

Prevalence of *Escherichia coli* in retail poultry feeds in Southeastern Nigeria

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ABSTRACT: Poultry feed manufacturing and distribution are confronted with feed microbial quality challenges arising from disease outbreaks traceable to contaminated feeds. Lack of feed biosecurity measures at sales depots, exposure of feeds for retailing, and unhygienic handling compromise the microbial quality of feeds. Feed could hence become a source of pathogenic microbes to poultry and humans. This study evaluated the prevalence of *Escherichia coli* in retail poultry feeds in three States of Southeastern Nigeria using a purposive sampling method. A total of 389 feed samples were collected from four feed types (broiler finisher, broiler starter, chick grower, and layer ration) belonging to 11 feed brands (coded A to K) from retail outlets in Umuahia (Abia State), Abakaliki (Ebonyi State) and Enugu (Enugu State) for the study. *Escherichia coli* was isolated from some of the feed samples following standard laboratory procedures. Samples from Abia were the most bacterial and *E. coli* positive (87.7 and 74.6%, respectively) while feed brands G and F (100%, respectively) followed by J and A (80.7 and 72.7%, respectively) were the most contaminated. No bacterial contaminant was detected in brand D while layer ration was the most contaminated feed type (76.8%). The source of samples was significantly related to the frequency of bacterial contamination (χ^2 : 68.473, $p < 0.000$ for State; and χ^2 : 92.765, $p < 0.000$ for feed brand). Feed brand and feed type did not affect *E. coli* colony count but colony counts were higher than the permissible level for coliforms in feeds, and values reported for feeds in intact bags. In conclusion, exposing feeds for retailing compromises feed microbial quality. Graded bagging of retail quantities would help to preserve the microbial quality of poultry feeds from factory to farm.

Keywords: Biosecurity, colony count, feed retailing, feed safety, microbial contamination.

INTRODUCTION

The attention of the world to food borne diseases was seriously aroused following the dioxin poisoning of pet foods in Belgium (Sapkota *et al.*, 2007), and the outbreak of bovine spongiform encephalitis (BSE) in Europe (Brown *et al.*, 2001; van Larebeke *et al.*, 2001; Turhington, 2014). These and other subsequent disease outbreaks (Sapkota *et al.*, 2007; Rivas *et al.*, 2015) attributed to microbial contaminants of animal feeds (Anderson *et al.*, 2016; Rama Prasad *et al.*, 2016) further highlighted the imperative of microbial quality of animal feed.

In Nigeria, poultry producers face enormous challenges such as poor output from enormous inputs, loss of income, incessant disease outbreaks, and ineffective medications (Apata, 2009; Ezekiel *et al.* 2011). These challenges are in part due to poor feed quality associated with feed contamination by pathogenic microorganisms such as bacteria and fungi (Okoli *et al.*, 2007; Sobczak *et al.*, 2016; Sule and Ilori, 2017), and highlight the importance of microbial feed safety in ensuring good profit for the farmers.

Measures to produce and deliver safe feed to chickens have continued to evolve in response to emerging feed, food safety, and health challenges (Jones, 2011; Rivas *et al.*, 2015). Setting and enforcing 'feed safety objectives' will greatly enhance the realization of safe feed for chickens (Al-Musawi *et al.*, 2016). To attain high feed safety standards, a proactive control system is very crucial (Rivas *et al.*, 2015; Rama Prasad *et al.*, 2016) and involves the implementation of not only best manufacturing practices but also best post-manufacturing handling (storage and distribution) practices (Turhington, 2014; Rivas *et al.*, 2015; Mahami *et al.*, 2019) aimed at curtailing microbial contamination of feeds.

At present, there is scanty and poorly enforced quality control regulations on the microbial safety of poultry feed from factory to farm and in most cases, feed millers, and distributors handle feeds without regard to microbial quality and safety of the products. Furthermore, the high cost of poultry feeds necessitates the retailing of feeds to resource poor farmers. This practice could further compromise the microbial quality of feeds by exposing feeds to environmental sources of microbial contaminants. The microbial quality of feeds in intact bags has been evaluated in a number of studies (Uwaezuoke and Ogbulie, 2008; Ezekiel *et al.*, 2011) but no study to our knowledge has evaluated the microbial quality of feeds exposed for retailing to resource poor farmers who constitute a significant proportion of poultry producers in Nigeria. The objective of this study was therefore to evaluate the prevalence of bacterial and *E. coli* contaminants in poultry feeds exposed for retailing in three States of Southeastern Nigeria.

MATERIALS AND METHODS

Study area/location

The study was carried out in three States of Southeastern Nigeria namely Abia, Ebonyi and Enugu States. South-eastern Nigeria covers about 76,358 km² east of the lower Niger and South of the Benue Valley. The region is located between latitudes 4 and 7°N of the Equator and longitudes 7 and 9°E (Okali *et al.*, 2001).

Sample collection

A total of 389 retail poultry feed samples from four feed types: broiler finisher, broiler starter, chick grower, and layer ration belonging to a variety of poultry feed brands coded A to K was collected from commercial poultry feed retail outlets in Umuahia (Abia State), Abakaliki (Ebonyi State) and Enugu (Enugu State) for the study using a purposive sampling technique. Locations that had high concentration of retail feed outlets, and retail outlets selling most of the feed brands and feed types were deliberately targeted for sampling in each State. This was to ensure that all available feed brands and feed types are well

represented in the pool of samples for each State. At each retail outlet, samples were collected from open (retail) feed bags into properly labelled, sterile, screw capped sample bottles. Where possible, feed samples were pooled from three retail feed bags of the same feed brand and feed type at each sampling point. All samples were transported in sterile polyethylene bags to the laboratory and stored under refrigeration until processed.

Isolation and identification of *Escherichia coli* bacteria

0.5 g of each feed sample was inoculated into thioglycolate broth in sterile bottles for initial enrichment and then incubated at 37°C for 24 h. Thereafter, a loop full of the enriched culture was inoculated onto MacConkey agar (MCA) plates and incubated at 37°C for 24 h. Colonies on MCA that were suggestive of *E. coli* by being round, raised, opaque, rose pink in colour (on account of lactose fermentation), and about 1 mm in diameter were carefully collected with a sterile metal loop, sub cultured in Eosin Methylene Blue (EMB) agar and then incubated at 37°C for 24 h. Colonies on MacConkey agar that were suggestive of other bacteria genera such as *Proteus* (colourless), *Klebsiella* (red/pink), and *Pseudomonas* (colourless), were also noted. Colonies on EMB agar with greenish metallic sheen indicative of *E. coli* were then subjected to the following confirmatory biochemical tests after gram staining: indole, methyl red, and glucose, lactose, and citrate fermentation (Quinn *et al.*, 2002; Shecho *et al.*, 2017). Colonies that were indole, and methyl red positive, glucose and lactose fermenting with acid and gas production, and non-citrate fermenting were accepted as *E. coli* colonies. Representative colonies were then transferred to nutrient agar and stored for further use.

Colony count of *Escherichia coli* in feed samples

1 g each of *E. coli* positive feed samples was homogenized in 9 ml of thioglycolate broth followed by serial (1:10) dilutions. The solutions were then incubated at 37°C for 24 h. Thereafter, aliquot of the 10⁻⁵ serial dilution was drawn using a standard dropper pipette and inoculated onto already prepared and solidified nutrient agar (NA) plates according to the pour plate method. The inoculants were then incubated at 37°C for 24 h. Colonies were counted using a colony counter and the total viable *E. coli* colony in a sample was calculated using the expression:

$$\text{Colony count (Cfu/ml)} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Dropper pipette volume}}$$

Statistical analysis

Data collected were presented using descriptive statistics and bar charts. Statistical tests for the association between

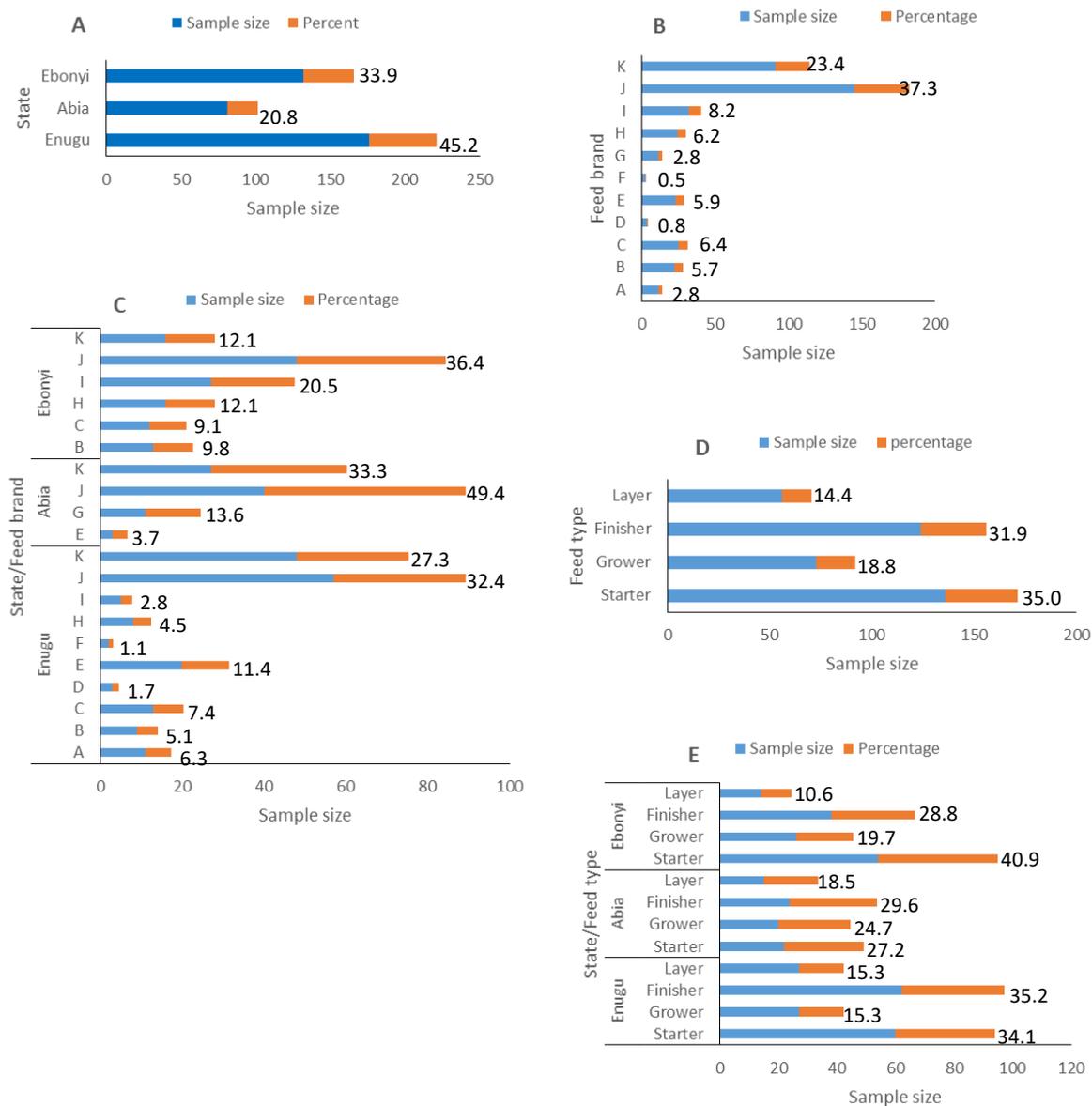


Figure 1. Distribution of feed sample. Panel A: according to state, Panel B: feed brand, Panel C: Feed brand by State, Panel D: feed type, Panel E: Feed type by State.

the rate of bacterial contamination of feed samples and sources of feed samples were performed by Chi-square analysis. Comparison between feed brands and feed types for colony count was done using Analysis of Variance and only feed brands which had all feed types positive for *E. coli* were included in this analysis. In all analyses, significance was accepted at $p \leq 0.05$.

RESULTS

A total of 389 samples were collected from feed types

(broiler finisher, broiler starter, chick grower, and layer ration) belonging to 11 feed brands (coded A to K) from retail outlets in Umuahia (Abia State), Abakaliki (Ebonyi State) and Enugu (Enugu State). The distribution of samples by state, feed brand and feed type (Figure 1) shows that 176 samples (45.2%) were from Enugu State, 81 (20.8%) from Abia State, and 132 (33.9%) from Ebonyi State (Panel A). The highest sample size of 145 (37.5%) was from brand J followed by brand K [$n = 91$ (23.4%)] while the least sample size was from brand F [$n = 2$ (0.5%)] (Panel B). For feed type (Panel C), 136 samples (35.0%) were of broiler starters while 124 (31.9%) were of

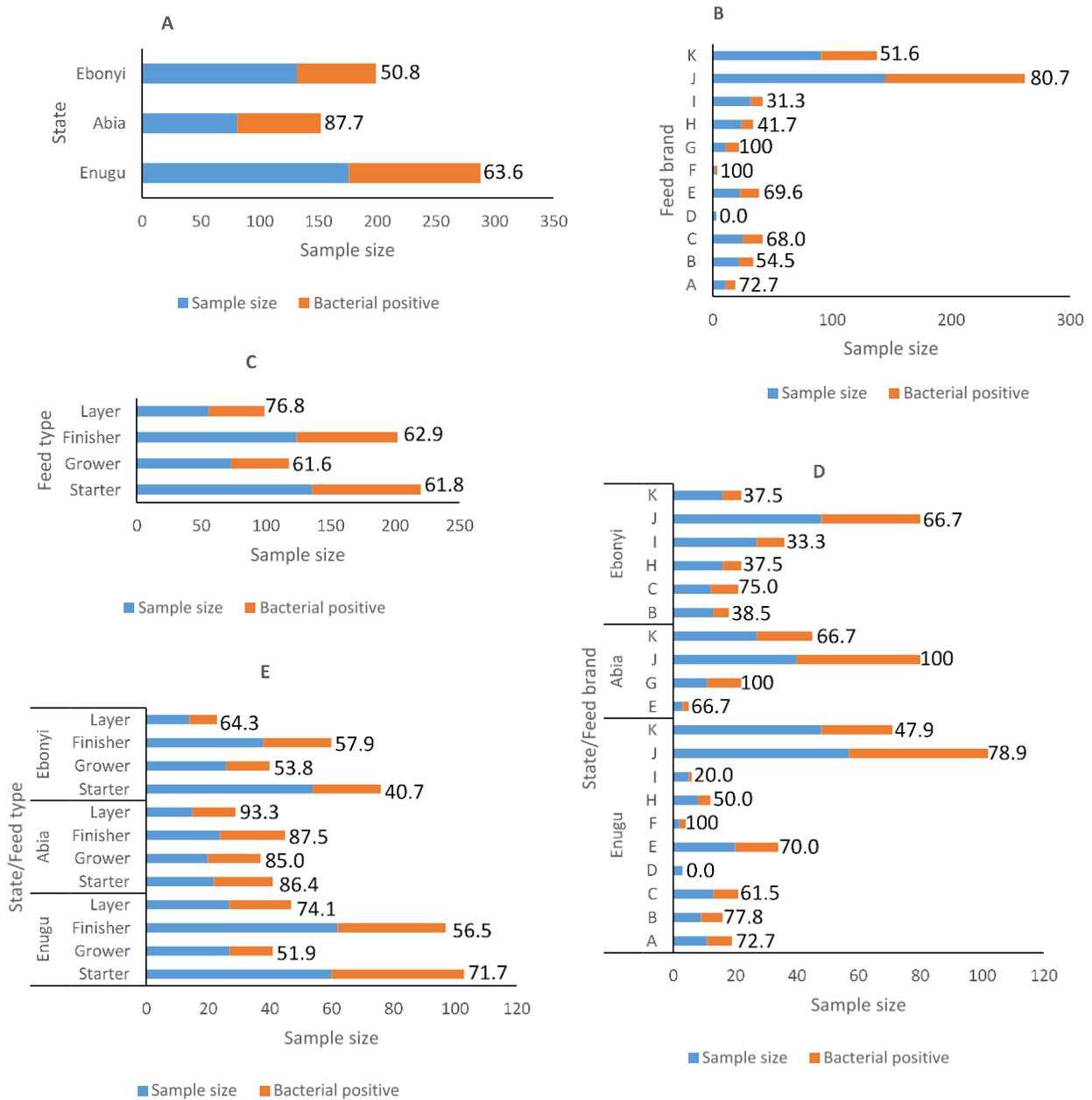


Figure 2. Distribution of bacterial positive feed samples. Panel A: according to state, Panel B: feed brand, Panel C: feed type, Panel D: Feed brand by State, Panel E: Feed type by State.

broiler finishers. Chick growers and layer rations yielded 73 (18.8%), and 56 (14.4%) samples, respectively. In Enugu State, brand J yielded the highest sample size of 57 (32.4%), while brand F yielded the least sample size of 2 (1.1%) (Panel D) while the highest sample size for Abia State was from brand J followed by brand K. Only three samples (3.7%) were from brand E. Brand J was the most sampled of the six brands encountered in Ebonyi State (n = 48, 36.4%) while brand B was the least sampled (n = 13, 9.8%). For feed type within States (Panel E), broiler finisher was the most sampled feed type in Enugu (n = 62,

35.2%), and Abia (n = 24, 29.6%) States while broiler starter was the most sampled in Ebonyi State (n = 54, 40.9%).

The distribution of bacterial contaminated samples according to State, feed brand and feed type is presented in Figure 2. Bacterial organisms suspected from colony appearance on MCA included staphylococcus, streptococcus, klebsieller, proteus, pseudomonas, and citrobacter. Feed samples from Abia State were the most contaminated at 87.7% (71/81) followed by samples from Enugu (n = 112, 63.6%) and Ebonyi State (n = 67, 50.8%)

(Panel A). Feed brands G, and F (100%, respectively) were the most contaminated brands followed by brands J and A (80.7 and 72.7%, respectively) while brand D was the least contaminated ($n = 0$, 0.0%) (Panel B). Layer ration was the most contaminated feed type ($n = 43$, 76.8%) (Panel C). The Chi-square test for association between State and frequency of bacterial contamination of feed samples revealed significant relationship (χ^2 : 69.473, $p < 0.000$). Feed brand was also significantly associated with frequency of bacterial contamination (χ^2 : 92.765, $p < 0.000$). There was no significant association between feed type and rate of bacterial contamination (χ^2 : 6.625, $p < 0.357$). Within Enugu State, brand F (100%) followed by J (78.9%), and B (77.8%) were the most contaminated while brand D (0.0%) was the least contaminated. Brands G and J (100%, respectively) were the most contaminated in Abia State while brand C (75.0%) was the most contaminated in Ebonyi State (Panel D). There was significant association between feed brand and rate of bacterial contamination within States (χ^2 : 34.972, $p < 0.010$ for Enugu State), (χ^2 : 23.329, $p < 0.001$ for Abia State) and (χ^2 : 22.004, $p < 0.015$ for Ebonyi State). The most contaminated feed type in Abia, Ebonyi, and Enugu States was layer ration (93.3, 64.3, and 74.1 %, respectively) (Panel E). Feed type was not significantly related to frequency of bacterial contamination within States (χ^2 : 2.083, $p < 0.912$; χ^2 : 9.484, $p < 0.148$; and χ^2 : 6.706, $p < 0.349$ for Abia, Ebonyi, and Enugu States, respectively).

Evaluation of feed types within brands for bacterial contamination showed that chick grower (100.0%), broiler finisher (55.6%), layer ration (89.7%), and broiler starter (62.1%) were the most contaminated in brands E, H, J, and K, respectively (Table 1). These feed brands had the full complements of the feed types. For brand A, layer ration was the most contaminated (100.0%) while for brands B and I, broiler finisher was more bacterial positive (75.0 and 37.5%, respectively) compared to other feed types. Broiler starter was the most contaminated feed type in brand C (78.6%) while for brand G, broiler starter and broiler finisher were more contaminated (100.0%, respectively). Chi square analysis yielded non-significant association between frequency of bacterial contamination and feed types within feed brands (χ^2 : 4.335, $p < 0.631$; χ^2 : 3.912, $p < 0.689$; χ^2 : 5.288, $p < 0.507$; and χ^2 : 4.414, $p < 0.621$ for brands E, H, J, and K, respectively).

Feed samples from Abia were the most *E. coli* positive (65.4%) compared to those from Ebonyi and Enugu States (Table 2). Feed brands F, and G (100.0 and 81.8%, respectively) were more *E. coli* contaminated than other brands while layer ration was the most *E. coli* positive feed type (41.1%).

Evaluation of feed brands within States for *E. coli* contamination (Table 3) showed that brand F was the most contaminated in Enugu State. Other brands had low levels of contamination with brands D and I being *E. coli* negative. In Abia State, brands G and J (81.8 and 77.5%, respectively) were more *E. coli* positive than other brands.

There was a low level of *E. coli* contamination of feed brands from Ebonyi State with brands B, H, and I being *E. coli* negative while brands C and J were 16.7 and 14.6% *E. coli* positive, respectively. Broiler starter (33.3%), layer ration (86.7%), and chick grower (15.4%) were the most *E. coli* positive feed types in Enugu, Abia, and Ebonyi States, respectively.

The distribution of *E. coli* positive feed types according to feed brands is presented in Table 4. Layer ration (50.0%) was the most *E. coli* contaminated in brand A while broiler finisher (37.5%) was the most contaminated in brand C. In brands B, F, and G, broiler finisher (12.5% for B, and 100.0% for F, and G, respectively) was the most *E. coli* positive. Feed types from brand I were *E. coli* negative while only broiler finisher was *E. coli* positive (11.1%) in brand H. Brands E, J, and K had all feed types positive for *E. coli* with chick grower (100.0%) in E; layer ration (51.7%) in J; and broiler starter (31.0%) in K being the most contaminated.

Escherichia coli colony count did not differ significantly ($p > 0.05$) between brands E, J and K and their feed types (Table 5). Colony count ranged between 24.18 ± 11.56 and 32.45 ± 4.03 colonies (2.24×10^8 and 2.67×10^8 CFU/ml) for these feed brands, while for feed types, it was between 24.29 ± 11.94 and 35.16 ± 6.35 colonies (2.08×10^8 to 2.76×10^8 CFU/ml). A lower *E. coli* load was observed in brand E (2.24×10^8 CFU/ml) compared to J, and K while broiler starter had higher *E. coli* load than other feed types (2.76×10^8 CFU/ml).

DISCUSSION

Differences in sample size for States, feed brands and feed type were due to differences in the level of retail poultry feed business in the sampled locations, the popularity of the different feed brands, the availability of the different brands for retailing, the level of demand for the different feed types by small scale poultry farmers (who buy in retail quantities) and the willingness of feed distributors to allow sampling. The higher sample size from Enugu State compared to other states was therefore a result of the higher number of retail feed outlets, greater varieties of feed brands, availability of the different feed types for retailing, and greater number of retail outlets that allowed sampling in the State. The higher number of samples obtained from brands J and K was because these brands were the most popular and all their feed types (broiler starter, chick grower, broiler finisher and layer ration) were available for retailing and sampling at most of the retail outlets. Other brands (A, B, C, D, F, G, H, and I) were new in the feed market, sporadic in distribution, and some of the feed types were out of stock or not retailled at most of the outlets sampled. The higher sample sizes obtained for broiler starter and broiler finisher was because these feed types were mostly demanded by small scale poultry farmers and therefore had assorted brands

Table 1. Distribution of bacterial positive feed samples according to feed type within feed brand

Feed brand	Feed type	Sample size	Bacterial positive (no.)	Percentage
A	Starter	NAS	ND	ND
	Grower	2	1	50.0
	Finisher	5	3	60.0
	Layer	4	4	100.0
B	Starter	14	6	42.9
	Grower	NAS	ND	ND
	Finisher	8	6	75.0
	Layer	NAS	ND	ND
C	Starter	14	11	78.6
	Grower	3	0	0.0
	Finisher	8	6	75.0
	Layer	NAS	ND	ND
D	Starter	3	0	0.0
	Grower	NAS	ND	ND
	Finisher	NAS	ND	ND
	Layer	NAS	ND	ND
E	Starter	10	8	80.0
	Grower	2	2	100.0
	Finisher	6	3	50.0
	Layer	5	3	60.0
F	Starter	1	1	100.0
	Grower	NAS	ND	ND
	Finisher	1	1	100.0
	Layer	NAS	ND	ND
G	Starter	2	2	100.0
	Grower	3	3	100.0
	Finisher	6	6	100.0
	Layer	NAS	ND	ND
H	Starter	11	4	36.4
	Grower	2	0	0.0
	Finisher	9	5	55.6
	Layer	2	1	50.0
I	Starter	10	3	30.0
	Grower	6	1	16.7
	Finisher	16	6	37.5
	Layer	NAS	ND	ND
J	Starter	42	31	73.8
	Grower	32	28	87.5
	Finisher	42	32	76.2
	Layer	29	26	89.7
K	Starter	29	18	62.1
	Grower	23	10	43.5
	Finisher	23	10	43.5
	Layer	16	9	56.3

NAS: not available for sampling; ND: not determined.

Table 2. Distribution of *E.coli* positive feed samples by state, feed brand, and feed type.

Source	Sample size	Sample bacterial status		<i>E. coli</i> positive as percentage of	
		Bacterial positive (no.)	<i>E. coli</i> positive (no.)	Bacterial positive	Sample size
States					
Enugu	176	112	50	44.6	28.4
Abia	81	71	53	74.6	65.4
Ebonyi	132	67	10	14.9	7.6
Feed brand					
A	11	8	3	37.5	27.3
B	22	12	2	16.7	9.1
C	25	17	7	41.2	28.0
D	3	0	0	0.0	0.0
E	23	16	10	62.5	43.5
F	2	2	2	100.0	100.0
G	11	11	9	81.8	81.8
H	24	10	1	10.0	4.2
I	32	10	0	0.0	0.0
J	145	117	59	50.4	40.7
K	91	47	28	59.6	30.8
Feed type					
Starter	136	84	38	45.2	27.9
Grower	73	45	20	44.4	27.4
Finisher	124	78	32	41.0	25.8
Layer	56	43	23	53.5	41.1

available for retailing and sampling. Broiler starter can be fed to all chick types (broiler, cockerel, and pullet chicks) during brooding phase, while broiler finisher is used to finish (fatten) chickens for the market. On the other hand, chick grower is for feeding of only pullet, and cockerel chicks during the growing phase while layer ration is for feeding laying chickens. In addition, a greater number of small scale poultry farmers engage in broiler production than laying chicken rearing due to the shorter duration and quicker returns from broiler chicken production.

The multi-bacterial contamination of feed samples in the present study agrees with previous studies (Okogun *et al.*, 2016; Mathew *et al.*, 2017; Mohammed *et al.*, 2021; Munoz *et al.*, 2021). Bacterial contaminants detected include *E. coli*, *Salmonella* sp., *Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp., *Lactobacillus* sp., *Proteus*, *Candida albicans*, and *Pseudomonas* sp. The higher bacterial contaminated samples from Abia State indicate that feeds retailed in this State were more exposed to bacterial contaminants compared to the other States. Factors that could predispose feeds to bacterial contamination include unhygienic and multiple handling during the piecemeal retailing (Turhington, 2014), long periods of exposure due to slow demand, contact with urine and/or faecal materials from rodents (Gopi *et al.*, 2017), insect infestation (Gopi *et al.*, 2017; Rossato *et al.*, 2019), and a myriad of other

environmental contaminants during the period of exposure for retailing. It has been reported that the odds of contamination of feed increase each time the feed is handled (Jones, 2011; Turhington, 2014). The observed higher bacterial contamination of brands F, G, J, and A could be due to unhygienic handling, long periods of exposure of their feed types as a result of low demand or the use of feed raw materials of poor microbial quality during compounding. The zero contamination of brand D was probably because the sampled feed type (broiler starter) was fast moving and do not take long to be disposed of. In addition, this brand is in pellets. The process of pelleting of feeds decontaminates feed raw materials (Furuta *et al.*, 1980; Blank *et al.*, 1996; Mahami *et al.*, 2019). The observed higher percentage of bacterial contamination of layer ration compared to other feed types agreed with Ngai *et al.* (2021) who reported higher prevalence of salmonella in layer mash compared to other feed types but contrary to Okogun *et al.* (2016) who reported higher bacterial contamination in broiler starter. The higher bacterial contamination of layer ration in the present study was probably due to low demand, long periods of exposure at the retail outlets, unhygienic handling, or the use of raw materials of poor microbial quality in feed compounding. The observed variation in the prevalence of bacterial contamination of feed brands

Table 3. Distribution of *Escherichia coli* positive feed samples by feed brand, and feed type within states

States	Parameters	Sample size	Sample contamination status		<i>E. coli</i> positive as percentage of	
			Bacterial positive (no.)	<i>E. coli</i> positive (no.)	Bacterial positive	Sample size
Feed brand						
Enugu	A	11	8	3	37.5	27.3
	B	9	7	2	28.6	22.2
	C	13	8	5	62.5	38.5
	D	3	0	0	0.0	0.0
	E	20	14	8	57.1	40.0
	F	2	2	2	100.0	100.0
	H	8	4	1	25.0	12.5
	I	5	1	0	0.0	0.0
	J	57	45	21	46.7	36.8
	K	48	23	8	34.8	16.7
Abia	E	3	2	2	100.0	66.7
	G	11	11	9	81.8	81.8
	J	40	40	31	77.5	77.5
	K	27	18	11	61.1	40.7
Ebonyi	B	13	5	0	0.0	0.0
	C	12	9	2	22.2	16.7
	H	16	6	0	0.0	0.0
	I	27	9	0	0.0	0.0
	J	48	32	7	21.9	14.6
K	16	6	1	16.7	6.3	
Feed type						
Enugu	Starter	60	43	20	46.5	33.3
	Grower	27	14	7	50.0	25.9
	Finisher	62	35	15	42.9	24.2
	Layer	27	20	8	40.0	29.6
Abia	Starter	22	19	14	73.7	63.6
	Grower	20	17	9	52.9	45.0
	Finisher	24	21	17	81.0	70.8
	Layer	15	14	13	92.9	86.7
Ebonyi	Starter	54	22	4	18.2	7.4
	Grower	26	14	4	28.6	15.4
	Finisher	38	22	0	0.0	0.0
	Layer	14	9	2	22.2	14.3

within States could be attributed to differences in feed biosecurity measures among operators in the feed business within States as well as the level of acceptance of the different brands which influences the rate of disposal and duration of exposure at sales outlets. Among brands A, B, C, F, G, and J (the most contaminated across States), brands A, B, C, F, and G, were new in the feed market during the period of this study and had not become

popular among small scale poultry farmers. Thus they were in low demand. Besides, brands A, B, F, and G are mashes, which unlike pellet feeds, do not undergo heat decontamination during compounding. Brand J on the other hand was the most retailed brand however, the layer ration of this brand once opened for retailing, could stay for a long time before disposal because very few layer chicken farmers demand retail feed quantities. In the present

Table 4. Distribution of *E. coli* positive feed samples according to feed type within feed brand.

Feed brand	Feed type	Sample size	Contamination status		<i>E. coli</i> positive as percentage of	
			Bacterial +ve (no.)	<i>E. coli</i> +ve (no.)	Bacterial +ve	Sample size
A	Starter	NAS	ND	ND	ND	ND
	Grower	2	1	0	0.0	0.0
	Finisher	5	3	1	33.3	16.7
	Layer	4	4	2	50.0	50.0
B	Starter	14	6	1	16.7	7.1
	Grower	NAS	ND	ND	ND	ND
	Finisher	8	6	1	16.7	12.5
	Layer	NAS	ND	ND	ND	ND
C	Starter	14	11	4	36.4	28.6
	Grower	3	0	0	0.0	0.0
	Finisher	8	6	3	50.0	37.5
	Layer	NAS	ND	ND	ND	ND
D	Starter	3	0	0	0.0	0.0
	Grower	NAS	ND	ND	ND	ND
	Finisher	NAS	ND	ND	ND	ND
	Layer	NAS	ND	ND	ND	ND
E	Starter	10	8	5	62.5	50.0
	Grower	2	2	2	100.0	100.0
	Finisher	6	3	1	33.3	16.7
	Layer	5	3	2	66.7	40.0
F	Starter	1	1	1	100.0	100.0
	Grower	NAS	ND	ND	ND	ND
	Finisher	1	1	1	100.0	100.0
	Layer	NAS	ND	ND	ND	ND
G	Starter	2	2	2	100.0	100.0
	Grower	3	3	1	33.3	33.3
	Finisher	6	6	6	100.0	100.0
	Layer	NAS	ND	ND	ND	ND
H	Starter	11	4	0	0.0	0.0
	Grower	2	0	0	0.0	0.0
	Finisher	9	5	1	20.0	11.1
	Layer	2	1	0	0.0	0.0
I	Starter	10	3	0	0.0	0.0
	Grower	6	1	0	0.0	0.0
	Finisher	16	6	0	0.0	0.0
	Layer	NAS	ND	ND	ND	ND
J	Starter	42	31	16	51.6	38.1
	Grower	32	28	14	50.0	43.8
	Finisher	42	32	14	43.8	33.3
	Layer	29	26	15	57.7	51.7
K	Starter	29	18	9	50.0	31.0
	Grower	23	10	3	30.0	13.0
	Finisher	23	10	4	40.0	17.4
	Layer	16	9	4	44.4	25.0

NAS: not available for sampling; ND: not determined.

Table 5. *Escherichia coli* load in positive feed brands and feed types.

Variable	Colony forming Units (CFU)	CFU/ml	Log CFU/ml
Feed brand			
E	24.18 ± 11.56	2.24 × 10 ⁸	8.25 ± 0.18
J	32.45 ± 4.03	2.67 × 10 ⁸	8.24 ± 0.06
K	32.13 ± 7.57	2.62 × 10 ⁸	8.22 ± 0.12
Feed type			
Finisher	24.29 ± 11.94	2.08 × 10 ⁸	8.21 ± 0.19
Grower	27.23 ± 9.86	2.73 × 10 ⁸	8.28 ± 0.15
Layer	31.64 ± 9.39	2.48 × 10 ⁸	8.23 ± 0.15
Starter	35.16 ± 6.35	2.76 × 10 ⁸	8.24 ± 0.10
Feed brand x feed type			
E x Finisher	15.00 ± 31.17	1.86 × 10 ⁸	8.27 ± 0.49
E x Grower	10.50 ± 22.04	1.30 × 10 ⁸	8.07 ± 0.34
E x Layer	20.00 ± 22.04	2.48 × 10 ⁸	8.39 ± 0.34
E x Starter	51.20 ± 13.94	3.33 × 10 ⁸	8.26 ± 0.22
J x Finisher	31.36 ± 8.35	2.23 × 10 ⁸	8.13 ± 0.13
J x Grower	38.87 ± 8.05	3.22 × 10 ⁸	8.36 ± 0.13
J x Layer	32.93 ± 8.05	2.85 × 10 ⁸	8.25 ± 0.13
J x Starter	26.63 ± 7.79	2.40 × 10 ⁸	8.24 ± 0.12
K x Finisher	26.50 ± 15.58	2.14 × 10 ⁸	8.21 ± 0.24
K x Grower	32.33 ± 18.00	3.66 × 10 ⁸	8.40 ± 0.28
K x Layer	42.00 ± 15.58	2.10 × 10 ⁸	8.04 ± 0.24
K x Starter	27.67 ± 10.39	2.57 × 10 ⁸	8.22 ± 0.16

study, layer ration was the most bacterial contaminated feed type within States. This is most likely the result of unhygienic handling and long period of exposure due to low demand for retail quantities by layer chicken farmers. The observed higher bacterial contamination of layer ration in brands A, and J; chick grower in brands E and G; broiler starter in brands C, F, G, and K; and broiler finisher in brands B, F, G, H, and I could be attributed to low demand, long period of exposure, and unhygienic handling at the retail outlets. The high incidence of bacterial contamination in almost all sampled feed types in the feed brands indicates that feed retailing compromises feed microbial quality. The significant association between the frequency of bacterial contamination and the source of feed samples (State and feed brand) agrees with Egbule (2022) and indicates that knowledge and application of feed biosecurity vary among operators (feed millers and distributors) across States and this impacts the microbial quality of feeds. It has been observed that some actors in the poultry feed value chain lack knowledge of feed hygiene and microbial safety of feeds (Egbule, 2022). The non-significant association between the frequency of bacterial contamination and feed types within States and feed brands indicates that all feed types exposed for retailing are equally prone to bacterial contamination.

The higher percentage of *E. coli* contaminated feed samples from Abia and Enugu States followed from the higher bacterial contamination of feeds from these States. Similarly, the higher *E. coli* contamination of brands F and G, and layer ration followed from the higher bacterial contamination of these brands and feed type. The low levels of *E. coli* detection in brands J, A, E, and C as well as in chick grower, broiler starter and broiler finisher which were highly bacterial positive indicate that other bacterial sp. were more important contaminants of these brands and feed types. The very high *E. coli* contamination of brand F from Enugu; and G, and J from Abia corresponded with the high bacterial contamination of these brands. Brands F and G are produced by local feed millers who use locally available raw materials of poor microbial quality in feed compounding. Many of these millers do not apply ingredient decontamination and feed quality enhancing technologies in their operations. Feed ingredients have been identified as major sources of contamination of poultry rations (Turhington, 2014; Gopi *et al.*, 2017; Mahami *et al.*, 2019). Locally sourced fish wastes and palm kernel cake have been reported to be highly contaminated with bacterial organisms (Okoli *et al.*, 2007). This in addition to prolonged exposure at the retail outlets, may have contributed to the high *E. coli* contamination

observed. The low level of *E. coli* detection in feed brands from Ebonyi State indicates that other bacterial sp. were more important contaminants of brands from this State.

The high *E. coli* contamination of broiler starter, layer ration, and chick grower in Enugu, Abia, and Ebonyi States, respectively (Table 3) and across feed brands (Table 4), agree with the findings of other studies (Okogun *et al.*, 2016; Ngia *et al.*, 2021; Egbule *et al.*, 2022). Variation in levels of bacterial contamination of feed types was attributed to differences in manufacturing standards, post compounding handling, nutrient composition of feeds and method of bacterial detection (Ngia *et al.*, 2021; Mohammed *et al.*, 2021).

The similarity in *E. coli* colony counts between feed brands, and feed types suggests similar sources and levels of *E. coli* contamination of the feed samples evaluated in this study. The result is at variance with Arotupin *et al.* (2007) who reported significantly higher colony count in broiler starter compared to other feed types, and higher colony count in chick grower compared to broiler finisher. Ngai *et al.* (2021) also reported highest microbial load in layer mash compared to other feed types. The aerobic counts in the present study were similar to the 1.0×10^8 to 7.9×10^8 cfu/g reported by Iram *et al.* (2020) for poultry feeds from farms in Karachi, Pakistan but higher than the range of 6.6×10^2 to 2.5×10^4 cfu/g reported by Arotupin *et al.* (2007) for commercial feeds in Akure, Nigeria; and the 10^3 to 10^6 cfu/g for compound feedstuff in Polland over a four year period (Kwiatk *et al.*, 2008). The values in this study were also higher than the 5.33×10^5 and 5.7×10^5 cfu/g reported for fresh composite feed and 5 weeks old samples from feed mills in Newzeland (Rivas *et al.*, 2015), as well as, the 5.0×10^3 to 1.76×10^6 cfu/g reported for feeds from poultry pens in Ilorin, Nigeria (Sule and Ilori, 2017). These differences may be attributed to the source of feed samples. The higher *E. coli* load obtained in the present study could result from *E. coli* contamination from a myriad of sources such as unhygienic handling, and contamination by urine and faeces from rodents and insects. Samples from feed factories and intact feed bags would normally be less contaminated than samples from feeds exposed for retailing. Egbule *et al.* (2022) detected higher bacterial contamination in feed samples from open markets compared to samples from feed stores. The colony counts in the present study were higher than the permissible coliform count of Log_{10} 4.0 in poultry feeds (Mahami *et al.*, 2019) indicating that exposure of feeds for retailing worsens the microbial quality of feeds and that feeds exposed for retailing are more heavily contaminated and are ready sources of microbial pathogens to poultry and ultimately to humans.

Conclusion

Exposure of poultry feeds for retail sales predisposes feeds to heavy microbial contamination with *E. coli* load

higher than the permissible level for coliforms for poultry feeds and values reported for samples from factories and intact feed bags. The practice of exposing poultry feeds for retailing should be discouraged by packaging graded retail quantities for small scale/backyard operations. In addition, stringent regulations for microbial feed quality monitoring should be enforced for feed industries, local millers and feed distributors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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