Research Journal of Biological Sciences 2 (2): 161-166, 2007

Microbiological Analyses of Freshly Laid and Stored Domestic Poultry Eggs in Selected Poultry Farms in Umuahia, Abia State, Nigeria

C.N. Obi and A.J. Igbokwe
Department of Microbiology, Michael Okpara University of Agriculture,
Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria

Abstract: Freshly laid and stored domestic fowl eggs of 7, 14 and 21 days old respectively were microbiologically analyzed for organisms on their shells, in the chalazae, albumen and yolks. All the egg parts were analyzed by plating unto Nutrient, McConkey and Sabourand's Dextrose agars respectively. Nine bacterial isolates which included *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Enterobacter, Bacillus, Alcaligenes, Proteus, Pseudomonas* and *Serratia* and five fungal genera namely *Aspergillus, Penicillium, Cladosporium, Monilia* and *Rhizopus* were isolated and identified in all the samples. The bacterial load was in the range with 3.3×10^{6}-1.5×10^{7} CFU mL^{-1} with the shell having the highest and the albumen the lowest load respectively. Result showed presence of pathogenic microbes in the sample analyzed and was concluded that domestic fowl eggs should not be consumed raw.

Key words: Domestic fowl eggs, raw, consumption, pathogens, microbes

INTRODUCTION

Eggs are complex biological systems produced primarily for reproduction in animals. The yolks containing the female germ cells are produced in the chicken's ovaries and are dropped into the mouth of the oviduct. As this happens, the egg is covered with layers egg white from albumen secreting cells with membranous tissue from other protein secreting cells and then finally with calcium and other minerals from mineral secreting cells near the bottom of the oviduct. This results in eggshell. Fertilized egg-yolks produce embryos (Adams and Moss, 1995). Fully mixed egg contains about 65% water, 12% proteins and 11% fat. Fresh egg has three structures, which are an outer waxy shell membrane, the shell and the inner shell membrane and each is effective to some degree of retarding the entry of microorganisms. Internally, lysozyme is present and this is quite effective against gram-positive bacteria. Egg white also contains avidin, which forms a complex with biotin thus making the vitamin unavailable to microbes.

Although considered as proteinous food, eggs contain every vitamin and mineral needed by human beings except Vitamin C (Kay and Sharon, 1968). Proper storage of eggs maintains the quality; however, both physical and chemical changes occur as eggs deteriorate. Physically, egg white becomes less viscous and more watery. Water from the egg white moves into the yolk thereby making it thinner. Evaporation of water takes place through the shell and Carbon (IV) Oxide CO_2) escapes causing an increase in the pH of the content. Due to this, the protein begins to breakdown and other changes occur too.

Owing to poor storage standards/ conditions of freshly laid poultry eggs, more complex spoilages are usually associated with freshly laid and poorly stored eggs. Poor treatment of freshly laid eggs results in the movement of bacteria into the shell leading to the rotting of eggs when the bacteria are in sufficient concentration. This is the commonest form of bacterial spoilage of eggs. Several kinds of bacterial rotting of eggs include green rots caused by *Pseudomonas* and *Acinetobacter;* black rot caused by *Proteus, Salmonella and Aeromonas;* pin rots caused by *Pseudomonas*; red spot caused by *Serratia,* custard rots caused by *Proteus vulgaris* and *Pseudomonas intermedium.* Several spoilage rots have been associated also with moulds like *Penicillium* and *Cladosporium* sp. Apart from the spoilage organisms of domestic fowl eggs, several pathogens have been isolated from domesticated fowl eggs. These include Salmonella and *Escherichia* sp. (Jones *et al*., 1991). Domestic fowl eggs form a basic food for the masses especially in the

Corresponding Author: C.N. Obi, Department of Microbiology, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria

aspect of protein and vitamins. Thus, in the local setting in Nigeria, eggs are treasured so much due to its use in feeding the infants, malnourished and the aged. This has led to the development of various egg recipes among the poor and the rich. Such include Mayonnaise, Eggnog, Baked egg custard, stuffed egg, fried, boiled and pouched eggs (Smittle, 1977).

The consumption of raw domestic fowl eggs is a common habit among many people and professions. Such habit is highly recommended and practiced by many musical artists who claim that drinking of raw eggs improves the quality of their voice thereby improving their performance. Some people have at one time or the other drunks raw eggs or mix the same with other drinks like malts as a way of enhancing blood-building process. This is very common among malnourished and/or anaemic patents. Previous studies revealed the presence of pathogens like Salmonella in raw eggs with high incidence in duck eggs (Jones *et al*., 1991). Apart from commercial poultry farms owned by the government and some individuals, many people in the local setting keep the native type domestic fowls in free-range which will lay eggs for use as food. The eggs are rarely sold in the market, as the quantity is never enough for the subsistence farmers who have several mouths to feed daily. Occasionally, the fowl eggs are sold in the market for use most times by several African Traditional Religionists who use the eggs for various kinds of sacrifices. Due to the low level of production of eggs traditionally, most people nowadays depend on the eggs produced by the exotic breeds of fowl foe their eggs production and consumption. Consequently, this research work carried out in 2005 aimed at the microbiological examination of freshly laid and stored commercially produced fowl eggs in selected commercial poultry farms and retailers in Umuahia Metropolis, Abia State, Nigeria.

MATERIALS AND METHODS

Sources of materials: Freshly laid eggs (a day old) and stored eggs of 7, 14 and 21 days respectively labeled A-D collected from four different Poultry farms in Umuahia metropolis were microbiologically analyzed for bacteria and fungal presence.

Media preparation: All the metallic and laboratory glas swares were sterilized by autoclaving at 121 °C for 15 min. The media (Nutrient, McConkey and Sabouraund's Dextrose Agars) were prepared the previous day by dissolving known quantities in corresponding volume of water according to the manufacturer's specification. The plates were prepared in duplicates and kept in the incubator to check for contamination while an uninnoculated plates was kept as control.

Sample collection Twenty egg samples labeled groups A-D and made up of five eggs per group were collected using sterile disposable hand gloves to prevent contamination by microorganisms, laboratory coats was worn. The samples were put into sterile glass beakers, covered with sterile aluminum foils and transfers into a cooler containing ice blocks. This was quickly taken to the National Root Crops Research Institute (NRCRI) laboratory and stored in the refrigerator till they were used.

Isolation and identification of organisms: From the samples collected, one egg was picked from sample A, was put into a sterile glass beaker and was washed with distilled water. After washing, 1ml of the water was inoculated on the plates so as to isolate the organisms on the surface of the sample. Also, another egg was broken with sterile blade and using a sterile forceps, the chalaza was picked, placed on the Nutrient and McConkey media and smears were made. The yolk of another egg was inoculated into the same media using a sterile Pasteur pipette. This was also carried out for the egg white. At the end, the plates were labeled $A_1 - A_4$ and incubated at 37 °C for 24 h. This procedure was carried out on the remaining samples (B-D). The Sabouraund's Dextrose agar used for the fungal isolation was fortified with 0.005% chloramphenicol to inhibit bacterial contaminants. The plates were inoculated in duplicates and incubated at 22 °C for 2-6days. Identification and characterization of the bacterial isolates were based on the methods described by Ogbulie *et al*. (1998) while morphological features, slide culture technique and slide mount in Lactophenol-cotton-blue of each fungal isolate was according to Barnett and Hunter (1972).

RESULTS AND DISCUSSION

A total of nine bacterial and five fungal genera were isolated from the egg samples analyzed in the research. Table 1-4 show the percentage occurrence of the nine bacterial isolates from the various parts of the egg analyzed; Table 5 shows the percentage occurrence of the fungal isolates from the samples while Table 6 shows the microbial loads of bacteria from the parts of the samples analyzed. All the bacterial isolates are found on the shells of 1-day-old eggs from the 4 samples of eggs analyzed.

The microbial isolates from the analyzed egg samples indicated the presence of both spoilage and pathogenic

Table 1: % occurrence of bacterial isolates in 0-day eggs

Bacterial isolates	*Bacillus*	*Staphylococcus aureus*	*Pseudomonas*	*Proteus*	*Serratia*	*Enterobacter*	*Escherichia coli*	*Alcaligenes*	*Salmonella typhi*
Samples:									
Shell									
A1	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+
% Occurrences	100%	100%	100%	100%	100%	100%	100%	100%	100%
Chalaza									
A_2	+	+	+	-	-	-	-	-	+
2	+	+	+	+	-	+	-	-	+
3	+	+	+	+	-	+	-	+	+
4	+	+	+	+	+	+	+	+	+
% Occurrence	100%	100%	100%	75%	25%	75%	25%	50%	100%
Albumen									
A_3	+	+	-	-	-	+	-	-	+
2	+	+	+	+	+	+	-	+	+
3	+	+	+	+	+	-	-	+	+
4	+	+	+	+	+	-	-	+	+
% Occurrence	100%	100%	75%	75%	75%	50%	0%	75%	100%
Egg yolk									
A_4	-	-	-	-	-	-	-	-	+
2	-	-	+	+	+	+	+	-	+
3	+	+	+	+	+	+	+	-	+
4	+	+	+	+	+	-	-	+	+
% Occurrence	50%	50%	75%	75%	75%	50%	50%	25%	100%°

Key: + Present;-Absent; A = 0day; A1 = Shell. A2 = Chalaza; A3 = Albumen; A4 = Egg yolk. 1, 2, 3 and 4 = poultry farms

Table 2: % Occurrence of bacterial isolates in 7day old eggs

Bacterial isolates	*Bacillus*	*Staphylococcus aureus*	*Pseudomonas*	*Proteus*	*Serratia*	*Escherichia coli*	*Enterobacter*	*Alcaligenes*	*Salmonella typhi*
Samples:									
Shell									
B1	+	+	+	+	+	-	-	-	+
2	+	+	+	+	+	-	-	-	+
3	+	+	+	+	+	-	+	-	+
4	+	+	+	+	+	-	-	+	+
% Occurrences	100%	100%	100%	100%	100%	0%	25%	25%	100%
Chalaza									
B_2	+	+	+	-	+	+	+	-	+
2	+	+	+	+	-	+	+	+	+
3	+	+	+	+	-	+	+	+	+
4	+	+	+	+	+	+	+	+	+
% Occurrence	100%	100%	100%	75%	50%	100%	100%	750%	100%
Albumen									
A_3	+	+	+	+	+	+	+	-	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	-	+	+
% Occurrence	100%	100%	100%	100%	100%	100%	75%	75%	100%
Egg yolk									
A_4	+	+	+	+	+	+	+	+	+
2	-	+	+	+	+	+	+	+	+
3	+	+	+	+	+	-	+	+	+
4	+	+	+	+	+	+	-	+	+
% Occurrence	75%	100%	100%	100%	100%	75%	75%	100%	100%

Key: + Present;-Absent; A = 0day; A1 = Shell. A2 = Chalaza; A3 = Albumen; A4 = Egg yolk. 1, 2, 3 and 4 = poultry farms

organisms. The result agrees with the earlier work of Braun and Fehlhaber (1995). The presence of *E. coli* and *Salmonella* in the eggs indicates faecal contamination probably from faecally contaminated poultry feeds or drinking water. Comparings 1 and 4, results showed an obvious ingress of microorganisms from the shells

Table 3: % Occurrence of bacterial isolates in 14 day old eggs

Bacterial isolates	*Bacillus*	*Staphylococcus aureus*	*Pseudomonas*	*Proteus*	*Serratia*	*Enterobacter*	*Escherichia coli*	*Alcaligenes*	*Salmonella typhi*
Samples:									
Shell									
B_1	+	+	+	+	+	-	-	-	+
2	+	+	+	+	+	-	-	-	+
3	+	+	+	+	+	-	+	-	+
4	+	+	+	+	+	-	-	+	+
%									
Occurrences	100%	100%	100%	100%	100%	0%	25%	25%	100%
Chalaza									
B_2	+	+	+	-	+	+	+	-	+
2	+	+	+	+	-	+	+	+	+
3	+	+	+	+	-	+	+	+	+
4	+	+	+	+	+	+	+	+	+
%									
Occurrence	100%	100%	100%	75%	50%	100%	100%	750%	100%
Albumen									
A_3	+	+	+	+	+	+	+	-	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	-	+	+
%									
Occurrence	100%	100%	100%	100%	100%	100%	75%	75%	100%
Egg yolk									
A_4	+	+	+	+	+	+	+	+	+
2	-	+	+	+	+	+	+	+	+
3	+	+	+	+	+	-	+	+	+
4	+	+	+	+	+	+	-	+	+
%									
Occurrence	75%	100%	100%	100%	100%	75%	75%	100%	100%

Key: + Present;-Absent; A = 0day; A1 = Shell. A2 = Chalaza; A3 = Albumen; A4 = Egg yolk. 1, 2, 3 and 4 = poultry farms

Table 4: % Occurrence of bacterial isolates in 21day old eggs

Bacterial isolates	*Bacillus*	*Staphylococcus aureus*	*Pseudomonas*	*Proteus*	*Serratia*	*Enterobacter*	*Escherichia coli*	*Alcaligenes*	*Salmonella typhi*
Samples:									
Shell									
B_1	+	+	+	+	+	-	-	-	+
2	+	+	+	+	+	-	-	-	+
3	+	+	+	+	+	-	+	-	+
4	+	+	+	+	+	-	-	+	+
%									
Occurrences	100%	100%	100%	100%	100%	0%	25%	25%	100%
Chalaza									
B_2	+	+	+	-	+	+	+	-	+
2	+	+	+	+	-	+	+	+	+
3	+	+	+	+	-	+	+	+	+
4	+	+	+	+	+	+	+	+	+
%									
Occurrence	100%	100%	100%	75%	50%	100%	100%	75%	100%
Albumen									
A_3	+	+	+	+	+	+	+	-	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	-	+	+
%									
Occurrence	100%	100%	100%	100%	100%	100%	75%	75%	100%
Egg yolk									
A_4	+	+	+	+	+	+	+	+	+
2	-	+	+	+	+	+	+	+	+
3	+	+	+	+	+	-	+	+	+
4	+	+	+	+	+	+	-	+	+
	75%	100%	100%	100%	100%	75%	75%	100%	100%

Key: + Present;-Absent; A = 0day; A1 = Shell. A2 = Chalaza; A3 = Albumen; A4 = Egg yolk. 1, 2, 3 and 4 = poultry farms

of 1day old eggs to the yolk of the same eggs. This movement from the shell to the yolk was probably due to a fall in the pressure as air escaped through the shell.

There was a gradual decrease in the contamination of the Albumin and yolk by all the bacterial isolates except *Bacillus, Staphylococcus* and *Salmonella* that appeared

Table 5: Percentage occurence of fungal isolates in all the egg samples

Fungal isolates	*Penicillium*	*Aspergillus*	*Cladosporium*	*Rhiozpus*	*Monilia*
A_1 Egg shell	+	+	-	+	-
B_1	+	+	+	+	-
C_1	+	+	-	+	-
D_1	+	+	+	+	+
A_2 Chalaza	+	+	-	+	+
B_2	+	+	+	-	-
C_2	-	+	-	-	-
D_2	+	+	+	+	+
A_3 Albumen	-	+	+	-	+
B_3	+	+	+	+	-
C_3	+	+	+	+	+
D_3	+	+	+	-	-
A_4 Egg yolk	+	+	+	+	+
B_4	+	+	+	+	+
C_4	+	+	+	+	+
D_4	+	+	+	-	+
% Occurrence	70%	100%	60%	55%	45%

A_1: Shell of 0-day; B_1: Shell of 7-days, C_1:Shell of 14 –days; D_1: Shell of 21-days, A_2: Chalaza 0f 0-day; B_2: Chalaza of 7-days; C_2: Chalaza of 14-days; D_2: Chalaza of 21-days, A_3: Albumen of 0-day; B_3: Albumen of 7 –days, C_3: Albumen of 14-days; D_3: Albumen of 21-days. A_4: Egg yolk of 0-day; B_4: Egg yolk of 7-days; C_4: Egg yolk of 14 –days; D_4: Egg yolk of 21 –days

Table 6: Bacterial load of different parts of the eggs

Egg shell	
A_1	1.3×10^7
B_1	1.23×10^7
C_1	1.50×10^7
D_1	1.20×10^7
Chalaza	
A_2	4.67×10^6
B_2	5.33×10^6
C_2	4.67×10^6
D_2	3.67×10^6
Albumen	
A_3	3.67×10^6
B_3	4.00×10^6
C_3	3.67×10^6
D_3	3.33×10^6
Yolk	
A_4	3.67×10^6
B_4	4.00×10^6
C_4	4.00×10^6
D_4	4.00×10^6

in the albumin of the four 1-day old egg samples (Table 2). *Salmonella* was the only organism found in all the analyzed portions of the four one-day old eggs. The presence of the bacterial and fungal isolates in the albumen and yolk of 1-day-old eggs could be due to contamination in the oviduct of the hen, with the chicken droppings or contaminated poultry feeds. Although all the four parts of the eggs were contaminated, there was gradual decrease in number from 1.37×10^7-3.67×10^6 CFU mL^{-1} for the shell, 1.23×10^7-4.00×10^6; 1.5×10^7-4.0×10^6 and 1.2×10^7-4.00×10^6 CFU mL^{-1} for the chalazae, albumen and the yolks respectively (Table 5). Interestingly, from Table 5, more bacteria were found in the yolk than in the albumen, which is a correlation with the report of Banwart (1980).

Looking at Table 1 to 4, results revealed that by the 21st day of storage, six out of the nine bacterial isolates have completely colonized the yolk of the four eggs while *Bacillus, Enterobacter* and *E. coli* respectively made a 75% appearance in the yolks of 7, 14 and 21 day old eggs. It is then obvious that given some more days, the last three organisms mentioned above would have found their way into the yolk of the remaining egg samples as it was reported by Braun and Fehlhaber (1995) that *Salmonella enteridis* could migrate from the albumen into the yolk within 24 h depending on the storage temperature and level of contamination. This implicitly implies that consumption of raw 21day old eggs creates direct access of the isolated bacteria into the body-a situation that could lead to several types of bacterial infections. In this instance, various strain of *E. coli* have been documented to be Enteropathogenic, Enterotoxigenic, Enterohemorrhagic and Enteroinvasive while *Salmonella* spp have been implicated in typhoid fever, bacteremia with focal lesions and enterocolitic consequent upon ingestion of food containing these organisms (Jawetz *et al*., 1995). Thus the consumption of 7-21rays old eggs without proper cooking increases the probability of occurrence of the afore-mentioned health problems.

From Table 5, *Aspergillus* was found on the shells, in the chalazae, albumen and yolks of all the eggs of 0-21days old. This is of great health importance since the presence of *Aspergillus* in all the egg samples from all the poultry farms analyzed hitherto points to the use of contaminated poultry feeds or poultry feeds raw materials or general low hygienic margins in these farms. Occurrence of *Aspergillus* is a threat to health due to the production of aflatoxins that have been found to be carcinogenic, teratogenic and mutagenic in humans and birds. Aflatoxins have also been found in cow milk following the consumption of contaminated cow feeds. Aflatoxins were discovered in 1960 when 100,000 turkey poults died from eating Fungus-infected peanut meal. The toxins are known to cause frame-shift mutation . From Table 6, *Penicillium* has a 70% occurrence level in all the egg samples analyzed followed by Cladosporium (60%), *Rhizopus* (55%) and *M onilia* (45%). These mentioned

fungi are spoilage in function and there presence in the eggs the insidious initiation and sustenance of various types of spoilages mentioned in the review. This will lead to great food and economic losses to the unsuspecting farm keepers and the consumers.

The presence of the spores of these fungi on and in the eggs could lead to several respiratory diseases like coccidioidomycosis, blastomycosis and histoblastomycosis when the fungal spores are inhaled by the humans and the birds. This condition is not less expected in the poultry farms as the eggs are simply kept on wooden shelves and at temperature suitable for fungal growths.

The proliferation of microbes in the inner part of the eggs especially of the 0 and 7day old eggs was possibly due to high level of contamination coupled with the abundant nutritional materials and growth factors in the egg. However, the incomplete colonization of the yolks by *Bacillus, Enterobacter* and *E.coli* after 21 days (Table 4) was obviously due to the bacteriostatic components of the egg such as lysozymes and avidin. The same could apply to *Penicillium, Cladosporium, Rhizopus* and *Monilia* growths respectively. However, under the prevailing poor storage conditions, the organisms will definitely overpower the intrinsic resistance.

CONCLUSION

The fore going studies have revealed that freshly laid and stored domestic fowl eggs used in the study were contaminated by consortia of microorganisms. They migrated and invaded the inner parts of the eggs due primarily to heavy contamination and then prevailing poor storage conditions. Thus, it is strongly recommended that the government should set quality control standards in the storage conditions of the eggs especially with respect to the installation of air-conditioning facilities in the ware houses to ensure that the required storage temperature of –1 °C is maintained. This would minimize moisture loss due to evaporation and ensure normal relative humidity. Furthermore, cross-contamination of freshly laid sterile eggs by contaminated poultry feeds and wash water must be checked and stopped entirely in order to prevent microbial migration into the eggs and subsequent spoilages that will ensure. This will also check aflatoxin production by *Aspergillus* in the eggs from contaminated poultry feeds. Since eggs pick odour and flavour due to the porousity of the shells, freshly laid eggs should be quickly coated with light mineral oil to prevent possible proteolysis and other organoleptical changes as advanced methods of egg preservation are missing in the local setting. The use of Cryogenic gas to effect rapid cooling of eggs should also be introduced in the poultry farms. Necessary microbiological analytical techniques should be incorporated in the poultry farms as part of the good manufacturing practice to ensure the release of wholesome products to the public. The general public should be well informed of the health threats associated with the raw consumption of poultry eggs. Installation of necessary storage facilities would ensure that eggs are in constant supply round the year due to its high nutritional values to the neonates, infants, the sick, the aged and the entire public.

REFERENCES

Adams, M.R. and M.O. Moss, 1995. Food Microbiology. Royal Society of Chemistry, Thomas Graham House Science Park, Cambridge, pp: 201.

Banwart, G.J., 1980. Food Microbiology. Avi publishing company, West Port conn. pp: 119-125.

Barnett, H.L. and B.B. Hunter, 1972. Illustrated Genera of Imperfect fungi. Macmillan publishing company. New York, pp: 10-12.

Braun, P. and K. Fehlhaber, 1995. Migration of *Salmonella enteritidis* rom the albumen into the egg yolk. Int. J. Food Microbiol., 25: 95-99.

Jawetz, E., J.L. Melnick and E.A. Adelberg, 1995. Medical Microbiology. Appleton and Lange, Norwalk, pp: 656.

Jones, F.T. R.C., D.V. Axtell, S.E. Rives, F.R. Scheideler, R.L. Tarver Jr., Walker and M.J. Wineland, 1991. A survey of Salmonella contamination in Modern Broiler Production. J. Food Protection, 54: 502-507.

Kay, Y.M. and L.R. Sharon, Food Science and You. Glencoe; Macmillan/McGraw-Hill, U.S.A., pp: 91.

Ogbulie, J.N., J.C. Uwaezuoke and S.I. Ogiehor, 1998. Microbiology Practical. Springfield Publishers, Owerri, pp: 162.

Smittle, R.B., 1977. Microbiology of Mayonnaise and Salad dressing: A Review of J. Food Protection. 40: 415-422.