DYNAMICS OF BACTERIAL CONTAMINATION AND ANTIBIOTIC RESISTANCE IN COMMERCIALLY- OR ORGANICALLY- FARMED CHICKEN EGGS

A Thesis

presented to

the Faculty of Natural and Applied Sciences

at Notre Dame University-Louaize

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

---

by

YASMEEN SALAH DAOU

JUNE 2021

Notre Dame University - Louaize

Faculty of Natural and Applied Sciences

Department of Sciences

We hereby approve the thesis of

Yasmeen Salah Daou

Candidate for the degree of Sciences

Dr. Pauline Aad — Supervisor, Chair

Dr. Sanaa Jehi — Committee Member

Dr. Esther Ghanem — Committee Member

Abstract

Eggs are one of nature's most nutritious and economical foods. Besides their nutritional value, antioxidant, anti-cancerous, antihypertensive activity, and immunomodulatory effects, chemical and microbial contamination of eggs pose a risk on human health. Chemical contamination is due to the abuse of antibiotics in poultry. Microbial contamination can be through horizontal or vertical transmission. Microbial contamination accompanies a high risk of spread of antibiotic resistance. Microbial contamination can be controlled by choosing the egg source wisely and by storing eggs carefully. A lot of studies have been conducted to find the most favorable farming type and storage temperature, however the outcomes appeared to be contradicting. In this study we tested the microbial and chemical contamination of eggs from different farming systems in Lebanon. We studied the microbial contamination of eggshell and content of commercial and organic eggs stored at room- and fridge temperature for 5 weeks. The isolated bacteria were subcultured on SS and TBX agar and then confirmed by API 20E; the serotoxicity of E.coli was tested using Antiserum Escherichia coli NONAVALENT. The antibiotic resistance was studied using disc diffusion method. Our result revealed less contamination risk on the commercial eggs in comparison to organic eggshells along with better eggshell quality and a lower pore count. No antibiotic residues were detected in the egg contents regardless of the farming system. Serotoxic E.coli, Enterobacter cloacae, and citrobacter freundii were isolated from organic eggshells. E.coli and Enterobacter cloacae seemed to also vertically contaminate the egg content of eggs stored for 2 weeks at room temperature. All eggs stored in the fridge showed eggshell contamination. All the isolated bacteria were sensitive to ENR and SPT, and resistant to Macrolide E. Isolated E. coli showed resistance to macrolides (100% to E and 71% to TL), tetracyclines (86% to O and 57% to DO), beta lactams (20%), colistin (57%), Aminoglycosides (71% to Gen and 29% to N), and COT(14%). Enterobacter cloacae appeared resistant to macrolides (100% to E and 50% to TL), betalactams (50%) and colistin (50%). Citrobacter Freundii was found resistant to betalactams,

tetracyclines (100% to O) and Colistin (50%). Our study shows that the farming system greatly affects the starting microbial population of eggs, where organic eggs constitute a greater risk than commercial eggs to bacterial infection, many times resistant to antimicrobials. Eggs should be cleaned properly, without compromising the pore or protective cuticle before storage.

Keywords: eggs, organic, commercial, antibiotic resistance

Acknowledgements

Working throughout the current situation in Lebanon was a big challenge. It would've have taken much more time if it wasn't for Dr. Pauline Aad, my thesis supervisor. Her flexibility and experience made this research possible even with the scarce materials available, and even after changing the whole topic. So primarily, I would like to thank Dr. Aad for her patience and flexibility. Again, I would like to thank her for teaching me how to think critically, and how to cope with any change and always have back up plans. Also, a big thank you goes to Mrs. Nada El Ghossein Maalouf and Dr. Elie Salem Sokhn, who helped me with their years of experience in the microbiology sector. I would also like to thank Dr. Kamil Rahme for giving us permission to access the lab multiple times even during the lock down. Last but not least, thank you to all the committee members, Dr. Sanaa Jehi and Dr. Esther Ghanem who helped me improve my work with their experience and valuable comments.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

RT: Room Temperature

AR: Antibiotic resistance

SPT : Spectinomycin

TL: Tylosine

COT: co-trimoxazole

E: Erythromycin

GEN: Gentamycin

DO: Doxycyclin

CT: Colistin

ENR: Enrofloxacin

AMP: Ampicillin

AML: Amoxycillin

N: Neomycin

Literature Review

I. The Chicken Egg: a Sterile or Tainted Feed

Eggs are one of nature's most nutritious and economical foods. They consist of 9.5% shell and membranes, 63% egg white, and 27.5% yolk (Cotterill and Geiger, 1977). The edible part of the egg comprises 74% water, 12% proteins,12% lipids, and <1% carbohydrates, vitamins, and minerals (Li-Chan and Kim, 2008). Proteins are distributed in the albumen, yolk, membrane, and shell(Abdou et al., 2013). Some egg proteins are only partly digested, thus naturally generating bioactive peptides without undergoing complete degradation into amino acids (Evenepoel et al., 1999). In the last decades, countless efforts have been made to better understand the biological activities of egg-derived hydrolytic peptides that can naturally occur throughout the digestive process (Evenepoel et al., 1999). Lipids are only found in the yolk; they constitute of 65% triglycerides, 30% phospholipids, 4% cholesterol, and <1%, carotenoids(Hatta et al., 2008). Micronutrients found in the egg constitute ~16%, 29%, 9% and 9% of the recommended daily intake of phosphorus, selenium, iron, and zinc, and 10% of the recommended daily intake of vitamin A, D, E, K, B2, B12, biotin and pantothenic acid (Seuss-Baum, 2007). Besides their exceptional nutritional quality, chicken eggs are known to have an antimicrobial effect. Various egg proteins, concentrated in the egg white and the vitelline membrane, exhibit antimicrobial activities (Kovacs-Nolan et al., 2005). Some of them, like lysozyme and avian beta-defensins, act directly by interacting with bacterial walls and permeabilizing them, causing bacterial death (Rehault-Godbert et al., 2011). Others, act indirectly either by diminishing the bioavailability of iron and vitamins required for some

microbial growth, like ovotransferrin and avidin, or by inhibiting microbial proteases that are virulent factors of infection, like Ovoinhibitor and cystatin (Rehault-Godbert et al., 2011). Da Silva et al. (2019) reported the presence of egg proteins, like AvBD11, OVAX, avidin, beta-microseminoprotein, not expressed in the human genome, making them a potential powerful anti-infectious agents against human enteric pathogens. Egg-derived peptides that are possibly formed after partial hydrolysis of proteins by exogenous proteases were reported to also have antimicrobial activity. Hydrolytic peptides obtained from lysozyme(Mine et al., 2004), ovotransferrin (Giansanti et al., 2005), ovomucin (Omana et al., 2010a), and cystatin (Blankenvoorde et al., 1998)have revealed a wide range of antimicrobial activities. Chicken eggs are also considered potential antioxidant foods. They have many naturally occurring antioxidant compounds like egg-white proteins ovalbumin, ovotransferrin and lysozyme; as well as egg yolk phosvitin, carotenoids and free aromatic amino acids(Nimalaratne and Wu, 2015a). Young et al. (2010) performed assays in a porcine model and succeeded to confirm the advantageous effect of egg yolk- derived proteins in reducing the production of pro-inflammatory cytokines in vivo. They presumed that a diet supplemented with egg yolk-proteins might serve as a novel strategy in intestinal oxidative stress reduction (Young et al., 2010). It is noteworthy that antioxidant-enriched eggs can be produced by feeding layers some lipophilic antioxidants such as vitamin E, carotenoids, selenium, iodine and others can be transferred from feed into egg yolk (Nimalaratne and Wu, 2015b). Eggs' anti-cancerous activity has also been studied. Using experimental tumors showed that, egg white lysozyme had tumor-inhibitory activity (Sava, 1989). Ovomucin and ovomucin-derived peptides were also found to possess anti-tumor activities via cytotoxic effects and activation of the immune system(Omana et al., 2010b).

Hydrolytic peptides from ovotransferrin also showed anti-cancerous activity when studied by Ibrahim and Kiyono (2009). Numerous yolk-derived peptides possessing anti-hypertensive activities have been described. Egg yolk oligopeptides were found to be a potential suppressant of the development of hypertension in spontaneously hypertensive rats (Yoshii et al., 2001). Also, ovotransferrin and egg white hydrolysates showed great potential as anti-hypertension agents in humans (Moon et al., 2017). Egg white ovotransferrin-derived tripeptide IRW showed to be active in reducing blood pressure in vivo after oral administration to hypertensive rats (Liao et al., 2016). In turn, Chen et al. (2017), discussed the degree to which ovotransferrin and ovotransferrin-derived peptides are viable treatment agents for endothelial dysfunction and the prevention of CVD. Besides their nutritional value, antioxidant/ anti-cancerous/ antihypertensive activity, and immunomodulatory effects, chemical (Domingo, 2014) and microbial (Musgrove, 2011) contamination of eggs pose a risk on human health.

II. Factors affecting bacterial contamination in eggs

Various intrinsic and extrinsic factors affect the chance of bacterial penetration of an eggshell, an opportunity that could be vertical or horizontal. Intrinsic factors comprise the chicken eggs' physical and chemical barriers. Extrinsic factors include the bacterial strains and number of organisms to which an egg is exposed, as well as the storage conditions including temperature, moisture and immersion.

1. Vertical and horizontal transmission

Vertical/ trans-ovarian transmission of bacteria takes place through the reproductive system of infected hens, usually those having a systemic infection (Keller et al., 1995) in the ovaries or an ascending infection from contaminated cloaca into the vagina and lower regions of the oviduct (Miyamoto T, 1997). In this type of transmission, the yolk, the albumen, and the membranes are contaminated straight away due to the bacterial infection in the reproductive organs, before the eggs are covered by the shell (Takehiko Yamamoto, 1996).

In the horizontal/ trans-shell transmission, the dominant route for bacterial infection of eggs, micro-organisms penetrate through the eggshell to possibly reach the internal egg (Bruce, 1994). The existence of diverse bacterial species on the eggshells surface embodies the risk of egg content contamination; a high microbial load on eggshells upswings the microbial penetration possibility (Smith et al., 2000). The microbial eggshell penetration process involves 3 steps: Cuticle and shell penetration, colonization of underlying membranes, and contamination of yolk and egg contents (Lock et al., 1992). The eggshell surface contamination may result either from the lower reproductive tract infection (uterus/shell gland). At the moment of its laying, the egg passes through the cloaca, a highly contaminated area where egg and feces co-exist, resulting in fecal contamination of the shell. However, even if eggs are germ-free during oviposition, their chances of contamination arise the moment they leave the oviduct (Board RG, 1995). After oviposition, the eggshell attains contamination from any surface that it contacts

2. Intrinsic Factors affecting egg quality

The cuticle, the mineralized shell, and shell membranes form the physical barrier that protects the egg content from contamination and invasion by microorganism. This physical barrier is particularly essential to prevent trans-shell contamination, in which microorganisms contaminate the egg after being laid by penetrating the shell, this being regarded as the prevalent route for bacterial infection of eggs (Bruce, 1994).

2.1. Cuticle

The cuticle coverage was found to be the key eggshell barrier against bacterial penetration (De Reu et al., 2006c). It is an organic-rich layer that coats the outer eggshell surface and restricts the movement of particles, water, and bacteria through shell pores, thus regulating egg shell permeability (Board and Halls, 1973). The cuticle covers about 10,000-17,000 pores, of about 15-65 µm diameter, significantly bigger than most micro-organisms. The cuticle is the egg's first defense against bacterial infection, it is not an inherent part of the true eggshell (Solomon, 1999). The epithelial cells lining the hen uterus deposit the cuticle during the last 1 h before oviposition (Nys et al., 1999). The cuticle, before oviposition, is moist and immature, and less effective in the avoidance of penetration than mature cuticle (Sparks and Board, 1985). The mature cuticle's thickness is variable, with a mean thickness ranging from 0.5 to 12.8 µm (Simons, 1971). It is distributed unevenly across the eggshell surface, with the egg poles habitually being covered by a quite thin layer(Sparks, 1994). In a study conducted by Board, R.G. and N.A. Halls 3.5% of eggs lacked a demonstrable cuticle, and 8.0% had no cuticle on the apex/ blunt end (Board and Halls, 1973). Scanning electron microscopy (SEM), showed

that the cuticle of > 98% eggs was absent or patchy throughout the laying cycle (Nascimento et al., 1992).Eggs with an absent/ partially absent cuticle are more susceptible to bacterial contamination, the lower the cuticle deposition the higher the susceptibility (Bain et al., 2013). The mature cuticle restricts bacterial invasion by filling the pores of the egg- shell. The cuticle's chemical composition is found to also have a significant role in restricting bacterial contamination. Rose-Martel et al. (2012) have identified numerous proteins having antimicrobial activity in the cuticle like lysozyme C, ovotransferrin, ovocalyxin-32, and ovocleidin-17. In their turn Wellman-Labadie et al. (2008b) also recognized C-type lysozyme, ovotransferrin and ovocalyxin-32 in the chicken eggshell and cuticle, and demonstrated that these proteins have an antimicrobial activity against Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis. Also, lipid components extracted from the cuticle have potent antimicrobial activity (Wellman-Labadie et al., 2010).

2.2. Outer Eggshell

The eggshell is a thin mineral structure that protects egg contents from mechanical influence, dehydration, and microorganism contamination. It allows the penetration of gases and H2O necessary for chick embryo development (Nys, 1999.; Hincke et al., 2012). The hen's eggshell has about 7,000 to 17,000 pores/ egg (Mayes and Takeballi, 1983), unbranched and coated with the cuticle (Board et al., 1979). Eggs with undamaged cuticle also have few (~10-20) uncovered pores, that in turn may provide gateways for bacteria into the egg contents (Svobodová and Tůmová, 2015). The pores on the eggshell are adequately wide, and permit the entry of microorganisms, making the eggshell an ineffective barrier against bacterial penetration. The quality of eggshells is determined by shell thickness which is measured in

terms of shell specific gravity and shell weight. Although the eggshells do not have an innate antibacterial activity, their quality matters, the thicker the shell the longer the time needed for bacteria to cross it. Eggshells of excellent quality with specific gravity >1.090 and high thickness appeared to be more resistant to penetration by Salmonella (Bain et al., 2013). Sauter and Petersen established that eggshells with high specific gravity were more resistant to penetration by Pseudomonas fluorescens(1969) and S. enteritidis (Sauter and Petersen, 1974). However, unlike porosity, the shell thickness appeared to be insignificant in determining the egg susceptibility to bacterial penetration and infection (Kraft et al., 1958b). Williams et al. (1968), reported no significant effect of shell thickness on the penetration with S. typhimurium. Similarly, Smeltzer et al. (1979) ,who studied the number of eggs penetrated on 3 poultry farms, indicated that eggshell thickness had no effect on the penetrability.

2.3. Eggshell Membrane

Eggshell membranes, their selves, add some defensive value to the eggshell; they improve the ability to keep bacteria out briefly. Bacteria are situated between the eggshell and the membranes at the initial stage of contamination due to horizontal transmission (Chen et al., 1996). The eggshell membranes act as filters and form a momentary barrier to bacterial penetration (Berrang et al., 1999), and this was revealed for Pseudomonas whose passage through the shell membrane was prevented for ~15- 20 h (Kraft et al., 1958a). The inner membrane, as compared with the outer membrane, is more effective in preventing bacterial entry, because it has a tighter meshwork (LIFSHITZ et al., 1964).

2.4. Chemical Defense

Around 520 proteins have been identified in eggshells (Mann et al., 2006) 148 in albumen (Mann, 2007), and 119 in the yolk (Mann and Mann, 2008). However, not all of these proteins have antimicrobial properties- 95 % of those identified proteins haven't been well tested (Réhault-Godbert et al., 2011). Antimicrobial proteins are indispensable they play inhibitory and bactericidal activities; they act via degrading microbial components, decreasing the bioavailability of iron and vitamins, or inhibiting the activity of bacterial proteases needed for bacterial invasion (D'Alba and 2015). The deposition of those proteins varies throughout the different compartments of the egg. According to (Gautron and Nys, 2006) antimicrobial proteins are most abundant in the albumen of the chicken egg and less present in the yolk and shell. Abdou (2013), reported that 90% of antimicrobial proteins are present in the egg white (50%) and egg yolk (40%), while the remaining 10% is distributed between the egg shell and egg shell membranes.

2.4.1. Egg white proteins

Antimicrobial proteins are deposited unevenly also within the albumen itself as following (Li-Chan and Kim, 2007) with the major albumen proteins being ovalbumin, followed by ovotransferrin and ovomucoid. Other proteins of the albumen include: ovomucin, lysozyme, avidin, cystatin, ovoinhibitor, and ovomacroglobulin (ovostatin) (Sugino, 1997).

Ovotransferrin (conalbumin) falls under the group of antimicrobial proteins that function by decreasing the bioavailability of iron and vitamins. It is an iron-binding protein; it chelates iron that is crucial for microorganisms' growth, thus depriving them from it. (Abdallah and Chahine, 1999). Pseudomonas spp., Escherichia coli, Streptococcus mutans appeared to be

sensitive to the antibacterial activity of ovotransferrin (Valenti et al., 1983). Further, Baron et al. (1999) demonstrated that ovotransferrin is the main anti-Salmonella enterica Enteritidis agent in eggwhite. Nevertheless, besides its iron binding property, ovotransferrin seemed also capable of exerting its antibacterial activity in a direct manner. Ovotransfferin was found to employ its antibacterial activity by penetrating bacterial outer membranes into the inner membranes limiting their permeability and dissipating the electrical potential (Aguilera et al., 2003). Ibrahim et al., (2000) verified that OTAP-92, A 92-amino acid ovotransferrin peptide, was able to kill Gram negative bacteria by crossing the bacterial outer membrane by self-promoted uptake, damaging the cytoplasmic membrane. Avidin falls under the same group as ovotransferrin. It is a vitamin chelator, its antibacterial activity depends on decreasing the bioavailability of vitamins. It is characterized by its strong binding affinity for biotin, which important for micro-organisms growth (Green, 1975). Avidin, thus hinders the growth of biotin-requiring bacteria. Korpela et al., (1984) reported that Avidin is capable of binding and suppressing the growth of several Gram negative and Gram positive bacteria: Escherichia coli K-12, Klebsiella pneumoniae, Serratia marcescens, Pseudomonas aeruginosa, Staphylococcus aureus, and Staphylococcus epidermis.

Cystatin, Ovomucoid and Ovoinhibitors fall under the group of antimicrobial proteins that act by inhibiting proteases necessary for microbial invasion. Ovomucoids and ovoinhibitors inhibit serine proteases. Ovomucoid acts inactivate trypsin, a serine protease and essential enzyme during multiplication of bacteria (Rzedzicki and Stępień-Pyśniak, 2009). Ovoinhibitor has been found to hinder serine proteinase in bacteria and fungi (Sugino, 1997). Cystatin in turn is a major cysteine protease inhibitor active against bacteria and viruses. It was reported

antiviral to rotavirus (Ebina and Tsukada, 1991), and antibacterial to Escherichia coli and Pseudomonas aeruginosa (Węsierska et al., 2005).

Lysozyme is an antimicrobial protein that acts by degrading microbial components. Its bacteriolytic activity depends on hydrolyzing the bond between N-acetyl-muramic acid and N-acetyl-glucosamine in bacterial cell wall peptidoglycan of some Gram positive bacteria (Rogers and Perkins, 1968). It was reported as effective against Clostridium botulinum and Listeria monocytogenes(Hughey and Johnson, 1987). Besides its bactericidal activity, lysozyme have been found to have antibacterial activity independent of its catalytic functions(Nash et al., 2006), and involving the exposure of antibacterial portions of the protein(Ibrahim et al., 2001).

2.4.2. Egg yolk proteins

Egg yolk consists of 16.6% proteins (Schade and Chacana, 2007) mostly in the form of lipoproteins (Sugino et al., 1997). It is made of large particles “globules” and smaller particles “granules” suspended in yellow fluid called plasma, which contains proteins(Romanoff and Romanoff, 1949).

Yolk granules contain High-density lipoproteins “lipovitellins”, phosvitin, and low-density lipoproteins(Burley and Cook, 1961). Lipovitellin and Phosvitin are metal binding proteins, they bind 90% of iron (Greengard et al., 1964)and 90% zinc (Tupper et al., 1954) in the yolk, respectively. Thus, they might have antimicrobial activity employed by decreasing the bioavailability of iron and vitamins. Sattar Khan et al., (2000) revealed that phosvitin under thermal stress was antibacterial against E. coli due to its metal-chelating capacity. Kassaify et al. (2005), identified that lipovitellins, on the other hand, possess no antibacterial activity; their

anti-adhesiveness is what appeared to inhibit Salmonella enteritidis, Salmonella typhimurium, and E. coli 0157:H7 colonization in-vitro.

The yolk plasma contains both a low-density and a water soluble lipoprotein fraction. The water soluble lipoprotein fraction includes α, β, and γ livetins (Li-Chan et al., 2017). γ – livetin is usually known as Immunoglobulin Y (IgY) (Leslie and Clem, 1969), and is the most predominant immunoglobulin present in the egg (Hamal et al., 2006). IgY revealed an antimicrobial activity dependent on inhibiting the activity of microbial proteases. It significantly inhibits the growth of bacteria by binding and immobilizing them thus limiting or constraining their growth and multiplication (Lee et al., 2000). Lee et al., (2002) demonstrated that salmonella-specific IgY bind antigens expressed on the Salmonella surface and alter its structure. Sui et al.,(2011) in turn evaluated the antibacterial activity of IgY specific to Listeria monocytogenes, to find out that specific IgY significantly decreased the growth of L. monocytogenes in liquid medium and food samples.

2.5. Eggshell as a physical and chemical barrier

Chicken eggshells are formed of 3.5% organic matrix, comprising eggshell membranes, cuticle, and others- embedded in calcium carbonate layer (Nys and Gautron, 2007). Eggshell matrix proteins include major egg white proteins like, ovalbumin (Hincke, 1995), bactericidal lysozyme (Hincke et al., 2000) and iron-binding ovotransferrin (Gautron et al., 2001b). Ubiquitous proteins like clusterin (Mann et al., 2003) and osteopontin (Hincke et al., 2008) are also part of this matrix. Further, eggshell expresses specific matrix proteins like Ovocleidin-17 (Hincke et al., 1995), ovocleidin-116 (Mann et al., 2002), Ovocalyxin-32 (Hincke et al., 2003), and ovocalyxin-36 (Gautron et al., 2007), Ovocalyxin-21 and Ovocalyxin-25 (Nys et al., 2004).

Mine et al., (2003) revealed that avian eggshell matrix proteins potentially possess an antimicrobial defense mechanism against Pseudomonas aureginosa, Bacillus cereus, and Staphylococcus aureus in-vitro.

Wellman-Labadie et al. (2008b) recognized C-type lysozyme, ovotransferrin in chicken eggshell and cuticle but also ovocalyxin-32, and demonstrated that these proteins have an antimicrobial activity against Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis. Ovocalyxin-32 belongs to the carboxypeptidase inhibitors family (Gautron et al., 2001a), it is antimicrobial by inhibiting microbial proteases. Xing et al., (2007) created a recombinant eggshell ovocalyxin-32 and reported it to significantly inhibit bovine carboxypeptidase, and inhibit Bacillus subtilis growth. However, OCX-32 was found to also be expressed in egg white at low levels (D'Ambrosio et al., 2008). Ovocleidin-17 is a C-type lectin-like protein (Mann and Siedler, 1999) that binds with high affinity to peptidoglycan, it was reported to inhibit the growth of Gram-positive bacteria by Wellman-Labadie et al. (2008a). Ovocalyxin-36 is 20–25% identical in sequence to mammalian proteins associated with the innate immune response (Gautron et al., 2007), it has been reported to exert antimicrobial effect by binding the bacterial lipopolysaccharides (Cordeiro et al., 2013). Besides the antimicrobial activity that specific matrix proteins possess, osteopontin has antimicrobial potential with its mammalian homologue being involved in the regulation of diverse cytokines production and natural killer T cells function (Diao et al., 2008). Ovocalyxin-25 appeared to express a protease inhibitor domain (Marie et al., 2015), and is potentially antibacterial. It is important to note that, also lipid components extracted from the cuticle revealed potent antimicrobial activity (Wellman-Labadie et al., 2010).

3. Extrinsic factors affecting egg quality

Aside from the egg quality and characteristics discussed above, the 2 main players in bacterial penetration are bacterial presence, and moisture that encourages bacterial invasion through the eggshell. The higher the number of bacteria on the shell or surrounding environment, the higher is the risk of trans-shell contamination (Schoeni et al., 1995). Increasing the inoculum in feces placed around the eggshell, before egg storage first at 35°C for 30 min and then at 4°C increased the frequency of membranes and contents contamination (Schoeni et al., 1995). As in for moisture, the importance of water in facilitating bacterial penetration through the cuticle and underlying shell was confirmed in a study conducted by Pardon. The study reported that Salmonella Typhimurium penetration is enhanced in the presence of water on the eggshell, but can also take place in the absence of water (Padron, 1990). Thus, apart from an egg's aspect and antimicrobial defenses some extrinsic factors can decide the risk of horizontal transmission of bacteria and impact the eggshell defense itself. The extrinsic factors that might affect the horizontal transmission process include: bacterial count and bacterial strains, egg housing, egg washing, and storage temperature and humidity.

3.1. Bacterial Count and Bacterial Strain

Naturally, gram positive bacteria, originating from dust, soil or feces, dominate eggshells' microflora, due to their tolerance for dry conditions (Board RG, 1995). According to Bruce and Johnson (1978), micrococci and Enterobacteriaceae constitute the foremost part of the hen egg's microflora, along with Staphylococcus spp., Streptococcus spp., and

pseudomonas spp. Eggshells are inclined to contamination with principal food poisoning bacteria like Enterobacteriaceae, Salmonella, Campylobacter, and Listeria (Jones and Musgrove, 2007). In Lebanon, a study conducted by Maamari and Na'was (2017) revealed contamination of eggshells by various members of the Enterobacteriaceae family, usually present in chicken feces, including Escherichia coli, Enterobacter cloacae, but excluding Salmonella spp. Most enteric bacteria: E. coli, Salmonella, Campylobacter spp., Listeria spp., Enterobacter spp., and Klebsiella spp. have been recovered from eggshell and contents (Adesiyun et al., 2006a). Egg contents' major contaminants are gram negative bacteria, like Escherichia coli, Salmonella, and Alcaligenes sp., (Reu et al., 2008). Also, some gram-positive bacteria like Staphylococcus lentus, Staphylococcus xylosus, and Bacillus sp. were also reported in contaminated egg content (Reu et al., 2008). Staphylococcus species appeared to be predominant in poultry houses air (Board RG, 1995), and according to Reu et al. (2008) it was the most prevalent in contaminated eggshells. Lukásová and Labounek (1976), proved that staphylococci penetrate through egg-shell structures into the egg content. Recently, Al-Natour et al. (2011), recognized that both Staphylococcus aureus and E. coli contaminate eggshells simultaneously, E. coli O157:H7 appeared to penetrate first and facilitate the invasion of Staphylococcus aureus.

Scientist studied the eggshell penetration ability of different pathogens such as Yersenia entercolitica, Campylobacter jejuni, and Salmonella serovars. Amin and Draughon (1990), studied the penetration ability of Y. enterocolitica, and reported that it is able to penetrate egg shells and infect egg contents. ALLEN and GRIFFITHS (2001), used luminescent Campylobacter jejuni ATCC 33291 to evaluate eggshell colonization and penetration; they were able to visualize the the luminescent bacteria on the exterior and interior surfaces of the egg membranes

signifying eggshell penetration. Fonseca et al. (2014), in their turn reported no recovery of *C. jejuni* in egg yolk of eggs inoculated with *C. jejuni* on the outer shell membrane, denying its penetration ability into the egg contents. Humphrey et al. (1991), while studying naturally infected flocks, reported the presence of many *Salmonella* serotypes, such as Enteritidis, Typhimurium and Hadar on eggshells, but only Enteritidis in the egg contents. However, it has been well demonstrated that various salmonella species are capable of penetrating through the egg not only Salmonella enteriditis(Williams and Dillard, 1968).

Some researchers considered the bacterial strain as an extrinsic factor affecting the eggshell penetration. They compared the ability of different bacterial strains to traverse the shell membranes. Sauter and Petersen (1974), found that among several salmonella strains *S. typhimurium* penetrated the highest proportion of eggs. Later studies showed that Salmonella enterica serovar Enteritidis and Salmonella typhimurium have the same eggshell penetration ability(Miyamoto et al., 1998), and so does S. Heidelberg (Schoeni et al., 1995). Salmonella Enteritidis, specifically, seem to conduct special mechanisms that allow them to survive and and grow inside the egg contents (Gantois et al., 2009). Yet, P. fluorescens crosses the shell membranes to infect the internal of the egg better than Salmonella enteritidis that usually survives on the egg exterior surface (Jones et al., 2002). Reu et al. (2007), *conducted* a broader comparative study on the penetration of 7 selected bacterial species usually recognized in egg contents using 2 different egg penetration models, and concluded that gram-negative, motile and non-clustering bacteria penetrate eggshells most frequently. Species that were found to often penetrate eggshells after 21 days of incubation in agar-filled eggs, included Pseudomonas sp. (60%), Alcaligenes sp. (58%), and Salmonella Enteritidis (43%) respectively (Reu et al., 2007).

Species that most frequently contaminated egg contents of intact eggs dipped in a bacterial suspension were reported to be Salmonella Enteritidis (33%) and Carnobacterium sp. (17.5%).

3.2. Egg Housing

The eggshell can be already contaminated or get contaminated while passing through the hen's cloaca, but the core contamination occurs the moment the egg is laid due to contact with dirty surfaces (Harry, 1963). As mentioned before, eggs are most prone to bacterial penetration 30 - 60 sec after being laid, before the hardening of the cuticle. The role of water in facilitating egg bacterial penetration and contamination becomes mostly significant in the egg immediately after oviposition, when the cuticle is moist and the egg's temperature is ~ 42°C, consequently warmer than the environment. Cooling of the egg leads to the contraction of its internal contents and, building a negative pressure inside the egg. This difference in pressure is balanced by the suction of air/ water from the external surface of the shell into the inside of the egg, which aids the passage of any bacteria from the egg surface into the egg through the pores (Haines and Moran, 1940a). Eggs laid on dirty house floors are more prone to internal bacterial contamination than those laid in nest boxes (Smeltzer et al., 1979). According to Padron (1990) 59% of eggs that were placed on Salmonella- contaminated nest box shavings for 10 min revealed eggshell and membrane penetration .

The type of housing system used has a major impact on the level of eggshell contamination of the newly laid eggs, indirectly influencing the risk of bacterial penetration in to the egg. Primarily, conventional cage systems also known as battery cage systems, that integrate wire floors for laying, were used in commercial egg production(Appleby et al., 2002a). Eggshells from deep litter systems display15 x more the bacteria and a higher amount of

prospective spoilage organisms than eggs from battery cage systems (Harry, 1963). Bacterial eggshell contamination is reported to be higher in eggs from litter floor houses than in eggs from wire floor houses. Litter floor houses had ~ 9x more bacteria in air, and ~ 20-30 x more aerobic bacteria on the shell than wire floor houses (Quarles et al., 1970).

However, Conventional cage housing/ battery cage systems have been prohibited since 2012 in the European Union, following Council Directive 1999/74/EC (Directive, 1999). Today, alternative housing systems are being used: furnished cages(Appleby et al., 2002b) and non-cage systems (LayWel, 2006). The effect of furnished cages and non-cage systems on egg hygiene was studied extensively, knowing that higher eggshell contamination increases the risk of microorganism penetration and consequently egg content contamination (De Reu et al., 2006c). Furnished cages allow laying hens to behave naturally, being equipped with nest box, perch, and dust-bathing area (Albentosa and Cooper, 2004). In furnished cages, unlike conventional battery cages, birds are not efficiently separated from their manure and the presence of perches may ruin bird's ability to walk over the droppings on the cage floor (Abrahamsson and Tauson, 1993). Furnished cages, provide a much smaller area for egg laying in comparison to conventional cages, and have a higher percentage of cracked and dirty eggs (Wall and Tauson, 2002). A study conducted by Mallet et al., (2006) on eggs hygiene in furnished cages compared to that in standard cages, indicated that the percentage of dirty eggs was higher in furnished cage designs, when eggs were laid outside the nest. Also, the bacterial load on the eggshell (total aerobic bacteria and enterococci) was higher in furnished cage designs (Mallet et al., 2006). However, the visually clean eggs, whether produced in furnished cages or in conventional cages, had similar bacterial counts on their eggshell (Mallet et al.,

2006). Thus, the positioning of perches, litter areas, and nests in relation to each other largely impacts the hygiene of the cage and eggs (Mallet et al., 2006). However, in 2008, Wall et al. stated that the quantities of dirty eggs were very similar in furnished and conventional cages (4.2% and 5.4% respectively), which indicates that well-designed furnished cages can reduce the proportion of dirty eggs and make it more similar to that in conventional cages (Wall et al., 2008). Even though bacterial eggshell contamination was considered as moderate in both furnished and conventional cages, bacterial counts on the eggshells of eggs produced in furnished cages were significantly higher as regards Enterococcus and total number of aerobic bacteria (Wall et al., 2008).

Non-cage housing systems are more animal-friendly than cage systems, offering hens more space and allowing them to perform natural behaviors(Shimmura et al., 2010). They include barns with litter covered ground floor, outdoor or free run systems, and single tier floor housing systems/ multiple-tier floor aviary systems (Windhorst, 2015). De Reu et al. (2005), compared bacterial eggshell contamination in conventional cages vs furnished cages vs aviary housing. In their study they showed that, shell contamination with aerobic bacteria didn't differ significantly between eggs laid in conventional cages and eggs laid in nest boxes of furnished cages (De Reu et al., 2005). On the other hand, they reported that the contamination with aerobic flora in eggs from non-cage aviary housing system, is higher than that in eggs from both conventional and furnished cage systems, with highest colony forming units reported for eggs laid on the floor of the aviaries (De Reu et al., 2005). 1 year later, De Reu et al. (2006b), detected no significant differences for the eggshell contamination with gram negative bacteria between eggs coming from organic aviaries and barns compared to eggs coming from

conventional cages. Thus contamination with aerobic bacteria is the only concern when it comes to the alternative housing systems. In 2009, (Reu et al.), confirmed a higher eggshell contamination with aerobic bacteria in eggs from non-cage systems than eggs from furnished cages, but no significant difference was reported in average eggshell contamination with enterobacteriaceae between both systems. Moreover, it is important to note that, there appeared to be no difference in shell contamination within each type of housing system between free range and organic flocks(Huneau-Salaün et al., 2010).

Regarding the eggshell structure, the eggshells of egg produced in non- cage systems is found to be not as good in quality as in cage eggs, this impaired shell structure of non-cage eggs increases the risk of bacterial penetration into the egg (EFSA). However, no large differences in egg content contamination was reported between eggs in furnished cages (~1.9%) and eggs in non-cage systems (~2.3%)(Reu et al., 2008). Eggshell weight was reported to be higher for eggs in cages (8.11 g) as compared to eggs in aviary systems with deep litter (Pištěková et al., 2006). Moreover, eggshells in furnished/ enriched cages appeared to be lighter in weight than eggshells from non-enriched cages(Lichovnikova and Zeman, 2008). However, in 2011 Tumova et al. reported contradictory results; they found eggshells on litter to be heavier than eggshells from conventional cages and enriched cages. They presumed that this may be due to difference in environmental conditions in the housing systems or difference in hens' genotype(Tumová et al., 2011). Studies showed that eggshell thickness also varied according to housing systems. Eggshell thickness appears to be higher in eggs from free-range systems than in conventional cages and floor cages(Mostert B.E., 1995). In 2001, Pavlovski et al. reported that eggs from free-range systems have thicker shells in comparison to eggs from cage housing systems(Pavlovski et

al., 2001). Similarly, noticeably higher eggshell thickness in eggs produced in alternative systems (barn, free range, organic) especially free range systems was described by Hidalgo et al. (2008) in comparison to eggs produced in cages(Hidalgo et al., 2008). In 2012 Ledvinka et al. studied the difference in eggshell thickness between litter and cage systems and found the eggshells from cages (0.355 mm) to be thinner to eggshells from litter systems (0.358 mm)(Ledvinka et al., 2012b). The eggshell strength appears to be highest for aviary eggs and weakest for free-range eggs, as indicated by Mertens et al. in their examination of the effects of the different housing systems (conventional and furnished cages, aviary, and free-range) on eggshell quality(Mertens et al., 2006). In 2014, Englmaierova et al. observed stronger egg shell quality in eggs from conventional cages than in eggs from enriched cages and aviaries(Englmaierová et al., 2014). They also indicated that the surface bacterial count and microbial contamination (Enterococcus/ Escherichia coli) was lower in eggs from cage systems (conventional ~4.05 log (CFU)/egg and furnished ~3.98 log CFU/egg) than in eggs laid on litter in aviaries (6.24 log CFU/egg)(Englmaierová et al., 2014). So, although the shell thickness was lower in eggs produced in cages, the shell strength was yet higher that of eggs produced in litter house systems. This may be due to ultra-structural features of the shells in cage eggs which are thought to support eggshell strength(Mertens et al., 2006; Ledvinka et al., 2012b).

The assumption is also related to the effect of housing system on pores density. Housing systems can also affect pore density; higher pore numbers were reported in cage eggs in comparison to litter housing system(Tumová et al., 2011).

3.3. Egg Processing

The presence of water plays a key role in the egg content contamination by fecal and other similar contaminants. It is less probable for bacteria to cross the egg-shell (pores/ cracks) in the absence of water (Board et al., 1979). Thus egg washing is a perilous step, since the way water is applied determines the movement of bacteria through the shell in to the egg contents, and the water quality is a key player in the egg washing process. Some countries support and utilize egg washing, while others do not. In Europe, washing of eggs is not allowed currently due to old reports of increased rates of spoilage for eggs washed under less than optimum conditions (Commission., 2008). Conversely, in the US, Australia, and Japan, egg washing is regarded as a safe routine that is always being improved (Hutchison et al., 2004). The major factors that affect egg washing and are to be considered include: wash water temperature, chemicals and pH, water quality.

Water temperature is a main player in the egg washing process, since temperature differentials, as mentioned before, influence bacterial invasion of the egg. Haines and Moran (1940b), reported that placing eggs in a cooler bacterial suspension creates positive pressure gradient that draws bacteria through the shell, while placing eggs in a bacterial suspension of higher temperature creates a negative hydrostatic pressure that diminishes the movement of solvent into the egg. Lorenz and Starr (1952), established that washing increases bacterial spoilage regardless of eggs' original condition, and that cold water produces more bacteria spoiled eggs than warm water. Even though wash water of higher temperature decreases bacterial penetration and results in less spoiled eggs, the increase in wash water temperature seemed to escalate the risk of cuticle damage and thermal cracking. Wesley and Beane (1967)

designated that wash water with a temperature > 45°C should be avoided. Besides the risk of cuticle damage and egg cracking, water temperatures ~ 49°C were found to increase the internal eggs' temperature by ~ 6.7-7.8°C, which generates an ideal environment for the growth of bacteria, including *Salmonella* Enteritidis (Caudill et al., 2010). Leclair et al. (1994), studied the impact of wash water temperature (38°C to 46°C) on S. typhimurium and Listeria monocytogenes. They reported an increase in inactivation of the pathogens with the increase in the water temperature..Also, LUCORE et al. (1997) studied the effect of 3 different wash water temperatures (15.5"C, 32.2"C and 48.9"C) on eggs' internal and external bacterial counts, while using a spray wash for a shorter than usual duration (10 s washing / 3 s rinsing). However, their results were contradictory to the basic idea that cool wash water increases bacterial penetration; the lowest temperature wash water used didn't seem to increase internal shell bacterial counts. Subsequently, Caudill et al. (2010) studied the potential of washing eggs using cool water in terms of egg quality and microbial growth within the egg. They determined that utilizing cold water in commercial shell egg processing at pH of ~10-12, lowers post processing egg temperatures thus speeding up cooling process. Also, they reported that washing with cold water caused no deterioration in egg shell quality, nor increase the presence of aerobic bacteria during 5 weeks of storage.

Wash water chemical composition and pH is a key player in the egg-washing process when it comes to chemical washing that includes washing with sanitizers, electrolyzed water, and ozone (O'Bryan et al., 2017). Wash water of pH (≥10.5) is linked to lower aerobic bacteria counts in wash water, thus better water quality (Bartlett et al., 1993). Also, wash water of high pH (10-10.5) is considered bactericidal to E. coli and Salmonella (Pearson et al., 1987). Jones et

al. (1995), were able to isolate Salmonella heidelberg from the shells of eggs washed at a pH below 10.2. Leclair et al. (1994), reported wash water of pH 10·5 and moderate temperature ~ 42°C, and wash water of pH 10.8 and high temperature ~47·4°C to be effective against L. monocytogenes and S. typhimurium.

Regardless of the pH and it's affectivity in killing various organisms, there exists a debate of whether or not chemical washing of eggs with sanitizers decrease their spoilage potential. Sanitizers can kill bacteria on the eggshell surfaces, but they cannot perform their antimicrobial action on bacteria that have already penetrated the pores of the shells. Rizk et al. (1966), identified that QAC and chlorine sanitizers were effective in removing Salmonella from the surface of artifically contaminated eggs, but couldn't kill Salmonella that penetrated deeply into the shell (blending).

On another note, the use of chemicals and sanitizers in wash water to reduce the bacterial load may cause physical damage to the egg shell and cuticle, and thus increase the risk of bacterial penetration through the eggshell. The different types of chemicals used in the wash water and their pH values cause different microstructural changes to the eggshell surfaces. Kim and Slavik (1996), studied the effect of egg washing with cetylpyridinium chloride (CPC) or trisodium phosphate (TSP) on eggshell surface microstructure; they identified that an increase in CPC concentration made the eggshell surface pitted and decreased the thickness of the cuticle layer, and an increase in TSP concentration made the cuticle layer to fissure, flake, and patched. They also reported that TSP- washed eggs were more porous and permeable than CPC washed eggs. 2 years later, WANG and SLAVIK (1998), studied the effect of washing with other various chemicals on bacterial penetration in eggs, stored at different temperatures. They

established that washing with Quaternary ammonium compound (QAC, pH 7.5), and sodium hypochlorite (NaOCl, 100 ppm, pH 7.5) at 43.3°C reduced bacterial penetration, while washing with sodium carbonate (Na2CO3, pH 12) facilitated bacterial penetration, during egg storage for 21 days. They stated that QAC and NaOCl at 100 ppm didn't destroy eggshell surfaces shielding the eggs from bacterial recontamination, while alkaline Na2CO3 altered the eggshell surface, allowing bacterial recontamination. Gole et al. (2014) reported that washing eggs with hydroxide and hypochlorite based solution (pH 12, 200 ppm, 40°C) damaged their cuticle and increased their permeability to *S.* Typhimurium in comparison with unwashed eggs.

Ozone (O3) is a strong antimicrobial agent that acts against microorganisms at low concentrations(Khadre et al., 2001). It had been studied extensively for potential uses in the food industry(Kim et al., 2003). In 2001, FDA permitted the use of aqueous or gaseous ozone as an antimicrobial in food (Leitner et al., 2001). Many researches have studied the effectiveness of washing with ozone on the eggshell contamination. Koidis et al. (2000), stated that washing Salmonella enteritidis artificially contaminated eggs with aqueous ozone decreased the S. enteritidis populations by 1 log units and 2log units at 22ºC and 4ºC respectively. However very few studies were conducted on egg processing with aqueous ozone; no studies were conducted on the effect of aqueous ozone on egg shell quality or microbial penetration. Researchers focused on studying egg processing with gaseous ozone probably due to its higher efficacy. Rodriguez Romo et al. (2007), reported that 3 minutes treatment with gaseous ozone (12 to 14% wt/wt O3 in O2 mix) rapidly decreased salmonella count on eggshells ~ 5 logs, and was also able to penetrate the egg and inactivate salmonella in egg yolk. Yuceer et al. (2015), in their turn, studied the effect of gaseous ozone on eggs' shelf life; they showed that it helped to

maintain egg internal egg quality and functional properties for a longer time during storage, but it only maintained egg shell quality at low concenrations (2ppm and 4 ppm), while it caused a detrimental effects on eggshell quality at high concentration (6 ppm).

Electrolyzed water (EOW) is produced via electrolysis of weak salt water solution, and can be acidic alkaline or neutral (Huang et al., 2008). Bialka et al. (2004), were the first to study its potential in washing eggs and compare it with sanitizers. They found the acidic EOW treatment to be more effective than alkaline EOW in killing Salmonella and Ecoli; yet the alkaline treatment remained crucial to remove the soil that may be present on the eggs. In comparison to sanitizer both had no impact on eggshell, and similar effect on the cuticle. More recently, Zang et al. (2019) studied the efficacy of slightly acidic EOW (SAEOW) in decontaminating eggs and inactivating salmonella Enteritidis and E. coli; it seemed to show greater bactericidal effect compared to acidic EOW, NaClO, and sterile deionized water. Prominently, SAEOW showed reduced corrosion of egg surfaces in comparison to acidic EOW. Most recently, Medina-Gudiño et al. (2020) analysed the antibactrial activity of neutral electrolyzed water on eggshells contaminated with Salmonella Enteriditis and E.coli; it appeared to reduce salmonella on artificially contaminated eggs by >1.45 Log10 CFU/egg, and E. coli on artificially contaminated eggs by >6.39 Log10 CFU/egg. It furthermore didn't show any interaction with the cuticle. However, it is important to mention that there are still no studies conducted on the effect of washing with EOW on egg bacterial penetration.

Besides the temperature and added chemicals, wash water quality is an essential factor that interferes with the egg washing process. Clean drinking water with low calcium and iron

levels should be used. Garibaldi and Bayne (1962), reported that rising iron content in wash water from 0.4 ppm to 10 ppm increased the Pseudomonas spoilage from 0.8% to 2.5% after 48 days storage at 13°C, while lowering iron content from 4.8 ppm to 0.2 ppm decreased it from 6.2% to 0.8%.

3.4. Egg Storage Conditions (temperature/ humidity/ time)

When eggs are moved from a cold environment to a warmer one, condensation also known as “sweating”, leads to a wet egg surface. The liquid on the surface of the egg and any associated contaminants can then be drawn from the shell surface into the pores and shell membranes. It has been known from a long time, that bacterial contamination of eggs’ internal is more probable in sweating eggs (Fromm and Margolf, 1958). De Reu et al. (2006a) studied the effect of eggshell condensation on bacterial penetration and egg contamination, and found that eggshell condensation enhances bacterial penetration through the eggshell while exerting no significant effect on whole egg contamination. Refrigerating eggs and storing them at low temperatures then placing them at room temperature, is thought to lead to the condensation of water droplets on the eggshell “sweating” and consequently bacterial penetration into the egg (Bruce J.). Nevertheless, refrigeration of freshly laid eggs appeared to reduce the risk of bacterial penetration. Miyamoto et al. (1998), studied the influence of egg freshness and refrigeration on eggshell penetration by Salmonella Enteritidis and Salmonella typhimurium. They found that salmonella seemed to penetrate mostly the shells of freshly laid eggs, and cooling (4°C-15 mins) prior to salmonella exposure suppressed penetration. Fajardo et al. (1995) in turn showed that eggs cooled at 0 °C were penetrated more than uncooled eggs.

As implied above, the risk of penetration is highest in freshly laid eggs and refrigeration right after the egg is laid- prior to contamination limits the penetration process. Yet the long term storage process of "possibly" contaminated eggs is a more delicate procedure. It is well known that as storage time of eggs increase the bacterial penetration increases, irrespective of the storage conditions. As eggs grow old their cuticle dehydrates and shrinks, exposing the pores and increasing the risk of bacterial penetration (Mayes and Takeballi, 1983).

Scientists have been trying to discover the optimal conditions for storage with the lowest risk for bacterial penetration and egg contamination. Old publications recommend the storage of eggs at 4- 5°C and 75-85% humidity for their preservation from microbial contamination (Stadelman, 1977). Similarly, up-to-date guidelines in USA state that $\leq$ $\mathbf{7.2°C}$ $(\mathbf{45°F})$ is the ambient temperature to maintain table eggs (Hester, 2017). In Lebanon, a more recent study conducted by Saleh et al. (2020), reported enterobacteriococcae contamination of the egg shell and yolk of eggs stored at 18°C and 24°C for 2 and 4 weeks, respectively. Yet, they noticed no contamination of eggs stored at 7°C (Saleh et al., 2020). Further, a lot of studies have also been conducted on storing eggs at room temperature (22°C-25°C), and the optimum storage conditions appeared to be dependent on whether or not the eggs are washed or not. Schoeni et al. (1995) studied washed eggshell permeability to different species of salmonella, when stored at different temperatures. Storage at 25°C revealed penetration of S. enteritidis, S. typhimurium, and S. heidelberg in 3 days. On the other hand, penetration was diminished in eggs stored at 4°C, only S. typhimurium was able to penetrate through the eggshells, but not the contents. They also monitored the growth of salmonella on the inside of the egg at different temperatures; salmonella seemed to grow most at 25°C , grow

well at 10°C, but rarely grow/ decrease in number at 4°C. LUBLIN et al. (2015), studied the penetrability of washed eggshells to *Salmonella* Infantis upon cold storage (5.5 ± 0.3°C) and storage at room temperature (25.5 ± 0.1°C). They noticed that penetration took place at both temperatures, but *Salmonella* Infantis seemed to multiply inside the eggs only at room temperature, after 4 weeks of storage. However, they noted that after 10 weeks of storage salmonella was also able to multiply inside eggs stored at 5.5 ± 0.3°C. Gole et al. (2014), studied the effect of temperature on the survival of *S.* Typhimurium on unwashed eggshell and its penetration at 20°C and 37°C. 20°C appeared to be more favorable than 37°C for *S.* Typhimurium survival on the eggshell surface 21 days post inoculation, and lower penetration was observed at 37°C maybe due to the reduced survival of *S.* Typhimurium on eggshell surface. Joshi et al. (2019), confirmed that washed and unwashed eggs react differently to storage temperature. Washed eggs showed higher microbial growth when stored at high temperature (25°C), while unwashed eggs showed higher microbial growth when stored at low temperature (7°C).

Unlike microbial growth, storage temperature seems to affect eggshell quality of washed and unwashed eggs in a similar manner. High storage temperature (25℃) lead to a more severe and 2 weeks earlier eggshell deterioration (starting week 2) in comparison with cool storage (5 and 10℃) , according to Joshi et al. (2019). However, washed eggshell quality appears to be more influenced by storage temperature, knowing that washing alters the cuticle layer (Joshi et al., 2019). As mentioned before, the cuticle is an indispensable part of an egg's physical defense system against bacterial penetration, its composition, thickness, and degree of coverage is affected by egg freshness. With the passage of time well-defined compositional

changes occur in the cuticle of freshly laid eggs(Rodriguez-Navarro et al., 2013). Scientists further considered the effect of storage temperature on the cuticle. Ball et al. (1975), reported that when eggs are stored at 24°C the cuticle deteriorates at a faster rate than at 5°C. Similarly, Liu et al. (2016), whom studied the effect of washing and storage temperature on eggshell quality, found that the deterioration rate of the cuticle upon egg storage at 25 °C was higher than at 7 °C.

III. Antimicrobials use and resistance development risks in poultry

Antimicrobial agents, with their capacity to prevent and treat infectious diseases, are utilized by livestock industries and poultry farms. They are mainly administered in feed or drinking water and rarely via individual injection and oral gavages (Löhren et al., 2009). They are used for therapy, prophylaxis, and growth stimulation. Antibiotics introduced to animals as growth factors are administered at low doses for long duration in comparison to those introduced for treatment purposes, increasing the risk of antimicrobial resistance; the use of antibiotics for growth promotion was banned in countries such as the European union, Mexico, Taiwan, Netherlands, Germany, Denmark, and Sweden (Maron et al., 2013).

1. Common Antimicrobials in Poultry

Antimicrobial groups usually used in poultry include: beta-lactams, polypeptides, aminoglycosides and aminocyclitols, macrolides and lincosamides, florfenicol, tetracyclines, sulfonamides, quinolones and fluoroquinolones and ionophores (Hofacre et al., 2013a).

Betalactams are bactericidal antibiotics (Cozens et al., 1986), known for their betalactam ring (Prescott, 2013). They are one of the most commonly used antimicrobials in veterinary and human medicine. Broad-spectrum penicillins are commonly given orally for preventative and therapeutic purposes in poultry, and are effective against many gram-negative, gram-positive bacteria, and anaerobic bacteria (Prescott, 2013). Penicillin is orally administered to poultry in order to prevent and treat necrotic enteritis, ulcerative enteritis, and intestinal spirochetosis (Giguère et al., 2013). Nowadays, penicillin G is used against Gram-positive bacterial infections like clostridial infections that cause necrotic enteritis (Gadbois et al., 2008), and against gram negative Pasteurella multocida (Sellyei et al., 2009).

Aminopenicillins act on gram positive and gram negative bacteria, but are supposedly more effective against Gram-negative infections. Amoxicillin has been reported efficacious against gram-positive clostridium staphylococcus and streptococcus, and against gram-negative Bordetella bronchiseptica, Escherichia coli, Proteus mirabilis, and Pasteurella, Salmonella and species(Anadón et al., 1996). Ampicillin trihydrate, administered via drinking water at a concentration ≥ 1.65 g/l for 4 days, showed worthy therapeutic effect against E.coli (Goren et al., 1981).

First-generation cephalosporins such as cephalexin are another type of beta-lactams used in poultry and act against Staphylococcus spp., Streptococcus spp., Escherichia coli, Proteus mirabilis, and Klebsiella spp. (Bui and Preuss, 2020). Third generation cephalosporin ceftiofur can be administered via subcutaneous injection to control of E. coli infections and navel infections in broilers (Schriemer et al., 1992). However, Their use in

broilers production is not recommended due to important public health considerations (Collignon et al., 2013).

Tetracyclines are also used widely in food animals, normally via feed or water, for disease prevention and treatment, and growth promotion (Chopra and Roberts, 2001). They are effective against a broad spectrum of gram-positive and gram-negative bacteria, Mycoplasma, Chlamydophila, and Rickettisa spp. (Chopra and Roberts, 2001). Tetracyclines are used for the treatment of chronic respiratory disease (Mycoplasma gallisepticum) (Glisson et al., 1989)and infectious synovitis (Mycoplasma synoviae)(Kleven and Anderson, 1971), as well as of fowl cholera (P. multocida). The tetracyclines routinely used in the poultry industry include chlortetracycline, tetracycline and oxytetracycline (Hagren et al., 2005). Oxytetracycline and tetracycline are used to treat *E. coli*-peritonitis in layers, and also chlortetracycline is used in layers to treat mycoplasmosis (Agunos et al., 2013). In Sudan, majority of animal farmers testified to use antibiotics, most commonly quinolones, in therapy and prophylaxis, while only 5% reported to use them for growth promotion (Eltayb et al., 2012). Quinolones have a bacteriostatic effect against a range of Gram-negative bacilli excluding Pseudomonas aeruginosa, and are ineffective against Gram-positive organisms (Anadón et al., 1990). In Europe, they are used to prevent mycoplasma and salmonella infections, and to treat Escherichia coli and Pasteurella multocida infections in poultry (World Health Organization. Division of et al., 1998). The use of Quinolones such as nalidixic acid or oxolinic acid can lead to rapid development of resistance (Aldred et al., 2014).

Fluoroquinolones are synthetic fluoride-containing quinolone derivatives, they act against gram-negative bacteria and may be effective against some gram-positive organisms

(Peterson, 2001). USA used to approve the use of Enrofloxacin and Sarafloxacin in poultry (World Health Organization. Division of et al., 1998). A study conducted by Stanley et al. (2001) showed that enrofloxacin successfully eliminated Mycoplasma gallisepticum infection in laying hens. Enrofloxacin also controls E. coli infections in chicken. In a study conducted by Glisson et al. (2004), enrofloxacin significantly reduced the mortality in chicken infected with colibacillosis. The use of **enrofloxacin** in broilers to treat colibacillosis caused no increase in resistant E. coli, but an increase in fluoroquinolone resistant Campylobacter jejuni (van Boven et al., 2003).

Amphenicols are broad-spectrum antimicrobials, habitually given to poultry(Yévenes et al., 2018). Chloramphenicol use in food animals in Europe is unapproved being genotoxic and also being implicated in the generation of aplastic anaemia in humans (Chain, 2014). Thiamphenicol and florfenicol are derivatives of chloramphenicol not reported to cause aplastic anemia (Yunis and Gross, 1975), but are still associated with dose dependent bone marrow suppression (Dowling, 2013b). Florfenicol is a better substituent for chloramphenicol having a broader spectrum of activity, lower minimum inhibitory concentration, and being active against many Chloramphenicol and thiampehnicol resistant strains (Syriopoulou et al., 1981). It is indicated for the treatment of respiratory infections it is bacteristatic for salmonellae and Escherichia coli and bactericidal for Haemophilus influenza, it also is slightly more active than chloramphenicol against, Mycoplasma hominis and Mycoplasma pneumoniae but less active against Ureaplasma urealyticum (Graham et al., 1988).

Macrolides act against Gram-positive bacteria such as Streptococcus spp. and Staphylococcus spp., but are only slightly effective against Gram-negative bacteria (Dinos, 2017). They are bacteriostatic but can be slowly bactericidal (Zhanel et al., 2001). Macrolides

erythromycin, tylosin and tilmicosin are commonly used in poultry(Hagren et al., 2005). Erythromycin acts primarily against Gram-positive cocci such as Staphylococcus aureus, and also against different pathogens such as Mycoplasma pneumonia (Shah, 1998). Erythromycin is administered to poultry in water in order to prevent and treat: staphylococcal or streptococcal infection, necrotic dermatitis, infectious coryza, and M. gallisepticum infection (Giguère, 2013b). Tylosin is effective in treating mycoplasma infection in laying hens (Kleven, 2008) and treating clinical and subclinical necrotic enteritis (Collier et al., 2003). However, resistance to tylosin restricts its efficacy in some Mycoplasma gallisepticum isolates (Migaki et al., 1993). On another note, tylosin administered in drinking water for 5 days seemed to immediately ease eggshell abnormalities linked with Mycoplasma synoviae infection(Catania et al., 2010). Tilmicosin controls mycoplasma infections, Pasteurella multocida and Ornithobacterium rhinotracheale bacterial infections (Abu-Basha et al., 2007). Administration of Tilmicosin is drinking water effectively treated experimentally induced Mycoplasma gallisepticum (Charleston et al., 1998). Lincomycin acts like macrolides on many Gram-positive and anaerobic bacteria. It is the only lincosamide approved for use in poultry, and its mainly used treat enteric infections (Lanckriet et al., 2010). Lincomycin-spectinomycin combination can be also administered orally to young chickens to control of mycoplasmal air sacculitis and complicated chronic respiratory disease caused by M. gallisepticum and E. coli (Giguère, 2013a).

Aminoglycosides and Aminocyclitols are bactericidal against gram-negative enterobacteriaceae and pseudomonas, and gram-positive staphylococci (Krause et al., 2016); they also act against mycobacteria (Ho et al., 1997). Aminoglycosides typically used in poultry include gentamicin, streptomycin, and neomycin (Dowling, 2013a). Streptomycin is partially

absorbed indicated for treating systemic E. coli infections (Dowling, 2013a). Neomycin has a local effect and is used to treat enteric infection, including colibacillosis (Marrett et al., 2000). Neomycin is occasionally administered orally in chickens to treat Salmonella infections (Smith and Tucker, 1978). Gentamicin is given to the chickens as prophylaxis and therapy against Salmonella typhymurium infection(Singh et al., 2019). Gentamicin is administered SC to 1- to 3-day old turkey poults and 1-day old chicks in the prevention and treatment of E. coli, P. aeruginosa, Arizona paracolon and Salmonella infections (Dowling, 2013a).

The **aminocyclitols** approved for use in poultry are spectinomycin and Hygromycin (Dowling, 2013a). Aminocyclitols usually administered to poultry include: Spectinomycin, hygromycin, and apramycin(Use). Hygromycin is administered in feed as an anthelmintic rather than as an antimicrobial (Foster III et al., 1960). Spectinomycin is used parenterally to control salmonellosis, pasteurellosis, E. coli, and Mycoplasma synoviae in young poults, but it can also be administered orally or in the water to control chronic respiratory disease and mycoplasma infections in chickens (Dowling, 2013a). Spectinomycin administered via drinking water, is effective in treating E. coli infections (Goren et al., 1988). Spectinomycin is also used in combination with lincomycin; this combination has been indicated, by Hamdy et al. (1979), as effectual against E. coli and Staphylococcus aureus. Apramycin is capable of treating of E. coli infections in poultry. According to Cracknell et al. (1986), it was reported to reduce mortality and improve the final weight in infected chickens. Leitner et al. (2001), in their turn, stated that apramycin acts by preventing the chicken gut colonization by E. coli.

Sulfonamides are bacteriostatic and act on gram-positive, gram-negative bacteria, protozoa, and coccidia (Löhren et al., 2009). They become bactericidal when combined with

diaminopyrimidines (Brumfitt and Hamilton-Miller, 1993). Sulfonamides are frequently used in the poultry industry for therapeutic, prophylactic, or growth-promoting purposes (Löhren et al., 2009). In 1947 and 1948 sulfonamides were indicated for the treatment of fowl cholera (Holtman and Fisher) and pullorum (Roberts et al.) in poultry. However, Sulfonamides such as Sulphaquinoxaline were reported to exert toxic effects on birds (Daft et al., 1989). Another common side effect of sulfonamide use is the decrease in egg production and the deterioration in eggshell quality (Hofacre et al., 2013b). In the US, a potentiated sulfonamide that is a combination of sulfadimethoxine and ormetoprim is seldom used, with a 5 day meat withdrawal period for birds under 16 weeks of age, to prevent mortality from coccidiosis and bacterial infections (Noack et al., 2019). Contrariwise, in Europe, the use of sulfonamides is banned, even for the prevention of coccidiosis in poultry (Noack et al., 2019).

Nitrofurans, identified by their 5-nitrofuran ring, are bacteriostatic antimicrobials effective in treating infections caused by Escherichia coli, Salmonella sp., and also some protozoans (Cooper and Kennedy, 2005). Furazolidone, furaltadone, nitrofurazone, and nifursol are the nitrofurans most commonly used in food animals (Barbosa et al., 2011). Nitrofuran derivatives are considered carcinogenic and genotoxic in mammalian cells(McCalla, 1983). Semicarbazide, an example of a nitrofurazone side-chain metabolite, is a strong carcinogen and mutagen that causes severe health hazards (Tian et al., 2014). The use of nitrofurans in livestock and poultry has been prohibited in many countries like Australia, Brazil, Philippines, Thailand, and the United States (Khong et al., 2004). In Europe it had been prohibited since (1993) for furaltadone and nitrofurazone and (1995) for furazolidone. However, nitrofurans

continued to be used illegally according to the Rapid Alert System for Food and Feed (Union, 2016).

Peptide antibiotics used in poultry sector include polymyxin E (colistin), bacitracin, and fosfomycin. Colistin is widely used in veterinary medicine; it is often used at low doses in feed to promote growth in countries where this practice is permitted (Kempf et al., 2013). It is utilized as therapy in case of gastrointestinal infections against non-invasive Enterobacteriaceae (EMA, 2016). In poultry, it is mainly used for treating colibacillosis since its use against Salmonella infections is prohibited; other drugs such as sulphonamides, tetracyclines and penicillins are undoubtedly more suitable for the treatment of colibacillosis (Löhren et al., 2009). Effectivity of colisitin to primary diarrheal disease due to E. coli is considered to be rare in poultry (Nolan et al., 2017). The use of colistin combined with other antimicrobials is outlawed (EMA, 2016). Bacitracin is given in the form of bacitracin methylene disalicylate or zinc bacitracin, to promote growth and to prevent and treat enteritis (Butaye et al., 2003). Administration of 55-100 ppm bacitracin in feed prevented necrotic enteritis (C. perfringens) in chickens, according to Wicker et al. (1977). Its use for growth promotion has been banned in the European Union since 2006 (Castanon, 2007). Fosfomycin, introduced orally, was reported to be successful in controlling experimental E. coli infection in broilers (Fernández et al., 1998). Yet, the use of fosfomycin in food animals is implausible, because of its high potential in treating multidrug resistant bacteria in humans, discussed by Ruiz Ramos and Salavert Lleti (2019).

Ionophores are extensively used in the poultry via feed to prevent diseases or to promote growth; monensin, lasalocid, salinomycin, narasin, and maduramycin are the

ionophores used in Europe(Novilla et al., 2017). Only monensin (in bovines) and salinomycin (in pigs) are effectively registered as growth promoters. They are primarily used to prevent coccidial infections (Dowling, 1992). Ionophores also act against Gram-positive bacteria, especially anaerobes such as Clostridium perfringens (Devriese et al., 1993). Narasin was reported to be effective in preventing necrotic enteritis in broilers (Brennan et al., 2001). According to Lanckriet et al. (2010), supplementation of the diet from the first day with salinomycin, narasin or maduramicin reduced necrotic enteritis lesions in birds. Ionophores were also found to have an anti-mycoplasma effect according to Stipkovits et al. (1984) who described this effect for lasalocid sodium. However, ionophores' effect against mycoplasma wasn't as efficient as other anti-mycoplasma drugs(Stipkovits et al., 1987)

Antibiotics used in the poultry industry in Lebanon exclude betalactams, sulphonamides, nutrofurans, and ionophores. The specific antibiotics used are listed in the Table 1. From the tetracyclines group, doxycycline is used. Doxycycline, a lipophilic Ab, highly penetrates into tissues and is reported to be more active in vivo than other tetracyclines (Riond and Riviere, 1988). Doxycycline's lipophilicity makes it deposit in higher concentrations, and last for longer periods than oxytetracyline in eggs when administered orally (Yoshimura et al., 1991b). Due to its high risk of deposition, the use of doxycycline in chicken is highly regulate, and its use in layers is banned in some regions like Europe (Anadón et al., 2018) and China (Yang et al., 2016).

2. The Misuse of Antimicrobials in the Poultry Industry

The use of antibiotics in poultry is firmly regulated by the Food and Drug Administration (FDA), which approves the use of drugs, in therapy and prophylaxis, after accessing their safety

in edible tissue (Act, 1958). For antibiotics approval, the FDA performs different toxicology tests, assigns the optimal antibiotic dosage, then ascribes a withdrawal period that maintains antibiotic residues in edible tissues within the tolerance level (Donoghue, 2003). However, antibiotic treatments are often overused or misused intentionally or most of the times unintentionally in poultry.

Many farmers are unaware of the danger of antibiotics, and their potential transfer to human consumers. Livestock farmers were found to have unlimited access to antibiotics while also disobeying withdrawal periods postulated by the FDA for antibiotic usage in food producing animals. A study conducted on farms in Uganada showed that 95% of farmers didn't obey withdrawal periods even though 80% were aware of their significance(Sasanya et al., 2005). In Sudan, while studying poultry farms, Sirdar et al (2012) reported that 49% of the farms surveyed were using antibiotic treatment and 59% had used it in past 3 months. In the same year, Eltayb et al. (2012) described that only 30% of farmers in Sudan were educated about antibiotic resistance, and around 50% were not aware of common zoonotic infections. In Maiduguri Metripolis Borno state in Nigeria, 85.7% of farmers seemed to use antibiotics for disease prevention. 97.1% of the farmers seemed not to be aware of the effect of antibiotic on public healthy and 82.9% did not know about antibiotics can residue in table eggs(Galad et al., 2018). In Bangladesh 39.1% of farmers were aware of antibiotic residues, yet 94.16% of farms were found to use antibiotics without obeying the withdrawal period. Besides, 49% of antibiotics found were in the watch groups, 8% in reserve groups (WHO AWaRe categorization), and 73% were those of critical importance in human medicine (Ferdous et al., 2019). To which extent the inappropriate use of antibiotic leads to the accumulation of antimicrobial residues in

animal tissue and animal products including meat and eggs is debatable in the literature. Further, the possibility of such a widespread use of antibiotics leading to the development of resistant bacterial strains further requires detailed observations and review.

3. Detection of Antibiotic Residues in Chicken and Eggs

It has been well stated that antimicrobial residues in food may result in side effects such as toxicity and allergic response in consumers, and may also lead to the development of antimicrobial resistance (AMR) among bacterial pathogens (Cerniglia et al., 2016). The presence of high concentrations of antimicrobial residues is being reported worldwide in chickens receiving therapeutic or prophylactic antibiotic regimes casually without considering recommended withdrawal times.

A lot of scientists targeted the antibiotic residue deposition in eggs. Al-Ghamdi et al (2000) reported that eggs from farms in the eastern province of Saudi Arabia contained tetracycline residues, and the residues exceeded the MRL in 14.4% of antibiotic positive eggs. Mehtabuddin et al. (2012) detected sulfonamide residues in 30% of egg samples collected from poultry farms in Rawalpindi/Islambad; in 10% of these samples however sulfonamide residues exceeded MRL. In Trinidad 6.5% of farm eggs, 6.1% of mall eggs1, and 15.0% of supermarket eggs appeared to be contaminated with antibiotic residues. 6.5% of the residues were sulfonamides, 3.8% were macrolides, and 2.7% were tetracyclines.(Adesiyun et al., 2005). Chowdhury et al. (2015) reported the presence of Tetracycline, amoxicillin, and ciprofloxacin residues in local (10.46 μg/g) and commercial (48.82 μg/g)eggs in Chittagong. In the traditional market of Yogyakarta City Widiasih et al. (2020) reported that 75% of antibiotic contaminated

eggs contained penicillin residues, 12.5% contained residues of aminoglycosides, and also 25% contained oxytetracycline residues.

To minimize the public health risk posed to consumers by antimicrobial residues in eggs, maximum residue levels (MRLs) were established for various antimicrobial agents in foods (WHO, 2016). Amjad et al., (2005) reported the presence of residual enrofloxacin , ciprofloxacin, flumequine and oxolinic acid in poultry products marketed in Pakistan. In Tehran, Iran oxytetracycline and enrofloxacin residues were identified in chicken from different farms. 95.55% of farms showed residues of oxytetracycline exceeding Maximum Residue Limits MRLs (Farahmand et al., 2006), and 24% of farms showed enrofloxacin residues exceeding MRLs (Farahmand et al., 2007). In algeria 85.51% of poultry meat samples from farms were tested positive to antibiotic residues. 75.81% of poultry meat samples were contaminated with β-lactam and/or tetracycline, 44.35% with macrolide and/or β-lactam, 36.29% with sulfonamide, and 13.71% with aminoglycoside(Titouche et al., 2013). In Chittagong district of Bangladesh, Sattar et al. (2014) identified residues of tetracycline, ciprofloxacin, enrofloxacin, and amoxicillin in chicken livers, kidneys, thigh muscles, and breast muscles. Jammoul and Darra (2019) screened for antibiotic residues in chicken samples collected from farms all around Lebanon. 77.5% of samples were contaminated with antibiotic residues, and out of them 53.75% revealed multidrug residues. The quinolone ciprofloxacin seemed to occur the most (32.5%), followed by the β-lactam amoxicillin (22.5%) and then tetracyclines (17.5%). Sarafloxacin, amoxicillin, and penicillin G residue levels appeared to exceed the Maximum Residue Limit (MRL) recommended according to the European Union EC.

Antibiotics, usually administered via feed or water to hens, get absorbed by the intestines then transported throughout the body via the blood circulation. Antibiotics reach the ovaries , the site of egg content formation and secretion, where they run the risk of residue deposition in the egg (Alaboudi, 2017). In fact, when the absorption of antibiotics in the gastrointestinal tract (GI tract) is higher, they are more bioavailable and consequently their chances in deposition in the egg rise.

Aminocyclitols and Aminoglycosides are poorly absorbed in the GI tract (Bennett et al., 2001) they have low chances of residing in eggs. Roudaut (1989b) showed that no residues were detected in eggs after 5 days of administration of 0.25 g/1 neomycin in the drinking water, (1 g/1) spectinomycin in feed, nor 1 g/1 DHS in drinking water. However, when DHS was administered intramuscularly around 1% of the dose administered was excreted via the eggs mostly the yolk (95%). Similarly when gentamicin was administered via subcutaneous and intramuscular injections to laying hens it deposited in egg yolk and albumen, with most residues recovered from the yolk (Filazi et al., 2005).

Chloramphenicol is absorbed rapidly but incompletely in the GI tract, and then it is rapidly distributed throughout the body (Anadón et al., 1994). Samouris et al. (1998), identified chloramphenicol residues in albumen and yolk of eggs from hens treated via feed for 5 days. Florfenicol is partially metabolized, it reaches it maximum plasma concentrations at around 0.63 hours after oral administration in chicken, and persists in edible tissues for around a week (Anadón et al., 2008). Xie et al. (2012), identified florfenicol and its metabolite florfenicol amine in eggs of layers treated orally for 5 days and reported that the residues persisted for around 10 days in the whole egg.

Orally administered penicillins are susceptible to hydrolysis in the GI tract (Prescott, 2013). Ampicillin however is quite stable in gastric acid and it is absorbed more than other penicillins (McKellar and Horspool, 1995). Still, ampicillin is more bioavailable in intramuscularly injected hens. Roudaut et al. (1987a) showed that ampicillin deposition and persistence in eggs of hens treated with 20mg/l ampicillin intramuscular injection was greater than that in eggs of hens treated orally with 1.5g/l ampicillin.

Cephalosporins are another subgroup of betalactams; the cephalosporin cefadroxil has high oral bioavailability in chicken, around 77.18% according to Elkomy et al. (2019). Cephalexin an example of another cephalosporin was found to have the ability deposit in egg yolk (60 ng/g) (Kitagawa et al., 1988).

Macrolides' bioavailability is high; they are distributed extensively after absorption and penetrate tissues well. Tilmicosin is rapidly absorbed and slowly eliminated after oral administration (Abu-Basha et al., 2007). Likewise, Erythromycin doses in plasma after oral administration are as high as those after intramuscular and subcutaneous injection (Goudah et al., 2004). Also, Tylosin's systemic bioavailability in broilers after oral administration, as reported by Sedeik (2018) is 90.29%.

Post oral administration macrolides can deposit in eggs. Yoshimura et al. (1979), compared antibiotic residues in eggs of layers treated with different macrolides via drinking water for 1 week. They stated that erythromycin and tylosin began to show in eggs the day after commencing medication, and that their levels increased in eggs on daily basis during treatment. Ji et al. (2019), in turn, analyzed the tilmicosin residues in eggs treated via drinking

water for 5 days, and reported maximal tilmicosin residues in albumen on the last day of treatment and in whole eggs and yolks on day 2 post treatment.

Quinolones, like oxolinic acid, have high oral bioavailability and are absorbed rapidly in the GI tract and distributed extensively to tissues (Hamamoto et al., 2001) . Quinolone residues can persist in chicken tissues and eggs for several days. Eggs of hens treated orally (water and feed) with oxolinic acid for 5 days appeared to contain residues, and residues persisted for a week or more after treatment discontinuation (Roudaut, 1998). Orally administered fluoroquinolones are quickly absorbed and bioavailable. Oral bioavailability of enrofloxacin and sarafloxacin in chickens were reported be around 64% (Anadón et al., 1995) and 59.6 +/- 13.8% (Ding et al., 2001), respectively. Chu et al. (2000), detected sarafloxacin residues in eggs of orally treated layers starting second day of dosing, and reported maximum levels at 24 h after drug withdrawal. Further,Lolo et al. (2005), identified gradually increasing enrofloxacin residues in eggs of hens treated orally and intramusculary until cessation of treatment.

Sulfonamides are moderately absorbed from the GI tract, depending on their solubility. Kiser et al. (1948), studied the blood concentrations of 7 sulfonamides introduced via feed. More recent studies focused on sulfonamide combinations like sulfadiazine along with trimethoprim that was reported to have oral bioavailability of around 80% for both components (Baert et al., 2003). Sulfonamides can deposit in eggs; Romváry and Simon (1992) identified sulfonamide residues in egg of layers treated via driniking water with sulfaquinoxaline alone or with sulfonamide mixture (sulfaquinoxaline, sulfadimidine, sulfamerazine). They indicated that sulfonamide residues persist in eggs 5.2-7.4 days post withdrawal of treatment. Likewise, Roudaut and Garnier (2002) reported that 0.9–1.4% of the dose of sulphadimidine and

sulphadimethoxine, administered to laying hens orally via water (5 days), was deposited in eggs (starting day 2). Atta and El-zeini (2001), identified trimethoprim and sulphadiazine residues in eggs of layers treated with trimethoprim and sulphadiazine combination via drinking water for 5 days. Maximum concentrations of the drugs, however, were obtained 1 day after drug withdrawal.

Tetracyclines' absorption in the GI tract is determined by their lipophilicity; the higher the lipohilicity the higher the absorption. Oxytetracycline is the least lipophilic, after oral administration its absorption half life time is around 0.49 ± 0.38 h and its bioavailability 12.13 ± 4.56% (Ziółkowski et al., 2015). Doxycycline is the most lipohilic, it is absorbed rapidly, and it has oral bioavailability of around 41.33 +/- 2.02% (Anadon et al., 1994). Tetracyclines can be deposited in the eggs of laying hens. Roudaut et al. (1987b), reported the presence of oxytetracycline residues in eggs of hens treated with 0.5 and 0.25 g/1 via drinking and in eggs of hens treated with 300ppm in feed for several days. 2 years later Roudaut et al. (1989) studied the deposition of tetracycline and chlortetracycline in eggs of layers treated orally, and identified that the deposition of tetracycline in eggs was 3-fold higher than the chlortetracycline deposition. They also reported that post treatment, tetracycline residues persisted in eggs 6-11 days, while chlortetracycline persisted 9 days. Yoshimura et al. (1991a), in turn, studied the deposition of doxycycline and oxytetracycline in eggs of layers treated via drinking water for a week, and reported that doxycycline deposits in higher concentrations in eggs and persists longer than oxytetracycline.

Colistin is not absorbed well from the GI tract (Gupta et al., 2009); its bioavalability is low after oral asministration but high in case of intramuscular subcutaneous injection. Colistin

residues were not identified in eggs of layers treated via drinking water but they were detectable until 7 days in eggs of layers treated via intramuscular injection (Roudaut, 1989a).

Nitrofuran drugs are introduced via water or feed for layer hens' therapy (Barbosa et al., 2012). They are metabolized rapidly in birds after oral administration, yet their metabolites bind to tissues and persist at low concentrations weeks post-treatment (Vass et al., 2008). McCracken et al. (2001) weren't able to detect furazolidone in eggs 4 days post treatment of layers via feed, but they reported the presence of its metabolite 3-amino-2-oxazolidinone (AOZ) up to 21 days post the withdrawal. McCracken and Kennedy (2007) also studied the deposition of furazolidone, furaltadone, nitrofurantoin, or nitrofurazone in eggs of layers after 1 week treatment without withdrawal, and reported the presence of both parent compounds and their metabolites, metabolites being at higher concentrations.

Ionophores like monensin (Henri et al., 2009), narasin (Catherman et al., 1991), and salinomycin(Atef et al., 1993) are rapidly absorbed in the GI tract of poultry and widely distributed to tissues. Monensin (Donoho et al., 1982) and narasin (Sweeney et al., 1996) are extensively metabolized and rapidly eliminated, while lasalocid persists in tissues for a longer period(Anadón and Martínez-Larrañaga, 2014). Kan et al. (1990) studied the residues of different coccidiostats in laying hens treated via feed for 7-14 days, and identified residues of all of them including ionophores narasin and salinomycin but not monensin in the eggs shortly after the treatment. Kennedy et al. (Kennedy et al., 1996) identified lasalocid residues in around 66% of eggs from hens treated via feed with 3.7% containing more than 40 ng/g; residues persisted in eggs for 10 days post withdrawal of treatment. Lasalocid is the ionophore that has the highest potential to accumulate and persist in eggs (Kennedy et al., 1998). In 1998 they

detected monensin, salinomycin and narasin in 3.7% , 1.2%, and 0.6% of eggs in concentrations less than 2.5 ng/g. However, lasalocid was detected in 66.4% of eggs at concentrations, ranging from 0.3 to 129 ng/g.

Antibiotic residues accumulate first in the egg's albumen if antibiotics are administered at the beginning of egg shell formation, when the egg is in the magnum that is depositing the albumen (Donoghue and Myers, 2000). In the yolk, antimicrobial deposition depends on antibiotic exposure length relative to yolk follicular growth, since yolk takes a long time to develop. In actively laying hen, numerous follicles at different developmental stages exist simultaneously, and before an egg is laid the yolk undergoes a stage of rapid growth, in which it increases in size exponentially over 10 days (Etches, 1996). Drugs deposited in the yolk will rapidly accumulate during yolk growth, and those residues can persist for days after cessation of treatment. A study conducted by Donghue et al. (1997) reported that ampicillin residues persisted in the yolk 6 days post treatment with intramuscular injection. Samouris et al. (1998), identified chloramphenicol residues in albumen 1 day before detecting it in yolk of eggs from layers treated via feed for 5 days. Residues persisted for a longer period in the yolk (7-9 days) than in albumen (3-4 days) after cessation of treatment. Xie et al. (2012), reported that florfenicol drug residues persisted for 7 days in albumen and 11 days in yolk post oral treatment (100.0 mg/ kg florfenicol). In a study conducted by Giorgi et al. (2001) thiamphenicol residues were first identified in maximum values in egg white 1 day after single oral administration (40 mg/kg), they then declined in the eggwhite and peaked in yolk on day 3 post the drug administration. Macrolide drugs triacetyloleandomycin, erythromycin, tylosin, and kitasamycin were also found to remain longer in yolk than in albumen after withdrawal of

drinking water treatment in hens according to Yoshimura et al. (1979). Ji et al. (2019), in turn, showed that tilmicosin residues were maximal on the last day of drinking water treatment regime in egg albumen and 2 days post treatment in the egg yolk. They reported that it took 24-44 days, and 19-49 days of withdrawal for tilmicosin to become undetectable in egg yolks and whites, respectively. Lolo et al. (2005), identified gradual increase in enrofloxacin residues in eggs of hens during oral and intramuscular treatment, and reported the persistence of metabolite ciprofloxacin for several days after cessation of treatment in the yolk. Similarly in a study conducted by Chu et al. (2000) sarafloxacin residues were found to persist post oral treatment for 2 days in albumen and 7 days in yolk. Sulfonamides, in a study conducted by Romváry and Simon (1992) were found to reach maximal levels at the last day of layers medication in the albumen and 3 days after withdrawal in in the yolk. Atta and El-zeini (2001)reported, after withdrawal of 5 day drinking water treatment in layers, Trimethoprim residues persisted around 5 - 7 in yolk and day 4 - 6 in albumen, while sulphadiazine residues persisted 4- 6 in yolk and day 5 -7 in albumen. Nagata et al. (1989) showed that Sulfadimethoxine and sulfamonomethoxine remained in egg yolk up to 8 days after withdrawal of 3 week dietary treatment. The tetracyclines: oxytetracycline, tetracycline, and chlortetracycline were reported to persist in the egg yolk for 3 days, 6-11 days, and 9 days post treatment, respectively (Roudaut et al., 1987b, 1989). Doxycycline in its turn deposits in higher concentrations and persists longer than oxytetracyline (Yoshimura et al. (1991a)).

The residue distribution and deposition pattern varies between albumen and yolk also according to drug's lipid solubility or protein binding ability, and physicochemical characteristics (molecular weight or pKa value). The different pH values of the egg white (pH=7.6–7.9) and yolk

(pH= 6.0) affect the dissolving capabilities of antimicrobial drugs with specific pKa values (Kan, 2003). Alaboudi et al. (2013) studied the distribution of Chlortetracycline and sulfanilamide residues between egg yolk and white, and stated that the antimicrobial residue prevalence were more prevalent in eggwhite than in yolk. However, in a few samples residues were identified in both yolk and white simultaneously. The levels of both chlortetracycline and sulfanilamide exceeded MRL in 1.2 and 4% of the positive samples respectively

4. Prevalence of bacteria in eggs and their antibiotic resistance

The overuse or misuse of antibiotics is reflected in the prevalence of resistance to antimicrobial agents. The occurrence of antimicrobial resistance (AMR) in bacterial pathogens, present in food, threatens food's safety and imposes a huge risk on public health. Resistance rates and antibiotic minimum inhibitory concentrations in gram negative bacteria isolated from conventional poultry farms, seem to be higher than those in bacteria isolated from poultry in organic keeping systems where the antibiotics use is restricted(Schwaiger et al., 2008a). Resistant bacteria can be transferred to humans through handling and consuming contaminated food products as discussed by Wang et al. (2012). The infection of humans with antimicrobial resistant bacteria from food threatens therapeutic failures in humans.

Papadopoulo et al. (1997) were the first to study the presumed transfer of antibiotic resistance from poultry (eggs) to humans. They reported the presence of Staphylococcus aureus resistant to penicillin-G, tetracycline, erythromycin, clindamycin, cefalosporins, oxacillin, gentamycin, chloramphenicol and tobramycin, Enterococcus faecalis resistant to

ampicillin, ciprofloxacin, clindamycin, gentamycin and tetracyclin, Escherichia coli resistant to tetracycline, erythromycin, ampicillin and cefalosporins, Enterobacter cloacae resistant to ampicillin, amoxycillin plus clavunalic acid, erythromycin and tetracycline, Pseudomonas stutzeri resistant to erythromycin and chloramphenicol, and Citrobacter freundii resistant to ampicillin, amoxicillin/ clavunalic acid, cefalosporins and co-trimoxazole. The prevalence of antibiotic resistance in eggs has been studied extensively since that research. In year 2000 (Chang) studied the presence and antibiotic resistance of Salmonella spp. in raw broilers and shell eggs in Korea. They reported that all Salmonella isolates showed multiple antibiotic resistance patterns. All isolates were resistant to penicillin and vancomycin, and most of them to erythromycin too. One strain showed resistance to all 12 antibiotics tested (ampicillin, amikacin, chloramphenicol, carbenicillin, cephalothin, ciprofloxacin, erythromycin, gentamicin, kanamycin, neomycin, tobramycin, penicillin, streptomycin, trimethoprim, tetracycline, and vancomycin.) A study conducted by Frye and Fedorka-Cray (2007) to determine the ceftiofour resistance in salmonella isolates from diagnostic laboratories, farms, and slaughter houses, between 1999 and 2003, reported a rise in resistance from 4.0% in 1999 to 18.8% in 2003. Isolates from diagnostic laboratories were found to have higher levels of resistance (18.5%) than those from farms (3.4%) and slaughter (7.1%); levels of resistance were lowest in eggs (3.6%). Adesiyun et al. (2006b) , in their turn concentrated on bacterial contamination and resistance in table eggs. They reported the contamination of 38.6% of egg samples with enteric microbes, excluding *E. coli*, *Salmonella*, *Campylobacter* spp. and *Listeria* spp. They recognized resistance to multiple antibiotics in 95.4% of the bacterial isolates, with highest resistance reported to

streptomycin (90.1%), tetracycline (51.9%) and kanamycin (30.5%). In their next study, they focused on the sensitivity *of Salmonella* spp. and *Escherichia coli* isolated from table eggs in sale outlets. They reported 88.1% of *E. coli* and 22.9% of salmonella isolates to be resistant to one or more antibiotic, with highest resistance reported to streptomycin 54.2% and tetracycline 35.9% and lowest resistance reported to gentamicin 11.5% and sulphamethoxazole/trimethoprim 9.4% (Adesiyun et al., 2007). According to their research 1.4% of *Salmonella* isolates and 46.6% of *E. coli* displayed multi-resistance. They denied any link between antibiotic-resistance frequency and egg isolation location or the egg source (Adesiyun et al., 2007). On the other hand, a study conducted by Alvarez-Fernandez et al. (2012) proved that the housing system influenced the average resistance in different E.coli strains isolated from eggs, with the highest resistance reported for eggs from conventional cages and barns followed by free range, and lowest resistance in eggs from organic and domestic production systems. Schwaiger et al. (2008b) investigated the prevalence and resistance of Gram-negative bacteria in laying hens and eggs from organic and conventional cages against 31 different antibiotics. They mainly detected E. coli and scarcely Salmonella and campylobacter in eggs. Organic E. coli isolates were highly susceptibile to amoxicillin/clavulanic acid, ampicillin, cefaclor, cefoxitin, cefuroxime, doxycycline, mezlocillin, neomycin and piperacillin. However, gentamicin and tobramycin appeared to be more effective on conventional isolates. In 2011, Arathy et al. (2011) studied the antimicrobial resistance profile of E coli isolated from the shell and yolk of commercial chicken eggs from several locations in Grenada. They reported resistance to one or more antibiotic in 64.7% of shell membrane isolates and 52.4% of yolk isolates, with highest level

of resistance recorded for ampicillin. 10.9% of the isolated showed multi-drug resistance. Also, Khan et al. (2015) studied the antibiogram of E. coli isolated from table eggs in Peshawar, Pakistan. They found E. coli to be most sensitiveto ciprofloxacin and enrofloxacin, slightly sensitive to amoxicillin and kanamycin , and completely resistant to tetracycline. In 2013, (Pyzik and Marek) were able to isolate and study the resistance of Staphylococcus aureus in the shell surfaces and contents of chicken eggs from laying farms in Lublin. S. aureus strains showed resistance to at least 1 tested antibiotic, yet 55.55% revealed resistance 5 more agents of the 17 antimicrobials tested. 66.66% of the strains were found to be resistant to erythromycin and tetracycline, 61.11% to oxytetracycline, 50% to penicillin G, and 44.44% to amoxicillin. In the same year, Han et al (2013) studied the antimicrobial susceptibility of Salmonella Enteritidis isolates from human patients, retail chicken, broiler farms, and egg production facilities. 56% of Salmonella Enteritidis isolates appeared resistant to 1 antimicrobial tested at least, and no isolates were found to be resistant to more than 3 antibiotics of those tested. Resistance to sulfisoxazole was 54%, to nalidixic acid 7.4%, and to streptomycin, ampicillin, and tetracycline 5.6%. In 2016, (Kemal et al.) estimated the levels and patterns of antibiotic resistance of Salmonella in chicken eggs. 5.3% salmonella existed on eggshells from the open market, while none were detected on eggshells from poultry farms. All salmonella isolates showed resistance to erythromycin and clindamycin, whereas all isolates were susceptible to ciprofloxacin and 87.5% to chloramphenicol; multiple antibiotic resistance was found in all isolates. In the same year, Kilonzo-Nthenge et al. (2016) investigated the prevalence of antimicrobial resistant Enterobacteriaceae in shell eggs purchased from small poultry farms and farmers'

markets. E. coli was detected on eggshells (11.9 %) and in shell contents (5.2%), Enterobacter (9.1 and 7.9% respectively), and Serratia (11.5 and 4.8% respectively). Salmonella was isolated from 3.6% of eggshells and absent in egg contents. Isolates from farm and market eggshells showed resistance to erythromycin (48.5 and 32.8%, respectively), ampicillin (44.8 and 17.2%), and tetracycline (29.9 and 17.2%). Multidrug resistance was reported in E. coli and Pantoea, and to a lesser extent in Salmonella and Klebsiella terrigena. A recent study conducted by Adesiyun, A.A., et al., (2020) also regarded the occurrence and resistance of Salmonella spp. and E. coli in table eggs from farms in Gauteng Province, South Africa. They focused on E.coli due to its higher prevalence in the table eggs, and found 71.4% of *E*. *coli* isolates resistant to one or more antibiotic. Highest resistance was recorded to therapeutic doxycycline, oxy-tetracycline, and amoxicillin.

Materials and methods

Given that Lebanese farming systems vary greatly between organic and commercial layers, and with the absence of clear guideline, guidance and regulatory oversight to commercialization of eggs including transport storage and consumption conditions, we found it essential to 1) compare the initial bacterial contamination between organic and commercial eggs, 2) study the dynamics of bacterial growth in the different egg compartments, as well as 3) analyze shell porosity evolution and potential trans-shell bacterial contamination. Furthermore, we aimed to 4) detect antibiotic residues, and 5) Antibiotic resistant bacteria in order to assess the best handling conditions of the egg from farm to kitchen.

1. Consumables and Materials

Consumables: sterile forceps, sterile conical tubes and petri dishes, sterile swabs and loops, sterile cork borers and syringes from Labise. Bacterial growth media, namely TBX Chromogenic agar (ISO 16649—2,3) CAT: 21151.00 from Deben Diagnostics Ltd CAT, Salmonella Shigella agar (SS agar) Cat. 1064.00 from CONDA, and Mueller Hington agar (MH agar) Cat. 1058.00 from CONDA, and nutrient broth CAT: 1216.00 from CONDA. Ecoli ATCC 25922 were purchased from microbiologics, Antiserum Escherichia coli NONAVALENT Ref 57411 from Bio-Rad, API 20E from BioMerieux, and Antibiotic disks from OXOID: Spectinomycin (SPT 100 ug)/ Tylosine (TL 15 ug)/ co-trimoxazole (COT 25 ug)/ Erythromycin (E 15 ug)/Gentamycin (GEN 50 ug)/ Doxycyclin (DO 30 ug)/ Colistin (CT 10 ug) / Enrofloxacin (ENR 5 ug)/ Ampicillin (AMP 10 ug)/ Amoxycillin (AML 25 ug)/ Neomycin (N 30 ug).

2. Sample collection and experimental design

A total of 22 organic, and another 22 commercial eggs were collected weekly (4 per time point), divided into 2 groups, and stored either in the fridge (4C) or at room temp (20-25 C). After 5 weeks of

sampling, eggs were transported carefully to the lab in a paper bag, at room temperature. In the lab, eggs were analyzed on spot as described in the following sections. The housing system from which the organic eggs were collected is considered semi-intensive, hens were allowed to roam on ground range during the day, but they were confined into a house at night. The house floor resembled that of a litter floor system, since it was rarely cleaned, and chickens laid eggs in nests. Day 0 organic eggs were collected one day before analysis in order to simulate the commercial conditions.

Store-bought eggs (commercial) were collected from a conventional cage system. They were purchased from the same seller, with an expiry date of 5 weeks and a time lag of one day (Day 0) between collection and commercialization.

3. Sample processing

Each egg was sequentially analyzed for porosity and bacterial contamination of the various compartments. Bacterial contamination was conducted by analyzing the egg shell, cleaning it, breaking it, separating the albumen from the yolk, and then analyzing each as described below. Further, day 0 eggs were analyzed for the presence of antibiotic residues using a microbiological assay as described below.

Porosity: Egg shell was observed for porosity by visualizing organic and commercial eggs through a high intensity lamp surrounded by a cardboard cone to expose the pores and photographed in a dark room at all-time points and storage conditions. Images were compared for porosity as determined by the light beam illumination diameter (pore size) and frequency (pore numbers).

Egg compartments: a sterile cotton swab was soaked in nutrient broth and then used to wipe the eggshell and collect any resident bacteria by inoculating a 6 mL nutrient broth in a conical tube. The eggshell was then disinfected by wiping with 70% ethanol. A sterile forceps was then used to crack the eggshell around the air sac area. Albumen and yolk were collected separately in a sterilized plastic egg

tray. Sterile loops were then used to homogenize each of the albumen and yolk and then inoculate a 5-6 ml of nutrient broth.

Antibiotic Residues: Antibiotic residues were assessed using an adapted microbiological assay (USDA ref). Briefly, MH agar was inoculated with E. coli ATCC25922. A crock borer was used to make 2 holes of 10 mm diameter. The homogenized yolk and albumen were added into the holes using sterile syringes in 2 replicates per sample, and then plates equilibrated at room temperature prior to incubation at 37 °C for 24 hours.

4. Bacterial growth, isolation, and identification

Growth media preparation and incubation: 1 L of nutrient broth was prepared by suspending 8g of the medium into 1L of distilled water followed by heating and stirring for 1 minute. 1.5 L of MH agar was prepared by suspending 57g of agar medium into 1.5 L of distilled water followed by heating and stirring for 1 minute. 900 mL of TBX agar was prepared by suspending 33g of agar medium in 900 mL of distilled water followed by heating and stirring for 1 minute. 900 mL of SS agar was prepared by suspending 54 grams of agar medium in 900 ml of distilled water followed by heating and stirring for 1 minute. All the prepared media were then autoclaved for 15 minutes at 121°C. The inoculated nutrient broth conical tubes were incubated at 37ºC for 48 hours in a shaker incubator. SS and TBX agar were used, respectively, to isolate any possible salmonella or E. coli growth. One loop full of each sample (eggshell, albumen, and yolk) was sub-cultured from the nutrient broth to SS and to TBX agar. The inoculated SS and TBX plates were then incubated at 37 °C for 24 hours.

API 20E testing: api 20E was used, following manufacturer guidelines, for further identification of typical Salmonella-like and E.coli-like colonies (Swanson and Collins, 1980). Briefly, suspected colonies were mixed in 5 ml of sterile water solution, homogenized, and then pipetted into the different wells on the

api strip. The api 20E strips were then incubated at 37°C for 24 hours, after which reagents were added and the results were read by referring to the reading table provided.

Antiserum testing for E.coli: E. coli I Nonavalent #57411 was also used on E.coli to test its serotoxicity. It can detect *Escherichia* coli serotypes 026, 055, 086, 0111, 0119, 0125, 0126, 0127, or 0128.

Antibiotic Resistance profile: The disc diffusion method was used to detect antibiotic resistance of the isolated bacteria. MH agar was prepared and inoculated with the isolated bacteria. Antibiotic discs were embedded in the agar and incubated overnight at 37° C, then observed for inhibition zones. The diameter of each inhibition zone was measured via a digital caliper and compared to the norms for determination of resistance/ susceptibility / sensitive status of bacteria.

Results

1. Effect of farming system and storage conditions on Eggshell porosity and density

The variability in porosity between organic and commercial eggs stored at different temperatures is demonstrated in figure 1. Pore density appeared to be higher in organic egg as compared to commercial eggs, regardless of the storage temperature. Organic eggshells also appeared to be thinner than commercial eggshells, as they allowed the passage of light. Fecal contamination of organic shells was apparent to the naked eye. At fridge temperature, organic and commercial eggshells, revealed wide pores in comparison to RT eggshells. Pore density was not affected by storage temperature. It is important to note that the porosity varied greatly between egg replicates, as indicated in figure 2. Week 3 organic- and Week 2 commercial eggshell replicates, regardless of their storage temperature, lacked uniformity in their porosity.

2. Detection of Antibiotic residues in eggs from different farming systems

The simple microbiological method adopted in our study to identify antibiotic residues in eggs from different farming systems, failed to detect any antibiotic residues in organic and commercial eggs. No inhibition zones were recognized on *E.coli* seeded Muller Hinton plates for the albumen and the yolk in both commercial and organic eggs as shown in Figure 3.

3. Effect of farming system and storage condition on Bacterial Growth

Bacterial growth was analyzed by swabbing the egg shell, taking a sample from the albumen and the yolk and culturing them in nutrient broth before streaking them on SS and TBX agar plates. As mentioned before 4 commercial and 4 organic eggs were studied at each time point, where 2 eggs were stored in the fridge and the other two were kept at RT. No bacterial growth was detected on commercial eggs, whereas the bacterial growth on organic eggshells is illustrated in figure 4. On day 0, bacterial

contamination was reported on 50% of the organic eggshells. On weeks 1, 2, and 3 bacterial growth was observed in 50% of the eggshells stored at room temperature. While, all the eggs stored in the fridge (100%) showed bacterial contamination on their eggshells on weeks 2 and 4. In our study, organic eggs that were stored for 2 weeks at room temperature- but not in the fridge- showed bacterial contamination of egg contents (Figure 4). 50% of the internally contaminated 2 week-old eggs revealed growth in the albumen, and the other 50% in the yolk. In 3 and 5 week-old eggs, no bacterial growth was detected.

The bacterial growth observed on SS and TBX is displayed in figure 5. Bacteria isolated on day 0 and week 4 from organic eggshells stored at RT grew in the form of white colonies on TBX and caused discoloration in SS agar. API 20E identified this growth as *enterobacter cloacae.* Bacteria isolated from the eggshells of week 1 and week 2 eggs stored at RT appeared as pink and white colonies on SS and TBX agar, respectively. API test showed this growth to be *citrobacter freundi.* Week 1, week 2, and week 4 eggshells of eggs stored at 4°C showed pink growth on SS agar and white/blue growth on TBX. All the blue growth on TBX was confirmed, by api 20E, to be E.coli. When the E.coli isolates were tested using E. coli I Nonavalent #57411 antiserum, 80 % of the confirmed E.coli tested positive with the antiserum test. They are suspected to be of the following serotypes: *Escherichia* coli serotypes 026, 055, 086, 0111, 0119, 0125, 0126, 0127, or 0128 .

4. Antibiotic resistance in isolated bacteria

Antibiotic resistance was measured using antibiotic disc diffusion method. The results are illustrated in figure 6. Isolated E. coli strains were most resistant to macrolides 100% and 71% resistance to E and TL, and also to tetracycline 86% and 57% resistance to O and DO. 71% and 29% of the E. coli isolates showed resistance to the aminoglycosides N and GEN, but all of the E. coli were 100% sensitive to the aminocyclitol SPT. 57% of the isolates showed resistance to Colistin, the peptide antibiotic. Only

20% of the *E. coli* isolates were resistant to the betalactams ampicillin and amoxicillin. 14% of the isolates showed resistance to COT. All *E. coli* isolates were sensitive to the fluorquinolone enrofloxacin.

The isolated *enterobacter cloacae* showed 100% resistance to the Macrolide E and 50% resistance to TL. 50% of the isolates showed resistance to betalactams AMP and AML. 50% of the isolates were resistant to the aminocyclitol CT, while no resistance was observed against aminoglycosides N and GEN. Enterobacter cloacae showed no resistance to all other tested antibiotics.

Citrobacter freundii were most resistant to Macrolides 100% and 50% to E and TL, respectively. All isolated colonies showed resistance to betalactam AML and 50% showed resistance to AMP. Besides, all colonies were found to be resistant to oxytetracycline while they showed sensitivity to Doxycycline. 50% of Citrobacter freundii showed resistance to Colistin. No resistance was recorded to all other tested antibiotics.

For all the isolated bacterial species, multidrug resistance was reported. All the isolates were resistant to at least two antibiotics at the same time. The multidrug resistance patterns are clear in Table 3.

Statistical analysis was carried out using ANOVA procedure and TUKEY test. In general No significant variation was observed in the resistance patterns. Variation was observed for N, ENR, and SPT $P<0.05$ in ANOVA. Yet, all isolated species appeared to be susceptible to ENR, SPT, and slightly susceptible to N. Tukey test revealed a difference in resistance to ENR between the *enterobacter cloacae* and *E.coli*.

Discussion

1. Effect of farming system and storage conditions on Eggshell porosity and density

The shell quality varied greatly between depending on the egg source, as reported in the results section. Organic eggshells collected from the littered house floor showed a higher in comparison to eggs in conventional cages. This indicates that litter floor system conditions seem to increase the eggshells' pore density and deteriorate the eggshell quality. The pores on the pore density in comparison to commercial eggshells collected from conventional cages. Tumová et al. (2011) reported similar results where a higher number of pores was reported in eggs from litter housing systems eggshell surface are usually covered by the protein rich cuticle, which extends up to 50 µm into pores thus plugging them to restrict bacterial access (Sparks and Board, 1984). According to Khan and Roberts (2014) cuticle deposition is significantly higher in cages eggs versus free-range eggs. Kusuda et al. (2011) proposed that the diversity in the cuticle structure can be affected by the nest environment and primarily its humidity, a difficult parameter to control in free-range systems. Besides their high pore density organic eggshells were thin and weak, susceptible to breaking by hand. Those results align with the findings of Pištěková et al. (2006) and Englmaierova et al. (2014) who reported heavier eggshells in conventional cage systems compared to litter floors. However, the housing system is not the only factor that affects the eggshell thickness. Tůmová et al. (2016) showed that interactions between housing, genotype and calcium supplementation in the diet lead to great variances in eggs weight and shell quality. Furthermore, Ledvinka et al. (2012a) and Englmaierova et al. (2014) found stronger shells in cages compared to litter. Nevertheless, the strength eggshells is not only affected by shell thickness but by many other factors like mineral density, mineral content, and spatial micro architectural arrangement (Tatara et al., 2015). Organic eggs showed remarkable fecal contamination on their shells, as identified by the naked eye. As it has been well established that eggshells from deep litter systems display 15 times more bacteria and a higher amount of prospective spoilage organisms than eggs from

cage systems (Harry, 1963). Quarles et al. (1970) noticed higher bacterial eggshell contamination of eggs from litter floor houses than in eggs from wire floor houses. They reported that litter floor houses had about 9 times more bacteria in air, and about 20-30 times more aerobic bacteria on the shell than wire floor houses. In our study storing eggs in the fridge seemed to widen the pores on the shell, which opposed Liu et al. (2016) findings on how storing eggs at room temperatures speeds up the drying and shrinkage of the cuticle and leads to bigger pore size. Refrigeration of eggs is well known to prevent cuticle drying, thus reducing pores exposure. However, there must be an interaction between different factors that affect cuticle dryness and pores widening. Many factors such as genetics, hen age, egg freshness, processing, and storage affects the cuticle quality (Liu et al., 2016). Unlike the housing system and the storage temperature, storage time seemed to have no impact on the number of pores. It also did not affect the pore size. The intra-replicate variability in the porosity of Week 3 organic- and Week 2 commercial eggshells, regardless of their storage temperature lines up with the results obtained by Haines and Moran (2009) where shells collected successively from the same hen varied widely in their porosity. In 1992, a study conducted by Nascimento et al. (1992) linked the increase in pore number with the bird's age. Further studies should be conducted to identify the factors affecting the porosity of eggshells and their overall quality.

2. Detection of Antibiotic residues in eggs from different farming systems

It has been well stated that antimicrobial residues in food may result in side effects such as toxicity and allergic response in consumers, and may also lead to the development of antimicrobial resistance (AMR) among bacterial pathogens (Cerniglia et al., 2016). A lot of scientists targeted the antibiotic residue deposition in eggs as mentioned in the literature. However, the simple microbiological assay we used in our study detected no antibiotic residues in commercial and organic eggs. This lack of response might be due to the low sensitivity of the method. Usually more specific identification

microorganisms that have high sensitivity to the majority of antibiotics are used to detect the presence of antibiotic residues in eggs and other food products. Microbiological assays used by the USDA include Swab Test on Premises (STOP) (JOHNSTON et al., 1981), Calf antibiotic and sulfonamide test (CAST) (Dey et al., 2005a) , and Fast antimicrobial screen test (FAST) (Dey et al., 2005b) that rely on *Bacillus subtilis* ATCC 6633 spores, *Bacillus megaterium* ATCC 9885, and *B. megaterium* ATCC 9885 as indicator organisms, respectively. Yet, all microbiological assays used for the screening of antimicrobial residues are not sensitive enough and not really specific due to the presence of inhibitory substances, especially in the albumen, which serve as natural defense against microbial contamination and proliferation. Microbiological assays require a post-screening step to determine the identity of any inhibitory substances (Gaugain-Juhel et al., 2009). Immunoassay or biochemical methods are used for confirmation. Immunoassays can detect very low concentrations of antibiotic residues. Various immunoassays that detect antibiotic residues in eggs are currently established including enzyme-linked immunosorbent assay, fluorescence immunoassay, radioimmunoassay, colloidal gold immunoassay, and chemiluminescence immunoassay (Ahmed et al., 2020). Ultra-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) techniques are also used extensively for confirmation of antibiotic residues in eggs; they can be considered semiquantitative, because they help identify analytes of interest and the information about the compliance of the analyzed samples with the MRL established. Multiresidue screening methods have also been developed like UHPLC/MS-MS method was that can semi-quantitatively determine antimicrobial residues from tetracyclines, aminoglycosides, quinolones, lincosamides, *β*-lactams, sulfonamides, and macrolides families in eggs(Caldeira et al., 2017). Altogether, our result could easily indicate that all farming conditions utilize antibiotics in levels below detectable range, in other words below the maximum residue limits (MRL), and thus are considered safe to consume.

3. Effect of farming system and storage condition on Bacterial Growth

In our study, bacterial growth was only detected on the eggshells of organic eggs. On day 0, bacterial contamination was reported on 50% of the organic eggshells. On weeks 1, 2, and 3 bacterial growth was observed in 50% of the eggshells stored at room temperature. While, all the eggs stored in the fridge (100%) showed bacterial contamination on their eggshells on weeks 2 and 4. Hence, eggs stored at 4°C show bacterial contamination more frequently. We can say that storing eggs at fridge temperature seemed to increase the risk of eggshell bacterial contamination. The poultry industry in various countries adopt different systems for treating and storage recommendations of the egg. In the USA and Canada, eggs are collected, washed with a disinfectant, waxed, and recommended to be stored in the fridge. In contrast, in Lebanon and Europe the eggs are collected, sometimes slightly wiped with a dry cloth, especially in organic systems, then stored at room temperature. Most of the studies conducted handled the effect of storage temperature on the microbial contamination in the egg contents. Nobody studied the effect of storage temperature on the eggshell contamination. However, the conducted studies succeeded to show that the temperature plays a key role in the growth and survival of bacteria in the egg white, and that refrigeration decreases bacterial growth and reduces the access of bacteria from the shell to the yolk (Guyot et al., 2013). This might explain the higher percentage of eggshell contamination of eggs stored in the fridge; the storage of eggs at low temperature seems to have reduced the access of bacteria into the egg. Both old (Stadelman, 1977) and recent (Hester, 2017) publications recommend the storage of eggs at fridge temperature $\leq 7.2°C\ (45°F)$. Some studies indicated that storage of eggs at low temperature is favored only in washed eggs, while unwashed eggs require storage at ambient temperatures (25°C) (Joshi et al., 2019). In Lebanon eggs are not washed, yet Saleh et al. (2020) reported bacterial growth on the egg shell and in the yolk of eggs stored at 18°C and 24°C for 2 and 4 weeks, but no contamination of eggs stored at 7°C. In accordance with Saleh et al. (2020) findings, in our study, organic eggs that were stored for 2 weeks at room temperature- but not in the fridge- showed bacterial contamination of egg contents (Figure 3).

50% of the internally contaminated 2 week-old eggs revealed growth in the albumen, and the other 50% in the yolk. The egg with the contaminated albumen showed no bacterial growth on its shell, thus we exclude the chance of bacterial penetration through the pores into the albumen. Besides, bacteria isolated from the albumen changed the color of SS and appeared white on TBX agar and was identified by API 20E as *enterobacter cloacae*. *Enterobacter cloacae* appears to have a very low penetration ability (14.2% of contamination at 21 weeks of storage) through the eggshell as reported by Smaniotto et al. (2017). Correspondingly, the egg with contaminated yolk showed contamination with a different bacterial species than on its shell. This contradicts any possibility of trans-shell contamination, where micro-organisms penetrate through the eggshell to possibly reach the internal egg (Bruce, 1994). Also, bacteria isolated from the yolk showed pink growth on SS and green/blue colonies and was confirmed as *E.coli* by API 20E. Even though *E.coli* is found to facilitate the penetration of different bacterial species into the egg contents, as mentioned in the literature review, the penetration ability of *E.coli* through the eggshell, similarly to enterobacter cloacae, seems to be low around 4.7% after 21 weeks of storage (Smaniotto et al., 2017). Thus, the internal contamination has nothing to do with the bacteria isolate from the eggshell. In both egg replicates the bacteria were probably transferred into the albumen via vertical transmission which takes place through hen's reproductive system (Keller et al., 1995). In this type of transmission, the yolk, the albumen, and the membranes are contaminated straight away due to bacteria in the reproductive organs, before the eggs are covered by the shell (Takehiko Yamamoto, 1996).

Enterobacter cloacae was isolated on day 0 and week 4 from organic eggshells stored at RT. This growth was expected, as mentioned in the literature review and in table 1, bacteria belonging to the Enterobacteriaceae family constitute a huge part of the egg's microflora. It is normal for avian eggs to acquire microflora during ovipositioning. Many researchers reported the growth of enterobacter cloacae in eggs. Papadopoulou et al. (1997) had isolated Enterobacter cloacae from table eggs in Greece.

Adesiyun et al. (2006b) identified enterobacter spp. in 12.5% of the egg samples that they studied, with 8.2% on eggshells and 3.3% in egg contents. *Enterobacter cloacae* is frequently isolated from humans and animals, but it is not considered to be an enteric pathogen, it may be an opportunistic pathogen (Guglielmetti and Bartoloni, 2003). Furthermore, citrobacter freundi was isolated from the eggshells of week 1 and week 2 eggs stored at RT. This growth was also expected, being a part of the Enterobacteriaceae family. Stepień-Pyśniak (2010), and while studying the occurrence of gram negative bacteria in hen eggs, indicated Citrobacter freundii among the most frequently isolated bacteria of the family Enterobacteriaceae after Salmonella and Escherichia coli.

Week 1, week 2, and week 4 eggshells of eggs stored at 4°C were contaminated with E.coli, a frequently reported egg shell and content contaminant, as mentioned in the literature. The recurrent and high isolation rate of pathogenic *E. coli* from eggshells is because *E. coli* are normal intestinal flora in humans and birds, and can easily contaminate egg shell through feces. These microorganisms are detected in both the shell and the content (Okorie-Kanu et al., 2016). When the E.coli isolates were tested using E. coli I Nonavalent #57411 antiserum, 80 % of the confirmed E.coli tested positive with the antiserum test. They are suspected to be of the *Escherichia* coli serotypes 026, 055, 086, 0111, 0119, 0125, 0126, 0127, or 0128 . Only 50% of the week 4 eggshells stores at room temperature showed no serotoxicity.

The absence of microbial eggshell contaminations in week 3 eggs, might have been due to the fact that no fecal contamination was identified by the naked eye on week 3 eggshells, as feces is one of the main sources of eggshell bacterial contamination (Board and Tranter, 2017). While the absence of microbial eggshell contaminations in 5 week-old eggs was probably due to the death of the bacteria**.** Bacterial growth was not detected in week 5 egg contents which negates bacterial penetration through the eggshell. Hence, absence of bacterial growth can be due to the death of the bacteria on the eggshell

from lack of nutrition or inconvenient growth temperature in case of cooling. However, the absence of bacterial growth inside the egg might be due to the antimicrobial proteins present in the albumen, discussed in detail in the literature. Bacteria might have penetrated the egg and got killed in the albumen.

4. Antibiotic resistance in isolated bacteria

Antibiotic resistance measured using antibiotic disc diffusion method, showed that the isolated E. coli strains had several resistance patterns. E. coli isolates were most resistant to macrolides 100% and 71% resistance to E and TL, and also to tetracycline 86% and 57% resistance to O and DO. Macrolides mainly act on gram positive bacteria and some gram negative bacteria, but some can relatively act on *Escherichia coli, Salmonella* spp and other Enterobacteriaceae. The resistance of E.coli to Macrolides is common, Phuc Nguyen et al. (2009) studied the resistance of E. coli, from 5 different countries, to Macrolides and reported E.coli as a reservoir for Macrolide resistance genes. E.coli was also found to have resistance genes for Tetracyclines, during a clinical trial(Tuckman et al., 2007). 71% and 29% of the E. coli isolates showed resistance to the aminoglycosides N and GEN, but all of the E. coli were 100% sensitive to the aminocyclitol SPT. 57% of the isolates showed resistance to Colistin, the peptide antibiotic. Only 20% of the *E. coli* isolates were resistant to the betalactams ampicillin and amoxicillin. 14% of the isolates showed resistance to COT. All *E. coli* isolates were sensitive to the fluorquinolone enrofloxacin. Our results support a study by Khan et al. (2015) in Pakistan reporting E. coli to be most sensitive to ciprofloxacin and enrofloxacin, slightly sensitive to amoxicillin, but completely resistant to tetracycline. Isolated *enterobacter cloacae* showed 100% resistance to the Macrolide E and 50% resistance to TL. 50% of the isolates showed resistance to betalactams AMP and AML. In the first study conducted on the antoibiotic resistance of bacteria isolated from eggs, Enterobacter cloacae showed resistance to ampicillin, amoxycillin, and erythromycin (Papadopoulou et

al., 1997). Also 50% of the isolates were resistant to the aminocyclitol CT, while no resistance was observed against aminoglycosides N and GEN. Enterobacter cloacae showed no resistance to all other tested antibiotics. *Citrobacter freundii* were most resistant to Macrolides 100% and 50% to E and TL, respectively. Also, all isolated colonies showed resistance to betalactam AML and 50% showed resistance to AMP. The resistance of *Citrobacter freundii* to macrolides and betalactams was not surprising, *Citrobacter freundii* has been reported, long ago, to be resistant to AMP and AML (Papadopoulou et al., 1997).Also, all colonies were found to be resistant to oxytetracycline while they showed sensitivity to Doxycycline. The higher resistance to oxytetracycline was surprising knowing the doxycycline is the antibiotic used in the poultry sector in Lebanon, as mentioned in the literature. 50% of *Citrobacter freundii* showed resistance to Colistin. In accordance with the researches discussed in the literature, multidrug resistance was obvious in all the isolated bacterial species isolated in our study.

Conclusion and Implications

Numerous studies have been conducted to determine the most favorable farming source and storage temperature for eggs. To date no clear vision of storage of eggs in Lebanon has been established or regulated, therefore this study aimed to determine whether or not there is an interaction between the farming system and best storage practices for eggs. Regarding the farming source, our study revealed that commercial egg laid by hens raised in conventional cages had clean eggshells while organic eggshells from semi-intensive housing systems were clearly contaminated with fecal matter, and thus microbes. Yet organic eggshells that appeared clean to the naked eye, showed no bacterial growth. We can say that the oviposition of organic eggs on dirty floors makes them more susceptible to microbial contamination. Regarding shell quality, organic eggshells showed lower thickness, lower pore density, and less strength in comparison to commercial eggshell- raising a higher risk of bacterial trans-shell contamination. Nevertheless, porosity showed a huge variability within the same source. It seems to be also affected by other factors yet to be studied, like hen age genetic and diet. Egg content contamination of organic eggs stored at RT was detected. It had nothing to do with transhsell contamination but vertical bacterial transmission, knowing that bacteria isolated from the content differed from that isolated from the shell. All together, we can say that organic eggs from semi-intensive housing systems, had higher bacterial contamination, low eggshell quality- high risk of trans-shell contamination, and showed vertical contamination of contents. Microbes contaminating organic eggs included *E. coli, Enterobacter cloacae* and *Citrobacter freundi*. Luckily, contamination with *Salmonella* was not identified, and the contaminating bacteria were in general not pathogenic. Thus, consuming them seemed to pose no serious risk on human health. However, it is important to note that 80% of E.coli strains seemed to be serotoxic. The isolated bacteria, however, seemed to be highly resistant to Macrolides and tetracyclines, and moderately resistant to peptide antibiotic colistin and betalactams.

Isolated E.coli additionally showed resistance to aminoglycosides Neomycin and gentamicin, and nitrofuran COT. E.coli species seemed to be the most multi-drug resistant. All isolates were sensitive to spectinomycin and enrofloxacin. However, antibiotic residues were not detected neither in commercial nor in organic eggs by microbiological assay used, indicating that chemical contaminations levels were below detectable range and are considered safe to consume. Concerning the storage conditions and in contrary to the usual guidelines that advise storing eggs in the fridge to decrease the risk of bacterial contamination, our study showed that frigid organic eggshells displayed higher eggshell contamination compared to eggs stored at room temperature. However, bacteria were unable to survive on eggshells post 4 weeks. We can say that, organic eggs/ unwashed eggs are better stored at room temperature and commercial/washed eggs are better stored in the fridge. In conclusion consumption of organic eggs pose a slightly higher health risk on consumers, especially during handling. We recommend storing these eggs at room temperature away from other food to avoid any cross contamination, and handled carefully with higher awareness on the possible risks. Proper disinfection before cooking or cracking is essential to preventing the egg shell bacterial population from inoculating the content during processing. Cooking, especially of organic eggs, should be long enough to prevent the multiresistant bacteria from further compromising the consumer.

Further attention should be taken to promote awareness on best practices to handle eggs to consumers, best nutritional and farming practices for farmers as well as develop proper government guidelines and oversight to the layer industry, be it farming and marketing.

Tables

Table 1: Bacterial contamination of egg compartments

Egg compartment	Bacteria	Reference
Eggshell	*Enterobacteriaceae family* *Micrococcus spp.* *Staphylococcus spp.* *Streptococcus spp.* *Pseudomonas spp.*	(Bruce and Johnson, 1978)
	Listeria spp. *Campylobacter spp.* *Enterobacter spp.* *Klesbsiella spp.* *Salmonella spp.* *Escherechia spp.*	(Adesiyun et al., 2006a)
Internal contents	*Yersenia entercolitica*	(Amin and Draughon, 1990)
	Salmonella Heidelberg	(Schoeni et al., 1995)
	Salmonella enteriditis *Salmonella typhimurium*	(Miyamoto et al., 1998)
	Campylobacter jejuni	(ALLEN and GRIFFITHS, 2001)
	Pseudomonas fluorescens	(Jones et al., 2002)
	Listeria spp. *Enterobacter spp.* *Klebsiella spp.*	(Adesiyun et al., 2006a)
	Alcaligenes sp. *Carnobacterium sp.*	(Reu et al., 2007)
	Staphylococcus lentus *Staphylococcus xylosus* *Bacillus spp.*	(Reu et al., 2008)
	Staphylococcus aureus *E. coli*	(Al-Natour et al., 2011)

Table 2: Antibiotics used in the poultry industry in Lebanon.

Antibiotic	Antibiotic family	Susceptible microorganisms	References
Doxycycline	Tetracycline	*Escherichia coli,* *Staphylococcus aureus* *Listeria monocytogenes* *Mycoplasma pneumoniae* *Rickettsia spp.* *Chlamydophila pneumoniae*	(Holmes and Charles, 2009)
Enrofloxacin	Fluoroquinolone	*Mycoplasma gallisepticum* *E. coli*	Stanley et al. (2001)
Florfenicol	Amphenicol	*Salmonellae* *Escherichia coli and* *Haemophilus influenza* *Mycoplasma hominis* *Mycoplasma pneumoniae* *Ureaplasma urealyticum*	(Graham et al., 1988)
Tylosin	Macrolide	*Mycoplasma spp.* *Clostridium perfringens*	(Collier et al., 2003; Kleven, 2008)
Tilmycosin		*Mycoplasma spp.* *Pasteurella multocida* *Ornithobacterium rhinotracheale*	(Charleston et al., 1998; Abu-Basha et al., 2007)
Erythromycin		*Staphylococcus aureus,* *Mycoplasma pneumonia* *M. gallisepticum* *Staphylococcus spp.* *Streptococcus spp.* *Haemophilus paragallinarum* infection	(Giguère, 2013b)
Neomycin	Aminoglycoside	*E. coli* *Salmonella*	(Marrett et al., 2000)
Gentamicin		*E. coli* *Pseudomonas aeruginosa* *Arizona paracolon* *Salmonella*	(Jernigan et al., 1988; Dowling, 2013a)
Colistin	Peptide	Non-invasive *Enterobacteriaceae*	(EMA, 2016)
Fosfomycin		*E. coli* *Salmonella spp.*	(Pérez et al., 2014)

Table 3: Antibiotic resistance profile of the isolated bacterial species

Bacteria	E15	N30*	AML25	AMP10	SPT100*	DO30	ENR5*	TL15	O30	CT10	COT25	GEN10
	R	**R**	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**S**	**R**
	R	**R**	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**S**	**S**
	R	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	**S**
E. coli	**R**	**S**	**R**	**R**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**
	R	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	**R**
	R	**R**	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**S**	**S**
	R	**S**	**R**	**R**	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**
Mean d	0.00	1.27	1.60	1.31	1.57	1.34	2.63	0.81	0.50	1.14	2.01	1.54
SD	0.00	0.15	0.81	0.90	0.24	0.48	0.14	0.42	0.87	0.09	0.90	0.34
E. cloacae	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
	R	**S**	**R**	**R**	**S**	**S**	**S**	**R**	**S**	**R**	**S**	**S**
Mean d	0.00	1.60	1.25	1.00	2.10	2.15	2.25	1.05	2.00	1.15	2.80	1.95
SD	0.00	0.14	1.77	1.41	0.14	0.78	0.21	1.48	0.00	0.21	0.56	0.21
C. Freundii	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**S**
	R	**S**	**R**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**S**	**S**
Mean d	0.35	1.55	0.60	0.45	1.90	2.00	2.30	0.70	1.30	1.15	1.75	2.10
SD	0.49	0.07	0.85	0.64	0.14	0.71	0.14	0.99	0.00	0.21	0.35	0.28

Figures

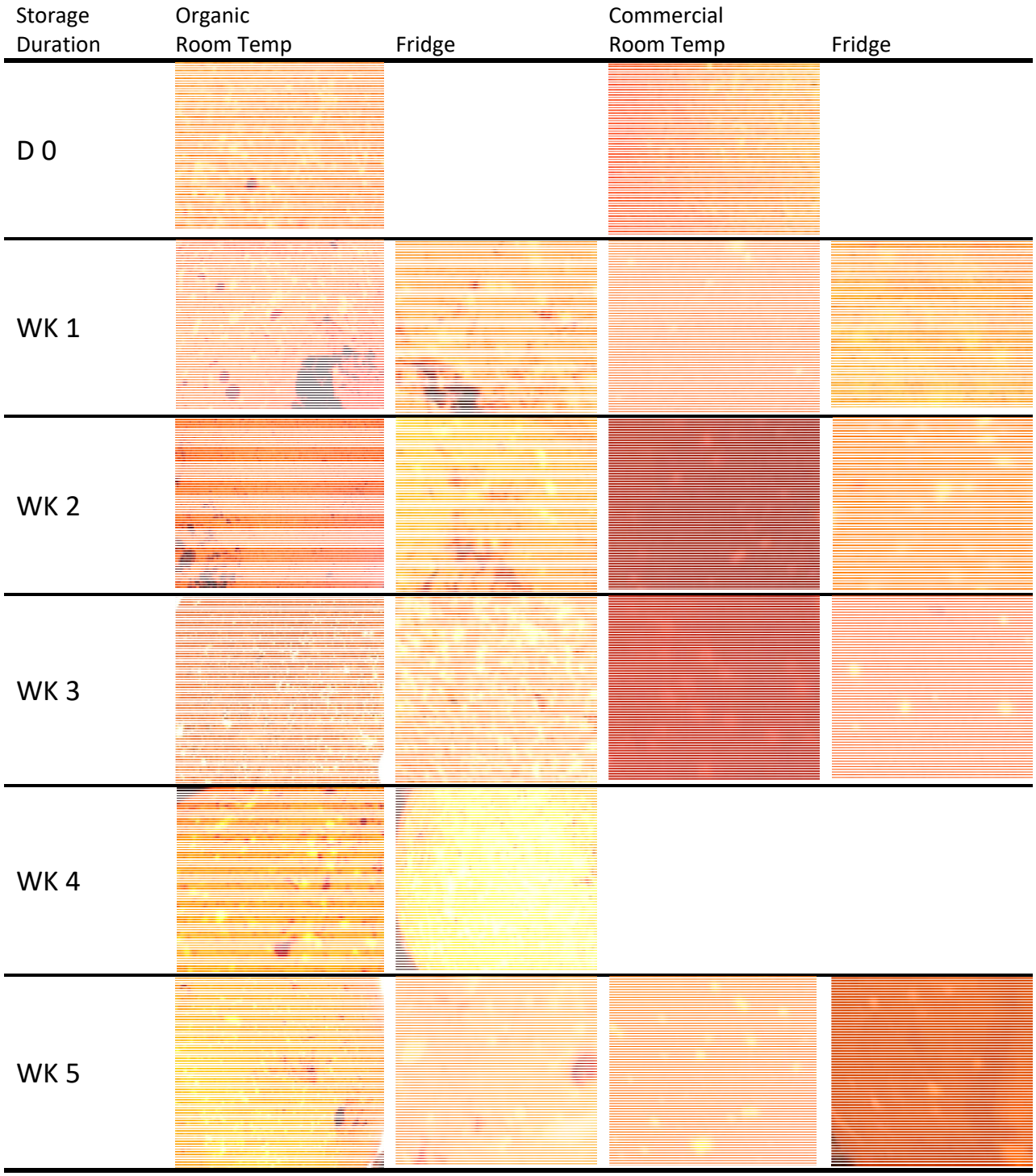

Figure 1: The variation in eggshell porosity

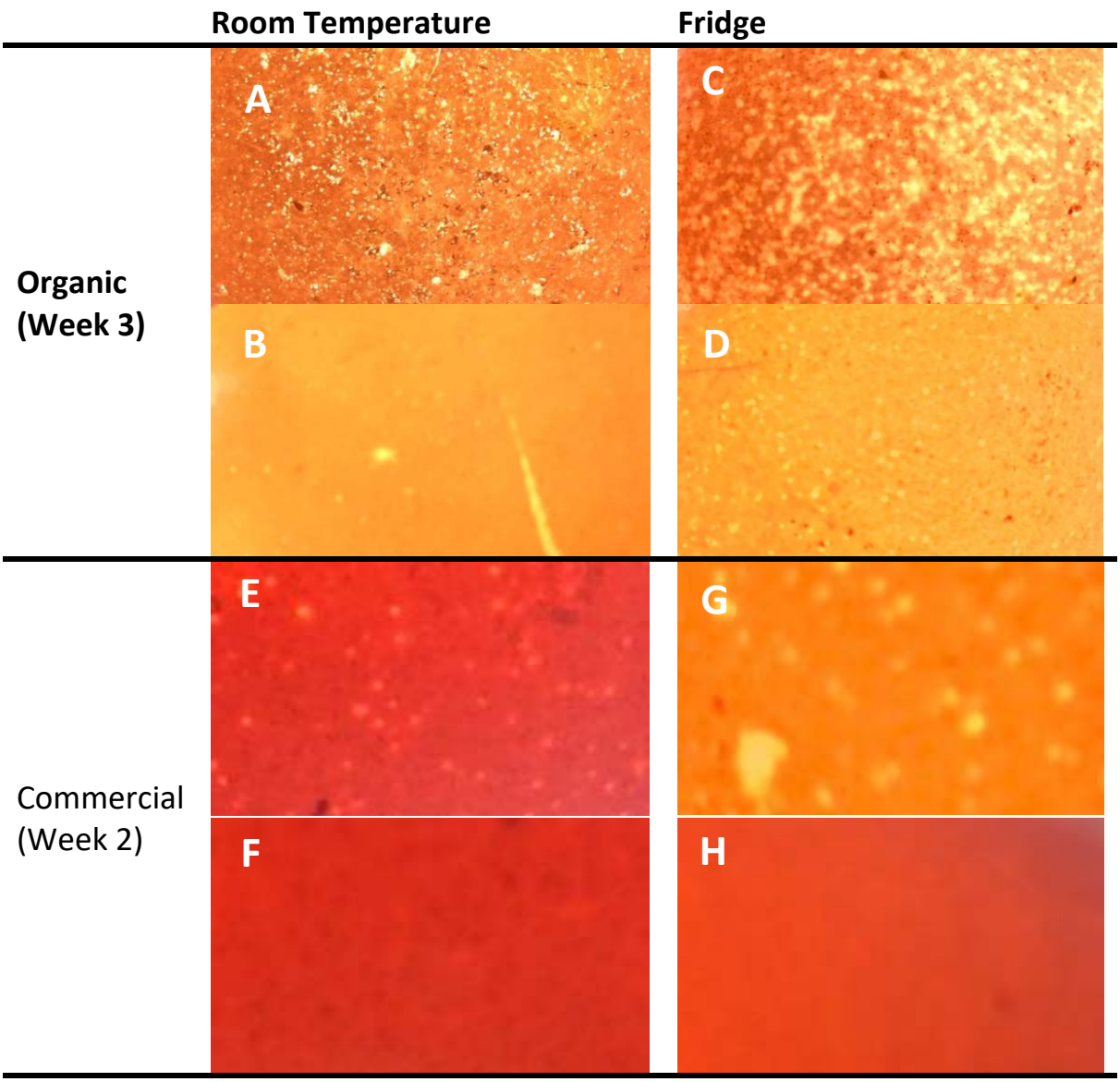

Figure 2: Replicate eggs shows a high variability of the samples, an indication of animal variability

Figure 3: Testing for antibiotic residues in egg contents

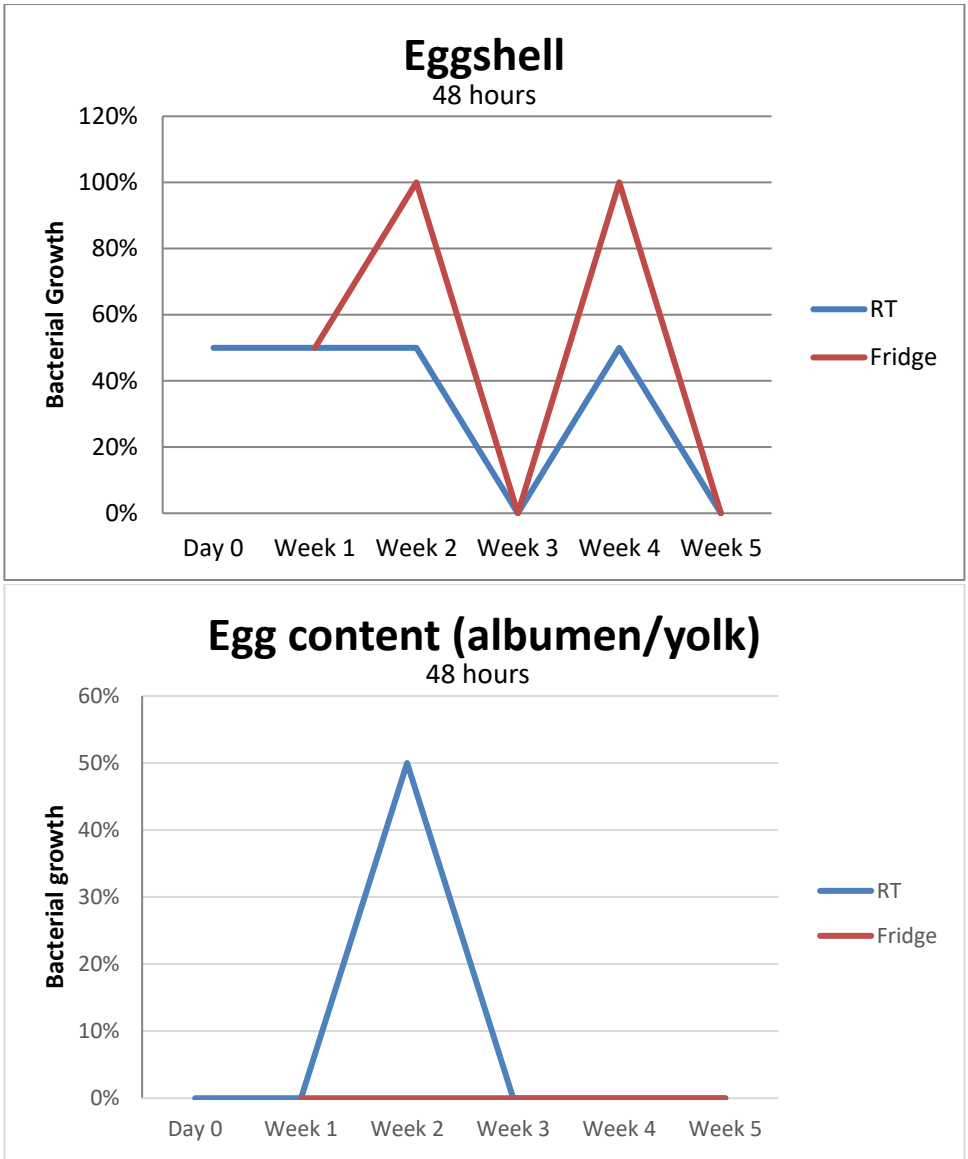

Figure 4: Bacterial Growth in Organic Eggs

Pink colonies-SS	Discoloration- SS	White colonies-TBX	Blue-Green colonies-TBX
			
Figure 5: Bacterial growth on SS and TBX			

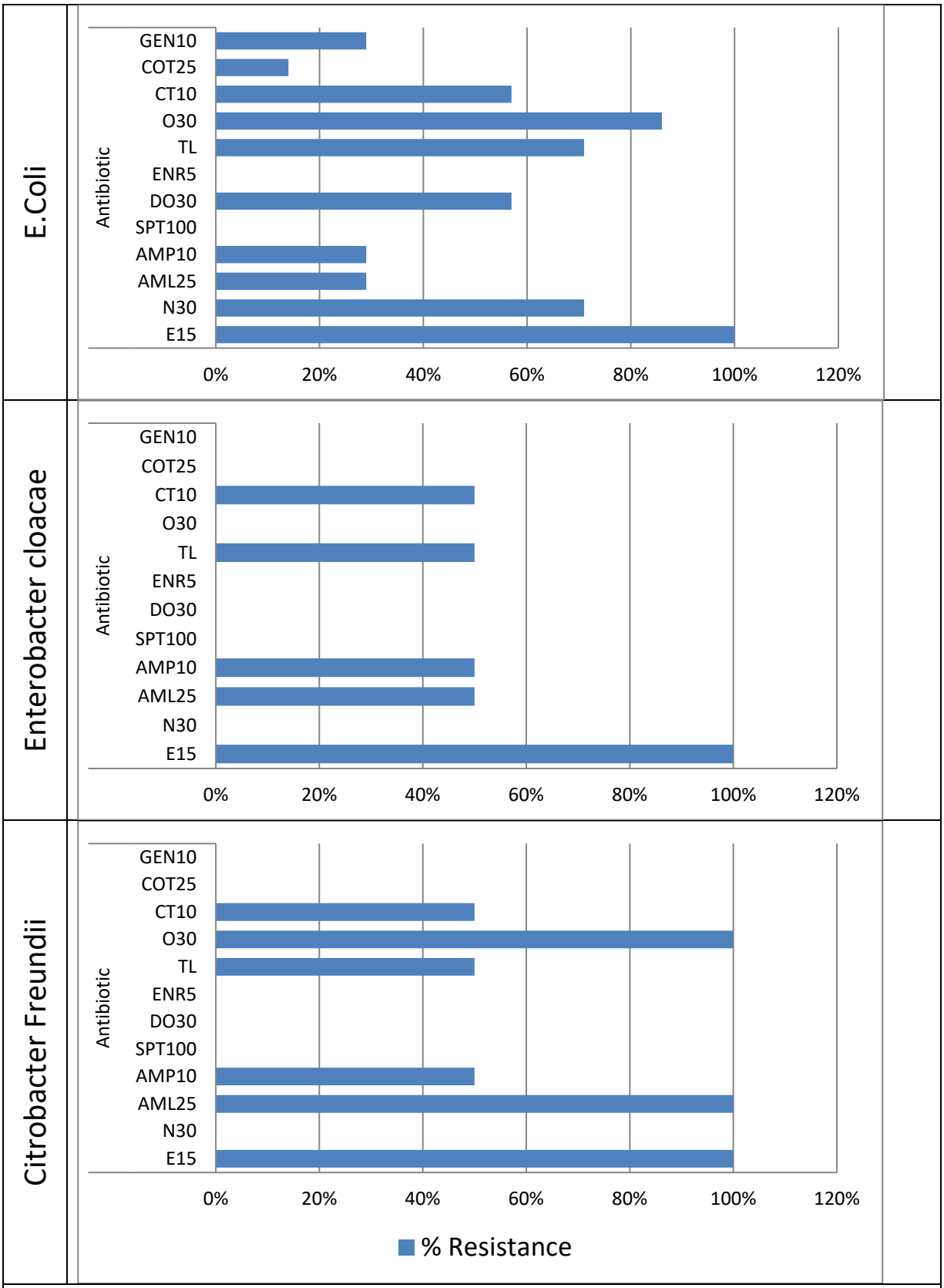

Figure 6: Antibiotic resistance of the isolated bacterial species

References

Abdallah, F. B., and J. M. Chahine. 1999. Transferrins, the mechanism of iron release by ovotransferrin. European journal of biochemistry 263: 912-920.

Abdou, A. M. 2013. Functional Proteins and Peptides of Hen's Egg Origin Bioactive Food Peptides in Health and Disease, . p 115-143.

Abdou, A. M., M. Kim, and K. Sato. 2013. Functional proteins and peptides of hen's egg origin. Bioactive food peptides in health and disease: 115-116.

Abrahamsson, P., and R. Tauson. 1993. Effect of Perches at Different Positions in Conventional Cages for Laying Hens of Two Different Strains. Acta Agriculturae Scandinavica Section A - Animal Science: 228-235.

Abu-Basha, E. A., N. M. Idkaidek, and A. F. Al-Shunnaq. 2007. Pharmacokinetics of tilmicosin (Provitil powder and Pulmotil liquid AC) oral formulations in chickens. Veterinary research communications 31: 477-485.

Act, U. 1958. Federal food, drug and cosmetic Act, as amended Title 21 US Code. US Washington: Governmental Printing Office.

Adesiyun, A. et al. 2005. Prevalence of antimicrobial residues in table eggs in Trinidad. Journal of food protection 68: 1501-1505.

Adesiyun, A. et al. 2006a. Frequency and antimicrobial resistance of enteric bacteria with spoilage potential isolated from table eggs. Food Research International - FOOD RES INT 39: 212-219.

Adesiyun, A. et al. 2006b. Frequency and antimicrobial resistance of enteric bacteria with spoilage potential isolated from table eggs. Food Research International 39: 212-219.

Adesiyun, A. et al. 2007. Antimicrobial resistance of Salmonella spp. and Escherichia coli isolated from table eggs. Food Control 18: 306-311.

Adesiyun, A. A., C. Nkuna, M. Mokgoatlheng-Mamogobo, K. Malepe, and L. Simanda. 2020. Food safety risk posed to consumers of table eggs from layer farms in Gauteng Province, South Africa: Prevalence of Salmonella species and Escherichia coli, antimicrobial residues, and antimicrobial resistant bacteria. Journal of Food Safety n/a.

Aguilera, O., L. M. Quiros, and J. F. Fierro. 2003. Transferrins selectively cause ion efflux through bacterial and artificial membranes. FEBS letters 548: 5-10.

Agunos, A., C. Carson, and D. Léger. 2013. Antimicrobial therapy of selected diseases in turkeys, laying hens, and minor poultry species in Canada. Can Vet J 54: 1041-1052.

Ahmed, S. et al. 2020. Current advances in immunoassays for the detection of antibiotics residues: a review. Food and Agricultural Immunology 31: 268-290.

Al-Ghamdi, M. S. et al. 2000. Residues of tetracycline compounds in poultry products in the eastern province of Saudi Arabia. Public Health 114: 300-304.

Al-Natour, M., A. Alaboudi, N. Al-Hatamelh, and T. Osaili. 2011. Escherichia coli O157:H7 Facilitates the Penetration of Staphylococcus aureus into Table Eggs. Journal of food science 77: M29-34.

Alaboudi, A., E. Abu-Basha, and I. Musallam. 2013. Chlortetracycline and sulfanilamide residues in table eggs: Prevalence, distribution between yolk and white and effect of refrigeration and heat treatment. Food Control 33: 281–286.

Alaboudi, A. R. 2017. Chapter 42 - Antimicrobial Residues in Table Eggs. In: P. Y. Hester (ed.) Egg Innovations and Strategies for Improvements. p 447-456. Academic Press, San Diego.

Albentosa, M., and J. Cooper. 2004. Effects of cage height and stocking density on the frequency of comfort behaviours performed by laying hens housed in furnished cages. Animal Welfare 13: 419-424.

Aldred, K. J., R. J. Kerns, and N. Osheroff. 2014. Mechanism of quinolone action and resistance. Biochemistry 53: 1565-1574.
ALLEN, K. J., and M. W. GRIFFITHS. 2001. Use of Luminescent Campylobacter jejuni ATCC 33291 To Assess Eggshell Colonization and Penetration in Fresh and Retail Eggs. Journal of food protection 64: 2058-2062.
Alvarez-Fernandez, E., J. Dominguez-Rodriguez, R. Capita, and C. Alonso-Calleja. 2012. Influence of housing systems on microbial load and antimicrobial resistance patterns of Escherichia coli isolates from eggs produced for human consumption. Journal of food protection 75: 847-853.
Amin, M. K., and F. A. Draughon. 1990. Infection of Shell Eggs With Yersinia Enterocolitica. Journal of food protection 53: 826-830.
Amjad, H., M. Naeem, J. Iqbal, and K. Khan. 2005. Estimation of selected residual antibiotics in poultry products available in local markets during summer. Journal- Chemical Society of Pakistan 27: 637-642.
Anadón, A., P. Bringas, M. R. Martinez-Larrañaga, and M. J. Diaz. 1994. Bioavailability, pharmacokinetics and residues of chloramphenicol in the chicken. Journal of veterinary pharmacology and therapeutics 17: 52-58.
Anadón, A. et al. 1995. Pharmacokinetics and residues of enrofloxacin in chickens. American journal of veterinary research 56: 501-506.
Anadón, A., and M. R. Martínez-Larrañaga. 2014. Veterinary Drugs Residues: Coccidiostats. In: Y. Motarjemi (ed.) Encyclopedia of Food Safety. p 63-75. Academic Press, Waltham.
Anadón, A., M. R. Martínez-Larrañaga, I. Ares, and M. A. Martínez. 2018. Regulatory aspects for the drugs and chemicals used in food-producing animals in the European Union Veterinary Toxicology. p 103-131. Elsevier.
Anadon, A. et al. 1994. Pharmacokinetics of doxycycline in broiler chickens. Avian pathology : journal of the W.V.P.A 23: 79-90.
Anadón, A. et al. 1996. Pharmacokinetics of amoxicillin in broiler chickens. Avian pathology : journal of the W.V.P.A 25: 449-458.
Anadón, A., M. R. Martinez-Larrañaga, M. J. Diaz, C. Velez, and P. Bringas. 1990. Pharmacokinetic and residue studies of quinolone compounds and olaquindox in poultry. Annales de recherches veterinaires. Annals of veterinary research 21 Suppl 1: 137s-144s.
Anadón, A. et al. 2008. Plasma and tissue depletion of florfenicol and florfenicol-amine in chickens. Journal of agricultural and food chemistry 56: 11049-11056.
Appleby, M. et al. 2002a. Development of furnished cages for laying hens. British poultry science 43: 489-500.
Appleby, M. C. et al. 2002b. Development of furnished cages for laying hens. British poultry science 43: 489-500.
Arathy, D. S. et al. 2011. Antimicrobial drug resistance in Escherichia coli isolated from commercial chicken eggs in Grenada, West Indies. The West Indian medical journal 60: 53-56.
Atef, M., A. Ramadan, S. A. Youssef, and K. Abo el-Sooud. 1993. Kinetic disposition, systemic bioavailability and tissue distribution of salinomycin in chickens. Res Vet Sci 54: 179-183.
Atta, A. H., and S. A. El-zeini. 2001. Depletion of trimethoprim and sulphadiazine from eggs of laying hens receiving trimethoprim/sulphadiazine combination. Food Control 12: 269-274.
Baert, K., S. De Baere, S. Croubels, and P. De Backer. 2003. Pharmacokinetics and oral bioavailability of sulfadiazine and trimethoprim in broiler chickens. Veterinary research communications 27: 301-309.
Bain, M. M. et al. 2013. Enhancing the egg's natural defence against bacterial penetration by increasing cuticle deposition. Animal Genetics 44: 661-668.

Ball, R. F., V. Logan, and J. F. Hill. 1975. Factors Affecting the Cuticle of the Egg as Measured by Intensity of Staining. Poultry Science 54: 1479-1484.
Barbosa, J., S. Ferreira, A. Pais, M. Silveria, and F. Ramos. 2011. Nitrofuran in poultry: use, control and residue analysis. Agricultural researche updates 1: 1-50.
Baron, F., M. Gautier, and G. Brule. 1999. Rapid growth of Salmonella enteritidis in egg white reconstituted from industrial egg white powder. Journal of food protection 62: 585-591.
Bartlett, F. M., J. M. Laird, C. L. Addison, and R. C. McKellar. 1993. The analysis of egg wash water for the rapid assessment of microbiological quality. Poult Sci 72: 1584-1591.
Bennett, M., J. Elliott, L. Sommerville, and M. Taylor. 2001. Drugs used in the treatment of bacterial, fungal, viral, and protozoal infections. The Veterinary Formulary: 135-218.
Berrang, M., N. Cox, J. Frank, and R. Buhr. 1999. Bacterial Penetration of the Eggshell and Shell Membranes of the Chicken Hatching Egg: A Review. The Journal of Applied Poultry Research 8: 499-504.
Bialka, K. L., A. Demirci, S. J. Knabel, P. H. Patterson, and V. M. Puri. 2004. Efficacy of electrolyzed oxidizing water for the microbial safety and quality of eggs. Poult Sci 83: 2071-2078.
Blankenvoorde, M. F. et al. 1998. Cystatin and cystatin-derived peptides have antibacterial activity against the pathogen Porphyromonas gingivalis. Biological chemistry 379: 1371-1375.
Board, R., and H. Tranter. 2017. The microbiology of eggs Egg science and technology. p 81-104. CRC Press.
Board, R. G., and N. A. Halls. 1973. The cuticle: A barrier to liquid and particle penetration of the shell of the hen's egg. British poultry science 14: 69-97.
Board, R. G., S. Loseby, and V. R. Miles. 1979. A note on microbial growth on hen egg-shells. British poultry science 20: 413-420.
Board RG, T. H. 1995. The microbiology of eggs. In: Stadelman WJ, Cotterill OJ (eds): Pages in egg science and technology. Food Products Press, New York. 81–103.
Brennan, J., R. Bagg, D. Barnum, J. Wilson, and P. Dick. 2001. Efficacy of narasin in the prevention of necrotic enteritis in broiler chickens. Avian Dis 45: 210-214.
Bruce, J., and E. M. Drysdale 1994. Trans-shell transmission. Microbiology of the Avian Egg. R. G. Board and R. Fuller, ed. Chapman & Hall, London, UK. : 63–91
Bruce, J., and A. Johnson. 1978. The bacterial flora of unhatched eggs. British poultry science 19: 681-689.
Bruce J., D. E. M. (1994) Trans-shell transmission In: Board R.G., Fuller R. (eds) Microbiology of the Avian Egg. Springer, Boston, MA: 63-91.
Brumfitt, W., and J. M. Hamilton-Miller. 1993. Reassessment of the rationale for the combinations of sulphonamides with diaminopyrimidines. Journal of chemotherapy (Florence, Italy) 5: 465-469.
Bui, T., and C. V. Preuss. 2020. Cephalosporins StatPearls, Treasure Island (FL).
Burley, R. W., and W. H. Cook. 1961. Isolation and composition of avian egg yolk granules and their constituent alpha- and beta-lipovitellins. Canadian journal of biochemistry and physiology 39: 1295-1307.
Butaye, P., L. A. Devriese, and F. Haesebrouck. 2003. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clinical microbiology reviews 16: 175-188.
Caldeira, L. G. M. et al. 2017. Validation of an UHPLC-MS/MS Method for Screening of Antimicrobial Residues in Eggs and Their Application to Analyses of Eggs from Laying Hens Subjected to Pharmacological Treatment. Journal of Analytical Methods in Chemistry 2017: 3259073.
Catania, S. et al. 2010. Treatment of eggshell abnormalities and reduced egg production caused by Mycoplasma synoviae infection. Avian Dis 54: 961-964.

Catherman, D. R. et al. 1991. Metabolism of narasin in chickens and Japanese quail. Poult Sci 70: 120-125.
Caudill, A. B. et al. 2010. The effects of commercial cool water washing of shell eggs on Haugh unit, vitelline membrane strength, aerobic microorganisms, and fungi. Poultry Science 89: 160-168.
Cerniglia, C. E., S. A. Pineiro, and S. F. Kotarski. 2016. An update discussion on the current assessment of the safety of veterinary antimicrobial drug residues in food with regard to their impact on the human intestinal microbiome. Drug Testing and Analysis 8: 539-548.
Chain, E. P. o. C. i. t. F. 2014. Scientific Opinion on Chloramphenicol in food and feed. EFSA Journal 12: 3907.
Chang, Y. H. 2000. Prevalence of Salmonella spp. in poultry broilers and shell eggs in Korea. Journal of food protection 63: 655-658.
Charleston, B., J. J. Gate, I. A. Aitken, and L. Reeve-Johnson. 1998. Assessment of the efficacy of tilmicosin as a treatment for Mycoplasma gallisepticum infections in chickens. Avian Pathology 27: 190-195.
Chen, J., R. C. Clarke, and M. W. Griffiths. 1996. Use of Luminescent Strains of Salmonella enteritidis To Monitor Contamination and Survival in Eggs. Journal of food protection 59: 915-921.
Chen, S., H. Jiang, H. Peng, and X. Wu. 2017. The Utility of Ovotransferrin and Ovotransferrin-Derived Peptides as Possible Candidates in the Clinical Treatment of Cardiovascular Diseases. 2017: 6504518.
Chopra, I., and M. Roberts. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and molecular biology reviews : MMBR 65: 232-260 ; second page, table of contents.
Chowdhury, S. et al. 2015. Antibiotic residues in milk and eggs of commercial and local farms at Chittagong, Bangladesh. Veterinary world 8: 467-471.
Chu, P. S., D. J. Donoghue, and B. Shaikh. 2000. Determination of total 14C residues of sarafloxacin in eggs of laying hens. Journal of agricultural and food chemistry 48: 6409-6411.
Collier, C. T., J. D. van der Klis, B. Deplancke, D. B. Anderson, and H. R. Gaskins. 2003. Effects of tylosin on bacterial mucolysis, Clostridium perfringens colonization, and intestinal barrier function in a chick model of necrotic enteritis. Antimicrob Agents Chemother 47: 3311-3317.
Collignon, P., F. M. Aarestrup, R. Irwin, and S. McEwen. 2013. Human deaths and third-generation cephalosporin use in poultry, Europe. Emerging infectious diseases 19: 1339-1340.
Commission., E. 1995. Regulation 1442/95/EC of the Commission of 26 June on amending Annexes I, II, III and IV of Council Regulation (EEC) No. 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Official J.
Commission., E. 2008. Commission Regulation (EC) No. 589/2008 of 23 June 2008 laying down detailed rules for implementing Council Regulation (EC) No. 1234/2007 as regards marketing standards for eggs. Official Journal of the European Union 163: 6-23.
Cooper, K. M., and D. G. Kennedy. 2005. Nitrofuran antibiotic metabolites detected at parts per million concentrations in retina of pigs--a new matrix for enhanced monitoring of nitrofuran abuse. The Analyst 130: 466-468.
Cordeiro, C. M., H. Esmaili, G. Ansah, and M. T. Hincke. 2013. Ovocalyxin-36 is a pattern recognition protein in chicken eggshell membranes. PloS one 8: e84112.
Cotterill, O., and G. Geiger. 1977. Egg product yield trends from shell eggs. Poultry Science 56: 1027-1031.
Cozens, R. M. et al. 1986. Evaluation of the bactericidal activity of beta-lactam antibiotics on slowly growing bacteria cultured in the chemostat. Antimicrob Agents Chemother 29: 797-802.

Cracknell, V. C., J. Andreotis, G. Facibeni, E. Owais, and G. Pradella. 1986. An evaluation of apramycin soluble powder for the treatment of naturally acquired Escherichia coli infections in broilers. Journal of veterinary pharmacology and therapeutics 9: 273-279.
D'Alba, L. S., Matthew D., and 2015. Mechanisms of antimicrobial defense in avian eggs. Journal of Ornithology 156: 399-408.
D'Ambrosio, C. et al. 2008. Exploring the chicken egg white proteome with combinatorial peptide ligand libraries. Journal of proteome research 7: 3461-3474.
Da Silva, M. et al. 2019. The Unique Features of Proteins Depicting the Chicken Amniotic Fluid. Molecular & cellular proteomics : MCP 18: S174-s190.
Daft, B. M., A. A. Bickford, and M. A. Hammarlund. 1989. Experimental and Field Sulfaquinoxaline Toxicosis in Leghorn Chickens. Avian Diseases 33: 30-34.
De Reu, K. et al. 2006a. Influence of eggshell condensation on eggshell penetration and whole egg contamination with Salmonella enterica serovar enteritidis. Journal of food protection 69: 1539-1545.
De Reu, K. et al. 2006b. Bacterial shell contamination in the egg collection chains of different housing systems for laying hens. British poultry science 47: 163-172.
De Reu, K. et al. 2005. Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. British poultry science 46: 149-155.
De Reu, K. et al. 2006c. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including Salmonella enteritidis. International journal of food microbiology 112: 253-260.
Devriese, L. A., G. Daube, J. Hommez, and F. Haesebrouck. 1993. In vitro susceptibility of Clostridium perfringens isolated from farm animals to growth-enhancing antibiotics. The Journal of applied bacteriology 75: 55-57.
Dey, B. P., R. P. Reamer, N. H. Thaker, and A. M. Thaler. 2005a. Calf antibiotic and sulfonamide test (CAST) for screening antibiotic and sulfonamide residues in calf carcasses. Journal of AOAC International 88: 440-446.
Dey, B. P., N. H. Thaker, S. A. Bright, and A. M. Thaler. 2005b. Fast antimicrobial screen test (FAST): improved screen test for detecting antimicrobial residues in meat tissue. Journal of AOAC International 88: 447-454.
Diao, H. et al. 2008. Osteopontin regulates development and function of invariant natural killer T cells. Proc Natl Acad Sci U S A 105: 15884-15889.
Ding, H., L. He, K. Fung, Z. Chen, and G. Qiao. 2001. Pharmacokinetics of sarafloxacin in pigs and broilers following intravenous, intramuscular, and oral single-dose applications. Journal of veterinary pharmacology and therapeutics 24: 303-308.
Dinos, G. P. 2017. The macrolide antibiotic renaissance. Br J Pharmacol 174: 2967-2983.
Directive, E. 1999. Council Directive 99/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. Official journal of the European Communities 203: 53-57.
Domingo, J. L. 2014. Health risks of human exposure to chemical contaminants through egg consumption: A review. Food Research International 56: 159-165.
Donoghue, D. J. 2003. Antibiotic residues in poultry tissues and eggs: human health concerns? Poult Sci 82: 618-621.
Donoghue, D. J. et al. 1997. Modeling drug residue uptake by eggs: yolks contain ampicillin residues even after drug withdrawal and nondetectability in the plasma. Poult Sci 76: 458-462.
Donoghue, D. J., and K. Myers. 2000. Imaging Residue Transfer into Egg Yolks. Journal of agricultural and food chemistry 48: 6428-6430.
Donoho, A. L., R. J. Herberg, L. L. Zornes, and R. L. Van Duyn. 1982. Excretion and tissue distribution of [14C]monensin in chickens. Journal of agricultural and food chemistry 30: 909-913.

Dowling, L. 1992. Ionophore toxicity in chickens: a review of pathology and diagnosis. Avian Pathology 21: 355-368.
Dowling, P. M. 2013a. Aminoglycosides and aminocyclitols. Antimicrobial Therapy in Veterinary Medicine 14: 233-255.
Dowling, P. M. 2013b. Chloramphenicol, Thiamphenicol, and Florfenicol Antimicrobial Therapy in Veterinary Medicine. p 269-277.
Ebina, T., and K. Tsukada. 1991. Protease inhibitors prevent the development of human rotavirus-induced diarrhea in suckling mice. Microbiology and immunology 35: 583-588.
EFSA. The welfare aspects of various systems of keeping laying hens (147 pages). Annex to the EFSA Journal (2005) 197: 1-23.
Elkomy, A., F. Elsayed, M. Elbadawy, M. Aboubakr, and E. Aboelftouh. 2019. ORAL BIOAVAILABILITY OF CEFADROXIL IN HEALTHY BROILER CHICKENS. 8: 168-175.
Eltayb, A., S. Barakat, G. Marrone, S. Shaddad, and C. Stalsby Lundborg. 2012. Antibiotic use and resistance in animal farming: a quantitative and qualitative study on knowledge and practices among farmers in Khartoum, Sudan. Zoonoses and public health 59: 330-338.
EMA. 2016. Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health.
Englmaierová, M., E. Tumová, V. Charvátová, and M. Skřivan. 2014. Effects of laying hens housing system on laying performance, egg quality characteristics, and egg microbial contamination. Czech Journal of Animal Science 59: 345-352.
Etches, R. J. 1996. Reproduction in poultry. CAB international.
Evenepoel, P. et al. 1999. Amount and fate of egg protein escaping assimilation in the small intestine of humans. The American journal of physiology 277: G935-943.
Fajardo, T. A., R. Anantheswaran, V. M. Puri, and S. J. Knabel. 1995. Penetration of Salmonella enteritidis into Eggs Subjected to Rapid Cooling. Journal of Food Protection® 58: 473-477.
Farahmand, S., M. Rahim, A. Salehzadeh, R. Nordehr, and G. Farid. 2006. Oxytetracycline Residue in Chicken Tissues from Tehran Slaughterhouses in Iran. Pakistan Journal of Nutrition 5.
Farahmand, S., A. Salehzadeh, R. Nordehr, M. Rahim, and G. Fariba. 2007. Enrofloxacin Residue in Chicken Tissues from Tehran Slaughterhouses in Iran. Pakistan Journal of Nutrition 6.
Ferdous, J. et al. 2019. Assessing farmers' perspective on antibiotic usage and management practices in small-scale layer farms of Mymensingh district, Bangladesh. Veterinary world 12: 1441-1447.
Fernández, A. et al. 1998. Efficacy of phosphomycin in the control of Escherichia coli infection of broiler chickens. Research in veterinary science 65: 201-204.
Filazi, A., U. T. Sireli, and O. Cadirci. 2005. Residues of gentamicin in eggs following medication of laying hens. British poultry science 46: 580-583.
Fonseca, B. B. et al. 2014. Campylobacter jejuni in commercial eggs. Braz J Microbiol 45: 76-79.
Foster III, R., C. Ryan, R. Turk, and J. Quisenberry. 1960. Continuous feeding of hygromycin as a poultry anthelmintic and its effect upon laying house performance. Poultry Science 39: 492-499.
Fromm, D., and P. H. Margolf. 1958. The Influence of Sweating and Washing on Weight Loss, Bacterial Contamination and Interior Physical Quality of 12-Day Old Shell Eggs*. Poultry Science 37: 1273-1278.
Frye, J. G., and P. J. Fedorka-Cray. 2007. Prevalence, distribution and characterisation of ceftiofur resistance in Salmonella enterica isolated from animals in the USA from 1999 to 2003. International journal of antimicrobial agents 30: 134-142.
Gadbois, P., J. J. Brennan, L. Bruce, J. B. Wilson, and J. J. Aramini. 2008. The role of penicillin G potassium in managing Clostridium perfringens in broiler chickens. Avian Dis 52: 407-411.

Galad, H. et al. 2018. Survey of Antimicrobial Residue in Table Eggs among Layer Poultry Farmers in Maiduguri Metropolis, Borno State. Asian Journal of Animal and Veterinary Advances 13: 101-108.
Gantois, I. et al. 2009. Mechanisms of egg contamination by Salmonella Enteritidis. FEMS Microbiology Reviews 33: 718-738.
Garibaldi, J., and H. Bayne. 1962. The effect of iron on the Pseudomonas spoilage of farm washed eggs. Poultry Science 41: 850-853.
Gaugain-Juhel, M. et al. 2009. Validation of a liquid chromatography-tandem mass spectrometry screening method to monitor 58 antibiotics in milk: a qualitative approach. Food additives and contaminants 26: 1459-1471.
Gautron, J. et al. 2001a. Ovocalyxin-32, a novel chicken eggshell matrix protein. isolation, amino acid sequencing, cloning, and immunocytochemical localization. J Biol Chem 276: 39243-39252.
Gautron, J. et al. 2001b. Ovotransferrin is a matrix protein of the hen eggshell membranes and basal calcified layer. Connect Tissue Res 42: 255-267.
Gautron, J. et al. 2007. Cloning of ovocalyxin-36, a novel chicken eggshell protein related to lipopolysaccharide-binding proteins, bactericidal permeability-increasing proteins, and plunc family proteins. J Biol Chem 282: 5273-5286.
Gautron, J., and Y. Nys. 2006. Eggshell Matrix Proteins and Natural Defenses of the Egg. .
Giansanti, F. et al. 2005. Antiviral activity of ovotransferrin derived peptides. Biochemical and biophysical research communications 331: 69-73.
Giguère, S. 2013a. Lincosamides, pleuromutilins, and streptogramins. Antimicrobial therapy in veterinary medicine: 179-190.
Giguère, S. 2013b. Macrolides, azalides, and ketolides. Antimicrobial therapy in veterinary medicine: 211.
Giguère, S., J. F. Prescott, and P. M. Dowling. 2013. Antimicrobial therapy in veterinary medicine. John Wiley & Sons.
Giorgi, M., M. Romani, M. Bagliacca, and G. Mengozzi. 2001. Determination of thiamphenicol residues in albumin and yolk of hen eggs. Journal of veterinary pharmacology and therapeutics 23: 397-399.
Glisson, J. R., I. H. Cheng, J. Brown, and R. G. Stewart. 1989. The effect of oxytetracycline on the severity of airsacculitis in chickens infected with Mycoplasma gallisepticum. Avian Dis 33: 750-752.
Glisson, J. R., C. L. Hofacre, and G. F. Mathis. 2004. Comparative efficacy of enrofloxacin, oxytetracycline, and sulfadimethoxine for the control of morbidity and mortality caused by Escherichia coli in broiler chickens. Avian Dis 48: 658-662.
Gole, V. C. et al. 2014. Effect of egg washing and correlation between eggshell characteristics and egg penetration by various Salmonella Typhimurium strains. PloS one 9: e90987-e90987.
Goren, E., W. A. de Jong, and P. Doornenbal. 1988. Therapeutic efficacy of medicating drinking water with spectinomycin and lincomycin-spectinomycin in experimental Escherichia coli infection in poultry. The veterinary quarterly 10: 191-197.
Goren, E., W. A. De Jong, and A. Van Solkema. 1981. Some pharmacokinetical aspects of ampicillin trihydrate and its therapeutic efficacy in experimental escherichia coli infection in poultry. Avian Pathology 10: 43-55.
Goudah, A., K. Abo El Sooud, and A. M. Abd El-Aty. 2004. Pharmacokinetics and tissue residue profiles of erythromycin in broiler chickens after different routes of administration. DTW. Deutsche tierarztliche Wochenschrift 111: 162-165.
Graham, R., D. Palmer, B. C. Pratt, and C. A. Hart. 1988. In vitro activity of florphenicol. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology 7: 691-694.
Green, N. M. 1975. Avidin. Advances in protein chemistry 29: 85-133.

Greengard, O., A. Sentenac, and N. Mendelsohn. 1964. PHOSVITIN, THE IRON CARRIER OF EGG YOLK. Biochimica et biophysica acta 90: 406-407.
Guglielmetti, P., and A. Bartoloni. 2003. ESCHERICHIA COLI | Occurrence and Epidemiology of Species other than Escherichia coli. In: B. Caballero (ed.) Encyclopedia of Food Sciences and Nutrition (Second Edition). p 2162-2166. Academic Press, Oxford.
Gupta, S. et al. 2009. Colistin and polymyxin B: a re-emergence. Indian J Crit Care Med 13: 49-53.
Guyot, N. et al. 2013. Antibacterial activity of egg white: influence of physico-chemical conditions. World's Poultry Science Journal 69: 124 p.
Hagren, V., P. Peippo, and T. Lovgren. 2005. 3 - Detecting and controlling veterinary drug residues in poultry. In: G. C. Mead (ed.) Food Safety Control in the Poultry Industry. p 44-82. Woodhead Publishing.
Haines, R. B., and T. Moran. 1940a. Porosity of, and bacterial invasion through, the shell of the hen's egg. The Journal of hygiene 40: 453-461.
Haines, R. B., and T. Moran. 1940b. Porosity of, and bacterial invasion through, the shell of the hen's egg. J Hyg (Lond) 40: 453-461.
Haines, R. B., and T. Moran. 2009. Porosity of, and bacterial invasion through, the shell of the hen's egg. Epidemiology and Infection 40: 453-461.
Hamal, K. R., S. C. Burgess, I. Y. Pevzner, and G. F. Erf. 2006. Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. Poult Sci 85: 1364-1372.
Hamamoto, K., R. Koike, and Y. Machida. 2001. The effect of soybean oil refuse powder used as vehicle on the absorption of oxolinic acid in chickens under fasting and nonfasting conditions and the correlation with in vitro dissolution. Journal of veterinary pharmacology and therapeutics 24: 333-341.
Hamdy, A. H., D. D. Kratzer, L. M. Paxton, and B. J. Roberts. 1979. Effect of a single injection of lincomycin, spectinomycin, and linco-spectin on early chick mortality caused by Escherichia coli and Staphylococcus aureus. Avian Dis 23: 164-173.
Han, J. et al. 2013. Evaluation of virulence and antimicrobial resistance in Salmonella enterica serovar Enteritidis isolates from humans and chicken- and egg-associated sources. Foodborne pathogens and disease 10: 1008-1015.
Harry, E. G. 1963. The relationship between egg spoilage and the environment of the egg when laid. British poultry science 4: 91-100.
Hatta, H., M. P. Kapoor, and L. R. Juneja. 2008. Bioactive components in egg yolk. Egg Bioscience and Biotechnology. New Jersey: John Wiley & Sons Inc: 185-237.
Henri, J., C. Burel, P. Sanders, and M. Laurentie. 2009. Bioavailability, distribution and depletion of monensin in chickens. Journal of veterinary pharmacology and therapeutics 32: 451-456.
Hester, P. Y. 2017. Chapter 12 - Effects of Temperature and Storage Conditions on Eggs. In: P. Y. Hester (ed.) Egg Innovations and Strategies for Improvements. p 125-134. Academic Press, San Diego.
Hidalgo, A., M. Rossi, F. Clerici, and S. Ratti. 2008. A market study on the quality characteristics of eggs from different housing systems. Food Chemistry 106: 1031-1038.
Hincke, M. T. 1995. Ovalbumin is a Component of the Chicken Eggshell Matrix. Connective Tissue Research 31: 227-233.
Hincke, M. T., Y. C. Chien, L. C. Gerstenfeld, and M. D. McKee. 2008. Colloidal-gold immunocytochemical localization of osteopontin in avian eggshell gland and eggshell. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 56: 467-476.
Hincke, M. T. et al. 2003. Purification of ovocalyxin-32, a novel chicken eggshell matrix protein. Connect Tissue Res 44 Suppl 1: 16-19.

Hincke, M. T. et al. 2000. Identification and localization of lysozyme as a component of eggshell membranes and eggshell matrix. Matrix biology : journal of the International Society for Matrix Biology 19: 443-453.
Hincke, M. T. et al. 2012. The eggshell: structure, composition and mineralization. Frontiers in bioscience (Landmark edition) 17: 1266-1280.
Hincke, M. T., C. P. W. Tsang, M. Courtney, V. Hill, and R. Narbaitz. 1995. Purification and immunochemistry of a soluble matrix protein of the chicken eggshell (ovocleidin 17). Calcified Tissue International 56: 578-583.
Ho, Y. I., C. Y. Chan, and A. F. Cheng. 1997. In-vitro activities of aminoglycoside-aminocyclitols against mycobacteria. The Journal of antimicrobial chemotherapy 40: 27-32.
Hofacre, C., J. Fricke, and T. Inglis. 2013a. Antimicrobial Drug Use in Poultry. p 569-587.
Hofacre, C. L., J. A. Fricke, and T. Inglis. 2013b. Antimicrobial drug use in poultry. Antimicrobial therapy in veterinary medicine 4.
Holmes, N., and P. Charles. 2009. Safety and Efficacy Review of Doxycycline. Clinical Medicine : Therapeutics 1.
Holtman, D. F., and G. Fisher. 1947. The Application of Sulfonamides to the Control of Typhoid in Poultry. Poultry Science 26: 478-488.
Huang, Y. U. R., Y.-C. Hung, S.-Y. Hsu, Y.-w. Huang, and D.-F. Hwang. 2008. Application of electrolyzed water in the food industry. Food Control 19: 329-345.
Hughey, V. L., and E. A. Johnson. 1987. Antimicrobial activity of lysozyme against bacteria involved in food spoilage and food-borne disease. Applied and environmental microbiology 53: 2165-2170.
Humphrey, T., A. Whitehead, A. Gawler, A. Henley, and B. Rowe. 1991. Numbers of Salmonella enteritidis in the contents of naturally contaminated hens' eggs. Epidemiology & Infection 106: 489-496.
Huneau-Salaün, A., V. Michel, D. Huonnic, L. Balaine, and S. Le Bouquin. 2010. Factors influencing bacterial eggshell contamination in conventional cages, furnished cages and free-range systems for laying hens under commercial conditions. British poultry science 51: 163-169.
Hutchison, M. L. et al. 2004. An assessment of the microbiological risks involved with egg washing under commercial conditions. Journal of food protection 67: 4-11.
Ibrahim, H., U. Thomas, and A. Pellegrini. 2001. A Helix-Loop-Helix Peptide at the Upper Lip of the Active Site Cleft of Lysozyme Confers Potent Antimicrobial Activity with Membrane Permeabilization Action. The Journal of biological chemistry 276: 43767-43774.
Ibrahim, H. R., and T. Kiyono. 2009. Novel anticancer activity of the autocleaved ovotransferrin against human colon and breast cancer cells. Journal of agricultural and food chemistry 57: 11383-11390.
Ibrahim, H. R., Y. Sugimoto, and T. Aoki. 2000. Ovotransferrin antimicrobial peptide (OTAP-92) kills bacteria through a membrane damage mechanism. Biochimica et biophysica acta 1523: 196-205.
Jammoul, and N. Darra. 2019. Evaluation of Antibiotics Residues in Chicken Meat Samples in Lebanon. Antibiotics 8: 69.
Jernigan, A. D., R. C. Wilson, R. C. Hatch, and D. T. Kemp. 1988. Pharmacokinetics of gentamicin after intravenous, intramuscular, and subcutaneous administration in cats. American journal of veterinary research 49: 32-35.
Ji, X. et al. 2019. Evaluation of Tilmicosin Contamination in Eggs Following Its Administration to Laying Hens and Subsequent Assessment of Dietary Risks to Chinese Consumers. Journal of Food Science 84.

JOHNSTON, R. W., R. H. REAMER, E. W. HARRIS, H. G. FUGATE, and B. SCHWAB. 1981. A New Screening Method for the Detection of Antibiotic Residues in Meat and Poultry Tissues. Journal of food protection 44: 828-831.
Jones, D. R., K. E. Anderson, P. A. Curtis, and F. T. Jones. 2002. Microbial contamination in inoculated shell eggs: I. Effects of layer strain and hen age1. Poultry Science 81: 715-720.
Jones, D. R., and M. T. Musgrove. 2007. Pathogen prevalence and microbial levels associated with restricted shell eggs. Journal of food protection 70: 2004-2007.
Jones, F. T., D. V. Rives, and J. B. Carey. 1995. Salmonella contamination in commercial eggs and an egg production facility. Poult Sci 74: 753-757.
Joshi, R. et al. 2019. Effects of the storage environment on the quality attributes of eggs with a washing treatment.
Kan, C. A., H. Gend, and M. Àerts. 1990. THE CARRY-OVER OF LOW LEVELS OF COCCIDIOSTATS FROM LAYERS FEED TO EGGS.
Kassaify, Z., E. Li, and Y. Mine. 2005. Identification of Antiadhesive Fraction(s) in Nonimmunized Egg Yolk Powder: In Vitro Study. Journal of agricultural and food chemistry 53: 4607-4614.
Keller, L. H., C. E. Benson, K. Krotec, and R. J. Eckroade. 1995. Salmonella enteritidis colonization of the reproductive tract and forming and freshly laid eggs of chickens. Infection and immunity 63: 2443-2449.
Kemal, J., B. Sibhat, S. Menkir, and D. Beyene. 2016. Prevalence, assessment, and antimicrobial resistance patterns of Salmonella from raw chicken eggs in Haramaya, Ethiopia. Journal of infection in developing countries 10: 1230-1235.
Kempf, I. et al. 2013. What do we know about resistance to colistin in Enterobacteriaceae in avian and pig production in Europe? International journal of antimicrobial agents 42: 379-383.
Kennedy, D. G., W. J. Blanchflower, P. J. Hughes, and W. J. McCaughey. 1996. The incidence and cause of lasalocid residues in eggs in Northern Ireland. Food additives and contaminants 13: 787-794.
Kennedy, D. G., P. J. Hughes, and W. J. Blanchflower. 1998. Ionophore residues in eggs in Northern Ireland: Incidence and cause. Food Additives & Contaminants 15: 535-541.
Khadre, M. A., A. E. Yousef, and J. G. Kim. 2001. Microbiological Aspects of Ozone Applications in Food: A Review. Journal of Food Science 66: 1242-1252.
Khan, A. et al. 2015. Isolation, Identification and Antibiogram of Escherichia coli from Table Eggs. Journal of Animal Health and Production 4: 1-5.
Khan, S., and J. Roberts. 2014. The eggshell cuticle of the laying hen. World's Poultry Science Journal 70: 693-708.
Khong, S. P. et al. 2004. Analysis of matrix-bound nitrofuran residues in worldwide-originated honeys by isotope dilution high-performance liquid chromatography-tandem mass spectrometry. Journal of agricultural and food chemistry 52: 5309-5315.
Kilonzo-Nthenge, A., S. N. Nahashon, S. Godwin, S. Liu, and D. Long. 2016. Prevalence and Antimicrobial Resistance of Enterobacteriaceae in Shell Eggs from Small-Scale Poultry Farms and Farmers' Markets. Journal of food protection 79: 2031-2037.
Kim, J. G., A. E. Yousef, and M. A. Khadre. 2003. Ozone and its current and future application in the food industry. Advances in food and nutrition research 45: 167-218.
Kim, J. W., and M. F. Slavik. 1996. Changes in Eggshell Surface Microstructure after Washing with Cetylpyridinium Chloride or Trisodium Phosphate. Journal of food protection 59: 859-863.
Kiser, J. S., M. K. Waters, M. I. Wirshup, and C. A. Bottorff. 1948. Sulfonamide Blood Concentrations in Chickens and Turkey Poults. Poultry Science 27: 219-222.
Kitagawa, T. et al. 1988. Sensitive enzyme immunoassay of cephalexin residues in milk, hen tissues, and eggs. Journal - Association of Official Analytical Chemists 71: 915-920.
Kleven, S. H. 2008. Control of avian mycoplasma infections in commercial poultry. Avian Dis 52: 367-374.

Kleven, S. H., and D. P. Anderson. 1971. In vitro Activity of Various Antibiotics against Mycoplasma synoviae. Avian Diseases 15: 551-557.
Koidis, P., M. Bori, and K. Vareltzis. 2000. Efficacy of ozone treatment to eliminate Salmonella enteritidis from eggshell surface. Archiv für lebensmittelhygiene 51: 4-6.
Korpela, J. 1984. Avidin, a high affinity biotin-binding protein, as a tool and subject of biological research. Medical biology 62: 5-26.
Kovacs-Nolan, J., M. Phillips, and Y. Mine. 2005. Advances in the value of eggs and egg components for human health. Journal of agricultural and food chemistry 53: 8421-8431.
Kraft, A. A., L. E. Elliott, and A. W. Brant. 1958a. The Shell Membrane as a Barrier to Bacterial Penetration of Eggs. Poultry Science 37: 238-240.
Kraft, A. A., E. H. McNally, and A. W. Brant. 1958b. Shell Quality and Bacterial Infection of Shell Eggs. Poultry Science 37: 638-644.
Krause, K. M., A. W. Serio, T. R. Kane, and L. E. Connolly. 2016. Aminoglycosides: An Overview. Cold Spring Harb Perspect Med 6: a027029.
Kusuda, S., A. Iwasawa, O. Doi, Y. Ohya, and N. Yoshizaki. 2011. Diversity of the Cuticle Layer of Avian Eggshells. The Journal of Poultry Science 48: 119-124.
Lanckriet, A. et al. 2010. The effect of commonly used anticoccidials and antibiotics in a subclinical necrotic enteritis model. Avian pathology : journal of the W.V.P.A 39: 63-68.
LayWel. 2006. Deliverable 2.3 Description of housing systems for Laying hens.
Leclair, K., H. Heggart, M. Oggel, F. M. Bartlett, and R. C. McKellar. 1994. Modelling the inactivation of Listeria monocytogenes and Salmonella typhimurium in simulated egg wash water. Food Microbiology 11: 345-353.
Ledvinka, Z., E. Tumová, M. Englmaierová, and M. Podsednicek. 2012a. Egg quality of three laying hen genotypes kept in conventional cages and on litter. Archiv fur Geflugelkunde 76: 38-43.
Ledvinka, Z., E. Tůmová, M. Englmaierová, and M. Podsedníček. 2012b. Egg quality of three laying hen genotypes kept in conventional cages and on litter. Arch Geflűgelkunde 76: 38-46.
Lee, E., H. Sunwoo, and J. Sim. 2000. Ovoglobulin IgY.
Lee, E. N., H. H. Sunwoo, K. Menninen, and J. S. Sim. 2002. In vitro studies of chicken egg yolk antibody (IgY) against Salmonella enteritidis and Salmonella typhimurium. Poult Sci 81: 632-641.
Leitner, G., R. Waiman, and E. D. Heller. 2001. The effect of apramycin on colonization of pathogenic Escherichia coli in the intestinal tract of chicks. The veterinary quarterly 23: 62-66.
Leslie, G. A., and L. W. Clem. 1969. Phylogen of immunoglobulin structure and function. 3. Immunoglobulins of the chicken. J Exp Med 130: 1337-1352.
Li-Chan, E., and H. O. Kim. 2007. Structure and Chemical Compositions of Eggs. p 1-95.
Li-Chan, E. C., and H.-O. Kim. 2008. Structure and chemical composition of eggs. Egg bioscience and biotechnology 10: 9780470181249.
Li-Chan, E. C., W. D. Powrie, and S. Nakai. 2017. The chemistry of eggs and egg products Egg science and technology. p 105-175. CRC Press.
Liao, W., S. Chakrabarti, S. T. Davidge, and J. Wu. 2016. Modulatory Effects of Egg White Ovotransferrin-Derived Tripeptide IRW (Ile-Arg-Trp) on Vascular Smooth Muscle Cells against Angiotensin II Stimulation. Journal of agricultural and food chemistry 64: 7342-7347.
Lichovnikova, M., and L. Zeman. 2008. Effect of housing system on the calcium requirement of laying hens and on eggshell quality. Czech Journal of Animal Science 53: 162-168.
LIFSHITZ, A., R. C. BAKER, and H. B. NAYLOR. 1964. The Relative Importance of Chicken Egg Exterior Structures in Resisting Bacterial Penetration. Journal of Food Science 29: 94-99.
Liu, Y.-C., T.-H. Chen, Y.-C. Wu, Y.-C. Lee, and F.-J. Tan. 2016. Effects of egg washing and storage temperature on the quality of eggshell cuticle and eggs. Food Chemistry 211: 687-693.

Lock, J. L., J. Dolman, and R. G. Board. 1992. Observations on the mode of bacterial infection of hens' eggs. FEMS microbiology letters 100: 71-73.
Löhren, U., A. Ricci, and T. Cummings. 2009. Guidelines for Antimicrobial Use in Poultry. p 126-142.
Lolo, M. et al. 2005. Study of enrofloxacin depletion in the eggs of laying hens using diphasic dialysis extraction/purification and determinative HPLC-MS analysis. Journal of agricultural and food chemistry 53: 2849-2852.
Lorenz, F., and P. B. Starr. 1952. Spoilage of washed eggs: 1. Effect of sprayed versus static water under different washing temperatures. Poultry Science 31: 204-214.
LUBLIN, A., I. MALER, S. MECHANI, R. PINTO, and S. SELA-SALDINGER. 2015. Survival of Salmonella enterica Serovar Infantis on and within Stored Table Eggs. Journal of food protection 78: 287-292.
LUCORE, L. A., F. T. JONES, K. E. ANDERSON, and P. A. CURTIS. 1997. Internal and External Bacterial Counts from Shells of Eggs Washed in a Commercial-Type Processor at Various Wash-Water Temperatures†. Journal of food protection 60: 1324-1328.
Lukásová, J., and M. Labounek. 1976. [Penetration of Staphylococci through the egg-shell]. Vet Med (Praha) 21: 215-220.
Maamari, J., and T. Na'was. 2017. CONTAMINATION OF THE SHELLS OF LEBANESE FREE RANGE CHICKEN EGGS WITH POTENTIAL PATHOGENS. International Journal of Advanced Research 5: 149-154.
Mallet, S., V. Guesdon, A. M. Ahmed, and Y. Nys. 2006. Comparison of eggshell hygiene in two housing systems: standard and furnished cages. British poultry science 47: 30-35.
Mann, K. 2007. The chicken egg white proteome. Proteomics 7: 3558-3568.
Mann, K. et al. 2003. Disulfide-linked heterodimeric clusterin is a component of the chicken eggshell matrix and egg white. Matrix biology : journal of the International Society for Matrix Biology 22: 397-407.
Mann, K., M. T. Hincke, and Y. Nys. 2002. Isolation of ovocleidin-116 from chicken eggshells, correction of its amino acid sequence and identification of disulfide bonds and glycosylated Asn. Matrix biology : journal of the International Society for Matrix Biology 21: 383-387.
Mann, K., B. Macek, and J. V. Olsen. 2006. Proteomic analysis of the acid-soluble organic matrix of the chicken calcified eggshell layer. Proteomics 6: 3801-3810.
Mann, K., and M. Mann. 2008. The chicken egg yolk plasma and granule proteomes. Proteomics 8: 178-191.
Mann, K., and F. Siedler. 1999. The amino sequence of ovocleidin 17, a major protein of the avian eggshell calcified layer. IUBMB Life 47: 997-1007.
Marie, P. et al. 2015. Quantitative proteomics and bioinformatic analysis provide new insight into protein function during avian eggshell biomineralization. Journal of proteomics 113: 178-193.
Maron, D. F., T. J. S. Smith, and K. E. Nachman. 2013. Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. Globalization and Health 9: 48.
Marrett, L. E., E. J. Robb, and R. K. Frank. 2000. Efficacy of neomycin sulfate water medication on the control of mortality associated with colibacillosis in growing turkeys. Poult Sci 79: 12-17.
Mayes, F. J., and M. A. Takeballi. 1983. Microbial Contamination of the Hen's Egg: A Review. Journal of food protection 46: 1092-1098.
McCalla, D. R. 1983. Mutagenicity of nitrofuran derivatives: Review. Environmental Mutagenesis 5: 745-765.
McCracken, R. J., and D. G. Kennedy. 2007. Detection, accumulation and distribution of nitrofuran residues in egg yolk, albumen and shell. Food additives and contaminants 24: 26-33.
McCracken, R. J., D. E. Spence, S. D. Floyd, and D. G. Kennedy. 2001. Evaluation of the residues of furazolidone and its metabolite, 3-amino-2-oxazolidinone (AOZ), in eggs. Food Additives & Contaminants 18: 954-959.

McKellar, Q. A., and L. J. Horspool. 1995. Stability of penicillin G, ampicillin, amikacin and oxytetracycline and their interactions with food in in vitro simulated equine gastrointestinal contents. Res Vet Sci 58: 227-231.
Medina-Gudiño, J. et al. 2020. Analysis of Neutral Electrolyzed Water anti-bacterial activity on contaminated eggshells with Salmonella enterica or Escherichia coli. International journal of food microbiology 320: 108538.
Mehtabuddin, A., T. Ahmad, S. Nadeem, Z. Tanveer, and J. Arshad. 2012. Sulfonamide residues determination in commercial poultry meat and eggs. J Anim Plant Sci 22: 473-478.
Mertens, K. et al. 2006. Monitoring of eggshell breakage and eggshell strength in different production chains of consumption eggs. Poult Sci 85: 1670-1677.
Migaki, T. T. et al. 1993. Efficacy of danofloxacin and tylosin in the control of mycoplasmosis in chicks infected with tylosin-susceptible or tylosin-resistant field isolates of Mycoplasma gallisepticum. Avian Dis 37: 508-514.
Mine, Y., F. Ma, and S. Lauriau. 2004. Antimicrobial peptides released by enzymatic hydrolysis of hen egg white lysozyme. Journal of agricultural and food chemistry 52: 1088-1094.
Mine, Y., C. Oberle, and Z. Kassaify. 2003. Eggshell matrix proteins as defense mechanism of avian eggs. Journal of agricultural and food chemistry 51: 249-253.
Miyamoto T, B. E., Tanaka T, Sasai K, Fukata T, Arakawa A. . 1997. Salmonella Enteritidis contamination of eggs from hens inoculated by vaginal, cloacal and intravenous routes. . Avian Diseases: 34, 463–465.
Miyamoto, T. et al. 1998. Salmonella penetration through eggshell associated with freshness of laid eggs and refrigeration. Journal of food protection 61: 350-353.
Moon, S. H., J. H. Lee, J. H. Kim, H. D. Paik, and D. U. Ahn. 2017. In vitro cytotoxic and ACE-inhibitory activities of promod 278P hydrolysate of ovotransferrin from chicken egg white. Poult Sci 96: 1982-1987.
Mostert B.E., B. E. H., Van der Walt J.C. . 1995. Influence of different housing systems on performance of hens of four laying strains. . South African Journal of Animal Science 25: 80–86.
Musgrove, M. T. 2011. 1 - Microbiology and safety of table eggs. In: F. Van Immerseel, Y. Nys and M. Bain (eds.) Improving the Safety and Quality of Eggs and Egg Products. p 3-33. Woodhead Publishing.
Nagata, T., M. Saeki, T. Iida, M. Kataoka, and R. Ino. 1989. Transfer of Dietary Sulfadimethoxine and Sulfamonomethoxine into Eggs and Their Disappearance from Eggs. Food Hygiene and Safety Science (Shokuhin Eiseigaku Zasshi) 30: 375-383_371.
Nascimento, V. P., S. Cranstoun, and S. E. Solomon. 1992. Relationship between shell structure and movement of Salmonella enteritidis across the eggshell wall. British poultry science 33: 37-48.
Nash, J. A., T. N. Ballard, T. E. Weaver, and H. T. Akinbi. 2006. The peptidoglycan-degrading property of lysozyme is not required for bactericidal activity in vivo. Journal of immunology (Baltimore, Md. : 1950) 177: 519-526.
Nimalaratne, C., and J. Wu. 2015a. Hen Egg as an Antioxidant Food Commodity: A Review. Nutrients 7: 8274-8293.
Nimalaratne, C., and J. Wu. 2015b. Hen Egg as an Antioxidant Food Commodity: A Review. Nutrients 7: 8274-8293.
Noack, S., H. Chapman, and P. Selzer. 2019. Anticoccidial drugs of the livestock industry. Parasitology Research 118.
Nolan, L. K., H. J. Barnes, J.-P. Vaillancourt, T. Abdul-Aziz, and C. M. Logue. 2017. Colibacillosis Diseases of Poultry. p 751-805.
Novilla, M. N., D. McClary, and S. B. Laudert. 2017. Chapter 29 - Ionophores. In: R. C. Gupta (ed.) Reproductive and Developmental Toxicology (Second Edition). p 503-518. Academic Press.

Nys, Y., and J. Gautron. 2007. Structure and Formation of the Eggshell. p 99-102.
Nys, Y., J. Gautron, J. M. Garcia-Ruiz, and M. T. Hincke. 2004. Avian eggshell mineralization: biochemical and functional characterization of matrix proteins. Comptes Rendus Palevol 3: 549-562.
Nys, Y., M. Hincke, J. Arias, J. Garcia-Ruiz, and S. Solomon. 1999. Avian eggshell mineralization. Poultry and Avian Biology Reviews 10: 143-166.
Nys, Y., M. T. Hincke, J. L. Arias, J. M. Garcia-Ruiz, and S. E. Solomon. . 1999. Avian eggshell mineralization. . Poult. Avian Biol. Rev.: 10:143–166.
O'Bryan, C. A., S. C. Ricke, and P. G. Crandall. 2017. Chapter 18 - Chemical and Physical Sanitation and Pasteurization Methods for Intact Shell Eggs. In: S. C. Ricke and R. K. Gast (eds.) Producing Safe Eggs. p 373-390. Academic Press, San Diego.
Okorie-Kanu, O. J., E. V. Ezenduka, C. O. Okorie-Kanu, L. C. Ugwu, and U. J. Nnamani. 2016. Occurrence and antimicrobial resistance of pathogenic Escherichia coli and Salmonella spp. in retail raw table eggs sold for human consumption in Enugu state, Nigeria. Veterinary world 9: 1312-1319.
Omana, D. A., J. Wang, and J. Wu. 2010a. Ovomucin–a glycoprotein with promising potential. Trends in food science & technology 21: 455-463.
Omana, D. A., J. Wang, and J. Wu. 2010b. Ovomucin – a glycoprotein with promising potential. Trends in Food Science & Technology 21: 455-463.
Padron, M. 1990. Salmonella typhimurium penetration through the eggshell of hatching eggs. Avian Dis 34: 463-465.
Papadopoulou, C. et al. 1997. Bacterial strains isolated from eggs and their resistance to currently used antibiotics: is there a health hazard for consumers? Comparative immunology, microbiology and infectious diseases 20: 35-40.
Pavlovski, Z., S. Hopic, and M. Lukic. 2001. Housing systems for layers and egg quality. Biotechnology in Animal Husbandry 17: 197-201.
Pearson, J., G. G. Southam, and R. A. Holley. 1987. Survival and transport of bacteria in egg washwater. Applied and environmental microbiology 53: 2060-2065.
Pérez, D. S., M. O. Tapia, and A. L. Soraci. 2014. Fosfomycin: Uses and potentialities in veterinary medicine. Open Vet J 4: 26-43.
Peterson, L. R. 2001. Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 33 Suppl 3: S180-186.
Phuc Nguyen, M. C. et al. 2009. Escherichia coli as reservoir for macrolide resistance genes. Emerging infectious diseases 15: 1648-1650.
Pištěková, V., M. Hovorka, V. Večerek, E. Strakova, and P. Suchý. 2006. The quality comparison of eggs laid by laying hens kept in battery cages and in a deep litter system. Czech Journal of Animal Science 51: 318-325.
Prescott, J. F. 2013. Beta-lactam Antibiotics Antimicrobial Therapy in Veterinary Medicine. p 133-152.
Pyzik, E., and A. Marek. 2013. Plasmid profile analysis and evaluation of antibiotic susceptibility of Staphylococcus aureus strains isolated from table chicken eggs. Polish journal of veterinary sciences 16: 307-312.
Quarles, C. L., R. F. Gentry, and G. O. Bressler. 1970. Bacterial Contamination in Poultry Houses and its Relationship to Egg Hatchability1. Poultry Science 49: 60-66.
Regulation, C. 1993. No. 2901/93 of 18 October 1993 amending Annexes I, II, III and IV to Regulation (EEC) No. 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Official Journal L 264: 1-4.
Rehault-Godbert, S. et al. 2011. Molecules involved in chemical defence of the chicken egg Improving the safety and quality of eggs and egg products. p 183-208. Elsevier.

Réhault-Godbert, S. et al. 2011. Molecules involved in chemical defence of the chicken egg. Improving the Safety and Quality of Eggs and Egg Products: Egg Chemistry, Production and Consumption: 183-208.
Reu, K. et al. 2007. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including Salmonella Enteritidis. International journal of food microbiology 112: 253-260.
Reu, K. et al. 2008. Estimation of the vertical and horizontal bacterial infection of hen's table eggs. World's Poultry Science Journal 64.
Reu, K. et al. 2009. Bacteriological contamination, dirt, and cracks of eggshells in furnished cages and noncage systems for laying hens: An international on-farm comparison. Poultry science 88: 2442-2448.
Riond, J. L., and J. E. Riviere. 1988. Pharmacology and toxicology of doxycycline. Veterinary and human toxicology 30: 431-443.
Rizk, S. S., J. C. Ayres, and A. A. Kraft. 1966. Disinfection of Eggs Artificially Inoculated with Salmonellae: 1. Application of Several Disinfectants1. Poultry Science 45: 764-769.
Roberts, E., L. E. Card, and J. O. Alberts. 1948. The Use of Sulfonamides in the Control of Pullorum Disease*. Poultry Science 27: 194-200.
Rodriguez-Navarro, A. B., N. Dominguez-Gasca, A. Munoz, and M. Ortega-Huertas. 2013. Change in the chicken eggshell cuticle with hen age and egg freshness. Poult Sci 92: 3026-3035.
Rodriguez Romo, L., M. Vurma, K. Lee, and A. Yousef. 2007. Research Note: Penetration of Ozone Gas Across the Shell of Hen Eggs. Ozone-science & Engineering - OZONE-SCI ENG 29: 147-150.
Rogers, H., and H. Perkins. 1968. The disruption of bacteria and the preparation of cell walls. Cell walls and membranes. E. and FN Spon Ltd., London: 196-205.
Romanoff, A. L., and A. J. Romanoff. 1949. The avian egg. The avian egg.: 134.
Romváry, A., and F. Simon. 1992. Sulfonamide residues in eggs. Acta Vet Hung 40: 99-106.
Rose-Martel, M., J. Du, and M. T. Hincke. 2012. Proteomic analysis provides new insight into the chicken eggshell cuticle. Journal of proteomics 75: 2697-2706.
Roudaut, B. 1989a. Depletion of colistin in eggs following medication of laying hens. The veterinary quarterly 11: 183-185.
Roudaut, B. 1989b. Residues of aminoglycoside antibiotics in eggs after medication of laying hens. British poultry science 30: 265-271.
Roudaut, B. 1998. Elimination of oxolinic acid in eggs after oral treatment of laying hens. British poultry science 39: 47-52.
Roudaut, B., and M. Garnier. 2002. Sulphonamide residues in eggs following drug administration via the drinking water. Food Additives & Contaminants 19: 373-378.
Roudaut, B., J. Moretain, and J. Boisseau. 1987a. Residues of ampicillin in eggs after oral and parenteral administration. Recueil de Medecine Veterinaire (France).
Roudaut, B., J. P. Moretain, and J. Boisseau. 1987b. Excretion of oxytetracycline in eggs after medication of laying hens. Food additives and contaminants 4: 297-307.
Roudaut, B., J. P. Moretain, and J. Boisseau. 1989. Excretion of tetracycline and chlortetracycline in eggs after oral medication of laying hens. Food additives and contaminants 6: 71-78.
Ruiz Ramos, J., and M. Salavert Lleti. 2019. Fosfomycin in infections caused by multidrug-resistant Gram-negative pathogens. Revista espanola de quimioterapia : publicacion oficial de la Sociedad Espanola de Quimioterapia 32 Suppl 1: 45-54.
Rzedzicki, J., and D. Stępień-Pyśniak. 2009. Antimicrobial defence mechanisms of chicken eggs and possibilities for their use in protecting human and animal health. Annales Universitatis Mariae Curie-Skłodowska. Sectio DD, Medicina Veterinaria 64: 1-8.

Saleh, G., N. El Darra, S. Kharroubi, and M. T. Farran. 2020. Influence of storage conditions on quality and safety of eggs collected from Lebanese farms. Food Control 111: 107058.
Samouris, G., H. Tsoukali-Papadopoulou, B. Nathanael, and V. Mirtsou-Fidani. 1998. Chlorampenicol residues in albumen and yolk of hen's eggs after experimental administration. Archiv fuer Gefluegelkunde (Germany).
Sasanya, J. J., J. W. O. Okeng, F. Ejobi, and M. Muganwa. 2005. Use of sulfonamides in layers in Kampala district, Uganda and sulfonamide residues in commercial eggs. Afr Health Sci 5: 33-39.
Sattar Khan, M. A. et al. 2000. Bactericidal action of egg yolk phosvitin against Escherichia coliunder thermal stress. Journal of agricultural and food chemistry 48: 1503-1506.
Sattar, S. et al. 2014. Antibiotic residues in broiler and layer meat in Chittagong district of Bangladesh. Veterinary world 7: 738-743.
Sauter, E. A., and C. F. Petersen. 1969. The effect of egg shell quality on penetration by Pseudomonas fluorescens. Poult Sci 48: 1525-1528.
Sauter, E. A., and C. F. Petersen. 1974. The Effect of Egg Shell Quality on Penetration by Various Salmonellae. Poultry Science 53: 2159-2162.
Sava, G. 1989. Reduction of B16 melanoma metastases by oral administration of egg-white lysozyme. Cancer chemotherapy and pharmacology 25: 221-222.
Schade, R., and P. A. Chacana. 2007. Livetin Fractions (IgY). In: R. Huopalahti, R. López-Fandiño, M. Anton and R. Schade (eds.) Bioactive Egg Compounds. p 25. Springer Berlin Heidelberg, Berlin, Heidelberg.
Schoeni, J. L., K. A. Glass, J. L. McDermott, and A. C. Wong. 1995. Growth and penetration of Salmonella enteritidis, Salmonella heidelberg and Salmonella typhimurium in eggs. International journal of food microbiology 24: 385-396.
Schriemer, T., J. Paulissen, and K. Dame. 1992. Evaluation of ceftiofur sodium for control of terminal bacterial infections in day-old broiler chickens. In: Proc 19th World's Poultry Congress. Amsterdam, The Netherlands. p 427.
Schwaiger, K., E.-M. V. Schmied, and J. Bauer. 2008a. Comparative Analysis of Antibiotic Resistance Characteristics of Gram-negative Bacteria Isolated from Laying Hens and Eggs in Conventional and Organic Keeping Systems in Bavaria, Germany. Zoonoses and public health 55: 331-341.
Schwaiger, K., E. M. Schmied, and J. Bauer. 2008b. Comparative analysis of antibiotic resistance characteristics of Gram-negative bacteria isolated from laying hens and eggs in conventional and organic keeping systems in Bavaria, Germany. Zoonoses and public health 55: 331-341.
Sedeik, M. 2018. Pharmacokinetics and Tissue Residues of Tylosin in Broiler Chickens.
Sellyei, B., Z. Varga, K. Szentesi-Samu, E. Kaszanyitzky, and T. Magyar. 2009. Antimicrobial susceptibility of Pasteurella multocida isolated from swine and poultry. Acta veterinaria Hungarica 57: 357-367.
Seuss-Baum, I. 2007. Nutritional evaluation of egg compounds Bioactive egg compounds. p 117-144. Springer.
Shah, F. 1998. Erythromycin. Pediatrics in review 19: 140-141.
Shimmura, T. et al. 2010. Multi-factorial investigation of various housing systems for laying hens. British poultry science 51: 31-42.
Simons, P. C. M. 1971. Ultrastructure of the hen eggshell and its physiological interpretation, Centre for Agricultural Publishing and Documentation, Wageningen.
Singh, T., A. Singh, N. Meena, and A. Kumar. 2019. Determination of gentamicin residues in chicken meat by high performance liquid chromatography. Indian Journal of Poultry Science 54: 155.
Sirdar, M. M., J. Picard, S. Bisschop, and B. Gummow. 2012. A questionnaire survey of poultry layer farmers in Khartoum State, Sudan, to study their antimicrobial awareness and usage patterns. The Onderstepoort journal of veterinary research 79: E1-8.

Smaniotto, B. et al. 2017. Age influence on quality of shell and penetration in bacterial laying lightweight eggs. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 69: 1301-1310.
Smeltzer, T. I., K. Orange, B. Peel, and G. Runge. 1979. Bacterial penetration in floor and nest box eggs from meat and layer birds. Aust Vet J 55: 592-593.
Smith, A., S. P. Rose, R. G. Wells, and V. Pirgozliev. 2000. The effect of changing the excreta moisture of caged laying hens on the excreta and microbial contamination of their egg shells. British poultry science 41: 168-173.
Smith, H. W., and J. F. Tucker. 1978. Oral administration of neomycin to chickens experimentally infected with Salmonella typhimurium. The Veterinary record 102: 354-356.
Solomon, S. E. 1999. Gordon Memorial Lecture. An egg ist ein ei, es un huevo, est un oeuf. British poultry science 40: 5-11.
Sparks, N., and R. Board. 1984. Cuticle, shell porosity and water uptake through hens' eggshells. British poultry science 25: 267-276.
Sparks, N. H., and R. G. Board. 1985. Bacterial penetration of the recently oviposited shell of hens' eggs. Aust Vet J 62: 169-170.
Sparks, N. H. C. 1994. Shell accessory materials: structure and function. In: R. G. Board and R. Fuller (eds.) Microbiology of the Avian Egg. p 25-42. Springer US, Boston, MA.
Stadelman, W. J., O. J. Cotterill. 1977. Egg Science and Technology.: 41-48.
Stanley, W., C. Hofacre, G. Speksnijder, S. Kleven, and S. Aggrey. 2001. Monitoring Mycoplasma gallisepticum and Mycoplasma synoviae Infection in Breeder Chickens after Treatment with Enrofloxacin. Avian diseases 45: 534-539.
Stepień-Pyśniak, D. 2010. Occurrence of gram-negative bacteria in hens' eggs depending on their source and storage conditions. Polish journal of veterinary sciences 13: 507-513.
Stipkovits, L., T. Kobulej, and Z. Varga. 1984. Efficacy of lasalocid sodium against mycoplasmas (preliminary communication). Acta veterinaria Hungarica 32: 127-129.
Stipkovits, L., T. Kobulej, Z. Varga, and S. Juhász. 1987. In vitro testing of the anti-mycoplasma effect of some anti-coccidial drugs. Veterinary Microbiology 15: 65-70.
Sugino, H., T. Nitoda, and L. Juneja. 1997. General chemical composition of hen eggs. CRC Press: Boca Raton, FL.
Sugino, H. N., T.; Juneja, L. R. . 1997. General chemical composition of hen eggs. In: C. Press (ed.) Hen Eggs, Their Basic and Applied Science: : Yamamoto, T.; Juneja, L. R.; Hatta, H.; Kim, M.

. p 13-24

New York.
Sui, J., L. Cao, and H. Lin. 2011. Antibacterial activity of egg yolk antibody (IgY) against Listeria monocytogenes and preliminary evaluation of its potential for food preservation. Journal of the science of food and agriculture 91: 1946-1950.
Svobodová, J., and E. Tůmová. 2015. Factors Affecting Microbial Contamination of Market Eggs: A Review. Scientia Agriculturae Bohemica 45: 226.
Swanson, E. C., and M. T. Collins. 1980. Use of the API 20E system to identify veterinary Enterobacteriaceae. Journal of clinical microbiology 12: 10-14.
Sweeney, D. J., A. Kennington, and A. L. Donoho. 1996. Tissue residues and metabolism of narasin in chicken. Journal of agricultural and food chemistry 44: 2829-2834.
Syriopoulou, V. P., A. L. Harding, D. A. Goldmann, and A. L. Smith. 1981. In vitro antibacterial activity of fluorinated analogs of chloramphenicol and thiamphenicol. Antimicrob Agents Chemother 19: 294-297.
Takehiko Yamamoto, L. R. J., Hajime Hatta, Mujo Kim. 1996. Hen Eggs: Basic and Applied Science. 180.

Tatara, M. R. et al. 2015. Interrelationships between morphological, densitometric and mechanical properties of eggs in Japanese quails (Coturnix Japonica). The Journal of Poultry Science 53: 51-57.
Tian, W.-R., Y.-X. Sang, and X.-H. Wang. 2014. Semicarbazide – from state-of-the-art analytical methods and exposure to toxicity: a review. Food Additives & Contaminants: Part A 31: 1850-1860.
Titouche, Y. et al. 2013. Screening of Antibiotics Residues in Poultry Meat by Microbiological Methods HAKEM (ex AKAM) A. Bulletin UASVM, Veterinary Medicine, 70(1)/2013 Print ISSN 1843-5270; Electronic ISSN 1843-5378 70: 1843-5378.
Tuckman, M. et al. 2007. Occurrence of tetracycline resistance genes among Escherichia coli isolates from the phase 3 clinical trials for tigecycline. Antimicrob Agents Chemother 51: 3205-3211.
Tumová, E., M. Englmaierová, Z. Ledvinka, and V. Charvátová. 2011. Interaction between housing system and genotype in relation to internal and external egg quality parameters. Czech Journal of Animal Science 56: 490-498.
Tůmová, E. et al. 2016. Interactions of genotype, housing and dietary calcium in layer performance, eggshell quality and tibia characteristics. South African Journal of Animal Science 46: 285-293.
Tupper, R., R. W. Watts, and A. Wormall. 1954. The incorporation of 65Zn into avian eggs. Biochem J 57: 245-255.
Union, E.-E. 2016. RASFF-The Rapid Alert System for Food and Feed: 2015 annual report. European Commission. Health and Food Safety Brexellas.
Use, S. A. G. o. A. o. t. C. f. M. P. f. V. Reflection paper onuse of aminoglycosides in animals in the European Union: development of resistance and impact on human and animal health. London: European Medicines Agency; 2018 Jun. 44 p, Report no. EMA/CVMP/AWP/721118/2014 44p.
Valenti, P. et al. 1983. Studies of the antimicrobial activity of ovotransferrin. International journal of tissue reactions 5: 97-105.
van Boven, M., K. T. Veldman, M. C. de Jong, and D. J. Mevius. 2003. Rapid selection of quinolone resistance in Campylobacter jejuni but not in Escherichia coli in individually housed broilers. The Journal of antimicrobial chemotherapy 52: 719-723.
Vass, M., K. Hruska, and M. Franek. 2008. Nitrofuran antibiotics: a review on the application, prohibition and residual analysis. Vet Med (Praha) 53: 469-500.
Wall, H., and R. Tauson. 2002. Egg Quality in Furnished Cages for Laying Hens—Effects of Crack Reduction Measures and Hybrid. Poultry Science 81: 340-348.
Wall, H., R. Tauson, and S. Sorgjerd. 2008. Bacterial Contamination of Eggshells in Furnished and Conventional Cages. Journal of Applied Poultry Research - J APPL POULTRY RES 17: 11-16.
Wang, H., J. C. McEntire, L. Zhang, X. Li, and M. Doyle. 2012. The transfer of antibiotic resistance from food to humans: facts, implications and future directions. Revue scientifique et technique (International Office of Epizootics) 31: 249-260.
WANG, H., and M. E. SLAVIK. 1998. Bacterial Penetration into Eggs Washed with Various Chemicals and Stored at Different Temperatures and Times. Journal of food protection 61: 276-279.
Wellman-Labadie, O., R. Lakshminarayanan, and M. T. Hincke. 2008a. Antimicrobial properties of avian eggshell-specific C-type lectin-like proteins. FEBS letters 582: 699-704.
Wellman-Labadie, O., S. Lemaire, K. Mann, J. Picman, and M. T. Hincke. 2010. Antimicrobial activity of lipophilic avian eggshell surface extracts. Journal of agricultural and food chemistry 58: 10156-10161.
Wellman-Labadie, O., J. Picman, and M. T. Hincke. 2008b. Antimicrobial activity of cuticle and outer eggshell protein extracts from three species of domestic birds. British poultry science 49: 133-143.
Węsierska, E. et al. 2005. Antimicrobial activity of chicken egg white cystatin. World Journal of Microbiology and Biotechnology 21: 59-64.

Wesley, R., and W. Beane. 1967. EFFECT OF VARIOUS WASH WATER AND INTERNAL EGG TEMPERATURES ON NUMBER OF THERMAL CHECKS OF OILED AND UNOILED EGGS. In: Poultry Science. p 1336-&.
WHO. 2016. Evaluation of certain veterinary drug residues in food. Eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization technical report series: 1-110.
Wicker, D. L., W. N. Iscrigg, and J. H. Trammell. 1977. The control and prevention of necrotic enteritis in broilers with zinc bacitracin. Poult Sci 56: 1229-1231.
Widiasih, D. et al. 2020. Detection of Antibiotic Residues in Chicken Meat and Eggs from Traditional Markets at Yogyakarta City Using Bioassay Method. Acta VETERINARIA Indonesiana: 1-6.
Williams, J., L. Dillard, and G. O. Hall. 1968. The penetration patterns of Salmonella typhimurium through the outer structures of chicken eggs. Avian Diseases 12: 445-466.
Williams, J. E., and L. H. Dillard. 1968. Penetration of chicken egg shells by members of the Arizona group. Avian Dis 12: 645-649.
Windhorst, H. 2015. Housing Systems in Laying Hen Husbandry.
World Health Organization. Division of, E., S. other Communicable Diseases, and Control. 1998. Use of quinolones in food animals and potential impact on human health : report and proceedings of a WHO meeting, Geneva, Switzerland, 2-5 June 1998. World Health Organization, Geneva.
Xie, K. et al. 2012. Residue Depletion of Florfenicol and its Metabolite Florfenicol Amine in Eggs. Journal of Animal and Veterinary Advances 11: 1012-1017.
Xing, J., O. Wellman-Labadie, J. Gautron, and M. T. Hincke. 2007. Recombinant eggshell ovocalyxin-32: expression, purification and biological activity of the glutathione S-transferase fusion protein. Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology 147: 172-177.
Yang, F. et al. 2016. Pharmacokinetics of doxycycline in laying hens after intravenous and oral administration. British poultry science 57: 576-580.
Yévenes, K. et al. 2018. Assessment of Three Antimicrobial Residue Concentrations in Broiler Chicken Droppings as a Potential Risk Factor for Public Health and Environment. Int J Environ Res Public Health 16: 24.
Yoshii, H. et al. 2001. Antihypertensive effect of ACE inhibitory oligopeptides from chicken egg yolks. Comparative biochemistry and physiology. Toxicology & pharmacology : CBP 128: 27-33.
Yoshimura, H., O. Itoh, K. Kondo, S. Yonezawa, and S. Nagura. 1979. Residues of macrolide antibiotics in eggs laid by hens given medicated drinking water. Annual Report of the National Veterinary Assay Laboratory (Japan).
Yoshimura, H. et al. 1991a. Residues of doxycycline and oxytetracycline in eggs after medication via drinking water to laying hens. Food additives and contaminants 8: 65-69.
Yoshimura, H. et al. 1991b. Residues of doxycycline and oxytetracycline in eggs after medication via drinking water to laying hens. Food Additives & Contaminants 8: 65-69.
Young, D., M. Z. Fan, and Y. Mine. 2010. Egg yolk peptides up-regulate glutathione synthesis and antioxidant enzyme activities in a porcine model of intestinal oxidative stress. Journal of agricultural and food chemistry 58: 7624-7633.
Yuceer, M., M. Aday, and C. Caner. 2015. Ozone treatment of shell eggs to preserve functional quality and enhance shelf life during storage. Journal of the science of food and agriculture.
Yunis, A. A., and M. A. Gross. 1975. Drug-induced inhibition of myeloid colony growth: protective effect of colony-stimulating factor. The Journal of laboratory and clinical medicine 86: 499-504.
Zang, Y. T. et al. 2019. Efficacy of slightly acidic electrolyzed water on the microbial safety and shelf life of shelled eggs. Poultry Science 98: 5932-5939.
Zhanel, G. G. et al. 2001. Review of Macrolides and Ketolides. Drugs 61: 443-498.

Ziółkowski, H. et al. 2015. Pharmacokinetics of oxytetracycline in broiler chickens following different routes of administration. The Veterinary Journal 208.