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Detection of Methicillin-Resistant *Staphylococci* (MRS) and *Salmonella* spp. in Consumer Egg Samples

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Abstract: Despite the excellent nutritional value, the egg can be an important vehicle for bacterial infections carried by food, among them, multiresistant *Staphylococci* and salmonellosis. The objective of this work was to detect the presence of Methicillin-Resistant *Staphylococcus* spp. (MRS) and *Salmonella* spp. Forty dozen eggs were obtained from three free-trade fairs in the Umuarama-PR; every dozen eggs comprised a sample. They were analyzed through enrichment, selective enrichment, plating, biochemical test and specific serology for the pathogens in consumption eggs. Twenty-eight *Staphylococcus* spp. were isolated from 13 samples of the yolk and 8 of the eggshell, being 47.62% phenotypically characterized as Methicillin-Resistant *Staphylococcus* (MRS) strains. Although considered a pathogen of high importance in public health and closely related to the product in question, no bacteria of the *Salmonella* genus were detected in any of the samples analyzed. *Staphylococci* are also pathogens of significant importance within Foodborne Diseases (DTAs), requiring more epidemiological information about outbreaks involving this microorganism. Also, the rational use of antimicrobials is necessary to avoid the emergence of resistant strains.

Keywords: *Staphylococci*, Salmonellosis, Multidrug Resistance, Chicken, Food

Introduction

Animal production has been improved through the use of antimicrobials. However, this practice has triggered the emergence and distribution of resistant microorganisms in animal products (Téo and Oliveira, 2005).

The egg is a food of high nutritional value. However, it can carry important pathogens, such as *Salmonella* spp., A bacterium widely studied in poultry farming and food analysis. Also, *Staphylococcus* spp. that have important resistant strains, such as Methicillin-Resistant *Staphylococcus* spp. (MRS) moreover, it can cause extremely severe infections in humans (Oliveira and Taham, 2011; Bond and Loeffler, 2012).

Contamination of egg contents may occur in the reproductive tract of the chicken before shell formation, leading to the production of already contaminated eggs, or after laying, by factors related to the environment and egg handling (Téo and Oliveira, 2005). The poor habits of preparation and consumption of raw or undercooked eggs are one of the main factors that make this food a potential contaminant for humans (Lacerda, 2011).

In eggs, research for *Staphylococcus* spp. is performed in liquid dehydrated and whole eggs, where the absence of this microorganism is required in 1 gram of the product (Brasil, 1990). According to (Menezes, 2013), this requirement is justified by the fact that the egg (fresh, liquid, or dehydrated) is used as an ingredient in several foods that may not undergo heat treatment. As the egg has high nutritional value, multiplication can occur if a microbial load is present, increasing the risk to humans.

Salmonellosis is considered worldwide as one of the main zoonoses of importance for public health, characterizing endemicity, high morbidity and above all, difficulty in adopting control measures related to its spread (Shinohara *et al*., 2008). When present in the

farm environment, the microorganism can infect birds and, consequently, their eggs (Braden, 2016), which may be associated with cases of foodborne infection in humans via Foodborne Diseases (Leal, 2011).

The objective was to detect the presence of *Staphylococcus* spp. and *Salmonella* spp., by conventional microbiological analysis specific for each pathogen, in eggs marketed in the free markets of the municipality of Umuarama-PR.

Materials and Methods

Forty dozen eggs were obtained from three free markets held weekly in the municipality of Umuarama-PR. The eggs were kept at room temperature for a maximum of five days since the analyses were always started on Mondays and the products were purchased on Wednesdays, Fridays and Sundays, at the several fairs visited. The analyses were presented at the facilities of the Animal Microbiology Laboratory of the State University of Maringá (UEM), Umuarama Regional Campus (CAU).

Each dozen eggs comprised one sample, totaling 40 samples analyzed. The dozens of eggs purchased met a pre-established criterion for the sanitary control involved. EGGS belonging to three categories were examined: Eggs without a sanitary inspection, eggs inspected by the Municipal Inspection Service (with the SIM seal) and eggs investigated by the Federal Inspection Service (with the SIF seal). All samples were qualitatively analyzed for the presence or absence of *Staphylococcus* spp. and *Salmonella* spp. in both yolk and shell.

The procedures were performed in a laminar flow cabin (chapel), minimizing the chances of contamination of the handled products. Initially, each egg had its surface washed in a sterile bag using 10 mL of 0.1% Buffered Peptone Water (APT). After this procedure, the wash liquid of the twelve eggs of the sample unit made up the sample of the shell to be processed, stored in a sterile container, corresponding to the initial pre-enrichment solution. It was incubated for 24 h at 37°C. Then the eggs were submerged in 70% alcohol for 10 min and after that time, they were dried in the laminar flow hood under UV light. The yolks were aseptically separated and homogenized.

For the detection of *Staphylococcus* spp., 25 mL of yolk were incubated in 225 mL of 0.1% peptone water for 24 h. Subsequently, nutrient agar was sown with 4.5% NaCl and the results analyzed after 24 and 48 h. Catalase, Gram stain and tube coagulase tests were also performed to characterize the bacteria. The typical colonies of *Staphylococcus* spp. were then seeded on Muller Hinton Agar for antimicrobial susceptibility testing with discs impregnated with penicillin, cefoxitin, oxacillin, erythromycin and clindamycin. The halos were evaluated, according to (CLSI, 2013).

For detection of *Salmonella* spp., after an incubation time of the pre-enrichment media, 1 mL of each broth (bud pool and eggshell wash pool) was transferred in 10 mL of Tetrationate Broth (incubated at 35°C for 24 h) and 0.1 mL in 10 mL Rappaport-Vassiliadis Broth (incubated at 42°C for 24 h). Samples from these selective enrichment media were then seeded in Petri dishes previously filled with XLD Agar (Xylose Lysine Deoxycholate Agar) and incubated at 35°C for 24 h. Suspected colonies identified by reading the XLD plates were seeded into test tubes for biochemical analysis with the Triple Sugar and Iron Agar (TSI) and Iron Lysine Agar (ILA) (Brazil, 2003). When the growth of characteristic colonies is suspected, the pathogen was confirmed by proof of specific polyvalent rapid serum agglutination (Paiva *et al*., 2006).

Results and Discussion

Methicillin-Resistant Staphylococci (MRS).

Of the 40 samples analyzed, 13 (32.5%) yolk and 8 (20%) eggshell samples were characterized as positive for the presence of *Staphylococcus* spp. In the remaining 27 (67.5%) yolk samples and 32 (80%) of the shell has not detected the presence of this microorganism.

A total of 28 strains of *Staphylococcus* spp. were isolated from 21 yolk and shell samples. The presence of bacterial monocross growth was verified in the 13 yolk samples, being three without an inspection, 6 with municipal inspection and 4 with a federal inspection. The monocross growth was also verified in one eggshell sample without inspection and one with municipal inspection, the rest of the eggshell samples were confirmed isolation of more than one bacterial type, being two bacterial strains isolated from 2 samples with municipal inspection and three samples with federal inspection. Federally, the fourth federally-inspected eggshell samples showed growth of 3 distinct bacterial strains of *Staphylococci*.

In the coagulase test, only one yolk isolate was detected as Coagulase-Positive *Staphylococcus* spp. (CoPS), the remaining 24 strains were characterized as Coagulase-Negative *Staphylococci* (CoNS).

Resistance to β-lactam antibiotics was found in 53.85% (n = 7) of the yolk isolates and 66.67% (n = 10) of the shell (Table 1 and Fig. 1). For phenotypic characterization of MRS strains, the associated resistance between penicillin and oxacillin/cefoxitin is required, being obtained in 38.46% of the isolated strains of the bud (n = 5) and 66.67% of the eggshell (n = 10), totaling 53.57% (n = 15) (tab. 1).

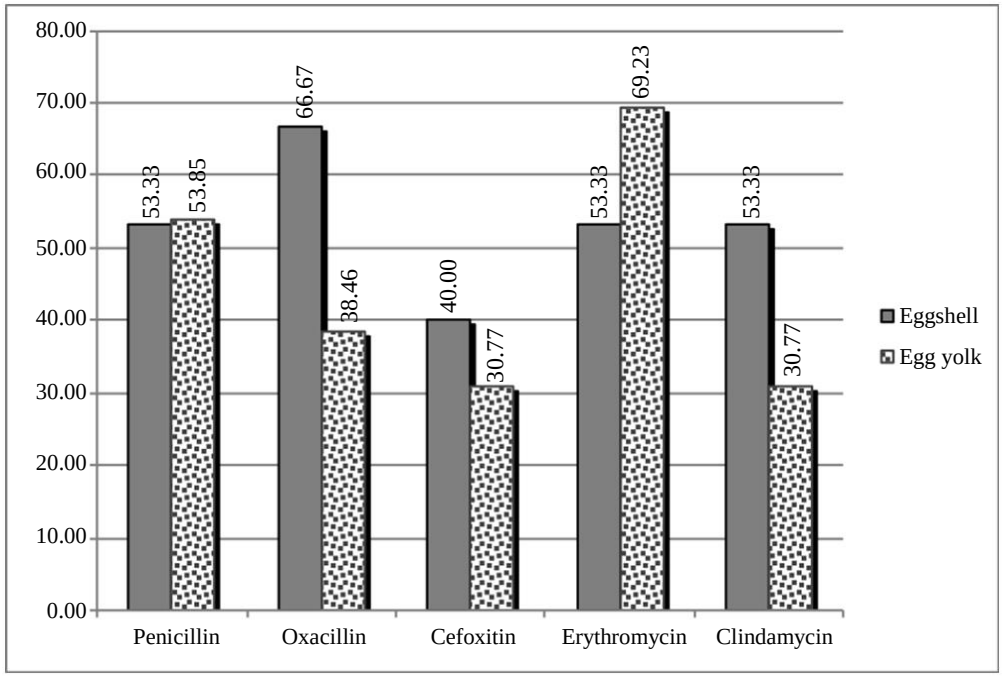

Fig. 1: Graphical representation of antibiotic resistance percentages of *Staphylococcus* spp. isolated from egg yolk and eggshell

Table 1: Characterization of resistance/susceptibility to β-lactams in *Staphylococcus* spp. isolates in Yolk and Shell in eggs of consumption in the municipality of Umuarama – PR, Brazil

	Resistant					
Sample	P/O/C	P/O	O	P	Susceptible	Total
Egg yolk	4	1	0	2	6	13
Eggshell	6	2	2	0	5	15

P: Penicillin; O: Oxacillin; C: Cefoxitin.

Table 2: Resistance phenotypes of *Staphylococcus* spp. isolated from egg yolk and eggshell

phenotype	shell	Yolk	Total	Phenotype %	Resistance
A	4	1	5	17,86	P/O/C E/Cl
B	2	1	3	10,71	P/O E/Cl
C	2	3	5	17,86	P/O/C
D	0	1	1	3,57	P E/Cl
E	0	1	1	3,57	P Cl
F	2	0	2	7,14	O Cl
G	2	6	8	28,57	E
I	3	0	3	10,71	-
Total	15	13	28		

P: penicillin; O: oxacillin; C: cefoxitin; E: erythromycin; Cl: clindamycin.

Resistance to erythromycin was found in 60.71% (n = 17) of the isolated bacterial strains, 69.23% (n = 9) in the yolk samples and 53.33% (n = 8) in the shell samples. Resistance to total clindamycin was 42.86% (n = 12), with percentage inversion when compared to erythromycin, with 30.77% (n = 4) of resistance in yolk isolates and 53.33% (n = 8) eggshell nodes (Fig. 1). Nine strains (32.14%) MLSb (*Staphylococcus* with resistance to Macrolides, Lincosamides and Streptogramins) were characterized, phenotypically detected with resistance to both antimicrobials, being three from the yolk and six from shell samples. Erythromycin resistance alone was found in 28.57% (n = 8) of the samples (6 yolks and two shells) and clindamycin resistance only in 10.71% (n = 10) (1 yolk and two shells). Eight bacterial strains showed sensitivity to both antibiotics.

Eight resistance phenotypes were found in the 25 isolated *Staphylococcus* (Table 2). The most prevalent phenotype comprised eight erythromycin-resistant bacteria (including CoPS), followed by five bacteria

resistant to all tested antibiotics and five resistant to β-lactam antibiotics.

The detection of *Staphylococcus* spp. in eggs of consumption draws attention because, according to (Santana *et al*., 2010), egg products have already been incriminated in food outbreaks involving staph.

The presence of this microorganism in the shell suggests the need for greater hygienic-sanitary control, aiming at observing aspects such as the shell quality, the washing and disinfection process and the proper storage.

And the fact that MRS strains have been found is fundamental since indiscriminate administration of antimicrobials in feeds as growth promoters accelerate the process of bacterial resistance and the presence of resistant strains in animal products threatens the efficacy of antimicrobials in human (Téo and Oliveira, 2005).

Salmonella spp.

The 40 dozen samples of surface (shell) and content (yolk) of eggs from free markets in the municipality of Umuarama-PR showed no contamination by *Salmonella* spp. Samples of eggs without a sanitary inspection, as well as eggs with municipal and federal inspection, were analyzed, all of which were negative for the presence of bacteria of the genus *Salmonella*. This fact demonstrates that the hygienic-sanitary standard of the birds and the places of laying involved (in all egg yielding properties evaluated) were adequate at the time of this research, regardless of the inspection seal granted or not to the product for the eggs traded at fairs in the municipality of Umuarama-PR, Brazil.

Results similar to this research, confirming the absence of *Salmonella* spp./25g of the egg sample were also described by (Vaz *et al*., 2012), in an analysis of 120 eggs from free-range and free-range farms. This research showed a higher degree of contamination by other microorganisms in the "rustic" eggs about the others. Still, none of the categories has detected the presence of *Salmonella* spp. Other research involving commercial egg analysis showed the absence of the pathogen in the 50 samples analyzed (Silva *et al*., 2004).

Kottwitz *et al*. (2008) analyzed 3,000 eggs from a cooperative with commercial posture integration in the state of Paraná, Brazil and obtained isolation of *Salmonella* spp. in eight of the 30 farms that participated in the research; however, none of the strains were detected from eggs and all samples were negative for the presence of *Salmonella Enteritidis* (the most important species associated with cases of human salmonellosis in the state of Paraná). Reinforcing this data, another field study indicates that the presence of *Salmonella Enteritidis* in eggs is considered low, despite the high incidence in lots of laying birds (Barancelli *et al*., 2012).

More recent studies have also obtained similar results to this research. Gomes Filho *et al*. (2014) reported the absence of the pathogen *Salmonella* spp. when analyzing eggs from farms with backyard chickens and eggs from free markets, in Fortaleza-CE, Brazil. The same negative result was reported in research that microbiologically analyzed 280 free-range eggs produced by family farmers in Seropédica, metropolitan region of Rio de Janeiro State, Brazil (Melo *et al*., 2015).

Unlike the present study, positive results for the presence of *Salmonella* spp. were found by (Dantas *et al*., 2006) when performing an experiment with 45 samples of non-inspected white eggs, obtained in Salvador-BA, Brazil, in which seven samples (15.5%) were positive for the pathogen. Of these, however, the only one showed contamination of the internal content (yolk) by *Salmonella* spp. More recent research reports positive results in five (1.47%) of the 340 egg samples analyzed from four supermarkets in Jaboticabal-SP, Brazil, but without isolation for *Salmonella Enteritidis* and *Salmonella Typhimurium* (Campello, 2012). In a study conducted in the United Kingdom, 1588 pool samples of six eggs from restaurants were analyzed, the *Salmonella* spp. were found in 6 (0.38%) samples when *Salmonella Enteritidis* was isolated in five of them (Little *et al*., 2008).

Still, regarding positive samples (Andrade *et al*., 2004) conducted a study in Goiânia-GO, Brazil, which randomly collected 816 eggs divided into 272 samples of commercial laying farms and retail outlets. The authors confirmed the presence of *Salmonella* spp. in five of the analyzed samples, four (1.48%) from backyard breeding and one (0.36%) from laying farm. These studies that reported the detection of the pathogen in egg samples to point to the importance of research in the area of foodborne diseases, in this case aiming at improving the sanitary and sanitary conditions of farms and establishments, thus collaborating to the food safety of consumers of these products of animal origin.

Conclusion

It is concluded from this work that the egg, despite having an excellent nutritional value, can be an important vehicle of multiresistant bacterial strains, presenting a risk to humans. *Staphylococci* are important pathogens of Foodborne Diseases, requiring further epidemiological information regarding outbreaks involving this microorganism. Also, the rational use of antimicrobials is necessary to prevent the emergence of multidrug-resistant strains.

Regarding the absence of *Salmonella* spp. in all samples analyzed, this work showed a similarity in the sanitary and hygienic standards in the breeding sites

of these eggs. However, due to the serious problem that this disease represents in a significant part of birds, as well as its zoonotic potential, characterized by the pathogen is one of the most dangerous causes of foodborne illnesses, the epidemiological surveillance of this pathogen is continuously needed, aiming at greater food security.

Author's Contributions

This section should state the contributions made by each author in the preparation, development and publication of this manuscript.

Ana Claudia Lemes Pavan and Rafaella da Silva: Conception, design and conduction of the study;

Vanessa Kelly Capoia Vignoto: Laboratory support.

Marcos Ferrante: Analysis and interpretation of data.

Sheila Rezler Wosiacki and Patrícia Marques Munhoz: Conception, design, drafting the manuscript and critical revision.

All the authors approved the final manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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