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Research Article

STUDIES ON MICROFLORA AND THEIR ROLE ON EGG SHELL CONTAMINATION AND INFECTION

B. Indhu[1], S. Muthusami[1] and N. Thirunavukkarasu[2]*

[1]Department of Zoology, Kandaswami Kandar's College, Velur, Namakkal, Tamilnadu, India.
[2]Department of Advanced Zoology and Biotechnology, Dr. Ambedkar Government Arts College (Autonomous), Vyasarpadi, Chennai-600 039, Tamilnadu, India.

ABSTRACT

The eggs are contaminated in many ways. The contamination detected was many microbial tests. The eggs were collected in farms mini markets, supermarkets, consumers. Samples were collected at 4 different kinds of units and the total number of samples collected was 62, each sample consisted of 4 eggs. From this study, we observed the different kinds of microbe in the different egg sample. The egg samples are egg white and egg shell. From the biochemical results and microscopic observation we can conclude the *Salmonella* sp., *E.coli*., *Campylobacter*, *Staphylococus aureus* and *Coliform*. Results from the microbial analysis of total count showed that eggs collected from forms and mini markets are more contaminated then eggs from supermarkets and consumers.

Keywords: Egg, Farms, Mini markets, Super markets, Consumers.

INTRODUCTION

The increasing consumer awareness of food safety issues has changed the public perception of a good egg from shell cleanliness and physical properties to that of microbial integrity. Microorganisms can contaminate eggs at different stages from production though processing to preparation and consumption (Barrow and Lovell, 1991). Different researchers reported on the penetration of bacteria through the eggshell with associated membranes and on whole egg contamination. Bacteria of the genus *Pseudomonas* have been shown to more readily penetrate into whole eggs of poor shell quality (Sauter and Peterson, 1969). For a number of reasons eggs have constituted an important part of the human diet for centuries and play a significant part in religious holidays.

Among these reasons are the high quality proteins of eggs, which makes them nutrition and the fact that the interior of the egg is protected by the shell, so that it is hard for Microorganisms to contaminate the egg (Svenska Lantagg, 2002). Finally hens are widely used domesticated animalsand relatively efficient feed converters.

Washing of eggshell is seen as a risk in most of the countries in the European Union because washing damages the outer cuticle of the shell. The cuticle offers a natural barrier to contamination and obstructs microorganisms to enter the interior of the egg. There are many bacteria that can contaminate the egg production system. The most common ones are *Salmonella*, *Campylobacter* and different coli forms (Garbutt, 1997). *Salmonella* are killed during heat treatment of food at 70°C for at least 10 minutes (Madigan and Martinko, 2006).

Campylobacter cause diseases in humans if the living bacteria are ingested. The incubation period is usually 3-5 days and the symptoms are almost the same as for 12 *Salmonella* besides abdominal pain (Garbutt, 1997). *Staphylococcus aureas* is involved in food poisoning the incubation period can be as short as 30 minutes. Approximately

100000 bacteria/gram food are required to produce enough enterotoxin to cause disease in humans (Smittskyddsinstitutet, 2006).

Coliform bacteria are fecal bacteria that indicate some kind of fecal contamination of the food (EFSA, 2005). *Escherichia coli* are a type of coliform bacteria that generally occur as a harmless member of the micro flora in the gut of animals. The infective dose of the harmless strains of *E.coli* is not interesting but pathogenic strains like 0157:H7 are extremely virulent and may have an infective dose as low as 10 organisms. *E.coli* 0157:H7 rises as inflammation of the colon and gives abdominal pain and diarrhea, in severe cases kidneys may be damaged as well as the brain due to lack of blood platelets. Food poisoning symptoms include nausea and diarrhea weakens the afflicted, and for the already sick, elderly or weak it can lead to critical conditions (Garbutt, 1997). Non host adapted strains of different pathogens require higher amount of bacteria to cause disease, while strains adapted to a certain host require less bacteria to cause disease (Adams & Moss 2008).

MATERIALS AND METHODS

Sample collection

4 samples were collected from 3 different grocery stores in Karur and 2 samples from 6 mini markets each. One mini market was situated at the countryside close to the city of Namakkal and the rest was concentrated to western Karur. In every sample 4 eggs were collected.

Isolation of Microorganisms from Samples

The temperature in which the eggs were stored at the farms and in the grocery stores and in mini markets was estimated. The method of ISO 6887-4:2003(E) each sample was placed in a stomacher bag with 100ml buffered peptone water and in the eggs were rubbed through for 1 minute. The eggs were removed from the Buffered Peptone water, their weighs were noticed and the stomacher bag with its diluents put in the Stomacher for 2 × 30 seconds.

Characterization of Microorganisms

Food laboratories was prepared and 0.1ml of the diluents was plated on Tryptic soy Agar (HiMedia) for total count and the rest of the material was incubated at 37°c for 3 hours in order to resuscitate injured bacteria. After the resuscitation the diluents were directly without any further dilution spread on violet Red Bile Agar (OXOID), Deoxycholate citrate agar (OXOID), campylobatcter agar (OXOID)and for all samples except farms, on Baird parker Agar (OXOID). Before plating on Baird parker Agar a dilution series had to be made. Plates with *Campylobacter* agar was incubated under micro aerobic conditions for 72 h at 42°C. All samples were plated on duplicate plates.

For further identification of coliforms and *E.coli*, colonies from the violet Red Bile Agar were transferred to test tubes with Lauryl Thryptose Broth (OXOID) and inverted Durham tubes and incubated for 24h at 37°C due to the manual of Microbiological Food Analysis for Food Laboratories. If gas was produced, 0.1 ml from each positive sample was transferred to test tubes with EC-MUG (HiMedia) with inverted Durham tubes and incubated for 24 h in water bath at 44.5°C.

Likewise, all the samples were characterized by Biochemical tests and microscope observation method. Based on their results the microorganisms were described in the table.

Physiological and biochemical characteristics of test organism gram staining

Gram staining

A clean glass slide was taken, a thin smear of each culture was made and heat fixed. The smear was folded with crystal violet for one minute and washed with distilled water. Then smear was flooded with Grams Iodine for 30 seconds and washed with distilled water, decolorized with 95% ethyl alcohol and washed with water immediately and flooded with safranin for 30 seconds and again washed with water. The smear was observed under the oil immersion objective.

Indole test

This test determines the ability of certain bacteria to decompose amino acid tryptohan to Indole, which accumulates in the medium and then tested with Kovac's Indole reagent. Culture was inoculated in 5ml of the medium and incubated at 37°c for 24 hours. 0.3ml of Kovac's Indole Reagent was added gently and allowed to stand for 10 minutes.

Methyl red test

This test is employed to detect the production of acid during fermentation of glucose and maintain the condition such that pH of the old culture is sustained below a value of 4.5 It is indicated by a change of colour of methyl red indicator, which is added at the end of the period incubation. 5ml of MR VP medium was inoculated with culture and

incubated for 24 hours at 37°C5 drops of methyl red indicator was added and read immediately.

Voges – proskauer test

Many bacteria ferment carbohydrate with the production of Acetyl Methyl Carbinol. The substances can be tested by a color reaction between diacetyl formed during the test by oxidation of acetyl methyl carbinol or its reduced product 2, 3 butylene glycol and guanidine group under alkaline condition. This test is usually done in conjugation with methyl red test. An organism of enterobacterial group is either methyl red positive or Voges – Proskauer negative, vice versa. 5ml of MRVP medium was inoculated with culture from young agar slope pure culture and incubated for 48hrs at 37°C. After incubation, 3ml if Barrit's reagent A and 1ml of Barrt's reagent B was added. The tubes were shaken and allowed to stand for 15 minutes and observed for colour change.

Citrate utilization test

This is a test to detect the ability of an organism to utilize citrate as the sole carbon source for its growth. Simmons's citrate agar slant was inoculated with culture and incubated for 48 hrs at 37°C.

Urease test

Some organism split the urea molecule into two, releasing carbon dioxide and ammonia. This indicates that the organism produces the enzyme, urease. The 24 hrs old culture is incubated tubes are examined for colour change.

RESULTS AND DISCUSSION

Results from the microbial analysis of total count showed that eggs collected from farms and mini markets are more contaminated than eggs from supermarkets and consumers.

From the Biochemical results and microscopic observation we can conclude the *Salmonella* sp., *E.coli*, *Campylobacter*, *Staphylococcus aureus* and *Coliform***.** *Salmonella* was occurred on more than 50% of forms 67% of mini markets 25% of supermarkets. The least amount of coliforms from consumers, as well as no *Campylobacter. Coliforms* was higher in mini markets than in forms. *E.coli* was higher from forms than from mini markets, 47% & 22%. *Campylobacter* since it was only found in a few only 50%.

The difference between the total bacterial number on egg shells from forms and supermarkets can be explained by the time the eggs been outside the contaminating environment the stables create. The transport of eggs were not done under refrigerated conditions in Karur but the Supermarkets, *Salmonella* does not grow when the temperature is below 6°C (Malorny et al., 2008). Consumers have significant lower amounts of *Salmonella* compared to farms, probably due to that many consumers in this study clean these eggs and store them in the refrigerator.

To prevent bacteria in food it was recommended to store it at a low temperature below 4°C no growth occurs. Handling factors important to reduce the bacterial contamination on and in egg are frequent gathering of eggs proper, cooling and humidity conditions, proper washing and that the eggs are handled with care to avoid breakage (Froning, 1998). The reason why *Salmonella* and *Campylobacter* was enriched before plated on the medium was due to that we wanted at egg shells and not only if they were present there.

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Table 1: Samples were collected at 4 different kinds of units and the total number of samples collected was 62, each sample consisted of 4 eggs

Unit	Number of units visited	Number of samples/ per visit	Total amount of samples
Farms	2	5	10
Mini markets	2	5	10
Super markets	2	5	10
Consumers	2	5	10

Table 2: Mean value of colony forming units per cm2 (CFU/cm2) on eggs from farms mini markets, supermarkets and consumers (n.d: no colony forming units detected)

Total count	*Salmonella*	*Coliforms*		*Campylobacter*	*S.aureus*
Farms	10 x 102	12	16 x 10^2	12	-
Mini markets	15 x 106	23 x 10^2	49 x 10^2	20	63 x 10^2
Super markets	9.2 x10^2	1.9	21	5.9	11 x 10^2
Consumers	79 x 102	n.d	11	n.d	2.3 x 10^2

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