INCIDENCE OF *SALMONELLA* AND *ESCHERICHIA COLI* IN POULTRY FEEDS

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Abstract

Raw poultry and meat products consumption remain the principal source of *Salmonella* and *E. coli* in many countries. *Salmonella* has been reported in a variety of chicken, turkey and other meat products. Poultry is frequently colonized with *Salmonella* without detectable symptoms. This constitutes health risk not only for the animal but also for humans. This study was designed to help in achieving one health. Eight different brands of poultry feed samples were aseptically collected from the different poultry farms and poultry market places in Anambra State. From the result, feed sample D (layer) had the highest viable bacteria count which was too numerous to count and the lowest was found in sample E which was $3.33x10^6$ CFU/g. The highest coliform count was found in sample H with $1.19 x10^7$ CFU/g and the lowest was seen in sample A with $3.7x10^6$ CFU/g. The bacteria viable count with the highest *Salmonella* was found in sample D (layer) and *Escherichia coli* in sample H. The bacteria percentage of *Salmonella* was 62.5% and *E. coli* was 100% from different feed samples. With the high presence of the pathogens in the feeds, there is need for good manufacturing practice, handling and retailing methods to enhance the microbiological quality of these feeds.

Keywords: *E. coli*, feeds, poultry, salmonella

Introduction

Poultry feeds are food materials used in growing birds. Poultry feeds are referred to as complete feeds as they are designed to contain all the nutrients required for proper growth as well as meat and egg production in birds. Poultry feeds are composed largely of grains such as corn, wheat or barley, oil seeds, cake meal (originating mainly from oil producing seeds such as soybeans), sunflower seeds, peanuts, cotton seed and protein products of animal origin such as fish meal, meat and bone meal, slaughter house offal's and feather meals (Bale *et al*., 2002). According to Cevger and Yalcin (2003), poultry feeds are essential source of energy needed to generate heat and to support the chemical reactions in which all physiological processes depended. Animal components of the poultry feeds possess high nutritional component

necessary for microbial growth especially when the environmental conditions are favourable (Madaki *et al*., 2019).

Raw poultry and meat products consumption remain the principal source of *Salmonella* and *E. coli* in many countries. *Salmonella* has been reported in a variety of chicken, turkey and other meat products, in addition to fresh produce such as lettuce and sprouts (Rajan *et al*., 2017). Studies have reported that poultry is found to be associated with 25% of outbreaks, illnesses, and hospitalizations caused by *Salmonella typhi*, a confirmed foodborne pathogen (Smadi and Sargeant, 2013; Chai *et al*., 2017). *Salmonella* is carried by different animals and may contaminate fresh water by direct or indirect contact, which may lead to contamination of fresh produce as well. Poultry is frequently colonized with *Salmonella* without detectable symptoms. As a result, it was suggested that poultry is the main human health risk factor, as it allows the bacteria to easily transmit from eggs and poultry meat to humans (Antunes *et al*., 2016) which defeats one health. The production of poultry feeds requires microbiological safety regulations to escape microbial contamination of the product. *Salmonella* infection remains a major public health concern worldwide. It contributes to the economic burden of both industrialized and underdeveloped countries through the costs associated with surveillance, prevention and treatment of disease (Crump *et al*., 2004). Gastroenteritis is the most common expression or symptom of *Salmonella* infection worldwide, followed by bacteremia and enteric fever (Majowicz *et al*., 2010). *Salmonella* are spread from poultry to humans, often through foods such as eggs and meat (Behravesh and Medus, 2008).

Escherichia coli is one of the other common microbial floras of gastrointestinal tract of poultry (Jawetz *et al*., 2004). Among the diseases caused by these microorganisms inhuman, some are often severe and sometimes lethal infections such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhoea of adults and children (Daini *et al*., 2005). Enteritis caused by *Escherichia coli* (colibacillosis) is an important disease in the poultry industry because of increased mortality and decreased performance (Barnes *et al*., 2003). Eating a. poultry product contaminated with *E. coli* is a major cause of disease in man and a setback to the achievement of one health. The aim of this research work is to determine the incidence of *Salmonella* and *Escherichia coli* in Poultry feeds.

Materials and Methods

Study Area

The present study was carried out at Applied Microbiology and Brewing laboratory in Nnamdi Azikiwe University Awka, Anambra State.

Collection of samples

Eight different brands of poultry feed samples were aseptically collected from different poultry farms and poultry markets in Anambra State. These samples

of different brands were labelled Sample A, Sample B, Sample C, Sample D, Sample E, Sample F, Sample G and Sample H were immediately taken to the laboratory.

Media

Different media such as Nutrient Agar (NA) (Oxoid, England), nutrient Broth (NB) , (Oxoid, England) Peptone water (Oxoid, England), Salmonella-Shigella Agar (SS) (Oxoid, England), Brilliant Green Agar (BGA) (Oxoid, England), EMB (eosin methylene blue) (Oxoid, England) and MacConkey (Oxoid, England), were used in this research.

Methods

The samples were aseptically transported at the same day to the laboratory for the analysis. Each sample was investigated for the occurrence of *Salmonella* and *Escherichia coli*.

Enrichment of bacteria present in feed samples

The peptone water was prepared by adding 6.75gm of peptone in 225ml of distilled water in 250ml glass flasks. The flasks were gently swirled and covered with aluminium foil. After wrapping the mouth and properly labelling, the flasks were autoclaved at 121ºC for 15mins. The flasks were removed from the autoclave and were kept at room temperature. Twenty-five gram was weighed from each feed sample and inoculated into the flasks containing 225ml peptone water for the enrichment of the bacteria. These flasks were then incubated at 37ºC for 24 hours in the incubator (Buchanan and Gibbons, 2007).

Culturing

For culturing, 1 ml of the Enriched media was aseptically introduced into 9 ml of sterile water and mixed properly to give good homogenate used as stock. A ten-fold serial dilution was made for samples in appropriate dilution tubes which were done until the 5th serial dilution. These appropriate dilutions were cultured by spread plate technique using sterile spreading rod on the Nutrient Agar media. These inoculated Nutrient Agar (NA) media were then incubated overnight at 37ºC in the incubator. Each plate was observed after 24 hrs for visible growth. The colonies were counted as the Total Viable Count (TVC).

For sub culturing, the colonies on the NA media were inoculated in the selective media, Brilliant Green Agar and *Salmonella-Shigella* Agar for the identification of *Salmonella*, MacConkey Agar for Coliforms and Eosin Methylene blue Agar for *Escherichia coli* from the same dilutions of the different feed samples and were incubated at 37ºC overnight. Also, the samples of the nutrient broth media were inoculated to all the selective media by weighing from the different feed samples. After inoculation of all of the samples to the selective media, the samples were incubated at 37ºC overnight. The bacterial load count of *Salmonella* and *Escherichia coli* were carried out.

Characterization Tests for Bacteria

Colonies to be identified were picked from each plate and kept on slants of nutrients agar medium for further biochemical analysis. Standard methods were used for the microscopic examination; Motility test, Indole test, Methyl red test, Voges Proskauer, Citrate test, Sugar fermentation test, Motility test and Oxidase test as described in Cheesbrough (2006)

Results

Characterization of *Escherichia coli* Isolates

Gram negative organisms which were rod-like, motile or non-motile, oxidation negative, indole positive, methyl-red positive, gas production from glucose and produce green metallic sheen on Eosine-methylene blue agar (EMB) were considered as *Escherichia coli* as shown in Table 1

Characterization of *Salmonella* Isolates

Table 2 showed the Gram staining characteristics which showed that the organisms were Gram negative rods. The colonies appeared pinkish with tiny black dots produced gas from glucose fermentation. They were also Indole negative, Oxidase negative, Methyl red test, Voges Proskauer negative, Citrate negative, and Catalase positive and are motile.

All Gram-negative organisms that showed these characteristics are considered as *Salmonella* species.

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Table 1: Biochemical Characteristic of *Escherichia coli* Isolates from the Feed samples (A-H)

Colony morphology	Microscopic Examination	Gram staining	Sugar test				Indole test	Methylred test	VP test	Citrate test	Catalase test	Motility test	Oxidase test	Identification
			G	L	S	M								
Dark blue-black colonies	Short rod in single	-	+	+	+	+	+	+	-	-	+	+	-	*Escherichia coli*
Dark blue-black colonies	Short rod in single	-	+	+	+	+	+	+	-	-	+	+	-	*Escherichia coli*
Dark blue-black colonies	Short rod in single	-	+	+	+	+	+	+	-	-	+	+	-	*Escherichia coli*
Dark blue-black colonies	Short rod in single	-	+	+	+	+	+	+	-	-	+	+	-	*Escherichia coli*
Dark blue-black colonies	Short rod in single	-	+	+	+	+	+	+	-	-	+	+	-	*Escherichia coli*
Dark blue-black colonies	Short rod in single	-	+	+	+	+	+	+	-	-	+	+	-	*Escherichia coli*
Dark blue-black colonies	Short rod in single	-	+	+	+	+	+	+	-	-	+	+	-	*Escherichia coli*
Dark blue-black colonies	Short rod in single	-	+	+	+	+	+	-	+	+	+	-	-	ND

Key: ND- Not detected, - : Negative, +: Positive, G=glucose, L=lactose, S=sucrose, M=mannitol

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Table 2: Biochemical Characteristics of *Salmonella* Isolates from the Feed samples (A-H)

Colony morphology	Microscopic Examination	Gram staining	Sugar test				Indole test	Methyl red test	VP test	Citrate test	Catalase test	Motility test	Oxidase test	Identification
			G	L	S	M								
Dark centered colonies	Short rod in single	-	+	-	-	+	+	+	-	-	+	+	-	*Salmonella specie*
Small circular colonies	Short rod in single	-	+	-	-	+	+	+	-	-	+	+	-	*Salmonella specie*
Small circular colonies	Short rod in single	-	+	-	-	+	+	+	-	-	+	+	-	*Salmonella specie*
Dark blue-black colonies	Short rod in single	-	+	-	-	+	+	+	-	+	+	+	-	*Salmonella specie*
Dark blue-black colonies	Short rod in single	-	+	-	-	+	+	+	-	-	+	+	-	*Salmonella specie*
Small circular colonies	Short rod in single	-	+	-	-	+	+	+	-	-	-	-	-	*Salmonella specie*
Dark blue-black colonies	Short rod in single	-	+	-	-	+	+	+	-	+	-	-	-	*Salmonella specie*
Small circular colonies	Short rod in single	-	+	-	-	+	+	-	+	+	+	+	+	ND

Key: ND- Not detected - :Negative +: Positive G=glucose, L=lactose, S=sucrose, M=mannitol

Total viable count (TVC) of different feed samples.

Table 3 represents the total viable count of bacteria of all of the samples on the Nutrient Agar media. The highest numbers of the total bacteria were present in the feed Sample D (Layer) which was numerous and the lowest number of total bacteria present in the feed Sample E (Starter) was 3.3×10^6 CFU/ml.

Table 3: Total viable count (TVC) of different feed samples

Different samples	**Mean of the colony of 10^5**	**TVC**
Sample A (Grower)	64	6.4×10^6
Sample B(Grower farm)	110	1.10×10^7
Sample C (Layer)	289	2.89×10^7
Sample D (Layer farm)	numerous	numerous
Sample E (Starter)	33	3.3×10^6
Sample F (Starter farm)	174	1.74×10^7
Sample G (Finisher)	42	4.2×10^6
Sample H (Finisher farm)	252	2.52×10^7

Coliform Bacterial Counts

The level of coliform bacteria count was in the range $3.7x10^6$ to $1.19\ x10^7$ CFU/g as presented in Table 4. Presumptive coliform test showed gas formation in the Durham tubes. This showed that coliform organisms were present. Confirmatory coliform test also gave a positive result, with green metallic sheen colony appearance. Gram staining characteristics showed the organisms were Gram negative. These microorganisms were considered as *Escherichia coli* an indicator organism for other coliforms and pathogenic organisms. The highest Coliform count was found in Sample H which is 1.19×10^7 and the lowest sample was found in Sample A which is 3.7×10^6.

Table 4: Coliform Bacterial Counts

Samples	Colony count	Colony forming unit
Sample A	37.0	3.7×10^6
Sample B	103	1.03×10^7
Sample C	50.0	5.0×10^6
Sample D	78.0	7.8×10^6
Sample E	53.0	5.3×10^6
Sample F	112	1.12×10^7
Sample G	87.0	8.7×10^6
Sample H	119	1.19×10^7

Bacterial load of different feed samples.

Table 5 showed the enumeration of *Salmonella* and *Escherichia coli* in the different feed samples with their contents. The total viable count (TVC) of *Salmonella* in the feed samples was found to be within the range of 0 to $2.75x10^7$ and that of *Escherichia coli* within the range of 0 to $2.67x10^7$. The highest number of *Salmonella* was found in Sample D (Layer) and that of *Escherichia coli* contamination was found in Sample H (Layer) feeds.

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Table 5: Bacterial load of different feed samples.

Samples	*Salmonella*	*E. coli*
Sample A (Grower)	No	4.4×10^6
Sample B (Grower farmer)	No	5.6×10^6
Sample C (Layer)	2.55×10^7	1.15×10^7
Sample D (Layer farm)	2.75×10^7	2.23×10^7
Sample E (Starter)	No	1.05×10^7
Sample F (Starter farm)	5.4×10^6	7.5×10^6
Sample G (Finisher)	3.2×10^6	3.9×10^6
Sample H (Finisher farm)	2.15×10^7	2.67×10^7

Bacterial Percentage and Range

From Table 6, out of 8 type samples, *Salmonella* were found in 5 samples and *Escherichia coli* were found in all samples. The percentage of total *Salmonella* isolates of the feed samples was 62.5%. On the other hand, the percentage of total *Escherichia coli* of the feed samples was 100%. The incidence of *Escherichia coli was* higher than that of *Salmonella*. The highest number of *Salmonella* contamination was given from sample D (Layer) poultry feeds and the highest number of *Escherichia coli* contamination was given from Sample H (Finisher) poultry farms.

Discussion

Animal feeds have been listed as one of the sources of microbes of farmed animals and poultry (Uwaezuoke and Ogbulie, 2008). All the 8 poultry feeds samples analysed in this study yielded isolates of *Escherichia coli*. This is of health concern since poultry (livestock) serve as a major food component to Nigerians.

Table 6: Percentage and range of *Salmonella* and *Escherichia coli* from the feed samples.

Bacteria	Percentage	Range
Salmonella	62.5%	0 to 2.75×10^7
Escherichia coli	100%	0 to 2.67×10^7

In this study, Coliform count in the poultry feeds range between $1.1x10^6$ to $5.0\ x10^6$ CFU/g and this could be attributed to contamination during drying machine milling (World Health Organization, 2008). Also, water used for washing materials could be a source of coliform contamination. Of great public health concern is the fact that *Escherichia coli* isolated could be of fecal origin. Fecal coliform is an indicator organism of possible presence of other pathogens such as *Salmonella, Shigella, Yersiniae* etc. The finding in this current study is not surprising since water from streams or even chlorinated tap water may be used in one way or the other during processing (Chowdhury *et al*., 2011). The relatively high numbers of coliforms isolated from livestock feed may constitute health hazard to livestock and man (Chowdhury *et al*., 2011).

Escherichia coli have long been recognized as a normal flora of the gastrointestinal tract of man and domestic animal, including birds. In this light, entero-toxigenic strains of *Escherichia coli* (ETEC) have been associated with epidemic and sporadic outbreak of infantile diarrhoea in humans and some diarrhoea diseases in livestock.

On the other hand, some of the samples analysed in this study yielded isolates of

*Salmonella sp*ecies. Out of the 8 samples analysed, 3 samples did not show the presence of *Salmonella* following confirming test, and the remaining 5 samples contained *Salmonella specie*s.

The total aerobic bacteria count was found to be high in this study which has a range of $3.3x10^6$ to $2.89x10^7$ cfu/g. This count is higher than in the work of Chowdhury *et al.* (2011) with $9.5x\ 10^5$ in layer feed sample. These significantly high numbers are invariably involved in the rapid contamination of poultry feed and products if they are not eliminated during processing.

This work also agrees with the work of Fakhruzzaman *et al*. (2014) which reported the isolation of *Salmonella* and *E. coli* from poultry feed and litter at significant amount. The widespread occurrence of *Salmonella* and *E. coli* in poultry feeds reveals a need for effective quality. control measures and hygiene in processing and handling of feeds (Fakhruzzaman *et al*., 2014). Monitoring of the route of the microbial contamination of animal production environment is an important first step in determining how such contaminants pass through the food chain (Madaki *et al*., 2019). In a similar research carried out by Madaki *et al*. (2019), the presence of *Pseudomonas*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* was reported and this is in line with what was observed in this research (Madaki *et al*., 2019).

In Nigeria, wastes from commercial poultry are not properly disposed and most rural farmers use these wastes as manure, which are often kept at the backyards before moving them to farms. These poultry wastes may serve as source of enteric organisms that harbour novel factor for birds that feed on such wastes as reported by Okoli *et al*. (2006). The result revealed the presence of *Salmonella* species and *E. coli* from the samples analysed (feed and droppings).

In this study, *Salmonella* was isolated from 62.5% of the feed samples while 100% of the feed samples contained *Escherichia coli*. The incidence of *Escherichia coli was* higher than that of *Salmonella* which was more than the incidence of 71.43% reported in a study on poultry feeds from farms and markets in Bangladesh, as reported by Chowdhury *et al*. (2011)

E. coli for example was reportedly associated in disease conditions such as colibacillosis which occurs in forms such as enteric and septicaemic colibacillosis whereas *Salmonella* is capable of causing acute and chronic infections in all or most types of birds and animals. The presence of *Salmonella* in the feed is also of public health importance, this is because, in general the transmission of *Salmonella* spp through the environment has been shown to be cyclic, and poultry feeds had been reportedly viewed as important links for contamination in poultry (Maciorowski *et al*., 2004) Although little is known about the relative significance of different sources of contamination of poultry feeds, it may depend partially upon the contamination levels of individual feed ingredients used in mixing the feed (Good Manufacturing Practices, 2008). With the high presence of the pathogens, there should be need for good manufacturing practice, handling and retailing methods to enhance the microbiological quality of these feeds.

Conclusion

This research provides relevant information on the incidence and contamination of poultry feed by *Salmonella* and *Escherichia co*li. The selected poultry feeds were found to be contaminated with aerobic bacteria such as: *Escherichia coli* and *Salmonella.*

The isolation and identification of the collected poultry feeds indicated *Salmonella* species having high number of contaminations in sample D (Layer) and that of *Escherichia coli* contamination was found in Sample H (Layer) feeds. The incidence of *Escherichia coli was* higher than that of *Salmonella*.

A better understanding of these will assist farmers and relevant stakeholders to reduce their infestation in poultry and thereby reducing the potential hazards and risks involved in transferring *Salmonella* to humans and consequently contracting human Salmonellosis, important foodborne diseases of public health concern.

The absence of *Salmonella* and *Escherichia coli* from other poultry feeds suggests that the food processing is well handled.

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Conflict of Interest

The authors declare no conflict of interest.

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