International Journal of Innovative Biosciences Research 12(1):10-19, Jan.-Mar., 2024

© SEAHI PUBLICATIONS, 2024 **www.seahipaj.org** **ISSN: 2467-8457**

Cultural and Metagenomics Characterization of Bacterial Associated With Selected Eggs of Domestic Birds in Bayelsa State, Nigeria

Otayoor, R.A[1*], Akpan, U. M[2], Dieware, G.K[2] and Abowei, J.F.N[1]

[1]Department of Biological Sciences,
Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

[2]Department of Science Laboratory Technology (Microbiology),
School of Applied Sciences,
Federal Polytechnic Ekowe, Bayelsa State, Nigeria

***Corresponding author email. richardotami3@gmail.com 07030810119**

ABSTRACT
Chicken eggs are cheap source of protein. There is a need to evaluate the bacterial characteristics of these birds' eggs. A total of 135 eggs from Ogbia, sagbama and Yenagoa poultry farms in Bayelsa state, were used. A rinks solution from the eggshell, extracted albumin and yolk were subjected to bacterial analysis. Cultural and metagenomics approach was used for the analysis/characterization. Result showed higher bacteria count of 67.0cfu/ml and 64.7cfu/ml on the eggs shell and yolk respectively, and no count was recorded in the albumin. The cultural dependent results obtained from this work recorded higher prevalence of bacteria species, with higher percentage abundance of *30.2%* for *Escherichia coli* followed by *Staphylococcus spp (20.4%).* The third most prevalent bacterium isolated *was Enterobacter spp (13.8%),* followed by *Citrobacter spp (10.2%), Pseudomonas spp (8.5%), bacillus spp (8.0%), Salmonella spp (6.7%),* and the least isolated bacterium generally was *Proteus spp (2.2%).* Operational Taxonomic Units (OTUs) contributing less than 1% of the total data was excluded, and the summarized metagenomic analysis of 16s/ITS1F gene sequencing showed *Escherichia. coli* (13.25%) with a higher percentage read count, followed by *Staphylococcus aureus* (10.65%), *Enterococcus faecalis* (9.15%) then *Enterobacter cloaca* (8.06%), for four (4) major bacteria. Bacteria identified were *Escherichia coli*, *Escherichia fergusoni*, *Salmonella enterica*, *Shigella sonnei*, *Enterococcus faecalis*, *Klepsiella grimorti*, *Staphylococcus aureus*, *Staphylococcus posteuri*, *Bacillus cereus*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, *Micrococcus caseclyticus*, *Enterobacter cloaca*, *Proteus mirabilis* and *Citrobacter freudi*. Eggs contain microorganism, despite their nutrition benefit. Good handling and hygiene practices in egg production firms should be encouraged.
Keywords: cultural, metagenomics, bacteria, domestic birds eggs, egg compartment.

INTRODUCTION
Egg exhibits a pronated and spheroid shape, characterized by one end being larger than the other (Hincke *et al.* 2012). This asymmetry is shown in female animals, with the egg displaying cylindrical symmetry along its long axis. The shell, the white, or albumen, and the yolk are the three main component of bird's eggs (Dudusola, 2010). Because chicken eggs are a cheap source of protein and they are consumed

frequently (Bashir et al., 2015). Hincke *et al.* (2010) examined an egg and saw that the egg yolk was inside, the white part suspends by tissues known as chalazae. Numerous communications have focused on the morphological variations that exist inside the shell, and their presence has been linked to dietary and environmental safety (EFSA, 2009).

Eggs have low economic cost and are of particular relevance from a nutritional standpoint because they contain significant amounts of important food nutrient and major elements (Sophie et al., 2019). Despite their nutraceutical values there are potential health issues associated with egg consumers due to microbial infestation (Abdul *et al*., 2012). It was recorded by Abdul *et al*., (2012) in a study that allowing eggs to be exposed to environmental conditions such as soil, dust, littered material, handling with the palm of workers, contact with bird fecal material, and dirty nesting materials, the eggs will be prone to different kinds of bacteria infestation. Bacteria would only infect or contaminate an egg and its content only when it overcomes the antibacterial effect of the egg albumen (Subramanian et al., 2005).

These bacteria are faced with varying effective defense mechanisms. This antibacterial defense mechanism is carried out by proteins, including ovo-transferrin, lysozyme, and avidin within the egg albumin (Grijspeerdt *et al.,* 2006). Despite the defense mechanisms recorded in eggs, there are potential microorganisms associated with eggs. Although there are numerous ways to identify these bacteria, one of which is the cultural dependent approach. However, the culture method can be time-consuming and labor-intensive (Hugenholtz, 2002). Recently, molecular techniques have made it possible to identify these bacteria more quickly. Therefore, there is a need to reevaluate the bacteria associated with selected domestic bird eggs in Bayelsa State using cultural and metagenomic approach.

MATERIALS AND METHOD

Sample collection

One hundred and thirty-five (135) eggs sample were collected aseptically, 3 each from 5 domestic birds in 3 different poultry farms located in Ogbia, sagbama and Yenagoa LGA *in* Bayelsa state *(Latitude: 4° 52' 3.9972. Longitude: 5° 53' 55.3704).* These poultry and livestock farms were represented as A, B, and C, for each farm location to protect the anonymity of the involved farms. Three (3) sample treatments and five (5) different egg types—local egg, layers egg, quail egg, duck fowl egg, and turkey egg—were used. These eggs sample were collected from the farms at different days of storage (days 1–7, 8–14, and 15–21). Each egg was deep into a 5mil prepared normal saline, right in the point of collection to obtain a rinks eggs shell solution.

Eggshell, York and albumin extraction

The rinks solution of the egg shell in the normal saline obtained at the site of collection was used. The eggs albumin and the yolk were aseptically extraction using a sterilized 100ml syringe. Using sterile forceps, the shells were punctured aseptically from the egg's blunt end.

Cultural technique

Five mil (5.0ml) albumin, yolk and the rinks solution were serially diluted separately. The microbial counts were enumerated by triplicate plating of 1-mL serially diluted sample solution from 10^{-3} dilution factor, and cultured onto a prepared 20ml nutrient agar plate and incubated at 37°C overnight. A drop of a prepared one gram each of itraconazole from 100ml of distilled water was added to prevent mixed growth of fungi. Bacteria colonies were counted and expressed in cfu/ml.

identification of isolates

Five (5) different positive isolates from each of the egg's types and compartment (shell: albumin: yolk), were randomly selected based on their morphology and colors, in a ratio of 3:0:2 for presumptive bacteria. These randomly selected isolates were each streaked for purity into the prepared agar plates and incubated at 37°C overnight to obtain pure bacterial culture for identification.

Phenotypic Characterization

The size, shape, color, edges, elevation, and structure of the colony were used to describe and isolate different species of bacteria. Every group of colonies that was morphologically distinguishable from the

others was isolated. Samples that were isolated were grown at their appropriate conditions and kept at 4°C.

Biochemical test

Catalase Test was carried out using 3% hydrogen peroxide to check for immediate resulting bubble as positive catalase. Indole Test was done using 3ml tryptone water, inoculated with pure culture and incubated at 35-37^{0}C for 48 hours after which 0.5ml of Kovac's reagent was added and checked for red coloration on the surface layer within 10 minutes, for positive indole. Lactose Test was carried out using phenol red lactose solution for 24-hour incubation period at a temperature range of 35–37 degrees Celsius and a color change from red to yellow was checked for as positive test. Coagulase Test was carried out to check for pathogenic staphylococcus species that produce coagulase enzymes, resulting to Clumping or coagulation within 10 seconds, after loop full of plasma was emulsified on the pure isolate, on a slide. Blood Medium was also used to test for pathogenicity by streaked pure isolate on a plate of blood agar for 24 hours, under B.O.D and checked for clear zone surrounding the colony which indicates lysis of the red blood cells or hemolysis (β hemolysis) as positive test. Kligler iron agar slant of butt of 20mm deep and a slope of 25mm long, by stabbing the butt and streaking the slope in a zig-zag pattern with the pure isolate and incubate at 35^{0}C 24h. A yellow slope and butt indicate lactose and glucose fermentation, red-pink slope and butt indicate no fermentation. Blacking along the stab line indicate H_2S production, while cracks and bubbles indicate gas production from glucose fermentation

Molecular analysis

Five mil (5.0ml) each of the sample's eggs albumin, yolk and rinks solution of the egg shell in an Eppendorf tube, were used for the molecular analysis. These samples obtained were sent out to inqaba biotec South African for molecular analysis. According to the directions, DNA was extracted using the Zymo Research for Bacterial DNA Kit for 16S Bacterial DNA. In a nutshell, DNA was extracted from lysed bacterial using the column approach. The metagenomic analysis of 16s/ITS1F gene sequencing was done with MiSeq system by illumina (www.illumina.com). Reads were processed through usearch (https://drive5.com/usearch) and taxonomic information was determined based on the Ribosomal Database Project's (http://rdp.cme.msu.edu/index.jsp) 16s database v16 or in the case of ITS1F, the RDP ITS V2 database. OTUs with a total data contribution of less than 1% were omitted from the analysis.

Statistical Analysis

Mean and standard deviation were calculated for all measured parameters. A one-way ANOVA was employed to compared means for variability or similarities. Turkey HSD Post Hoc test was used to separate mean were variability occurred. The analysis was done by the use of SPSS version 20.0.

RESULTS

Cultural dependent analysis of sample eggs compartments

The experimental results show that bacteria colony count from the shell was higher than the yolk, while the albumin has no or fewer count. Result obtained (table 1, 2 & 3) shows that higher bacteria count was recorded on eggs shell collected in ogbia, sagbama and Yenagoa with a mean and standard deviation values ranging from 30.7±2.5–67.0±5.0x10^3, 33.7±1.5–61.7±5.0x10^3, 30.0±0.6–66.3±6.1x10^3 respectively, followed by the egg yolk 27.7±4.0–64.7±9.9x10^3, 27.3±2.1–55.7±5.9x10^3, 21.7±2.1–53.7±6.7x10^3. There was no (0.0±0.0) or fewer (0.3±0.6) bacteria count from the albumin in all the farms. A significant difference was recorded among egg compartment at $p<0.05$

Table 1 Mean and standard deviation for the bacteria colonies from Ogbia L.G.A

		Bacteria (x 10^3 cfu/ml)		
Storage days	Egg type	Egg Shell	Egg Albumin	Egg Yolk
	L.F.E	33.0±3.0^b	0.0±0.0^a	27.7±4.0^c
1-7days	P.L.E	33.3±6.2^b	0.0±0.0^a	32.3±1.5^c
	Q.B.E	30.7±2.5^b	0.0±0.0^a	28.7±1.5^b
	D.F.E	41.0±7.5^b	0.0±0.0^a	35.7±4.2^c
	T.B.E	39.3±7.2^b	0.0±0.0^a	32.7±6.7^c
	L.F.E	46.7±5.1^c	0.0±0.0^a	43.7±5.1^c
8-14days	P.L.E	46.0±6.6^b	0.0±0.0^a	41.3±3.5^c
	Q.B.E	48.0±3.6^b	0.0±0.0^a	41.7±3.8^c
	D.F.E	46.3±6.1^b	0.0±0.0^a	43.3±3.5^b
	T.B.E	48.7±9.0^b	0.0±0.6^a	37.6±3.8^c
	L.F.E	62.0±9.0^c	0.0±0.0^a	57.7±11.0^c
15-21days	P.L.E	67.0±5.0^c	0.0±0.6^a	64.7±9.9^c
	Q.B.E	62.7±4.2^b	0.0±0.0^a	53.3±3.5^c
	D.F.E	61.0±10.5^b	0.0±0.0^a	54.0±7.2^c
	T.B.E	60.0±5.3^b	0.0±0.0^a	49.0±9.2^c

L.F.E = local fowl egg, P.L.E= poultry layers eggs, Q.B.E= quail bird eggs, D.F.E= duct fowl eggs and T.B.E= turkey bird eggs. Different superscript along column indicates a significant difference among treated value at p<0.05.

Table 2 Mean and standard deviation for the bacteria colonies from Sagbama L.G.A

		Bacteria (x 10^3 cfu/ml)		
Storage days	Egg type	Egg Shell	Egg Albumin	Egg Yolk
	L.F.E	36.7±2.1^d	0.0±0.6^b	33.3±1.5^d
1-7days	P.L.E	34.3±1.5^d	0.0±0.6^b	29.3±3.1^a
	Q.B.E	33.7±4.5^a	0.0±0.0^b	28.7±1.5^c
	D.F.E	33.7±1.5^c	0.0±0.6^b	28.3±2.9^c
	T.B.E	33.7±3.5^c	0.0±0.0^b	27.3±2.1^a
	L.F.E	44.3±5.1^c	0.0±0.6^b	39.7±2.1^c
8-14days	P.L.E	44.3±2.5^c	0.0±0.0^b	41.3±3.1^c
	Q.B.E	41.7±4.5^a	0.0±0.0^b	37.0±2.0^a
	D.F.E	44.7±4.5^c	0.0±0.6^b	38.0±1.7a
	T.B.E	45.3±3.5^c	0.0±0.0^b	39.3±5.5^b
	L.F.E	61.7±5.0^a	0.0±0.0^b	54.7±5.5^c
15-21days	P.L.E	52.0±7.2^a	0.3±0.6^b	39.7±11.6^c
	Q.B.E	56.7±7.8^a	0.0±0.0^b	50.0±10.4^c
	D.F.E	60.3±3.1^a	0.0±0.6^b	55.7±5.9^c
	T.B.E	55.0±12.3^a	0.0±0.0^b	45.7±16.2^c

L.F.E = local fowl egg, P.L.E= poultry layers eggs, Q.B.E= quail bird eggs, D.F.E= duct fowl eggs and T.B.E= turkey bird eggs. Different superscript along column indicates a significant difference treated value at p<0.05.

Table 3 Mean and standard deviation for the bacteria colonies from Yanegoa L.G.A

		Bacteria (x 10^3 cfu/ml)		
Storage days	Egg type	Egg Shell	Egg Albumin	Egg Yolk
	L.F.E	30.0±1.7[a]	0.0±0.6[b]	21.7±2.1[e]
1-7days	P.L.E	33.3±8.4[a]	0.0±0.6[b]	26.3±6.5[c]
	Q.B.E	28.0±6.2[a]	0.0±0.0[b]	23.7±4.0[a]
	D.F.E	39.7±4.1[a]	0.0±0.6[b]	35.0±4.6[c]
	T.B.E	39.3±7.2[a]	0.0±0.0[b]	32.7±6.7[c]
	L.F.E	47.0±3.0[c]	0.0±0.6[b]	42.3±3.5[d]
8-14days	P.L.E	48.7±5.7[c]	0.0±0.0[b]	39.3±3.1[d]
	Q.B.E	48.0±4.6[c]	0.0±0.0[b]	40.7±2.3[d]
	D.F.E	48.3±3.5[c]	0.0±0.6[b]	40.7±4.5[d]
	T.B.E	49.3±5.9[c]	0.0±0.0[b]	38.3±6.4[d]
	L.F.E	61.3±9.0[e]	0.0±0.0[b]	53.0±8.5[c]
15-21days	P.L.E	66.3±6.1[e]	0.0±0.6[b]	53.7±6.7[c]
	Q.B.E	61.0±5.0[e]	0.0±0.0[b]	48.3±1.5[c]
	D.F.E	61.3±4.6[e]	0.0±0.6[b]	49.7±7.8[c]
	T.B.E	56.3±4.9[c]	0.0±0.0[b]	43.7±9.5[d]

L.F.E = local fowl egg, P.L.E= poultry layers eggs, Q.B.E= quail bird eggs, D.F.E= duct fowl eggs and T.B.E= turkey bird eggs. Different superscript along column indicates a significant difference among sample values at $p<0.05$.

From results shown in table 4, for all the pure isolates observed morphologically from their growth media, based on elevation, shapes margin and their gram reactions microscopically, shows that *Citrobacter spp, Proteus spp, Escherichia coli, Staphylococcus spp, Enterobacter spp, Salmonella spp, pseudomonas spp and bacillus spp* were present. These bacteria characterized were mostly from the family *Enterobacteriaceae, Pseudomonadaceae, Bacillaceae* and *Staphylococcaceae*. A higher prevalence of *Enterobacteriaceae was recorded, which include Citrobacter spp, Proteus spp, Escherichia coli, Enterobacter spp, and Salmonella spp.* Other bacteria identified is *pseudomonas spp,* belonging to the family *Pseudomonadaceae, Staphylococcus spp*; belonging to the family *Staphylococcaceae*, and *Bacillus spp,* from the family *Bacillaceae*.

Table 4; Morphological and biochemical characterization of the identification bacteria

COLONY MORPHOLOGY	Grey Colony	creamy & moist	colorless colony	Creamy Yellow & circular	Milkish in color	Round & moist	Light green colony	colorless & circular
Gram reaction	-ve Rod	-ve Rod	-ve Rod	+ve cocci	-ve Rod	-ve Rod	-ve Rod	+ve cocci
Eleviation	convex	Flat	convex	Convex	convex	Convex	umbonate	convex
Margin	entire	serrated	entire	Entire	entire	Entire	undulate	smooth
Shape	circular	circular	circular	Irregular	circular	Irregular	circular	Circular
BICHEMICAL TEST								
Cat.	–	+	–	+	–	–	+	+
Ind.	+	+	+	–	-	-	-	-
Lactose	-	-	+	+	+	-	-	+
Pathogenicty Test								
Coagulation	–	–	–	+	–	–	–	–
Blood (heamolysis)	–	–	–	+	–	–	+	+
Kia Medium								
Slope	R	R	Y	Y	Y	R	Y	Y
Butt	Y	Y	Y	Y	Y	Y	Y	Y
H_2S	+	+	-	+	-	+	-	-
Gas	+	+	+	+	+	+	+	+
IDENTIFIED BACTERIA	*Citrobacter spp*	*Proteus spp*	*Escherichia coli*	*Staphylococcus spp*	*Enterobacter spp*	*Salmonella spp*	*Pseudomonas spp*	*Bacillus spp*

Cat=Catalase. Ind= Indole. Coag=Coagulates. Lac=Lactose. Glu=Glucose N=Nutrien Agar.

Percentage (%) prevalence and abundance of the identified microorganism as recorded in table 5, shows that bacteria are associated with commercial eggs. *Escherichia coli (30.2%),* was recorded having higher percentage (%) prevalence or abundance. The second bacterium generally isolated was *Staphylococcus*

spp (20.4%). The third prevalent bacterium isolated *was Enterobacter spp (13.8%),* followed by *Citrobacter spp (10.2%), Pseudomonas spp (8.5%), bacillus spp (8.0%), Salmonella spp (6.7%),* and the least isolated bacterium generally was *Proteus spp (2.2%).*

Table 5: Percentage frequency of the identified Bacteria isolated from egg samples

BACTARIA ISOLATE	Ogbia Farms [(TN×100)/75]				Sagbama farms [(TN ×100)/75]				Yenagoa farms [(TN ×100)/75]				[(O+SA+Y)×100/225]
	S	A	Y	% FRQ.	S	A	Y	% FRQ.	S	A	Y	% FRQ.	OVERALL %
Citrobacter spp	8	-	2	13.3%	7	-	1	10.7%	5	-	-	6.7%	10.2%
Proteus spp	3	-	-	4.0%	-	-	-	0.0%	2	-	-	2.7%	2.2%
Escherichia Coli	18	-	5	30.7%	15	-	6	28.0%	19	-	5	32.0%	30.2%
Staphylococcus spp	11	-	5	21.3%	12	-	2	18.7%	14	-	2	21.3%	20.4%
Enterobacter spp	6	-	3	12.0%	10	-	1	14.5%	8	-	3	14.7%	13.8%
Salmonella spp	2	-	-	2.7%	4	-	1	6.7%	8	-	-	10.7%	6.7%
Pseudomona spp	5	-	2	9.3%	7	-	1	10.7%	4	-	-	5.2%	8.5%
Bacillus spp	4	-	1	6.7%	8	-	-	10.7%	5	-	-	6.7%	8.0%
TOTAL				**100%**				**100%**				**100%**	**100%**

O=Ogbia, SA= sagbama, Y= Yenagoa, S= shell, A= albumin, Y=York, TN= total number.

Metagenomic results for the sample eggs and compartments

The bacterial metagenomic result (table 6), showed that all isolates associated with the sample egg compartments belong to two phyla; *Pseudomonadota* and *Bacillota*, two classes; *Gammaproteobacterial* and *Bacilli*, four order; *Enterobacterales, Lactobacillales, Bacillale* and *Pseudomonadales,* five family; *Enterobacteraceae, Enterococcaceae, Staphylococcaceae, Bacillaceae* and *Pseudomonaceae,* fifteen species: *Escherichia. coli, Escherichia fergusoni*, *Salmonella enterica*, *Shigella sonnei*, *Enterococcus faecalis, Klepsiella grimorti*, *Staphylococcus aureus*, *Staphylococcus posteuri*, *Bacillus cereus*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, *Micrococcus caseclyticus*, *Enterobacter cloaca*, *Proteus mirabilis* and *Citrobacter freudi*. The *Escherichia. coli* was isolated from shell egg of poultry layers eggs, *Escherichia fergusoni* from the Yolk of local fowl eggs, *Salmonella enterica* from shells of poultry layers eggs, *Shigella sonnei* from Yolk of local fowl eggs, *Enterococcus faecalis* from shell of local fowl eggs, *Klepsiella grimorti* from the Yolk of quail birds eggs, *Staphylococcus aureus* from shells of poultry layers eggs, *Staphylococcus posteuri* from Yolk of duck fowl eggs, *Bacillus cereus* from shell of poultry layers eggs, *Bacillus pumilus* from Yolk of local fowl eggs, *Pseudomonas aeruginosa* from shell of quail birds, *Micrococcus caseclyticus* from Yolk of Turkey, *Enterobacter cloaca* from shells of local fowl eggs, *Proteus mirabilis* from shells of duck fowl eggs and *Citrobacter freudi* from shell of turkey eggs. Operational Taxonomic Units (OTUs) contributing less than 1% of the total data was excluded, and the summarized metagenomic analysis of 16s/ITS1F gene sequencing (table 6) showed that *Escherichia. coli* (13.25%) recorded a higher percentage read count, followed by *Staphylococcus aureus* (10.65%), *Enterococcus faecalis* (9.15%) then *Enterobacter cloaca* (8.06%), for four (4) major bacteria. Fungi percentage read count recorded higher percentage of *Mucor racemosus* (20.07%), followed by *Rhizopus stolonifera* (18.64%), *Penicillum albican* (10.39%) then *Aspergillus flavur* (5.38%).

Table 6 Bacteria Metagenomics of sampled eggs compartment collected from Sagbama, Ogbia and Yenagoa.

Taxa.	Shell	Albumin	Yolk	PLE	LE	QE	DE	TE
Phylum	*Pseudomonadota*	—	*Pseudomonadota*	+	+	+	+	+
	Bacillota	—	*Bacillota*	+	+	+	+	+
Class	*Gammaproteobacterial*	—	*Gammaproteo-bacterial*	+	+	+	+	+
	Bacilli	—	*Bacilli*	+	+	+	+	+
Order	*Enterobacterales*	—	*Enterobacterales*	+	+	+	+	+
	Lactobacillales	—	*Lactobacillales*	+	+	+	+	+
	Bacillale	—	*Bacillale*	+	+	+	+	+
	Pseudomonadales	—	*Pseudomonadales*	—	—	+	—	—
family	*Enterobacteraceae*	—	*Enterobacteraceae*	+	+	+	+	+
	Enterococcaceae	—		—	+	—	—	—
	Staphylococcaceae	—	*Staphylococcaceae*	+	—	—	+	—
	Bacillaceae	—	*Bacillaceae*	+	+	—	—	—
	Pseudomonaceae	—		—	—	+	—	—
Genus / Species	*Escherichia. coli*	—	*Escherichia fergusoni*	**Ec/Sh**	**Ef/Yk**	—	—	—
	Salmonella enterica	—	*Shigella sonnei*	**Ss/Yk**	**Se/Sh**	—	—	—
	Enterococcus feacalis	—	*Klepsiella grimorti*		**Ef/Sh**	**Kg/Yk**		
	Staphylococcus aureus	—	*Staphylococcus posteuri*	**Sa/sh**	—	—	**Sp/Yk**	—
	Bacillus cereus	—	*Bacillus pumilus*	**Bc/Sh**	**Bp/Yk**	—	—	—
	Pseudomonas aerugenosa	—	*Micrococcus caseclyticus*	—	—	**Pa/Sh**	—	**Mc/Yk**
	Enterobacter cloaca	—	—	—	**Ec/Sh**	—	—	—
	Proteus mirabilis	—	—	—	—	—	**Pm/Sh**	—
	Citrobacter freudi	—	—	—	—	—	—	**Cf/Sh**

Keys; — = Absent or not involve, + = present or confirm**,** Ec/Sh= *Escherichia. coli* isolated from shell egg, Ef/Yk= *Escherichia fergusoni* from yolk, Ss/Yk= *Shigella sonnei* from yolk, Se/Sh= *Salmonella enterica* from shell, Ef/Sh= *Enterococcus faecalis* from shell, Kg/Yk= *Klepsiella grimorti* from yolk, Sa/sh= *Staphylococcus aureus* from shell, Sp/Yk= *Staphylococcus posteuri* from york, Bc/Sh=*Bacillus cereus* from shell, Bp/Yk= *Bacillus pumilus* from yolk, Pa/Sh= *Pseudomonas aeruginosa* from shell, Mc/Yk= *Micrococcus caseclyticus* from yolk, Ec/Sh= *Enterobacter cloaca* from shell, Pm/Sh= *Proteus mirabilis* from shell, Cf/Sh= *Citrobacter freudi* from shell.

DISCUSSION

The cultural dependent procedure revealed the bacteria associated with sampled eggs at species level, and the result revealed the present of *Citrobacter spp, Proteus spp, Escherichia coli, Staphylococcus spp, Enterobacter spp, Salmonella spp, pseudomonas spp and bacillus spp*. These bacteria identified were mostly from the family *Enterobacteriaceae, Pseudomonadaceae, Bacillaceae* and *Staphylococcaceae*. This result is similar to a work carried out by Arathy *et al*., (2009), which isolated bacteria from table eggs and find out that majority of the bacteria isolated belong to family; *Enterobacteriaceae*. Some of the identified bacteria are normal flora, others opportunistic and a few pathogenic (Musgrove, et al., 2004).

The cultural dependent results obtained from this work recorded higher prevalence of bacteria species, with higher percentage abundance of *30.2%,* for *Escherichia coli* followed by *Staphylococcus spp (20.4%).* The third prevalent bacterium isolated *was Enterobacter spp (13.8%),* followed by *Citrobacter spp (10.2%), Pseudomonas spp (8.5%), bacillus spp (8.0%), Salmonella spp (6.7%),* and the least isolated bacterium generally was *Proteus spp (2.2%).* The presence of *Salmonella spp* in this study is the same with a recent work conducted by Guard-Petter (2001), which stated that *Salmonella spp* causes of food-borne infection known as salmonellosis.

Following metagenomic description of sample bacterial (15) focusing the 16S rRNA gene, phenotypic characterization of the bacteria isolates was carried out for assumptive confirmation. The identified bacteria include *Escherichia. coli, Escherichia fergusoni, Salmonella enterica, Shigella sonnei,*

Enterococcus faecalis, Klepsiella grimorti, Staphylococcus aureus, Staphylococcus posteuri, Bacillus cereus, Bacillus pumilus, Pseudomonas aeruginosa, Micrococcus caseclyticus, Enterobacter cloaca, Proteus mirabilis and *Citrobacter freudi*. Although *Staphylococcus posteuri* is typically thought of as a food-poisoning bacterium (Hennekinne et al., 2012), reports of it are common since the disease it causes is mild (Paparella et al., 2018). The use of specific food products is frequently linked to *S. aureus* foodborne disease (Madahi et al., 2014). *S. aureus* contamination of dairy products has already been documented (Guven et al., 2010). By avoiding the oxidation of unsaturated free fatty acids, *Staphylococcus posteuri* has been shown to be able to reduce nitrite, improve colour stability, and prevent rancidity (Talon and Leroy, 2006; Mainar et al., 2017). According to numerous reports, coagulase-negative staphylococci frequently predominate in food and egg products, and could aid in biodefence against pathogenic organisms (Lorenzo et al., 2015; Mainar et al., 2017).

The results also showed a number of Enterobacteriaceae bacteria, which are frequently found in the intestines of animals and are able to live in a variety of settings (Martinson et al., 2019; Schierack et al., 2007). They may, however, also result in a range of nosocomial and community-acquired (foodborne) diseases (Bereket et al., 2012). Animal feces have frequently been observed to include Klebsiella as a contaminant (Gundogan et al., 2013). Salmonella and Shigella are frequently transferred to food by an unhygienic food handler (Garedew et al., 2016). Salmonella is a prevalent bacterial pathogen in instances of foodborne disease with laboratory confirmation, and it can cause bacteremia, typhoid fever, and gastroenteritis (USDAFSIS, 2015). Several studies reported the prevalence of *S. enteritidis*, and it was recorded that *Salmonella* species infected eggs from most sites of poultry farm that is not highly clean (Arathy et al., 2009). Human foodborne sickness is caused by the endospore-forming bacterium *B. cereus* and *pumilus* (Tewari et al., 2015).

However, it should be understood that a decline in diversity does not equate to a decline in density. There may be more bacteria in the egg compartment, but they would likely be less diverse, meaning that they would mostly consist of a small number of morphologies rather than a wide range (Xu, 2006). It should also be mentioned that the difference between albumin/yolk diversity and shell diversity was statistically significant ($P > 0.05$). Furthermore, it should be highlighted that no bacterial morphologies, or colonies, were produced by the albumin samples (Koch, 2002 & 2003). This is in line with theories put up in prior research that suggested the albumin may be resistant to contamination because it contains lysozyme, is alkaline in nature, and is viscous, all of which would slow down bacterial motility (Wu et al., 2006). As a result, the diversity seen in the yolk may be less than that in the membrane because the albumin that surrounds the yolk prevents additional contamination of the internal egg components (Kojima & Blair, 2004). In this situation, albumin may stop germs from migrating from the yolk to the egg's outer layers.

CONCLUSION

The visible pollutants on the bird eggs included feces and dirt. A sign of microbial contamination is present as a result of the environment and continuous exposure to laying materials. Depending on where the egg was sampled, there is a considerable variance in the richness of bacterial. Consequently, the outermost layer of the egg shell, being the initial point of contact with the environment, has higher potential to a wider array of bacteria compared to the underneath layers. The observed decline in diversity and abundance from the outer shell to the inner membrane, and further from the membrane to the internal structures such as the yolk and albumin, could perhaps indicate a combination of vertical contamination and the development of resistance mechanisms. The cuticle serves as a barrier that effectively limit the diversity and abundance of microbes towards the central region of the egg. Microbial effect on eggs should be routinely checked. This practice is not quite common in Nigeria. Therefore, regulations for assessing quality eggs before retail-sales should be made available.

REFERENCES

Hincke MT, Nys Y and Gautron J (2010). The Role of matrix proteins in eggshell formation. *Journal of Poultry Science*, 47 (3), 208-219.

Hincke MT, Nys Y, Gautron J, Mann K, Rodriguez-Navarro AB and McKee MD (2012). The eggshell: structure, composition and mineralization. *Frontiers in Biosciences special edition on Biomineralization.* 17, 1266-1280.

Dudusola, IO. (2010). Comparative evaluation of internal and external qualities of eggs from quail and guinea fowl. *International Research Journal of Plant Science* 1, 112-115.

Bashir, L., Ossai, PC., Shittu, OK., Abubakar, AN. and Caleb, T. (2015). Comparison of the nutritional value of egg yolk and egg albumin from domestic chicken, guinea fowl, and hybrid chicken. *American Journal of Experimental Agriculture* 6, 310-316.

Abdul, ASB; Sabry, AH; and Baling, AE (2012). Microbial Quality and Content Aflatoxins of Commercially Available Eggs in Taif, Saudi Arabia, *Afric. J. Micro. Res*. 6(13), 3337- 3342.

European Food Safety Authority (EFSA), (2005). Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to the Microbiological risks on washing of Table Eggs. *The EFSA Journal*, 269, 1-39.

Sophie R.G, Nicolas G., and Yves N. (2019). The Golden Egg: Nutritional Value, Bioactivities, and Emerging Benefits for Human Health. *Nutrients.* 11(3): 684.

Subramanian, S., Braham, O.C. Rupali, P. Zachariah, A. Mathews, M.S. and Mathai, D. (2005). Disseminated histoplasmosis. *J. Assoc. Phys. India*, 53, 185-189.

Grijspeerdt K, De Reu K, Messens W, Heyndrickx A, Uyttendaele M, Debevere J and Herman L. (2006). Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including Salmonella enteritidis. *Int J Food Microbiol*, 112(3)**,** 253-260.

Hugenholtz P, (2002). Exploring prokaryotic diversity in the genomic era. *Genome Biol.* 3:1–8.

Arathy, S; Vanpee, G; Belot, G; Vanessa, M; Claude, D; and Ravindra, NS (2009). Bacterial Contamination of Commercial Chicken Eggs in Canada, West Indies. *West Ind. Vet*. J. 9(2), 4-7.

Musgrove, M. T., Jones, D. R. and Northcutt J. K (2004): Identification of Enterobacteriaceae from washed and unwashed commercial shell eggs. *J. Food Prot.*, 67, 2613–2616.

Guard-Petter J. (2001). The chicken, the egg and Salmonella enteritidis. *Environmental microbiology.* 3(7); 421-430.

Guven, K., Mutlu, M. B., Gulbandilar, A., and Cakir, P. (2010). Occurrence and characterization of Staphylococcus aureus isolated from meat and dairy products consumed in Turkey. *J. Food Safety* 30, 196–212.

Hennekinne, J. A., De Buyser, M. K., and Dragacci, S. (2012). Staphylococcus aureus and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiol*. Rev. 36, 815–836.

Madahi, H., Rostami, F., Rahimi, E., and Dehkordi, F. S. (2014). Prevalence of enterotoxigenic Staphylococcus aureus isolated from chicken nugget in Iran. Jundishapur. *J. Microbiol*. 7:e10237.

Paparella, A., Serio, A., Rossi, C., Mazzarrino, G., and López, C. C. (2018). "Food-Borne transmission of staphylococci," in Pet-To-Man Travelling Staphylococci, ed. V. Savini (*Cambridge: Academic Press*), 71–94.

Mainar, M. S., Stavropoulou, D. A., and Leroy, F. (2017). Exploring the metabolic heterogeneity of coagulase-negative staphylococci to improve the quality and safety of fermented meats: a review. *Int. J. Food Microbiol*. 247, 24–37.

Talon, R., and Leroy, S. (2006). "Latest developments in meat bacterial starters," *in Advanced Technologies for Meat Processing,* eds L. M. L. Nollet and F. Toldra (New York, NY: CRC Press), 401–418.

Lorenzo, J. M., Bermúdez, R., Domínguez, R., Guiotto, A., Franco, D., and Purriños, L. (2015). Physicochemical and microbial changes during the manufacturing process of dry-cured lacón

salted with potassium, calcium and magnesium chloride as a partial replacement for sodium chloride. *Food Contr*. 50, 763–769.

Martinson, J. N. V., Pinkham, N. V., Peters, G. W., Cho, H., Heng, J., and Rauch, M. (2019). Rethinking gut microbiome residency and the Enterobacteriaceae in healthy human adults. *J. ISME*. 13, 2306–2318.

Schierack, P., Walk, N., Reiter, K., Weyrauch, K., and Wieler, L. (2007). Composition of intestinal Enterobacteriaceae populations of healthy domestic pigs. *Microbiology* 153, 3830–3837.

Bereket, W., Hemalatha, K., Getenet, B., Wondwossen, T., Solomon, A., and Zeynudin, A., (2012). Update on bacterial nosocomial infections. *Eur. Rev. Med. Pharmacol*. Sci. 16, 1039–1044.

Gundogan, N., Ataol, O., and Ozturk, F. (2013). Determination of virulence factors in Staphylococcus aureus, Enterococcus faecalis and Enterococcus faecium isolated from meat and milk products. *J. Food Safety* 33, 387–393.

Garedew, L., Hagos, Z., Zegeye, B., and Addis, Z. (2016). The detection and antimicrobial susceptibility profile of Shigella isolates from meat and swab samples at butchers' shops in Gondar town, Northwest Ethiopia. *J. Infect. Public Health* 9, 348–355.

United States Department of Agriculture, Food Safety and In- spection Service (USDA-FSIS) (2015). Salmonella enteritidis risk assessment. Available from: http://www. fsis.usda.gov /ophs/ risk/ Accessed Oct. 20.

Tewari, A., Singh, S. P., and Singh, R. (2015). Incidence and enterotoxigenic profile of Bacillus cereus in meat and meat products of Uttarakhand, India. *J. Food Sci. Technol*. 52, 1796–1801.

Xu J., (2006). "Microbial ecology in the age of genomics and metagenomics: concepts, tools, and recent advances". *Mol Ecol*., 15 (7), 1713–31.

Koch, A.L. (2003). "Bacterial wall as target for attack: past, present, and future research". *Clin Microbiol Rev.*, 16 (4), 673–87.

Wu M, Roberts J, Kim S, Koch D and DeLisa M (2006). "Collective bacterial dynamics revealed using a three-dimensional population-scale defocused particle tracking technique". *Appl Environ Microbiol.*, 72 (7), 4987–94.

Kojima S and Blair DF (2004). "The bacterial flagellar motor: structure and function of a complex molecular machine". *Int Rev Cytol. International Review of Cytology*, 233, 93–134.