

Microbiological Quality and Antibiotic Susceptibility of Isolates from Fish and Poultry Feeds

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Abstract: The microbiological quality and antibiotic susceptibility of bacterial isolates from fish and poultry feeds used in Imo State, South Eastern Nigeria was studied between January and July, 2015. A total of 13 different feeds comprising 3 fish feeds and 10 poultry feeds were collected from different marketers Imo State and examined bacteriologically by culture technique. Seven genera of bacteria: *Staphylococcus* species, *Bacillus* species, *Campylobacter jejuni*, *Listeria* species, *Proteus mirabilis*, *Escherichia coli*, and *Salmonella* species, as well as 2 genera of fungi: *Aspergillus* species and *Penicillium* species were isolated from the feeds. Statistical analysis of the data showed significant difference ($p < 0.05$) in the bacteriological and mycological quality between local and industrial processed feeds. There was no significant difference ($p > 0.05$) in the bacteriological and mycological quality between fish and poultry feeds. Antibiotic susceptibility test revealed that Ciprofloxacin exhibited the highest antibacterial effects (grow inhibition) on most of the bacterial isolates than other antibiotics used. This study has revealed that fish and poultry feeds are potential sources of microbial infections for fish and poultry as well as human handlers and consumers of fish and poultry products in Imo State. The Government and public health operators in the State should educate the population on protective measures and also carry out routine supervision and regulation of production and use of these products.

Keywords: Microbiological Quality, Antibigram Bacteria Fish, Poultry Feeds

1. Introduction

Poultry is the fastest growing source of meat in Nigeria. It contributes over $\frac{1}{4}$ of all the meat produced and consumed in the country. Similarly, fish farming is expanding rapidly in the country, especially in Southern Nigeria. Globally, over $\frac{1}{5}$ of humans depend on fish as their primary source of protein. In Jamaica, Aiken *et al.*, (2002) reported that reported that annual production of food fish from aquaculture rose from a low of 0.1 t in 1976 to just over 5,000 t in 2000. Elsewhere, Klinger and Floyd (2009) stated that fish culture as a hobby or business is well established in Florida. According to the World Fish Centre (2003), fish is a key ingredient on the global menu, a vital factor in the global environmental balance and an important basis for livelihoods worldwide. The concentration of cholesterol in red meat is high, for this reason, nutritionists and health workers encourage adults to eat more of fish and poultry meat than

red meat. This has contributed immensely to increased utilization of poultry and fish as well as interest in poultry and fish farming. Poultry provides eggs and meat for human consumption. It includes all domesticated birds used for these purpose but in Nigeria the word most commonly refers to chickens. Different brands of poultry and fish feeds exist in Nigeria for different functions. These include Growers, Finishers, Layers starter among others. Feeds supply livestock with nutrients required for their body functions. Quality feed for livestock is very important for the maintenance of physiological functions and defense mechanism against diseases and infections. Feed quality is specified on basis of the nutritional value of every individual feed component. Micro-organisms such as bacteria and fungi growing on livestock feed affect their quality.

Animal feeds are sometimes exposed to contamination from environmental pollution, handling processes and other sources. Some may contain endogenous toxins arising principally from

specific primary and secondary substances produced by fodder plants. Feed toxins include compounds of plant and microbial origin. Feed materials often contain non-nutritional contaminants that may reduce the nutritional value or exert adverse health effects in animals. Fungi spores, may remain dominant in processed feeds until more favourable condition which support their proliferation. Contamination of feeds with moulds or bacteria may decrease their nutritional value and affects animal health. Contaminated feeds may help to distribute pathogens to humans especially in tropical countries where temperature and relative humidity are high.

Poultry feeds have been implicated in various bacterial diseases with varied pathological manifestations. *Salmonella* infections of poultry, has been reported to be of critical importance in Nigeria. 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 (Ohalet *et al.*, 2012).

2. Materials and Methods

Study area.

This study was carried out in Imo State, South – Eastern Nigeria. Imo State lies between latitudes 5°30' and 6°15' North, longitude 6° 38' and 7° 18' East. It is bound on the West by Delta State, on the North by Anambra State, on the South by Rivers State and on the East by Abia State. It comprises of 3 geo – political zones: Orlu, Owerri and Okigwe.

Imo State is densely populated with men and women of all ages. About 30% of the working class dwellers in Imo State are crop and animal farmers. Fish and livestock are widely produced, marketed and consumed in different parts of Imo State.

3. Study Samples

The samples used for this study are industrial and locally produced livestock and fish feeds: Top Feed Grower Mash (Top GM), Top Feed Layer Mash (Top LM), Top Feed Broiler Finisher (Top BF), Livestock Layer Mash (LS LM), Life Stock Broiler Starter (LS BS), Livestock Broiler Finisher (LS BF), Guinea Broiler Starter (Guinea BS), Guinea Broiler Finisher (Guinea BF), Guinea Layer Mash (Guinea LM). Local Poultry Feed (Local PF), Vita Fish Starter (Vita FS), Vita Fish Finisher (Vita FF) and Euro Fish Finisher (Euro FF). The samples were purchased from standard shops and open markets in Owerri and Orlu, Imo State. Five samples (3 from Owerri and 2 from Orlu) of each feed were purchased from different marketers. They were transported in sterile containers to the Microbiology Laboratory, Imo State University Teaching Hospital Orlu and examined within 2 hours.

3.1. Microbiological Quality of Test Samples

Samples of the selected poultry fish feed were examined for bacteriological and mycological quality by culture technique, using streaking and spread - plate methods as in

Chesbrough (2002), Obiajuru and Ozumba (2009). The media used were Mueller Hinton agar, Chocolate agar, MacConkey agar, Campylobacter selective agar, Salmonella & Shigella agar, Listeria Selective agar and Saboraud Dextrose agar. One set of Saboraud dextrose agar plates were incubated at 37°C for 24 hours and another set was incubated at room temperature in a fume cupboard and examined daily for 7days. Chocolate agar plates were incubated anaerobically at 37°C for 24 hours using anaerobic gas jar and other inoculated plates were incubated aerobically at 37°C for 24 hours. The incubated plates were examined for microbial growth. Those showing no microbial growth were re – incubated for another 24 hours and examined for microbial growth. Discrete colonies of each isolate on the different plates were sub cultured on fresh agar plates and incubated at 37°C for 24 hours to obtain pure cultures of all isolates. Pure culture of the isolates were identified and used for antimicrobial susceptibility tests.

3.2. Microbial Plate Counts

Two samples of each selected poultry and fish feed were used to determine the total heterotrophic bacterial and fungal counts. The total bacterial and fungal counts were determined using spread - plate and colony counter as in Obiajuru and Ozumba (2009). Each sample was processed by serial dilution by dissolving 1g of each sample in 9ml maximum recovery diluent to obtain 1 in 10 dilution. 1ml of this was diluted in 9ml of the diluent and so on to obtain 1 in 10², 1 in 10³ 1 in 10⁶ dilutions. 0.1ml of the various dilutions of each sample was inoculated on Mueller Hinton agar plates using spread plate technique. The inoculated plates were incubated for 24 hours at 37°C. They were examined and all bacterial colonies were counted using colony counter. Acceptable colony counts were those ranging from 30 to 300 cfu.

3.3. Antibiotic Susceptibility of Test Isolates

The antimicrobial agents used for this study are commercially prepared single antibiotic discs containing Ciprofloxacin (Ciproxin) 5µg and Levofloxacin (Levomax) 5µg, and laboratory prepared single discs containing Rifampicin 10µg, Ampiclox 30µg, Erythromycin 10µg and Neomycin 30µg and Oxytetracycline (Doxycycline) 30µg. The antibiotics used to prepare the discs and the commercially prepared discs were obtained from accredited pharmaceutical shops and suppliers of Laboratory reagents in Orlu and Owerri. The bacterial isolates were subjected to antibiotic susceptibility test using disc paper diffusion method as in Chesbrough (2002), Obiajuru and Ozumba, (2009). The discs were placed 16mm away from each other, using sterile forceps. The plates were incubated at 37°C overnight and examined for bacterial growth inhibition. The zone of growth inhibition around each sensitive antibiotic disc was measured using a transparent metric rule.

3.4. Method of Statistical Analysis

The data obtained from this study were analysed using

simple percentage analysis, Chi square and Analysis of variance (ANOVA) as in Philip, (2002).

4. Results

Microbiological quality of fish and poultry feeds.

The study showed that 7 genera of bacteria: Staphylococcus species, Bacillus species, Campylobacter jejuni, Listeria species, Proteus mirabilis, Escherichia coli, Salmonella species and 2 genera of fungi: Penicillium species and Aspergillus species, were involved in contamination of fish and poultry feeds sold and used by farmers in Imo State. Table 1 summarized the prevalence of bacteria and fungi in fish and poultry feeds. As shown, the most prevalent bacteria in poultry feeds was Escherichia coli (29.2%) while the least prevalent bacteria was Bacillus (10.8%). The most prevalent fungus was Penicillium species (9.2%). The total heterotrophic Bacterial (THB) and Fungal (THF) counts of fish and poultry feeds used in this study are summarized in tables 2 and 3 respectively. Analysis of the data using chi square showed significant difference ($p < 0.05$) in the bacteriological and mycological quality between local and industrial processed poultry feeds. There was no significant difference ($p > 0.05$) in the bacteriological quality between fish and poultry feeds. As shown, the highest bacterial counts (1.08×10^6 and 1.74×10^5) were observed in

locally processed poultry feeds while the least bacterial count (1.1×10^4) was observed on Guinea Broiler Starter feed. Similarly, the highest heterotrophic fungal count (8.4×10^4) was observed in locally processed poultry feed while the least fungal count (3.7×10^3) was observed on industrially processed poultry feed (Top GM feed).

Table 4 shows the antibacterial effects (growth inhibitory) of selected antibiotics on the test bacterial isolates while Fig 1 summarizes their susceptibility pattern. As shown, Ciprofloxacin inhibited growth of 90.9% Staphylococcus aureus, 85.7% Bacillus species, 83.3% Listeria and Proteus species, 78.9% Escherichia coli, 70% Campylobacter jejuni, and 66.7% Salmonella species. The mean zone of growth inhibition exhibited the on the isolates by Ciprofloxacin ranged from 20.0mm (Bacillus and Proteus species) to 25.4mm on Listeria species. Erythromycin inhibited growth of only 10.5% Escherichia coli and 25% Proteus mirabilis with mean zones of growth inhibition 15mm and 12mm respectively. Rifampicin exhibited 100% growth inhibitory effects on Bacillus species with mean zone of growth inhibition 18.5mm.

All Bacillus species, Staphylococcus aureus, Salmonella, Listeria species and Campylobacter isolates were resistant to Erythromycin. Analysis of the data using Chi – square shows significant difference ($p < 0.05$) in the susceptibility pattern between fluoroquinolones and amynoglycosides / macrolides.

Table 1. Prevalence of Bacteria and fungi species in poultry and fish feeds.

Sample	Number Exam	Number Infected (%)							Fungi	
		Staphylococcus aureus	Bacillus species	Campylobacter jejuni	Listeria species	Escherichia coli	Proteus mirabilis	Salmonella sp	Penicillium	Aspergillus sp
Top GM	5	-	-	2	1	3	2	1	2	-
Top LM	5	-	2	-	-	2	-	1	1	1
Top BF	5	2	-	1	2	1	1	-	-	-
LS BS	5	-	1	-	-	2	1	2	1	1
LS BF	5	2	-	-	2	2	-	1	-	-
LS LM	5	1	2	-	1	2	-	2	-	-
Vita FF	5	-	-	-	-	2	2	1	-	-
Vita FS	5	1	-	1	2	1	1	-	-	-
Euro FF	5	-	1	1	-	2	2	-	-	-
Guinea BS	5	2	-	2	-	-	-	1	-	-
Guinea BF	5	1	-	1	2	1	1	-	1	1
Guinea LM	5	2	-	-	2	-	1	-	-	1
Local PF	5	-	1	2	-	1	1	-	1	-
Total	65	11 (16.9)	7 (10.8)	10 (15.4)	12 (18.5)	19 (29.2)	12 (18.5)	9 (13.8)	6 (9.2)	4 (6.2)

$P < 0.05$.

Table 2. Total Heterotrophic Bacterial count.

Sample	Dilution Factor	Number of Colonies			Total Heterotrophic Bacterial count (cfu/ml)
		Plate 1	Plate 2	Mean	
Top BF	$1/10^3$	167	161	164	1.64×10^5
Top BF	$1/10^2$	132	124	128	1.28×10^4
Top LM	$1/10^2$	205	201	203	2.03×10^4
Top LM	$1/10^2$	108	118	113	1.13×10^4
Top GM	$1/10^3$	159	157	158	1.58×10^5
Top GM	$1/10^3$	99	93	96	9.6×10^4
L/S BS	$1/10^2$	184	180	182	1.82×10^4
L/S BS	$1/10^3$	84	92	88	8.8×10^4
L/S B/F	$1/10^2$	171	169	170	1.70×10^4
L/S B/F	$1/10^3$	69	53	61	6.1×10^4
L/S LM	$1/10^3$	123	129	126	1.26×10^5

Sample	Dilution Factor	Number of Colonies			Total Heterotrophic Bacterial count (cfu/ml)
		Plate 1	Plate 2	Mean	
L/S LM	1/10 ³	104	112	108	1.08 x 10 ⁵
VITA FF	1/10 ²	102	108	105	1.05 x 10 ⁴
VITA FF	1/10 ³	71	77	74	7.4 x 10 ⁴
VITA FS	1/10 ²	93	87	90	9.0 x 10 ⁴
VITA FS	1/10 ³	60	72	66	6.6 x 10 ⁴
EURO FF	1/10 ²	110	106	108	1.08 x 10 ⁴
EURO FF	1/10 ²	137	123	130	1.3 x 10 ⁴
GUINEA BS	1/10 ³	120	114	117	1.17 x 10 ⁵
GUINEA BS	1/10 ²	115	105	110	1.1 x 10 ⁴
GUINEA BF	1/10 ³	150	146	148	1.48 x 10 ⁵
GUINEA BF	1/10 ²	140	152	146	1.46 x 10 ⁴
GUINEA LM	1/10 ³	100	104	102	1.02 x 10 ⁵
GUINEA LM	1/10 ³	131	125	128	1.28 x 10 ⁵
LOCAL PF	1/10 ³	173	175	174	1.74 x 10 ⁵
LOCAL PF	1/10 ⁴	101	115	108	1.08 x 10 ⁶

P < 0.05.

Table 3. Total Heterotrophic Fungal count.

Sample	Dilution Factor	Number of Colonies			Total Heterotrophic Fungal count (cfu/ml)
		Plate 1	Plate 2	Mean	
Top GM	1/10 ²	44	42	43	4.3 x 10 ³
Top GM	1/10 ²	35	39	37	3.7 x 10 ³
Top LM	1/10 ²	45	47	46	4.6 x 10 ³
LS BS	1/10 ²	63	67	65	6.5 x 10 ³
Guinea B/F	1/10 ²	58	62	60	6.0 x 10 ³
Guinea LM	1/10 ²	34	38	36	3.6 x 10 ³
LOCAL PF	1/10 ³	81	87	84	8.4 x 10 ⁴

P < 0.05.

Table 4. Antibacterial Effects of Selected Antibiotics on Test Isolates.

Isolates	Number Tested	Mean Zone of Growth Inhibition (mm)						
		Ciproxin	Levofloxacin	Rifampicin	Ampiclox	Erythromycin	Doxycyclin	Neomycin
Staphylococcus aureus	11	22.5	26.0	23.2	15.0	0.0	12.0	14.0
Bacillus species	7	20.0	22.5	18.5	12.5	0.0	10.0	12.0
Campylobacter jejuni	10	21.6	20.4	24.5	10.4	0.0	0.0	0.0
Listeria species	12	25.4	24.5	18.0	12.6	0.0	10.5	0.0
Escherichia coli	19	24.0	28.2	22.5	18.5	15.0	12.2	15.5
Proteus mirabilis	12	20.0	26.4	20.0	20.0	12.0	14.0	12.5
Salmonella species	9	20.5	20.0	16.5	10.5	0.0	0.0	14.0

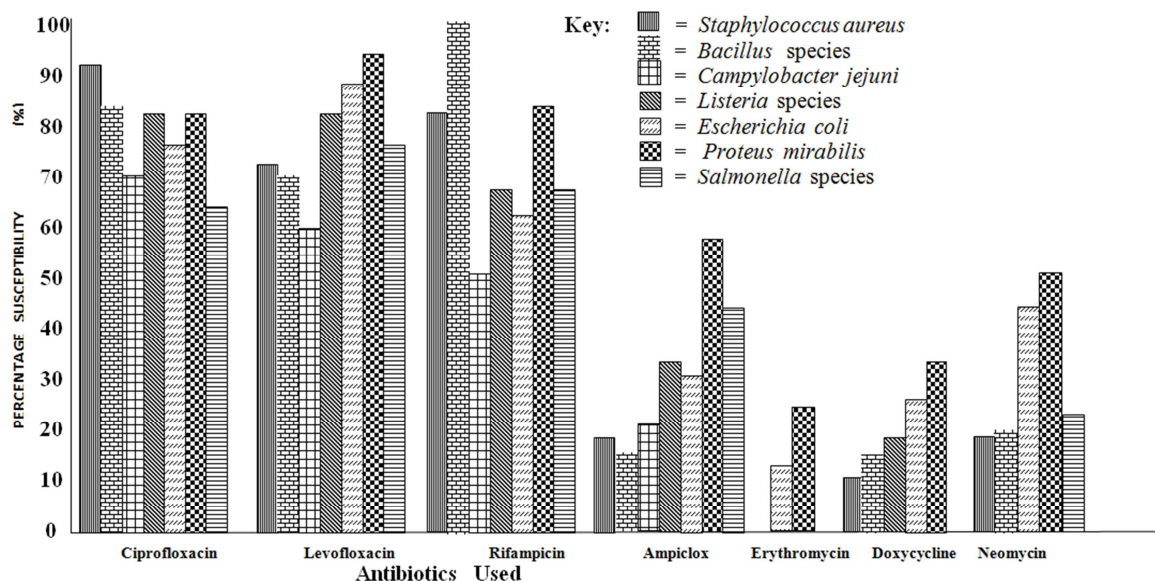


Fig. 1. Antibiotic Susceptibility of the Test Isolates.

5. Discussion of Findings

Human and livestock food materials contain bacteria and/or fungi, some are normal flora of the raw materials used in production of food, others are contaminants introduced during processing and handlings. Some of these organisms are responsible for spoilage and poisoning of human and livestock food. The present study implicated 7 genera of bacteria: (*Staphylococcus* species, *Bacillus* species, *Campylobacter jejuni*, *Listeria* species, *Escherichia coli*, *Salmonella* species and *Proteus*) and 2 genera of fungi (*Aspergillus* and *Penicillium*). This finding agrees with report of previous workers (Uwaezuoke, *et al.*, 2000) who reported the presence of bacteria species in livestock feeds. Some of the bacteria isolated in the present study are responsible for human infections via the gastrointestinal tract. *Salmonella* species causes typhoid and paratyphoid fever in humans. Previous studies (Obiajuru *et al.*, 2015) reported emergence of campylobacteriosis in Imo State and related this findings to increasing prevalence of *Campylobacter jejuni* in public water, fish, beef and chicken sold in the area. The microorganisms isolated from fish and poultry feeds in the present study are capable of infecting both fish and poultry birds that consume the feeds. When ingested by these farm animals, the organisms grow and multiply in the livestock which in turn will be consumed by humans. Thus, not only the livestock are at risk but also humans who feed on the infected livestock. Public health authorities should therefore monitor and regulate production, marketing and use of livestock feeds to control possibility of infection and disease outbreak through it. Human food borne infections is a growing public Health problem in many parts of the world especially the developing Countries like Nigeria. Thus Public Health inspectors should include inspection of Fish and livestock feeds in their routine duties. Feeds contaminated with pathogenic microbes such as *Salmonella*, *Campylobacter* and *Vibrio* species should be decontaminated before use or destroyed outright.

The fungal isolates (*Aspergillus* and *Penicillium*) in the present study are among organisms commonly implicated in the fermentation and steeping of cereals for the production of livestock feeds and human food. This finding agrees with previous workers (Banigo and Mueller, 1972), who isolated *Fusarium* species, *Aspergillus*, *Penicillium*, etc. in the fermentation and steeping of cereals for the production of human food and livestock feeds. It is likely that these fungi contaminated the test samples through the raw materials used for the production or the techniques used in processing of these materials into final products. The health implications of these fungi are many. *Aspergillus* species cause infection which resemble tuberculosis in symptoms but does not respond to usual treatments for tuberculosis. In rural communities and places without adequate diagnostic facilities for fungal infections, health workers treat such patients as tuberculosis patients only to end up wasting drugs

and money and finally losing the patient. Furthermore, *Aspergillus* produce aflatoxins which cause varied diseases including blood poisoning in humans. Diagnosis and treatment of human victims of aflatoxins is not easily achieved and often, cases terminate in death of patient. Muller, (1990) reported that Toxin producing fungi are dangerous, especially those producing aflatoxins, patulins, and trichotecens which are strongly carcinogenic and mutagenic.

The antibiotics used in this study are commonly used clinically in Imo State to treat infected humans. Some of the bacterial isolates exhibited remarkable degree of antibiotic resistance. One of the challenges facing clinicians in developing countries is management of antibiotic resistant bacterial infections. A number of factors such as beta lactamase production and drug abuse among other factors are responsible for this growing health challenge. Humans who are directly and indirectly in contact with these feeds contaminated with antibiotic resistant bacteria are at risk of acquiring antibiotic resistant bacteria infection. Previous workers (Gill and Best, 1998) reported that antibiotic resistant bacteria have been transferred to humans from farm produce. Other workers (Austin and Austin, 1989; Ogbulie and Okpokwasili, 1998) observed that most antibiotics approved for use in aquaculture and animal husbandry by Food and Drug Administration Authorities in some countries are also used to treat humans. These workers observed that regular exposure of micro-organisms to small doses of antibiotics via farm animals result to emergence of antibiotic resistant strains which in turn infect humans leading to treatment failure, delays, waste of drugs and resources.. The antibacterial effects of the sensitive antibiotics on the bacterial isolates in the present study are similar to that observed on clinical bacterial isolates in previous studies (Obiajuru and Chukuezi, 2011, Anyadoh – Nwadike, 2015). This finding shows that irrespective of the growing prevalence of antibiotic resistant bacteria in the country, hope is not lost on the efficacy of drugs commonly used in Nigeria to eliminate bacteria found in humans, fish and poultry feeds. Humans and livestock exposed to bacterial infections emanating from contaminated livestock feeds will benefit from many antibiotics such as the fluoroquinolones and amino-glycosides. The manufacturers of the feeds should take advantage of the study and use the antibiotics only when necessary and in the right dose to reduce the risk of developing antibiotic resistant strains.

The present study has shown that fish and poultry feed are potential sources of livestock and human infections. Apart from livestock, people at risk include farmers and consumers of fish and poultry products. Improved farm management practice, genetic selection, improved feeding and use of antibiotic therapeutic agents to treat bacterial diseases of farm animals, will play major roles to control the infections. Government should also legislate and monitor production, marketing and use of fish and poultry feeds to ensure quality and standard. Regular seminars and workshops should be

organized for farmers, marketers and local manufacturers of fish and poultry feeds to educate them on possible health hazards associated with these feeds and possible control measures.

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