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Identification of *Salmonella* species in chicken eggs sold in Port Harcourt

Oridikitorusinyaa O.*, Amaechi G. and Emmanuel O. O.

Department of Microbiology, Rivers State University, P.M.B. 5080, Port Harcourt, Nigeria.

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ABSTRACT

Chicken eggs are a staple food consumed by people worldwide, but they can serve as reservoirs for foodborne pathogens, including various *Salmonella* species, posing significant risks to public health. This study investigated the prevalence and incidence of *Salmonella* in chicken eggs sourced from 7 poultry farmhouses and 7 supermarket retailers in Ozuoba axis of Obio/Akpor Local Government Area, Port Harcourt, Rivers State, Nigeria. Total Heterotrophic Bacteria (THB) counts for egg samples showed higher counts on the eggshell surface (ESS) compared to the inner content (INC) and also showed higher *Salmonella* counts on the ESS compared to INC. Prevalence/percentage occurrence of *Salmonella enteritidis*, *S. enterica*, *S. typhimurium*, *S. gallinarum* and *S. parataphi* were found to be 28%, 24%, 20%, 16% and 12%, respectively. Additionally, other bacteria species were identified from nutrient agar plates predominantly found on eggshell surfaces such as *Escherichia coli*, *Bacillus* sp., *Staphylococcus* sp. and *Streptococcus* sp. Furthermore, the nutritional composition of egg components and their pH levels that influence bacterial growth was examined. The results suggest that while egg white contains antimicrobial proteins inhibiting bacterial growth, egg yolk's rich nutrient content may support microbial proliferation if contaminated. These findings emphasize the importance of stringent food safety regulations, including proper hygiene practices during egg production, handling, and distribution, to mitigate the risk of *Salmonella* contamination. Public health interventions such as consumer education on safe food handling practices and regular monitoring of foodborne pathogens in chicken eggs are essential for preventing foodborne illnesses and protecting public health in the Port Harcourt metropolis and beyond.

Keywords: *Salmonella*, chicken eggs, foodborne disease, food safety, public health.

*Corresponding author. Email: oridikitorusinyaa.odike@ust.edu.ng. Tel: 08133776346.

INTRODUCTION

Foodborne diseases are of great public health concern in the modern world. In developing countries, the greater populace is largely affected by foodborne infections (Adeyanyu et al., 2014). Foodborne disease, apart from affecting the health and well-being of individuals, equally affects the social and economic productivity of the countries. The main factors contributing to the increased burden of foodborne diseases, especially in Africa, are the people's poor hygiene practices (Clasen et al., 2007; Crump et al., 2004). Poor personal hygiene among food handlers, coupled with the inadequate handling of poultry products in farms could be possible sources of microbial pathogens that cause foodborne infections (Alfred et al., 2019; Genigeorgis et al., 2006; Gründling et al., 2004). Foodborne diseases transmitted through the consumption of microbial-contaminated food have been a significant worldwide public health concern. *Salmonella* remains one of the notable foodborne bacterial pathogens. It is associated with poultry and poultry products, including eggs (Tafida et al., 2013; Desta and Mekonne 2015).

Salmonellosis is one of the most important foodborne bacterial zoonotic diseases worldwide. It also causes great economic loss in poultry, especially young chickens in terms of heavy morbidity and mortality. Poultry eggs are frequently involved in the transmission of *Salmonella*. Among the serovars of *Salmonella*, Typhimurium, enterica and enteritidis have been of great concern from

egg-borne human salmonellosis (Jone, 2018; Ben-Salem et al., 2017). *Salmonellosis* is usually associated with the ingestion of salmonella-contaminated food products. Animal-derived foods, particularly chicken and poultry products are the most common transmission source of *Salmonella* to humans (Arslam et al., 2010; ICMSF, 2002). When a significant quantity of *Salmonella* has been ingested, it will colonize the infected human's intestinal tract, triggering a range of clinical manifestations. *Salmonella* infections are often accompanied by various symptoms, including gastroenteritis, bacteremia, and typhoid fever (Dewey-Mattia et al., 2018; Iwamoto, 2015). Multiple Salmonellosis outbreaks related to chicken and poultry product consumption have been reported in recent years, implying that these products constitute the primary vehicle for *Salmonella* transmission (Bata et al., 2016). The intestinal tract is the primary reservoir of *Salmonella* in poultry birds, leading to contamination of chicken eggs in the cloacal region through horizontal route. Trans-ovarian transmission from infected chickens is another important route of contamination of chicken eggs, leading to egg-borne salmonellosis. *Salmonella* is a common pathogen found in poultry, and it can be transmitted to the eggs during the laying process. If eggs are not properly handled, stored, or cooked, the bacteria can survive and cause salmonellosis in humans when the contaminated eggs are consumed. Symptoms of salmonellosis include diarrhoea, abdominal cramps, fever, nausea, and vomiting. In severe cases, the infection can spread beyond the gastrointestinal tract and become life-threatening (Olovo et al., 2019).

Poultry farming is one of the most widespread food industries worldwide. Chicken is the most commonly farmed species, with over 90 million tons of meat and eggs produced yearly. Chicken eggs are one of the best sources of high-quality protein, along with important vitamins and minerals (Akeem et al., 2019). In both developed and developing countries, increased egg production and consumption could significantly improve the nutritional needs of adults and children. Eggs are also an economical source of nutrients for a healthy diet and life, which is particularly crucial for the mental development of growing children (Amini et al., 2010).

In Nigeria, typhoid fever is among the major widespread diseases affecting both young children and young adults as a result of many interrelated factors, such as inadequate facilities for processing human wastes and indiscriminate use of antibiotics (Hawker et al., 2019; Hu and Kopecko, 2003). Morbidity associated with illness due to *Salmonella* continues to increase, occasionally resulting in death (Olovo et al., 2019; Campbell, 2008). A more accurate figure of salmonellosis is difficult to determine because normally only large outbreaks are investigated whereas sporadic cases are under-reported (Hansen-Wester et al., 2002). On the other hand, non-typhoidal cases account for 1.3 billion cases with 3 million deaths (Raufu et al., 2014). The infectious dose of Salmonella depends upon the serovar, bacteria strain, growth condition and host susceptibility (Harakeh et al., 2005). Furthermore, host factors controlling susceptibility to infection include the condition of the intestinal tract, age and underlying illnesses or immune efficiencies (Raufu et al., 2014).

Due to the high frequency of consumption of poultry eggs and poultry products in Nigeria, Port Harcourt precisely, there is a possible risk of infection and change in the susceptibilities of the different *Salmonella* species that could be isolated from these poultry eggs and negatively impact the health of consumers. Hence, it is necessary to isolate, evaluate, characterize and identify *Salmonella* present in chicken eggs to have scientific proof of the risks associated with the consumption of contaminated chicken eggs, so that the general public can be advised on health matters related to the consumption of contaminated chicken eggs.

MATERIALS AND METHODS

Description of the study area

The study was undertaken in Obia/Akpor Local Government Area of Rivers State, with coordinates of Latitude 40 52l 50ll N, Longitude 60 58l 39ll E. The study area was chosen because poultry farming and fish farming businesses are carried out in this Local Government Area (Figure 1).

Sample collection

Fourteen (14) edible fresh chicken eggs were randomly collected from an egg retailer and farmhouse in the Ozuoba axis of Obio/Akpor Local Government Area, Rivers State, Nigeria, 7 each from the poultry farmhouse and supermarket retailer. The eggs were collected into sterile bags using sterile nylon gloves and transferred in refrigerated containers to the microbiology laboratory, at Rivers State University for bacteriological analysis according to the method described by Tafida et al. (2013). Samples not analyzed on the day of collection were stored in the refrigerator (4^0C) until analyzed (Raufu et al., 2014). During the collection of egg samples, it was ensured that cross-contamination was minimized by ensuring that all tools and containers used for collecting samples were properly sanitized before use.

Media and diluent preparation

All the media and diluent - Nutrient Agar (NA),

Figure 1. Map of Obio/Akpor Local Government Area.

Salmonella-Shigella Agar (SSA), MacConkey Agar (MCA) and Normal Saline - were prepared according to the manufacturer's specifications and instructions.

Microbiological analysis of chicken egg samples

Preparation of egg content for culturing

The egg surface was sterilized by immersion in 70% alcohol for 2 minutes, air-dried for 8 minutes and then cracked with a sterile laboratory knife. Egg contents were pipetted using a sterile pipette, poured into a sterile beaker and homogenized uniformly, according to Ogu and Akinnibasu (2019).

Enrichment and isolation of Salmonella

Pre-enrichments were performed as a standard method for isolation of *salmonella* from egg samples, 1ml was added to 9ml (1:10 dilution) in buffered peptone water (BPW) and was prepared from homogenized egg content, then using a sterile pipette, dropping 0.1ml of pre-enrichment on to salmonella-shigella agar (SSA) (Oxoid) and spread evenly on the sterile dried SSA and incubated in an inverted position for 24 hours at 37^0C (Amini et al., 2010).

Enumeration of Total Heterotrophic Bacteria (THB) counts of the egg samples

The spread plate technique described by Olovo et al. (2019) was employed to determine the total bacterial load of the fresh eggs sample. A sterile 1ml pipette was selected to aseptically drop 0.1ml of the inoculums from 10^{-5} and 10^{-6} dilutions onto properly dried nutrient agar plates in duplicates and evenly spread all over the surface of the agar using a glass spreader. All inoculated plates were immediately incubated at 35 – 37^0C for 24 hours in an inverted position (upside down) to prevent drops of condensations from collecting on the inoculated surface. The bacterial colonies that developed after the incubation period were enumerated and recorded as Colony Forming Units per gram (CFU/g) of the egg samples.

Sub-culture of isolates

The streak plate technique described by Cheesbrough (2010) was employed to obtain pure isolates for identification and characterization. Each of the distinct colonies formed was streaked on already prepared sterile dried NA, MCA and SSA plates using a sterile inoculating loop. The streaked NA, MCA and SSA plates were incubated at 37^0C for 24 hours. After the incubation period, pure cultures were obtained.

Isolation and identification of Salmonella species

Isolates of *Salmonella* sp. were recovered from fresh egg samples according to the method of Njie and Mochaiwa (2014) with slight modifications. The smooth and opaque or colourless, pink to record colonies with black centers formed after 24 hours at 37^0C incubation from SSA plates were suspected to be *salmonella* species. It was thereafter identified from the colony characteristics such as size, shape, elevation and edge, surface texture, opacity and colour development on various selective

media: microscopic observation after gram staining; and the *salmonella* species were subjected to various biochemical tests such as indole, catalase oxidase, sugar fermentation that, methyl-red, Voges Proskauer, motality, hydrogen sulphide production, etc. The identification of bacterial isolates followed Bergy's manual of determinative bacteriology described by Jones (2018).

Purification, maintenance and preservation of isolates

Ten per cent (10%) glycerol solution was prepared, dispensed in McCartney bottles and autoclaved at 121^0C for 15 minutes, and allowed to cool; discrete colonies were purified by repeated sub-culture onto nutrient agar. Pure cultures were inoculated into nutrient agar slants, then stored and kept in the refrigerator at 4^0C for further testing, as described by Kiin-Kabari et al. (2011).

Gram's staining/microscopy

A thin smear of bacterial isolates was made by emulsifying a small portion of the investigated bacteria picked from the grown colony of 24 hours pure culture into a drop of sterile distilled water on a grease-free glass slide using a wire-loop by passing it over the Bunsen burner flame. The smear was allowed to air-dry and heat-fixed by passing it slightly over a flame or Bunsen burner. The slide was carefully placed on a staining rack flooded with crystal violet (primary stain) for 60 seconds and gently rinsed with slow-running tap water at an angle of 45^0. Gram's iodine solution (mordant) was applied for 60 seconds. The smear was gently rinsed with slow running tap water. The smear was decolonized with ethanol for 30 seconds and rinsed with enough slow-running water. The smear was counter-stained with safrani for 30 seconds and then rinsed with slow-running water at an angle of 45^0. The smear was allowed to drain water and blot dry by placing it back on the staining rack. The stained slide was examined microscopically using an oil immersion objection lens (x100). Gram-positive bacteria appear purple/blue, while gram-negative bacteria appear pink/red under the microscope.

Biochemical test

Catalase test

A drop of 30% hydrogen peroxide was placed onto the center of a slide, and a sterile wire loop was used to pick a small portion of the bacteria to be identified into the hydrogen peroxide. Immediately, gas bubble formation or foaming indicates a positive result.

Oxidase test

A piece of filter paper was placed into a clean petri dish, and 2 or 3 drops of freshly prepared oxidase reagent were added. A small portion of the investigated bacteria was smeared on the filter paper using a glass rod. A blue-purple colour indicates a positive result.

Citrate utilization test

This test detects the ability of an organism to use citrate as a sole source of carbon and energy. 2.4 g of citrate agar was dissolved in 100 ml of distilled water; 10 ml of citrate medium was dispensed into each tube and covered, then sterilized and allowed to cool in a slanted position. The tubes were inoculated with the test organism using a sterile wire loop across the surface. A change from green to blue indicates utilization of the citrate and a positive result.

Urease test

A slant of urea agar was aseptically inoculated with the organism to be investigated using a sterile wire loop incubated at 37^0C for 24 hours and observed for colour change. A red-pink colour is indicative of a positive test result.

Indole test

The investigated organism to be identified was inoculated into trytophane broth for 48 hours at 37^0C, and 5 drops of Kovac's reagent were added. A deep red colour indicates a positive result, while yellow colourations indicate a negative result.

Methyl red test

Five (5) millimetres of glucose phosphate broth (1g glucose, 0.5% KH, Po4, 0.5% peptone and 100 ml distilled water) was dispensed into clean test tubes and sterilized. The tubes were inoculated with the test organisms and incubated at 37^0C for 48 hours. At the end of incubation, a few drops of methyl red solution were added to each test and observed for colour changes. A red colour indicates a positive reaction.

Voges Proskauer test

Five (5) millimetres of glucose phosphate was dispensed into clean test tubes and sterilized; the tubes were then

inoculated with the test organisms and incubated at 37^0C for 48 hours. After incubation, 6% α-naphthol and 6% sodium hydroxide were added to about 1ml of both cultures.

Sugar fermentation test

Sugar indicator was prepared using peptone water medium containing 1% fermentable sugar and 0.1% phenol red; 10 millilitres of sugar was dispensed into each of the test tubes; the dirham tube which would trap the gas if produced was carefully inverted; The test tubes inoculated with a loopful of 24 hour old culture of the test organisms were autoclaved and incubate for 2-7 days at 36 ± 1^0C and observed daily for acid and gas production. Yellow colouration indicates acid production while gas production was indicated by displacement of the medium in the dirham tube.

Sample preparation

The eggs were carefully separated into three main components: shell, albumen (white), and yolk. This separation was done manually, ensuring no cross-contamination between components. The albumen and yolk were homogenized separately using a laboratory blender to ensure uniformity in the samples. The eggshells were thoroughly cleaned of adhering albumen, rinsed with distilled water, and dried in an oven at 105°C for 24 hours to remove all moisture. Once dried, the shells were ground into a fine powder using a laboratory mill to prepare them for further analysis.

Moisture content

Moisture content was determined using the standard oven-drying method. Precisely weighed samples (approximately 5g) of each egg component were placed in pre-weighed aluminium dishes. These were then dried in an oven at 105°C for 24 hours. After drying, the samples were cooled in a desiccator and reweighed. The difference in weight before and after drying was used to calculate the moisture content as a percentage of the original sample weight.

Protein analysis

The Kjeldahl method, the standard for protein determination in foods, was used. This method involves three steps:

Digestion: Samples were digested with concentrated sulfuric acid in the presence of a catalyst, converting organic nitrogen to ammonium sulfate.
Distillation: The digested sample was made alkaline with sodium hydroxide, and the liberated ammonia was distilled into a boric acid solution.
Titration: The ammonia in the distillate was titrated with standardized hydrochloric acid.
The nitrogen content was then multiplied by the factor 6.25 (as protein is assumed to contain 16% nitrogen) to estimate the total protein content.

Lipid analysis

The Folch method was employed for total lipid extraction. Samples were homogenized with a 2:1 (v/v) mixture of chloroform and methanol. The homogenate was filtered, and the filtrate was washed with 0.9% sodium chloride solution to separate the phases. The chloroform layer containing the lipids was collected and evaporated under nitrogen. The residue was weighed to determine the total lipid content.

Ash content

Samples were placed in pre-weighed crucibles and incinerated in a muffle furnace at 550°C for 12 hours. This process burned off all organic matter, leaving only the inorganic mineral content (ash). The crucibles were cooled in a desiccator and reweighed. The weight of the remaining ash was used to calculate the total mineral content of the samples.

Carbohydrate content

Carbohydrate content was calculated by difference. The sum of the percentages of moisture, protein, lipid, and ash was subtracted from 100%. This method assumes that these four components and carbohydrates constitute the entire mass of the sample.

Mineral analysis

The ash samples were digested with concentrated nitric acid to solubilize the minerals. The resulting solution was then analyzed using Atomic Absorption Spectrophotometry (AAS). This technique measures the concentrations of individual minerals based on their characteristic absorption of light at specific wavelengths. The analysis focused on calcium and magnesium, which are particularly important in eggshell composition, as well as other minerals like phosphorus, potassium, and sodium.

pH measurement

The pH of the egg white and yolk was measured directly using a calibrated pH meter. Measurements were taken at room temperature (approximately 25°C) to ensure consistency across samples.

Vitamin analysis

High-Performance Liquid Chromatography (HPLC) was used for vitamin analysis. This method separates, identifies, and quantifies each component in a mixture. Samples were prepared by liquid-liquid extraction to isolate the vitamins. The extracted samples were then injected into the HPLC system, which separated the components based on their interactions with the chromatographic column. Detectors at the end of the column measured the quantity of each vitamin as it eluted. The analysis focused on vitamins A, D, E, and B12, particularly important in egg nutrition.

RESULTS

Table 1 shows the Total Heterotrophic Bacteria (THB) counts for egg samples from 7 poultry farmhouses and 7 supermarket retailers. The samples were taken from the eggshell surface (ESS) and the inner content of the eggshell (INC). ESS and INC samples were diluted to 10^{-4}. The result shows that the ESS counts are generally higher than the INC counts, which is expected as the outer surface is more exposed to contamination. The THB counts vary across different sources, with supermarket retailer F showing the highest ESS count (9.50 x10^4 CFU/g) and Supermarket Retailer C showing the lowest ESS count (1.20 x10^4 CFU/g).

As with Table 2, analyses were performed from both the eggshell surface (ESS) and the inner content of the eggshell (INC), with ESS samples diluted to 10^{-4} and INC samples also diluted to 10^{-4} similar to the THB counts, the *Salmonella* counts are generally higher on the eggshell surface than in the inner content. Supermarket Retailer C shows the highest ESS *Salmonella* count (9.3 x 10^4 CFU/g), While Supermarket Retailer F has the lowest ESS count (1.1 x 10^4 CFU/g). The presence of *Salmonella* in all samples is a concern from a food safety perspective.

The result also shows the prevalence rate and percentage occurrence of Salmonella sp. from chicken eggs. *Salmonella enteritidis* was found to be more prevalent: 28.0% in all the egg samples, followed by *Salmonella enterica*: 24.0%; *Salmonella typhimurium*: 20.0%; *Salmonella gallinarum:* 16.0%, and *Salmonella paratphi* has the lowest occurrence rate: 12.0% (Table 3). *Salmonella* serotypes identified from the study were *Salmonella typhimurium, S. enteritidis, S. gallinarum, S. typhi* and *S. paratyphi*. *Streptococcus* sp., *Bacillus* sp., *Staphylococcus* sp. and *Escherichia coli* were also seen and identified in the nutrient agar plates (Figure 2). *Staphylococcus* sp. was predominantly found on the fresh eggshell surface. *Salmonella* sp. was higher in the inner contents of the eggs, followed by *E. coli* and *Staphylococcus aureus*. This result aligns with the findings of Fàbrega and Vila (2013) and Fagbamila et al. (2017).

Table1. Total Heterotrophic Bacteria (THB) counts of the egg samples (CFU/g).

Sources of Egg	ESS	INC
Poultry Farmhouse A	2.20 x 10^4^	2.5 x 10^3^
Poultry Farmhouse B	3.15 x 10^4^	1.8 x 10^3^
Poultry Farmhouse C	1.75 x 10^4^	9.2 x 10^3^
Poultry Farmhouse D	5.60 x 10^4^	3.7 x 10^3^
Poultry Farmhouse E	8.90 x 10^4^	6.1 x 10^3^
Poultry Farmhouse F	4.30 x 10^4^	2.9 x 10^3^
Poultry Farmhouse G	7.80 x 10^4^	5.3 x 10^3^
Supermarket Retailer A	4.60 x 10^4^	1.8 x 10^3^
Supermarket Retailer B	2.90 x 10^4^	3.5 x 10^3^
Supermarket Retailer C	1.20 x 10^4^	7.6 x 10^3^
Supermarket Retailer D	6.70 x 10^4^	4.2 x 10^3^
Supermarket Retailer E	3.80 x 10^4^	2.1 x 10^3^
Supermarket Retailer F	9.50 x 10^4^	8.3 x 10^3^
Supermarket Retailer G	5.20 x 10^4^	3.9 x 10^3^

Key: ESS - Eggshell Surface Content; **INC** - Inner Content of Eggshell.

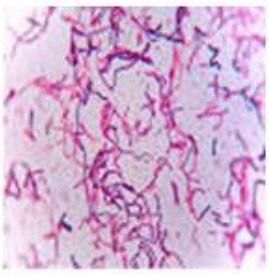
Table 2. Total *Salmonella* counts of egg samples from poultry farmhouse and supermarket retailers (CFU/g).

Sources of Egg	ESS	INC
Poultry Farmhouse A	3.4 x 10^4^	1.5 x 10^3^
Poultry Farmhouse B	2.8 x 10^4^	9.7 x 10^3^
Poultry Farmhouse C	1.6 x 10^4^	5.2 x 10^3^
Poultry Farmhouse D	4.9 x 10^4^	2.3 x 10^3^
Poultry Farmhouse E	7.2 x 10^4^	3.8 x 10^3^
Poultry Farmhouse F	5.5 x 10^4^	1.9 x 10^3^
Poultry Farmhouse G	8.1 x 10^4^	4.6 x 10^3^
Supermarket Retailer A	2.8 x 10^4^	1.3 x 10^3^
Supermarket Retailer B	3.7 x 10^4^	1.7 x 10^3^
Supermarket Retailer C	9.3 x 10^4^	4.1 x 10^3^
Supermarket Retailer D	5.6 x 10^4^	2.8 x 10^3^
Supermarket Retailer E	4.2 x 10^4^	1.5 x 10^3^
Supermarket Retailer F	1.1 x 10^4^	6.9 x 10^3^
Supermarket Retailer G	6.5 x 10^4^	3.2 x 10^3^

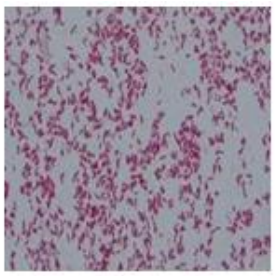
Key: ESS - Eggshell Surface Content; **INC** - Inner Content of Eggshell.

Table 3. Prevalence rate and percentage occurrence of *Salmonella* sp. from chicken eggs.

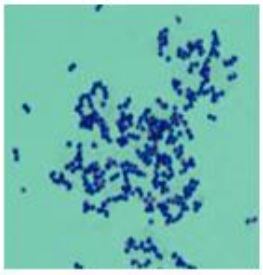
***Salmonella* sp**	**Prevalence rate**	**Percentage occurrence (%)**
S. enteritidis	14	28.0
S. enterica	12	24.0
S. typhimurium	10	20.0
S. gallinarum	8	16.0
S. paratyphi	6	12.0
Total	**50**	**100**



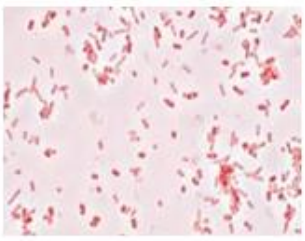
Microscopic appearance of *Bacillus* sp.



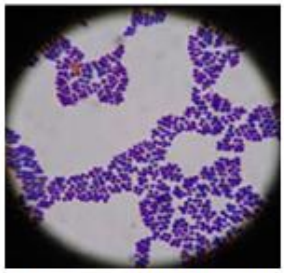
Microscopic appearance of *E. coli*



Microscopic appearance of *Streptococcus* sp.



Microscopic appearance of *Salmonella* sp.



Microscopic appearance of *Staphylococcus* sp.

Figure 2. Microscopic appearances of organisms found.

Table 4 shows the biochemical identification of isolates, *S. enteritidis, S. enterica, S. typhimurium, S. gallinarum* and *S. paratyphi,* including other organisms such as *Escherichia coli, Bacillus* sp*., Staphylococcus* sp*.* and *Streptococcus* sp*.*

Table 5 shows the nutritional composition of the eggs. The eggshell is primarily made up of calcium carbonate, magnesium carbonate, calcium phosphate, organic matter and moisture content, and has a pH level of 7.4-7.8. These components create an environment conducive to bacterial growth when combined with environmental contaminants. On the other hand, egg white (albumen) consists mostly of water, proteins, carbohydrates, lipids and ash (minerals) and has a pH level of 7.6-8.5. It also contains antimicrobial proteins such as lysozyme, ovotransferrin, ovomucoid and avidin in specific concentrations, which generally inhibit bacterial growth. This explains why there are lower bacterial counts in the egg white compared to other parts of the egg.

Egg yolk contains lesser percentage of water compared to the albumen and is rich in lipids (fats), proteins, carbohydrates and minerals, and has a pH level of 6.0-6.5. It also contains important vitamins such as A, D, E, and B_{12}, which can support bacterial growth if contamination occurs.

Table 4. Biochemical identification of isolates.

SS	CAT	OXI	SA	CIT	STT	URS	MR	VP	IND	MOT	GLBL	LA	MAN	MAL	XY	Probable Bacteria
SED	+ve	-ve	-	-ve	-	-	+	-	+	+	A	N	A	AG	N	*Salmonella*
SEC	+	-ve	-	-ve	-	-	+	-	+	+	AG	A	A	N	A	*Escherichia coli*
STM	+ve	+	+	+	-	-	-	+	-	+	AG	N	A	AG	A	*Bacillus sp*
SGM	+	-	-	+	+ve	+	-	+	-	-	A	A	N	A	A	*Staphylococcus* sp
SPP	-	-	-ve	-ve	-ve	-ve	+	+ve	-	-	A	A	N	A	A	*Streptococcus* sp

Key: SS - *Salmonella* Serovars, **SED** - *S. enteritidis*, **SEC** - *S. enterica*, **STM** - *S. typhimurium*, **SGM** - *S. gallinarum* **SPP** - *S. paratyphi*, **CAT** - Catalase, **OXI** - Oxidase, **SH** - Starch Hydrolysis, **CIT -** Citrate, **STT** - Salt Tolerance, **URS** - Urease, **MR** - Methyl Red, **VP** - Voges Proskauer, **IND** - Indole, **MOT** - Motility, **GLU** - Glucose, **LAC** - Lactose, **MAN** - Mannitoal, **MAL** - Maltose, **XY** -Xylose, **+ve** - Positive, **-ve** - Negative, **A** - Acid, **AG** - Acid/Gas, **N** - Neutral/Negative.

Table 5. Nutritional composition of egg and it concentrations.

Eggshell	Egg white (albumen)	Egg yolk	Vitamins in egg yolk
Calcium carbonate: 95-97%	Water: 87-89%	Water: 48-52%	Vitamin A: 140-160 µg/100g
Magnesium carbonate: 1-2%	Protein: 10-12%	Lipids: 32-35%	Vitamin D: 2-2.5 µg/100g
Calcium phosphate: 1-2%	Carbohydrates: 0.8-1%	Protein: 16-18%	Vitamin E: 2.5-3 mg/100g
Organic matter: 2-3%	Lipids: 0.02-0.3%	Carbohydrates: 0.2-1%	Vitamin B12: 1.5-2 µg/100g
Moisture content: 1-4%	Ash: 0.5-0.6%	Minerals: 1.5-2%	
pH: 7.4-7.8	pH: 7.6-8.5	pH: 6.0-6.5	

DISCUSSION

Foodborne diseases are of great public health concern in the modern world. In developing countries, the greater populace is largely affected by foodborne infections (Adeyanyu et al., 2014; Abdissa et al., 2017; Aditi et al., 2017). Foodborne disease, apart from affecting the health and well-being of individuals, equally affects the social and economic productivity of the countries. The main factors contributing to the increased burden of foodborne diseases, especially in Africa, are the

people's poor hygiene practices (Barika et al., 2020; Buzby, 2009). Poor personal hygiene among food handlers coupled with the inadequate handling of poultry products in farms could be possible sources of acquiring microbial pathogens that cause foodborne infections (Alfred et al., 2019; Brenner et al., 2000). In this current study of the incidence of *Salmonella* in chicken eggs collected from poultry farms in Rivers State, the findings of the results show that the total heterotrophic bacteria count of the eggshell surface of the supermarket was higher than the total heterotrophic bacteria count of the poultry farmhouse. Higher heterotrophic bacteria counts were recorded on eggshell surfaces for the poultry farmhouse and supermarket than the total heterotrophic bacteria count obtained from the inner content of the eggshell of the various locations. The high bacterial count observed in the eggshell surface for the poultry farmhouse could be attributed to the environment, where the harvested eggs are exposed to chicken excrete and waste dump, and the supermarket could be attributed to the poor sanitary condition of the retailers handling the eggs in the supermarket before re-selling it to the final consumers. This finding aligned with that of Alfred et al. (2019). Results showed that the total *Salmonella* count of the supermarket retailers was higher than the total *Salmonella* count of poultry farmhouses. The result also showed that the total *Salmonella* count from the eggshell surface was higher than the total *Salmonella* count of the inner content of the eggshell, respectively. The high *Salmonella* count observed on the egg surface could be attributed to the high rate of chicken excretion found in poultry farms where the eggs were harvested. While the low rate of *Salmonella* sp. recorded in the inner content could be linked to the low access rate of microorganisms entering the eggs. Several studies have been conducted on the incidence of *Salmonella* sp. associated with chicken eggs. According to Adeyanju and Ishola (2014) and Chiu et al. (2002) in their research, high bacteria counts were recorded on egg surfaces, which was attributed to the poor sanitary hygiene condition of the environment where the egg samples were collected.

The total heterotrophic bacteria (THB) counts were generally higher on the eggshell surface compared to the inner content of the eggs (Table 1). This aligns with the findings by Alfred et al. (2019), who also observed higher bacterial counts on egg surfaces. The authors attribute this to "poor sanitary condition of the retailers handling the eggs in the supermarket before re-selling it to the final consumers". For *Salmonella* specifically, counts were also higher on the eggshell surface compared to the inner content (Table 2). This finding could be attributed to the high rate of chicken excreta found on the eggs in poultry farms where the eggs were harvested. This is consistent with Arslan and Eyi (2010), who noted that the intestinal tract of poultry is a primary reservoir for *Salmonella*, leading to contamination of the eggshell through horizontal transmission. Regarding *Salmonella* prevalence, *S. enteritidis* was found to be the most prevalent (28.0%) followed by *S. enterica* (24.0 %), *S. typhimurium* (20.0%), *S. gallinarum* (16.0%), and *S. paratphi* has the lowest occurrence rate: 12.0% as shown in Table 3. This aligns with Akeem et al. (2019) findings, who described these serovars as "opportunistic pathogen[s] mainly affecting children, pregnant women, and aged and immune-challenged individuals" (Galanis *et al*., 2006). The higher prevalence of *Salmonella* on eggshells compared to inner contents suggests contamination is largely occurring post-laying. This point to the importance of proper handling and hygiene practices in poultry farms and retail environments. The warm, humid conditions of poultry farms provide an ideal environment for bacterial growth on eggshells. Meanwhile, this study does not provide specific physicochemical data; it's worth noting that eggshells are porous and can allow bacterial penetration under certain conditions. Factors like temperature, humidity, and pH can influence bacterial survival and growth on eggshells. For example, *Salmonella* can survive on eggshells at room temperature for weeks (Gast, 2003). The inner egg contents are generally protected by various antimicrobial components in the albumen, including lysozyme and ovotransferrin. However, the detection of *Salmonella* in inner contents, albeit at lower levels, suggests some penetration is occurring. This could be due to microscopic cracks or other factors compromising the egg's natural defences.

To more fully understand the conditions supporting bacterial growth, future studies should include measurements of temperature, humidity, pH, and other relevant parameters both in the farm and retail environments. Additionally, examining the nutrient composition of eggshells and inner contents in relation to bacterial growth could provide valuable insights for improving egg safety.

CONCLUSION AND RECOMMENDATION

In conclusion, foodborne diseases caused by bacterial contamination in eggs are a significant public health concern, particularly in developing countries. Poor hygiene practices among food handlers and inadequate handling of poultry products contribute to the increased burden of these diseases. The study found higher bacterial counts on the surface of eggshells compared to the inner contents, indicating potential contamination during post-laying.

Salmonella was the most prevalent pathogen detected on eggshells, with *S. enteritidis, S. enterica* and *S. typhimurium* being the dominant serovars. This highlights the importance of proper handling and hygiene practices in poultry farms and retail environments to prevent

bacterial contamination. The composition of eggshells provides a suitable substrate for bacterial growth, while antimicrobial proteins present in egg whites inhibit bacterial growth to some extent. However, *Salmonella* was still detected in inner contents at lower levels, suggesting some penetration may occur due to microscopic cracks or other factors compromising the natural defences of eggs. Future studies should consider measuring temperature, humidity, pH levels, and other relevant parameters in both farm and retail environments to gain a better understanding of conditions that support bacterial growth. Analyzing the nutrient composition of eggshells and their inner contents could also provide insights for improving egg safety measures.

In light of these findings, it is crucial to emphasize proper hygiene practices among food handlers and implement effective control measures throughout the production chain to reduce the risk of foodborne infections associated with eggs. Thus the study recommends enhancing consumer education on proper food handling and cooking practices to reduce the risk of foodborne illnesses associated with *Salmonella*-contaminated eggs. Regulatory authorities should collaborate with poultry farms to enforce food safety regulations and standards, ensuring compliance with best practices. Regularly assess and monitor antibiotic usage on poultry farms. Antibiotics should be administered judiciously to prevent the development of antibiotic-resistant *Salmonella* strains.

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