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Microbiological Assessment of Poultry Feeds within Ilorin, Nigeria

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Abstract

The poultry feeds were obtained from 20 different poultry pens and their microbial contents were assessed. The antibiotics resistance patterns of the bacterial isolates were also determined. The bacterial count ranged from 5.0×10^3 to 1.76×10^6 cfu/g while the fungal count ranged from 3.5×10^4 to 1.9×10^5 cfu/g. The bacterial species isolated were *Streptococcus salivarius, Streptococcus pyogenes, Micrococcus luteus, Micrococcus varians, Micrococcus roseus, Staphylococcus aureus, Staphylococcus saprophyticus* and *Staphylococcus hominis*, while the fungal species isolated were *Saccharomyces cerevisisae, Fusarium oxysporum*, *Penicillium* sp., *Humicola grisea, Aspergillus fumigatus, Hansenula* sp. and *Humicola fuscoatra*. All the bacterial isolates were resistant to ceftazidime and cefuroxime and all the isolates were resistant to at least three antibiotics. Ofloxacin produced the highest zone of inhibition, followed by gentamicin, and then erythromycin. The presence of some pathogenic microorganisms in the poultry feeds revealed high level of contaminations. It is recommended that poultry feeds should be made from good quality grains and it should be prevented from environmental or other contamination.

Keywords: antibiotics, assessment, contaminants, microbial content, poultry feeds

Introduction

Poultry is the second most widely eaten meat in the world, accounting for about 38% of the world meat (Raloff, 2003). For the development of healthy poultry, the poultry farmers should formulate a feed that will give the best possible result at the least possible cost (Lossli *et al*., 1999). Poultry feeds are food materials used in raising poultry birds and are designed to contain all the nutritional materials needed for proper growth, meat and egg production in birds. Antibiotics such as bacitracin, tetracycline, oxytetracycline, chlorotetracycline have been incorporated into poultry feed formulations usually at low prophylactic level to prevent minor diseases and enhance efficient growth (Smith, 2005). The feeds for poultry production are composed largely of grains such as corn, wheat or barley, oil seeds, cake meal, sunflower seeds, peanuts, cotton seed and protein products of animal origin such as fish meal, meat and bone meal, slaughter house offal's and feather meals (Bale *et al*., 2002).

The poultry industries rely on the supply of ready-to use feed from feed mills (Aganaga *et al*., 2000). These packaged feeds from feed mills constitute the main source of feeds for poultry farmers. Livestock (poultry) get infected when pathogenic organism passes to the susceptible animal through feeding (Barnes *et al*., 2003). Consequently, poultry feed has been implicated in several poultry diseases with varied pathological manifestations. These diseases might be of viral (Avian influenza, Newcastle disease), bacterial (Salmonellosis and infectious coryza) or fungal origin. The involvement of poultry feeds in the transmission of aflatoxicosis, which is the most prevalent and economically significant mycotoxin, represents also the main concern to the poultry farmers and extended consumers (Aliyu *et al*., 2016).

From an ecological point of view, harvested grains are not only ingredients for livestock diets, but can act as substrate for the transmission of vectors of simple unicellular prokaryotic and eukaryotic organisms. Feeds may contain diverse microflora that is acquired from environmental sources, including dust, soil, water, and insects. Feed materials may be inoculated at any time during growing, harvesting, processing, storage and transportation of the feed.

The microorganisms can affect feed quality negatively including reducing dry matter and nutrients, causing musty or sour odours, and causing caking of the feed and producing toxins. Finally, feed can act as a carrier of animal and human pathogens.

The type of feed, processing treatments and storage conditions are some of the factors that influence the population levels and types of microorganisms present in poultry feeds (Zhao and Xiuping, 2014).

In Nigeria, Obi and Ozugbo (2007), Uwaezuoke and Ogbule (2008), Adebayo-Ttayo and Ettah (2010) independently reported the isolation of pathogenic bacterial genera and species in the poultry feed samples sold in Western and Eastern parts of the country.

The current research was conducted to determine the quality of poultry feeds collected from various poultry pens

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within Ilorin (Nigeria). Hence, it is necessary to assess the hygiene and safety of these feeds. Therefore, the objectives of the research were to determine the bacterial and fungal loads of poultry feeds; to isolate, to characterize, and to identify the bacterial and fungal species in these products; to determine the absence or presence, as well as the count of specific bacterial pathogens present in poultry feeds and to determine the antibiotic susceptibility patterns of the bacterial isolates.

Materials and Methods

Collection of poultry feeds and the counting of microorganisms

The poultry feeds were collected from 20 different poultries located in Ilorin metropolis. They were aseptically collected using sterile spatula into sterile black polythene bags. The collected feeds were coded A to T.

One gramme of the feed sample was serially diluted and 1 ml aliquot was inoculated into sterile Petri dishes using pour plate technique. Nutrient agar was used for the bacteriological analysis.

For fungal counts, 0.1 ml of the serially diluted aliquot was plated using spread plate method. The sterile potato dextrose agar supplemented with streptomycin was used for fungal isolation. After incubation, the colonies were counted and expressed in cfu/g (Fawole and Oso, 2004).

Total and faecal coliform counts

Isolation of total and faecal coliforms were carried out by inoculating 0.1 ml of aliquot from 10^{-1} to 10^{-3} dilutions on MacConkey agar (MA) and eosin methylene blue agar (EMB) plates respectively using spread plate method. After the incubation period, typical pinkish colonies was counted and recorded as total coliform on MA, while colonies with greenish metallic sheen were counted and recorded as faecal coliform (*E. coli*). The colonies were further confirmed by biochemical tests (Fawole and Oso, 2004).

Isolation of specific pathogenic bacteria

In order to observe the pathogenic bacteria *Staphylococcus aureus, Salmonella, Shigella* and *Pseudomonas aeruginosa* mannitol salt agar, Salmonella-Shigella agar and cetrimide agar respectively were used. Plating of 0.1 ml aliquot from 10^{-1} to 10^{-3} dilutions was done using spread plate method. After incubation, typical colonies were observed on the media and they were further confirmed by suitable biochemical tests (Collins and Lyne, 1970; Willey *et al*., 2008).

Then, isolation of pure culture of microorganisms was performed by subculturing until pure culture was obtained. The pure cultures were then stocked in agar slant and kept in a refrigerator at 4-8 °C (Fawole and Oso, 2004).

Characterization and identification of isolates

The bacterial isolates were characterized and identified mainly on the basis of their colonial morphology, cellular morphology and biochemical reactions. Identification was based on standard texts such as Cowan and Steel (2005).

The fungal isolates were identified based on their macroscopic and microscopic features and making reference to standard texts (Onions *et al*., 1981).

Antibiotics susceptibility test

Normal saline broth culture of each bacterium was prepared and standardized using 0.5 MacFarland's standard. The standardized inocula were then used to seed the surface of a plate of Mueller Hinton agar and antibiotic disc was placed on the surface of the inoculated medium. Incubation was done at 37 °C for 24 hours after which the diameter of the zone of inhibition was measured in mm (CLSI, 2005).

Statistical analyses

Statistical analysis package SPSS 15.0 was used to determine the mean, the range and the standard deviation. The differences within the means were expressed using one way analysis of variance (SPSS, 2010) and the means were compared by Duncan's test, $\alpha \leq 0.05$.

Results

Microbial counts of poultry feeds

The bacterial and fungal counts of the poultry feeds ranged from 5.0×10^3 to 1.76×10^6 cfu/g and 3.5×10^4 to 1.59×10^5 cfu/g respectively (Table 1). The total coliform and *Staphylococcus aureus* counts ranged from zero to 3.0×10^5 cfu/g and 1.9×10^3 to 5.5×10^5 cfu/g respectively. There was a complete absence of faecal coliform, *Salmonella, Shigella* and *Pseudomonas aeruginosa* from the poultry feeds (Table 2).

Characterization and identification of isolates

After characterization, the bacterial isolates *Streptococcus salivarius*, *Streptococcus pyogenes*, *Mirococcus luteus, Micrococcus varians, Micrococcus roseus, Staphylococcus aureus, Staphylococcus saprophyticus* and *Staphylococcus hominis* were identified (Table 3). Likewise, fungi such as

Table 1. Total bacterial and fungal counts of poultry feeds

Sampling sites	Counts (cfu/g) × 10^4	
	Bacteria	Fungi
A	22.0±3.0^{c}	13.3±2.0^{de}
B	10.5±1.0^{b}	15.8±2.0^{ef}
C	4.9±1.0^{ab}	15.9±3.0^{ef}
D	3.9±0.5^{ab}	12.4±2.0^{cde}
E	0.5±0.0^{a}	9.3±2.0^{bc}
F	3.3±0.3^{ab}	19.0±4.0^{f}
G	0.8±0.1^{a}	8.5±1.0^{bc}
H	10.0±2.0^{b}	6.0±1.0^{ab}
I	21.0±2.0^{c}	7.7±2.0^{b}
J	40.0±5.0^{d}	8.6±2.0^{bc}
K	176±10.0^{j}	9.0±2.0^{bc}
L	5.0±1.0^{ab}	7.5±1.0^{b}
M	92.0±8.0^{h}	9.9±3.0^{bcd}
N	39.0±4.0^{d}	10.1±3.0^{bcd}
O	49.0±4.0^{ef}	6.1±1.0^{ab}
P	56.0±5.0^{f}	8.1±2.0^{b}
Q	41.0±4.0^{d}	8.6±2.0^{bc}
R	63.0±5.0^{g}	8.1±2.0^{b}
S	44.0±4.0^{de}	3.5±0.0^{a}
T	51.0±5.0^{ef}	6.1±1.0^{ab}

Values followed by the same superscript within the same column are not significantly different at $\alpha \leq 0.05$ based on Duncan's multiple range test

Table 2. The pathogenic bacteria in the poultry feeds

Samples	Counts (cfu/g) × 10^3				
	Total coliform	Faecal coliform	*S. aureus*	*Salmonella/Shigella*	*P. aeruginosa*
A	9.1±1.0^{ab}	0.0±0.0^{a}	2.9±0.2^{a}	0.0±0.0^{a}	0.0±0.0^{a}
B	41.0±4.0^{de}	0.0±0.0^{a}	3.2±0.2^{a}	0.0±0.0^{a}	0.0±0.0^{a}
C	32.0±3.0^{cd}	0.0±0.0^{a}	3.0±0.2^{a}	0.0±0.0^{a}	0.0±0.0^{a}
D	32.0±5.0^{cd}	0.0±0.0^{a}	12±2.0^{c}	0.0±0.0^{a}	0.0±0.0^{a}
E	1.1±0.2^{a}	0.0±0.0^{a}	2.9±0.3^{a}	0.0±0.0^{a}	0.0±0.0^{a}
F	100.0±10.0^{g}	0.0±0.0^{a}	3.2±0.2^{a}	0.0±0.0^{a}	0.0±0.0^{a}
G	0.0±0.0^{a}	0.0±0.0^{a}	3.8±0.2^{ab}	0.0±0.0^{a}	0.0±0.0^{a}
H	0.0±0.0^{a}	0.0±0.0^{a}	55±5.0^{e}	0.0±0.0^{a}	0.0±0.0^{a}
I	21.0±3.0^{bc}	0.0±0.0^{a}	2.9±0.2^{a}	0.0±0.0^{a}	0.0±0.0^{a}
J	52.0±5.0^{e}	0.0±0.0^{a}	2.8±0.2^{a}	0.0±0.0^{a}	0.0±0.0^{a}
K	200.0±1.5^{h}	0.0±0.0^{a}	7.2±1.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}
L	8.2±1.0^{ab}	0.0±0.0^{a}	51±5.0^{d}	0.0±0.0^{a}	0.0±0.0^{a}
M	20.0±2.0^{bc}	0.0±0.0^{a}	2.6±0.1^{a}	0.0±0.0^{a}	0.0±0.0^{a}
N	32±3.0^{cd}	0.0±0.0^{a}	1.9±0.2^{a}	0.0±0.0^{a}	0.0±0.0^{a}
O	300.0±30.0^{i}	0.0±0.0^{a}	3.4±0.2^{a}	0.0±0.0^{a}	0.0±0.0^{a}
P	55.0±5.0^{e}	0.0±0.0^{a}	12±2.0^{c}	0.0±0.0^{a}	0.0±0.0^{a}
Q	27.0±3.0^{cd}	0.0±0.0^{a}	2.6±0.1^{a}	0.0±0.0^{a}	0.0±0.0^{a}
R	56.0±6.0^{e}	0.0±0.0^{a}	2.8±0.2^{a}	0.0±0.0^{a}	0.0±0.0^{a}
S	200.0±20.0^{h}	0.0±0.0^{a}	3.7±0.3^{ab}	0.0±0.0^{a}	0.0±0.0^{a}
T	75.0±5.0^{f}	0.0±0.0^{a}	83.0±5.0^{f}	0.0±0.0^{a}	0.0±0.0^{a}

Values followed by the same superscript within the same column are not significantly different at $\alpha \leq 0.05$ based on Duncan's multiple range test

Table 3. The characterization and the identification of bacterial isolates

Bacterial isolates	Gram reaction	Cell shape	Cells' arrangement	Motility	Oxidase	Catalase	Coagulase	Haemolysis	Starch hydrolysis	Citrate	Indole	Urease	Glucose	Lactrose	Sucrose	Maltose	Mannitol	Xylose	Arabinose	Cellobiose	Adonitol	Inositol	Fructose	OF	Gelatin	Probable identity
1	+	C	Cl	-	-	+	+	γ	-	-	+	-	Ag	A	A	A	A	-	+	-	-	+	A	F	-	*Staphylococcus aureus*
2	+	C	Cl	-	-	+	-	γ	+	-	+	+	Ag	-	-	-	-	-	+	-	+	+	Ag	F	+	*Staphylococcus saprophyticus*
3	+	C	Ch	-	+	-	-	β	+	-	-	+	A	A	A	A	-	-	-	-	-	-	-	O	+	*Streptococcus pyogenes*
4	+	C	Cl	-	+	-	-	-	+	-	-	+	A	A	A	A	-	+	-	-	-	+	-	F	+	*Streptococcus salivarius*
5	+	C	S	-	+	+	-	β	+	+	+	-	Ag	Ag	Ag	Ag	-	-	+	+	+	+	Ag	O	-	*Micrococcus luteus*
6	+	C	Cl	-	+	+	-	α	+	-	+	+	Ag	Ag	-	Ag	-	+	+	+	+	+	Ag	F	-	*Micrococcus varians*
7	+	C	S	-	+	+	-	α	+	-	+	+	Ag	Ag	Ag	Ag	Ag	+	+	-	-	+	-	-	+	*Micrococcus roseus*
8	+	C	S	-	-	+	-	γ	+	+	+	+	Ag	Ag	A	Ag	-	+	+	-	-	+	Ag	F	-	*Staphylococcus hominis*

Table 4. The occurrence of bacterial isolates in the poultry feeds

Sampling sites	B1	B2	B3	B4	B5	B6	B7	B8
A	+	+	-	-	-	-	-	+
B	+	+	-	-	-	-	-	-
C	+	-	+	-	-	-	-	-
D	+	-	+	-	-	-	-	-
E	+	+	-	-	-	-	-	-
F	+	+	-	+	-	-	-	+
G	+	+	+	+	-	-	-	+

H	-	+	+	+	-	-	-	+
I	+	+	+	-	-	-	-	-
J	+	+	+	-	-	-	-	-
K	+	-	+	-	+	-	+	+
L	+	-	+	-	+	+	-	+
M	+	+	+	-	+	+	+	-
N	+	+	+	+	-	+	+	-
O	+	+	+	-	+	+	-	-
P	+	+	+	-	+	+	-	-
Q	+	+	+	+	-	-	-	-
R	+	-	-	+	+	+	-	-
S	+	+	+	+	+	+	-	-
T	+	+	+	-	-	+	-	-

Keys:+ = isolated; - = Not isolated; B1 = *Staphylococcus aureus*; B2 = *Staphylococcus saprophyticus;* B3 = *Streptococcus pneumoniae*; B4 = *Streptococcus salivarius*; B5 = *Micrococcus luteus*; B6 = *Micrococcus varians*; B7 = *Micrococcus roseus;* B8 = *Staphylococcus hominis*

Table 5. The occurrence of fungal isolates in the poultry feeds

Sampling sites	F1	F2	F3	F4	F5	F6	F7
A	+	+	-	-	-	-	-
B	+	-	+	-	-	-	-
C	+	+	-	+	-	-	-
D	+	+	-	+	+	+	+
E	+	+	+	-	-	+	+
F	+	+	+	-	+	-	+
G	+	-	+	-	-	-	+
H	+	+	-	-	-	+	+
I	+	+	-	+	+	+	-
J	+	+	-	+	-	+	-
K	+	+	+	+	-	+	+
L	+	+	-	-	+	+	+
M	+	-	+	+	+	-	+
N	+	-	+	+	+	+	+
O	+	-	-	+	+	+	+
P	+	+	+	+	+	-	+
Q	+	+	+	+	+	-	-
R	+	+	-	-	-	+	+
S	+	+	+	+	+	+	+
T	+	+	+	+	-	-	+

Keys:+ = isolated; - = Not isolated; F1 = *Saccharomyces cerevisiae;* F2 = *Fusarium oxysporum*; F= *Penicillium* sp.; F4 = *Humicola grisea*; F5=*Aspergillus fumigatus*; F6=*Hansenula* sp.; F7=*Humicola fuscoatra*

Table 6. The antibiotics susceptibility patterns of bacterial isolates

Bacterial isolates	Diameter of zone of inhibition (mm)							
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
Staphylococcus aureus	-	-	23	-	15	-	24	-
Staphylococcus saprophyticus	-	-	20	-	-	-	24	-
Streptococcus pyogenes	-	-	21	-	24	-	30	-
Streptococcus salivarius	-	22	30	-	27	-	28	36
Micrococcus luteus	-	-	20	-	31	32	34	-
Micrococcus varians	-	-	18	-	18	-	26	-
Micrococcus roseus	-	-	15	-	22	-	34	-
Staphylococcus hominis	-	-	18	-	-	-	22	-

Keys: - absence of zone of inhibition; CAZ, Ceftazidime 30 µg; CRX, Cefuroxime 30 µg; GEN, Gentamicin 10 µg; CTR, Ceftriaxone 30 µg; ERY, Erythromycin 5µg; CXC, Cloxicillin 5 µg ;OFL, Ofloxacin 5 µg; AUG, Amoxycillin-Clavulinate 30 µg

Saccharomyces cerevisiae, Fusarium oxysporum, *Penicillium* sp., *Humicola grisea, Aspergillus fumigatus, Hansenula* sp. and *Humicola fuscoatra* were identified, based on their macroscopic and microscopic features.

Antibiotics susceptibility patterns of the bacterial isolates

All the bacterial isolates were susceptible to gentamicin and ofloxacin, but resistant to ceftazidime and cefuroxime. Each bacterial isolate was resistance to at least 3 of the antibiotics used (Table 6).

Discussion

Animal feed has been listed as one of the main sources of microbes in farm animals and poultry. The high occurrence of fungal and bacterial species is of public health concern and this may indicate obvious health hazard in terms of direct consumption of contaminated feed or their toxins by farm animals (Aliyu *et al*., 2016). The high counts of fungi in the current study indicate that more attention is needed in the storage strategies employed by the poultry feed manufacturers, or with the warehouse condition, as well as with handling of products and duration of storage. Arotupin *et al*. (2007) obtained bacterial and fungal count in the range of 6.6×10^2 - 2.5×10^4 and $1.5 \times 10^2 - 7.4 \times 102$ cfu/g respectively.

All the poultry feed samples examined showed the presence of microorganisms which included: *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, *Streptococcus salivarius*, *Micrococcus luteus, Micrococcus varians*, *Micrococcus roseus* and *Staphylococcus hominis*. The presence of these microorganisms in the poultry feeds suggest that the feeds contain sufficient nutrients for the growth of the isolated organisms. The activities of these microorganisms on the feeds under the study may cause degradation, thereby reducing the nutrients for the livestock. The present findings are in agreement with the report of Aganaga *et al*. (2000) on poultry feeds and the sensitivity pattern of the associated microorganisms. These microorganisms may probably have originated from the raw materials from which the feeds were produced. In addition, most of the isolated microorganisms owned their origin from air and soils (Arotupin and Akinyosoye, 2001). Hancock *et al*. (1998) reported microbial contamination of poultry feeds of plant and animal origin to be due to climatic conditions encountered, harvesting, processing, storage and transport technologies employed.

The test for specific bacterial pathogens revealed the high presence of total coliforms and *Staphylococcus aureus*. The presence of *Staphylococcus aureus*, a normal floral of the skin and nose suggests improper handling practices (Hancock *et al*., 1998), while members of total coliforms had probably environmental origin. Dhand *et al*. (1998) and Hancock *et al*. (1998) separately implicated *Micrococcus luteus* and *Staphylococcus aureus* in the microbial infection outbreak of poultry farming. The isolation of toxigenic mould, *Aspergillus fumigatus*, should be viewed with serious concern. This organism has been documented to be the most dominant of all the fungi in respect of aflatoxin production in poultry feeds (Henzler and Opitz, 1992).

Most of the bacterial isolates were resistant to at least one or more antibiotics especially ceftazidime, cefuroxime, ceftriaxone, cloxacillin and augmentin. However, they were susceptible to gentamicin and ofloxacin to different extents. Khan *et al*. (2002) reported the isolation of erythromycin resistant Staphylococci, Enterococcci, and Streptococci from litters samples collected from poultry houses that added antibiotics to their feeds.

Aseptic handling and good storage condition which may prevent frequent exposure to the atmosphere is the basic step needed to minimize the contamination of feed product. High quality ingredients such as grains should be used for the manufacture of the poultry feeds.

Conclusions

The poultry feeds analysed in the hereby study contained high counts of bacteria and fungi. Specific pathogenic bacteria test revealed the presence of total coliforms and *Staphylococcus aureus* in addition to the isolation of aflatoxigenic fungus, *Aspergillus fumigatus*, in the poultry feeds. The poultry feeds investigated were free of faecal coliform, *Salmonella, Shigellla*, and *Pseudomonas* sp.

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