



Bacterial Contamination of Chicken Eggs from Poultry Farms and Retail Markets in the New Juaben Municipality, Ghana

**Matthew Glover Addo^{1*}, Manomba-Asamoah Raissa¹, Kingsley Badu¹
and Samuel Agordzo¹**

¹*Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors MGA and KB managed the conceptualization, supervision, validation, writing, review and editing. Author MAR did the data duration, investigation, methodology and writing. Author SA managed the analyses of the study and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This cross-sectional study seeks to investigate the bacterial contamination of poultry products (eggs) produced from selected farms and sold within the major markets in the New Juaben Municipality in the Eastern region of Ghana. A total of 100 eggs were sampled randomly from October to December, 2018 from five poultry farms and five retail markets in the municipality for microbial evaluation. The collected swabs of eggshells and its contents collected at various times of the day were cultured on suitable media and standard microbiological test were performed to identify the isolated organisms. The entire eggshell samples were positive for bacteria with only 13 (13%) having growth in the egg content. The genera of bacteria recorded are *Staphylococcus*, *Escherichia*, *Klebsiella*, *Proteus* and *Citrobacter*. The mean total viable count of bacteria from both shell and content sampled from poultry farms and retail markets were 13.2×10^7 cfu/ml and 8.0×10^7 cfu/ml ($p > 0.05$) respectively. These were higher than the acceptable value of 10×10^5 gm/cfu. The study also showed that the level of microbial contamination of the eggs was highest in the

*Corresponding author: E-mail: maddosci@yahoo.co.uk;

afternoon as compared to the morning and evening. The bacteria isolates and the sheer numbers from both the eggshells and the egg content confirm the poor hygienic conditions under which both the poultry farms and markets were kept. Even though serious human pathogens were not prevalent in this study, it is recommended that strict hygienic protocols be adhered to on the poultry farms and in the markets.

Keywords: Egg; microbial contamination; poultry farms; retail markets; bacteria; Ghana.

1. INTRODUCTION

The egg produced by chicken is undoubtedly an important source of nutrients including proteins, vitamins, zinc, phosphorus among others and it remains the single major source of cholesterol among the foods consumed in the USA [1]. In Ghana, poultry farmers keep the birds for either the eggs, meat or both. In order to ensure a continuous supply of high-quality eggs, several improvements have been made in enriching the omega3, lutein and folate components [2]. Apart from humans, microorganisms especially bacteria also find the egg highly nutritious thereby feeding on it. These microorganisms can survive on the shell of the egg and even cross the shell and membrane to feed on the yolk and other eggshell contents. Microbial contamination of poultry and poultry products such as the egg significantly impacts the industry by way of economic loss and at the same time results in infections and illnesses [3]. While *Campylobacter* is the major bacteria associated with the foodborne disease, *Salmonella*, on the other hand, is the most common bacterial cause of poultry and egg-related illnesses [4]. Apart from *Salmonella enteritidis*, other bacteria associated with the contamination of chicken and their eggs include *Listeria monocytogens*, *Campylobacter jejuni* and *Escherichia coli* [5]. These bacteria are capable of contaminating both the eggshell and its content. Internal (shell content) contamination may occur before oviposition when the eggshell is not fully formed and the reproductive organs are infected. The eggshell contamination can result from contact of the eggshell with the colonized gut containing contaminated faeces before or during oviposition [6]. Under the intensive system of poultry keeping, eggs laid by the hen are collected in the morning, afternoon and evenings. Eggs collected in the morning spend several hours in the house from the previous evening after the day's collection up until the following morning before collection. It is therefore expected that eggs collected in the morning should have higher bacterial contamination as compared to those collected in the afternoon and evening [7]. The

increase in human population and lack of good sanitation practices in the New Juaben Municipality in the Eastern region of Ghana has led to an increase in bacterial diseases, especially, Typhoid. Poultry farms in the municipality are also facing similar sanitation problems. This leaves the eggs produced by the poultry not safe for consumption if not well washed and thoroughly cooked. The objective of this study therefore, is to investigate the contamination of chicken eggs from poultry farms and retail markets in the New Juaben Municipality of Ghana.

2. MATERIALS AND METHODS

2.1 Sample Sites and Sample Collection

Samples were collected from five poultry farms (PF) and coded as; PF₁, PF₂, PF₃, PF₄ and PF₅, as well as five retail markets (RM) at RM₁, RM₂, RM₃, RM₄ and RM₅ (Table 1) together with interviews using structured questionnaires for assessing factors such as hygiene. In total, 100 eggs were randomly selected from the poultry farms and the retailers in the markets in the New Juaben Municipality of Ghana from October to December, 2018. Sampling of the eggs was conducted in the morning, afternoon and evening across the study sites. Each of the eggs was kept in a separate sterile plastic ziplock bag and transported on ice to the Microbiology laboratory of the Regional Hospital in Koforidua for processing within 2 hours.

2.2 Cultures for the Eggshell

The surfaces of each of the eggshells were swabbed using separate sterile cotton swabs sticks that were soaked in 0.1% peptone water. These swabs were inoculated into different tubes containing 5ml of 0.1% peptone water. These test tubes were labelled using coded names of the locations (farms and markets) as well as the time of collection. The tubes were then incubated at 37°C for 18-24 hours. A pool was made using each of the cotton swabs on well-labelled media (Blood Agar, Muller Hinton Agar, and

MacConkey Agar) and streaked. Subsequently, 1ml each of the samples incubated in the 0.1% peptone water was transferred into 9ml of selenite broth and incubated 18-24 hours. Using the streaking method, the selenite broth with its content was plated on separate Salmonella-Shigella (S-S) agar. The S-S agar plates were incubated at 37°C for 24 hours and observed for growth.

2.3 Cultures for the Eggshell Content

Before cracking the eggshells to assess their contents, 70% ethanol was used to disinfect the shells. The eggs were cracked-open gently with a sterile spatula and their contents (yolk and albumin) were carefully emptied into separate and well labelled sterile glass Petri-dishes. The contents of the Petri dishes were then homogenized with the aid of sterile cotton swab sticks. Pre-enrichment and subsequent enrichment techniques were repeated for the eggshell content as previously done for the eggshell.

2.4 Isolation and Identification of Microorganisms

After the 18-24hr incubation period, standard bacteriological techniques including morphology (shape, size, colour, and outline) of the colony-forming units, Gram's staining and biochemical (Coagulase, Urease, Indole and Citrate) tests were performed to identify the isolated microorganisms.

2.5 Determination of Total Viable Count (TVC)

For us to enumerate the total viable organisms, serial dilutions of 10⁻¹ to 10⁻⁶ concentrations of each test sample were prepared using 1ml of the 0.1% peptone water containing dissolved swabs from the eggshell and eggshell content. This was followed by transferring 1ml of each serial dilution into tubes containing 9ml of molten agar. The tubes were gently wrapped in between the palm to obtain a homogenous mixture. The mixtures were immediately poured onto sterile glass Petri-dishes and incubated at 37°C for 24 hours. The plates showing the growth of colonies between 30-300 were selected and counted.

2.6 Data Analysis

The data obtained were entered into Microsoft Excel and analyzed using IBM SPSS statistics

software version 20.0. Variations in microbial counts at different sites were analyzed using Analysis of Variance (ANOVA). The level of significance was determined at an alpha level of 0.05.

3. RESULTS

3.1 Isolation and Characterization of Microorganisms

3.1.1 Isolation of microorganisms

A total of one hundred eggs were sampled from both farms and retail markets. Bacteria of different genera were isolated from 13% of the eggshell contents whereas all the eggshells (100%) had bacteria isolated from them. In all, 110 bacterial isolates were isolated and these can be grouped into five (5) genera; Staphylococcus, Escherichia, Klebsiella, Proteus and Citrobacter (Table 2).

Table 1. Sampling locations and their corresponding codes

Sampling locations			
Poultry farms	Code	Retail markets	Code
Ben	PF ₁	Jumapo	RM ₁
Akyeme	PF ₂	Oyoko	RM ₂
Mark	PF ₃	Agartha	RM ₃
Bempomaa	PF ₄	Central	RM ₄
Waveland	PF ₅	Adweso	RM ₅

3.1.2 Biochemical characterization

Various biochemical tests such as catalase test, indole, urease, coagulase, citrate and glucose utilization were done to identify the suspected isolates present in the agar plates. All the suspected organisms were able to utilise glucose and also produce catalase but failed to utilise citrate. *Citrobacter diversus* (A6) showed variable reaction with the enzyme urease (Table 2).

3.2 Microorganisms Isolated from Eggshell Surfaces and Eggshell Contents

The eggs sampled from both the poultry farms and retail markets were contaminated with the microorganisms either on the eggshell or egg content. *Coagulase Negative Staphylococcus* was the most abundant and was predominantly

isolated from the eggshell surface 72.7% (n=80). *Staphylococcus aureus* 2.7% (n=3) and *Proteus mirabilis* 0.9% (n=1) were isolated from only the eggshell surface representing (Table 3).

3.3 Isolates from the Poultry Farms and the Retail Markets

Eggs obtained from all the poultry farms were contaminated with bacteria. *Staphylococcus* sp. was identified as the predominant microorganisms contaminating eggshell surfaces in all the five farms particularly PF₁, PF₂ and PF₅ where it was single handedly contaminate the shell surfaces (Table 4). Apart from *Staphylococcus* spp., *Klebsiella* spp. (PF₃), *Escherichia coli*, *Proteus mirabilis* and *Citrobacter diversus* (PF₄) were also encountered on eggshell surface. Among them, *Citrobacter diversus* and *Proteus mirabilis* were the least abundant microorganism associated with the eggshell surfaces. Egg content was also contaminated by microorganisms. *Escherichia coli* was considered as most abundant bacteria from egg content, contaminating major farms (Table 4) while *Citrobacter* spp. being the least abundant. PF₂ was devoid of egg content contamination.

Microorganisms were isolated and characterized from the sampled eggs bought from the five selected retail markets. *Staphylococcus* sp. was dominant microflora, invading on egg shell surface in four (RM₁-RM₄) out of five retail market particularly RM₂, RM₃ and RM₄ where it was single handedly contaminate the shell surface (Table 5). Complete absence of *Staphylococcus* sp. was observed from RM₅. RM₅ market had *Escherichia coli*, *Citrobacter* sp. and *Klebsiella* sp. on the eggshell surface. Contamination in egg content was noticed only from RM₂ market. It was contaminated with *Klebsiella* sp.

3.4 Comparing the Bacterial Contamination between Poultry Farms and Retail Markets

Our study also compared the level of bacterial contamination on the eggs between the poultry farms and the retail markets. We obtained a total mean bacterial count of 13.2×10⁷cfu/ml and 8.0 ×10⁷cfu/ml (p>0.05) from the farms and the markets respectively. These values represented the mean bacterial count from eggs sampled in the morning, afternoon and evening from the two sources (results not shown).

Table 2. Cultural, morphological and biochemical characteristics of bacterial isolates

Characteristics	Suspected organism						
	A1	A2	A3	A4	A5	A6	A7
Cultural							
Elevation	Convex	Convex	Raised	Convex	Convex	Convex	Flat
Margin	Entire	Entire	Entire	Entire	Serrated	Entire	Serrated
Colour	Cream	Cream	Beige	Greyish	Cream	Cream	Cream
Shape	Round	Round	Rod	Rod	Rod	Rod	Circular
Morphology							
Gram Stain	+	+	-	-	-	-	-
Spore Stain	-	-	-	-	-	-	-
Cell Type	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Cell Arrangement	Single	Single	Single	Single	Single	Single	Single
Biochemical							
Catalase	+	+	+	+	+	+	+
Indole	-	-	+	-	+	+	-
Urease	+	-	-	+	-	+/-	-
Coagulase	-	-	-	-	-	-	-
Citrate	+	-	-	-	-	-	+
Glucose	+	+	+	+	+	+	+
Number n (%)	82 (74.5)	3 (2.7)	9 (8.1)	9 (8.1)	4 (3.6)	2 (1.8)	1 (0.9)

Legend: + = positive - = negative +/- = variable

A1 - *Coagulase Neg. Staphylococcus*; A2 - *Staphylococcus aureus*; A3 - *Escherichia coli*; A4 - *Klebsiella* sp.; A5 - *Citrobacter* sp.; A6 - *Citrobacter diversus*; A7 - *Proteus mirabilis*

Table 3. Number of bacterial species isolated from both eggshell surface and eggshell content

Isolate	Number of Isolates from Eggshell content n (%)	Number of Isolates from Eggshell surface n (%)	Total Number of isolates n (%)
Coagulase Neg. <i>Staphylococcus</i>	2 (1.8)	80 (72.7)	82 (74.5)
<i>Staphylococcus aureus</i>	0 (0.0)	3 (2.7)	3 (2.7)
<i>Escherichia coli</i>	5 (4.5)	4 (3.6)	9 (8.1)
<i>Klebsiella</i> sp.	4 (3.6)	5 (4.5)	9 (8.1)
<i>Citrobacter</i> sp.	1 (0.9)	3 (2.7)	4 (3.6)
<i>Citrobacter diversus</i>	1 (0.9)	1 (0.9)	2 (1.8)
<i>Proteus mirabilis</i>	0 (0.0)	1 (0.9)	1 (0.9)
Total			110

Table 4. Distribution of the bacteria isolated from the five poultry farms

Poultry farms	Eggshell surface	Number n (%)	Eggshell content	Number n (%)
PF ₁	<i>Staphylococcus</i>	11 (10)	<i>Escherichia coli</i>	1 (0.9)
			<i>Staphylococcus</i>	1 (0.9)
PF ₂	<i>Staphylococcus</i>	15 (13.6)		
PF ₃	<i>Staphylococcus</i>	3 (2.7)	<i>Klebsiella</i> sp.	2 (1.8)
PF ₄	<i>Staphylococcus</i>	5 (4.5)	<i>Escherichia coli</i>	3 (2.7)
			<i>Escherichia coli</i>	3 (2.7)
			<i>Proteus mirabilis</i>	1 (0.9)
			<i>C. diversus</i>	1 (0.9)
PF ₅	<i>Staphylococcus</i>	15 (13.6)	<i>Escherichia coli</i>	1 (1.0)
			<i>Staphylococcus</i>	1 (1.0)

Table 5. Distribution of the bacterial isolates in the five retail markets

Retail market	Eggshell surface	Number n (%)	Eggshell content	Number n (%)
RM ₁	<i>Staphylococcus</i>	10 (9.0)	-	-
			<i>Klebsiella</i> sp.	1 (0.9)
RM ₂	<i>Staphylococcus</i>	6 (5.4)	<i>Klebsiella</i> sp.	2 (1.8)
RM ₃	<i>Staphylococcus</i>	12 (10.9)	-	-
RM ₄	<i>Staphylococcus</i>	11 (10.0)	-	-
RM ₅	<i>Escherichia coli</i>	1 (0.9)	-	-
			<i>Citrobacter</i> sp.	3 (2.7)
			<i>Klebsiella</i> sp.	1 (0.9)
			<i>Klebsiella</i> sp.	1 (0.9)

3.5 Bacterial Growth across Poultry Farms

The mean total viable count (TVC) for the eggs samples from each of the Poultry Farms are shown in Fig. 1. The result showed that the level of microbial contamination of the eggs was highest in the afternoon as compared to the morning and evening samples. In the morning, mean total viable count (TVC) recorded from the five farms (PF₁, PF₂, PF₃, PF₄ and PF₅,) were (8.64×10⁶: 7.2×10⁶) cfu/ml, (6.38×10⁶: 0.00 ×10⁶) cfu/ml, (5.56×10⁶: 4.67×10⁶) cfu/ml, (6.23×10⁶:5.10×10⁶) cfu/ml and (6.56×10⁶:

5.50×10⁶) cfu/ml respectively for eggshell surfaces and eggshell contents (Fig. 1) while the afternoon samples recorded mean TVC of (8.56×10⁶: 5.85×10⁶) cfu/ml, (7.80×10⁶: 0.00×10⁶) cfu/ml, (6.48×10⁶: 3.17×10⁶) cfu/ml, (7.95×10⁶: 5.17×10⁶) cfu/ml and (7.2×10⁶: 4.40×10⁶) cfu/ml. The bacteriological examinations conducted on the eggs obtained in the evening revealed growth on only the eggshell surfaces from four farms. It was 7.74×10⁶, 7.49×10⁶, 6.03×10⁶ and 7.14×10⁶ g/cfu respectively for PF₁, PF₂, PF₄ and PF₅ (Fig. 1). PF₃ however, recorded no bacterial growth on both the eggshell surface and eggshell content.

3.6 Bacterial Growth across Retail Markets

The morning eggs samples from three markets recorded bacterial growth on the shell surfaces only while those of RM₁ and RM₂, recorded bacterial growth on both the shell surface and the shell content (6.90×10⁶:4.31×10⁶) and (2.45×10⁶:1.93×10⁶) cfu/ml respectively (Fig. 2). The afternoon samples also resulted in the isolation of organisms only from the shell surfaces of the eggs obtained from the RM₁, RM₃, RM₄ and RM₅ with a mean total viable bacterial counts of 1.74×10⁶ cfu/ml, 8.57×10⁶ cfu/ml, 7.23×10⁶ cfu/ml and 6.23×10⁶ cfu/ml respectively. RM₂ market however, recorded isolates from both the eggshell and its content, with a mean total viable count of 1.74×10⁶ g/cfu and 5.24×10⁶ g/cfu respectively (Fig. 2). The evening samples showed that, the eggs from three (RM₂, RM₃ and RM₄) markets recorded bacterial count and these were isolated from the shell surfaces with a mean total viable count of 4.0×10⁶ cfu/ml, 6.4×10⁶ cfu/ml and 7.4×10⁶ cfu/ml respectively. None of the market showed bacterial growth in evening sampled egg content.

4. DISCUSSION

Freshly laid eggs are generally expected to be sterile [8]. Comprehensive studies, however, have indicated that microorganisms can be found

on the eggshell and egg content [9]. Contamination of chicken eggs can occur in a number of ways. Prior to being laid, chicken eggs may become horizontally infected, constituting movement of bacteria into the developing egg, while the egg is still in the oviduct of the hen. Bacterial contamination can also occur through vertical infection during the laying process. Hens are a common carrier of a number of bacteria and many of which, like Salmonella, exist in the alimentary canals. Eggs can be contaminated by these bacteria as they are deposited through the cloaca, a structure which serves as the end of the reproductive, urinary, and intestinal tract. In this study, some level of bacterial contaminations were isolated on both the eggshell surfaces and in the eggshell contents.

The microbial isolates were mainly; coagulase-negative Staphylococcus (74.5%), Staphylococcus aureus (2.7%), Escherichia coli (8.2%), Klebsiella sp. (8.2%), Proteus mirabilis (0.9%), Citrobacter sp. (3.6%), and Citrobacter diversus (1.8%). Even though some studies have shown that Salmonella enteritidis is mainly associated with egg contamination [10], in this study, however, Salmonella sp. was not isolated from the eggs which is in accordance with the work of EL-Kholy et al. [11] in Egypt. This difference in prevalence may partly be due to differences in sample size and the randomly sampled eggs used between the different

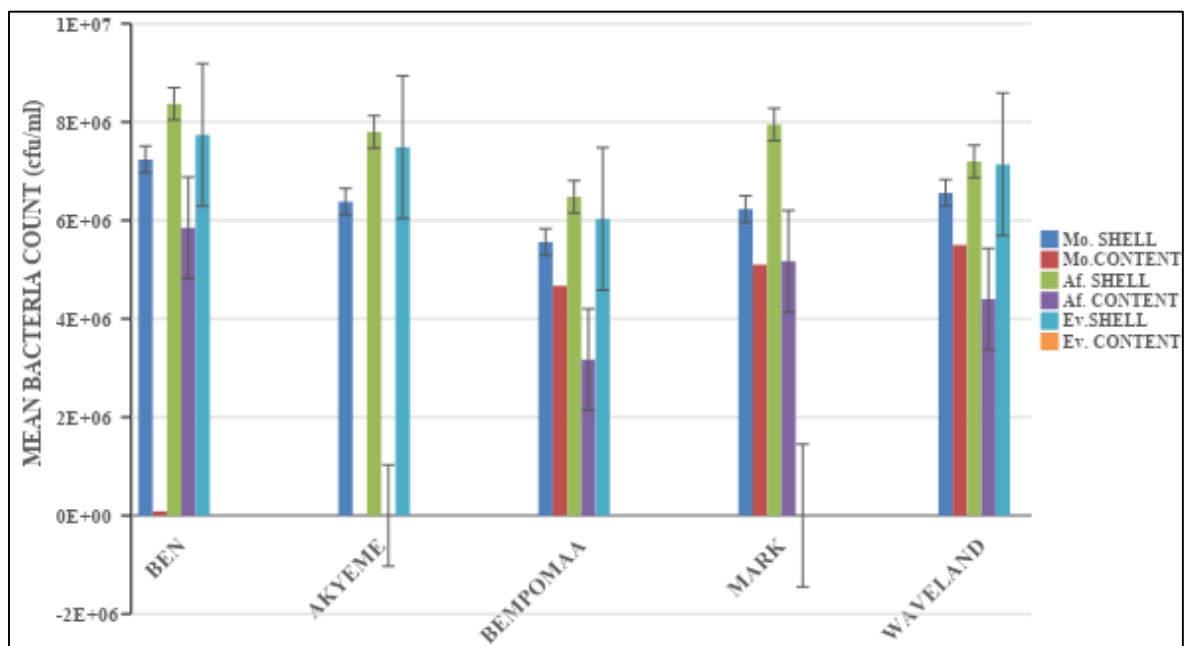


Fig. 1. Comparison of the bacterial load at different times of the day

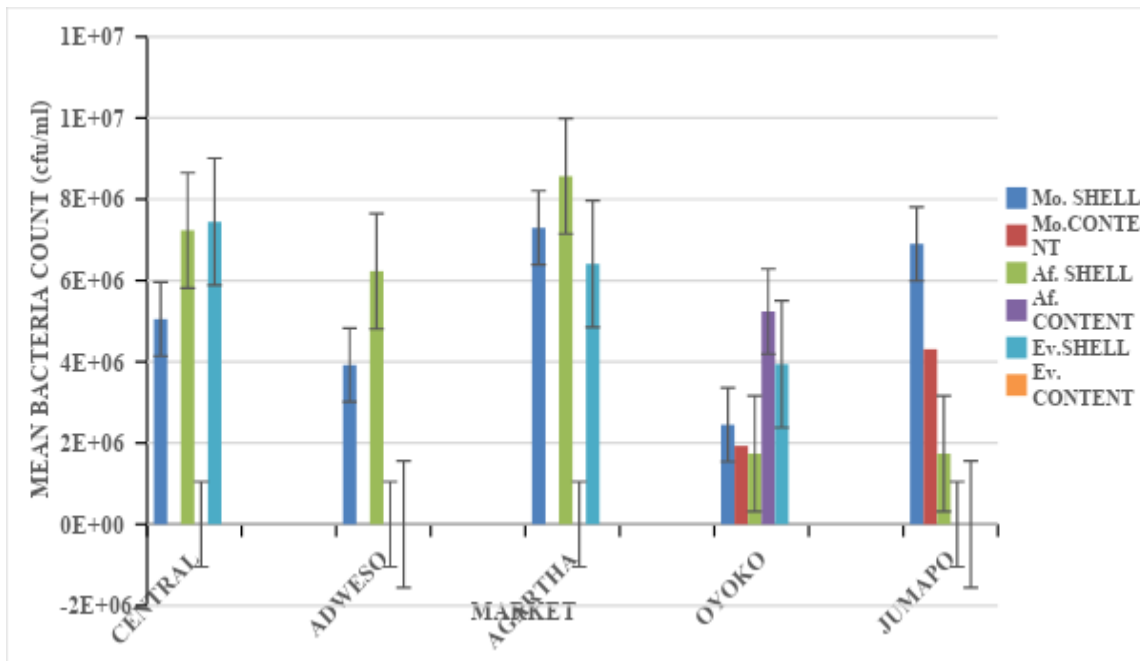


Fig. 2. Comparison of the bacterial load at different times of the day

studies. This may also be due to the fact that the eggs received from all the farms and the retail markets were free of salmonellosis. Our results is also supported by Ansah et al. [12] who reported the presence of Staphylococcus and Escherichia coli, in their studies conducted in the northern region of Ghana.

In a similar study conducted in Nigeria, Salihu et al. [13] also reported the isolation of Escherichia coli, Staphylococcus aureus, Klebsiella sp. and Citrobacter diversus from table egg. Generally, the isolation of coliforms is attributed to faecal contamination and poor hygienic conditions [14]. The isolation of *E. coli*, *Klebsiella spp.*, and *Citrobacter spp* either on the eggshell surface or in the eggshell content from both retail market and poultry farms reveals the poor hygienic conditions under which these farms were kept. Mahdavi et al. [14] reports that, the isolation of coliforms is attributed to faecal contamination and poor hygienic conditions.

This is in agreement with the work of Musgrove et al. [15] who reported that *Klebsiella sp.* is one of the most frequently isolated Gram-negative bacteria found on eggs. The presence of *Klebsiella spp.* in this study, may attributed to poor hygiene conditions which can lead to significant public health concern. Under these conditions, consumers, especially children, and immunologically impaired individuals are at risk

of developing diarrhoea, urinary tract infections, and other severe illnesses if the eggs are not washed well and cooked properly before consuming [10]. For example, *Klebsiella pneumoniae* is known to cause arthritis, meningitis, septicemia in neonates [16]. *Proteus mirabilis* is known to cause kidney stone disease upon altering the pH of urine to an alkaline state [17]. *Citrobacter* can cause a wide spectrum of infections in humans, such as infections in the urinary tract, respiratory tract, wounds, bone, peritoneum, endocardium, meninges, and bloodstream [18]. Among the various sites of infection, the urinary tract is the most common, followed by the abdomen, skin/soft tissues (including surgical site infection), and pneumonia [19]. In addition, the presence of *Klebsiella sp.* and *Citrobacter sp.* can lead to spoilage of the egg [12]. Bacterial contamination of the external and internal contents of the egg is influenced by several factors which are categorized into two as vertical and horizontal contamination [8]. *Klebsiella spp.* were isolated from the sampled eggs from both markets and poultry farms.

In this study, we determined the effect of time of egg collection on bacterial contamination of eggs. The time of the day in the collection of the eggs was found to be significantly associated with bacterial contamination. In most of the cases, even though not significant ($p > 0.05$), the afternoon samples recorded the highest mean

microbial load on the eggshell for all the five farms and three markets. Mesophilic microorganisms grow best at their optimum temperature of 25°C-40°C and this explains why the microbial load is highest in the afternoon as compared to the morning and evening. It is in agreement with the finding of Theron et al. (2003) who observed much higher organism counts in both the egg contents and on the eggshells, which had been subjected to high temperature shocks [20].

5. CONCLUSION

Despite the high nutritional values of egg, contaminations with coliforms and other pathogenic bacteria may result in critical health complications. The bacteria isolates and the sheer numbers from both the eggshells and the egg content confirm the poor hygienic conditions under which both the poultry farms and markets were kept. Even though serious human pathogens were not prevalent in this study, it is recommended that strict hygienic protocols be adhered to on the poultry farms and in the markets. The findings of this study will help inform policy on the intervention measures used to address bacterial contamination in poultry farms and the retail markets.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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