Eurasian Journal
of Veterinary Sciences

www.eurasianjvetsci.org

RESEARCH ARTICLE

Determination of *Salmonella* spp. presence and antibiotic resistance in egg and egg products

Hilal Keskinoğlu[1], Göknur Terzi Gülel[1]

[1]Ondokuz Mayıs University, Veterinary Faculty, Department of Food Hygiene and Technology, Samsun, Turkey

Received:31.05.2020, Accepted: 03.11.2020
*goknurterzi@yahoo.com.tr

Yumurta ve Ürünlerinde *Salmonella* spp. varlığı ve antibiyotik dirençliliğinin belirlenmesi

Eurasian J Vet Sci, 2022, 38, 1, 24-31
DOI: 10.15312/EurasianJVetSci.2022.361

Öz

Amaç: Bu çalışmanın amacı, yumurta ve yumurta ürünlerinde *Salmonella* spp varlığını belirlemek ve elde edilen izolatların antibiyotik direnç profilleri ve minimum inhibitör konsantrasyon (MİK) değerlerini belirlemektir.

Gereç ve Yöntem: Çalışmada 100 yumurta (35 köy yumurtası, 35 konvansiyonel yumurta ve 30 organik yumurta) ve 100 yumurta ürünü (30 yumurta tozu, 70 pastörize sıvı yumurta) olmak üzere toplam 200 numune Samsun, Türkiye'den toplandı. Yumurta kabuğu ve yumurta içi örnekleri üç yumurta bir araya getirilecek ayrı ayrı analiz edidi. *Salmonella* spp. izolasyon ve identifikasyonu ISO 6579 tarafından önerilen yönteme göre yapıldı. Elde edilen *Salmonella* spp. izolatlarının çeşitli antibiyotiklere karşı antimikrobiyal duyarlılığı ve MİK değerleri VITEK 2 AST-GN38 kartları kullanılarak VITEK 2 kompakt sistemi ile gerçekleştirildi.

Bulgular: İncelenen 100 yumurtanın (organik yumurta içi) ikisinde (%2) ve 100 yumurta ürününün (pastörize likit yumurta) birinde (%1) *Salmonella* spp. pozitif bulundu. Toplam 11 izolat *oriC* geninin varlığı yönünden PCR tekniğiyle *Salmonella* spp. olarak doğrulandı. Antibiyotik direnç testleri sonucunda en yüksek direnç amikasin, enrofloksasin, gentamisin, tobramisin, sefaleksine (%100), ardından nitrofurantoin (%81,8), tetrasiklin (%63.6), ampisilin (%54,5), piperasilin (%54,5), sefpodoksim (%54,5), ve imipeneme (%9,09) karşı bulundu. Bununla birlikte amoksisilin, marbofloksasin ve trimetoprim/sülfametoksazole karşı direnç tespit edilemedi. Sonuçta üç numuneden elde edilen 11 *Salmonella* spp. izolatının 11'inin (11/11, %100) üç veya daha fazla antimikrobiyal ajana karşı çoklu direnç gösterdiği görüldü.

Öneri: Yumurta ve yumurta ürünlerinin tüketimine bağlı Salmonelloz riskini en aza indirmek için iyi hijyen uygulamaları, iyi üretim uygulamaları ve pastörizasyon tekniklerinin uygulanması önerilmektedir.

Anahtar kelimeler: *Salmonella* spp, yumurta, yumurta ürünleri, moleküler doğrulama.

Abstract

Aim: The aim of this study was to determine the presence of *Salmonella* spp. in egg and egg products and to determine antibiotic resistance profiles and minimum inhibitory concentration (MIC) values of isolates.

Materials and Methods: A total of 200 samples including 100 eggs (35 village eggs, 35 conventional eggs and 30 organic eggs) and 100 egg products (30 egg powders, 70 pasteurized liquid eggs) were collected from Samsun, Turkey. Eggshell and egg contents samples were processed separately by pooling three eggs together. The isolation and identification of *Salmonella* spp. was done according to the method proposed by ISO 6579. The antimicrobial susceptibility of *Salmonella* spp. isolates to various antibiotics and MIC values was performed by VITEK 2 compact system using VITEK 2 AST-GN38 cards.

Results: *Salmonella* spp. were found in two of 100 (2%) eggs (organic egg contents) and one of 100 (1%) egg products (pasteurized liquid egg). A total of 11 isolates were confirmed by PCR techniques as *Salmonella* spp. with the presence of *oriC* gene. The highest resistance was against amikacin, enrofloxacin, gentamicin, tobramycin, cephalexin (100%), followed by nitrofurantoin (81.8%), tetracycline (63.6%), ampicillin (54.5%), piperacillin (54.5%), cefpodoxime (54.5%), and imipenem (9.09%). However, there was no resistance to amoxicillin, marbofloxacin and trimethoprim/sulfamethoxazole. The results showed that 11/11 (100%) of *Salmonella* spp. from three sample showed multi-drug resistance against three or more antibiotic agents.

Conclusion: It is recommended to implement good hygiene practices, good production practices and pasteurization techniques to minimize the risk of Salmonellosis due to the consumption of eggs and egg products.

Keywords: *Salmonella* spp, egg, egg products, molecular confirmation

Introduction

Eggs are an important source of easily digestible, highly nutrient protein besides they contain many trace elements, essential amino acids, fats, water-soluble vitamins and minerals needed by different human organism (Joel et al 2010). Eggs and egg products are primarily utilized in the food industry sector, especially in products like cakes, pasta, mayonnaise, salad dressing, confectionery, and ice cream, in which eggs are used for the purposes of coagulating, emulsifying, yeasting, thickening, softening, moisture retention, flavour and colour adding as well as increasing the nutritional value of products (Asgar and Abbas 2012). Liquid, frozen and dry eggs, which are among egg products, are widely used in the food industry. In Turkey, chicken egg production was reported to be 19 billion 800 million units in 2020 (TUIK 2020), and the average number of egg consumption per person was 224 pieces in 2018 (Yumbir 2018).

Salmonella species are gram-negative bacteria that classified within the Enterobacteriaceae family, they appear to be 2-5 µm long, 0.5-1.5 µm wide, rod-shaped, non-spore, unencapsulated (including microcapsule), active with most peritric flagellas, facultative anaerobe, fermentative, catalase-positive and oxidase negative (Andino and Hanning 2015). *Salmonella* causes food-borne infections as a result of consumption the raw or undercooked eggs, poultry, red meat, and its products. Some foods such as homemade sauces, tiramisu, homemade ice cream, mayonnaise, cookie dough are also risky sources for *Salmonella* since raw eggs can be used (De Knegt et al 2015). According to CDC data, it was reported that each year 1.2 million salmonellosis induced diseases occur in the United States, of this number, 23.000 people are hospitalized. From them, 450 are dying and the annual medical expenses are estimated to be 365 million dollars (CDC 2021).

Antimicrobial agents are used in the prevention and treatment of bacterial infections in poultry. Antibiotic resistance is a major problem worldwide. Unconscious antibiotic use in poultry causes the emergence of antibiotic-resistant bacteria. Antibiotics used in poultry include beta-lactams (penicillin G, amoxicillin, ampicillin, ceftiofur), polypeptides (bacitracin), aminoglycosides (gentamicin, neomycin, streptomycin), macrolides (erythromycin, tylosin, tilmicosin, tiamulin), lincosamides (lincomycin), tetracyclines (chlortetracycline, tetracycline, oxytetracycline), sulphonamides, fluoroquinolones and ionophores (Diaz-Sanchez et al 2015, Landoli and Albarellos 2015).

The aim of this study was to investigate the presence of *Salmonella* spp. in eggs and egg products (egg powder, pasteurized liquid egg) obtained from various markets, supermarkets and businesses in the province of Samsun, Turkey, also to confirm the obtained isolates with PCR and to find out the resistance and minimum inhibitor concentrations (MIC) against various antibiotics with VITEK 2 compact system.

Material and Methods

Sample collection

A total of 200 samples including 100 eggs and 100 egg products were collected in Samsun province, Turkey between October 2017 and May 2019. 35 village egg samples were collected from seven different village, 35 traditional eggs were collected from seven different market, 30 organic eggs were collected from four different brands of three markets as well as 100 egg products (30 egg powders, 70 pasteurized liquid eggs) were collected from three different brands of three different companies. After all the samples were brought to the laboratory as soon as possible in the cold chain. Three eggs were pooled and accepted as one sample. Pooled sampling is an efficient method for detecting *Salmonella* especially when prevalence and contamination level risks are low. In addition pooling can also increase efficiency in time and labor and reduce overall testing costs (Pasquali et al 2014). The presence of *Salmonella* spp. was researched on both the eggshell and egg contents. The eggshell was disinfected with 70% alcohol to determine the contamination of eggs (egg contents), then the eggs were broken into a sterile container.

Isolation and identification of Salmonella spp.

All samples were analyzed for the presence of *Salmonella* spp. in eggs and egg products using the standard bacteriological method recommended by ISO 6579 (The International Organization for Standardization (ISO 2017). Briefly, 25 g of samples were taken into sterile stomacher bags and 225 mL of Buffered Peptone Water (BPW) (Merck, Germany) was added and homogenized by stomacher (Interscience Bagmixer 400, StNom, France) for 2-3 minutes. In order to determine the contamination on the eggshell, eggs were placed in a sterile sample bag and 225 ml BPW was added and washed for 2-3 minutes. The eggs were taken out and incubated in rinsed liquid for 24 hours at 37 °C. Obtained suspect colonies with smooth edges and black centres from Xylose-Lysine Deoxycholate Agar (XLD) (Merck, Germany) were confirmed by biochemical tests (Triple Sugar Iron Agar, Lysine Iron Agar, Indol, Methyl Red–Voges-Proskauer and urea test) (ISO 2017).

PCR method

Genomic DNA was extracted by using the boiling method (Seel et al 2016). The primer pairs (*oriC*) described by Widjojoatmodjo et al (1991) were used to detect *Salmonella* spp. (Table 1). PCR reactions were performed in a 25 µl volume containing 1X PCR buffer (500 mM KCl, 200mM TrisHCl), 0.1 mM dNTPs, 1.5 mM MgCl2, 2 U Taq DNA polymerase, 0.5 µM *oriC*-R primer, 0.5 µM *oriC*-F primer and 3 µl template DNA.

Reactions were performed in a thermal cycler (Bio-Rad, USA) with initial denaturation for 5 min at 94 °C, which was followed by 35 cycles for 1 min at 94 °C, annealing for 1 min at 53 °C, extension for 1 min at 72 °C and final extension at 72 °C for 10 min. The amplified product was electrophoresed on a 1.5% (w/v) agarose gel containing 10 mg/ml ethidium bromide (Merck, Germany) at 80 V for 45 min. *OriC* gene positive isolates were visualized by UV transillumination (Wise-UVWuv-L50, Daihan Scientific, Seoul, Korea) at 163 bp. S. Enteritidis ATCC 13076 was used as a reference strain (Figure 1).

Antimicrobial susceptibility testing

The antibiotic susceptibility test against the *Salmonella* spp. isolates was carried out using AST-GN38 (bioMérieux, Fransa) cards with VITEK 2 Compact (bioMérieux, France) according to the manufacturer's instructions. The antibiotics and concentrations used in this study are presented in Table 3. For this purpose, *Salmonella* spp. isolates were subcultured in Tryptic Soy Broth (TSB) at 37 °C for 24 hours. Then, they were incubated at Tryptone Soy Agar (TSA) at 37 °C for 24 hours.

Then, suspected *Salmonella* spp colonies were selected and suspended in sterile tubes containing 3 ml of 0.45% physiological saline (PSS) and its density was adjusted to 0.5 McFarland (1.5 x 108 CFU/ml) (Biosan, Latvia). Bacterial suspensions and AST-GN38 test cards were loaded in a cassette, and then loaded into the VITEK 2 Compact system and turbidity was automatically measured. The obtained results were evaluated according to the Clinical and Laboratory Standards Institute (CLSI 2021).

Results

In this study, a total of 20 suspicious colonies were detected from 200 samples by the conventional method established by ISO 6579. Eleven of the 20 suspicious colonies were found to be positive for *Salmonella* spp. by the PCR method. Of the obtained 11 isolates, six were from organic egg contents and five from the pasteurized liquid eggs.

Table 1. The sequences of primers used in this study

Target gene	Primer sequence	PCR product (bp)	Reference
oriC primer 1	5'-TTA TTA GGA TCG CGC CAG GC-3'	163 bp	Widjojoatmodjo et al (1991)
oriC primer 2	5'-AAA GAA TAA CCG TTG TTC AC-3'		

Table 2. Prevalence of *Salmonella* spp. isolates in egg and egg products

Type of samples	Classical culture technique (ISO 6579)		PCR (*oriC* gene)	
	Sample	Isolate	Sample	Isolate
Egg samples				
Conventional egg content (n=35)	-	-	-	-
Conventional eggshell (n=35)	-	-	-	-
Village egg content (n=35)	-	-	-	-
Village eggshell (n=35)	-	-	-	-
Organic egg content (n=30)	3	11	2	6
Organic eggshell (n=30)	1	4	-	-
Egg product samples				
Egg powder (n=30)	-	-	-	-
Pasteurized liquid egg (n=70)	1	5	1	5
Total (n=200)	5	20	3	11

According to this, two of 100 (2%) egg samples and one of 70 (1.4%) pasteurized liquid egg samples were positive for *Salmonella* spp. None of the egg powder and eggshell samples were found to be positive for *Salmonella* spp. while egg content samples were positive by 2%. Although one of the 35 conventional and 35 village egg samples were contaminated by *Salmonella* spp., two of the 30 (6.6%) organic egg samples were positive for *Salmonella* spp. (Table 2).

In our study, *Salmonella* spp. was found in one (1.4%) of the 70 pasteurized liquid eggs. As a result of antibiotic resistance tests, it was found that 11 isolates were resistant to at least one antibiotic. The highest resistance was against amikacin, enrofloxacin, gentamicin, tobramycin, cephalexin (100%), followed by nitrofurantoin (81.8%), tetracycline (63.6%), ampicillin (54.5%), piperacillin (54.5%), cefpodoxime (54.5%) and imipenem (9.09%).

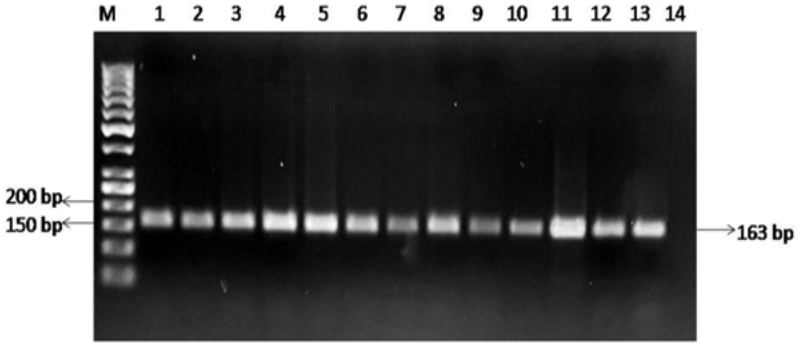

Figure 1. Electrophorese image of *oriC* gene (163 bp) of *Salmonella* spp by PCR.
M: 50 bp DNA ladder, Lane 1-2: Positive control (S. Enteritidis ATCC 13076), Lane 3-13: *oriC* gene positive isolates, Lane 14: Negative control (deionized water)

Table 3. Antimicrobial resistance profiles of *Salmonella* spp. isolates

Antibiotics (dose, µg/ml)	MIC value (µg/ml)	No. of isolates (%)		
		R (%)	I (%)	S (%)
Amikacin (AMK)(8, 16, 64)	2	11(100%)	0(0%)	0(0%)
Amoxicillin(AMX)(4/2,16/8, 32/16)	2	0(0%)	0(0%)	11(100%)
Ampicillin (AMP)(4, 8, 32)	2 - 4	6 (54.5%)	0(0%)	5(45.5%)
Enrofloxacin (ENR)(0.25, 1, 4)	0.12 - 1	11(100%)	0(0%)	0(0%)
Gentamicin (GEN)(4, 16, 32)	1	11(100%)	0(0%)	0(0%)
Imipenem (IPM)(2, 4, 16)	1 - 16	1(9.09%)	2(18.18%)	8(72.7%)
Marbofloxacin (MFX)(1, 2)	0.5 - 1	0(0%)	0(0%)	11(100%)
Nitrofurantoin (NIT)(16, 32, 64)	64- 512	9(81.8%)	2(18.18%)	0(0%)
Piperacillin (PIP)(4, 16, 32, 64)	4 -16	6(54.5%)	0(0%)	5 (45.5%)
Cefalexin (LEX)(8, 32, 64)	4 -16	11(100%)	0(0%)	0(0%)
Cefpodoxime (CPD)(0.5, 1, 4)	0.25 - 2	6(54.5%)	0(0%)	5(45.5%)
Ceftiofur (CEF)(1, 2)	1 - 4	0(0%)	1(9,09%)	10(90,9%)
Cefpirome (CPR)(2, 8, 64)	1	0(0%)	6(54.5%)	5(45.5%)
Tetracycline (TET)(2, 4, 8)	1- 16	7(63.6%)	0(0%)	4(36.4%)
Tobramycin (TOB)(8, 16, 64)	1	11(100%)	0(0%)	0(0%)
Trimethoprim/sulfamethoxazole (TMP/SMX)(1/19, 4/76, 16/304)	20	0(0%)	0(0%)	11(100%)
Chloramphenicol (CHL)(4, 16, 32)	4 - 16	0(0%)	6(54.5%)	5(45.5%)

R: Resistant, I: Intermediate, S: Susceptible

Table 4 Antimicrobial resistance patterns of the *Salmonella* spp isolates and MIC values

No of isolates	AMP	AMX	PIP	LEX	CPD	CEF	CPR	IPM	AMK	GEN	TOB	ENR	MFX	TET	NIT	CHL	TMP/SMX
1. Organic chicken egg content	<=2 (R)	<=2 (S)	16 (R)	16 (R)	2 (R)	4 (I)	<=1 (I)	<=1 (S)	<=2 (R)	<=1 (R)	<=1 (R)	1 (R)	1 (S)	>=16 (R)	>=512 (R)	16 (I)	<=20 (S)
2. Organic chicken egg content	<=2 (R)	<=2 (S)	8 (R)	16 (R)	2 (R)	2 (S)	<=1 (I)	<=1 (S)	<=2 (R)	<=1 (R)	<=1 (R)	1 (R)	1 (S)	>=16 (R)	>=512 (R)	16 (I)	<=20 (S)
3. Organic chicken egg content	4 (R)	<=2 (S)	16 (R)	16 (R)	2 (R)	2 (S)	<=1 (I)	<=1 (S)	<=2 (R)	<=1 (R)	<=1 (R)	1 (R)	1 (S)	>=16 (R)	>=512 (R)	16 (I)	<=20 (S)
4. Organic chicken egg content	<=2 (R)	<=2 (S)	8 (R)	8 (R)	2 (R)	2 (S)	<=1 (I)	<=1 (S)	<=2 (R)	<=1 (R)	<=1 (R)	1 (R)	1 (S)	>=16 (R)	>=512 (R)	16 (I)	<=20 (S)
5. Organic chicken egg content	<=2 (R)	<=2 (S)	16 (R)	16 (R)	2 (R)	2 (S)	<=1 (I)	2 (I)	<=2 (R)	<=1 (R)	<=1 (R)	1 (R)	1 (S)	>=16 (R)	256 (R)	16 (I)	<=20 (S)
6. Organic chicken egg content	4 (R)	<=2 (S)	16 (R)	8 (R)	2 (R)	2 (S)	<=1 (I)	<=1 (S)	<=2 (R)	<=1 (R)	<=1 (R)	1 (R)	1 (S)	>=16 (R)	>=512 (R)	16 (I)	<=20 (S)
7. Pasteurized liquid egg	<=2 (S)	<=2 (S)	<=4 (S)	<=4 (R)	0,5 (S)	<=1 (S)	<=1 (S)	2 (I)	<=2 (R)	<=1 (R)	<=1 (R)	<=0,12 (R)	<=0,5 (S)	<=1 (S)	64 (I)	4 (S)	<=20 (S)
8. Pasteurized liquid egg	<=2 (S)	<=2 (S)	<=4 (S)	16 (R)	<=0,25 (S)	<=1 (S)	<=1 (S)	>=16 (R)	<=2 (R)	<=1 (R)	<=1 (R)	<=0,12 (R)	<=0,5 (S)	>=16 (R)	128 (R)	8 (S)	<=20 (S)
9. Pasteurized liquid egg	<=2 (S)	<=2 (S)	<=4 (S)	16 (R)	0,5 (S)	<=1 (S)	<=1 (S)	<=1 (S)	<=2 (R)	<=1 (R)	<=1 (R)	<=0,12 (R)	<=0,5 (S)	<=1 (S)	128 (R)	4 (S)	<=20 (S)
10. Pasteurized liquid egg	<=2 (S)	<=2 (S)	<=4 (S)	8 (R)	<=0,25 (S)	<=1 (S)	<=1 (S)	<=1 (S)	<=2 (R)	<=1 (R)	<=1 (R)	<=0,12 (R)	<=0,5 (S)	<=1 (S)	128 (R)	4 (S)	<=20 (S)
11. Pasteurized liquid egg	<=2 (S)	<=2 (S)	<=4 (S)	8 (R)	<=0,25 (S)	<=1 (S)	<=1 (S)	<=1 (S)	<=2 (R)	<=1 (R)	<=1 (R)	<=0,12 (R)	<=0,5 (S)	<=1 (S)	64 (I)	4 (S)	<=20 (S)

Ampicillin (AMP), Amoxicillin (AMX), Piperacillin (PIP), Cefalexin (LEX), Cefpodoxime (CPD), Ceftiofur (CEF), Cefpirome (CPR), Imipenem (IPM), Amikacin (AMK), Gentamicin (GEN), Tobramycin (TOB), Enrofloxacin (ENR), Marbofloxacin (MFX), Tetracycline (TET), Nitrofurantoin (NIT), Chloramphenicol (CHL), Trimethoprim/sulfamethoxazole (TMP/SMX)

However, there was no resistance to amoxicillin, marbofloxacin, and trimethoprim/sulfamethoxazole. All of the isolates (100%) showed multiple drug resistance to three or more antibiotic agents. Antimicrobial resistance profiles of *Salmonella* spp. isolates and MIC values were shown in Table 3 and Table 4.

Discussion

In this study, the incidence of *Salmonella* spp. in eggs was found at 2%. In studies conducted by different researchers, the incidence of *Salmonella* spp. was found varied from 0% to 28% in eggs (Karim et al 2017, Pesavento et al 2017, Karadal et al 2018). This difference occurs due to many factors including environmental conditions of the poultry habitat, quality of feed, used litters, and hygienic criteria of the poultry housing. In particular, animal feeds used in poultry that contains fish powder additives, can cause *Salmonella* infections. Additionally, the contamination resulting from packaging may cause the cold chain to break during transportation and a small crack in the egg can also lead the bacterial agents to enter the egg (Cardoso et al 2021).

While *Salmonella* spp. was not isolated on the shells of the eggs in our study, however in other studies *Salmonella* spp. positivity rates were detected in the eggshells from many countries. Most of these conducted studies have reported *Salmonella* contamination to be higher on eggshell (Karim et al 2017). On the contrary, some studies reported no *Salmonella* spp. positivity rates in the eggshells similar to our study (Harsha et al 2011).

The contamination of eggs with *Salmonella* species bacteria occurs in two ways. The first of these is the transmission of the egg with contaminated feces during or after ovulation and it is called horizontal transmission. The second one is the contamination of egg contents as a sequel to poultry reproductive organs infection, which could happen before eggshell formation, and this is called direct contamination (vertical transmission) (Gantois et al 2009). Some researchers have reported horizontal transmission as the most important way to contaminate eggshells (Bichler et al. 1996). Whereas, other researchers have stated that vertical transmission is also very important and plays a critical role in this matter (Guard-Petter 2001).

In this study, while *Salmonella* spp. was not isolated in conventional and village eggs, 2 of the organic eggs (egg contents) (2/30, 6.6%) were found to be *Salmonella* spp. positive. In organic production, chicken has open walking and closed areas, so they can easily walk around without being given any chemicals, hormones, or antibiotics (Hoogenboom et al 2008). Consumers prefer organic products since this poultry is grown in appropriate breeding systems and no synthetic feed additives and genetically modified feed are used. While some researchers stated that organic products are healthier, other researchers emphasize that there are no important differences in quality between organic and conventional production methods (Konca et al 2010). Similar to the results of our study, *Salmonella* spp. incidence was found to be higher in organic eggs when compared with conventional eggs in a study conducted in South Korea (Lee et al 2013). The worrying results we had by finding *Salmonella* spp. in organic eggs of our study point to the fact that contamination may have occurred possibly due to feeding system, transportation, or cross-contamination.

Today egg products are widely used in the pastry industry in various forms, especially as frozen, dried, and pasteurized liquid eggs. Egg products are often preferred due to their practical use in industry and their long-term preservation. In our study, *Salmonella* spp. was found in one (1.4%) of the 70 pasteurized liquid eggs. Similar to the results of our research, Hara-Kudo and Takatori (2009) found *Salmonella* spp. in 1.7% of the pasteurized liquid eggs they examined in Japan. On the other hand, Dogruer et al (2015) did not find *Salmonella* spp. in 40 pasteurized liquid eggs they examined in Konya. In liquid eggs, pasteurization is made against microbial contamination, especially *Salmonella* spp. Previously, it has been reported that no *Salmonella* spp. was found in liquid eggs following an efficient pasteurization process (Board 2000). On the contrary, *Salmonella* spp. was found in pasteurized liquid eggs in our study. This can be due to insufficient pasteurization, cross-contamination after pasteurization, or breakage of cold chain during transportation.

In our study, *Salmonella* spp. was not found in the analysed egg powder samples. This result shows that heat treatment applied during egg powder production is sufficient and that cross-contamination did not occur after production. Unlike to results of our study, some researchers found *Salmonella* spp. in egg powder samples (Sidik et al 2015). Researchers reported that *Salmonella* could enter the egg from unwashed eggshells, and stay alive during production as a result of insufficient heat application, also *Salmonella* spp. could be detected as a result of cross-contamination (Jones et al 2012). Moreover there are also studies that washing eggshell may increase the entry of bacteria from eggshell due to damage to the cuticle layer and opening of pore plug (Wang and Slavik 1998, Samiullah et al 2013).

Antimicrobial agents are generally used to protect and treat bacterial infections in the poultry industry (Landoni and Albarellos 2015). In the present study, high resistance against amikacin, enrofloxacin, gentamicin, tobramycin, cephalexin, nitrofurantoin, and tetracycline could happen as a negative result stemming from the fact that these antibiotics are generally used by veterinaries for treatment. Similar to the results of our study, Tessema et al (2017) reported that 8 (72%) of the 11 isolates obtained from chicken eggs in Ethiopia were resistant against more than one antibiotic type, besides that the most common resistance was against tetracycline (72.7%) and ampicillin (72.7%). Gentamicin is an aminoglycoside antibiotic frequently used in veterinary medicine against gram-negative and some gram-positive bacteria. The main use of gentamicin in poultry is through SC injection, and it's applied daily sometimes for different broilers and layers of chickens. When gentamicin is applied in poultry via IM or SC, it leaves residue in egg yolk and albumen (Goetting et al 2011). In our study, all of the *Salmonella* spp. isolates (100%) were found to be resistant against gentamicin. Lower than the results of our study, Maka et al (2015) reported that 1.6% of their *Salmonella* spp. isolates were resistant to gentamicin.

One of the most widely used antibiotics in poultry production is tetracyclines. Tetracyclines are bacteriostatic antibiotics that inhibit the protein synthesis of bacteria (Landoni and Albarellos 2015). In this study, *Salmonella* spp. isolates were found to be highly (63.6%) resistant to tetracycline. As a result of using tetracycline in chickens, these agents could accumulate in the egg and so the residues of the antibiotics may appear in egg albumen faster than the yolk (Goetting et al 2011). Our results are in agree with Harsha et al (2001) who reported that 63.63% of *Salmonella* spp. isolates obtained from eggs were resistant to tetracycline. However, it is different from our study that Telli et al (2018) found 37.2% of *Salmonella* spp. isolates were resistant to tetracycline in chicken meat.

Antibiotic resistance is a major problem threaten public health concern worldwide (Ventola 2015). In this study, our results showed that 100% of the isolates were multi-drug resistant by showing resistance to three or more antibiotics. Yildirim et al (2011) reported that 97% of their *Salmonella* isolates were multi-drug resistant. The high resistance of our *Salmonella* spp. isolates to amikacin, enrofloxacin, gentamicin, tobramycin, cephalexin nitrofurantoin, tetracycline, ampicillin, piperacillin, and cefpodoxime may indicate that most of these antimicrobial agents are used unconsciously to support growth and are also used as a treatment in poultry. Moreover there are many complicated antibiotic resistance ways for the transfer of the resistance, and some of them still remain unknown (Sultan et al 2018). Some studies have proven that the resistance of the pathogens is present even after 20 years of restriction (Birkegård et al 2019).

In our country, with the "Regulation on the Control of *Salmonella* and Other Identified Foodborne Zoonotic Factors" in order to reduce the risks and incidence of *Salmonella* and other foodborne zoonotic agents to public health in poultry, procedures and principles that will take appropriate and effective measures for the detection and control of the food pathogens during production, processing and distribution stages, including feed, are being arranged. With this regulation, the use of antimicrobials in poultry is subjected to government control by the appropriate ministry. So to check the antimicrobial resistance of *Salmonella* in poultry, *Salmonella* isolates are being collected and monitored from selected different animal populations (Official Journal 2014).

Conclusion

As a conclusion of this study, *Salmonella* spp. detection is a significant public health problem. At the same time, the presence of *Salmonella* spp. indicates post-production cross-contamination of heat-treated foods, given the fact that our detected *Salmonella* spp. isolates show multiple resistance to most of the antimicrobial agents used in veterinary and clinical medicine, causing delays in treatment and loss of workforce. For this reason, the use of antibiotics in poultry farms should be under control with strict rules and inspections. Also, it is recommended to apply good production and hygiene practices, as well as pasteurization techniques, during the preparation of eggs and egg products.

Acknowledgement

This study was presented as a poster presentation at the V. International Congress on Natural and Health Sciences in Adana, Turkey (December 13-15, 2019) and was published as a summary text in the congress book. The study was part of a master thesis.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

Funding

This study was supported by the Scientific Research Projects Project Programs, Ondokuz Mayis University (Project Number: PYO.VET.1904.17.024).

References

Andino A, Hanning I, 2015. *Salmonella* enterica: survival, colonization, and virulence differences among serovars. Sci World J, 2015 (520179), 1-16.

Asgar A, Abbas M, 2012. Dried egg powder utilization, a new frontier in bakery products. Agr Biol J N Am, 3(12), 493-505.

Bichler LA, Kabambi V, Nagaraja DVM, Halvorson DA, 1996. *Salmonella* Enteritidis in eggs, cloacal swab specimens, and internal organs of experimentally infected white leghorn chickens. Am J Vet Res 57, 489-495.

Birkegård AC, Græsbøll K, Clasen J, Halasa T, et al., 2019. Continuing occurrence of vancomycin resistance determinants in Danish pig farms 20 years after removing exposure to avoparcin. Vet Microbiol, 232, 84-88.

Board RG, 2000. Eggs and egg products, In: The Microbiological Safety and Quality of Food, Eds; Lund BM, Baird-Parker TC, Gould GW, first edition, Aspen Publishers Inc., Maryland, USA, pp; 590-619.

Cardoso MJ, Nicolau AI, Borda D, Nielsen L, et al., 2021. *Salmonella* in eggs: From shopping to consumption-A review providing an evidence-based analysis of risk factors. Compr Rev Food Sci Food Saf, 20(3), 2716-2741.

CDC, 2021. Snapshots of *Salmonella* serotypes. Centers for Disease Control and Prevention. Accessible at: https://www.cdc.gov/salmonella/reportspubs/salmonella-atlas/serotype-snapshots.html. Accessed on July 10, 2021.

CLSI, 2021. Performance Standards for Antimicrobial Susceptibility Testing. M100-E31, 31st ed. Clinical and Laboratory Standards Institute, Wayne, PA.

De Knegt LV, Pires SM, Hald T, 2015. Attributing foodborne salmonellosis in humans to animal reservoirs in the European Union using a multi-country stochastic model. Epidemiol Infect, 143(6),1175-1186.

Diaz-Sanchez S, Moscoso S, Solís de los Santos F, Andino A, et al., 2015. Antibiotic use in poultry: A driving force for organic poultry production. Food Prot Trends, 35(6), 440–447.

Dogruer Y, Telli N, Telli AE, Kahraman HA, et al., 2015. Comparison of pasteurized liquid egg with shell eggs in terms of some quality characteristics. Eurasian J Vet Sci, 31(3), 177-183.

Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, et al., 2009. Mechanisms of egg contamination by *Salmonella* Enteritidis. FEMS Microbiol Rev, 33(4), 718-738.

Goetting V, Lee KA, Tell LA, 2011. Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: A review of the literature. J Vet Pharmacol Therap, 34(6), 521-556.

Guard-Petter J, Henzler DJ, Rahman MM, Carlson RW, 1997. On-farm monitoring of mouse-invasive *Salmonella* enterica serovar Enteritidis and a model for its association with the production of contaminated eggs. Appl Environ Microb, 63, 1588–1593.

Hara-Kudo Y, Takatori K, 2009. Microbial quality of liquid egg and *Salmonella* infection status in Japan. Food Hyg Soc Japan, 50(1), 34-40.

Harsha HT, Reshmi R, Varghese R, Divya PS, et al., 2011. Prevalence and antibiotic resistance of *Salmonella* from the eggs of commercial samples. J Infect Dis, 1(3), 93-100.

Hoogenboom LAP, Bokhorst JG, Northolt MD, van de Vijver LPL, et al., 2008. Contaminants and microorganisms in Dutch organic food products: A comparison with conventional products. Food Addit Contam, 25(10),1195-1207.

ISO, 2017. International Organization for Standardization 6579-1 Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Part 1. Detection of *Salmonella* spp.

Joel N, Udobi CE, Amaechi N, 2010. Effect of oven drying on the functional and nutritional properties of whole egg and its components. Afr J food Sci, 4(5), 254-257.

Jones DR, Anderson KE, Guard JY, 2012. Prevalence of coliforms, *Salmonella*, Listeria, and Campylobacter associated with eggs and the environment of conventional cage and free-range egg production. Poultry Sci, 91(5), 1195-1202.

Karadal F, Ertas Onmaz N, Hizlisoy H, Yildirim Y, et al., 2018. The microbiological quality of the village and market eggs sold at retail in Nigde and Kayseri. Erciyes Univ Vet Fak Derg, 15(1), 51-57.

Karim MR, Giasuddin M, Abdus Samad M, Showkot Mahmud M, et al., 2017. Prevalence of *Salmonella* spp. in poultry and poultry products in Dhaka, Bangladesh. Int J Anim Biol, 3(4), 18-22.

Konca Y, Buyukkilic S, Metin J, Adkinsonay AY, et al., 2010. Comparison of some animal product properties obtained from organic and conventional animals. Turkiye I. Organik Hayvancılık Kongresi, Kayseri, pp; 1-10.

Landoli MF, Albarellos G, 2015. The use of antimicrobial agents in broiler chickens. Vet J, 205,21–27.

Lee SK, Chon WJ, Song YK, Hyeon YJ, et al., 2013. Prevalence, characterization, and antimicrobial susceptibility of *Salmonella* Gallinarum isolated from eggs produced in conventional or organic farms in South Korea. Poult Sci ,92(10), 2789-2797.

Maka L, Mackiw E, Sciezynska H, Popowska M, 2015. Occurrence and antimicrobial resistance of *Salmonella* spp. isolated from food other than meat in Poland. Ann Agr Env Med, 22(3), 403-408.

Official Journal, 2014. Regulation on the control of *Salmonella* and other specified food-borne zoonotic agents. Official Journal, 27.03.2014, 28954.

Pasquali F, De Cesare A, Valero A, Olsen JE, et al., 2014. Improvement of sampling plans for *Salmonella* detection in pooled table eggs by use of real-time PCR. Int J Food Microbiol, 184, 31-34.

Pesavento G, Calonico C, Runfola M, Lo Nostro A, 2017. Free-range and organic farming: eggshell contamination by mesophilic bacteria and unusual pathogens. Poult Sci J, 26(4), 509-517.

Samiullah KK, Roberts JR, Sexton M, May D, Kiermeier A, 2013. Effects of egg shell quality and washing on *Salmonella* Infantis penetration. Int J Food Microbiol, 165(2),77-83.

Sidik KR., Lukman, DW, Wibawan IWT, 2015. *Salmonella* detection in egg powder ımported to Indonesia through port of tanjung priok, jakarta and its resistance against antibiotics. Appl J Hygiene, 4(1), 12-17.

Seel SK, Kabir SML, Islam MA, 2016. Molecular detection and characterization of *Salmonella* spp. isolated from fresh fishes sold in selected upazila markets of Bangladesh. J Vet Med, 14(2), 283-28.

Sultan I, Rahman S, Jan AT, Siddiqui MT, et al., 2018. Antibiotics, resistome and resistance rechanisms: A bacterial perspective. Front Microbiol, 21(9),1-16.

Telli AE, Biçer Y, Kahraman HA, Telli N , et al., 2018. Presence and antibiotic resistance of *Salmonella* spp. isolated from chicken meat and giblets consumed in Konya, Turkey. Eurasian J Vet Sci, 34(3) 164-170.

Tessema K, Bedu H, Ejo M, Hiko A, 2017. Prevalence and antibiotic resistance of *Salmonella* species isolated from chicken eggs by standard bacteriological method. J Vet Sci Technol, 8(1), 1-5.

TUIK, 2020. Turkish Statistical Institute. Animal statistics. Accessible at: https://turkstatweb.tuik.gov.tr. Accessed on July 10, 2021.

Ventola CL, 2015. The antibiotic resistance crisis: Part 1: Causes and threats. P T, 40(4), 277-283.

Wang H, Slavik MF, 1998. Bacterial penetration into eggs washed with various chemicals and stored at different temperatures and times. J Food Prot, 61, 276-279.

Widjojoatmodjo MN, Fluit AC, Torensma R, Keller BH, et al., 1991. Evaluation of the magnetic immuno-PCR assay for rapid detection of *Salmonella*. Eur J Clin Microbiol Infect Dis, 10(11), 935-938.

Yildirim Y, Gonulalan Z, Pamuk S, Ertas N, 2011. Incidence and antibiotic resistance of *Salmonella* spp. on raw chicken carcasses. Food Res Int, 44, 725-728.

Yumbir, 2018. Yumurta tavukçuluğu verileri, Yumbir. Accessible at: https://www.yum-bir.org/UserFiles/File/yumurta-veriler2019web.pdf. Accessed on July 10, 2021.

Author Contributions

Motivation / Concept: Goknur Terzi Gulel
Design: Goknur Terzi Gulel
Control/Supervision: Goknur Terzi Gulel
Data Collection and / or Processing: Hilal Keskinoglu, Goknur Terzi Gulel
Analysis and / or Interpretation: Hilal Keskinoglu, Goknur Terzi Gulel
Literature Review: Hilal Keskinoglu, Goknur Terzi Gulel
Writing the Article: Hilal Keskinoglu, Goknur Terzi Gulel
Critical Review: Goknur Terzi Gulel

Ethical Approval

An ethical statement was received from the author that th data, information and documents presented in this article were obtained within the framework of academic and ethical rules and that all information, documents, evaluations and results were presented in accordance with scientific ethics rules.

CITE THIS ARTICLE: *Keskinoğlu H, Terzi Gürel G, 2022. Determination of Salmonella spp. presence and antibiotic resistance in egg and egg products. Eurasian J Vet Sci, 38, 1, 24-31.*