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Research article

Salmonella spp. isolation from different types of egg samples and antimicrobial resistance of these strains in Argentina

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Abstract

We studied the serovars present and the performance of two selective plating media, Hektoen enteric agar (HE) and Xylose-Lysine-Desoxycholate (XLD) agar, as well as the time of tetrathionate broth incubation (one and five days) used for *Salmonella* isolation in different samples of egg and egg packs collected from supermarkets in Argentina. We also determined the occurrence of bacterial growth inhibitors in egg content and the antibiotic resistance profile of the isolated strains. *Salmonella* spp. was detected in 29 samples (1.8%) from 1,643 different types of egg and egg pack samples analyzed. Eight serovars of *Salmonella* were isolated: *S.* ser. Typhimurium (ST), *S.* ser. Enteritidis (SE), *S.* ser. Agona, *S.* ser. Westhampton, *S.* ser. Brandenburg, *S.* ser. Gallinarum (biovar Gallinarum), *S.* ser. Muenchen, and *S.* ser. Montevideo. The most frequent were ST and SE; both were present in eggshells, yolk, and albumen. The increase in the incubation time in tetrathionate broth affected the isolation of *Salmonella* spp. from eggshell and pool of yolk and albumen with different values of relative sensitivity and accuracy (p<0.05). Furthermore, there was a significant difference between the two-plating media at the time of tetrathionate broth incubation in eggshell for the relative sensitivity (p<0.05). The agreement between XLD and HE agar in relation to the time of tetrathionate broth incubation depended on the type of samples studied. The occurrence of bacterial growth inhibitors in yolk and albumen depended on bacteria assayed. However, these were presented in yolk and albumen from the same pool of egg content. There were 2 and 8 different patterns of resistance among *Salmonella* strains isolated, and 87% of these strains were multidrug-resistant. It is necessary to understand the epidemiology of *Salmonella* from eggs and to alert public health to minimize the prevalence of antibiotic resistance of *Salmonella* spp. in Argentina.

Keywords: Antibiotic, Bacterial growth inhibitor, Culture media, Eggs, *Salmonella*

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Introduction

Eggs and egg products are nutritious foods that form an important part of the human diet. However, consuming eggs has been associated with negative health impacts. Eggs and egg products that are improperly handled can be a source of foodborne diseases, such as salmonellosis. *Salmonella* spp. is a major food-borne bacterial pathogen, with poultry and poultry products being a primary source of

infection in humans (Wang et al., 2023). Poultry producers are faced with intensifying pressures from public health authorities, elected officials, and consumers regarding food safety issues about this bacterium (Baum and Kincheloe, 2022).

Although the relative contribution of food animal sources to human *Salmonella* infection varies between regions and countries, eggs are the major vehicle of these bacteria (Cardoso et al., 2021). *Salmonella* spp. can be transmitted in eggs through vertical or horizontal means. Vertical transmission occurs when both the inside and outside of an egg's shell get infected. Internal contamination can result from an infection in the reproductive organs that enters the eggshell or from direct contamination of the egg contents before laying. Before reaching the yolk, the bacteria inside the egg need to overcome antibiotic elements in the egg white and yolk membranes (Gantois et al., 2009; Khatun et al., 2022).

The principal *Salmonella* serovar associated with infections linked to eggs and egg products in the UK, most European countries, and North America is *Salmonella* ser. Enteritidis (SE). However, other serovars have also been implicated in several egg-associated outbreaks, most notably *Salmonella* ser. Typhimurium (ST) exhibits a range of phage types. In parts of the world where SE historically has not penetrated laying hen breeding flocks, egg-related salmonellosis is a problem associated specifically with non-SE serovars (Threlfall et al., 2014; Agbaje et al., 2021; Cardoso et al., 2021).

Salmonella spp. can be found in food using various methods, but failure in this process is not uncommon despite the availability of a wide range of testing materials. This bacterium may not be present after the enrichment step, even if the sample was contaminated. The main risk lies in the sampling process. If the *Salmonella* spp. dominates the microorganisms in the enrichment broth, it will be present in all tests. However, if *Salmonella* spp. is outnumbered by other bacteria, detection may be purely down to luck. The choice of plating media becomes crucial in this scenario. Using the right plating media can make it easier to detect sporadic colonies of *Salmonella* spp., depending on the type of competing bacteria. Unfortunately, we never know the composition of the microorganisms in advance, and as a result, we won't know the appropriate plating media either (Soria et al., 2012; Waltman and Gast, 2016; Gast and Porter, 2019).

The poultry business, on the other hand, uses antimicrobials to improve growth, feed efficiency, and lower bacterial illness. Antimicrobials are used in layer hens to treat and avoid bacterial infections. Antibiotic residues in animal-derived food pose a risk to human health because they can cause direct toxicity, such as cancer and allergic reactions. Additionally, low levels of antibiotic exposure can change the microflora and lead to the development of resistance, which can lead to the failure of antibiotic therapy in clinical settings. These residues include metabolites, conjugates, and residues that attach to macromolecules. They can also include substances produced from the parent drug (or both) (Ghimpețeanu et al., 2022; Abreu et al., 2023; Singer, 2023).

With 314 eggs per capita, Argentina stands in fourth place in the ranking of countries in 2022 (Ruiz, 2023). Outbreaks of foodborne diseases are associated with the consumption of raw eggs in this country, eaten in the form of homemade mayonnaise or undercooked hens' eggs. Although SE and ST are the most isolated serovars from eggs in Argentina (Eiguer et al., 1990; Caffer and Eiguer, 1994; Favier et al., 2013; Freije et al., 2019), there are no reports about the performance of different culture media on *Salmonella* spp. isolation in natural contamination from egg samples and the profiles of antibiotic sensitivity of different strains isolated from this matrix. So, it is considered necessary to carry out effective control of poultry products, as well as permanent surveillance of salmonellosis. Thus, the objectives of this study were to investigate the serovars present, evaluate the performance of two differential plating media, and determine the optimal incubation time for tetrathionate broth used in isolating *Salmonella* from egg and egg pack samples obtained from supermarkets in Argentina. Additionally, identify any substances in the eggs that inhibit bacterial growth and analyze the antibiotic resistance profile of the isolated strains.

Materials and methods

Sampling

Eggs were collected from 113 supermarkets situated in 14 cities (11 counties) of Entre Rios, Argentina. Sixty eggs were taken from each

supermarket. Those eggs were taken from a pack of six, but when the supermarket did not have this presentation, eggs were taken from a 30-egg fiber carton. After purchasing at the supermarkets, eggs were transported at room temperature to the INTA Poultry Health Laboratory (Concepcion del Uruguay, Entre Rios).

Egg packaging, eggshell, and egg content samples

Five thousand four hundred twenty-four eggs were analyzed: 3,475 were white eggshells (64%), and 1,949 eggs were brown eggshells (36%). From 60 eggs from each supermarket, eight samples of 6 intact eggs (a total of 48) were selected for this study; the rest were discarded. Analyzed eggs were packed in different packs: 6-egg fiber cartons (60.6%), 6-egg polystyrene bags (17.7%), six eggs wrapped in newspaper (10.2%), 30-egg fiber cartons (6.2%, in samples of 6 eggs) and 6-egg polystyrene foam cartons (5.3%). Four samples of 6 eggs were used to obtain four pools of egg packing (EP) and four pools of eggshell (ES) samples, and the other four samples of 6 eggs were used to obtain pools of 6 egg contents per supermarket. When the egg pack was a 30-egg fiber carton, four samples of 25 g were taken from each pack. The egg content was collected after sterilizing the egg surface by immersion in 70% ethyl alcohol for 10 min and then in boiling water for 5 sec (Gast, 1993; Himathongkham et al., 1999). The eggs were aseptically broken, and the contents from six eggs were pooled [mixture of yolk and albumen (YA)] or separated into pools of 6 yolks or albumen. The pools were stomached (Stomacher 400 circulator, Seward, UK) for 2 min at 230 rpm at room temperature (25 ± 2 °C).

Salmonella spp. isolation from egg contents, eggshells, and egg packaging

Different pools of EP (452), ES (452), YA (451), yolk (144), and albumen (144) were analyzed for the presence of *Salmonella* spp. based on the Bacteriological Analytical Manual from the U.S. Food and Drugs Administration (Andrews et al., 2023). Twenty-five grams of ES, EP, or 25 ml of egg content (pool of yolk, albumen, or YA) samples were pre-enriched in 225 mL of tryptic soy broth (TSB; Merck, Darmstadt, Germany) with ferrous sulfate (TSBF, 35 mg of ferrous sulfate added to 1,000 mL of TSB). The mixture was incubated at 35 ± 2 °C for 18-24 h. One milliliter of incubated broth was transferred to 10 mL of tetrathionate broth base (Acumedia-Neogen, Lansing, MI, USA) in addition to 20 mL/L of iodine potassium iodide solution (6 g of iodine; 5 g of potassium iodide; 20 mL of demineralized water), brilliant green 0.1% (Sigma, Steinheim, Germany), and 40 mg/mL of novobiocin (Sigma), and incubated at 35 ± 2 °C for five days. At days 1 (TT1) and 5 (TT5), a loopful of selective enrichment broth was streaked onto xylose lysine desoxycholate agar (XLD, Oxoid Ltd., Basingstoke, UK) and Hektoen enteric agar (HE, Acumedia-Neogen, Lansing, MI, USA) and incubated at 35 ± 2 °C for 18-24 h. Two colonies of presumptive *Salmonella* were biochemically confirmed using triple-sugar iron agar (Acumedia-Neogen, Lansing, MI, USA), lysine iron agar (Merck, Darmstadt, Germany), Simmons citrate (Merck, Darmstadt, Germany), sulfide indole motility medium (Merck, Darmstadt, Germany), phenylalanine agar (Hi-Media, Bombay, India) and ortho-nitrophenyl-β-galactoside (ONPG) test (Britania, Buenos Aires, Argentina). All *Salmonella* spp. isolations were preserved on nutritive (Acumedia-Neogen) slants agar until serotyping confirmation. The serotyping was carried out according to the White-Kauffmann-Le Minor scheme, with somatic (AgO) and flagellar (AgH) antigens (Grimont and Weill, 2007).

Bacterial growth inhibitors in albumen and yolk samples

The presence of bacterial growth inhibitors was assayed on Mueller-Hinton agar (Britania, Argentina) plates. Bacteria used in this assay were ST 06/11, SE PT 1, and *Salmonella* ser. Gallinarum biovar Gallinarum (SG) 03/121 and biovar Pullorum (SP) 90/142. The strains belong to the collections from the Laboratory of Bacteriology of the EEA INTA Balcarce (Buenos Aires, Argentina). On the other hand, *Bacillus subtillis* ATCC 6633, belonging to the American Type Culture Collection (ATCC), was used as positive control testing because this bacterium is used to study antimicrobial residues in different tissues (United States Department of Agriculture, 2011).

Each strain was grown in TSB (Acumedia-Neogen) for 16-18 h at 35 ± 2 °C, equivalent to 1×10^8 cfu/mL. The plates of Mueller-Hinton agar were thoroughly swabbed uniformly with a sterile cotton swab, which was dipped before into the suspension of the test organisms, one quadrant

at a time, to achieve a confluent growth. Inoculated plates were allowed to dry for 15 min. Afterwards, holes of about 10 mm diameter were punched out in agar plate. The holes were filled with 200 µL of egg content (albumen or yolk) and incubated at 35 ± 2 °C for 18-24 h. The inhibition zones were determined for which the growth of a test microorganism was inhibited with a zone of at least 1 mm. Data on the presence of bacterial growth inhibitors was compared with *Salmonella* spp. isolation results.

Antibiotic susceptibility test

The antibiotic susceptibility test was performed by the standard disk diffusion method (CLSI, 2022) in Mueller-Hinton agar (Difco). *Salmonella* strains were screened for resistance to antibiotics: amikacin (30 µg), ampicillin (10 µg), amoxicillin/clavulanic acid (30 µg), cephalothin (30 µg), cefixime (5 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5µg), doxycycline (30 µg), enrofloxacin (5 µg), florfenicol (30 µg), fosfomycin (50µg), gentamycin (10 µg), imipenem (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), neomycin (30 µg), norfloxacin (10 µg), streptomycin (300 µg), tigecycline (15µg),trimethoprim/sulfamethoxazole (25 µg), and tetracycline (30µg). All the antimicrobial disks were purchased from Oxoid. *Escherichia coli* ATCC 25922 was used as control. The susceptibility of the isolates to the antibiotics was determined by measuring the zone of inhibition. They were interpreted as susceptible, intermediate, or resistant, as previously described (ANLIS and Malbrán, 2021), and for tigecycline and the rest of the antibiotics (except for neomycin and erythromycin), as previously described (CLSI, 2014, 2020, 2022). *Staphylococcus aureus* breakpoints were adopted from (CLSI, 2022) and (Zurita et al., 1989) for erythromycin and neomycin, respectively. *Pseudomonas* spp. breakpoints were adopted from CLSI (CLSI, 2015) for colistin, as no Enterobacterales criteria for disc diffusion antibiotic-resistance assay has been defined either by CLSI or the European Committee on Antimicrobial Susceptibility Testing (EUCAST), as it was proposed by Bueno et al. (Bueno et al., 2024).

An isolate was classified as multidrug-resistant (MDR) if it resulted in non-sensitive (intermediate + resistant) to at least one agent in three or more antimicrobial categories. Extensively drug-resistant (XDR) bacteria were defined as non-susceptibility to at least one agent in all but one or two fewer antimicrobial categories. Only acquired antimicrobial resistance was taken into consideration in creating definitions for MDR and XDR; intrinsic (natural) resistance was not addressed (Magiorakos et al., 2012).

Analysis of performance criteria and statistical analysis

Relative sensitivity (RSe) and accuracy (RAc), and agreement (Kappa coefficient) of differential-selective agars, used for *Salmonella* spp. isolation in egg and egg pack samples were analyzed as previously described (Soria et al., 2011). Relative true positive was defined when a sample was positive to *Salmonella* spp. in at least one differential-selective agar. Relative true negative was defined as samples where *Salmonella* spp. was not detected in any differential-selective agar. The agreement of *Salmonella* isolation results on TT1 and TT5 was calculated using the Kappa coefficient. On the other hand, the same parameter was used to study the agreement of bacterial growth inhibitors between albumen and yolk for each *Salmonella* strain. Kappa coefficients were summarized, according to Dawson and Trap (Dawson and Trap, 2004), as an excellent agreement (0.93 to 1.00), a very good agreement (0.81 to 0.92), a good agreement (0.61 to 0.80), a fair agreement (0.41 to 0.60), a slight agreement (0.21 to 0.40), a poor agreement (0.01 to 0.20), and no agreement (p<0.01). Z test was used to test the statistical significance of kappa coefficients.

Results

***Salmonella* spp. contamination in egg and egg packs, and distribution of *Salmonella* serovars**

Table 1 shows *Salmonella* spp. isolation in different samples from egg and egg packs collected from supermarkets in Entre Rios, Argentina, according to cities and type of sample eggs for consumption. Out of 1,643 samples analyzed in the supermarkets, 29 samples were positive for *Salmonella* spp., belonging to 16 supermarkets from 7 cities (4, 4, and 3 cities for EP, ES, and egg content, respectively).

Table 1: *Salmonella* spp. isolation in different samples from egg and egg packs collected from supermarkets in Entre Rios, Argentina, according to cities and type of samples.

Counties of Entre Rios	Cities	N° positive samples/ N ° of samples analyzed					
		Total samples	Egg packs	Eggshell	Yolk-Albumen	Albumen	Yolk
Colon	Colon	1/64	1/20	0/20	0/20	0/2	0/2
	San Jose	0/64	0/20	0/20	0/20	0/2	0/2
	Villa Elisa	0/176	0/48	0/48	0/48	0/16	0/16
Concordia	Concordia	2/204	0/68	2/68	0/68	-	-
Federación	Federación	0/96	0/24	0/24	0/24	0/12	0/12
Federal	Federal	0/80	0/20	0/20	0/20	0/10	0/10
Gualeguay	Gualeguay	0/144	0/36	0/36	0/36	0/18	0/18
Gualeguaychu	Gualeguaychu	2/192	1/48	0/48	1/48	0/24	0/24
Islas del Ibicuy	Villa Paranacito	0/48	0/12	0/12	0/12	0/6	0/6
San Salvador	San Salvador	18/112	0/28	1/28	4/28	6/14	7/14
Tala	Rosario del Tala	2/48	1/12	1/12	0/12	0/6	0/6
Uruguay	Basavilbaso	1/80	0/20	1/20	0/20	0/10	0/10
	Concepcion del Uruguay	3/191	2/60	0/60	1/59	0/6	0/6
Villaguay	Villaguay	0/144	0/36	0/36	0/36	0/18	0/18
Total		29/1,643	5/452	5/452	6/451	6/144	7/144

Five samples of EP and ES were positive for this bacterium. In reference to the pool of albumen, yolk, and YA samples, 6, 7, and 6 samples were positive for *Salmonella* spp., respectively. From them, forth YA samples and all albumen and yolk. Sixty-seven *Salmonella* strains were isolated from 29 samples and were typified into eight serovars (Table 2); the most frequent were ST (66.5%; 1.2% of samples) and SE (14%; 0.2% of samples). The first was isolated mainly in egg contents, followed by ES. This serovar was isolated from 4 pools of albumen and four pools of yolk belonging to the same egg pools. However, only one sample was positive for ST in YA and their corresponding albumen sample. *S.* ser Enteritidis was present in EP, ES and YA. On the other hand, *S.* ser. Westhampton and *S.* ser. Agona were isolated in the same sample of EP but from TT1 and TT5, respectively.

Table 2: Distribution of *Salmonella* serovars observed in isolates obtained from egg and egg packs from supermarkets in Entre Ríos, Argentina.

***Salmonella* serovars**	**Number of positive samples (%)**	**Number of positive samples for *Salmonella* contamination per sample type**				
		Egg pack	Eggshell	Yolk-albumen	Yolk	Albumen
Agona	2 (0.12)	2	0	0	0	0
Brandenburg	1 (0.06)	0	1	0	0	0
Enteritidis	4 (0.24)	2	1	1	0	0
Gallinarum (biovar Gallinarum)	1 (0.06)	0	0	1	0	0
Muenchen	1 (0.06)	1	0	0	0	0
Montevideo	1 (0.06)	0	1	0	0	0
Typhimurium	19 (1.16)	0	2	4	7	6
Westhampton	1 (0.12)	1	0	0	0	0

samples belonged to one county of Entre Rios.

Respect to *Salmonella* spp. isolation at different times of incubation in tetrathionate broth (TT1 and TT5), two more samples were positive in TT1 (Table 3). Twenty-eight (41.8%) and 39 (58.2%) strains were isolated from TT1 and TT5, respectively (Data not shown). The increase in the incubation time in TT broth did not have any effect on the isolation of this bacteria for yolk or albumen pools. The agreement between TT1 and TT5 was good for EP and YA, respectively. However, there was no agreement for ES samples.

Analysis of performance criteria for selective-differential plating media

The RSe and RAc for selective-differential plating media (HE and XLD agar) used in eggs and egg packaging for *Salmonella* isolation at TT1 and TT5 are shown in Table 4. The RSe and RAc did not show any significant differences (p>0.05) in relation to selective plating media and time of tetrathionate incubation in EP and YA. In reference to ES, RSe showed a significant

difference between the two-plating media at TT1 and TT5. The RSe of XLD agar was reduced from 0.60 in TT1 to 0 in TT5, while this parameter increased in HE agar at TT5. The RAc did not show any significant differences (p>0.05) in relation to selective plating media and time of tetrathionate incubation. On the other hand, the RSe increased on TT5 with respect to TT1 for HE agar in albumen samples (p<0.05), while RSe was 1 in both times of incubation in XLD agar. The last medium had greater RSe and RAc than HE agar (p<0.05) at TT1. However, there was no statistical difference in TT5. On the other hand, HE agar increased the RSe and RAc at TT5 in yolk samples. However, XLD agar increased RSe at TT5, but the RAc did not change with the time incubation of tetrathionate broth.

Table 3: Agreement (kappa coefficient) and *Salmonella* spp. isolation from egg and egg packs at two incubation times in tetrathionate broth (TT1 and TT5) from supermarkets of Entre Ríos, Argentina[1].

Type of sample	*Salmonella* spp. positive samples spp. at two incubation times in tetrathionate broth		Kappa coefficient (Standard error; confidence interval)
	TT1	TT5	
Egg pack	4	4	0.74 (0.399-1.000)
Eggshell	3	2	0.00
Yolk-albumen	5	4	0.66 (0.2836-1.000)
Albumen	6	6	1.00 (1.000-1.000)
Yolk	7	7	1.00 (1.000-1.000)
Total	25	23	

1. The tetrathionate broth was separated considering the different times of streaking in the selective plating media at d 1 (TT1) or 5 (TT5) of incubation of this broth.

Table 4: Relative sensitivity (RSe), accuracy (RAc), and Kappa coefficient for selective-differential plating media (HE -Hektoen enteric agar-, XLD-Xylose lysine desoxycholate agar-) used in eggs and egg packs collected from supermarkets of Entre Ríos, Argentina, for *Salmonella* isolation at two incubation times in tetrathionate broth (TT1 and TT5)[1]. Values in parentheses indicate a 95% confidence interval for the respective parameter.

Type of sample	Media	RSe*		RAc*		Kappa coefficient (Standard error; confidence interval)	
		TT1	TT5	TT1	TT5	TT1	TT5
Egg pack	HE	$0.80^{a,X}$ (0.354-0.956)	$0.60^{a,X}$ (0.223-0.881)	$0.99^{a,X}$ (0.988-0.999)	$0.99^{a,X}$ (0.984-0.998)	0.66 (0.200-1.000)	0.66 (0.201-1.000)
	XLD	$0.60^{a,X}$ (0.223-0.881)	$0.40^{a,X}$ (0.118-0.777)	$0.99^{a,X}$ (0.984-0.998)	$0.99^{a,X}$ (0.980-0.997)		
Eggshell	HE	$0.0^{a,X}$ (0.004-0.459)	$0.40^{b,X}$ (0.118-0.777)	$0.98^{a,X}$ (0.974-0.995)	$0.99^{a,X}$ (0.980-0.997)	0.00	0.00
	XLD	$0.60^{a,Y}$ (0.223-0.881)	$0.0^{b,Y}$ (0.004-0.459)	$0.99^{a,X}$ (0.980-0.997)	$0.98^{a,X}$ (0.974-0.995)		
Pool yolk-albumen	HE	$0.43^{a,X}$ (0.157-0.755)	$0.43^{a,X}$ (0.157-0.755)	$0.99^{a,X}$ (0.977-0.996)	$0.99^{a,X}$ (0.977-0.996)	0.33 (0.000-0.987)	0.86 (0.574-1.00)
	XLD	$0.57^{a,X}$ (0.245-0.843)	$0.57^{a,X}$ (0.245-0.843)	$0.99^{a,X}$ (0.980-0.997)	$0.99^{a,X}$ (0.980-0.997)		
Pool albumen	HE	$0.0^{a,X}$ (0.004-0.410)	$0.66^{b,X}$ (0.290-0.901)	$0.96^{a,X}$ (0.912-0.980)	$0.98^{a,X}$ (0.951-0.996)	0.00	0.79 (0.508-1.000)
	XLD	$1.0^{a,Y}$ (0.590-0.996)	$1.0^{a,X}$ (0.590-0.996)	$1.0^{a,Y}$ (0.975-0.999)	$1.0^{a,X}$ (0.975-0.999)		
Pool yolk	HE	0.29^{aX} (0.085-0.650)	$1.0^{b,X}$ (0.630-0.996)	$0.96^{a,X}$ (0.921-0.985)	$1.0^{b,X}$ (0.975-0.999)	0.00	1.00 (1.000-1.000)
	XLD	$0.71^{a,Y}$ (0.349-0.915)	$0.81^{a,X}$ (0.473-0.968)	$0.98^{a,X}$ (0.951-0.996)	$0.99^{a,X}$ (0.962-0.998)		

^{a,b}Values followed by different lowercase letters in the same row for each parameter are significantly different (p<0.05).
$^{Y-X}$Values followed by different uppercase letters in the same column for each sample type are significantly different (p<0.05).
[1] The tetrathionate broth was separated considering the different times of streaking in the selective plating media at d 1 (TT1) or 5 (TT5) of incubation of this broth.

With respect to the agreement between HE and XLD agar in relation to the time of tetrathionate broth incubation (TT1 and TT5), a good agreement was observed in EP samples. However, there was no agreement at both times of incubation of this broth in the ES. Although we observed no agreement to albumen or yolk samples on TT1, this parameter was good and excellent for albumen or yolk in TT5, respectively. On the other hand, the agreement between HE and XLD agar was slight and very good on TT1 and TT5 in YA, respectively (Table 4).

Antimicrobial resistance profile

The susceptibility patterns of 67 *Salmonella* strains, isolated from egg and egg packs from the supermarket of Entre Rios, Argentina, which belonged to ST (52 strains), SE (9 strains), *S.* ser. Agona (2 strains), SG (1 strain), *S.* ser. Muenchen (1 strain), *S.* ser. Brandenburg (1 strain), and *S.* ser. Westhampton (1 strain) indicated that none of *Salmonella* strains were resistant against amoxicillin/clavulanic acid. The antimicrobial susceptibility patterns of the ST and SE strains isolated are detailed in Table 5. Most ST strains were resistant to streptomycin, while SE strains showed resistance to cephalothin. There was a rate of intermediate strains higher than 50% for 6 and 3 antibiotics in ST and SE strains, respectively.

Table 5: Antimicrobial susceptibility patterns of *Salmonella* ser. Typhimurium and *Salmonella* ser. Enteritidis strains isolated from egg and egg packs from the supermarket of Entre Rios, Argentina.

	Percentages of strains Susceptible (S), Intermediate (I), and Resistant (R)					
	***Salmonella* ser. Typhimurium (N= 52)**			***Salmonella* ser. Enteritidis (N= 9)**		
	S	**I**	**R**	**S**	**I**	**R**
Ampicillin	46.2	40.4	13.4	77.8	11.1	11.1
Amoxicillin/clavulanic acid	100.0	0.0	0.0	100.0	0.0	0.0
Nalidixic acid	92.3	1.9	5.8	88.9	11.1	0.0
Ciprofloxacin	90.4	7.7	1.9	100.0	0.0	0.0
Enrofloxacin	88.5	11.5	0.0	77.8	22.2	0.0
Norfloxacin	98.1	0.0	1.9	100.0	0.0	0.0
Doxycycline	13.5	86.5	0.0	77.8	22.2	0.0
Tetracycline	5.8	51.9	42.3	100.0	0.0	0.0
Chloramphenicol	94.2	0.0	5.8	88.9	0.0	11.1
Florfenicol	44.2	48.1	7.7	66.7	22.2	11.1
Amikacin	80.8	15.4	3.8	33.6	55.6	11.1
Streptomycin	0.0	1.9	98.1	100.0	0.0	0.0
Gentamycin	96.2	3.8	0.0	100.0	0.0	0.0
Kanamycin	36.5	55.8	7.7	66.7	22.2	11.1
Neomycin	3.8	78.8	17.4	11.1	66.7	22.2
Cephalothin	0.0	21.2	78.8	0.0	11.1	88.9
Cefixime	75.0	9.6	15.4	77.8	0.0	22.2
Cefotaxime	21.2	55.7	23.1	22.3	33.3	44.4
Cefoxitin	88.5	7.7	3.8	100.0	0.0	0.0
Ceftazidime	63.5	28.8	7.7	77.8	22.2	0.0
Imipenem	50.0	48.1	1.9	33.3	66.7	0.0
Trimethoprim/sulfamethoxazole	78.8	19.3	1.9	100.0	0.0	0.0
Tigecycline	38.5	61.5	0.0	0.0	100.0	0.0
Colistin	51.9	46.2	1.9	100.0	0.0	0.0
Fosfomycin	100.0	0.0	0.0	88.9	0.0	11.1

On the other hand, one *S.* ser. Agona isolate was resistant to cephalothin, cefotaxime, kanamycin, amikacin, neomycin, and doxycycline. *S.* ser. Muenchen strain was resistant to cephalothin, cefexime, cefotaxime, kanamycin, amikacin, and neomycin, while *S.* ser. Westhampton strain was resistant to cephalothin, cefotaxime, streptomycin, kanamycin, amikacin, neomycin,

and nalidixic acid. *S.* ser. Brandenburg strain was only resistant to florfenicol, and the SG strain presented intermediate susceptibility to cephalothin, cefotaxime, and ciprofloxacin.

The 87% of tested strains (58/67) were MDR, but none of them were XDR. The resistance patterns encountered among *Salmonella* serotypes from eggs indicated between 2 and 8 different patterns of resistance in SE and ST. Regarding ST, the most frequent patterns of resistance were streptomycin-cephalothin (9 strains/47 total strains). On the other hand, *S.* ser. Agona, *S.* ser. Westhampton, and *S.* ser. Muenchen strains showed patterns of resistance between 5 and 7 antibiotics (data not shown).

Bacterial growth inhibitors in albumen and yolk from eggs collected from supermarkets

The incidence of bacterial growth inhibitors in albumen or yolk pools is shown in Table 6. This value depended on the bacteria assayed. *Bacillus subtilis* ATCC 6633 was 100% inhibited for albumen pool samples, while no yolk pool samples inhibited the growth of this bacteria. The SP strain was not inhibited by albumen or yolk pool samples, so the agreement was not calculated for this strain. However, the growth inhibition substances in the SG strain were present in double the number of albumen (44) than in yolk (22) pool samples. On the other hand, ST strain growth was inhibited by 4 and 2 albumen and yolk pool samples, respectively, while the SE strain was only inhibited for albumen pool samples (1.8%). The agreement of bacterial growth inhibitors between albumen and yolk pool samples was fair (0.5484; IC 95%: 0.3975-0.6993), good (0.6585; IC 95%: 0.1895-1.000), and no agreement to SG, ST, and SE strains, respectively. Bacterial growth inhibitors detected in yolk pool samples were also detected in a pool of albumen from the same egg pool sample. On the other hand, one albumen pool sample was positive for ST isolation. However, it only showed SG strain growth inhibition. The rest of the yolk or albumen pool samples, which showed *Salmonella* growth inhibition, were negative to *Salmonella* spp. isolation.

Table 6: Incidence of bacterial growth inhibitors in albumen and yolk samples from eggs collected from supermarkets of Entre Ríos, Argentina.

Strains	N° positive samples/N° total of samples	
	Albumen	Yolk
Salmonella ser. Typhimurium INTA 06/11	4/112	2/112
Salmonella ser. Gallinarum biovar Gallinarum INTA 03/121	44/112	22/112
Salmonella ser. Gallinarum biovar Pullorum INTA 90/142	0/112	0/112
Salmonella ser. Enteritidis INTA PT1	2/112	0/112
Bacillus subtilis ATCC 6633	112/112	0/112

Discussion

In the present study, *Salmonella* contamination was studied in different samples of eggs and EP collected from the supermarket of Entre Rios, Argentina. There is no previously published data for *Salmonella* spp. isolation in these kinds of samples in this state. In this study, 35 mg/L of ferrous sulfate in the pre-enrichment step was used, and different serotypes of *Salmonella* from egg contents were isolated. Using the same culture method, Soria et al. (2012) reported that the detection limit of different *Salmonella* strains in egg content was 5–54 cfu/25 mL. It is known that controlling the iron content in poultry diets is an effective way to prevent large-scale growth of *Salmonella* spp. in the intestine (Tan et al., 2021). Different studies have shown that iron bioavailability determines the growth rates of *Salmonella* cells in liquid eggs (Gast and Holt, 1995; Guillén and Cebrián, 2022). Furthermore, Cogan et al. (2001) reported that iron in the form of ferrous sulfate promotes the growth of Gram-negative bacteria in eggs, and supplementation at levels of 35 mg/L in nonselective broth effectively promotes *Salmonella* isolation in raw eggs.

Gantois et al. (2008) demonstrated that SE and ST strains colonize the reproductive organs better than *Salmonella* belonging to the other serotypes and could survive in the egg albumen during egg formation. Although different authors

reported that SE and/or ST were the only serovars isolated from egg content (Begum et al., 2010; Favier et al., 2013), others reported the isolation of other serovars (Akhtar et al., 2010; Betancor et al., 2010). Although ST was the most prevalent serovar, three serotypes were isolated in egg content from our study: ST, SE, and SG. This finding contrasts with Martelli and Davies, who reviewed the information available on egg contamination and reported that ST was not often isolated from table eggs and that contamination of table eggs with SE and other serovars was more frequent (Martelli and Davies, 2012). Furthermore, the presence of SG in our study is in contrast to De Oliveira et al. results, who demonstrated that eggs laid by contaminated hens with SG might not be contaminated with this biovar and the dissemination of the disease is strongly related to the presence of dead birds in the cage (De Oliveira et al., 2005).

In reference to EP and ES, different serovars were isolated in our study. Furthermore, *S.* ser. Muenchen and *S.* ser. Westhampton were isolated from the same sample of EP, according to the day of selective enrichment (TT1 and TT5). Although De Franceschi and colleagues did not find *Salmonella* spp. in ES from 7,760 eggs collected in farms located in the province of Buenos Aires, Argentina (De Franceschi et al. 1999), other authors reported SE, ST, *S.* ser. Infantis, *S.* ser. Montevideo, *S.* ser. Derby, *S.* ser. Livingstone and/or *S.* ser. Cerro in ES (Murchie et al., 2007; Sasaki et al., 2011; Sodagari et al., 2019) and SE in egg pack (Viora et al., 1993). So, *Salmonella* spp. on the surface of eggs and packaging can contaminate hands and protective clothing and can be spread onto other food (Oldham Council, 2008).

It was reported that a longer incubation time than 24 h was more important (more positive results after 48 h) for selective enrichment medium in chicken meat, poultry, and poultry environmental samples (Waltman et al., 1991; Kuijpers AFA et al., 2008) and it can be used to define false-negative probabilities for cultural gold standard methods for *Salmonella* detection (Sullivan et al., 2020). In our study, we found a statistical difference for *Salmonella* spp. isolation between TT1 and TT5 in one plating media for yolk or albumen pool samples and in the two-plating media for ES samples. Despite, Soria and coworkers (Soria et al., 2012) did not find any statistical difference in YA pool samples, different reports showed that the combination of the two media clearly would decrease the number of false negative results, although with a little extra cost (Petersen, 1997; Waltman and Gast, 2016). This was observed especially for ES samples, where there was no agreement between HE and XLD agar at TT1 and TT5.

Multiple antibiotic resistances have been reported in *Salmonella* isolates from various authors (Yang et al., 2002; Suresh et al., 2006; Graziani et al., 2008; Nair et al., 2018; Hai et al., 2020). Although antibiotic resistance determinants have been circulating within the microbial genome for millennia, largely predating the manufacture and use of antibiotics by human beings (Karam et al., 2016; Nair et al., 2018). In our study, the high number of MDR strains (87%) was due to the high rate of intermediate strains for different antibiotics. On the other hand, 88% of SE, noticed in hatching eggs, litter, feed, drinkers, bird rinse, and ceca, were reported to be resistant to multiple drugs, including ampicillin, nalidixic acid, and tetracycline (Nair et al., 2018). The resistance patterns encountered in the present work among *Salmonella* serotypes were 2 and 8 to SE and ST, respectively. Other authors reported five different drug resistance patterns in SE (Graziani et al., 2008; Abou Elez et al., 2021). In reference to elevated resistance patterns shown in ST in the present study, Wang et al. (Wang et al., 2017) reported that the most frequently observed (63%) antibiotic resistance patterns in ST were 4 to 8 different patterns. So, *Salmonella* strains isolated in the study might have originated from environments where antimicrobials are often used (Suresh et al., 2006; Nair et al., 2018; Castro-Vargas et al., 2020).

On the other hand, Akoachere et al. (2009), Esaki et al. (2004), and Castro-Vargas et al. (2020) reported low or intermediate resistance to quinolones groups in *Salmonella* serotypes isolated from food-animals and poultry samples, which was similar to the results obtained in the present study. In contrast, Shang et al. (2018) reported a 97.6% resistance to nalidixic acid. In reference to the tetracycline group, the results of the present study were similar to other reports (Hoszowski and Wasyl, 2002; Miko et al., 2005). On the other hand, the high resistance to streptomycin in ST was also found by other authors (Hoszowski and Wasyl, 2002; Graziani et

al., 2008; Wang et al., 2017).

In the present work, the incidence of bacterial growth inhibitions was serovars and biovars dependent, and the results on SE agreed with De Franceschi et al. (1999), who noted that albumen inhibited SE growth like bactericidal and bacteriostatic until the 7th day and until the 15th day post-lay, respectively, while yolk samples were negative for this effect. It is known that eggs possess physical and biological defense systems to protect the embryo against the invasion and multiplication of microorganisms. Albumen and yolk also contain several proteins with demonstrated antimicrobial activities (Kovacs-Nolan et al., 2005; Jabalera et al., 2022). Furthermore, egg whites produced by old hens are less prone to contamination by *Salmonella* spp., and the relative concentration of egg white antimicrobial proteins increases with hen age (Jabalera et al., 2022). Although *Salmonella* penetration experiments demonstrated that non-contaminated eggs display significantly higher concentrations of antimicrobial proteins (Jabalera et al., 2022) and the chemical nature of the inhibitors has not been identified in our assays, the different incidences in the yolk or albumen permits speculated that they are mainly from antibiotics. The distribution of them into eggs is different. The amount of drug excreted into the egg yolk mainly depends on the lipid solubility of the drug. Tetracycline and chlortetracycline are excreted preferentially into the yolk (Roudaut et al., 1989; Kan and Petz, 2000), and residues of macrolides, quinolones and nitrofurans are predominantly present in albumen (Kan and Petz, 2000; Alm El Dein and Elbearon, 2010). On the other hand, many yolk or albumen pool samples, which showed *Salmonella* growth inhibitors of different strains, were negative for *Salmonella* spp. isolation in our study. The inhibitors can be diluted to insignificance through the pre-enrichment step used for *Salmonella* spp. isolation (Wang and Hammack, 2014). However, the possibility that they could reduce the viable count and the rate of isolation of this bacterium cannot be excluded.

Conclusions

The present study shows that the most prevalent serovars, ST and SE, represent a potential health problem. It emphasizes the need to continue the consumer's education on proper food handling and cooking practices to decrease the risk of transmission of *Salmonella* spp. from eggs. The effect of increasing the incubation time in tetrathionate broth and the use of different selective plating media on *Salmonella* isolation depends on the types of egg samples. The occurrence of bacterial growth inhibitors is higher in albumen than in yolk pool samples, and their effects on the reduction of the rate of *Salmonella* isolation cannot be excluded. The different multidrug resistant patterns of *Salmonella* isolated strains are extremely important local microbiologic data to make a better choice of the antibiotic to be used. So, the knowledge of the rate of isolation and characteristics of the isolated strains can contribute to the understanding of the epidemiology of this pathogen and alert public health organisms about risks to human health to minimize the prevalence and antibiotic resistance of *Salmonella* species in Argentina.

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References

Abou Elez, R.M.M., Elsohaby, I., El-Gazzar, N., Tolba, H.M.N., Abdelfatah, E.N., Abdellatif, S.S. et al., 2021. Antimicrobial resistance of *Salmonella enteritidis* and *Salmonella typhimurium* isolated from laying hens, table eggs, and humans with respect to antimicrobial activity of biosynthesized silver nanoparticles. Animals 11, 3554. https://doi.org/10.3390/ani11123554

Abreu, R., Semedo-Lemsaddek, T., Cunha, E., Tavares, L., Oliveira, M., 2023. Antimicrobial drug resistance in poultry production: Current status and innovative strategies for bacterial control. Microorganisms 11, 953. https://doi.org/10.3390/microorganisms11040953

Agbaje, M., Ayo-Ajayi, P., Kehinde, O., Omoshaba, E., Dipeolu, M., Fasina, F.O., 2021. *Salmonella* characterization in poultry eggs sold in farms and markets in relation to handling and biosecurity practices in Ogun state, Nigeria. Antibiotics 10, 773.

https://doi.org/10.3390/antibiotics10070773

Akhtar, F., Hussain, I., Khan, A., Rahman, S.U., 2010. Prevalence and antibiogram studies of *Salmonella* Enteritidis isolated from human and poultry sources. Pakistan Veterinary Journal 30, 25–28.

Akoachere, J.F.T., Tanih, N.F., Ndip, L.M., Ndip, R.N., 2009. Phenotypic characterization of *Salmonella* Typhimurium isolates from food-animals and abattoir drains in Buea, Cameroon. Journal of Health, Population, and Nutrition 27, 612–618. https://doi.org/10.3329/jhpn.v27i5.3637

Alm El Dein, A., Elbearon, E., 2010. Antibiotic residue in eggs of laying hens following injection with gentamicin. New York Science Journal 3, 135–140.

Andrews, W., Wang, H., Jacobson, A., Ge, B., Zhan, G., Hammack, T., 2023. *Salmonella*. In bacteriological analytical manual. Food and Drug Administration. https://www.fda.gov/media/172194/download?attachment

Anlis, D.C.G. Malbrán, W.-A., 2021. Protocolo de Trabajo de la Red Nacional de Vigilancia de la Resistencia a los Antimicrobianos WHONET Argentina. http://sgc.anlis.gob. ar/handle/123456789/2374

Baum, M.D., Kincheloe, J., 2022. Consumers, USDA, industry, scientists, and voters all say it's time to make poultry safer. https://www.foodsafetynews.com/2022/04/consumers-usda-industry-scientists-and-voters-all-say-its-time-to-make-poultry-safer/

Begum, K., Reza, T., Haque, M., Hossain, A., Hassan, F.K., Hasan, S.N. et al., 2010. Isolation, identification, and antibiotic resistance pattern of *Salmonella* sp. from chicken eggs, intestines, and environmental samples. Bangladesh Pharmaceutical Journal 13, 23–27.

Betancor, L., Pereira, M., Martinez, A., Giossa, G., Fookes, M., Flores, K. et al., 2010. Prevalence of *Salmonella enterica* in poultry and eggs in Uruguay during an epidemic due to *Salmonella enterica* serovar enteritidis. Journal of Clinical Microbiology 48, 2413–2423. https://doi.org/10.1128/JCM.02137-09

Bueno, D.J., Rodríguez, F.I., Machado, L.C., Soria, M.A., Procura, F., Gómez, S.C. et al., 2024. Study of *Salmonella* spp. from Cage Papers Belonging to Pet Birds in an Argentinean Canary Breeder Championship. Animals 14, 1207. https://doi.org/10.3390/ANI14081207

Caffer, M.I., Eiguer, T., 1994. *Salmonella enteritidis* in Argentina. International Journal of Food Microbiology 21, 15–19. https://doi.org/10.1016/0168-1605(94)90195-3

Cardoso, M.J., Nicolau, A.I., Borda, D., Nielsen, L., Maia, R.L., Møretrø, T. et al., 2021. Salmonella in eggs: From shopping to consumption—A review providing an evidence-based analysis of risk factors. Comprehensive Reviews in Food Science and Food Safety 20, 2716–2741. https://doi.org/10.1111/1541-4337.12753

Castro-Vargas, R.E., Herrera-Sánchez, M.P., Rodríguez-Hernández, R., Rondón-Barragán, I.S., 2020. Antibiotic resistance in *Salmonella* spp. isolated from poultry: A global overview. Veterinary World 13, 2070. https://doi.org/10.14202/VETWORLD.2020.2070-2084

Clinical and Laboratory Standards Institute, 2014. Performance standards for antimicrobial susceptibility testing. (24th ed), CLSI Supplement M100.

Clinical and Laboratory Standards Institute, 2015. Performance standards for antimicrobial susceptibility testing. (29th ed), CLSI Supplement M100.

Clinical and Laboratory Standards Institute, 2020. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. (5th ed), CLSI supplement VET01S.

Clinical and Laboratory Standards Institute, 2022. Performance standards for antimicrobial susceptibility testing. (32nd ed), CLSI Supplement M100.

Cogan, T.A., Domingue, G., Lappin-Scott, H.M., Benson, C.E., Woodward, M.J., Humphrey, T.J., 2001. Growth of *Salmonella enteritidis* in artificially contaminated eggs: The effects of inoculum size and suspending media. International Journal of Food Microbiology 70, 131–141. https://doi.org/10.1016/S0168-1605(01)00540-2

Dawson, B., Trap, R.G., 2004. Basic and clinical biostatistics. (4th ed), New York: McGraw-Hill Companies, Inc.

De Franceschi, M., Viora, S., Anselmo, R., Barrios, H., 1999. Salmonella Enteritidis en huevos frescos. Revista de Medicina Veterinaria 79, 5–7.

De Oliveira, G.H., Junior, A.B., Fernandes, A.C., 2005. Experimental infection of laying hens with *Salmonella enterica* serovar Gallinarum. Brazilian Journal of Microbiology 36, 51–56. https://doi.org/10.1590/S1517-83822005000100011

Eiguer, T., Caffer, M.I., Fronchkowsky, G.B., 1990. Significance of *Salmonella enteritidis* in outbreaks of diseases transmitted by foods in Argentina. Revista Argentina de Microbiologia 22, 31–36.

Esaki, H., Morioka, A., Ishihara, K., Kojima, A., Shiroki, S., Tamura, Y. et al., 2004. Antimicrobial susceptibility of *Salmonella* isolated from cattle, swine, and poultry (2001–2002): Report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. Journal of Antimicrobial Chemotherapy 53, 266–270. https://doi.org/10.1093/JAC/DKH081

Favier, G.I., Lucero Estrada, C.S.M., Lazarte Otero, V., Escudero, M.E., 2013. Prevalence, antimicrobial susceptibility, and molecular characterization by PCR and pulsed-field gel electrophoresis (PFGE) of *Salmonella* spp. isolated from foods of animal origin in San Luis, Argentina. Food Control 29, 49–54. https://doi.org/10.1016/J.FOODCONT.2012.05.056

Freije, J.A., Comba, E.R., Caffer, M.I., Alcain, Á., Pidone, C.L., 2019. Aislamiento de *Salmonella* spp. a partir de huevos de gallina para consumo comercializados en la ciudad de Casilda, Santa Fe, Argentina. FAVE Sección Ciencias Veterinarias 18, 23–25. https://doi.org/10.14409/FAVECV.V18I1.8277

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T.J. et al., 2009. Mechanisms of egg contamination by *Salmonella enteritidis*. FEMS Microbiology Reviews 33, 718–738. https://doi.org/10.1111/J.1574-6976.2008.00161.X

Gantois, I., Eeckhaut, V., Pasmans, F., Haesebrouck, F., Ducatelle, R., Van Immerseel, F., 2008. A comparative study on the pathogenesis of egg contamination by different serotypes of *Salmonella*. Avian Pathology Journal of the W.V.P.A 37, 399–406. https://doi.org/10.1080/03079450802216611

Gast, R.K., 1993. Immersion in boiling water to disinfect egg shells before culturing egg contents for *Salmonella enteritidis*. Journal of Food Protection 56, 533–535. https://doi.org/10.4315/0362-028X-56.6.533

Gast, R.K., Holt, P.S., 1995. Differences in the multiplication of *Salmonella enteritidis* strains in liquid whole egg: Implications for detecting contaminated eggs from commercial laying flocks. Poultry Science 74, 893–897. https://doi.org/10.3382/PS.0740893

Gast, R.K., Porter, R.E., 2019. *Salmonella* Infections. Diseases of Poultry 717–753. https://doi.org/10.1002/

9781119371199.CH16

Ghimpeţeanu, O.M., Pogurschi, E.N., Popa, D.C., Dragomir, N., Drăgotoiu, T., Mihai, O.D. et al., 2022. Antibiotic use in livestock and residues in food—A public health threat: A review. Foods 11, 1430. https://doi.org/10.3390/FOODS11101430

Graziani, C., Busani, L., Dionisi, A.M., Lucarelli, C., Owczarek, S., Ricci, A. et al., 2008. Antimicrobial resistance in *Salmonella enterica* serovar Typhimurium from human and animal sources in Italy. Veterinary Microbiology 128, 414–418. https://doi.org/10.1016/J.VETMIC.2007.10.017

Grimont, P.A., Weill, F.X., 2007. Antigenic formulae of the Salmonella serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*. www.pasteur.fr/sites/default/files/veng_0.pdf

Guillén, S., Cebrián, G., 2022. Relationship between iron bioavailability and *Salmonella* Typhimurium fitness in raw and pasteurized liquid whole egg. Food Microbiology 104, 104008. https://doi.org/10.1016/J.FM.2022.104008

Hai, D., Yin, X., Lu, Z., Lv, F., Zhao, H., Bie, X., 2020. Occurrence, drug resistance, and virulence genes of *Salmonella* isolated from chicken and eggs. Food Control 113, 107109. https://doi.org/10.1016/J.FOODCONT.2020.107109

Himathongkham, S., Riemann, H., Ernst, R., 1999. Efficacy of disinfection of shell eggs externally contaminated with *Salmonella enteritidis*: Implications for egg testing. International Journal of Food Microbiology 49, 161–167. https://doi.org/10.1016/S0168-1605(99)00092-6

Hoszowski, A., Wasyl, D., 2002. Salmonella serovars found in animals and feeding stuff in 2001 and their antimicrobial resistance. Bulletin of the Veterinary Institute in Puławy 46, 165-178.

Jabalera, Y., Dominguez-Gasca, N., Muñoz, A., Hincke, M., Jimenez-Lopez, C., Rodriguez-Navarro, A.B., 2022. Antimicrobial defenses of table eggs: Importance of antibacterial proteins in egg white as a function of hen age in an extended production cycle. Food Microbiology 107, 104068. https://doi.org/10.1016/J.FM.2022.104068

Kan, C.A., Petz, M., 2000. Residues of veterinary drugs in eggs and their distribution between yolk and white. Journal of Agricultural and Food Chemistry 48, 6397–6403. https://doi.org/10.1021/JF000145P

Karam, G., Chastre, J., Wilcox, M.H., Vincent, J.L., 2016. Antibiotic strategies in the era of multidrug resistance. Critical Care 20, 1–9. https://doi.org/10.1186/S13054-016-1320-7/TABLES/2

Khatun, M.F., Khan, M.A.S., Ahmed, M.F., Rahman, M.M., Rahman, S.R., 2022. Assessment of foodborne transmission of *Salmonella enteritidis* in hens and eggs in Bangladesh. Veterinary Medicine and Science 8, 2032. https://doi.org/10.1002/VMS3.874

Kovacs-Nolan, J., Phillips, M., Mine, Y., 2005. Advances in the value of eggs and egg components for human health. Journal of Agricultural and Food Chemistry 53, 8421–8431. https://doi.org/10.1021/JF050964F

Kuijpers, A.F.A, Veenman, C., van de Kassteele, J., Mooijman, K.A., 2008. EU interlaboratory comparison study food-II bacteriological detection of Salmonella in minced beef 330604010. RIVM Report. https://www.rivm.nl/bibliotheek/rapporten/330604010.html

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G. et al., 2012. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection 18, 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Martelli, F., Davies, R.H., 2012. Salmonella serovars isolated from table eggs: An overview. Food Research International 45, 745–754. https://doi.org/10.1016/J.FOODRES.2011.03.054

Miko, A., Pries, K., Schroeter, A., Helmuth, R., 2005. Molecular mechanisms of resistance in multidrug-resistant serovars of *Salmonella enterica* isolated from foods in Germany. The Journal of Antimicrobial Chemotherapy 56, 1025–1033. https://doi.org/10.1093/JAC/DKI365

Murchie, L., Whyte, P., Xia, B., Horrigan, S., Kelly, L., Madden, R.H., 2007. Prevalence of *Salmonella* in grade A whole shell eggs on the island of Ireland. Journal of Food Protection 70, 1238–1240. https://doi.org/10.4315/0362-028X-70.5.1238

Nair, D.V.T., Venkitanarayanan, K., Johny, A.K., 2018. Antibiotic-resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. Foods 7, 167. https://doi.org/10.3390/FOODS7100167

Oldham Council, 2008. *Salmonella* and the use of raw eggs and the recommended use of pasteurized eggs. Business Advisory Sheet 7. https://www.oldham.gov.uk/download/downloads/id/325/salmonella_and_the_use_of_raw_eggs

Petersen, L., 1997. A comparison of EF-18 agar and modified brilliant green agar with lutensit for isolation of *Salmonella* from poultry samples. Acta Veterinaria Scandinavica 38, 79–85. https://doi.org/10.1186/BF03548510

Roudaut, B., Moretain, J.P., Boisseau, J., 1989. Excretion of tetracycline and chlortetracycline in eggs after oral medication of laying hens. Food Additives and Contaminants 6, 71–78. https://doi.org/10.1080/02652038909373740

Ruiz, B., 2023. Argentina becomes fourth highest for egg consumption. https://www.wattagnet.com/blogs/blog/15537174/Argentina-becomes-fourth-highest-for-egg-consumption

Sasaki, Y., Tsujiyama, Y., Asai, T., Noda, Y., Katayama, S., Yamada, Y., 2011. Salmonella prevalence in commercial raw shell eggs in Japan: A survey. Epidemiology and Infection 139, 1060–1064. https://doi.org/10.1017/S0950268810002153

Shang, K., Wei, B., Kang, M., 2018. Distribution and dissemination of antimicrobial-resistant *Salmonella* in broiler farms with or without enrofloxacin use. BMC Veterinary Research 14, 1–14. https://doi.org/10.1186/S12917-018-1590-1/FIGURES/2

Singer, R.S., 2023. Estimates of on-farm antimicrobial usage in egg production in the United States, 2016–2021. Frontiers in Veterinary Science 10, 1135377. https://doi.org/10.3389/FVETS.2023.1135377

Sodagari, H.R., Mohammed, A.B., Wang, P., O'Dea, M., Abraham, S., Robertson, I. et al., 2019. Non-typhoidal *Salmonella* contamination in egg shells and contents from retail in Western Australia: Serovar diversity, multilocus sequence types, and phenotypic and genomic characterizations of antimicrobial resistance. International Journal of Food Microbiology 308, 108305. https://doi.org/10.1016/J.IJFOODMICRO.2019.108305

Soria, M.A., Soria, M.C., Bueno, D.J., 2012. A comparative study of culture methods and polymerase chain reaction for *Salmonella* detection in egg content. Poultry Science 91, 2668–2676. https://doi.org/10.3382/PS.2012-02253

Soria, M.C., Soria, M.A., Bueno, D.J., Colazo, J.L., 2011. A comparative study of culture methods and polymerase chain reaction assay for *Salmonella* detection in poultry feed. Poultry Science 90, 2606–2618. https://doi.org/10.3382/PS.2011-01548

Sullivan, G., Guo, X., Tokman, J.I., Roof, S., Trmcic, A., Baker, R.C. et al., 2020. Extended enrichment procedures can be used to define false-negative probabilities for cultural gold standard methods for *Salmonella* detection, facilitating comparisons between gold standard and alternative methods. Journal of Food Protection 83, 1030–1037. https://doi.org/10.4315/0362-028X.JFP-19-422

Suresh, T., Hatha, A.A.M., Sreenivasan, D., Sangeetha, N., Lashmanaperumalsamy, P., 2006. Prevalence and antimicrobial resistance of *Salmonella enteritidis* and other salmonellas in the eggs and egg-storing trays from retail markets of Coimbatore, South India. Food Microbiology 23, 294–299. https://doi.org/10.1016/J.FM.2005.04.001

Tan, Z., Lu, P., Adewole, D., Diarra, M.S., Gong, J., Yang, C., 2021. Iron requirement in the infection of *Salmonella* and its relevance to poultry health. Journal of Applied Poultry Research 30, 100101. https://doi.org/10.1016/J.JAPR.2020.09.016

Threlfall, E.J., Wain, J., Peters, T., Lane, C., De Pinna, E., Little, C.L. et al., 2014. Egg-borne infections of humans with *Salmonella*: Not only an *S. enteritidis* problem. World's Poultry Science Journal 70, 15–26. https://doi.org/10.1017/S0043933914000026

United States Department of Agriculture, 2011. Bioassay for the detection, identification, and quantitation of antimicrobial residues in meat and poultry tissue. https://www.fsis.usda.gov/sites/default/files/media_file/2021-03/MLG_34_03.pdf

Viora, S., Anselmo, R., Barrios, H., De Franceschi, M., 1993. Salmonella Enteritidis en maples. Revista de Medicina Veterinaria 74, 96–99.

Waltman, W., Gast, R., 2016. A laboratory manual for the isolation, identification, and characterization of avian pathogens. (6th ed.), Athens, GA: American Association of Avian Pathologists.

Waltman, W.D., Horne, A.M., Pirkle, C., Dickson, T.G., 1991. Use of delayed secondary enrichment for the isolation of *Salmonella* in poultry and poultry environments. Avian Diseases 35, 88–92. https://doi.org/10.2307/1591299

Wang, H., Hammack, T., 2014. *Salmonella* detection by classical cultural techniques. Encyclopedia of Food Microbiology 332–338.

Wang, J., Li, Y., Xu, X., Liang, B., Wu, F., Yang, X. et al., 2017. Antimicrobial resistance of *Salmonella enterica* serovar Typhimurium in Shanghai, China. Frontiers in Microbiology 8. https://doi.org/10.3389/FMICB.2017.00510

Wang, J., Vaddu, S., Bhumanapalli, S., Mishra, A., Applegate, T., Singh, M. et al., 2023. A systematic review and meta-analysis of the sources of *Salmonella* in poultry production (pre-harvest) and their relative contributions to the microbial risk of poultry meat. Poultry Science 102, 102566. https://doi.org/10.1016/J.PSJ.2023.102566

Yang, S.J., Park, K.Y., Kim, S.H., No, K.M., Besser, T.E., Yoo, H.S. et al., 2002. Antimicrobial resistance in *Salmonella enterica* serovars Enteritidis and Typhimurium isolated from animals in Korea: Comparison of phenotypic and genotypic resistance characterization. Veterinary Microbiology 86, 295–301.https://doi.org/10.1016/S0378-1135(02)00009-3

Zurita, A., Montes, S., Rivas, R., Moraga, B., 1989. Mastitis clínica II: Actividad "*in vitro*" de espiramicina, lincomicina y neomicina y efectividad "in vitro" de la asociación de estos antibióticos mas corticoide. Monografías de Medicina Veterinaria.