Microbial Contaminants Associated with Commercial Poultry Feeds in Ilaro, Nigeria

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ABSTRACT

The quality of poultry feeds plays important role in the productivity of poultry farmers, contaminated feeds would negatively affect the poultry yield and directly affects human well-being. The study was designed to determine the microbial loads and types of bacteria and moulds associated with poultry feeds available for commercial purpose in Ilaro, Ogun State. A total of thirty-two (32) feed samples of four (4) categories; Layers mash, Growers mash, Broiler starter and Broiler finisher, were collected from different millers; Reality feed, Poultry consult services, Cornerstone and Biacom Agro. The microbial contaminants associated with the feeds were determined using pour plate technique. Isolates were identified using conventional technique. Antibiotic susceptibility was determined using agar disk-diffusion technique. The microbial loads varied from categories of feed and milers. The total viable bacterial counts ranged from $7.6x10^3$cfu/g – $5.3x10^5$cfu/g. The presence of coliforms was recorded in all the samples. Total coliform counts ranged $7.5x10^3$cfu/g – $5.1x10^5$cfu/g. Presence of *Salmonella* was recorded in samples GMR, GMP, LMP and BSP. Only sample GMR showed the presence of *Shigella*. Presence of moulds was recorded in all the feed samples. The bacteria associated with the feeds include; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* species, *Bacillus* species, *Proteus* sp., *Salmonella* species and *Shigella* species, while the mpoulds were *Aspergillus*, *Fusarium*, *Rhizopus* and *Penicillin*. Varying degrees of antibiotic susceptibility was showed by the bacteria. The presence of these organisms in the sampled feeds is of great concern to poultry farming and revenue diversification agenda of the present federal government in Nigeria.

Keywords: Bacteria, feed, microbial load, moulds, quality, sample

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1. INTRODUCTION

Poultry are domesticated birds kept for egg laying or meat production by man. These birds are also referred to as livestock, they include domestic fowl, duck, geese, turkey, guinea fowl, pigeons and ostriches (Barakat, 2004). . Poultry meat is the second most widely eaten meat in the world, accounting for about 38% of the world meat (Raloff, 2003). Livestock poultry get infected when pathogenic organisms passes to the susceptible animal through feeding (Barnes *et al*., 2003). The diseases of poultry is like the disease of other animals, these diseases may be caused by pathogenic organisms, nutritional deficiency and from wound. The quality of feeds of great importance to poultry business and productivity. Poultry feeds are composed largely of grains such as corn, wheat or barley, oil seeds, cake meat (originating mainly from oil producing seeds such as soybeans), sunflower seeds, peanuts, cotton seeds and protein

products of animal origin such as fish meal, meat and bone meal, (Bale, Sekoni & Kwanashie, 2002). Since these feeds are expected to be the sole sources of nutrition of the birds, they usually contain essential mineral and vitamin additives. The poultry feeds are essential source of energy needed to support the chemical reaction and to generate heat in which all physiological processes depends on. Poultry feeds, consisting of plants and animal origin are commonly contaminated with microorganisms mostly bacteria and fungi (Cevgar & Yalcin, 2003). Feeds can be source of contamination of the birds if fed with feeds contaminated with pathogenic bacteria or moulds. Most of the bacteria contaminants that are associated with feeds depend on the environment where the raw materials are obtained, due to field or post-field contamination of the raw materials.

However, the microbial loads and types of contaminants depend on the function of the materials, location of its origin, climatic conditions encountered, harvesting process, processing, storage, transport, technologies employed and packaging materials. The impact of general environmental and handling circumstances including the nature and extent of quality control also measures on the level of microbial contamination (D' Mello, 2006). According to Okogun, Jemikalajal & Ebhohimen (2016), feeds have been shown to be a major vector for transmission of *Salmonella* and other bacteria to the farm and processing plant.

The presence of *Escherichia coli, Bacillus cereus, Bacillus subtilis, Kebsiella* species, *Pseudomonas aeruginosa, Erwinia herbicola, Salmonella* sp., *Enterococcus faecalis, Proteus, Aspergillus flavus, A. parasiticus*, *Aspergillus niger*, *Rhizopus stolonifer*, *Geotrichum candidum*, *Penicillium* sp. *and Fusarium* sp. has been implicated in poultry feeds in various authors (D' Mello, 2006; Arotupin *et al*., 2007; Omojasola & Kayode, 2015; Okogun *et al*., 2016). Favourable storage temperature and relative humidity of the environment are some of the factors that usually encourage the growth and development of microorganisms in feeds.

In order to prevent the above mentioned pathogenic organisms from getting into the body of poultry, attention should be given to the factors that influence their infectious spread. Apart from pathogenic organisms, toxigenic moulds have been implicated in poultry feeds in tropical regions of the worlds. The presence of moulds and mycotoxins in poultry feeds are usually associated with materials such as cereal and oil seeds used in their production. Moulds contamination of feed raw materials can occur in the field (during pre-harvest) and post-harvest; during storage (Krnjaja, Kayode & Awojobi, 2008; Davies & Wales, 2010). Feeds may be contaminated by pathogens at any point in the production, storage, preparation processes.

In developing countries animal feeds are not subjected to the same stringent microbiological quality criteria and standards as those of human food (Omojasola & Kayode, 2015). Some of the feed Millers depend on raw material whose quality is in doubt in order to save cost, which eventually lead to the production of poor quality feeds. The study aimed at determining the microbial quality of commercial poultry feeds available in Ilaro, Yewa-South Local Government, Ogun State.

2. MATERIAL AND METHODS

Collection of Samples

A total of 32 samples of different categories of commercial poultry feeds; Broiler starter, Broiler finisher, Layers mash, and Growers mash from four (4) different Millers were collected from different trade outlets in Ilaro using sterile plastic containers. All samples were aseptically brought to the laboratory where bacteriological and mycological analyses were carried out within 2 hours collection.

Isolation of Microorganisms

The bacteria and moulds associated with the feed samples were determined using pour plate technique as described by Edema *et al.,* (2008). A Ten-fold serial dilution of the samples was carried. Dilutions 10^{-2}, and 10^{-4} were plated. Nutrient agar was used total bacterial counts, Salmonella/Shigella agar for *Salmonella* and *Shigella*, MacConkey agar for total coliform counts and Potato dextrose agar (PDA) incorporated with streptomycin was used for moulds isolation. All the plates were incubated at 37^{o}C for 24-48 hours except PDA that were incubated at 27^{0} C for 72 hours. The isolates were enumerated and sub-cultured in order to get pure cultures. The pure isolates were maintained on agar slant and kept at refrigerated temperature (4^{o}C) before further analysis.

Identification of Bacterial Isolates

The bacterial isolates were identified using conventional method, on the basis of cultural characteristics, cellular characteristics and Biochemical characteristics which include; sugar fermentation, indole, catalase, oxidase, citrate utilization, coagulase as described by Cheesbrough (2000). Bergeys manual of systematic bacteriology was used as reference for identification of the isolates.

Identification of Fungal Isolates

The development of the colony was observed considering the size, color and texture of the colony and reverse color. The fungal isolates were stained with lactophenol cotton blue so as to observe the microscopic feature. Fungal atlas was used as reference of identification (John and Mishra, 2017)

Determination of Antibiotic Susceptibility

The bacterial isolates were screened for anti-microbial resistance profile using the Agar disk-diffusion method as described by Sherris *et al*., (1996). 0.1ml of 18-24 hours old bacterial isolates was pipetted on the surface of nutrient agar plates under aseptic condition. The inoculum was streaked on the entire surface of the plate with a sterile inoculating loop. The plates were inverted and allowed to dry before disks impregnated with known concentrations of antibiotics were placed on the surface with sterile forceps. The plates were then allowed to stand for a pre-diffusion period of about an hour before being incubated at 37OC in an inverted manner. After incubation, diameter of zone of inhibition was used as parameter to measure the isolates susceptibility, the ones without zones of inhibition was recorded as resistant according to the methods recommended by Clinical and Laboratory Standards Institute (CLSI) 2006. Antibiotics and their concentrations used are as follows; Erythromyin (5mg), Chloramphenicol (30mg), Ceftriazone (30mg), Nitrofurantoin (200 mg), Cotrimoxazole 25mg), Ofloxacin (5mg), Gentamycin (10mg), Amoxycillin (30mg), Augmentin (30mg), Ciprofloxacin (10mg), Streptomycin (10mg), Pefloxacin (5mg) and Tetracycline (30mg).

3. RESULTS AND DISCUSSION

3.1 Results

A total of seven (7) bacteria genera were isolated from four (4) different poultry feed samples. The microbial loads of the samples were shown in Table 1, the total viable counts ranged from $7.6x10^3$ - $5.3x10^5$ cfu/g. The highest total plate count ($5.3x10^5$ cfu/g) was recorded as sample GMR while the least count ($7.6x10^3$) was in sample BFB. The Total coliform count also ranged from $7.5x10^3$ - $5.1x10^5$ cfu/g, the highest total coliform count ($5.1x10^5$ cfu/g) was recorded in sample LMR while the least count ($7.5x10^3$) was in BFP. The total Salmonella Shigella Agar count ranged from $5.0x10^3$ - $1.9x10^4$ cfu/g, the highest total plate count ($1.9x104^4$ cfu/g) was recorded in sample LMR while the least count ($5.0X10^3$ cfu/g) was in sample GMR. The overall identification criteria was employed as shown in Table 2 based on their colonial morphology, cell characteristics (Gram staining) and bio-chemical tests (indole, sugar fermentation, catalase, coagulase), which indicated the isolation of more bacteria than fungal species.

The probable organisms were *Staphylococcus aureus*, *Pseudomonas* sp., *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Bacillus* sp., and *Proteus* sp.

The colonial morphology, microscopic characteristics and probable fungi of the isolates were identified as shown in Table 3, where *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *and Fusarium* sp. were recorded as the probable organisms.

Table 4 indicates the prevalence of the organisms where *Staphylococcus aureus* was present in all the poultry feed samples, *E. coli* was also present in all the poultry feed samples except GMR, LMP, BSP, BFP and BFC, *Salmonella and Shigella* were present in samples GMR, GMP, LMP, LMB, and BFR, Proteus sp. was present in GMC, BSR, BSC and BSB while *Bacillus* sp. was found in sample GMR, GMB, LMR, LMC, BSC and BFC. The fungal species isolated includes *Aspergillus* species. *which* was present in all the poultry feed samples except BFR and BFC, *Penicillium* sp. which was present in sample LMR, LMB, BSP and BFP, *Fusarium* sp. which was present in GMB, LMC, BSP BSB and BFC and *Rhizopus* sp. present in almost all the feed samples.

The susceptibility of both Gram-positive and Gram-negative isolates to Augmentin (30µg), Ceftriaxone (30µg), Nitrofurantan (200µg), Gentamycin (10µg), Ofloxacin (5µg), Amoxycillin (30µg), Ciprofloxacin (10µg), Tetracycline (30µg), Pefloxacin (5µg), Streptomycin (10µg), Chloramphenicol (30µg), Cotrimoxazole (25µg) and Erythromycin (5µg) was as shown in Tables 5 and 6. The organisms showed a varying degrees of susceptibility of antibiotics. As showed in table 5, *Staphylococcus aureus and Bacillus* were both resistant to Ceftriaxone and Cotrimoxazole. *Staphylococcus* was totally resistant to Amoxycillin, Streptomycin and Pefloxacin while *Bacillus* was also resistant to Erythromycin and Chloramphenicol. *Staphylococcus* aureus and *Bacillus* sp. were both susceptible to Ofloxacin and Ciprofloxacin, while *Bacillus* was susceptible to Pefloxacin, Streptomycin and Amoxycillin. Ciprofloxacin was more active against the Gram positive bacteria isolates and recorded the highest zone of inhibition.

More so, all the Gram-negative bacteria isolates were resistant to Nitrofuranton, Likewise all organism were resistant to Amoxycillin except *Salmonella* sp. *Pseudomonas* sp. was susceptible to Pefloxacin, while *Proteus* sp. was susceptible to Gentamycin. The highest zone of inhibition (16mm) was recorded by Gentamycin against *Shigella* sp.

TABLE 1: Microbial Load of the Feed Samples

Sample code	N.A (cfu/g)	M.A (cfu/g)	SSA (cfu/g)	PDA (cfu/g)
GMR	5.3×10^5	1.3×10^4	5.0×10^3	4.2×10^4
GMP	2.0×10^5	2.1×10^4	9.2×10^3	1.9×10^4
GMC	8.9×10^4	9.2×10^3	1.6×10^4	1.0×10^4
GMB	2.8×10^5	7.7×10^3	1.0×10^4	7.6×10^3
LMR	1.7×10^5	5.1×10^5	1.9×10^4	7.5×10^3
LMP	1.3×10^5	5.1×10^3	1.0×10^4	7.6×10^3
LMC	6.6×10^4	6.0×10^3	8.3×10^3	7.6×10^3
LMB	8.9×10^4	5.8×10^3	7.6×10^3	1.3×10^4
BSR	3.1×10^5	1.2×10^4	1.3×10^4	7.6×10^3
BSP	4.6×10^5	7.6×10^3	1.3×10^4	1.8×10^4
BSC	2.7×10^5	1.6×10^4	7.7×10^3	1.7×10^4
BSB	7.0×10^4	7.6×10^3	7.7×10^3	1.8×10^4
BFR	7.7×10^4	1.2×10^4	1.3×10^4	7.6×10^3
BFP	1.2×10^5	7.5×10^3	1.0×10^4	1.0×10^4
BFC	1.3×10^4	1.0×10^4	1.3×10^4	1.1×10^4
BFB	7.6×10^3	1.0×10^4	7.5×10^3	1.0×10^4

Key:

GMR= Growers mash reality
GMP= Growers mash poultry consult
GMC= Growers mash cornerstone
BFR= Broilers finisher reality
BFP=Broilers finisher poultry consult
BFC= Broiler finisher corner stone
BFB= Broiler finisher Biacom
BSP= Broiler starter Reality
BSC= Broiler starter cornerstone
BSB= Broiler starter biacom
LMP=Layers mash poulty
LMR=Layers mash Reality
LMC=Layers mash biacom
LMB= Layers mash biacom
GMB= Grower mash biacom
BSR= Broiler starter reality

Table 2: Identification of bacterial isolates

	Colonial Morphology						Cell Morphol			Biochemical test						
Isolat	Form	Size	Colour	Opacity	Margin	Consistency	Gram Reaction	Shape	Arrangement	Calatase	Coagulase	Indole	Glucose	Sucrose	Lactose	Probable I
A	Circular	Tin	Yellowis	Opaque	Entire	Butryou	+	Cocc	Cluster	+	+	-	+A	+A	+A	*S. aureus*
B	Spherical	Lar	Whitish	Opaque	Entire	Mucoid	-	Rod	Scattere	+	-	+	+A	+AG	+AG	*E. coli*
C	Circular	Lar	Creamy	Opaque	Undula	Dry	+	Rod	Cluster	+	-	-	+A	+AG	-	*Bacillus* sp
D	Circular	Lar	Creamy	Opaque	Entire	Mucoid	-	Rod	Scattere	+	-	-	+AG	+AG	+AG	*Klebsiella* s
E	Circular	Tin	Creamy	Opaque	Entire	Butryou	-	Rod	Cluster	+	-	-	+G	-	-	*Proteus* sp
F	Circular	Lar	Greenish	Opaque	Entire	Butryou	-	Rod	Cluster	+	-	+	+A	-	-	*Pseudomo* sp.
G	Spherical	Tin	Colourle With bla center	Opaque	Entire	Mucoid	-	Rod	Cluster	+	-	-	+AG	+G	-	*Salmonella*
H	Circular	Lar	Colourle	Transpar	Entire	Mucoid	-	Rod	Cluster	+	-	-	+A	+A	+A	*Shigella* sp

Key: + = positive - = negative AG = Acid Gas Production

TABLE 3: Identification 0f Fungal Isolates

Isolate codes	Colony Morphology	Microscope characteristics	Probable fungi
GM_d	Wooly, velvety, but whitish later turned black fungal colony with yellowish reverse side	Septate with unbranched conidiophores. Double sterigmata covered the entire vesicles to form radiate head	*Aspergillus* nger.
GM_f	Powdery whitish surface but later turned bluish-green with whitish reverse side and edges	Branched septate hyphae with flask shaped sterigmata. The conidia is unbranched with a penicillater bluish appearance	*Penicillium* sp.
BS_c	Creamy powdery growth that later turned black	Aseptate hyphae, unbranched sporangiospores are from the food of rhizoids that enlarged in a cup-shaped form with the mycellial region	*Rhizopus* sp.
BF_b	Fluffy creamy growth that later turned pinkish with a yellowish reverse side	Septate with branched conidiophore, curved sporandiospore and oblong conidia,	*Fusarium* spp.

TABLE 4: Microbial Isolates Associated With Various Poultry Feed Samples

Sample code	Bacteria isolates	Fungal isolates
GMR	*S. aureus, Salmonella* sp.., *Shigella and Bacillus* sp.	*Aspergillus and Rhizopus* sp.
GMP	*S. aureus, E. coli, Salmonella* sp.	*Aspergillus niger*
GMC	*S. aureus, Proteus* sp., *E. coli*	*Aspergillus niger, and Rhizopus*
GMB	*S. aureus, Bacillus* sp. *E. coli and Pseudomonas* sp.	*Aspergillus niger and Fusarium* sp.
LMR	*S. aureus, Bacillus sp., and E. coli.*	*Aspergillus niger and Penicillum* sp.
LMP	*S. aureus, Salmonella and Pseudomonas* sp.	*Aspergillus and Rhizopus*
LMC	*S. aureus and Bacillus* sp.	*Aspergillus, Rhizopus and Fusarium* sp.
LMB	*S. aureus, E. coli*	*Aspergillus* sp.
BSR	*S. aureus, E. coli, , Proteus* sp.	*Aspergillus and Rhizopus* sp.
BSP	*S. aureus, Salmonella and Pseudomonas*	*Aspergillus, Penicillum and Fusarium* sp.
BSC	*S. aureus, Bacillus sp. Proteus and Pseudomonas* sp.	*Aspergillus and Rhizopus* sp.
BSB	*S. aureus, E. coli, Bacillus and Proteus* sp.	*Aspergillus and Fusarium* sp.
BFR	*S. aureus and E. coli*	*Rhizopus* sp.
BFP	*S. aureus and Pseudomonas* sp.	*Aspergillus* sp. *and Penicillum* sp.
BFC	*S. aureus and Bacillus* spp.	*Fusarium* sp. *and Rhizopus* sp.
BFB	*S. aureus, E. coli, Pseudomonas* sp. *and Bacillus* sp.	*Aspergillus* sp. *and Rhizopus* sp.

TABLE 5: Gram Negative Isolates Antibiotic Susceptibility

INHIBITION (MM) ISOLATE CODE				ORGANISM	ANTIBIOTIC ZONE OF					
	CPX	TET	PFX		AUG	COT	NIT	GEN	OFL	AMX
B				*Escherichia coli*		12	8	R	4	6
R	3	R	7							
F				*Pseudomonas* sp.			R	R	R	11
13	R	12	R	15						
G				*Salmonella sp.*		5	7	R	R	9
15	13	3	R							
H				*Shigella* sp.			R	5	R	16
3	R	13	7	R						
E				*Proteus* sp.			R	8	4	5
7	R	5	R	R						

Key: AUG = Augmentin
COT = Cenftriazone
NIT = Nitrofuranton
GEN = Gentamycin
OFL = Ofloxacin
AMX = Amoxycilin
CPX = Ciprofloxacin
TET = Tetracycline
PFX = Pefloxacin

TABLE 5: Gram Positive Isolates Antibiotic Susceptibility

INHIBITION (MM) ISOLATE CODE				ORGANISM	ANTIBIOTIC ZONE OF					
					AMX	OFL	STR	CHL	CRO	GEN	PEX
COT	CPX	ERY									
A				*Staphylococcus aureus*	R	16	R	8	R	10	
R	R	14	13								
C				*Bacillus* sp.		8	15	13	R	R	
14	16	R	15	R							

Key: STR = Streptomycin CHL = Choramphenicol COT = Cotimoxazole ERY = Erythromycin CPX = Ciprofloxacin AMX = Amoxycilin OFL = Ofloxacin CRO = Cotrimoxazole PEX = Pefloxacin

4. DISCUSSION

This study revealed that seven (7) bacterial and four fungal genera were isolated in the feed sample analyzed and time factor did not affect the bacterial and fungal isolates in the feed. Crumps *et al*., (2000) stated that poultry feed is a major vector for transmission of *Salmonella* and other bacteria. This study has shown that most poultry feeds are contaminated with various microbes in Ilaro Ogun State. Other bacteria isolates obtained were *Staphylococcus aureus, Proteus* sp., *Escherichia coli, Pseudomonas* species and *Shigella* sp. This does not agree with the reports Veldman *et al*., (1995), who stated that the family Enterobacteriaceae are the common contamination of poultry feeds. The isolation of *E. coli* may be attributed to faecal contamination during the preparation of these feeds, while the presence of *Proteus* sp. *and Pseudomonas* may be due to the exposure of the feeds to the unsanitary environment which allowed such organisms with pathetic potential to proliferate and contaminate the feeds. The presence of *Staphylococcus aureus* may suggest both bad manufacturing practice and contamination through holding. Studies elsewhere have indicated such magnitude of *Staphylococcus* aureus contamination to be hazardous. In this study, Growers mash, Broiler starter, and Layers mash grew the highest contamination of microbes. This may be attributed to high protein contents of these marshes which also serve as growth factors for the contaminating organisms. Poor exposure of poultry feed to low temperature and organic acid has been found to enhance bacteria growth in feeds which result to poor production in poultry. Maciorowski, Jones, Pillai & Riche (2004) have earlier reported that exposure of feeds to a temperature of 85.7^{o}C would successfully eliminate the heat resistance bacterial spores, the presence of spore formers in the feeds might be due to inability of feeds to be subjected to heat treatment. The occurrence of *Aspergillus, Fusarium and Penicillium* could be could be due to pre-harvest or post-harvest contamination of the feed raw materials, presence of these mould are known for their toxigenic potential (Faparusi & Alagamba, 2018). More so, the presence of these moulds in the feeds was in agreement with the findings of Osho, Awoniyi & Adebayo, 2007).

5. CONCLUSION

The poultry feeds showed the presence of some pathogenic and opportunistic bacteria. The organisms could pose a great threat to poultry famers and their source of livelihood. The fungal isolates were also found in significant number and these organisms; *Fusarium, Fusarium* and *Aspergillus* could be mycotoxin producers. Routine microbiological examination, good manufacturing practice, handling and retailing methods need to be improved to enhance the microbiologically quality of these products and also to improve the production performance in poultry management.

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