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Original Article

Frequency Of Isolation Of Salmonella From Commercial Poultry Feeds And Their Anti-Microbial Resistance Profiles, Imo State, Nigeria

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Abstract:
This study was conducted to determine the frequency of isolation of salmonella and their microbial resistance profiles across different commercial poultry feeds sold in Imo State, Nigeria. Thirty-six bulk feed samples were colleted from 154 bag across different feed types and brands which included Guinea (GF), Top (TF), Vital (VF), Extra (EF), Animal care (AF) and livestock (LF) feeds. The salmonella isolated were tested against 14 anti-microbial drugs using the disc diffusion method. Bacterial load enumeration of the samples indicated a range of <30 colony forming unit (CFU) to overgrowth at 10^4 serial dilutions. Eight feed samples (22.2%) which cuts across the entire feed brands expect EF were positive for salmonella. The highest prevalence of 28.8% and 25.0% were recorded for LF and TF respectively, while VF, GF and AF had 11.1 and 10.0% respectively. Salmonella isolates showed high rates of resistance (51-100%) against nitrofurantoin, ampicillin, tetracycline and ceftriazole, while moderate rates (31-50%) were recorded for chloramphenicol, oxfloxacin and cotrimoxazole. Low resistance rates (1-30%) were on the other hand recorded against ciprofloxacin and amoxycillin clavulanate (Augumentine), whereas zero resistance was demonstrated against pefloxacin, gentamycin, streptomycin and nalidixic. Commercial feeds form important channels for the dissemination of multi-drug resistant salmonella in Imo State, Nigeria.
Key Words: Salmonella, poultry feed, anti-microbial drugs, resistance profile, Nigeria

Introduction:
Factors such as quality feed, sound health management and rearing environment are indispensable complements to genetic techniques for livestock improvement in the tropics.(1) This is because farm animals are vulnerable to numerous diseases of economic and public health importance. Prominent among these are salmonella infections of poultry, which have been shown to be of critical importance especially in Nigeria (2-4). Several studies have shown that sources of salmonella infection in poultry include contaminated products, feeds and feed ingredients, human wastes, mouse and rat droppings among others.(5-8)

Hygienic production of poultry feeds is therefore important and involves the processing of feeds under health hazard free conditions. This usually starts from the harvesting of the feed ingredients, to the storage, processing, packaging transporting and eventual marketing of the bagged feeds at the various sales outlets from where the farmers collect to feed their animals.(9) In developed countries, measures such as Hazard Analysis and Critical Control Point (HACCP) program are been adopted to control salmonella and other pathogenic micro-organism to near zero tolerance in poultry feeds. In developing countries such as Nigeria however, such programs are not in place and there are no reliable data on the prevalence and anti-microbial susceptibility of salmonella isolates from poultry feed.(10)

Overall, the challenge for animal nutritionist and commercial feed producers anywhere is to consistently monitor all segment of feed production, and measure those variables that are good indicators of quality control against pathogenic organisms such as salmonella. This is imperative since commercial feeds and feed ingredients are major potential routes of disease dissemination outside the control of the farmer.(11, 12) Furthermore, since commercial feeds are usually sourced from wide geographical areas, they remain potentially, major vehicles for the introduction of bacteria harboring novel resistance factors to a local farm environment.(13)

Antibiotic resistance among bacteria genera is a worldwide problem.(14)

The rate at which resistance arise among bacterial populations has been reported to be contingent on the extent of use of a particular antibiotics in a particular environment.(15) The salmonella organism contributed by the different raw materials used in compounding a commercial feed may harbor resistance factors reflecting antibiotic use in their area of origin.

This study was designed to investigate the frequency of occurrence of salmonella from commercial poultry feeds and their microbial resistance profile in Owerri, Imo State, Nigeria.

Materials and Methods:

Study area: The study was carried out in Imo State, Nigeria. The geographical and agro-climatic characteristic of the area has been described.(14) The characteristic of poultry production has also been reported by Okoli et al.(13,14). Commercial poultry farmers in the area usually purchase their feeds from dealers on any of the popular commercial feed brands. Most large-scale operators produce their own feeds from feed raw materials purchased from dealers. Water is obtained from public taps where available or from streams or harvested rainwater. Self-medication is very rampant among the farmers with some of them also using human preparation for the poultry disease problems.(16)

Table 1: Distribution of commercial feeds sample type collected for isolation of salmonella in Imo State, Nigeria.

		Feed type in bags				
Visit	Feed Brand	GM	LM	BS	BF	Total Bags
1st	LF	-	-	4	-	4
	VF	4	4	4	4	16
	TF	4	4	4	4	16
	GF	4	4	4	4	16
	EF	-	-	4	4	8
	AF	-	-	4	-	4
		3	3	6	4	
		Total bulk=16 Bulk samples				74 bags
2nd	LF	-	-	-	-	
	VF	4	4	-	-	8
	TF	-	-	4	-	4
	GF	4	4	4	4	16
	EF	4	4	-	4	12
	AF	-	-	-	-	-
		3	3	2	2	
		Total Bulk= 10 bulk sample.				40 bags
3rd	LF					
	VF	-	4	4	4	12
	TF	4	4	4	-	12
	GF	4	4	-	-	8
	EF	-	4	-	-	4
	AF	4	-	-	-	4
		3	4	2	1	
		Total Bulk= 10 bulk sample				40 bags
		Grand Total = 36 bulk sample made up of 154 bags of feed				

Key: BS-Broiler starter, LM- Layer mash, GM-Grower mash, BF-Broiler finisher, LF-Livestock feed, VF-Vital feed, TF-Top feed, GF-Guinea feed, EF-Extra feed, AF-Animal care feed

Sample collection: A total of 36 bulked samples were collected from a list of 6 purposively selected commercial feed brands sold in different feed outlets in Owerri, Imo State, Nigeria between the months of July and September 2004. Each selected feed outlet was visited three times for samples collection during the study period. The commercial six feed brands were livestock (LF), Extra (EF), Top (TF), Guinea (GF), Animal care (AF) and vital (VF) feed (Table 1). Altogether, the 36 bulked samples were obtained by sapling 154 feed bags of different types, which included grower, layer, broiler starter and finisher masher. A standard commercial feedbag in the state weighs 25 kg.Each feed brand was sampled by carefully opening 4

randomly selected bags of the same feed type and collecting about 3 g using a sterile universal bottle. These were thereafter homogenized to obtain a representative bulk sample of about 12 g of the feed type. The samples were for feed brand and type. They were transported to the laboratory for analysis within 2 hours of collection.

Bacterial load enumeration: This was carried out at Imo State Environment Protection Agency (ISEPA) Microbiology Laboratory. A 4 fold serial dilution of the homogenized samples as described by Ogbulie and Okpokwasili (17) was prepared for each sample. This involved adding 5 g of the feed sample in 45 ml of sterile deionized water and mixing thoroughly. 0.1 ml aliquot of the appropriate dilution was drawn and inoculated unto nutrient agar. After over night incubation, the bacterial load was enumerated using a colony counter (Suntex^{R}) to count the colony-forming unit (CFU).

Bacterial isolation: Aliquots of the serially diluted samples were enriched in peptone water and after over night incubation at 37^{0}C, these were then sub-cultured onto selenite broth for selective growth according to method of Cheesbrough.(18) Suspected salmonella growths (deep orange colored broth), were subsequently sub-cultured onto MacConkey agar and incubated overnight at 37^{0}C. Non-lactose fermenting colonies suggestive of salmonella were further subjected biochemical tests, which included Simmon citrate, Indole and Urease tests (19) to confirm salmonella isolation.

Susceptibility testing: The confirmed salmonella isolates were screened for anti-microbial resistance using the disc diffusion method (20) according to the methods recommended by the National Committee for Clinical Laboratory Standard Guidelines.(21) The disc diffusion method is widely recognized to work well with rapidly growing facultatively anaerobic and aerobic organisms such as Enterbacteriaceae.(21)

Commercial antibiotics discs used in the study included CH, Chloramphenicol (30 µg); CR, Ceftriazole (30 µg); NI, Nitrofurantoin (200 µg); CO, Cotrimoxazole (30 µg); OF, Oxfloxacin (10 µg); GN, Gentamycin (10 µg); AU, Amoxycillin clavulanate (30 µg); NA, Nalidixic acid (30 µg); CP, Ciprofloxacin (10 µg); SP, Streptomycin (10 µg); PF, Pefloxacin (10 µg); AM, Ampicillin (30 µg), TE, Tetracycline (25 µg) and CE, Cephalexin (15 µg). The susceptibility data were recorded qualitatively as resistant or sensitive. The isolates resistant to individual drugs and anti-microbial pattern were computed according to species and origin.

Statistical analysis: The data collected was analyzed for prevalence of the isolates and their anti-microbial resistance profile using simple descriptive statistics such as means, percentages and histograms.

Results:

Bacterial load: Table 2a and b showed the bacterial load in the different commercial feed brands. At 4 serial dilutions, 2 samples of grower mash (GF and VF) had overgrowth while 5 samples recorded it among the layer mashes. Three of these were recorded for VF and one each for GF and TF. Across the broiler mashes (Table 2b) one sample each from GF and VF had overgrowth, while another from TF had >300 CFU. Similarly, one sample each from GF, VF and EF recorded overgrowth in broiler finisher.

Salmonella prevalence: Table 3 showed that 8(22.2%) of the 36 bulk samples had salmonella isolates. Across the feed types, 40.0 and 25.0% of these came from layer and broiler finisher mashes respectively. None of the grower mashes yielded salmonella isolates. Table 4 showed that 28.8 and 25.0% of these isolates came from livestock and top feeds respectively

while the rest brands recorded from 10 to 11.1% isolations.

Anti-microbial resistance: Figure I showed that the salmonella isolates registered high rate of resistance (51-100%) to nitrofurantoin, ampicillin, tetracycline, and ceftriazole, while moderate rate (31-50%) were recorded against chloramphenicol, oxfloxacin and cotrimoxazole. Low resistance rates (1-30%) were on the other hand returned for ciprofloxacin and amoxycillin clavulanate, while zero resistance were recorded against pefloxacin, gentamycin, streptomycin and nalidixic acid

Table 2: Bacterial load enumeration in different commercial feed brands fold in Imo State, Nigeria

A								
	Grower Mash				Layer Mash			
Sample	GF	VF	EF	TF	GF	VF	EF	TF
1		Overgrowth				Overgrowth		
2	<30							<30
3	<30				>300			
4		<30			Overgrowth			
5			>300				<30	
6	<30					Overgrowth		
7				<30		Overgrowth		
8	Overgrowth						<30	
9								Overgrowth
10								<30
B								
	Broiler Starter				Broiler Finisher			
Sample	GF	VF	TF	AF	GF	VF	EF	TF
1							<30	
2				<30			<30	
3			>300		Overgrowth		>300	
4	<30					Overgrowth		
5		No growth						<30
6			<30				Over-growth	
7	Overgrowth							
8			>300				<30	
9		<30						
10		<30						

Key: GF-Guinea feed, VF-Vital feed, EF-Extra feed, TF-Top feed, AF-Animal care feed, OG-Overgrowth.

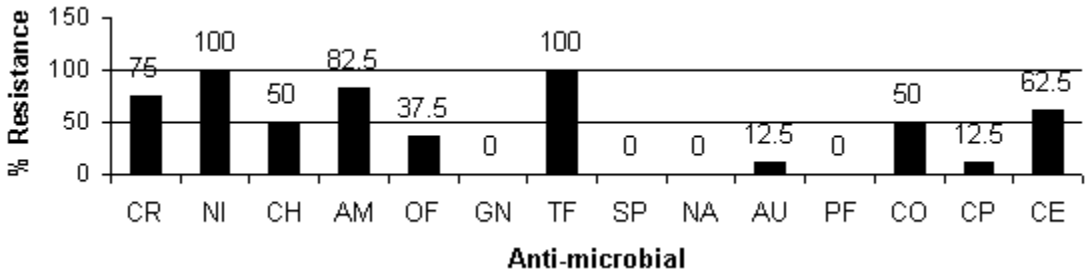
Table 3: Frequency of isolation of salmonella from different type of commercial poultry feeds sold in Owerri, Imo State.

Source	Number of samples	Number (%) infected
Grower mash	8	0 (0.0%)
Layer mash	10	2 (20.0)
Broiler starter	10	4 (40.0)
Broiler finisher	8	2 (25.0)
Total	36	8 (22.2)

Table 4: Frequency of isolation of salmonella from different type of commercial poultry feed sold on Owerri, Imo State.

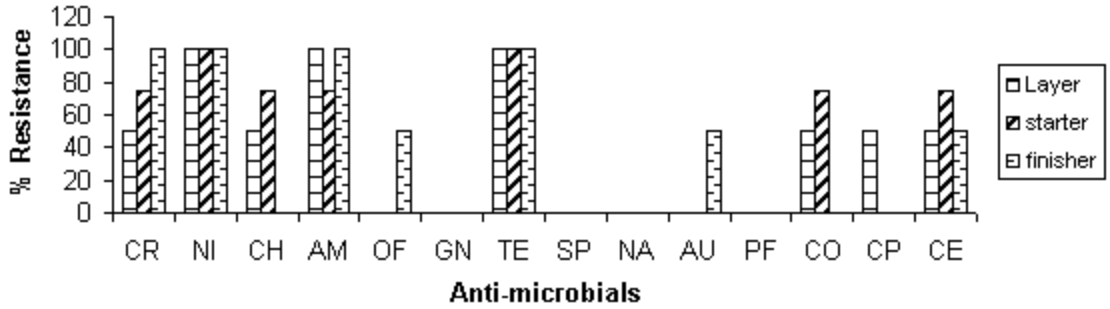
Source	Number of samples	Number (%) infected
Guinea feed	10	1 (10.0%)
Vital feed	9	1 (11.1)
Top feed	1	2 (25.0)
Animal care feed	1	1 (10.0)
Livestock feed	7	2 (28.8)
Total	36	8 (22.2)

Fig I: Anti-microbial resistance of salmonella isolated from commercial poultry feed sold in Imo State, Nigeria.



CR-Ceftriazole, NI-Nitrofurantoin, CH-Chloramphenicol, AM-Ampicillin, OF-Oxfloxacin, GN-Gentamycin, TE-Tetracycline SP-Streptomycin, NA-Nalidixic acid, PF-Pefloxacin, AU-Amoxycillin clavulanate, CO-Cotrimoxazole, CP-Ciprofloxacin, CE-Cephalexin

Fig II: Comparison of anti microbial resistance in salmonella isolates from layer, broiler starter and broiler finisher mash sold in Imo State, Nigeria.



Key as in Fig I

Figure II shows the comparison of anti-microbial resistance of salmonella isolates from the different commercial poultry feed types. Isolates from broiler finisher mashes returned 100% resistance to tetracycline, nitrofurantoin, ampicillin and ceftriazole. Similarly isolates from layer and broiler starter mashes returned 100% resistance to nitrofurantoin and tetracycline, while those from layer mash only recorded 100% resistance against tetracycline, ampicillin and nitrofurantoin.

Discussion:

Monitoring of microbial contamination of animal production environment is an important first step in determining how such contaminants especially salmonella pass through the food chain.(22) This is because, in general the transmission of salmonella spp through the environment has been shown to cyclic, and poultry feeds have historically been viewed as important links for contamination in poultry.(23,24) 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.(25)

The obvious disparity in bacterial load of the feeds analyzed in this study may be reflecting this since animal protein sources have been shown to harbor heavier bacterial growth than other feed raw materials, especially locally processed fish wastes.(25) It is probable that high incidence of bacterial overgrowth recorded in the layer mashes is due to the use of such fish wastes in commercial feed mixing. It would seem from the present results that guinea and vital feeds are particularly prone to this practice. Chemical amendment, heat treatment, irradiation and careful sourcing of materials are proven methods of reducing bacterial loads in feed ingredient.(24)

Table 5: Resistance pattern of salmonella isolates from various commercial poultry feeds sold in Owerri, Imo State.

	Pattern	Frequency		Source
1	CR-NI-TE	3	1	BS (TF)
2	CR-NI-TE-AM	4	1	LM (EF)
3	CR-NI-TE-AM-OF	5	1	BF (TF)
4	CR-NI-TE-AM-CE-AU	6	1	BF (EF)
5	CH-NI-TE-AM-CE-CO	6	1	BS (VF)
6	CH-CR-NI-TE-AM-CE-OF	7	1	LM (GF)
7	CH-NI-TE-AM-CE-CO-CP	7	1	BS (AF)
8	CH-CR-NI-TE-AM-CO-CE-OF	8	1	BS (LF)

The present 22.2% prevalence of salmonella in bulk commercial feed samples sold in Imo State, Nigeria is of economic and public health significance, since commercial feed remain sources of infection outside the control of the poultry farmer.(11,12,24) The higher prevalence of salmonella in Extra and Top feed samples probably reflect the level of bio-security and hygienic practices in these establishments. Decomposing fecal pellets from wild life and vermin that are attracted to the feed milling environment are important transmitters of salmonella spp to feed supply.(7,26) The control of such vermin and scavengers and grains feeders in addition to ingredient are final products treatment could make a different between salmonella contamination of the final product from different firms.

Again the observed higher salmonella prevalence in broiler starter, layer and broiler finisher mashes probably reflects the contamination picture of ingredients used in producing them. The higher performance needed in broiler and egg production requires inclusion of animal proteins in these mashes, usually to elicit the animal protein factor effect in the birds.(27) The use of

these animal protein ingredients especially cheap locally processed fish wastes has been reported to be important vehicles for bacterial contamination of poultry feed ingredients.(25) Such products should therefore, be subjected to appropriate anti-bacterial treatment before inclusion in poultry feeds.

The present results of anti-microbial resistance of salmonella isolates from poultry feeds highlight again the severally reported multi-drugs resistance of bacterial of the Enterbacteriaceae family in Imo State.(13,14,28-30) The present data is however, of particular public health interest since some of the isolated organisms could be zoonotic. While these organisms were not identified to genera level, unpublished field data by Anyanwu (31) and Okoli (32) suggest that *S. enteritidis, S. typhimurium* and *S montevideo* are involved in poultry contamination in this study area.

The 37.5% resistance recorded for oxfloxacin is again of interest since fluoroquinolones are currently the drug of choice in the treatment of both human and animal salmonellosis in the study area. The low to zero resistance of other quinolones and aminoglycosides (gentamycin and streptomycin) are in agreement with earlier results on *E. coli* resistance in the study area.(13,14,29,30) These earlier works also highlighted similar high resistances against the cheap, readily available first line antibiotics such as ampicillin, tetracycline, nitrofurantoin, and cotrimoxazole among others recorded in the present study.

Conclusion:

The relatively high prevalence of salmonella in commercial feeds recorded in this study highlights the need for institution of salmonella monitoring measures and regulation in the feed industry in Nigeria.

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