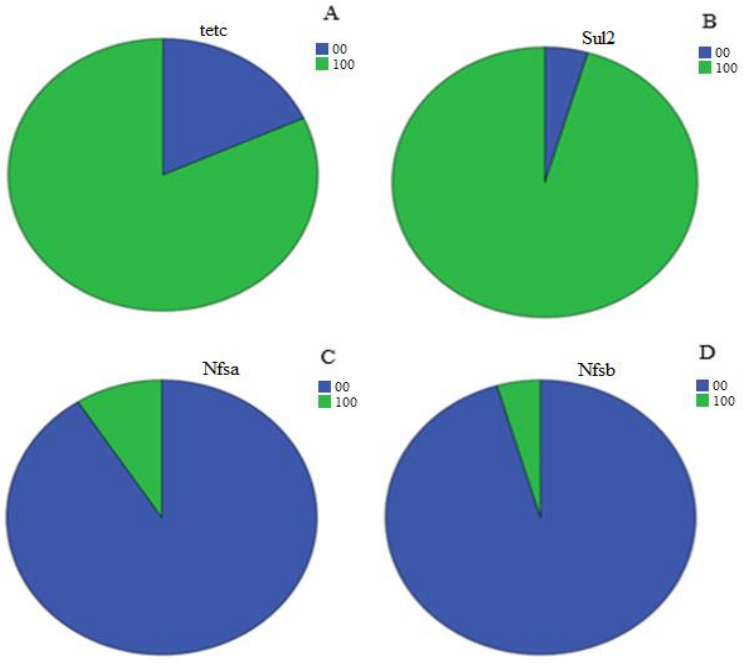

Figure 4. Frequency of presence of different genes resistant to tetracycline, sulfonamide and nitrofurantoin in drug-resistant Salmonella isolated from eggs

of samples of multidrug-resistant Salmonella, is connected to the source of resistance. Figure 3. According to linked studies, the number of cases of salmonella infection worldwide each year is expected to be 9.38 million, with 15,000 deaths resulting from the infection (11). According to data from epidemiological monitoring studies, chicken and poultry products are not only recognized as the leading reservoirs but also as the primary causes of human salmonellosis (12), and egg white has distinctive physical and biochemical features. It is distinctive and offers a sophisticated antibacterial environment for antigenic resistance (13). It should be noted that Salmonella enterica serovar Typhimurium, Salmonella Indiana, and Salmonella enterica enteritidis serotypes are serotypes linked to human infections globally (14), which is consistent with the findings of the current investigation.

Studies have shown that it will be challenging to successfully eradicate this infection through vaccination and other interventions if the eggs are produced by laying hens that are Salmonella-infected. As a result, given that eggs are regarded as a healthy and important source of protein, this could result in the development of food-borne illnesses in people as well as the contamination of chickens that hatch from infected eggs (15). Human nutrition plays a significant influence on one of the primary components of food items, and food security is crucial (16), demonstrating the significance of the current study in reducing the harm caused by contamination and antibiotic resistance. Contrarily, numerous Salmonella serotypes are frequently recovered from eggs, particularly non-typhoidal Salmonella (NTS) strains, which have been identified as the major pathogens in outbreaks of food-borne Salmonellosis (17), which is consistent with the current investigation.

18 of the 20 bacterial samples tested positive for antibiotic resistance using the disk diffusion method, according to the study's findings, including tetracycline (90%), doxycycline (90%), and chlortetracycline (90%) Recognized. The recent global spread of Haplotype 58 in S. typhi epidemiology is a novel and concerning development (H58). H58 is the predominant S. Typhi haplotype in many areas of South and Southeast Asia and sub-Saharan Africa, and its spread in a region is linked to the spread of illnesses and epidemics. H58 strains are frequently multidrug resistant, although the underlying reasons of the global H58 remain unknown. Concerningly, the rise of H58-S strains that are extraordinarily drug-resistant (XDR). TDRy/XDRol S is becoming more prevalent in Typhi that are resistant to fluoroquinolones, ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, and third-generation cephalosporins. This poses a serious threat to the effectiveness of current therapies, and if additional resistance arises, there is a danger of infections becoming incurable (18), which is consistent with the results of the current study.

In Iran, there is little information about the occurrence of multi-drug resistant Salmonella series and the role of integrins in antibiotic resistance (19) and antibiotics have been an effective way to control bacterial infections (20). However, the incorrect use and overuse of these substances have led to the benefits of a selection of microbial resistance (AMR) (21). A study showed that the use of antibiotics in poultry farms reached 19.6% (18,100/92,100). 140), beta-lactams, cephalosporins and fluoroquinolones are the most common antibiotics used in the poultry industry (22).

Veterinary antibacterial drugs ranged in concentration from 18 to 188 mg/kg per

nation in relation to the total biomass of food-producing animals, according to data gathered in 10 European countries. Tetracyclines made up 48% of all sales of veterinary antibiotics, followed by sulfonamides and trimethoprim (17% as a sulfonamide or in combination) and β-lactams (16%). (23). In order to sustain protein synthesis, these protective proteins discharge tetracycline from the ribosome (24).

23 genes (60%) of all tet resistance genes are related to efflux pump coding genes. These genes are mostly found in gram negatives. Out of 23 genes encoding efflux pumps, 4 genes were found in Gram-positive bacteria, 3 genes in Streptomyces and 16 genes in Gram-negative bacteria (25). 11 genes out of all tet resistance genes were related to genes. It encodes a cytoplasmic protein that protects the ribosome from the action of tetracycline. Also known as plasmid-encoded sulfonamide resistance genes, sul1, sul2, and sul3 generate dihydropteroate synthetase (DHP) and promote resistance to sulfonamides (26). It relates to resistance. Integrin 1 is present, whereas Sul2 is typically transferrable on big, high-resistance plasmids or small, non-conjugating plasmids. The plasmid is also the source of the sul3 gene, another sulfonamide resistance gene (27). In order to check imported eggs for veterinary drug residues, the CFIA in Canada undertook research for Quon. Three hundred eighty-six of these samples, including tetracycline, were examined for the presence of several antibiotics. Nine of these samples tested positive for tetracycline (26, 28), confirming the focus of our analysis.

Figure 3 shows that the Sul2 gene was found in 95.5% of the samples in this investigation that were resistant to multiple drugs, and that the Nfsa and Nfsb genes were the cause of the Salmonella resistance to nitrofurantoin. In the research of Xie et al., in West Africa (Lumé) Regarding the effect of antibiotic residues on the microbial control of the studied foods, in total 90 samples, 18.89% were positive in terms of antibiotic residues and 99.51% were positive in terms of the relationship between antibiotic residues and the sample source (0.05 P<) (29) which confirms our research.

The grouping of strains resistant to multiple drugs based on the presence of different types of antibiotic resistance genes with the cluster analysis method in Figure 5 showed that cutting the dendrogram from the 18-unit distance classified the strains into three sub-clusters. These strains had all three resistance genes to sul2, nfsa, nfsb and did not have tetc. The second microcluster included strains 7 and 10, which had three tetc, nfsa, and sul2 genes, and the strains were under the third cluster with only tetc. Figure 5.

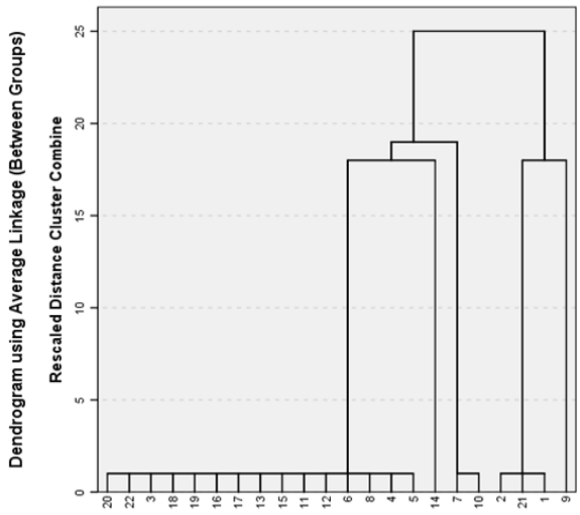

Figure 5. Cluster analysis of Salmonella multidrug-resistant strains for tetracycline, sulfonamide and nitrofurantoin antibiotic-resistant genes

Salmonella has been linked to numerous studies that have linked it to chicken and items associated with poultry, particularly eggs (30), and there is a lot of genetic variability among Salmonella isolates from various sources (31). Laboudi et al. (2013) conducted research on the antibiotic residues of chlortetracycline and sulfonamides in eggs in Jordan. The PREMI test was used to check 500 egg samples for antibiotic residues. Using the

HPLC approach, the amount of antibiotic residue and its composition were both identified. Of 500 egg samples, 64 cases (12.8%) had antibiotic residues. Additionally, during production, storage, and handling, the prevalence and features of Salmonella in chicken products, particularly eggs, exhibit dynamic fluctuation. (32). As a result, it is crucial to continuously monitor the presence of this group of pathogenic agents in eggs in order to ensure food safety (33). For example, Sirdar et al. 2012's study on the examination of antibiotic residues in eggs in Sudan included a total of 933 eggs from 175 farms that were screened using the microbial growth inhibition method. Antibiotic residues were found in many regions and during various times of the year, and no discernible variation in occurrence (p=0.57) between time periods was found (34). In the study conducted in 2014 by Ehsani and Hashemi, which looked at antibiotic residues such as tetracycline and sulfonamides in eggs in Iran. Paying (Urmia). Bacillus subtilis was present when the disk diffusion method for the microbial inhibition test was used to reveal that 25 samples (12.5%) of the processed eggs tested positive for antibiotics, and they are all macrolides (35). The findings of a study on antibiotic residues in chicken and eggs from traditional markets in Indonesia (Yogyakarta) in 2019 by Widiasih et al. revealed that 8.33% (2 out of 24 samples) of the chicken samples had the residual antibiotic tetracycline. Of the egg samples, 75% (18 out of 24 samples) had traces of the antibiotic penicillin. Tetracycline and aminoglycoside antibiotics that were still detected in egg samples were both 12.5% (3 out of 24 cases), which supports the findings of the current investigation.

Recommendations

The expression of detected genes in the studied strains under antibiotic treatment should be examined. The origin of resistance genes should be also examined in terms of plasmids or genomes to control them by extinction of them. The presence of other genes related to resistance sources should be examined. Health monitoring on trade and transfer of egg and poultry products (Salmonella quarantine) should be performed. Searching for the possibility of sterilizing eggs by non-chemical methods such as gamma rays or new sterile materials such as anolyte should be done.

Conclusion

In the present study, 830 egg samples from 37 Izani brands were extracted from the yolk source of Salmonella and after confirmation by differential phenotypic methods and resistance to tetracycline, sulfonamide and nitrofurantoin antibiotics, 22 multidrug-resistant strains were identified. DNA from the strains was extracted, and using PCR with specified primers, the presence of tetA, tetB, tetC, tetD, tetG, tetE, tetH, sul I, sul II, sul III, nfsA, and nfsB genes with DNA (ATCC14028) control was investigated to investigate the molecular mechanism of multidrug resistance The findings showed that tetc was present in 81% of the samples and absent from 19% of the samples among the genes of teta, tetb, tetc, tetd, tete, tetf, tetg, and teth. There were no other tetracycline resistance genes discovered in the samples. Genes related to sulfonamide resistance by PCR showed that among the Sul1, Sul2, and Sul3 genes, sulfonamide resistance of Sul2 was observed in 95.5% of the samples and was not seen in 4.5% of the samples. Nitrofurantoin-resistant genes in the samples showed that 9.1% of the samples included nfsa gene and 4.5% included nfsb gene and the rest of the samples did not have any nitrofurantoin resistance gene. The similarity of resistance genes in different samples indicates their transmission through the exchange of eggs and food sources between poultry farmers, which is also considered as a health threat to humans. Monitoring on trade and the transfer of egg and poultry products can be the most important method in controlling Salmonella.

Author's contribution

Mohammad Saram and Arash Chaichi Nosrati developed the study concept and design. Leila Modiri acquired the data. Khossrow Issazadeh and Mohammad Saram analyzed and interpreted the data, and wrote the first draft of the manuscript. All authors contributed to the intellectual content, manuscript editing and read and approved the final manuscript.

Informed consent

Questionaires were filled with the participans' satisfaction and written consent was obtained from the participants in this study.

Funding/financial support

There is no funding.

Conflict of interest

The authors declare that they have no conflict of interests.

References

1. Khalil AT, Iqrar I, Bashir S, Ali M, Khalil AH, Shinwari ZK. Preemptive and Proactive Strategies for Food Control and Biosecurity. Food Safety and Preservation. 2018;3(1):39-58. https://doi.org/10.1016/B978-0-12-814956-0.00003-2
2. Tadesse G, Gebremedhin EZ. Prevalence of Salmonella in raw animal products in Ethiopia: a meta-analysis. BMC Res Notes. 2015;8(1):163-170. https://doi.org/10.1186/s13104-015-1127-7
3. Lee K. & Runyon M. & Herrman T. & Phillips RW. & Hsieh JJ. Review of Salmonella detection and identification methods: Aspects of rapid emergency response and food safety. Food Control. 2015;47(1):264-276. https://doi.org/10.1016/j.foodcont.2014.07.011
4. Li W, Li H, Zheng S, Wang Z, Sheng H, Shi C, Shi X, Niu Q, Yang B. Prevalence, Serotype, Antibiotic Susceptibility, and Genotype of Salmonella in Eggs from Poultry Farms and Marketplaces in Yangling, Shaanxi Province, China. Front Microbiol. 2020;11(1):1482-1496. https://doi.org/10.3389/fmicb.2020.01482
5. Ashtiani MT, Monajemzadeh M, Kashi L. Trends in antimicrobial resistance of fecal Shigella and Salmonella isolates in Tehran, Iran. Indian J Pathol Microbiol. 2009;52(1):52-5. https://doi.org/10.4103/0377-4929.44964
6. Sulieman AME, Dafallah FE, Abdel-Rahman EH, Alshammari NI, Shommo SA, Ibrahim SE. Isolation, Identification and Characterization of Salmonella spp. from Chicken purchased at Wad Madani City, Gezira State, Sudan. Advancements in Life Sciences. 2020;8(1):98-102. http://www.als-journal.com/8117-20/
7. Rodrigues GL, Panzenhagen P, Ferrari RG, Paschoalin VMF, Conte-Junior CA. Antimicrobial Resistance in Nontyphoidal *Salmonella* Isolates from Human and Swine Sources in Brazil: A Systematic Review of the Past Three Decades. Microb Drug Resist. 2020;26(10):1260-1270. https://doi.org/10.1089/mdr.2019.0475
8. Al-Abadi IK, Al-Mayah AA. Observations on the pathogenesis of orally inoculated Salmonella typhimurium in a newly hatched broiler chicks. Iraqi Poultry Sciences Journal. 2013;7(1):42-51. https://search.emarefa.net/detail/BIM-759007
9. Antunes P, Réu C, Sousa JC, Peixe L, Pestana N. Incidence of Salmonella from poultry products and their susceptibility to antimicrobial agents. Int J Food Microbiol. 2003;82(2):97-103. https://doi.org/10.1016/s0168-1605(02)00251-9
10. Cowen P, Currier RW, Steele JH. A Short History of One Health in the United States. Vet Herit. 2016;39(1):1-15. https://pubmed.ncbi.nlm.nih.gov/27344860/
11. Patchanee P, Eiamsam-Ang T, Vanaseang J, Boonkhot P, Tadee P. Determination of regional relationships among Salmonella spp. isolated from retail pork circulating in the Chiang Mai municipality area using a WGS data approach. Int J Food Microbiol. 2017;254(1):18-24. https://doi.org/10.1016/j.ijfoodmicro.2017.05.006
12. McWhorter AR, Chousalkar KK. From hatch to egg grading: monitoring of *Salmonella* shedding in free-range egg production systems. Vet Res. 2019;50(1):58-70. https://doi.org/10.1186/s13567-019-0677-4
13. Huang X, Zhou X, Jia B, Li N, Jia J, He M, He Y, Qin X, Cui Y, Shi C, Liu Y, Shi X. Transcriptional Sequencing Uncovers Survival Mechanisms of Salmonella enterica Serovar Enteritidis in Antibacterial Egg White. ASM Journals. 2019;4(1):e00700-18. https://doi.org/10.1128/mSphere.00700-18
14. Moffatt CRM, Musto J, Pingault N, Combs B, Miller M, Stafford R, Gregory J, Polkinghorne BG, Kirk MD. Recovery of Salmonella enterica from Australian Layer and Processing Environments Following Outbreaks Linked to Eggs. Foodborne Pathog Dis. 2017;14(8):478-482. https://doi.org/10.1089/fpd.2016.2268
15. Kouam MK, Biekop MH, Katte B, Teguia A. Salmonella status of table eggs in commercial layer farms in Menoua Division, West region of Cameroon. Food Control. 2018;85(1):345-349. https://doi.org/10.1016/j.foodcont.2017.09.037
16. Song J, Gao X, Galán JE. Structure and function of the Salmonella Typhi chimaeric A(2)B(5) typhoid

toxin. Nature. 2013;499(7458):350-4. https://doi.org/10.1038/nature12377
17. Tajbakhsh M, Hendriksen RS, Nochi Z, Zali MR, Aarestrup FM, Garcia-Migura L. Antimicrobial resistance in Salmonella spp. recovered from patients admitted to six different hospitals in Tehran, Iran from 2007 to 2008. Folia Microbiol (Praha). 2012;57(2):91-7. https://doi.org/10.1007/s12223-012-0099-4
18. Cortés V, Sevilla-Navarro S, García C, Marín C, Catalá-Gregori P. Monitoring antimicrobial resistance trends in Salmonella spp. from poultry in Eastern Spain. Poult Sci. 2022;101(6):101832. https://doi.org/10.1016/j.psj.2022.101832
19. Siriken B, Al G, Erol I. Prevalence and Antibiotic Resistance of *Salmonella* Enteritidis and *Salmonella* Typhimurium in Ground Beef and Meatball Samples in Samsun, Turkey. Microb Drug Resist. 2020;26(2):136-144. https://doi.org/10.1089/mdr.2018.0481
20. Fardsanei F, Soltan Dallal MM, Douraghi M, Zahraei Salehi T, Mahmoodi M, Memariani H, Nikkhahi F. Genetic diversity and virulence genes of Salmonella enterica subspecies enterica serotype Enteritidis isolated from meats and eggs. Microb Pathog. 2017;107(1):451-456. https://doi.org/10.1016/j.micpath.2017.04.026
21. Mąka Ł, Popowska M. Antimicrobial resistance of Salmonella spp. isolated from food. Rocz Panstw Zakl Hig. 2016;67(4):343-358. https://pubmed.ncbi.nlm.nih.gov/27922740/
22. Cortez AL, Carvalho AC, Ikuno AA, Bürger KP, Vidal-Martins AM. Identification of Salmonella spp. isolates from chicken abattoirs by multiplex-PCR. *Research in veterinary science*. 2006;81(3):340-344. https://doi.org/10.1016/j.rvsc.2006.03.006
23. van Hoek AH, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJ. Acquired antibiotic resistance genes: an overview. Front Microbiol. 2011;2(1):203. https://doi.org/10.3389/fmicb.2011.00203
24. Trobos M, Christensen H, Sunde M, Nordentoft S, Agerso Y, Simonsen GS, Hammerum AM, Olsen JE. Characterization of sulphonamide-resistant Escherichia coli using comparison of sul2 gene sequences and multilocus sequence typing. Microbiology (Reading). 2009;155(Pt 3):831-836. https://doi.org/10.1099/mic.0.024190-0
25. Antunes P, Machado J, Sousa JC, Peixe L. Dissemination of sulfonamide resistance genes (sul1, sul2, and sul3) in Portuguese Salmonella enterica strains and relation with integrons. Antimicrob Agents Chemother. 2005;49(2):836-9. https://doi.org/10.1128/AAC.49.2.836-839.2005
26. Quon DJ. Monitoring of domestic and imported eggs for veterinary drug residues by the Canadian Food Inspection Agency. J Agric Food Chem. 2000;48(12):6421-7. https://doi.org/10.1021/jf000144x
27. Pérez-Rodríguez M, Pellerano RG, Pezza L, Pezza HR. An overview of the main foodstuff sample preparation technologies for tetracycline residue determination. Talanta. 2018;182(1):1-21. https://doi.org/10.1016/j.talanta.2018.01
28. Sánchez-Salazar E, Gudiño ME, Sevillano G, Zurita J, Guerrero-López R, Jaramillo K, Calero-Cáceres W. Antibiotic resistance of Salmonella strains from layer poultry farms in central Ecuador. J Appl Microbiol. 2020;128(5):1347-1354. https://doi.org/10.1111/jam.14562
29. Xie T, Wu G, He X, Lai Z, Zhang H, Zhao J. Antimicrobial resistance and genetic diversity of *Salmonella enterica* from eggs. Food Sci Nutr. 2019;7(9):2847-2853. https://doi.org/10.1002/fsn3.1126
30. Li X, Liu L, Li Q, Xu G, Zheng J. *Salmonella* Contamination in Layer Farms in China: Detection and Genetic Analysis. J Poult Sci. 2018;55(1):1-9. https://doi.org/10.2141/jpsa.0160144
31. Denagamage T, Jayarao B, Patterson P, Wallner-Pendleton E, Kariyawasam S. Risk Factors Associated with Salmonella in Laying Hen Farms: Systematic Review of Observational Studies. Avian Dis. 2015;59(2):291-302. https://doi.org/10.1637/10997-120214-Reg
32. laboudi A, Basha EA, Musallam I. Chlortetracycline and sulfanilamide residues in table eggs: Prevalence, distribution between yolk and white and effect of refrigeration and heat treatment. Food control. 2013;33(1):281-286. https://doi.org/10.1016/j.foodcont.2013.03.014
33. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM; International Collaboration on Enteric Disease 'Burden of Illness' Studies. The global burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis. 2010;50(6):882-9. https://doi.org/10.1086/650733
34. Sirdar MM, Picard J, Bisschop S, Jambalang AR, Gummow B. A survey of antimicrobial residues in table eggs in Khartoum State, Sudan, 2007-2008. Onderstepoort J Vet Res. 2012;79(1):1-9. https://doi.org/10.4102/ojvr.v79i1.360
35. Ehsani A, Hashemi M. Determination of Antibacterial Drug Residues in Commercial Eggs Distributed in Urmia, Iran. J. Food Qual. Hazards Control. 2015;2(2):61-65. http://jfqhc.ssu.ac.ir/article-1-147-en.html
36. Widiasih DA, Drastini Y, Yudhabuntara D, Maya FLRD, Sivalingham PL, Susetya H, et al. Detection of Antibiotic Residues in Chicken Meat and Eggs from Traditional Markets at Yogyakarta City Using Bioassay Method. Acta Vet Indones. 2019;7(4):1-6. https://doi.org/10.29244/avi.0.0.1-6