



## MICROORGANISMS ASSOCIATED WITH POULTRY DUNGS AND EGG SHELLS

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### ABSTRACT

*Poultry products were analyzed for their microbiological and antibiotic susceptibility pattern that can influence their quality. Egg shell and poultry dung samples were collected from poultry farms, in Akungba-Akoko, Ondo State and Lagos city, Lagos State, Nigeria. The samples were collected in triplicates during the study. They were subjected to microbiological investigation in the laboratory using standard methods. The bacterial load for the sample sources studied ranged from  $1 \times 10^3$  cfu/mL to  $160 \times 10^3$  cfu/mL in egg shell sources and  $290 \times 10^3$  cfu/mL to  $550 \times 10^3$  cfu/mL in poultry dung sources. The bacterial isolates from the samples include *Staphylococcus* spp., *Bacillus* spp., *Escherichia coli*, *Salmonella* spp., *Serratia* spp., *Micrococcus* spp., *Pseudomonas* spp. and *Enterobacter* spp. Similarly, the fungal isolates were *Rhizopus* spp., *Aspergillus* spp., *Candida* spp. and *Saccharomyces* spp. Microbiological analysis of the egg shell samples implies that consumption of raw and undercooked eggs was unsafe as a result of heavy contamination with harmful microorganisms including pathogens that could cause human infections. Antibiotic susceptibility test showed that *Serratia* spp., *Salmonella* spp. and *Escherichia* spp. were highly susceptible to most of the antibiotics used including Ampicillin (AMP)  $10 \mu\text{g}$ , Augmentin (AUG)  $30 \mu\text{g}$ , Gentamycin (GEN)  $10 \mu\text{g}$ , Ofloxacin (OFL)  $5 \mu\text{g}$ , Streptomycin (STR)  $10 \mu\text{g}$ , Chloramphenicol (CHL)  $10 \mu\text{g}$ , Cotrimoxazole (COT)  $25 \mu\text{g}$ , Sparfloxacin (SPFX)  $5 \mu\text{g}$ , Perfloxacin (PEF)  $5 \mu\text{g}$ , Erythromycin (ERY)  $5 \mu\text{g}$ , and Ciprofloxacin (CPX)  $5 \mu\text{g}$ , while *Micrococcus* species recorded the highest level of antibiotics resistance. Results obtained from this study can be used as a benchmark for environmental study and food safety.*

**Key words:** Ecology, Microorganisms, Nigeria, Poultry, Quality.

### INTRODUCTION

Poultry products, particularly eggs are valuable nutritional food supplements for human beings (Moeller et al., 2000; Ruxton and Derbyshire, 2009). Meat and poultry consumption has been on the rise in the United States with per capita of poultry products increasing 6.5 fold some decades ago. Hence, people are at risk for exposure to *Salmonella* and related

microbes through contaminated foods (Foley-Steven et al., 2011; Gómez-Aldapa et al., 2012). This is also experienced in some parts of the world. Commercial poultry is one of the fastest growing sectors of the animal agricultural industry (Foley-Steven et al., 2011). There are diverse forms of microorganisms in nature and their existence depends on some environmental

conditions including temperature, pH and nutrient substrates that influence the microbial group found in an ecological niche. This includes poultry farm products like egg and the environment in which they are produced which is grossly populated by microbes in their waste droppings. The nature of microorganisms which colonize this ecological zone both affect quality of egg produced and the final consumers, which is human. Navidghasemizadet al.,(2013) in their study stressed the effect of contaminants on left over egg contents that adversely influence their physicochemical properties.

Eggs are fertilized products of many arthropods including birds and fowls. Egg are laid by females of many different species, including birds, reptiles , amphibians and fish and have probably been eaten by mankind for millennia. Eggs laid by snake generally have leathery shells which often adhere to one another depending on the species, turtles and tortoises lay hard or soft eggs. In an average laying hen, the process of shell formation takes around 20 hours. Pigmentation is added to the shell by papillae lining the oviduct. Colors and patterns depend on species since eggs are usually laid blunt and first (Hunton, 2005). An egg shell is the outer covering of a hard shell eggs and of some eggs with soft outer coats. The chicken egg shell is 95.97% calcium carbonate crystals which are stabilized by protein matrix (Nysetal., 2004).

There is paucity of information on bacterial contamination on the shell of eggs meant for human consumption. The shell may be infected when passing through the vent, but many researchers suggest that the main contamination occurs within a short period after laying due to contact with dirty surfaces (Ghaniei, 2012; Madadi and Azari, 2012; Navidghasemizadet al., 2013). Careful preservation of edible eggs is

extremely important as an improperly handled egg can cause severe bacterial food poisoning. The simplest method to preserve egg is to treat it with salt. Salt draws water out of bacteria and molds which prevents their growth. Temperature, humidity, air movement storage time, handling and tainting can all have adverse effects on interior quality. These factors, if not controlled can cause loss of moisture in eggs (Navidghasemizadet al., 2013).

The contents of freshly laid eggs are usually sterile and contain few organisms capable of causing spoilage even when the shells are slightly dirty or strained. The main cause of spoilage by bacteria is the watching of dirty eggs before marketing. When the egg is washed, organisms from water usually bacteria can penetrate the shell. Once inside they multiply and eventually spoil the egg, causing green, black and red rots. When eggs are kept dry no such way is provided for bacteria to penetrate the shell. Mould spores normally present on egg shells may, if sufficient time with adverse conditions elapses germinate and grow; penetrating the shell and causing spoilage (De Reuet al., 2008). On the other hand, there are some perturbations in the ecological environment that affect negatively the survival and quality of eggs laid by higher organisms (Relyea, 2005).

Microorganisms like mould and yeasts have been found involved in egg spoilage. While bacteria of the family enterobacteriaceae mainly associated with the intestines of animal and humans has also been found to cause egg spoilage. However, contamination from air water and soil could increase the presence of microorganism on egg and other poultry products. The group of bacteria involved in egg spoilage includes *Pseudomonas* spp., *Salmonella* spp., *Campylobacter* spp., *Escherichia coli* and many others. Fungi that can cause spoilage include species of *Aspergillus* spp. and

**Saccharomyces**spp. Although, any microorganism can be seen due to contamination from environment. All these organisms are pathogenic to human health (Notermans *et al.*, 1990; Humphrey, 1994; Gantois *et al.*, 2009; Freston, 2011).

In assessing the quality of eggs, candling is the only method of testing internally and externally without breaking them. It consists of inspecting an egg with a beam of light that makes the interior quality visible. A very simple form of candling is placing a candle in a dark room and positioning an egg in front of the flame and looking at the interior quality. However, to protect eggs from spoilage, it should be stored at low temperature. The ideal temperature is 13°C or lower usually between 10°C and 13°C. Egg should not be kept at room temperature where it is not too cold or too dry to prevent deterioration (Notermans *et al.*, 1990; De Reu *et al.*, 2008). The objective of this study was to determine the nature of microorganisms populating egg shell and poultry dung sources. The knowledge of their antibiotic susceptibility pattern assists in the control measures of cases of infection emanating from consumption of poultry products.

## **MATERIALS AND METHOD**

### **MATERIALS USED**

Media used for the study were adequately sterilized. The media generally used for the study includes, Nutrients Agar (NA), Salmonella-Shigella agar, MacConkey agar, Malt extract agar, De Man Rogosa Sharpe (MRS) Agar. Reagents such as Gram staining reagents, hydrogen peroxide, phenol red, Lactophenol, cotton blue stain, peptone and motility broth were also used during the study. Fermentative sugars such as sucrose, glucose, fructose, maltose, raffinose and lactose were also used for biochemical test.

### **SAMPLE COLLECTION**

The samples: egg shell (chicken egg), and poultry dung were collected from Araromi quarters, Akungba-Akoko, Ondo State and Lagos, Nigeria. The sample sources, the first portion mainly for home consumption was Farm (A). This constitutes fresh, which is laid within a day and old eggs, which are more than five days and poultry dung collected at off Supare junction, Araromi quarters in Akungba-Akoko, Ondo State and Oshodi area in Lagos, Nigeria. The other source, mainly for commercial purpose was Farm (B). This constitutes fresh and old eggs and poultry dung at Iyana Ipaja area of Lagos state Nigeria. Aseptic measures were taken during collection by the use of sterile gloves and covered containers. The samples were properly labeled with all necessary information such as date of collection and sample name for Laboratory analysis.

### **SUB - CULTURING AND IDENTIFICATION OF ISOLATES**

The bacterial colonies were sub-cultured by picking them into freshly prepared NA plates. This was done with a flamed and cooled wire loop used to pick isolates from stock culture. Pure colonies were obtained by streaking representative discrete colonies of different morphological types which appear on the plates. The plates were incubated at 37°C for 24 hours. Discrete bacteria colonies were further transferred into NA slants and then kept in the refrigerator. The fungal colonies were sub-cultured by picking them with needle and transferred into Malt Extract Agar (MEA) medium and then incubated at 25°C for 5 days. Pure culture of fungi were transferred to malt extract agar slants and kept at room temperature.

The colonies on the nutrients agar and malt extract agar were observed directly. For bacteria, the shape, elevation, colour, surface and edges of the colonies were

observed. The shape was either circular, irregular, rhizoid, filamentous or punctiform. The elevation was either flat, raised or convex. The edge was either entire, lobate, undiluted or rhizoid. The surface was either smooth or rough, dull or glistening. The colour of the colonies on the agar was also recorded as either cream, or milk colour or any other colour. For fungi, the changes in colour of the colonies of sub-cultured plates were recorded for 3 days.

Serial dilutions of stock culture were prepared from samples of egg shell and poultry dung. Appropriate diluents (i.e diluents  $10^{-2}$  and  $10^{-5}$ ) were used for inoculation of plates for the estimation of bacterial counts by a pour plate method technique. Inoculated plates of nutrient agar were incubated at  $37^{\circ}\text{C}$  for 18-24 hours. Distinct colonies on plates were randomly selected for sub-culture on sterile nutrient agar and incubated for another 18-24 hours. Standard microbiological techniques were used to identify the bacterial isolates. These include their cultural characteristics in solid media, Gram staining reaction, catalase test, indole test, motility test, and sugar fermentation.

### **FUNGAL ISOLATES**

Lacto-phenol in cotton blue stain was used to identify isolated fungi. This reagent stains the cytoplasm of the fungi. Using sterilized inoculating needle, small mycelium of fungi was transferred from a young culture into the drop of the stain on the slide. The hyphae and the preparation was covered with cover slip carefully. The slide was then viewed under the microscope using x40 objective lens to know the type of hyphae, spores, colour of the spores, among others.

### **PREPARATION OF INOCULUM**

Cultures of isolated organisms on nutrient agar slant were used to prepare the inocula using a sterile wire

loop. Approximately one isolated colony was transferred into 10ml of sterile distilled water contained in the test tube and then mixed properly. The liquid served as source of inoculum containing approximate  $10^6$  CFU/ml of bacterial suspension.

### **INOCULATION OF PLATING MEDIUM**

A sterile cotton wool swab stick was dipped into the properly mixed inoculum and excess fluid was removed by rotating the swab against the inside wall of the test tube. The swab was used to inoculate the surface of the prepared nutrient agar plates which was constituted according to the manufacturers specifications. The spreading was done in three different planes by rotating the pane at approximately 60 degree each time. This was to ensure that every part of the plate was inoculated. The plates were then kept in the refrigerator for at least 30 minutes.

### **PLACEMENT OF DISC**

The antibiotic susceptibility test was performed using the agar-disc diffusion method as described by Kaiser (2012). A pair of forceps was flamed until red hot and cooled. Using this, the antibiotics disc were picked and placed on the inoculated plates. The discs were pressed down on the agar so as to be in complete contact with agar. The plates were then incubated at  $37^{\circ}\text{C}$  for 18 hours. After this the diameter of zone of inhibition around each antibiotic disc were measured to the nearest millimeter and interpreted according to the current CLSI standard (2008 and 2012).

### **RESULTS**

The results obtained for this study show different types of microorganisms isolated from egg and poultry dung samples. This includes bacteria and fungi. The Bacterial isolates include *Micrococcus* spp., *Salmonella* spp., *Citrobacter* spp.,

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**Pseudomonas** spp., **Bacillus** spp., **Staphylococcus** spp., **Escherichia coli** and **Serratias** spp. The fungi include **Aspergillus** spp., **Rhizopus** spp., **Candida** spp. and **Saccharomyces** spp.

Tables 1 shows the microbial population of the Egg shell and poultry dung from sample sources. The bacterial load for the sample sources studied ranged from low of  $1 \times 10^3$  cfu/mL to  $160 \times 10^3$  cfu/mL in egg shell sources and  $290 \times 10^3$  cfu/mL to a high of  $550 \times 10^3$  cfu/mL from poultry dung sources. Statistically, the Standard Deviation of 150.459297 in poultry dung sources is

significantly higher than that of eggs showing 44.7385503 which have nearer numerical value to sample sources range. The sample sources were generally populated with large amounts of bacterial and fungi species.

In Tables 2 and 3, the Antibiotics susceptibility pattern of bacteria isolates during the study was determined. The test showed that **Serratias** spp., **Salmonella** spp. and **Escherichia** spp. were highly susceptible to most of the antibiotics used while **Micrococcus** species recorded the highest level of resistance to the antibiotics.

**TABLE 1: Total bacterial counts of poultry dungs and egg shells**

Poultry dung		Egg shell	
SAMPLES	Bacterial counts (X10 <sup>3</sup> ) CFU/ml	SAMPLES	Bacterial counts (X10 <sup>3</sup> ) CFU/ml
SFA1	510	SFA7	29
SFA2	290	SFAB1	25
SFA4	210	SFA3	8
SFA6	420	SFA5	11
SFA8	470	SFB1	84
SFAB2	525	SFB2	160
SFAB3	330	SFB3	34
SFB6	520	SFB4	47
SFB7	550	SFB5	16
PFBC1	390	SFB8	1
PFBC2	545	SFBC1	4
PFBC3	76	SFBC2	44
Standard Deviation	150.5	Standard Deviation	44.7

Legend: SFA – FARMA, in Akungba-Akoko, Ondo State,

SFAB – FARMA, in Lagos, Nigeria

PFBC- FARM B, poultry dung at Iyana Ipaja area of Lagos, Nigeria.

SFBC – FARM B, Farm at Iyana Ipaja area of Lagos, Nigeria.

Table 2: Antibiotic sensitivity profile of Gram negative bacteria

Isolate	AMP	AUG	CHL	CPX	GEN	PEF	OFL	SPFX	COT	STR
<b>Serratiaspp</b>	14 (R)	16 (S)	15 (S)	12 (R)	15 (S)	12 (R)	12 (R)	15 (S)	12 (R)	0 (R)
<b>Serratiaspp</b>	14 (R)	12 (R)	11 (R)	19 (S)	18 (S)	12 (R)	17 (S)	14 (R)	14 (R)	0 (R)
<b>Bacillus spp.</b>	13 (R)	19 (S)	19 (S)	19 (S)	13 (R)	18 (S)	17 (S)	15 (S)	17 (S)	0 (R)
<b>Micrococcuspp.</b>	0 (R)	0 (R)	12 (R)	12 (R)	0 (R)	0 (R)	0 (R)	15 (S)	14 (R)	0 (R)
<b>Enterobacterspp.</b>	13 (R)	14 (R)	16 (S)	14 (R)	18 (S)	19 (S)	20 (S)	17 (S)	14 (R)	17 (S)
<b>Salmonellaspp.</b>	16 (S)	15 (S)	16 (S)	11 (R)	14 (R)	17 (S)	17 (S)	14 (R)	13 (R)	19 (S)
<b>Pseudomonasspp.</b>	0 (R)	0 (R)	11 (R)	14 (R)	0 (R)	16 (S)	14 (R)	15 (S)	0 (R)	0 (R)
<b>Pseudomonasspp.</b>	14 (R)	0 (R)	0 (R)	17 (S)	0 (R)	20 (S)	0 (R)	20 (S)	16 (S)	0 (R)
<b>Escherichia coli</b>	0 (R)	0 (R)	12 (R)	18 (S)	0 (R)	20 (S)	0 (R)	12 (R)	14 (R)	0 (R)
<b>Escherichia coli</b>	11 (R)	12 (R)	20 (S)	0 (R)	12 (R)	16 (S)	14 (R)	16 (S)	0 (R)	11 (R)
<b>Escherichia coli</b>	13 (R)	0 (R)	14 (R)	18 (S)	0 (R)	0 (R)	0 (R)	19 (S)	0 (R)	0 (R)
<b>Citrobacterspp.</b>	13 (R)	18 (S)	11 (R)	16 (S)	14 (R)	12 (R)	17 (S)	15 (S)	0 (R)	14 (R)
<b>Escherichia coli</b>	10 (R)	15 (S)	17 (S)	15 (S)	20 (S)	20 (S)	19 (S)	17 (S)	15 (S)	20 (S)
<b>Escherichia coli</b>	10 (R)	0 (R)	10 (R)	19 (S)	0 (R)	10 (R)	20 (S)	12 (R)	15 (S)	0 (R)
<b>Citrobacterspp.</b>	0 (R)	0 (R)	15 (S)	20 (S)	10 (R)	0 (R)	0 (R)	18 (S)	0 (R)	0 (R)
<b>Micrococcuspp.</b>	10 (R)	15 (S)	15 (S)	17 (S)	16 (S)	16 (S)	15 (S)	20 (S)	10 (R)	16 (S)

LEGEND: S – Sensitive, R – Resistant

Table 3: Antibiotic sensitivity profile of Gram positive bacteria

Isolate	AMP	CPX	ERY	GEN	PEF	STR	COT
<b>Serratiaspp.</b>	13 (R)	15 (S)	14 (R)	0 (R)	20 (S)	13 (R)	10 (R)
<b>Micrococcus spp.</b>	0 (R)	0 (R)	16 (S)	14 (R)	0 (R)	0 (R)	0 (R)
<b>Micrococcus spp.</b>	0 (R)	16 (S)	16 (S)	0 (R)	16 (S)	0 (R)	0 (R)
<b>Bacillus spp.</b>	0 (R)	16 (S)	14 (R)	0 (R)	19 (S)	13 (R)	0 (R)
<b>Aerococcuspp.</b>	10 (R)	12 (R)	14 (R)	17 (S)	17 (S)	14 (R)	16 (S)
<b>Bacillus spp.</b>	14 (R)	16 (S)	14 (R)	0 (R)	19 (S)	14 (R)	0 (R)
<b>Citrobacter spp.</b>	17 (S)	14 (R)	16 (S)	15 (S)	16 (S)	14 (R)	18 (S)
<b>Micrococcus spp.</b>	13 (R)	15 (S)	16 (S)	14 (R)	12 (R)	14 (R)	12 (R)
<b>Staphylococcus spp.</b>	15 (S)	15 (R)	15 (S)	14 (R)	15 (S)	14 (R)	16 (S)

LEGEND: S – Sensitive, R – Resistant, FA – Farm A source, FB and PFBC – Farm B source

**Antibiotics used:** Ampicillin (AMP) 10µg, Augmentin (AUG) 30µg, Gentamycin (GEN) 10 µg, Ofloxacin (OFL) 5 µg, Streptomycin (STR) 10 µg, Chloramphenicol (CHL) 10µg, Cotrimoxazole (COT) 25µg, Erythromycin (ERY) 5µg, and Ciprofloxacin (CPX) 5µg, Sparfloxacin (SPFX) 5µg, Perfloxacin (PEF) 5µg.

## DISCUSSION

Different species of bacteria and fungi from the egg shell and poultry dung sources were isolated and identified during this study. This variation in microbial species is in correlation with the report of Theron *et al.*, (2003) and Yohannes *et al.*, (2014) which described the presence of different species of microorganism in poultry products, particularly, eggs as contaminants. The bacterial load for the sample sources studied ranged from low of  $1 \times 10^3$  cfu/mL to  $160 \times 10^3$  cfu/mL in egg shell sources and  $290 \times 10^3$  cfu/mL to a high of  $550 \times 10^3$  cfu/mL from poultry dung sources. The egg shell and poultry dung sample were populated with various forms of bacterial species including *Bacillus* spp., *Serratia* spp., *Escherichia coli*, *Staphylococcus* spp., *Citrobacter* spp. and *Enterobacter* spp. The Fungal species encountered were *Aspergillus* spp., *Rhizopus* spp. and *Saccharomyces* spp. Many investigators (Gantoiset *et al.*, 2009; Freston, 2011; Madadi and Azari, 2012; Navidghasemizad *et al.*, 2013) have isolated *Salmonella* spp. and *Pseudomonas* spp. as the dominant bacteria from egg shell and is the most important bacteria closely associated with poultry and poultry products. Humphrey (1994) similarly showed related forms of bacteria on egg shell in his previous study. As with any food

of animal origin, eggs may be contaminated with organisms which are potentially pathogen for humans.

Egg shells that are contaminated or coated with poultry dungs may favour microbial growth as demonstrated in this study. Some environmental conditions can favour the proliferation of these microbes on egg shell surfaces and even penetrate into the inner content at certain point. This can limit productivity. Though poultry dung is often used for some agricultural farm manure including aquaculture feed for fishes, care should be taken in doing this. Subasinghe (2009) in related study showed that the increasing animal diseases may bring some constraint to the development and management of the animal sector in agriculture. Therefore, microbiological analysis of egg shell samples show that the consumption of raw and undercooked egg is unsafe because not all eggs are free from microorganisms which can cause infection in the body. In order to limit these problems, it is advisable to clean egg shell surfaces to some reasonable extent before packaging for sale. Since some of the organisms encountered during the study include coliforms and some pathogenic microbes, it is important to embark on adequate preservation and inculcate cautionary measures in the handling of poultry products.

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