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Sequential Pathogenicity Study of Enteric Bacteria Isolated from Chicken Feeds on Broiler Chickens

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Abstract
This study was carried out to investigate the pathogenic potentials of enteric bacteria isolated from chicken feeds sold in Anambra State, Nigeria. A total of 1,536 of different chicken feed samples were collected and screened for the presence of enteric bacteria using pour plate technique. The isolates obtained were characterized and identified. The pathogenic potentials of the isolates on broiler chickens were investigated by challenging the chickens orally using 0.5 ml of the inoculum (10^8cells/ml). All chickens were kept under complete observation for 4 weeks for pathological signs and symptoms, mortalities and gross morphological lesions of the internal organs. *Escherichia coli* O157:H7 SS52 (EC), *Salmonella* ser. Typhimurium U288 (ST), *Escherichia coli* SEC470 (ES), *Salmonella* ser. Enteritidis YU39 (SY) and *Salmonella* ser. Enteritidis FM366 (SE) were isolated from the feed samples. There were significant pathological lesions among the infected chickens, and these decreased significantly ($P<0.05$) as the age of the chickens increased.The histopathological examination of infected organs revealed congestion, mononuclear infiltration, hemorrhage, necrosis and significant destruction of the organs. The mean plate counts of EC was highest followed by SE, and ST was the least among the plated organs. The isolates showed obvious pathological features among the infected chickens, and these obvious pathological features were pronounced among the chicks mostly the day old chicks.

INTRODUCTION

The chicken industry has played a significant role in man's civilization in many ways and has gone through a phase of rapid development and commercialization (Olugbemi *et al*., 2004). The term "chicken" used in agriculture generally refers to all domestic fowls kept for egg laying or meat production (Onyeze *et al*., 2013).

The chicken industries rely on the supply of ready-to- use feed from feed mills, and these feeds undergo many processes before used by the chicken rearers (Aganaga *et al*., 2000). D' Mello (2006), reported that the impact of the general environmental and handling circumstances including the nature and extent of quality control measures determine the level of microbial contamination in chicken feeds.

High rate of chicken diseases and death have been traced from consumption of contaminated feeds (Onyeze *et al*., 2013). Some of the bacteria contaminants associated with commercially produced chicken feeds such as *Escherichia coli* and *Salmonella* species have been reported (D' Mello, 2006; Arotupin *et al*., 2007; Onyeze *et al*., 2013). Various types of chicken diseases which also affect humans have been traced from the contamination of feeds and chicken products by microorganisms, mainly enteric bacteria (Onyeze *et al*., 2013).

Previous studies focused on physiochemical properties and microorganisms associated with poultry feeds (Arotupin *et al.,* 2007; Chowdhuri *et al*., 2011; Habtamu *et al*., 2011; Onyeze *et al*., 2013), but little or no information have been published on the actual strains of pathogenic enteric bacteria associated with chicken feeds in Nigeria. There is also still paucity of information in characterizing the pathogenic features of enteric bacteria associated with chicken feeds, hence chicken diseases, economic losses; food borne diseases and death rates is of public concern. The objective of this study is to evaluate the sequential pathogenic features of enteric bacteria isolated from chicken feeds sold in Anambra State, Nigeria.

MATERIALS AND METHODS

Collection of Samples: A total of 1536 commercially produced poultry feed samples were aseptically collected from three major chains of distributors; wholesalers, retailers and consumers. The feed types which included X (756 samples), Y (756 samples) and Z (756 samples) were aseptically collected from twenty-one (21) major towns located within the twenty-one (21) Local Government Areas of Anambra State. One cup of the feed sample was aseptically collected from each feed type by randomly collecting one Table spoon of the feed sample from each bag containing the feed type. The feed samples were mixed and homogenized to generate a representative sample for each feed type. The representative feed samples were collected from Broiler starter (128 samples), Grower mash (128 samples), Broiler finisher (128 samples) and Layer mash (128 samples) for each feed type (X, Y and Z) using aluminum foil. The samples were carefully labeled, classified based on the sources of collection and transported to the laboratory for analysis within 1 h.

Culture and Isolation of Enteric Bacteria: This was carried out using the modified method of Arotupin *et al*.

(2007). One gram (1.0g) of each sample was first measured and dissolved in 10ml of sterile distilled water prior to serial dilution. One milliliter aliquot was aseptically transferred into a sterile test tube containing 9.0 ml of the diluent (distilled water) and from this; ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the sample was plated on *Salmonella-Shigella* agar (SSA/Biotech) for *Salmonella* and *Shigella* species and MacConkey agar (MA/Biotech) for coliforms and non coliforms. All the plates in triplicates were incubated inverted at 44.5˚C for 24 h for *E. coli* and 37˚C for 24 h for other enteric bacteria.

Characterization and Identification of the Isolates: The isolates were subcultured on nutrient agar (Biotech), incubated invertedly at 37˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions (Arotupin *et al.,* 2007), biochemical reactions (Arotupin *et al.,* 2007; Uwaezuoke and Ogbulie, 2008) and molecular characterization (Habtamu *et al.,* 2011; Gabriela *et al.,* 2014). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

Pathogenicity study: A total of eighty (80) chickens which included twenty (20) day-old broiler chicks of mixed sex, twenty (20) one-week old broiler chicks of mixed, twenty (20) two-weeks old broiler chicks of mixed sex and twenty (20) adult layer chickens obtained from Mrs. Chukwumaeze's chicken farm were used for evaluation of pathogenicity of the isolates. The chicks were kept in separate, thoroughly cleaned and disinfected houses and provided with feeds and water adlibitum. All the chicks were vaccinated against Newcastle disease using Lasota vaccine strains at 6 and 19 days of age, against infectious bronchitis using live H120 strain at 6 days old and also against avian influenza (A1) disease using inactivated H5N1virus vaccine strain at 7 days old. All the vaccines were given via eye drop instillation except (A1) vaccine which was given through subcutaneous route at the back of the neck.

Challenging the chickens with the test organisms: This was carried out using the method of Wafaa *et al.* (2012). Broth cultures of the isolates were centrifuged at 3000 r.p.m for 10 minutes. The sediments were diluted with sterile phosphate buffer saline (PBS) and adjusted to 10^8 cells/ml using McFarland matching standard prepared by mixing 0.6 ml of 1 % $BaCl_2.2H_2O$ and 99.4 ml of 1 % concentrated H_2SO_4 . Then each group (Day-old, One week, Two weeks and Adult layers) was inoculated orally with 0.5 ml of the inoculum while five (5) chickens from each group were only given distilled water as control.

Examination of infected chickens: The infected chickens were carefully observed for obvious pathological signs such as anorexia (loss of appetite), weakness (lack of physical strength), simple diarrhea, whitish diarrhea, bloody diarrhea and respiratory distress (difficulty in breathing) for a period of 4 weeks. The number of deaths was also observed. After 4 weeks, some of the infected chickens were sacrificed and gross examination of the morphologies of the internal organs and the intestines such as omphalitis (inflamed navel), air sacculitis (inflamed air sac), pericarditis (inflamed pericardium), perihepatitis (inflamed peritoneal covering the liver), hepatomegaly (liver enlargement), liver congestion, lung haemorrhage, eroded intestine, bloody intestine and fluid accumulation in the intestine was carried out (Wafaa *et al*., 2012)

Re-isolation of the challenged organisms from the infected chickens: The internal organs (Liver, Spleen, Lung and Heart) of the infected chickens were harvested and portions were macerated in peptone water and serial diluted using ten-fold serial dilution. Samples were inoculated into tetrathionate broth and peptone water, incubated at 37˚C for 24 h, then plated onto *Salmonella-Shigella* agar and MacConkey agar, and incubated at 37˚C for 24 h for *Salmonella* species and at 44.5˚C for 24 h for *E. coli*. The standard plate counts were carried out after 24 h. Cloacae swabs were also taken from the adult layered chicken and plated on MacConkey agar and *Salmonella-Shigella* agar and incubated at 37˚C for 24h for *Salmonella* species and at 44.5˚C for 24 h for *E. coli*. The standard plate counts was carried out after 24 h (Wafaa *et al.,* 2012).

Detection of the challenged organisms in the eggs laid by the infected chickens: The eggs of the infected adult layers were harvested and the albumins were aseptically collected using sterile syringe. One milliliter of the collected sample was aseptically plated on MacConkey agar and incubated at 44.5˚C for 24 h for *E. coli,* and also plated on *Salmonella-Shigella* agar and incubated at 37˚C for 24 h for *Salmonella* species (Wafaa *et al.,* 2012)

Histopathological study of the internal organs of infected chickens: This was carried out using the modified method of Mohkber *et al.* (2011). This study was done in Zoology Department, University of Nigeria, Nsukka. After 4 weeks, the chickens were sacrificed. The Liver, Lung, Spleen and Heart tissues were removed, portion of these tissues were washed and stored in Bouins solution for histopathological study.

RESULTS

Number of Samples that Showed Positive Results: Out of 1536 chicken feed samples collected from major towns located within the twenty-one (21) Local Government Areas of Anambra State, 934 (60.81%) samples were positive to enteric bacteria (Table1).

Characterization and identification of the Isolates from Feed Samples: The morphological characteristics of the isolates are shown in Table 2. Isolates 5, 7 and 11 were isolated from *Salmonella-Shigella* agar (SSA) and

they exhibited similar morphological characteristics on SSA plates. In addition, isolates E and G exhibited similar morphological characteristics on MacConkey agar (MA) plates. The isolates were further characterized using their biochemical and molecular characteristics as shown in Tables 3 and 4 respectively.

Obvious pathological Signs and Symptoms Associated with the Challenge Enteric Bacterial Isolates: The obvious pathological signs of the challenge enteric bacteria isolated on the infected chickens are shown in Tables 5, 6 and 7. Anorexia was common in both chickens infected by *E. coli* O157:H7SS52 and *Salmonella* species, and significantly ($P<0.05$) observed most in day-old chicks infected by *E.coli* O157:H7 SS52 and *Salmonella* species and one-week old chicks infected by *Salmonella* ser. Typhimurium U288. Bloody diarrhoea was significantly ($P<0.05$) observed mostly on those chicks infected by *Salmonella* ser. Typhimurium U288 and *E. coli* O157:H7 SS52. Omphalitis was only observed in adult-layers infected with *E. coli* O157:H7 SS52. Respiratory distress was only seen in those chicks infected by *E. coli* O157:H7 SS52. Death was also recorded among the infected chicks, and significantly ($P<0.05$) observed most on those chicks infected by *S.* ser. Enteritidis FM366. Diarrhoea and weakness were common among the infected chicks, and significantly ($P<0.05$) observed most on those chicks infected by *S.* ser. Enteritidis FM366. No pathological sign was observed in adult chickens infected by *Salmonella* species but diarrhoea was observed in two-fifth ($^2/_5$) of the adult chickens infected by *E.coli* O157:H7 SS52

Gross Pathological Features of Internal Organs and Intestines of Infected Chickens: The gross pathology of the internal organs and intestines of the infected chickens are shown in Tables 8, 9 and 10. Air sacculitis, pericarditis and lung haemorrhage were only seen in those chickens infected by *E. coli* O157:H7 SS52, and they were significantly ($P<0.05$) decreased as the age of the chickens increased. Bloody intestine and liver congestion were only seen in those chicks infected by *Salmonella* species. The liver congestion was non-significantly higher in *S.* ser. Enteritidis FM366 while the occurrence of bloody intestine was mostly observed in those chicks infected with *Salmonella* ser. Typhimurium U288 and *E. coli* O157:H7 SS52. Eroded intestine was mostly observed in those chicks infected by *Salmonella* species and was significantly ($P<0.05$) higher in *S.* ser. Typhimurium U288. Fluid accumulation in the intestines of the infected chickens was common pathological features seen among the infected chickens.

Total Mean Viable Plate Counts of the Challenge Isolates from the Samples Collected from the Cloaca of Infected Adult Layer Chickens: The total mean viable plate counts of the challenge enteric bacteria in the cloaca of infected adult-layer chickens are shown in Table 11. The result revealed that the total mean viable plate counts of *E. coli* O157:H7 SS52 was the highest while *S.* ser. Typhimurium U288 was the least. The total mean viable plate counts of infected adult-layer chicken was significantly ($P<0.05$) higher than the control adult-layer chicken.

Prevalence of the Challenge Isolates in the Eggs laid by the Infected Adult- Layer Chickens: The prevalence of the challenge enteric bacteria in the eggs laid by the infected adult-layer chickens are shown in Table 12. Out of ninety (90) eggs laid by the infected chickens, fifty-three (53) eggs were positive for enteric bacteria, of which *E. coli* O157:H7 SS52 was significantly ($P<0.05$) most and *S.* ser. Typhimurium U288 was the least. Twenty-one (21) eggs out of 90 eggs from the control chickens were positive for enteric bacteria, and *E. coli* O157:H7 SS52 was significantly ($P<0.05$) most and *S.* ser. Tphimurium U288 was the least. The result showed that the number of eggs that were positive to enteric bacteria was significantly ($P<0.05$) higher in the infected egg samples than the samples from the control.

Total Mean Viable Plate Counts of the Challenge Isolates from Internal Organs of Infected Chickens: The total mean plate counts (TMPCs) of enteric bacteria from the internal organs are shown in Tables 13 and 14. The results showed that the total mean plate counts of *Salmonella* species were higher in the liver than the total mean plate counts of *E. coli* O157:H7 SS52, and the total mean plate count of *S.*ser. Enteritidis FM366 in the liver was non-significantly higher than the total mean plate count of *S.* ser. Typhimurium U288. The total mean plate counts (TMPCs) of *E. coli* O157:H7 SS52 were significantly ($P<0.05$) most in the heart and least in the lungs. There was generally significant ($P<0.05$) decrease in the total mean plate counts of the enteric bacteria as the age of the chickens increased. The results also showed that those chicks infected by *Salmonella* species were not able to produce well developed spleen within the period of 2 weeks, but the total mean plate counts of *S.*ser. Enteritidis FM366 in adult chicken was significantly ($P<0.05$) higher than TMPC of *S.* ser. Typhimurium U288. The infected organs were able to show significant ($P<0.05$) growth of enteric bacteria as compared to the control organs from the chicks/adult chickens that had no visible growth.

Pathological Features of Internal Organs of Infected Chickens: The pathological features of the infected chickens after histological examination of the infected organs are shown in plates 1–26. The results showed that damages to the infected organs such as severe destruction of liver archtecture, congestion of central vein of the liver, loss of liver parenchyma cells, enlargement of sinusoids of the liver, severe destruction of myofibres and oblique fibres of the heart, vascular thrombosis of the lungs, oedema and multifocal haemorrhage of the alveoli of the lungs were more prominent in the chicks than the adult chickens. Also the severity of damages done to these infected organs decreased as the age of the chickens increased. The organs from non infected chickens

showed normal lungs, liver, heart and spleen morphologies, with their features intact and prominent.

Table 1: Types and sources of chicken feed samples that was positive to enteric bacteria

Type of feed	Positive sample (%)	Negative sample (%)	Total (%)
X	294 (57.42)	218(42.58)	512(33.33)
Y	312(60.94)	200(39.06)	512(33.33)
Z	328(64.06)	184(35.94)	512(33.33)
Total	934(60.81)	602(39.19)	1536(99.99)

Table 2: Morphological characteristics of the isolates from chicken feed samples

Parameter \ Isolate	E	G	5	7	11
Appearance on agar plate	Red colony on MA	Red colony on MA	Colourless with black center on SSA	Colourless and dark at the center on SSA	Colourless and dark at the center on SSA
Edge	Entire	Entire	Entire	Entire	Entire
Size (mm)	1.00	1.20	2.20	1.40	1.60
Consistency	Soft	Soft	Soft	Soft	Soft
Optical property	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation	Slightly raised	Convex	Slightly raised	Slightly raised	Slightly raised
Pigmentation	–	–	–	–	–
Gram Reaction	–	–	–	–	–
Shape	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+

SSA = *Salmonella-Shigella* Agar
MA = MacConkey Agar
+ = Positive – = Negative

Table 3: Characteristics and identities of the enteric isolates from the chicken feed samples

Parameter \ Isolate	E	G	5	7	11
Indole production	+	+	–	–	–
Hydrogen Sulphide	–	–	+	+	+
Ornithine decarboxylase	–	–	–	–	–
Methyl Red	+	+	+	+	+
Voges-Proskauer	–	–	–	–	–
Citrate Utilization	–	–	+	+	+
Catalase	+	+	+	+	+
Urease	–	–	–	–	–
Glucose	+	+	+	+	+
Maltose	+	+	+	+	+
Dulcitol	–	–	+	+	+
Lactose	+	+	–	–	–
Xylose	+	+/–	+/–	+	+
Arabinose	+	+	+	+	–
Inositol	–	–	+	–	–
Mucate	–	–	–	+	+

E – *Escherichia coli* G – *Escherichia coli*
5 – *Salmonella* species 7 –*Salmonella* species
11 – *Salmonella* species + = Positive – = Negative

Table 4: Molecular identities of the isolates

Isolate	Max score	Total score	Query Cover	Gap	Identity	Accession Number	Description
E	2856	2967	100%	0%	100%	CO010304.1	*Escherichia coli* strain 0157:H7 str SS52 Complete genome
G	1297	1297	100%	0%	96%	CP007594.1	*Escherichia coli* strain SEC470 Complete genome
5	2193	4386	100%	0%	98%	CP003836.1	*Salmonella enterica* subsp. enterica serovar Typhimurium str U288 Complete genome
7	660	660	100%	0%	96%	NG03836.1	*Salmonella enterica* subsp. enterica serovar Enteritidis str FM366 Complete genome
11	2844	2844	100%	0%	100%	CP011428.1	*Salmonella enterica* subsp. enterica serovar Enteritidis str YU39 Complete genome

Table 5: Obvious pathological signs of *E. coli* O157:H7 SS52 on the infected chickens

Pathological Sign	DOC (%)	OWC (%)	TWC (%)	AC (%)	C_1 (%)	C_2 (%)	Total (%)
Anorexia	4 (8.33)	4 (8.33)	2 (4.17)	0 (0)	0 (0)	0 (0)	10 (20.83)
Weakness	3 (6.25)	1 (2.08)	1 (2.08)	0 (0)	0 (0)	0 (0)	5 (10.42)
Bloody Diarrhoea	5 (10.42)	3 (6.25)	3 (6.25)	0 (0)	0 (0)	0 (0)	11 (22.92)
Omphalitis	0 (0)	0 (0)	0 (0)	2 (4.17)	0 (0)	0 (0)	2 (4.17)
Diarrhoea	0 (0)	2 (4.17)	2 (4.17)	2 (4.17)	0 (0)	0 (0)	6 (12.50)
Respiratory Distress	4 (8.33)	2 (4.17)	2 (4.17)	0 (0)	0 (0)	0 (0)	8 (16.67)
Death	3 (6.25)	2 (4.17)	1 (2.08)	0 (0)	0 (0)	0 (0)	6 (12.50)

DOC — Day-Old Chick, OWC — One-Week Old Chock, TWC — Two-Week Old Chick, AC — Adult Chicken, C_1 — Normal Chick, C_2 — Normal Adult Chicken

Table 6: Obvious pathological signs of *Salmonella* ser. Typhimurium U288 on the infected chickens

Pathological Sign	DOC (%)	OWC (%)	TWC (%)	AC (%)	C_1 (%)	C_2 (%)	Total (%)
Anorexia	4 (10.00)	4 (10.00)	3 (7.50)	0 (0)	0 (0)	0 (0)	11 (27.50)
Weakness	2 (5.00)	3 (7.50)	2 (5.00)	0 (0)	0 (0)	0 (0)	7 (17.50)
Bloody Diarrhoea	5 (12.50)	5 (12.50)	5 (12.50)	0 (0.00)	0 (0)	0 (0)	15 (37.50)
Omphalitis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Diarrhoea	0 (0)	0 (0.00)	0 (0.00)	0 (0)	0 (0)	0 (0)	0 (0.00)
Respiratory Distress	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Death	2 (5.00)	3 (7.50)	2 (5.00)	0 (0)	0 (0)	0 (0)	7 (17.50)

DOC — Day-Old Chick, OWC — One-Week Old Chock, TWC — Two-Week Old Chick, Ac — Adult Chicken, C_1 — Normal Chick, C_2 — Normal Adult Chicken

Table 7: Obvious pathological signs of *Salmonella* ser. Enteritidis FM366 on the infected chickens

Pathological Sign	DOC (%)	OWC (%)	TWC (%)	AC (%)	C_1 (%)	C_2 (%)	Total (%)
Anorexia	4 (10.00)	2 (5.00)	2 (5.00)	0 (0)	0 (0)	0 (0)	8 (20.00)
Weakness	3 (7.50)	3 (7.50)	2 (5.00)	0 (0)	0 (0)	0 (0)	8 (20.00)
Bloody Diarrhoea	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Omphalitis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Diarrhoea	5 (2.50)	5 (0)	5 (2.50)	0 (0)	0 (0)	0 (0)	15 (37.50)
Respiratory Distress	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Death	3 (7.50)	3 (7.50)	3 (7.50)	0 (0)	0 (0)	0 (0)	9 (22.50)

DOC — Day-Old Chick, OWC — One-Week Old Chock, TWC — Two-Week Old Chick, Ac — Adult Chicken, C_1 — Normal Chick, C_2 — Normal Adult Chicken

Table 8: Gross pathology of the internal organs of chickens infected by *E. coli* O157:H7 SS52

Pathological Feature	DOC (%)	OWC (%)	TWC (%)	AC (%)	C_1 (%)	C_2 (%)	Total (%)
Air sacculitis	5 (6.25)	3 (3.75)	3 (3.75)	1 (1.25)	0 (0)	0 (0)	12 (15.00)
Liver congestion	1 (1.25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.25)
Pericarditis	5 (6.25)	5 (6.25)	4 (5.00)	1 (1.25)	0 (0)	0 (0)	16 (20.00)
Perhepatitis	3 (3.75)	2 (2.50)	2 (2.50)	1 (1.25)	0 (0)	0 (0)	8 (10.00)
Hepatomegaly	2 (2.50)	2 (2.50)	2 (2.50)	1 (1.25)	0 (0)	0 (0)	7 (8.75)
Lung haemorrhage	4 (5.00)	3 (3.75)	3 (3.75)	1 (1.25)	0 (0)	0 (0)	11 (13.75)
Bloody intestine	3 (3.75)	2 (2.50)	1 (1.25)	0 (0)	0 (0)	0 (0)	6 (7.50)
FAI	5 (6.25)	5 (6.25)	5 (6.25)	1 (1.25)	0 (0)	0 (0)	16 (20.00)
Eroded intestine	1 (1.25)	1 (1.25)	1 (1.25)	0 (0)	0 (0)	0 (0)	4 (5.00)

DOC — Day-Old Chick, OWC — One-Week Old Chock, TWC — Two-Week Old Chick, Ac — Adult Chicken, C_1 — Normal Chick, C_2 — Normal Adult Chicken, FAI — Fluid Accumulation in the Intestine

Table 9: Gross pathology of the internal organs of chickens infected by *Salmonella* ser. Typhimurium U288

Clinical Feature	DOC (%)	OWC (%)	TWC (%)	AC (%)	C_1 (%)	C_2 (%)	Total (%)
Air sacculitis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Liver conjestion	3 (3.75)	3 (3.75)	3 (3.75)	0 (0)	0 (0)	0 (0)	9 (11.25)
Pericarditis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Perhepatitis	5 (6.25)	5 (6.25)	4 (5.00)	2 (2.50)	0 (0)	0 (0)	16 (20.00)
Hepatomegaly	5 (6.25)	5 (6.25)	4 (5.00)	2 (2.50)	0 (0)	0 (0)	16 (20.00)
Lung haemorrhage	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Bloody intestine	4 (5.00)	5 (6.25)	3 (3.75)	0 (0)	0 (0)	0 (0)	12 (15.00)
FAI	5 (6.25)	5 (6.25)	5 (6.25)	0 (0)	0 (0)	0 (0)	15 (18.75)
Eroded intestine	5 (6.25)	4 (5.00)	3 (3.75)	0 (0)	0 (0)	0 (0)	12 (15.00)

DOC — Day-Old Chick, OWC — One-Week Old Chock, TWC — Two-Week Old Chick, AC — Adult Chicken, C_1 — Normal Chick, C_2 — Normal Adult Chicken, FAI — Fluid Accumulation in the Intestine

Table 10: Gross pathology of the internal organs of chickens infected by *Salmonella* ser. Enteritidis FM366

Clinical Feature	DOC (%)	OWC (%)	TWC (%)	AC (%)	C_1 (%)	C_2 (%)	Total (%)
Air sacculitis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Liver conjestion	5 (7.14)	5 (7.14)	5 (7.14)	0 (0)	0 (0)	0 (0)	15 (21.43)
Pericarditis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Perhepatitis	5 (7.14)	5 (7.14)	5 (7.14)	0 (0.00)	0 (0)	0 (0)	15 (21.43)
Hepatomegaly	5 (7.14)	5 (7.14)	3 (4.29)	2 (2.86)	0 (0)	0 (0)	15 (21.43)
Lung haemorrhage	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Bloody intestine	0 (0.00)	0 (0.00)	0 (0.00)	0 (0)	0 (0)	0 (0)	0 (0.00)
FAI	5 (7.14)	5 (7.14)	5 (7.14)	1 (1.43)	0 (0)	0 (0)	16 (22.86)
Eroded intestine	4 (5.71)	3 (4.29)	1 (1.43)	0 (0)	0 (0)	0 (0)	8 (11.43)

DOC — Day-Old Chick, OWC — One-Week Old Chock, TWC — Two-Week Old Chick, Ac — Adult Chicken, C_1 — Normal Chick, C_2 — Normal Adult Chicken, FAI — Fluid Accumulation in the Intestine

Table 11: Total mean viable plate counts of the challenge enteric bacteria in the cloacae of infected adult-layer chickens

Enteric Bacterium	Infected chicken (x 10^8 CFU/g)	Control (x 10^8 CFU/g)
EC	67.00 ± 3.61	24.00 ± 4.36
ST	13.00 ± 2.65	2.00 ± 2.00
SE	31.00 ± 2.00	7.00 ± 1.73

EC ---------- *E. coli* O157:H7 SS52
ST---------- *S.* ser. Typhimurium U288
SE---------- *S.* ser. Enteritidis FM366

Table 12: Prevalence of the challenge enteric bacteria in the eggs lay by the infected adult-layer chickens

Enteric Bacterium	Infected Chicken (n)		Control (n)	
	P (%)	N (%)	P (%)	N (%)
EC	26 (86.67)	4 (13.33)	11 (36.67)	19 (63.33)
ST	4 (13.33)	26 (86.67)	1 (3.33)	29 (96.67)
SE	23 (76.67)	7 (23.33)	9 (30)	21 (70)
Total	53 (66.67)	37 (33.33)	21 (23.33)	69 (66.67)

P — Positive, N — Negative, n — Number of eggs used

Table 13: Total mean viable plate counts of *E. coli* O157:H7 SS52 isolated from the internal organs of infected chickens

Chicken	Liver (x 10^8CFU/g)	Lungs (x 10^8CFU/g)	Heart (x 10^8CFU/g)
Day-Old	28.00 ± 3.61	24.00 ± 3.61	39.00 ± 4.58
One-Week Old	22.00 ± 4.58	17.00 ± 2.65	29.00 ± 3.06
Two-Week Old	16.00 ± 4.36	13.00 ± 1.00	22.00 ± 2.00
Adult	5.00 ± 2.65	3.00 ± 1.73	7.00 ± 2.65
Control (chick)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Control (adult)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 14: Total mean viable plate counts of *Salmonella* species isolated from the liver of infected chickens

Chicken	ST (x 10^8CFU/g)		SE (x 10^8CFU/g)	
	Liver	Spleen	Liver	Spleen
Day-Old	36.00 ± 4.63	A	43.00 ± 1.73	A
One-Week Old	29.00 ± 1.73	A	31.00 ± 3.00	A
Two-Week Old	24.00 ± 4.58	ND	26.00 ± 1.73	A
Adult	11.00 ± 1.73	14.00 ± 2.65	13.00 ± 1.73	19.00 ± 2.65
C_1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

A — Absent, ND — Not well Developed, C_1— Normal chick, C_2— Normal adult chicken
EC ---------- *E. coli* O157:H7 SS52
ST---------- *S.* ser. Typhimurium U288
SE---------- *S.* ser. Enteritidis FM366

DISCUSSION

The presence of enteric bacteria in the feed samples could be traced from the management practices of mill, dust, feed ingredients, and transportation of the feeds, poor handling and sanitary conditions attributed to the feed samples. Similar findings were reported by many researchers (Immerseel *et al.,* 2002; Alshawabkeh, 2006; Maciorowski *et al.,* 2007). Researchers had shown that animal housing and transportation of equipments can also harbour enteric bacteria and this contributes to the contamination of chicken feeds (Primm, 2008). Maciorowski *et al.* (2007) also stated that the high prevalence and high populations of enteric bacteria in animal wastes was evidence that manure could be a principal source of enteric pathogens to chicken industry. Chicken feeds contaminated by enteric bacteria pathogenic to humans can contribute to human food-borne illness through the feed-food-human chain. This shows that the production of chicken feeds requires microbiological safety regulations to escape microbial contamination of the product.

The significant pathological signs associated with the organisms corroborated the findings of Zende *et al.* (2013). Gross pathological lesions were seen on the internal organs and intestines of infected chickens. The occurrence of air sacculitis, pericarditis and lungs haemorrhage among those chickens infected by *E. coli* could be due to the organism's capability of invading the lungs and hearts of the infected chickens. *Escherichia coli* is considered as a member of normal microflora of the poultry intestine, but certain strains, such as those designated as avian pathogenic *Escherichia coli* (APEC), can spread into various internal organs and cause infections characterized by systemic fatal disease (La Ragione and Woodward, 2002). The wide distribution of *E.coli* O157:H7 SS52 in the lungs and hearts of infected chickens could probably indicate concurrent extra-intestinal infections. *Escherichia coli* O157:H7 SS52 is a common avian pathogen mainly associated with extra-intestinal infections collectively known as colibacillosis (Dia-de-Silveira *et al.,* 2002). *Escherichia coli* O157:H7 SS52 produces serine proteases (EspP), an accessory virulence factor that is plasmid mediated which can exacerbate some disease conditions (Schmidt *et al.,* 2001).

Liver congestion, eroded and bloody intestine associated with organisms was due to the affinity of these organisms to liver cells and their proliferative capabilities in the intestine. In the intestine, the toxins produced by

the organisms were able to cause secretion and retention of fluids in the intestinal loops and especially in the caeca. The intestines were pale and some were darker and distended, particularly the caeca that were filled with fluid containing gas bubbles. Diarrhea and weakness were common among the infected chickens. Similar conclusion was drawn by many researchers (Dia-de-Silveira *et al.,* 2002; Dashe *et al.,* 2013; Nur *et al.,* 2014).

The presence of *E.coli* O157:H7 SS52, *S.* serovar Typhimurium U288 and *S.* serovar Enteritidis FM366 in the cloacae of the infected chickens supported the findings of many researchers (Dho-Moulin and Fairbrother, 2009; Wiriya *et al.,* 2011; Dashe *et al.,* 2013; Nur *et al.,* 2014). Cloaca serves as a common junction for the passage of materials removed from the body of infected and non-infected chickens. The highest occurrence of *E. coli* O157:H7 SS52 in the cloaca suggests that cloaca is an important site for detection of *E. coli* strains. Similar finding was stated by Dia-de-Silveira *et al.* (2002).

The detection of *E. coli* O157:H7 SS52*, S.* serovar Typhimurium U288 and *S.* serovar Enteritidis FM366 from the eggs laid by the infected adult-layer chickens supported the findings of many researchers (Gebreyes *et al.,* 2000; Daly *et al.,* 2002; Shahilah *et al.,* 2010; Wiriya *et al.,* 2011). Rahman *et al.* (2006) reported that poultry were considered as important sources of food borne diseases and the illnesses were associated with the consumption of contaminated eggs. Barnes *et al.* (2007) also reported that laying chicken infected by enteric bacteria might infect the internal egg before shell formation, and faecal contamination of the egg shell was possible during the passage of the egg through the cloaca and after the egg is being laid. *E. coli* O157:H7 SS52 were mostly detected from the egg samples analyzed in this study. This could be due to the fact that *E. coli* strains was mostly contracted through faecal contamination and most commonly encountered in the cloaca of chickens. Similar findings were reported by many researchers (Trampel *et al.,* 2007; Sahilah *et al.,* 2010; Wiriya *et al.,* 2011)

The significant mean viable plate counts of *E. coli* O157:H7 SS52*, S.* serovar Typhimurium U288 and *S.* serovar Enteritidis FM366 recorded from the internal organs of the infected chickens supported the reports of many researchers (Masdooq *et al.,* 2008; Dashe *et al.,* 2013). The presence of these enteric bacteria in the liver, heart, spleen and lungs, suggest that the organs contain sufficient nutrients and favourable environment for the growth of the enteric bacteria. The activities of the invaded organisms on the organs might cause degradation of the nutrients, obstruction of the lumen of the organs, deterioration and deformation of the organs, thereby producing pathological lesions that can clinically manifest on the infected chickens. This is in consonance with the report of Dashe *et al.* (2013). The study also revealed that *Salmonella* species were mostly detected in the liver of the infected chickens. This supports the report of Barrow *et al.* (2004) that *Salmonella* species lodge and multiply in those organs rich in reticuloendothelial tissues, mainly the liver. Dashe *et al.* (2013) also reported that the distribution of *S.* serovar Enteritidis strains occurred mostly in the liver of apparently sick chickens.

The mean viable plate counts of *E. coli* O157:H7 SS52 occurred most in the heart. This is in consonance with the report of Dashe *et al.* (2013). Kabir *et al.* (2010) reported that when *E. coli* reaches the vascular system, the internal organs and the heart are infected. The significant decrease in the mean viable plate counts of enteric bacteria as the age of the chickens increased observed in this study, was due to increase in maturation of the immune cells and organs as the chickens continually responds to natural gut colonization. Studies have shown that interleuklin-17 (IL-17) inductions in response to enteric infection and colonization of gut microbiota were observed in 16-day-old chickens and above. Interleuklin-17 receptors have been found on dendritic cells, macrophages and T-lymphocytes (Nogvales, 2008; Magdalena *et al.,* 2011).

The histopathological changes observed in this work were encountered in the results of other researchers at different time intervals on experimental chickens (Wigley *et al.,* 2005; Haider *et al.,* 2008; Mohkber *et al.,* 2011). The infiltration of mononuclear cells observed in the liver was also encountered by other researchers (Goncalves *et al.,* 2002; Adehan *et al.,* 2006; Mohkber *et al.,* 2011). The multifocal haemorrhage and infiltration of the red blood cells associated with the infected organs corroborated by hemosiderosis of the infected organs (Mohkber *et al.,* 2011). Many studies have shown that hemosiderosis associated with the infected organs is known to be caused by increase in the rate of destruction of red blood cells in these organs (Miyazaki *et al.,* 2001; Milud *et al.,* 2013)

The current work has revealed that the severity of damages done to the infected organs decreased as the age of the chickens increased. Several studies have shown that the development of microbiota and maturation of the chicken gut immune system leads to increased resistance to invasion of the organs by the pathogens (Bar-Shira and Friedman, 2006; Magdalena *et al.,* 2011).

CONCLUSION

This study has revealed the presence of *Escherichia coli*O157:H7 SS52*, E. coli* SEC470 *Salmonella* serovar Typhimurium U288, *Salmonella* serovar Enteritidis FM366 and *Salmonella* Enteritidis YU39 in the chicken feed samples randomly collected from 21 major towns in twenty-one Local Government Areas of Anambra State, of which the occurrences of E. coli SEC470 and S. serovar Enteritidis YU39 were negligible due to very low counts of the isolates from the studied samples. The broiler chickens of different ages and layer chickens that were

orally infected by these organisms, exhibited different pathological signs, gross leisions, obvious pathological features due to septicemic infection, and deaths.

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