Isolation, Characterization and Identification of Bacteria Associated with Freshly Collected Quail Eggs in Ado Ekiti, Nigeria

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ABSTRACT
Quail eggs are considered a delicacy which are taken raw by most consumers with the aim of deriving all the nutritional constituent and medicinal benefit. This research focused on the microbial load of the sampled eggs and the identity of the isolated organisms. Sixty (60) eggs were collected from 5 different farms, analyzed microbiologically and the organisms identified. It was found that 18.33% of the quail eggs were sterile, 28.33% of the eggs carried coliform and 11.67% of the eggs carried salmonella. The average microbial load of the quail eggs for the farms ranged from 0.0CFU/ml in sterile eggs to 2.87x10^5 CFU/ml in heavily loaded eggs. All of the farm have coliform while 4 out of the 5 farms produced eggs that carries salmonella. The organisms isolated in the eggs are Salmonella sp, Escherichia coli, Klebsiella sp, Staphylococcus aureus, Pseudomonas sp and Proteus.

Keyword: Quail egg, Salmonella, MacConkey agar, Coliforms, Microbial load.

INTRODUCTION:
Eggs have been used as food by human beings since antiquity [13]. Egg yolk and whole egg store significant amount of protein and choline [11] and are widely used in cookery. Due to their protein content, the United State Department of Agriculture categorize eggs as meat within the food guild pyramid. Eggs gave a good combination of nutrient, supplies all the essential amino acid for human and provide several vitamins and minerals [19]. Quail Eggs are considered a delicacy in many part of the world, including Europe and North America. In Japanese cuisine, they are sometimes used raw or cooked as “Tamago” in Sushi and often found in bento lunches [4]. Today in Nigeria, it is no longer news for people who live on Quails Eggs, individuals who feed on the eggs do that for the claimed medicinal properties and packed nutrient [12]. Eggs and egg products have been incriminated in foodborne outbreaks [5]. Raw eggs can be contaminated with Salmonella, either presenting as shell contamination due to contact with feces after laying (migration of the organism through the shell is possible) or as egg content contamination due to colonization of the hen’s oviduct [6]. Shell defects have been shown to increase the risk of microbial contamination of eggs [16]. A major health concern that comes into heart irrespective of the medicinal usefulness and nutritional value of quail eggs is the mode through which people are encourage to take the eggs. Most consumers take the eggs raw in order to extract all nutritional quality. It is of public health concern because FSEP (2013) [9] reported that Salmonella may infect a laying flock without causing clinical symptoms but might contaminate eggs. Therefore, it is imperative to look into the microbial load and the type of organisms present in the eggs.

MATERIALS AND METHOD
Quail eggs used for this research were bought from 5 quail farms around Ado Ekiti, between January and February 2015, (Farm 1, Farm 2, Farm 3, farm 4, Farm 5) the samples were transferred to microbiology laboratory, Ado Ekiti, within two hours of collection, where it was analyzed.

Determination of microbial load of the eggs
The shells of the quail’s eggs were disinfected with 70% ethanol and aseptically separated the shells from the eggs. Five (5ml) of the homogenized egg content were introduced into 45 ml sterile buffer peptone water. One (1 ml) was inoculated on Salmonella-Shigella agar (Biomark), Nutrient agar (Biomark) and MacConkey agar (Biomark) in triplicates. The pour plate method was employed. It was incubated for 24 hours at 37°C temperature in an incubator (Royalcare England. DNP 9022A).

Enumeration of microbial population
After incubation at room temperature for 24h, colonies on Nutrient agar plates were counted using the colony counter and the mean was recorded as total viable count (TVC). Enumeration of Salmonella was performed on Salmonella-Shigella agar plates. Total coliforms were determined on MacConkey agar plates.

Biochemical identification
The colonies were purified on nutrient agar plates followed by biochemical identification of colonies according to Macfaddin (1980) [15].

RESULTS
A total of 60 quail eggs were collected from five different farms located around Ado Ekiti, Ekiti state Nigeria. A total of 7 eggs carried Salmonella, 17 of the eggs carried coliforms while only 11 of the eggs were sterile. The percentage of the eggs with...
salmonella is 11.67%, coliform constituted 28.33%, while about 18.33% of the eggs were sterile.

Table 1 showed the microbial load of the freshly collected quail eggs from five different farms in Ado Ekiti over a period of four weeks. The result showed that farm 3 had the all-time highest microbial load over the period of the four weeks with values ranging from $2.87 \times 10^2$ to $1.43 \times 10^2$ CFU/ml. Farm 1 had all the egg analyzed in week 1 sterile, but with mild microbial load subsequently. Coliforms were found in all of the farms from which eggs are collected. However, farm 3 had the highest coliform presence in their eggs just as the microbial load. The coliform level is lowest in Farm 5. It was observed that the salmonella was isolated from quail eggs from all farms except Farm 2 where there was no salmonella growth.

Table 1: Microbial load of Quail eggs from 5 different farms (x 10^2 CFU/ml)

<table>
<thead>
<tr>
<th>Week</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
<th>Farm 4</th>
<th>Farm 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1.36</td>
<td>2.87</td>
<td>0.51</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>0.58</td>
<td>2.48</td>
<td>1.70</td>
<td>1.28</td>
</tr>
<tr>
<td>3</td>
<td>0.75</td>
<td>2.06</td>
<td>2.75</td>
<td>0.81</td>
<td>0.77</td>
</tr>
<tr>
<td>4</td>
<td>0.70</td>
<td>0.82</td>
<td>1.43</td>
<td>1.36</td>
<td>0.77</td>
</tr>
</tbody>
</table>

The result of the biochemical test showed that Organisms present in the quail eggs samples are, Salmonella sp, Escherichia coli, Klebsiella sp, Staphylococcus aureus, Pseudomonas sp, and Proteus, as shown in table 2.

Table 2: Biochemical / Identification Test

<table>
<thead>
<tr>
<th>CULTURAL CHARACTERISTICS</th>
<th>GRAM REACTION</th>
<th>COLOUR</th>
<th>CATALASE</th>
<th>COAGUASE</th>
<th>MOTILITY</th>
<th>INDOLE</th>
<th>CITRATE</th>
<th>OXIDASE</th>
<th>METHYL RED</th>
<th>VOGES-PROSKAUER</th>
<th>UREASE</th>
<th>PROBABLE IDENTIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round convex colonies</td>
<td>- Rod</td>
<td>MS</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>E. coli</td>
</tr>
<tr>
<td>flat, greenish colonies</td>
<td>- Rod</td>
<td>G</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>P. aerugenosa</td>
</tr>
<tr>
<td>Yellow and smooth colony</td>
<td>+ Cocci</td>
<td>Y</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Mucoid, pink colony on MacConkey agar</td>
<td>- Rod</td>
<td>P</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Klebsiella sp</td>
<td></td>
</tr>
<tr>
<td>smooth round convex pale</td>
<td>- Rod</td>
<td>P</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Salmonella sp</td>
</tr>
<tr>
<td>swarm at certain intervals and produce a pattern of concentric rings.</td>
<td>- Rod</td>
<td>Cr</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Proteus sp</td>
<td></td>
</tr>
</tbody>
</table>

Y= Yellow, MS= Metallic Sheen, Cr=Cream, G= Green, P=Pale, ND= Not Determined.
DISCUSSION

Eggs and eggs products had been described as one of the main food vehicles associated with foodborne outbreaks [5]. Stepień-Pyśniak (2010) [18] reported that an important factor influencing quantitative bacterial contamination of eggs depends on the number of bacterial cells on the shells and in the content of the egg and on the rate at which they multiply within it. The contamination may have resulted from both horizontal and vertical infection, since contamination in eggs are as a result of exposure to environmental conditions (for example, soil, dust and dirty nesting materials), eggs become contaminated with different types of microorganisms [7, 17]. Furthermore, these microorganisms may contaminate the egg contents either by penetration or withdrawal through pores of the shells, and also through the trans-ovarian route. Some other factors such as environ-mental temperature and humidity influence the bacterial penetration and thus, enhance the infection and spoilage [1]. The source of the organisms present in the freshly collected eggs may have resulted from the quail bird [10] and by extension from the poultry feed and water the quail bird consumed [3]. Raw eggs can be contaminated with Salmonella, either presenting as shell contamination due to contact with feces after laying (migration of the organism through the shell is possible) or as egg content contamination due to colonization of the hen’s oviduct [6]. The number of quail eggs observed with Salmonella was 11.67%. In an earlier report of Erdoúrul and Akiroúlu (2002) [8], it was reported that the quail eggs used in the research had 5.69% Salmonella. Lammerding (1994) [14], reported that the food products most commonly identified as vehicles for transmission of Salmonella include raw eggs and under processed egg products, this is an indication that the eggs could serve as a source of Salmonella infection to the consumers especially those taking the eggs raw.

Coliforms were also present in the eggs analyzed: Escherichia coli, and Klebsiella sp, there are presences of other Gram negative organisms: Pseudomonas sp and Proteus, while Staphylococcus aureus was the only gram positive organism found in the quail eggs analyzed. The report of the presence of coliform and other organisms in eggs is in line with what was reported by Stepień-Pyśniak (2010) [18] for hen eggs. Alvarez-Fernandez et al., (2012) [2] also reported the presence of coliform in stored quail eggs. It was reported that the coliform bacterial, and other gram negative microorganisms can get their ways into the eggs through the pores on the egg shell. The presence of coliform in the quail eggs suggest that quail eggs could be of potential health hazard to the public who consume the eggs raw. This can also predispose people to infection as a result of the organisms in the eggs. In the light of the potential risk associated with the type of organisms present in quail eggs sold around Ado Ekiti metropolis, it is preferable not to take the quail eggs raw.

REFERENCES:

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