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ISOLATION AND CHARACTERIZATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) FROM TABLE EGGS

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ABSTRACT

Eggs continue to be significant in terms of the world economy and human nutrition and routine non-therapeutic antimicrobial use and overcrowding in animal farming may facilitate the propagation of Methicillin-Resistant *Staphylococcus aureus* (MRSA). The aim of this study was to estimate the prevalence of MR Staphylococcus aureus (MRSA) in table eggs production. Three hundred and fifty eggs represent 35 samples from balady egg and 35 sample from poultry farm egg (each sample 5 egg) were tested for isolation of *Staphylococcus aureus*. These findings are important for local risk assessments concerning possible human foodborne infections via cross-contamination of eggs. *S. aureus* was isolated from balady and poultry farm egg shell in percentage of 57.1 and 80 %, also *S. aureus* could be isolated from the content of balady and poultry farm egg in percentages of 74.4 and 85.8 %, respectively. In addition, *S. aureus* was identified by the coagulase test and our findings showed that 37.1 and 51.4% of examined samples of balady and poultry farm, respectively were coagulase positive *S. aureus*. Notably, 34.3% and 48.6% of coagulase positive *S. aureus* isolates were isolated from balady and poultry farm egg contents, respectively. The results revealed that the incidence of coagulase negative staphylococci on examined balady egg shell and contents were 20 and 40%, respectively. Coagulase negative staphylococci was isolated in a percentage of 10 (28.6%) and 13 (37.1%) from examined poultry farms egg shells and contents. Identification *mecA* gene carriage, hence MRSA, using polymerase chain reaction (PCR) revealed that 3 from 10 samples were positive for *mecA* by using (PCR) in percentage of (30%). Risk of egg borne disease strongly increases because of unhygienic conditions of egg production and improper practices of egg handling, including also storage times and temperatures. If all the necessary precautions are not taken during the poultry production, marketing and processing chains in that case poultry meat and eggs can be contaminated by infectious agents that are harmful to humans.

Key words: MRSA – Table egg

INTRODUCTION

Table eggs are nutritionally important food consumed globally. Despite being protected inside the hard shell and a semipermeable membrane, the egg contents may be contaminated with microbes and thus become a possible carrier of infectious agents to humans. Also, table eggs are consumed worldwide and are considered the most nutritious inexpensive source of protein that can be part of a healthy diet. However poultry may carry bacteria that can cause illness, infected birds do not usually appear sick and even unbroken clean fresh shell eggs may contain harmful bacteria. (Barbara and Ron, 2010). Special attention has been paid for raw or undercooked eggs because the hens act as natural reservoirs of a variety of pathogens. The contamination occurs through the shell; but humidity, temperature and storage time are critical for migration of bacteria from the surface of the shell to the inner structures of the egg (Evêncio *et al.,* 2012). Egg is an excellent source of choline and selenium and a good source of vitamin B12, riboflavin and phosphorus. The yolk contains different vitamins such as A, D, E and K as well as folic acid and zinc (ENC, 2004). The extent of egg spoilage due to effect of microorganisms is very high which result in big economic losses (Saif *et al., 2009;* Howard *et al.,* 2011). At the beginning, the microbial load is very low but it increases when the shell acquires at oviposition, a few are from the vent and others from the nesting materials and feces. Besides these egg can be contaminated from different stages like during collection, handling, storage and transportation.

A number of medically significant bacterial species have already been reported from table eggs. More important is the presence of antimicrobial-resistant bacterial strains in this food source. There is now considerable evidence that transfer of antimicrobial

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resistance from food-producing animals to humans directly via the food chain is a likely route of spread, transmission by direct handling or close contact between infected animals and humans, transmission via contaminated animal products, particularly but not exclusively food products. The World Organization for Animal Health (OIE) has developed a list of antibiotics categorized by the need for their use in animal treatment; Thus these antibiotics may all affect bacteria in both animal and human treatment settings (Wooldridge, 2012).

Staphylococcus aureus strains are now regarded as zoonotic agents. In pastoral settings where human-animal interaction is intimate, multi-drug resistant microorganisms have become an emerging zoonotic issue of public health concern. The study of *S. aureus* prevalence, antimicrobial resistance and clonal lineages in humans, animals and food in African settings has great relevance, taking into consideration the high diversity of ethnicities, cultures and food habits that determine the lifestyle of the people (Asiimwe *et al.,* 2017).

Staphylococcus is considered to be a normal flora of chickens, isolated from the skin and feathers as well as in the respiratory and intestinal tracts (Casey *et al.,* 2007). However, some of the common forms of Staphylococci are associated with poultry infections. Increasing attention has been given to the role of poultry and poultry products, including eggs, as a potential source of infections in humans induced by antibiotic-resistant Staphylococcus strains (Abulreesh and Organji 2011). *S. aureus* is a medically significant bacterial species responsible for diverse types of infections ranging from superficial skin and soft-tissue infections to fasciitis, otitis media, necrotizing pneumonia, and urinary infections (Tamarapu *et al.,* 2001; Harris *et al.,* 2002). In addition, *S. aureus* is one of the major causes of food poisoning due to their ability to produce heat-stable enterotoxins that may remain protected in the food environment and cause foodborne illness (Bergdoll *et al.,* 1967; Argudı´n *et al., 2010).* There are over 20 types of staphylococcal enterotoxins (SE), two of them (SEA and SEB), that are best characterized and considered super antigens as they may bind MHC II molecules on the surface of antigen-presenting cells and may stimulate massive T cell proliferation and ultimately leading to toxic shock (McCormick *et al.,* 2001; Le Loir *et al.,* 2003; Rahimi *et al.,* 2013; Kadariya *et al.,* 2014).

Methicillin-Resistant *S. aureus* (MRSA) shows resistance against almost all b-lactam antibiotics, including penicillin and cephalosporins. Resistance against b-lactam antibiotics is due to bacterial ability to produce an altered form of penicillin-binding proteins (PBP), that is, PBP2a that has lowered affinity for b-lactam drugs. The *mecA* gene located on the chromosomal DNA of MRSA strains encodes PBP2a. This *mecA* gene is a part of staphylococcal cassette chromosome *mec* (*SCCmec*), a mobile genetic element that may be horizontally transferred among strains of *S. aureus*, causing dissemination of antimicrobial resistance genes among the isolates. Strains devoid of *SCCmec* are sensitive to methicillin and are termed as methicillin-sensitive *S. aureus* (Stapleton and Taylor, 2002).

There is a very limited data available on the presence of *S. aureus* in table eggs (Abdullah, 2010; Pyzik *et al.,* 2014). These studies primarily focused on bacterial detection and antimicrobial susceptibility testing. The presence of MRSA in table eggs will not only indicate a risk of foodborne illness, but also a source of dissemination of antimicrobial-resistant strains to humans and the environment (De Reu *et al.,* 2005; Woodridge, 2012). It is very important for public health to ensure the quality and safety of eggs. However, foodborne illness due to the ingestion of eggs contaminated with pathogens occasionally occurs all over the world. Therefore, it is necessary to enhance the surveillance and risk assessment of egg safety.

Methicillin-Resistant *S. aureus* (MRSA) was identified in 1962 and, together with certain species of Enterococcus, are currently considered as global pandemic threats (CDC 2013; Rossolini *et al.,* 2014). MRSA is a major cause of severe healthcare-associated (HA) infections. Although during the last decade the incidence of HA invasive infections has dropped, the incidence of community- associated MRSA (CA-MRSA) infections has risen among the general population. Moreover, CA-MRSA, livestock-associated MRSA (LA-MRSA) and HA-MRSA (HA-MRSA) can be found in foods intended for human consumption (Sergelidis and Angelidis 2017). The contamination sources for foods, especially animal-origin foods, may be livestock as well as humans involved in animal husbandry and food-processing. Since the early 1990s, MRSA strains have emerged, which are involved in community-associated infections (nonhospital related) in humans in many countries (community-associated, methicillin-resistant *S. aureus,* CA-MRSA) (Deurenberg *et al.,* 2007). Moreover, in recent years, the isolation of MRSA from livestock (livestock-associated, methicillin-resistant *S. aureus*, LA-MRSA) and companion animals has also been reported (Wendlandt *et al.* 2013; Vincze *et al.,* 2014). HA-MRSA and CA-MRSA are believed to predominantly affect humans and, in general, are not involved in live- stock infections. However, humans may harbour LA-MRSA, especially in cases where there is occupational contact with affected livestock (Cuny *et al.,* 2015). Furthermore, overlaps between these different MRSA reservoirs have been reported, including nosocomial infections by CA-MRSA

(Skov and Jensen 2009) and isolation of LA-MRSA from the hospital environment (Van Rijen *et al.*, 2008). The incidence of invasive HA-MRSA infection seems to be declining (Rossolini *et al.*, 2014), while a rapid rise of CA-MRSA infections has been observed among the general population (CDC 2013). The traditional epidemiological classification of MRSA into HA-MRSA, CA-MRSA and LA MRSA may no longer be valid because there are considerable overlaps of identical clones between these groups (*Bal et al., 2016*).

The objective of the present study was aimed to isolate and identify the MRSA from egg shell and egg contents.

MATERIALS AND METHODS

Sample collection: A total of 350 random eggs, representing 70 samples, (35 from balady egg, and 35 from poultry farms) were collected from, poultry farms, groceries, framers, supermarkets and rural area located in Assiut Governorate, Egypt. Each egg sample (composed of 5 eggs) was placed in a sterile plastic bag and dispatched to the laboratory with a minimum of delay where they were prepared and examined.

Preparation of samples:
Egg shells: Egg shells were tested by a surface rinse method as described by Moats (1980).

Egg contents: The egg sample was prepared for evacuation of its content according to Speck (1976).

Isolation and Identification of *S. aureus* from table egg according to *Bennett and Lancette (2001):* All the samples were prepared and enriched on Staphlococci broth for 20h at 35 ^{o}C and then inoculated onto Baird Parker Medium (Oxide, Basingstoke, England), and incubated aerobically at 37 ^{o}C for 24 h. The isolates were identified using established microbiological methods which included colony morphology, Gram staining and biochemical testing [catalase, coagulase and sugar fermentation (glucose, sucrose, lactose and mannotol)].

Identification and characterization of coagulase positive and negative Staphylococcus Species: the isolates were identified according to (ISO, 2003b).

Coagulase test according to *(ISO, 2003b):*
Five colonies typical and atypical were selected from each plate. The selected colonies inoculated into 5ml Brain Heart Infusion broth. The tubes were incubated at 37ºC for 24 hours. From which 0.1 ml was transferred to tubes containing 0.3 ml of sterile citrated rabbit plasma. Inoculated tubes were incubated at 37ºC and examined for clot formation after 4 hours.

Isolation of antibiotic resistant *S. aureus*: (CLSI /NCCLS (2001)
Antimicrobial susceptibility pattern of *S. aureus* isolates were determined by using disk diffusion assay following the guidelines of Clinical and Laboratory Standard Institute. The pre-incubated 24 hours cultures of *S. aureus* were diluted in sterile buffer peptone water and matched with the 0.5 MacFarlane turbidity standards to get 1 ×10 ^{8} cfu/ml as total count. Bacterial suspensions were spread on Muller-Hinton agar (Merck, Germany). The antibiotic discs (Methicillin 1µm) were placed over the lawn and incubated at 37 ^{o}C for 18-24 hours. The clear zone around each antibiotic disc was measured in millimeter according to zone size interpretation chart modified from NCCLS (2001).

Disc content in µm	Antimicrobial agent	Diameter of zone of inhibition to nearest mm.		
		Resistant	Intermediate	Susceptible
1	Methicillin	10	11-12	13

Detection of *mecA* gene by Polymerase chain reaction (PCR):
DNA extraction. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{o}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer: Primers used were supplied from Metabion (Germany), the following table showing **primers sequences, target genes, amplicon sizes and cycling conditions**:

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondar denaturation	Annealing	Extension		
mecA	GTA GAA ATG ACT GAA CGT CCG ATA A / CCA ATT CCA CAT TGT TTC GGT CTA A	310	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**McClure *et al.*, 2006**

PCR amplification. Primers were utilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products.

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the uniplex PCR products Gelpilot 100 bp DNA ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

RESULTS

Table 1: Incidence of isolated *staphylococcal* species in the examined table eggs shell samples.

Type of samples	No. of samples analyzed	No. of Positive samples	%
Balady egg shells	**35**	**20**	**57.1**
Poultry farm egg shells	**35**	**28**	**80**

Table 2: Incidence of isolated *staphylococcal* species in the examined hen's eggs content samples.

Type of samples	No. of samples analyzed	No. of Positive samples	%
Balady egg contents	**35**	**26**	**78.4**
Poultry farm egg conents	**35**	**30**	**85.8**

Table 3: Occurrence of *S. aureus* and coagulase negative staphylococci from examined samples.

Examined samples	No. of examined samples	Coagulase positive *S. aureus*		Coagulase negative staphylococci		Total	
		No.	%	No.	%	No.	%
Balady egg shells	**35**	**13**	**37.1**	**7**	**20**	**20**	**57.1**
Poultry farm egg shells	**35**	**18**	**51.4**	**10**	**28.6**	**28**	**80**
Balady egg contents	**35**	**12**	**34.3**	**14**	**40**	**26**	**74.3**
Poultry farm egg contents	**35**	**17**	**48.6**	**13**	**37.1**	**30**	**85.8**
Total	**140**	**60**	**42.9**	**44**	**31.4**	**104**	**74.3**

Table 4: Antimicrobial susceptible profile of *S. aureus* isolated from examined samples.

S. aureus strains isolated from	Resistant strains		Intermediate strains		Sensitive strains	
	No.	Freq.%	No.	Freq.%	No.	Freq.%
Balady egg shells	0	0	3	23.1 %	10	77%
Poultry farm egg shells	3	16.7 %	5	27.8 %	10	55.6%
Balady egg contents	0	0	3	25%	9	75 %
Poultry farm egg contents	7	41.2%	2	11.8 %	8	47%

Table 5: Freqency % of *mecA* gene in the examined of table egg samples by using PCR technique.

Examined samples	No. of examined samples	*mecA*	
		No.	*%*
Balady egg shells	0	0	0%
Poultry farm egg shells	3	1	33.3%
Balady egg contents	0	0	0%
Poultry farm egg contents	7	2	28.6%

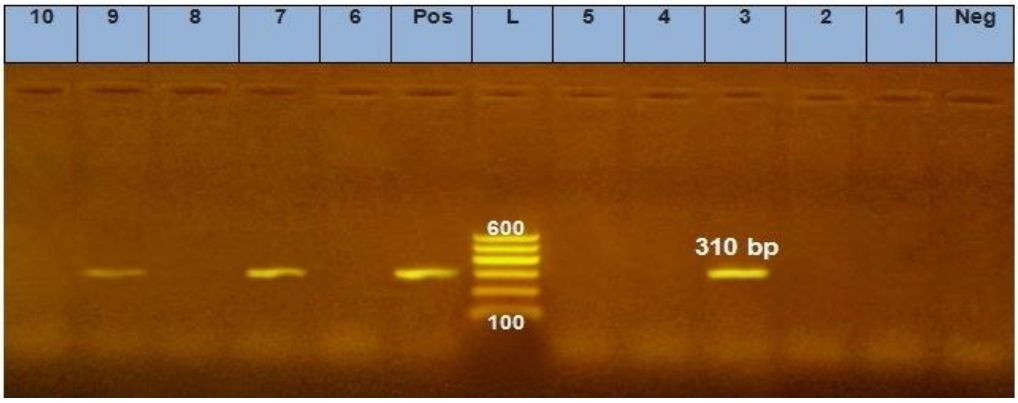

Fig. 1: PCR result of mecA gene among Coagulase positive *S. aureus* isolates. Lane L: ladder, lane pos: control positive, lane neg: control negative lane 3,7 ,9 (+ve mecA). lane 1,2,4,5,6,8,10 (-ve mecA).

DISCUSSION

Humans have been fighting the battle against antimicrobial resistance among bacteria; the disease caused by resistant *S. aureus* strains among those on prolonged antibiotic therapy may be difficult to be controlled. Of further concern is dissemination of these resistant bacteria to food, the environment, and animals. Antimicrobial resistance profiles of the *S.aureus* strains isolated from egg samples were comparable to those reports published in other parts of Egypt in humans, which is an alarming situation (Taj *et al.,* 2010). Injudicious and uncontrolled use of antimicrobials while treating farm animals and antimicrobial use in poultry feed may be possible explanations for MRSA in eggs in this study, since previous experimental studies on table eggs have already proved bacterial penetration of egg shells (Berrang *et al.,* 1999). Therefore, resistant bacterial strains may easily enter the egg and contaminate them. An additional explanation for the results of this study may be transfer of human antimicrobial resistant strains to poultry.

The requirements for the table eggs include high nutritional value and digestibility as well as safety of use in the everyday diet of millions of people worldwide. Taking these criteria into account, the presence of pathogenic bacteria in food, including table chicken eggs, may pose a serious health problem (food poisoning and foodborne infections), considering the fact that many foods containing eggs or egg products undergo no thermal treatment, or thermal treatment insufficient to neutralize these pathogens (Baumann–Popczyk and Sadkowska–Todys 2012, Pyzik and Marek 2012). At the time of

laying, the eggs are sterile due to natural chemicals and physical defenses against microbial infection, but on exposure to environmental conditions as temperature and length of storage eggs were contaminated by different types of microorganisms which cause spoilage and public health problems (Abd Elhady and Emara, 1997). The egg shell acts as a natural packing material for the egg contents, preventing the penetration of harmful bacteria.

The present study revealed a high rate of total Staphylococcus contamination of table chicken eggs, which were 20 (57.1 %) and 28 (80%) isolates from shell of balady and poultry farm egg and 26 (74.4) and 30 (85.8) strains from contents of balady and poultry farm eggs respectively (Table 1&2). Samah *et al.* (2015) showed the presence of Coagulase Positive Staphylococci both on the shell and in the contents, 80 isolates were detected with an overall prevalence rate of 40%. Isolation rates were 29(14.5%), 15 (7.5%), and 36 (18%) from the shell, contents, and both shell and contents, respectively. Higher prevalence rates were recorded by (Stepień *et al.,* 2009) when they reported the isolation of Coagulase Positive Staphylococci from eggs with a rate of 45.7%, of which 2.5%, 38.7%, and 58.8% were detected from white, yolk, and on the shell, respectively. One of the typical features that distinguish the more pathogenic Staphylococcus strains from the less pathogenic is the ability to produce free coagulase and bound coagulase (clumping factor). This feature is also considered as one of the virulence factors (Bannerman 2003, MacFaddin 2000). During storage on farms or in the packing centers, as well as during transport, poor handling practices can lead to the disruption of the egg's physical barriers. The presence of cracks increases the risk of bacterial contamination of the broken egg and of other eggs if the cracked ones leak, affecting the quality of the shell and that of the egg contents (EFSA, 2014).

A total 104 staphylococcal isolates were isolated from 140 samples of balady and poultry farm egg shell and contents in present study (Table 3). 13(37.1%) and 18(51.4%) isolates were identified as coagulase positive *S. aureus* from the shell of balady and poultry farm respectively. While, 12 (34.3%) and 17 (48.6%) isolates were identified as coagulase positive *S. aureus* from contents of balady and poultry farm egg. In addition, the obtained results showed that 7 (20%), 10(28.6%), 14(40%) and 13(37.1%) from isolate of shell and contents of balady and poultry farm, respectively, were contaminated with coagulase negative staphylococci. It is clear that percentage of CNS was low 44 (31.4%) compared to CPS 60 (42.9%) and this is lower than results obtained by Goja *et al.* (2013); Yurdakul *et al.* (2013); Piyali and Pranab (2016), the latter found that the percentage of CNS (60%) was higher than that of CPS (40%). Also, higher incidence (52.00%) of the unacceptable samples was associated with coagulase positive staphylococci could be detected by Eman and Saad (2015) and Shimaa El-Nagar *et al.,* 2017 revealed that staphylococci were detected in 26.7% of table eggs; 16.7% for *S. aureus* and 10% for CNS. In addition, Syed *et al.* (2018) could detect that 21.3% (64/300) of samples were contaminated with Staphylococcus which were isolated from the three parts of table egg and they found that the maximum number of staphylococci were isolated from egg yolk that represented 37.5% (24/64), followed by 34.4% (22/64) from egg white, and 28.1% (18/64) from the inner membrane of egg shells.

The number of CNS isolated in this study could be justified by the fact that CNS are found abundantly in the normal teat skin flora and mucosa of humans and animals while some are free living in the environment (Addis *et al.,* 2011). Staphylococci can be divided into two groups according to the production of Coagulase enzyme, which is capable of coagulating blood plasma. The synthesis of this enzyme is restricted to some species in the genus, among which *S. aureus.* The other Staphylococci that do not synthesize coagulase are referred to as Coagulase Negative Staphylococci (Koneman, 1997).

The present study was to establish the presence of methicillin-susceptible *S. aureus* MRSA in table egg so detailed analysis of the isolated *S. aureus* strains positive for *mecA* gene among coagulase positive *S. aureus* isolates demonstrated that only three out of the 10 isolated strains in percentage of 30% containing *mecA* (Table 5). Syed *et al.* (2018) showed that prevalence of MRSA in table eggs was 11% (33/300). *S. aureus* strains, especially those resistant to methicillin (MRSA) have been regarded as zoonotic agents and there is growing genuine concern about the likely transmission of MRSA between animals and humans from close interaction or from handling and/or consuming MRSA infected animal products (Garcia-Alvarez *et al.,* 2011). In settings where humans depend on animals and their products for food and livelihood, such as in pastoral Africa, contact is intimate and multi-drug resistant microorganisms have become an emerging veterinary and zoonotic issue of public health concern (Kasozi *et al.,* 2014 and Kamau *et al.,* 2013). Additionally, *S. aureus* is known to be the third most reported cause of food-borne diseases in the world (Normanno *et al.,* 2007).

Staphylococci are most common bacteria contaminating eggshells. Contamination is more likely linked with cracked egg, dirty shells and storage in contaminated surroundings. It can be contaminated during formation and laying process (Abdullah, 2010). The eggshell contamination

increasing the chances of egg contents contamination by penetration (Messens *et al.,* 2006). Bacterial contamination can happen at three main parts of egg (egg yolk, albumen and shell membrane / egg shell) (Bahrouz and Al-Jaff 2005). *S. aureus* is one of most common foodborne pathogens (Akbar and Anal, 2013a; Ghasemian, 2011; Akbar and anal, 2011). Eggs and egg products were responsible for 11% of all cases of staphylococcal food poisoning (Haeghebaert *et al.*, 2002).

Detection of the *mecA* gene, therefore, would be a good way of standardizing identification of MRSA across laboratories. The results are similar to those in another study comparing methods for the detection of MRSA on isolates from foods of animal origin in Italy by Corrente *et al.* (2007). and they concluded that analysis for MRSA in isolates from food of animal origin is better done with the mecA gene-specific PCR rather than conventional phenotypic assays (Corrente *et al*., 2007). The *mecA* gene was detected as 28.6% and 33.3% among *S. aureus* isolates from poultry farm egg contents and shells, respectively. Electrophoresis analysis of PCR amplification products using genus specific primer (*mecA gene*) showed that (Fig. 1). (Lane L: ladder, lane pos: control positive, lane neg: control negative lane 3,7 ,9 (+ve mecA). lane 1,2,4,5,6,8,10 (-ve mecA). Shimaa El-Nagar *et al.* (2017) could detect the presence of gene *mecA* in higher percentages (66.7 %) of examined samples.

The absence of standard structures and drainage system in the market and the relatively high humidity could have contributed to the high microbial growth. It was also found out that most retailers do not store eggs in refrigerators, thus the eggs are exposed to weather conditions, resulting in their contamination. The isolated microbes could cause severe health problems like, diarrhea, nausea and abdominal pain, since they are pathogenic (Adday *et al.,* 2009). *S. aureus* strains from processed poultry are thought to be human strains endemic to the processing plant or from the hands of workers in the plant. The literature varies as to the 61 origin of processing plant strains with biotyping indicating the passage of human staphylococcal strains to poultry in processing plants; plasmid profiling indicates that endemic strains in the processing plant are introduced by incoming birds.

Eggs have natural defense system against the contaminating microbes, such ascuticle, calcium hard shell and shell membrane (Jerzy and Dagmara, 2009). The albumen contains several egg white proteins that have antimicrobial properties, especially the lysozyme. Ovomucoid is another proteinase that inhibits the ability of bacterial to use the protein in albumen. Furthermore, the pH in albumen which is about 9–10 and the viscosities of the egg white are not suitable for microbial growth (*Froning, 1998*). Egg can be contaminated at both egg shell and egg contents by a variety of microbes with a wide range of pathogens such as Campylobacter jejuni, Listeria monocytogenes, Escherichia coli, Yersinia enterocolitica and especially salmonella (Ricke *et al.*, 2001; Board and Tranter, 1995).

Consumers are at a high risk of infection because most of the organisms isolated are pathogenic to humans. Ensuring good hygienic standards at the various markets and farm houses in the metropolis is a shared responsibility between stakeholders, government, consumers and retailers. Retailers in particular should be impressed upon to endeavour to store and retail their eggs under refrigerated or good sanitary conditions to reduce microbial contaminations**.**

Backyards flocks are reared under limited or no veterinary supervision. In such production systems, antimicrobials are freely used as feed or water additives (Otalu *et al.,* 2011). These practices can facilitate the emergence and spread of antibiotic resistant pathogens among birds with possible transmission to humans. Backyard chickens are extensively reared in close proximity to human dwellings and therefore play an important role in environmental contamination, in addition to serving as significant vehicles for the transfer of pathogens to humans by way of handling of live birds or consumption of contaminated meat and other poultry products. (Suleiman *et al.,* 2013). Eggs produced from backyard house hold chickens in Egyptian villages are commonly used for own consumption or to be sold in local markets, (most commonly used unwashed).

The eggs from supermarkets were not stored at refrigerated temperatures and the level of contamination in eggs collected from these sites was considerable. Thus, we suggest that supermarkets should store eggs at refrigerated temperatures to control the contamination of table eggs. The results in this study also indicate that contamination may occur at the farm level rather than during handling. The data underscore the need for optimum hygienic conditions at the farm level to decrease the bacterial load in commercial chicken eggs. Despite the difference in the type of bacterial growth and different number of isolates in each group, this difference was statistically non-significant. This indicates that all types of table eggs in the current study are not handled in healthy conditions.

CONCLUSION

The presence of MRSA in eggs is a matter of food safety and alternatively a source of dissemination of resistant strains to humans through the food chain. Antimicrobial resistance of these strains against commonly used antibiotics to cure human diseases is of public health concern. Proper sanitation in the poultry farms, improved storage conditions, as well as judicious and controlled use of antibiotics in the poultry industry are all important to control bacterial carriage and health risks by contaminated table eggs. We conclude from this study that eggs are exposed to contamination due to bad storage conditions in storehouse, wrong show in market, dirty table, high temperature, dust, hand touching, and all other surrounding pollution state, also consumers should keep egg in refrigerator and cooked egg well to kill bacteria. Finally the trade people must be transport egg from good source and good hen farms because the type of rearing (cage or floor) greatly effect on quality of egg and also from countries empty from dangerous zoonotic diseases. So, this study holds a great importance to understand the present risks of table egg borne diseases on human health and will help to take necessary measures to reduce the risk by creating public awareness, improving knowledge in rural women, good hygiene practices, thorough cooking, provision of vaccines and essential medicines and development of linkages with the different agencies.

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عزل وتوصيف الميكروب المكور العنقودي الذهبي المقاوم للميثيسلين من بيض المائدة

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يعتبر البيض غذاء متكامل يحتوى على معظم العناصر الغذائية الأساسية التي يحتاجها جسم الإنسان فى جميع المراحل العمرية وفى نفس الوقت يعتبر بيض الدجاج وسط جيد لنمو وتكاثر الميكروبات المختلفة التي تمثل خطورة على صحة الإنسان ومن هذه الميكروبات ميكروب المكور العنقودي الذهبي المقاوم للميثيسلين. لذا تضمنت الدراسة جمع 350 بيضة عشوائية من بيض الدجاج بواقع 175 بيضه من بيض الدجاج البلدي و 175 بيضه من بيض دجاج المزارع لمعرفة مدى تواجد ميكروب المكور العنقودي الذهبي و المكور العنقودي الذهبي الايجابي لاختبار البلازما و المكور العنقودي الذهبي المقاوم للميثيسلين. أسفرت النتائج عن تواجد المكور العنقودي الذهبي في قشرة بيض الدجاج البلدي بنسبة 57.1% و قشرة بيض دجاج المزارع بنسبة 80% و بنسبة 74.4 % 26 (عينة) و85.8% (30عينة) من المحتوى الداخلي لبيض الدجاج البلدي والمزارع علي التوالي. تم عزل ميكروب المكور العنقودي الذهبي الايجابي لاختبار البلازما من 13 عينة بنسبة (37.1%) من عينات قشرة بيض الدجاج البلدي ومن 18عينة بنسبة (51.4%) من قشرة بيض دجاج المزارع. كما أمكن عزله بنسبة (34.3%) و بنسبة (48.6%) من عينات المحتوي الداخلي لبيض الدجاج البلدى ودجاج المزارع على التوالي. التعرف على جين (*mecA*) للمكور العنقودي الذهبي المقاوم للميثيسلين عن طريق إستخدام تفاعل البلمره المتسلسل وأسفرت النتائج عن تواجد ميكروب المكور العنقودي الذهبي المقاوم للميثيسلين بنسبة إيجابية 1 (33.3%) و2 (28.6%) من عينات قشرة بيض دجاج المزارع و من عينات المحتوي الداخلي لبيض دجاج المزارع. هذا وقد تم مناقشة النتائج مع ذكر الشروط الصحية الواجب إتباعها لمنع تلوث البيض بميكروب المكور العنقودي الذهبي المقاوم للميثيسلين وذلك لحماية صحة المستهلك. هذا وقد أوصت الدراسة ببذل المزيد من الجهد لزيادة إنتاج البيض بطريقة آمنة عن طريق إتباع الطرق الصحية فى إنتاج بيض الدجاج ومنع التلوث فى كل من بيض الدجاج البلدي والمزارع .