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(RESEARCH ARTICLE)

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Detection and public health risks of *Salmonella spp* and other pathogens in eggs

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Abstract

Salmonellosis and some other illnesses have been documented to be acquired through consumption of infected raw or undercooked foods such as eggs. Large population of human and animals around the Federal Capital Territory (F.C.T.), Abuja could be at risk due to the high consumption of this important food materials. Therefore, the aim of this study was to isolate and identify *Salmonella spp* and the related bacteria pathogens from the eggshells and the egg contents of samples obtained from five (5) deep liter farms within the FCT, Abuja, Nigeria. A total number of 150 samples of eggs, 30 each from the selected deep liter farms viz; Lugbe, Abaji, Kuje, Gwagwalada and Madalla were analyzed for the presence of *Salmonella spp*. and other pathogens using conventional culture method. The results revealed that 20.8% of the total bacteria count from all the egg content were contaminated with *Salmonella spp.* Other bacteria pathogens like *E. coli*, *Staphylococcus aureus* were isolated from both the egg shells and the egg contents. The observation was similar to literatures but the occurrence of these pathogens in the egg content was higher than expected.

Therefore, the present study provides a recent data-generation of the prevalence of *S. typhimurium* and other pathogens in eggs at farm level in the selected locations in the FCT, Nigeria. It is important to remember that necessary control is required at all levels in the egg production chain and strict hygiene is needed to avoid epidemic resulting from the consumption of eggs.

Keywords: Egg; *Salmonella*; Prevalence; Isolation; FCT

1. Introduction

Globally, *Salmonella* species and other bacteria pathogens have been reported to be one of the most important food-borne pathogens that are responsible for several health challenges (Gargiulo *et al*., 2022). Among these pathogens, Salmonella species, *E. coli*, Shigella, Staphylococcus are the most often associated with food-borne outbreaks in all the occurrence throughout the world (Sinclair *et al*., 2022). Although, Salmonella like *S. enteritidis* and *S. typhimurium* are often self-limiting when associated with gastroenteritis as well as assuming an asymptomatic carrier state in many situations in farm animals, their presence is viewed often as indication of potential risk due to the possibility of transfer of resistance genes (Habib *et al*., 2023). In Nigeria, a wide variety of animal species have been reported to be reservoir of these important pathogens. High mobility of human and the domestic animals also add to the geographical spread that often characterized these pathogens (Howard *et al.,* 2012). Several literatures have reported that human infections with Salmonella could occur from the consumption of eggs and egg products, especially when the products are consumed raw or undercooked (Argüello, *et al*., 2013). Pigs, cattle and poultry meat, as well as environmental contamination are reported to be sources of continuous spread of important pathogenic diseases. Specifically, egg-associated infections are reported to be high because eggs are often used as an inexpensive food source in the form of shell eggs, liquid, frozen, and/or dried products (Galiş *et al*., 2013). Being a ready source of a reliable source of rich nutrition and serving many functions as ingredients in other products, eggs are important food materials and as such

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have wide spread applications thereby extending high risks where there are contaminations (Majowicz *et al*., 2010). Studies that will give knowledge of not only the use of eggs as a food source but also the safety of this product become very important. Studies also shows that eggshells and egg contents can be contaminated by some pathogenic and spoilage bacteria in a variety of ways which include during egg formation in the hen reproductive system, routine farm activities that exposes the eggs to contact with the environmental conditions etc. (Gast *et al*., 2020). This has led to several outbreaks of salmonellosis and other related cases where the consumption of eggs was the source of the infection. Egg contamination by *Salmonella* and related microorganisms can occur via penetration of the bacterium through the eggshell from the colonized gut or from contaminated faeces during or after the eggs are laid (Jones *et al*., 2015).

Figure 1 Deep liter Poultry Production System

Deep litter system of poultry production is the commonest in Nigeria owing to being easier to start-up and manage when compared with the capitally intensive battery cage system. In the former, several birds are kept together in a unit area with beddings such as saw dust or wood shavings underneath (Figure 1) that absorbs faeces from the birds (Gole *et al.*, 2017). In the present study areas, not much have been reported about the incidence and prevalence of bacterial contamination of food sources such as eggs. Therefore, the present study was designed to isolate and identify *Salmonella spp* and other food pathogenic bacteria from the eggshells and egg contents of some egg samples obtained from some farms around the FCT, Abuja, Nigeria.

2. Materials and Methods

2.1. Sample Collection

2.1.1. Study Area and Sampling Procedures

The egg sample for this study were collected randomly from farms located in Lugbe, Abaji, Kuje, Gwagwalada and Madalla respectively (Fig. 2). The sampling involved collection of eggs from both deep liter and battery cage systems which were produced at industrial scale levels. In all, a total a total of 150 eggs were aseptically collected, placed in sterile bags and transported to the microbiology Laboratory for analysis (Li *et al*., 2020).

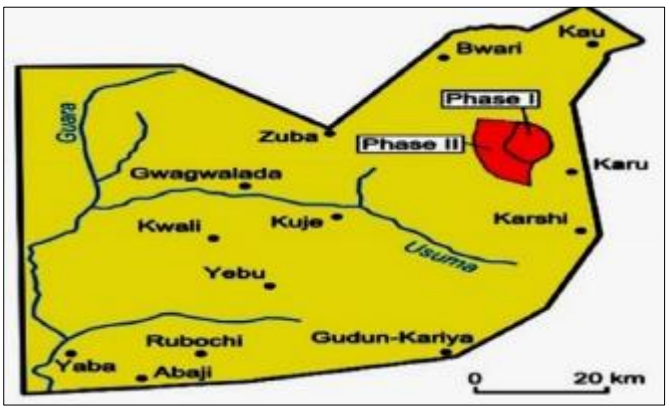

Figure 2 Map Showing The Federal Capital Teretory

2.2. Microbiological Analysis

2.2.1. Detection of Pathogens from the Egg Rinsate

Each of the egg in each sample were transferred aseptically to pre-sterilized glass bottles containing 250 ml of sterile Peptone Water and mixed gently and incubated at 37 °C for 24 hours. Afterwards, 1ml of the egg rinsate (Figure 3) was used for the enumeration and isolation of the respective bacteria.

Figure 3 Preparation of Egg Rinsate (Eggshell Rinsing)

2.2.2. Detection of pathogens from the egg contents

After the immersion of the eggs in the sterile peptone water, they were removed from, cleaned with 70% ethanol the eggs were carefully broken in sterile glass Petri dish (Fig. 4A).

A B

Figure 4 Serial Dilution Protocol

Afterwards, 1ml from the respective dilution and egg content was transferred to 9ml of peptone water and then serially diluted to 10^{-6}. Another 1ml was added to 9ml selenite–cystine broth and serially diluted to 10-6. From the 10^{-3} and 10^{-5} dilutions, 0.1ml was transferred to Brilliant Green and Phenol Red agar, Nutrient agar, Eosin methylene blue and Bismuth Sulphite Agar respectively and the respective media were inoculated at 37 °C for 24- 48 hours (Onyeneho and Hedberg, 2013). After the incubation period, the suspected colonies were sub-cultured to appropriate media and then incubated at 37 °C for 24 h and afterwards purified by further sub-culturing before being subjected to primary biochemical screening tests, such as Triple Sugar Iron agar, motility, Indole, H_2S production in Sulfide-Indole-Motility, Urease test. (Schirone *et al*., 2019).

3. Results and Discussions

Globally, poultry and poultry products have been identified as one of the major sources of foodborne diseases and pathogen transmission (Havelaar *et al*., 2013). Commonly encountered pathogen related to eggs is Salmonella. Several effort have been made by the egg production industries to improve egg safety and reduce the incidence of bacterial infections due to Salmonella and the related bacteria. This approach includes good manufacturing practice as well as Hazard analysis critical control point (HACCP) system. Notwithstanding, there is need for research to be carried out in order to generate data to support the presence of egg-related foodborne pathogens around the world. This research was conducted in this regard.

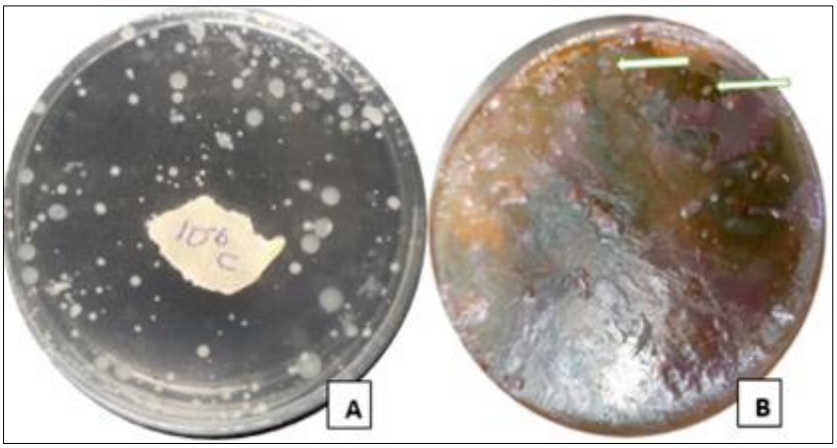

Figure 5 Colonies of Important Bacteria Pathogens on Media; A= Total Aerobic Count, B= E. coli colonies (Arrows) with Metallic Sheen

Table 1 Mean Aerobic Bacteria Count of Egg Rinsate

Sampling Location	TVC	TCC	TSC	E. coli	Staph
Lugbe	2.3x10^5	3.1x10^3	1.4x10^1	4.5x10^1	2.1x10^1
Abaji	2.0x10^4	4.2x10^2	6.7x10^1	2.3x10^1	ND
Kuje	4.3x10^4	2.7x10^1	3.2x10^1	1.6x10^1	1.1x10^1
Gwagwalada	3.3x10^3	4.1x10^1	1.5x10^1	ND	5
Madalla	2.3x10^4	3.6x10^2	1.0x10^1	5.4x10^1	3.5x10^1

Key: TVC=Total Viable Count; TCC=Total Coliform Count; TSC=Total Salmonella Count; Staph. = Staphylococcus Count; ND=Not Detected

The total aerobic viable count of bacteria from the egg rinsate generally ranged from 3.3x10^3 in Gwagwalada to 2.3x10^5 in Lugbe. The total coliform count however ranged from 2.7x10^1 in Kuje to 3.1x103 in Lugbe. Similarly, *Escherichia coli* count ranged from not been detected in Gwagwalada to 5.4x10^1 in Madalla. The The total *Salmonella typhimurium* occurred in n the range of 1.0x10^1 CFU/mL in Madalla to 6.7x10^1 CFU/mL in Abaji. Total Staphylococcus count ranged from not been detected in Abaji samples to 3.5x10^1 CFU/mL in Madalla. Abaji had the highest occurrence of Salmonella pathogen in the study as shown in Figure 6.

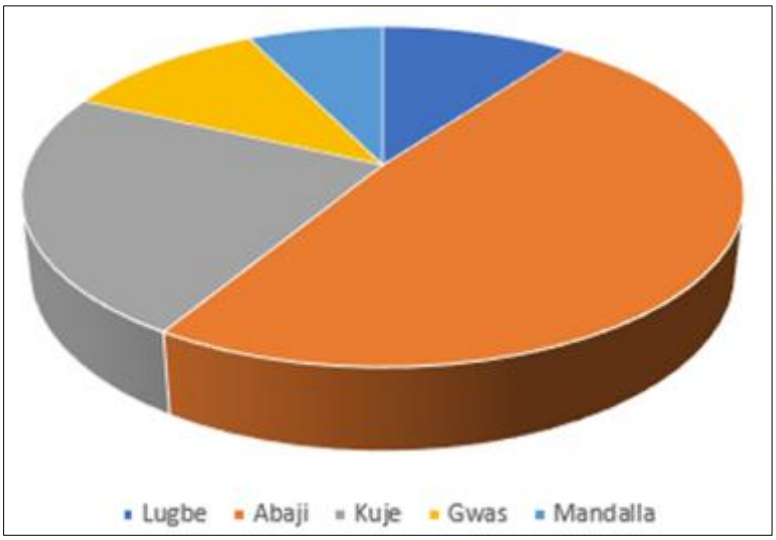

Figure 6 The Mean Distribution of *Salmonella typhimurium* in the Studied Sites

Table 2 Mean Aerobic Bacteria Count of Egg Contents

Sampling Location	TVC	TCC	TSC	E. coli	Staph
Lugbe	5.2x10^1	3.5x10^1	8	3	ND
Abaji	4.3x10^1	2.3x10^1	2	2	4
Kuje	3.2x10^1	1.3x10^1	9	ND	2
Gwagwalada	2.5x10^1	1.5x10^1	1.2x 10^1	5	ND
Madalla	2.1x10^1	9	5	ND	7

Key: TVC=Total Viable Count; TCC=Total Coliform Count; TSC=Total Salmonella Count; Staph. = Staphylococcus Count; ND=Not Detected

The mean occurrence of the bacteria in the studied areas as obtained from the egg contents is as shown on Table 2. Madalla had the lowest total viable count of 2.1x10^1 CFU/mL while the highest count of 5.2x10^1. Andalla also recorded the lowest coliform count of 9 CFU/mL while the highest of 3.5x10^1 CFU/mL was recorded in Lugbe sample. The total Salmonella count of 2 CFU/mL was recorded in Abaji as the lowest in the study as against the highest of 1.2x10^1 in Gwagwalada. Therefore, this location had the highest occurrence of Salmonella in the content of the eggs in the studied area (Figure 2). None were recorded for *Escherichia coli* in both Kuje and Madalla samples while 5 CFU/mL was recorded in Gwagwalada samples.

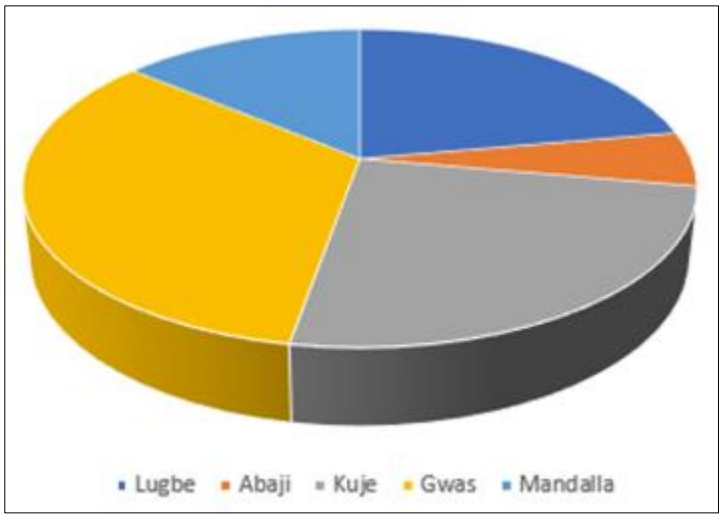

Figure 7 The Mean Relative Occurrence of Salmonella typhimurium from the Egg Content

Epidemiological records have suggested that there is a direct link between the presence of *Salmonella* in poultry products and salmonellosis occurrence in humans. It is worth noting therefore that the high occurrence of Salmonella in this study demand that surveillance studies be put in place to avert the occurrence of disease associated with the consumption of this important poultry product. It was clearly demonstrated from this study that eggs were obviously contaminated by *Salmonella* and other pathogenic bacteria. In a similar study by Gast *et al*. (2014) on the "Horizontal transmission of *Salmonella enteritidis* in experimentally infected laying hens housed in conventional or enriched cages". However, *Salmonella* was not isolated from some sites. This might be due to the fact that variations in the management approach affect bacterial contamination rates. Frequent changes of poultry beddings have been reported to reduce incidence of bacterial contamination of eggs (Adesiyun *et al.,* 2014). The rates for contamination of eggs with *Salmonella* and other pathogen associated with poultry eggs obtained from the studied sites is different from those reported by Scallan *et al*., 2011 who reported lower counts from similar samples. This might be due to the level of hygiene practice at the farm as well as after egg collection. The proportion of Salmonella in the egg content according to our study is shown in the Figures 8.The percentage of Salmonella in the egg content was 20.8%.

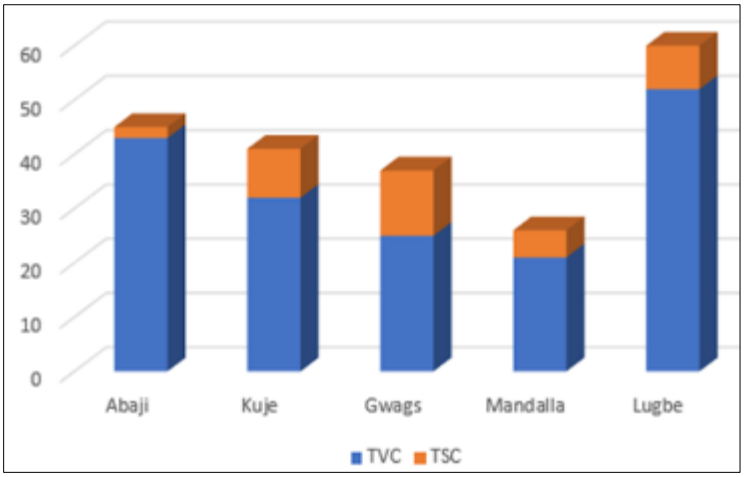

Figure 8 The proportion of Salmonella in the Egg Content

Worrisome scenario in this study was that *Salmonella* was isolated from both eggshells (egg rinsate) as well as from the contents. This is contrary to the similar studies carried out by Chousalkar *et al*., 2018, where *Salmonella* were isolated only from commercial eggshells …. which made it not consistent with the findings of our study. As seen in this study and similar occurrence from other countries, *Salmonella* has been isolated only from shells but there are also reports of contamination of the egg contents in few cases (Moffatt and Musto, 2013). The contamination of eggs especially the contents, represent a high risk for the consumers, in the sense that some consumers prefers undercooked to un-boiled eggs for one reason or the other. By this, they can directly be infected by the associated bacteria and even cross-

contamination of other foodstuffs can occur (Gast *et al*., 2019). The occurrence of possible Salmonellosis outbreak through contaminated eggs with *Salmonella spp* and other pathogens showed the importance of proper hygiene practice on the farm and during transportation. In previous reports such as those by Pande *et al.,* 2016 Salmonella was the dominant bacteria that was isolated from eggs as the most important pathogen, but in our study, other important bacteria such as *E. coli* and *Staphylococcus sp*. were isolated. Among the food sources meant for human consumption, eggs are considered as one of the main sources of infection of Salmonella bacteria (Rouger *et al*., 2017). This is primarily due to the fact that egg contents are an ideal growth medium for bacteria and other microorganisms that could be pathogenic to humans. Research finding have recorded that Gram-positive bacteria predominate the eggshell, while gram negative bacteria possess the ability to overcome the antimicrobial substances in the egg content and are therefore found more in the content (Oluwasile *et al*., 2014). The major ways by which eggs become contaminated by Salmonella bacteria have been suggested to include direct contamination which occur during the egg formation process in the reproductive tract areas like the ovary and oviduct, as well as the indirect contamination which occur after the eggs are laid. The various surfaces of eggs can be contaminated by the materials used as beddings and liter that often have direct contact with Salmonella-laden *faeces* (Raspoet *et al*., 2014). The long contact between faecal material and the eggshell though sometimes unavoidable could strengthen entrance of microorganisms into the egg. Also, defects in eggshells such as thin shells, increased shell pore numbers, translucency, etc. may allow the entry of food-borne pathogens into the egg contents. Research findings have suggested that egg washing can reduce the microbial load on the eggshell surface thereby lowering the rate of penetration of Salmonella across the eggshell and decrease the incidence of food poisoning due to the contamination. This agrees with the work of Li *et al*., (2007) in their studies on *Salmonella* populations and prevalence in layer faeces from commercial high-rise houses and characterization of the *Salmonella* Isolates by serotyping, antibiotic resistance analysis, and pulsed field gel electrophoresis. It is also argued that egg-washing chemicals can damage the cuticle layer of the eggshell, resulting in moisture loss, and deterioration of the internal quality of the egg. Worthy of note is that, egg washing may encourage the transmission of Salmonella across the eggshell barrier instead of preventing them especially, when the post-washing storage and drying conditions are not adhered to (Crump *et al*., 2015). It is thus important to note that, the contamination and persistence of Salmonella bacteria in chicken eggs presents some level of epidemiological feature of the organism that can eventually be transmitted to human.

The occurrence of *E. coli* from the farm eggs were also documented. Record of Food borne diseases have been classified as one of the important issues worldwide. In this regard, *E. coli* is considered the most prevalent food borne pathogen that has gained increased attention in recent years especially in the developing countries like Nigeria. In the present study, the presence of *E. coli* from both eggshell and egg content is in line with the study of Van *et al*., 2015. However, the prevalence in this finding is higher than some reports from previous works. In another study by Ferens and Hovde (2011) on *Escherichia coli* O157: H7: Animal reservoir and sources of human infection, *E. coli* was isolated from faeces of poultry sample at different farms in Nigeria that may shows contamination of cloacae of chicken. The differences that was observed in the studies could be attributed to differences in sampling and isolation procedures, the type or choice of culture media used, faecal contact to egg through the bedding, the general hygiene status of the poultry operation, the methods and study design as well as the season variation in which samples were collected (Ventola, 2015). It is worthy of note that *E. coli* is a common inhabitant of the intestinal tract of chickens and other poultry species. Therefore, the oral-faecal transmission between the same and different poultry species is a possibility. The presence of the bacterium is an indication of possible disease incidence to both human and animal around. Poultry workers could be prone to contract *E. coli* infections through inhalation of infected dust during routine farm work. Also, through many ways, *E. coli* can be transmitted to new environments such as by beetle, flies, insects, mites, rats, and wild birds (Salihu *et al*., 2015).

Also, the occurrence of *Staphylococcus aureus* in this study is noteworthy. To our knowledge, this study presents an important information regarding the risks associated with the consumption of raw eggs. The results revealed that the occurrence of *S. aureus* in both eggshells as well as egg content from poultry farms studied. This is similar to the previous studies of Igbinosa *et al*., (2016b). Many factors could be responsible for this observation which include sample size, sampling seasons, and the isolation method used. The presence of *S. aureus* in eggshell and egg contents point to the low hygiene status of the farms concern. Certain reports especially under experimental study condition, *S. aureus* isolates have been shown to penetrate the eggshell (Jaja *et al*., 2018). The fact that as low concentration as 1 µg of the bacterium toxin can cause food poisoning, keeping eggshells and the environment clean generally should be considered as one of the effective ways to reduce the incidence of foodborne illness associated with *S. aureus* (Kadariya *et al*., 2014).

Igbinosa *et al*., 2016a) also found similar results that the detection rates of *S. aureus* in food stuffs could be as a result of none adherence to basic agricultural hygiene practice. Expectedly, the presence of the bacterium is higher in the eggshell rinsates than from the egg content. This could be attributed to the antimicrobial defense system in the egg content as

well as the physical barriers poses by the egg shell (Diarra *et al.,* 2014). Cooking processes such as boiling and frying might be able to eliminate the pathogens. However, consumption of raw eggs is a serious issue that needed to be educated about. Also, for the fact that eggs are used as composite of other important food preparations, high presence of this bacterium can easily lead to cross-contamination. Particularly important to note is that there are remarkable number of virulence factors in *S*. *aureus* that play an important role in causing enterotoxin poisoning (Geiger *et al*., 2012).

It has been reported that egg-related outbreaks from several pathogenic bacteria such as those isolated in this study were commonly as a result of breakdowns in controlling or adhering to certain hygiene measurements along the farm to fork continuum as reported in similar work by Nakamura *et al.,* 2013. This has become more worrisome where these bacteria become multidrug resistance.

4. Conclusion and Recommendations

Although there are several international poultry control programs being put place in developing countries, they have not really transformed into concrete control of egg-related illnesses unlike in the developed countries which have resulted in significant decreases. Such programs like on-farm monitoring/ quality control system, diverting contaminated eggs for processing, culling infected flocks, cleaning and disinfection of sheds, maintaining cold chain of eggs, and vaccination of flocks needed to be encouraged. Given the results in this study, it is recommended that these controlling mechanisms should be done carefully in all African countries including Nigeria. It is also worthy of note that the results of the present study will provide more recent data information regarding the prevalence of *S. typhimurium* and related foodborne pathogens in eggs at farm level in the FCT, Nigeria. It is also important to recall that *Salmonella* was isolated from both eggshells (rinsate) and egg content in this study. This carries some weights in the presentation of how important urgent control measures should be put in place at all levels in the egg production and supply chain. The contamination of eggs by Salmonella and related pathogens is a complex issue affected by every stage of the egg production, from farm to the customer. Based on the results in this research, it can be concluded that there is need to carry out detection of Salmonella and other pathogens associated with eggs from other location in Nigeria. It is also paramount to explore other methods for detection of Salmonella and related pathogens from eggs such as PCR-based techniques. Then, based on adequate detection methods, better ways to prevent this pathogenic microorganism can be deduced in order to prevent the health associated risks. Although some scientific research finding opined that it is not yet practicable to produce eggs void of Salmonella bacteria, it is possible to reduce further the incidence to a better level by adopting options such as adequate routine disposal methods and disinfections. Going by the recent lifestyle interest in the use of raw foods such as eggs, there is need to educate the populace of the dangers associated with this practice. Further research is also required in order to explore the most hygienic protocol for the production of wholesome poultry products that would contain very minimal level of bacteria through the use of environmental-controlled factors, re-education of all stakeholders in egg production. It is further recommended here that the common *egg safety facts* be adopted by all the stakeholders in the egg matters such as everyone who uses, sells, or distributes eggs. These facts include: cooking eggs and food containing eggs until they are hot and the temperature is able to kill associated bacteria that may be present; By the rule of the thumb, obviously dirty eggs should be separated and thrown away because washing makes it easier for bacteria to penetrate thereby contaminating the content; Also, cracked eggs should promptly be discarded because such cracked pore are standard entry path to several contaminating microorganisms; All eggs should be handled with high level of care against contamination as other perishable foods like dairy products, chicken and sea foods; all food containing egg should be used before the best-before date; The rural folks who use eggs in raw forms should be advised to seek alternative recipes that do not require raw eggs; last but not the least, eggs should be left inside cartons with their best before dates while in the fridge so that the date can be tracked adequately

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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