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Microbiological quality of commercially available poultry feeds sold in parts of Eastern Nigeria

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ABSTRACT: Food poisoning and infection by bacterial and fungal genera pose obvious health threat to both animals and humans. Pfizer, Guinea, Extra, Top, NOM and Master brands of feed sold in Owerri Metropolis were analysed for their bacterial and fungal quality. The genera of bacteria and fungi isolated and their percentage occurrence were *Listeria* spp (10.4%), *Staphylococcus* spp. (50%), *Bacillus* spp (27.1%), *Pseudomonas* spp. (12.5%), *Escherichia coli* (29.2%) and *Salmonella* spp. (25%), *Aspergillus* spp (56.3%), *Fusarium* spp (58.3%), *Penicillium* spp. (62.5%) and *Rhizopus* spp (89.6 %). The mean bacterial and fungal counts vary with samples while statistical analysis for the goodness of fit revealed no significant difference between the observed and expected values at 1.0% level. @JASEM

In the tropics, intensive animal husbandry utilizes a minimum of land and labour resources while providing an economically profitable resource of high quality protein for human consumption. In an effort to achieve rapid animal growth to meet the increasing demand for animal, large quantities of nitrogenous waste fortified with other supplements such as spent grain, cassava waste, bone meal etc. are compounded as animal feed (Okpokwasili and Ogbulie, 1993). Reports by Gill and Best (1998) and Ruff (1992) have listed animal feed as one of the sources of micro organisms to animals. Specifically, some of the additives have been incriminated amongst the principal sources of bacteria of public health concern (Ogbulie and Okpokwasili 1998). Various types of farmed animal diseases such as diarrhoeal diseases like bacillary dysentery, amoebic dysentery, fowl cholerae etc., Salmonellosis, staphylococcosis, colibacillosis, erysipelas, listeriosis etc have been traced to the contamination of animal feed (Healing and Greenwood, 1991). A potential and more deadly hazard has been associated with the consumption of microbial toxins of bacterial and fungal origin in feed (Hesseltine, 1984; Betina, 1989; Gilbert, 1995). Studies elsewhere have associated some animal feeds with toxigenic strains of fungi and bacteria of public health concern (Bilgram *et al*., 1995; White and Torman,1995). Considering the health hazard posed to animal/poultry and the unsuspecting consumers of such contaminated feeds and its overwhelming socioeconomic impact, it is petinent to undertake this study. This research is therefore designed to identify and characterize the bacterial and fungal flora of commercially available poultry feeds sold in Owerri metropolis.

MATERIALS AND METHODS

Sample Collection: Duplicate sample of six different brands of poultry feeds namely Pfizer, Master, Top, NOM, Guinea and Extra feeds were aseptically collected from 2 major markets in Owerri Municipality. Sample collection was done using sterile Durham's bottles and a repeat duplicate sample were collected after a two weeks interval to determine whether time factor affected microbial composition. All samples were aseptically transported to the laboratory where bacteriological and mycological analysis were carried out within 2 hours of sample collection.

Microbiological Analysis: The bacteriological and mycological quality of the six brands of animal feed were assayed with a view to establishing their autochthonous flora. The feed samples were processed as described by Ogbulie and Okpokwasili (1999) by homogenizing 5 grams of each sample in 45ml of sterile physiological saline and the fungal and bacterial load enumerated using Nutrient Agar; coliform, staphylococcal, fungal and fastidious organisms were assayed using MacConkey, Mannitol salt, potato dextrose and blood agar respectively. Except for the potato dextrose agar plates that were incubated at 37^0C for 72 hours, other plates were incubated at 37^0C for 24 hours. Reported plate counts were those that had between 30-300 colony forming units (cfu). Representative isolates were picked, subcultured for purification and characterized using standard techniques (Cowan, 1993; Cruickshank, 1975; Cheesbrough, 1984; Holt, 1984; Baron *et al*., 1994). This characterization process involved incubation at certain temperature condition, colony morphology, cell morphology, motility, oxidase, catalase, urease, H_2S production, citrate utilization, indole, nitrate reduction, coagulase, sugar fermentation, methyl red and Voges – Proskauer tests.

Statistical Analysis: The Chi-square test statistic at the 1.0% level as described by Araoye (2003) was

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employed to determine the significant deference between the observed and the expected data. The null hypothesis assumed that there was no significant difference between the observed data from the expected data. The mean bacterial count (cfu/ml) for each duplicate was used in this analysis and the (row-1) (column-1) gave the degree of freedom (df).

RESULTS

Table 1 shows the bacterial and fungal isolates recovered from the six brands of poultry feeds and their respective percentage occurrence. The overall sample specific percentage occurrence of the isolates revealed that fungal species occurred more than bacterial species with *Apergillus* spp*, Fusarium* spp, *Penicillium* spp and *Rhizopus* spp accounting for 56.3%, 58.3%, 62.5% and 89.6% respectively whereas bacterial species of coagulase-negative staphylococci, *Escherichia coli*, *Bacillus* spp, *Salmonella* spp, *Staphylococcus aureus*, *Pseudomonas* spp and *Listeria* spp accounted for 35.4%, 29.2%, 27.1%, 25% 14.6% 12.5% and 10.4% respectively.

Table 1: Percentage Occurrence of Bacterial and Fungal Isolates Contaminating Brands of Poultry Feeds

Isolates	PF N = 8 n (%)	GF N = 8 n (%)	EF N = 8 n (%)	TF N = 8 n (%)	NF N = 8 n (%)	MF N = 8 n (%)	Percentage Overall N-48 n (%)
Listeria spp	2 (25)	0	1(12.5)	0	0	2(25)	5(10.4)
Staphylococcus aureus	3 (37.5)	1(12.5)	2(25)	0	0	1(12.5)	7(14.6)
Coag. Neg. Staph	2 (25)	4(50)	1(12.5)	3(37.5)	5(62.5)	2(25)	17(35.4)
Bacillus spp	3(37.5)	2(25)	2(25)	1(12.5)	3(37.5)	2(25)	13(27.1)
Pseudomonas spp	1(12.5)	0	0	2(25)	1(12.5)	2(25)	6(12.5)
Escherichia coli	2(25)	3(37.5)	4(50)	2(25)	2(25)	1(12.5)	14(29.2)
Salmonella spp	*2(25)*	3(37.5)	1(12.5)	1(12.5)	2(25)	3(37.5)	12(25)
Aspergillus flavus	5(62.5)	3(37.5)	6(75)	3(37.5)	4(50)	6(75)	27(56.3)
Fusarium spp	3(37.5)	2(25)	5(62.5)	6(75)	5(62.5)	7(87.5)	28(58.3)
Penicillium spp	6(75)	4(50)	3(37.5)	7(87.5)	4(50)	6(75)	30(62.5)
Rhizopus spp	8(100)	6(75)	6(75)	8(100)	7(87.5)	8(100)	43(89.6)

KEY: PF = Pfizer feed; NF: = NOM feed
GF = Guinea feed; MF = Master feed
EF = Extra feed; N = Number of samples
TF = Top feed.; n = Number of isolates
Coag-neg. staph = coagulase negative Staphylocci.

Identification criteria employing cultural and biochemical tests as shown in tables 2 and 3 indicated the isolation of more bacteria than fungal species. However high preponderance of fungal species were isolated from the six brands of poultry feeds.

The data in Table 4, shows the mean bacterial and fungal counts of the feed-types. The study revealed that the counts recorded after two weeks interval were similar to the ones recorded previously. The means of the first duplicate was therefore used in the table. The source of the feeds did not in any way affect the mean counts obtained. The two markets of Relief and Ekeukwu had aerobic counts of 10^7 order magnitude for Guinea and Extra feeds and 10^4 order for Master, Top, Pfizer and NOM feeds. Coliform counts of 10^6 order magnitude was obtained for Guinea and Extra feeds whereas 10^4 order magnitude was obtained for Master, Top, Pfizer and NOM feeds. Staphylococcal counts of 10^5 order magnitude was obtained for Guniea and Extra while 10^3 order magnitude was obtained for Master, Top, Pfizer and NOM feeds. Total fungal counts revealed that 10^8 order was obtained for Guinea, Extra and Pfizer whereas 10^7 order was obtained for Master, Top and NOM feeds. The statistical analysis carried out as described by Araoye (2003) revealed a high significant difference ($P<0.01$) between the observed data and the expected values.

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Table 2: Biochemical properties of isolates

Gram reaction And Cultural characteristics	Motility	Catalase	Oxidase	Coagulase	Indole	Methyl-Red	Voges-Proskauer	Citrate Utilization	H_2S Production	Nitrate Reduction	Urea hydrolysis	Sugar Fermentation: Glucose	Sucrose	Lactose	Maltose	Mannitol	Organisms
GP; short bacilli (formed small, grey, droplet-like small zone of beta-haemolysis on blood agar	+	+	-	ND	-	-	+	-	-	-	-	**A**	**A**	**A**	**A**	-	*Listeria spp*
GP cocci in clusters (yellow and smooth on mannitol salt agar	-	+	-	+	-	+	+	-	-	+	+	A	A	A	A	A	*Staphylococcus aureus*
GP cocci in clusters (yellow & smooth on mannitol salt agar)	-	+	-	-	-	+	+	-	-	+	+	A	A	A	A	-	*Coagulase-negative staphylococci*
***GP** bacilli* (Large cream with rhizoid – like edge on nutrient agar)	+	+	-	ND	-	-	+	+	+	+	-	A	A	A	A	-	*Bacillius spp*
GN small rods (flat, greenish colonies)	+	+	+	ND	-	-	-	+	-	+	+	A	-	-	-	-	*Pseudomonas spp*
GN small rods (round convex colonies)	+	+	-	ND	+	+	-	-	-	+	-	A/G	-	A	A	A	*Escherichia coli*
GN small rods (smooth round convex pale Colonies on MacConkey)	+	+	-	ND	-	+	-	-	+	+	-	A/G	-	-	A	A	*Salmonella spp*

Key: GP = Gram Positive; GN = Gram Negative; ND = Not done; A = Acid Production; AG = Acid and gas production; + = positive; - = negative

Table 3: Identification of fungal isolates

Serial No Of Isolates	Macroscopic characteristics and texture	Microscopic Characteristic	Organism
1.	Greamy powdery growth that later turned black	Aseptate hyphae, unbranched sporangiospores are from the foot of rhizoids that enlarged in a cup-shaped form with the mycellial region	*Rhizopus spp*
2.	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 penicillate or bluish appearance.	*Penicillium spp*
3.	Fluffy creamy growth that later turned pinkish with a yellowish reverse side	Septate with branched conidiophore and oblong conidia.	*Fusarium spp*
4.	Velvety, wooly, whitish but later turned black fungal colony with yellowish reverse side.	Septate with Unbranched conidiophores. Double sterigmata covered the entire vesicles to form radiate head.	*Aspergillus spp*

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Table 4: Mean Bacterial Count of Poultry Feed Samples in Owerri

SAMPLE	SOURCE	TOTAL AEROBIC COUNT	COLIFORM COUNT	STAPHYLO-COCCAL COUNT	TOTAL FUNGAL COUNT
Guinea	Relief Market	2.60×10^7	5.5×10^6	7.0×10^5	1.6×10^8
Feed	Ekeukwu	1.90×10^7	6.0×10^6	8.0×10^5	2.0×10^8
Extra feed	Relief Market	2.7×10^7	7.0×10^6	6.0×10^5	1.0×10^8
	Ekeukwu	3.5×10^7	1.5×10^6	5.0×10^5	3.0×10^8
Master feed	Relief Market	4.5×10^4	3.0×10^4	7×10^3	8.0×10^7
	Ekeukwu	6.8×10^4	4.5×10^4	3.0×10^3	6.0×10^7
Top feed	Relief Market	9.9×10^4	4.5×10^4	6.0×10^3	1.4×10^7
	Ekeukwu	6.6×10^4	3.8×10^4	5.0×10^3	1.9×10^7
Pfizer feed	Relief Market	8.0×10^4	5.0×10^4	3.0×10^3	2.4×10^8
	Ekeukwu	7.5×10^4	3.7×10^4	4.0×10^3	1.9×10^8
	Relief Market	6.5×10^4	3.5×10^4	2.0×10^3	3.0×10^7
Nom feed	Ekeukwu	7.8×10^4	4.7×10^4	2.0×10^3	3.6×10^7

DISCUSSION

This study revealed that six bacterial and four fungal genera were isolated in the feed sample analysed and time factor did not affect the bacterial and fungal isolates in the feeds. Higher fungal counts were obtained in the brands of poultry feed and this corroborates the report of studies carried out elsewhere (Andrews and Pitt, 1986 and Ogbulie, 1995). The high level of fungi obtained in this study can be associated with the low water activity of animal feed and the physiology of the contaminating fungal genera. Animal feeds have been listed as one of the sources of microbes of farmed animals and poultry. Thus the high fungal and bacterial recovery may indicate a potential hazard to the animal. The high occurrence of fungal and bacterial species of public health concern may indicate obvious health hazard in terms of direct consumption of bacteriological or fungal contaminated feed or their toxins by farmed animal (Frazier and Westhoff, 1978). Also the staphylococcal counts raging from 2.0×10^3 to 8.0×10^5 may suggest both bad manufacturing practice and contamination through handling. Studies elsewhere have indicated such high magnitude of staphylococcal contamination to be hazardious (Bergdoll, 1979; Edson *et al.,* 1981).

The source of these organisms may vary extensively. While bacterial genera may have originated from nitrogenous waste products used in compounding animal feeds such as dung, chicken excreta etc as reported by Ogbulie (1995), fungal species may have resulted from carry-over of over seasoned fungal species from the field. Also handling and other post harvest process may contribute amongst the primary sources of contamination. Most of the fungal species have been isolated from cereals (Pitt, *et al.,* 1994) and the physiological adaptation of these fungal genera may have supported their survival.

On the other hand, the presence of *Listeria, Bacillus, Pseudomonas, Escherichia coli* and *Salmonella* species may suggest faecal as well as environmental contamination. Some of these organisms are well known pathogens of birds and farmed animals. (Mallinson, 1984). For instance, *E. coli* is implicated in disease conditions such as colibacillosis which occurs in forms such as enteric and septicaemic colibacillosis whereas *Salmonella*, *Listeria* and *Staphylococcus aureus* are capable of producing acute and chronic infections in all or most types of birds and animals (Mallinson, 1984). The study showed that the percentage occurrence of *Listeria* spp was 10.4%. These organisms can survive and multiply at refrigerator temperatures and in a wide range of pH, hence even a small amount of contamination may be significant (Linnan *et al.,* 1988).

The occurrence of *Aspergillus, Fusarium* and *Penicillium* could be as a result of their high pathogenicity as reported by researchers elsewhere (Pitt *et al.*, 1994). These organisms although on their own can cause several poultry and farmed animal infections, they also produce mycotoxins that are also of public health importance to both humans and their farmed animals. The socio-economic and health implication of these findings are enormous. Economically, the presence of these bacterial and fungal genera has been reported to overwhelmingly affect the viability of some animal husbandry undertaking and agriculture in general (Misra *et al*., 1995; Fajardo *et al.,* 1995; Ogbulie, 1995). With the high colonization of bacteria and fungi of public health concern in poultry feeds, good manufacturing practice, handling and retailing methods need to be improved to enhance the microbiological quality of these products

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