Assessment of *Salmonella* Contamination of Feed Raw Materials and Their Anti-microbial Resistance Profiles in 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 selected feed raw materials sold in Imo State, Nigeria. Three hundred and sixty (360) bulk samples were collected across different feed raw materials which include animal proteins-foreign fish meal (FFM) and local fish meal (LFM), plant proteins-groundnut cake (GNC) and soybean meal (SBM), fiber sources-palm kernel cake (PKC) and wheat offal (WO), energy grain-maize (MZ) and Minerals-bone meal (BM). The *salmonella* isolated were tested against 14 anti-microbial agent using disc diffusion method. Bacterial load enumeration of the samples indicated a range of >300 to overgrowth of colony forming unit (CFU) at 4 serial dilution. One hundred and twenty (120) samples (33.33%) were positive for *salmonella* isolates with fiber sources and animal protein recording 56.00% and 50.91% prevalence, respectively. Across the individual raw material types, it recorded LFM (90.0%), WO (60.0%), PKC (50.0%), SBM (40.0%) and GNC (28.67%) prevalence while non were isolated from maize and bone meal. Salmonella isolates showed a high rate of resistance to ampicillin (100%), tetracycline and nitrofurantoin (78.6%) and cotrimoxazole (50%), and moderate rate of 42.6%, 35.7%, and 21.4% against cephalexin, streptomycin and ceftriazole, and ciprofloxacin respectively, while low rates of 7.1% were recorded for amoxycillin clavulanate and pefloxacin and 14.39% for oxfloxacin, nalidixic acid and chloramphenicol. The present study showed that feed ingredients sold in Owerri form important vehicles for the introduction of multi-drug resistant *salmonella* organisms into poultry feeds. It is therefore, recommended that feed raw materials should be hygienically processed before inclusion in livestock feeds. [Life Science Journal. 2006;3(4): 75-80] (ISSN: 1097-8135).

Keywords: *salmonella*; feed materials; livestock; antibiotics; drug resistance; Nigeria

Abbreviations: AP: animal protein; BM: bone meal; CFU: colony forming unit; FB: fiber sources; FFM: foreign fish meal; GNC: groundnut cake; ISEPA: Imo State Environment Protection Agency; LFM: local fish meal; MZ: maize; PKC: palm kernel cake; PP: plant protein; SBM: soybean meal; WO: wheat offal

1 Introduction

There is a close relationship between the quality of livestock feed and that of animal products offered for human consumption. This quality is primarily nutritional, but it is also technological, organoleptic and sanitary. Although feed contributes to animal health by preventing dietary deficiencies and optimizing physiological functions, it can also lead to dysfunctions and negatively influence the sanitary quality of animal products when not properly processed[1]. Feeds can serve as important source of food borne diseases in animal food products and has therefore remained an important public health threat worldwide[2]. However, many factors are involved in this public health threat. Kan[3], for example stated that feeds and feed ingredients are possible materials since residues of organochlorine pesticides in poultry and eggs are due to their presence in feedstuffs. Similarly, there is evidence that poultry feeds are important sources of many microbial contaminants including *salmonella* in poultry[4-7]. Prominent among these microbial contaminants are *salmonella* strains, which have been showed to be of critical importance in the Nigerian poultry industry[8,9]. It has been shown that infection in poultry can result from one *salmonella* organism per grams of feed[10] and even one organism per 15 grams of feed[11].

Strict hygienic measures should therefore be applied to the production, processing and distribution of raw materials used as feedstuffs so as to pre-

vent contamination with pathogenic microbes and other undesirables[5]. Hygienic production of animal feeds however involves the processing of feeds under a health hazard free condition[12]. This usually starts from the harvesting, milling, processing, packaging, transportation and eventual marking of the bagged products at the various sales outlets from where the farmer collects to feed his animals[13].

Intensive feeding of poultry in the tropics involves the use of unconventional blending of feed components such as industrial wastes, cereal by-products, poultry waste, animal blood and others containing microbial genera of questionable quantity and quality[14]. Bains and Mackenzie[15] correlated high mortality in infected broiler flocks with increased incidence of *salmonella* in the grain constituents of broiler ration. Vaughn *et al*[16] also found 27% of protein feed ingredient meals collected at mills to carry one or more serotypes of *salmonella*.

A recent study by Okoli *et al*[17] determined that 22.20% of commercial poultry feed samples analyzed in Owerri, Nigeria contained *salmonella* isolates. It is however necessary to understand the major contaminating feed components that of finished feeds in the area in order to restrict sanitization treatment on them. Such information is important a developing economy like Nigeria where it may not be economically feasible to effect whole feed treatment.

The antibiotic resistance among bacterial general is a global problem[5]. 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[18]. Thus *salmonella* and other organisms contributed by the different raw materials used in compounding commercial feeds may harbor resistance factors reflecting antibiotic use in their areas of origin[19]. There is however scarcity of published information about anti-microbial resistance of bacterial isolates form farm animals and farm environments in southeastern Nigeria[19-24]. Furthermore, the fact that avian salmonellosis is a disease of major economic and public health importance demands that its prevalence and anti-microbial resistance profile in different feedstuffs should be understood at any given time in an animal production area.

This study was designed to investigate the prevalence of *salmonella* organism in feed raw materials and their microbial resistance profile in Owerri, Imo State, Nigeria.

2 Materials and Methods

2.1 Study area

The study was carried out in Imo State, Nigeria. The agro-climatic characteristics as well as poultry production systems in the area have been described[5]. The study was carried out during the rainy season months of July to September of 2004. A preliminary field survey was carried out to identify reputable commercial poultry feed sellers in Owerri. These sellers were informed of the nature and purpose of the research and based on the preliminary survey, a list of 8 feed raw materials sold at the outlets which included animal protein-foreign fish meal (FFM) and local fish meal (LFM), minerals-bone meal (BM), fiber sources-wheat offal (WO) and palm kernel cake (PKC), plant protein-soybean meal (SBM) and groundnut cake (GNC) and energy grains-maize (MZ) were purposively selected for the study. The materials were sampled at random across the three months using method described by Okoli[5].

2.2 Sample collection

A total of 360 bulked samples were collected from chosen feed raw materials selling outlets. Each selected sites was visited 3 times corresponding to once every month for sample collection. During the visits, samples were collected as shown in Table 1.

Table 1. Distribution of feed raw material sample types collected for isolation of *salmonella* in Imo State, Nigeria

Visits	FFM	LFM	BM	WO	PKC	SBM	GNC	MZ	Total
July	20	20	10	10	10	20	40	20	160
August	20	20	20	10	20	10	10	10	100
September	10	20	20	10	10	10	20	10	100
Total	50	60	50	30	20	40	70	40	360

Each of the feed raw materials were sampled by carefully opening 3 randomly selected bags that contained the same feedstuff type and collecting about 3 g from each with the aid of sterile universal bottles. These were homogenized to obtain a representative bulk sample of about 12 g of the sample types for analysis. The samples were taken to the laboratory for analysis within two hours of their collection.

2.3 Bacterial load enumeration

These were carried out at Imo State Environment Protection Agency (ISEPA) Microbiology Laboratory. Four-fold serial dilution of the homog-

enized samples as described by Ogbulie and Okpokwasili[25], was prepared for each sample and involved adding 5 g of the sample in 45 ml of sterile deionized water and mixing thoroughly. Thereafter, 0.1 ml of the appropriate dilution was drawn and inoculated onto nutrient agar. After overnight incubation, the bacterial load was enumerated using the colony counter (Suntex^r) to count the colony forming units (CFU).

2.4 Bacterial isolation

Aliquots of the serially diluted samples were enriched in peptone water after overnight incubation at 37 ℃. These were cultured onto then sub selenite broth for selective growth according to method of Cheesbrough[26]. They were subsequently subculture onto MacConkey agar and incubated overnight at 37 ℃. Non-lactose fermenting colonies suggestive of *salmonella* organism were subjected to biochemical test, which included Simmon citrate, indole and urease tests among others to confirm *salmonella* isolation[27].

2.5 Susceptibility testing

The confirmed *salmonella* isolates were screened for anti-microbial resistance profile using the disc diffusion method[28] according to the methods recommended by the National Committee for Clinical Laboratory Standards Guidelines[29]. This was done by streaking the surface of nutrient agar plates uniformly with the organisms. Thereafter, the plates were inverted and left to dry on the bench for 30 minutes before discs (Optun Lab.^{R}) impregnated with known concentrations of anti-microbial substances were placed on the surface with sterile forceps. The plates were then allowed to stand for a pre-diffusion period of about 1 hour before being incubated at 37 ℃ overnight with the lid uppermost. The disc diffusion method is widely recognized to work well with rapidly growing facultatively anaerobic and aerobic organisms such as Enterbacteriaceae[29].

Fourteen anti-microbial drugs were tested against the *salmonella* isolates. They included chloramphenicol (30 μg, CR), ceftriazole (30 μg, CF), nitrofurantoin (200 μg, NI), cotrimoxazole (30 μg, CO), oxfloxacin (10 μg, OF), gentamycin (10 μg, GN), amoxycillin clavulanate (30 μg, AU), nalidixic acid (10 μg, NA), ciprofloxacin (10 μg, CP), streptomycin (10 μg, ST), pefloxacin (10 μg, PF), ampicillin (30 μg, AM), tetracycline (25 μg, TE) and cephalexin (15 μg, CE).

2.6 Statistical analysis

The susceptibility data were recorded qualitatively as resistant or sensitive. The isolates resistant to individual drugs and anti-microbial pattern were computed. The data collected was analyzed using simple descriptive statistics such as percentage and histograms.

3 Results

Results of bacterial load enumeration showed that all of the samples yielded overgrowth or >300 cfu at 4 serial dilution.

3.1 *Salmonella* prevalence

Table 2 showed that 120 (33.33%) of the 360 bulked samples had *salmonella* isolates. Across the feed raw materials groups, fiber sources and animal protein recorded 56.00% and 50.91% prevalence, respectively and was followed by the 32.73% rate obtained in plant proteins, while *salmonella* organism were not isolated from energy grains and mineral groups. Across the individual feed raw materials (Table 3), LFM recorded 90.00% prevalence and was followed by the 60.00%, 50.00% and 40.00% recorded for WO, PKC and SBM, respectively.

Table 2. Frequency of isolation of *salmonella* from the different feed raw material types

Feed type	No. of samples	No. isolated	Percentage
Animal protein	110	56.0	50.91
Plant protein	110	36.0	32.73
Energy grain	40	0.0	0.00
Fiber source	50	28.0	56.00
Mineral	50	0.0	0.00
Total	360	120	33.33

Table 3. Frequency of *salmonella* isolation from various feed raw materials components

Materials	No. of samples	*Salmonella* isolation	% Prevalence
FFM	50	2	4.00
LFM	60	54	90.00
SBM	40	16	40.00
WO	30	18	60.00
GNC	70	20	28.57
BM	50	0.0	0.00
MZ	40	0.0	0.00
PKC	20	10	50.00
Total	360	120	33.33

3.2 Anti-microbial resistance

Figure 1 showed that the *salmonella* isolates recorded high rate of resistance (51 – 100%) to ampicillin, nitrofurantoin and tetracycline, while moderate rate (31 – 50%) were recorded against cotrimoxazole, cephalexin and streptomycin. The

organisms were however lowly resistant to the other antibiotics, with augumentine and pefloxacin recording 7.1% and oxfloxacin, gentamycin, nalidixic acid and chloramphenicol, while ciprofloxacin and ceftriazole returned 21.4%, respectively.

Figure 2 showed a comparison of the anti-microbial resistance of *salmonella* isolates from different poultry feed raw materials groups namely plant protein (PP), animal protein (AP) and fiber sources (FB). Isolates from PP, AP and FB recorded 100% resistance against ampicillin, while PP also singly recorded 100% resistance against nitrofurantoin. Similarly, isolates from AP returned 83.3% resistance to tetracycline and nitrofurantoin, while FB resistance levels were generally low with 0.0% resistance being recorded against oxfloxacin, gentamycin, augumentine and chloramphenicol.

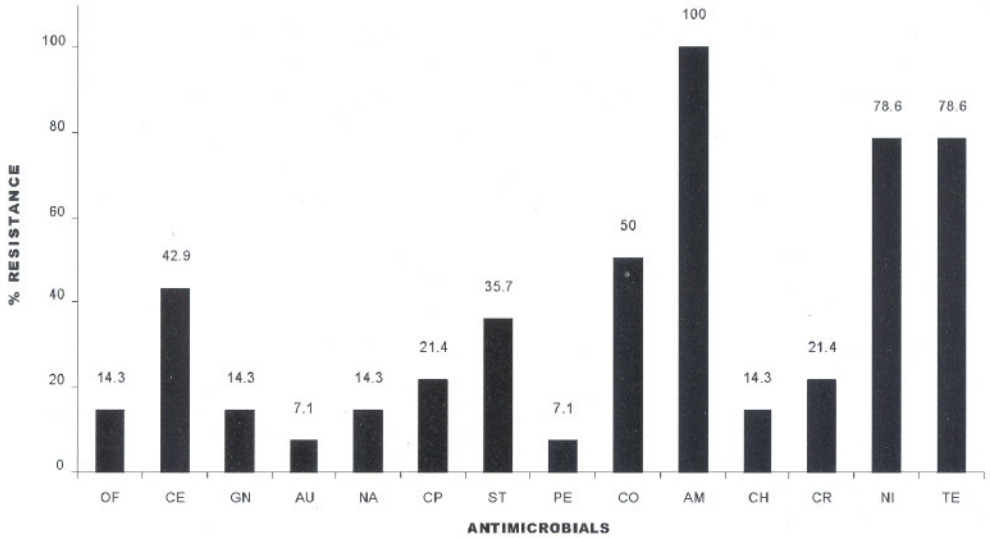

Figure 1. Histogram showing the rate of occurance of antimcrobial resistance patterns of *salmonalla* isolated from poultry feed raw materials in Owerri, Imo State, Nigeria

Key: OF-Oxfloxacin, CE-Cephalexin, GN-Gentamycin, Au-Amoxycillin clavulanate, CP-Ciprofloxacin, ST-Streptomycin, PE-Pefloxacin, CO-Cotrimoxazole, AM-Ampicillin, CH-Chloramphenicol, CR-Ceftriazole, NI-Nitrofurantoin, TE-Tetracycline.

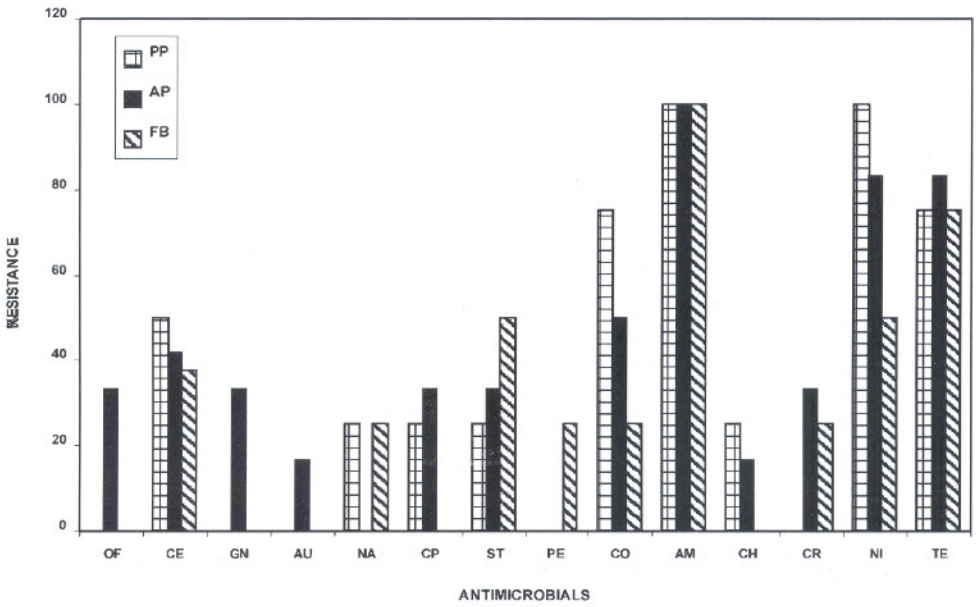

Figure 2. Comparison of antimicrobial resistance rates of *salmonella* isolates from poultryfeed rawmaterials of plant protein, animal protein and fibre sources. Key: As in Figure 1.

4 Disscusion

The high microbial contamination observed in LFM, GNC and PKC is in consonance with[30] which regarded these protein raw materials as "high risk ingredient" readily contaminated by microbes. These high densities of bacterial growth may be due to post-processing handling state of these ingredients. This is in consonance with the report of Butcher and Miles[31], which indicated that high temperatures in ground grains and oil meals encourage moisture migration and condensation inside the storage containers thus promoting bacterial as well as fungal growths. Reports by Bastianelli and Le Bas[1] and Cheesbrough[26] have also shown that tropical countries such as Nigeria are more prone to microbial and fungal contaminations of poultry feed raw materials.

The overall 33.33% prevalence of *salmonella* organism recorded in this study is of economic and public health importance[9,32]. Vaughn *et al*[16], Wilson[30] and MAFF[33] had earlier reported that in UK, 27% of protein feed ingredients carry one or more serotypes of *salmonella*. According to Dupree and Hurner[34], Ogbulie[14] and Ndujihe[35], *salmonella* in commercial feeds may have originated from some of the raw materials used in compounding them. Prevalence rates across the different raw material groups and types were unevenly distributed with local fish meal, recording 90%, while bone meal and maize had none. The observed difference in the prevalence rate of foreign and local fish meals may be attributed to the high level of hygiene employed in processing and handling of the former. The different weather condition experienced during the different seasons in the tropics as well as pre-harvest, harvest and post harvest practices and the bionomics of the organisms are also known to influence pathogenic contamination of local feedstuff[19]. Furthermore, there are program such as those of National Marine fisheries Services (NMFES) that monitors the quality of fish ingredients produced for export[31].

Similarly, considering the level of heat employed the processing of WO and PKC, the high degree of isolation may suggest handling and post-production sources of contamination[20]. The zero prevalence rate observed in BM could be attributed to high temperatures necessary for the ashing techniques employed in preparing the ingredients. The very low moisture content of the finished products may also not be able to support the growth of *salmonella*. While these organisms were not identified to genera level, unpublished field data by Anyanwu[36] and Okoli[32] suggest that *S. enteritidis*, *S. typhimurium* and *S. montevideo* are involved in poultry contamination in this study area.

The present result of anti-microbial resistance of *salmonella* isolated from feed raw materials highlight again the already established multi-drug resistance of bacteria of the Enterbacteriaceae family in Imo State[5,21,19,23]. The 36.7% resistance recorded for streptomycin and 21.4% against ciprofloxacin are again of public health interest since aminoglycosides and fluoroquinolones are currently the drugs of choice in the treatment of both human and animal salmonellosis in the study area. This work again highlighted the high resistance profiles of *salmonella* organism in Imo State against the cheap, readily available first line anti-microbial drugs such as cotrimoxazole, tetracycline, nitrofurantoin and ampicillin among others.

5 Conclusion

The result of the study confirms that feed ingredients are important vehicles for introduction of *salmonella* organisms in finished poultry feeds in Imo state. The high prevalence rate of *salmonella* isolates in this study highlights the need for the institution of *salmonella* monitoring measures programs in the Nigerian feed industry. LFM and fiber sources should be carefully sourced and sanitized before inclusion in animal feeds.

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