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ARTICLE

Antibiogram of Aerobic Bacteria Isolated from Poultry Feeds in Zaria, Nigeria

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ABSTRACT

Poultry feeds are known to constitute a source of bacterial infection to birds. This study was conducted to determine the presence and antimicrobial resistance of aerobic bacteria in poultry feeds in Zaria, Nigeria. Fifty-two feed samples were collected from twelve different sources which included self-compounded poultry feeds, commercial poultry feeds at sales point and feeds from feed mills. The samples were analyzed by plating unto Blood, MacConkey, Eosin Methylene Blue and Brilliant Green Agar. Bacteria isolated from the samples were *Citrobacter diversus, Escherichia coli, Klebsiella pneumoniae, Morganella morganii, Salmonella typhi and Staphylocuccus aureus*. *Citrobacter diversus* 50(92.59 %) was the most frequently isolated bacterium, followed by *Escherichia coli* 39(72.22 %), *Morganella morganii* 16(29.62 %), *Klebsiella pneumoniae* 13(24.07 %), *Salmonella typhi* 4(7 .40%) and *Staphylococcus aureus* 1(1.85 %).The isolates were tested against 10 anti-microbial agents:amoxycillin/clavulanic acid, chloramphenicol, ciprofloxacin, enrofloxacin, erythromycin, gentamicin, neomycin, nitrofurantoin, sulphamethoxazole/trimethoprim and tetracycline, using the Kirby-Bauer disc diffusion method. The result of this research showed the presence of aerobic bacteria and also the presence of resistant strains of bacteria to certain antibiotics in poultry feed samples.Strict hygienic practices and biosecurity measures should be instituted and maintained both at feed mills and poultry farms so as to eliminate contamination of poultry feeds.

Key words: Poultry feed, Aerobic bacteria, Antibiotic resistance

INTRODUCTION

Poultry feeds are food materials formulated to contain all nutritional needs for proper growth, meat and egg production in birds (Obi and Ozugbo, 2007). Thus, there are various types of feeds depending on what they are designed to achieve in the birds e.g. starters, growers, finishers and layers (Fagbenro and Adebayo, 2000). An important part of raising poultry is feeding. Feeding accounts for the major cost of production and good nutrition is reflected in the bird's performance and its products (Fagbenro and Adebayo, 2000). Poultry feed ingredients include energy concentrates such as corn, oats, wheat, barley, sorghum, and milling by-products. Protein concentrates include soybean meal and other oilseed meals (peanut, sesame,

safflower, sunflower), cottonseed meal, animal protein sources (meat and bone meal, dried whey, fish meal), grain legumes such as dry beans and field peas, and alfalfa (Fagbenro and Adebayo, 2000). In an effort to achieve fast growth rate to meet increasing demand for poultry meat and other poultry products, large quantities of nitrogenous waste fortified with othersupplements such as spent grain, cassava waste, bone meal and blood meal are compounded as feed (Okpokwasili and Ogbuile, 1993).

Reports by Gill and Best (1998) and Ruff (1992) have listed animal feed as one of the sources of microrganisms (including bacteria) to animals. Various animal diseases like yersiniosis and campylobacteriosis have been traced to contaminated animal feed (Healing and Greenwood, 1991). During 1968, frozen chickens from a packing station in Cheshire, England, were implicated in a large outbreak of infection with *Salmonella enterica* serotype Virchow (Semple *et al.,* 1968). Investigation showed that the hatchery and the majority of rearing farms that supplied the packing station contained chickens colonized with *S. enterica* serotype Virchow, and the organism was isolated from feed fed to the chickens (Pennington *et al.,* 1968).

The presence of bacteria in poultry feeds could pose great economic problem to the poultry farmer in terms of morbidity and mortality in the poultry flock and also the increased cost of production due to purchase of antibiotics in an attempt to treat the bacterial disease that may ensue. This study was therefore designed to achieve isolation, identification and antimicrobial susceptibility of aerobic bacteria from poultry feeds in Zaria, Nigeria.

MATERIALS and METHODS

Preliminary survey

A preliminary field survey was carried out to identify commercial poultry feed mills and depots; then private farms involved in self-compounding of feeds in Zaria. Two commercial feed mills,five commercial poultry feed retail outlets and five farms involved in self-compounding of poultry feed were identified.

Study area

This study was carried out in Zaria metropolis, situated in the Northern Guinea Savannah Zone of Nigeria between latitudes 11 ^{0}N and 12 ^{0}N and longitudes of 7 ^{0}E and 8 ^{0}E.

Sample collection

A total of 52 samples were collected from the list of 12 selected poultry feed sources identified following preliminary field survey. Each selected poultry feed source was visited twice for sample collection during the study period (between the months of November, 2011 and March, 2012).Paired sample collection was done to determine whether time factor affected bacterial composition. In all, 52 samples were obtained by random sampling without replacement.

Each feed type (chick mash, broiler starter, grower, broiler finisher and layer mash) was sampled by carefully opening a bag of each and collecting about 20 grams using a sterile polyethene bag and a repeat sample was collected after two weeks. The samples were labelled and aseptically transported to the Microbiology laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, within two hours of collection for microbiological analysis.

Culture, isolation and identification of bacteria

In the laboratory,five grammes of each sample was homogenized in 45 ml of peptone water and incubated for 24 hours at 37 ^{0}C. Bacterial presence was demonstrated by subculturing from the enrichment cultures ontoblood agar, MacConkey agar, brilliant green agar and eosin methylene blue agar for preliminary identification. The blood agar was to allow any bacterium present to grow being an enriched medium. This medium also served to show the presence of haemolytic *Staphylococcus* by discolourations around the colonies where β haemolysis was observed because there was

complete clearance around colonies. MacConkey and briliant green agar served to differentiate the lactose from non-lactose fermenters that were present. Colonies of lactose fermenters (*Escherichia coli, Citrobacter diversus* and *Klebsiella pneumoniae*) on MacConkey agar appeared pinkish while colonies of the non-lactose fermenters (*Salmonella typhi* and *Morganella morganii*) appeared colourless. The opposite was true with brilliant green agar as colonies of lactose fermenters appeared colourless to yellowish while those of non-lactose fermenters appeared pinkish to reddish. In addition, colonies of *Klebsiella* on both media also appeared large, heaping and moist. Eosin methylene blue agar distinguished *E. coli* where the colonies appeared small to medium-sized with raised dark centres and the characteristic greenish sheen around colonies.

Secondary cultures of individual bacteria were obtained on brilliant green agar and eosin methylene blue to get pure cultures of suspected members of the family *Enterobacteriaceae.* Growths obtained after incubation were described macroscopically, stating their size, colour, shape, effect on media, smell, texture. After isolation, Gram staining and biochemical characterization, the individual species were identified using the interpretative key of some common genera of bacteria by Cowan, 1993 and Cheesbrough, 2006.

Antibiotic sensitivity tests

Antibiotic sensitivity tests were carried out on the various bacteria isolated. This was done using the disc diffusion method as explained by Kirby-Bauer (Bauer *et al.,* 1966) and the interpretation of results was by the standards laid down by the Clinical and Laboratory Standards Institute (CLSI), 2010. The *in-vitro* antibiotic testing of bacteria was done on Mueller-Hinton agar where an aliquot of the bacteria was made to an equivalent concentration of 10^8 cfu/ml by MacFarland's method; this was smeared on the surface of the medium before placing the antibiotic discs and incubating for 24 hours before taking the readings of the diameter zones of inhibition using a centimeter rule.

RESULTS

The bacteria were isolated with the following frequencies: *Citrobacter diversus* 50(92.59 %) was the most frequently isolated bacterium comprising 16 isolates from feed mills, 17 from self-compounded feed and 17 from retail outlets. This was followed by *Escherichia coli* 39 (72.22 %) comprising 12 isolates from feed mills, 18 from self-compounded feeds and 9 from retail outlets. *Morganella morganii* 16(29.62 %) was next and it composed of 6 isolates from feed mills, 6 from self-compounded feeds and 4 from retail outlets. The next bacterium was *Klebsiella pneumoniae* 13(24.07 %) comprising 5 isolates from feed mills, 4 from self-compounded feeds and 4 from retail outlets. Next was *Salmonella typhi* 4 (7.40%) all of which were from self-compounded feeds; and *Staphylococcus aureus* 1(1.85 %) from one of the feed mills.

Table II showed the results of Gram's reaction and biochemical tests on the bacterial isolates. All the bacteria were Gram negative except *Staphylococcus aureus* that was Gram positive. All the bacteria were fermenters on oxitative/fermentative medium; only *Salmonella typhi* produced H_2Sontriple sugar iron agar and all the bacteria produced gas from glucose fermentation except *Salmonella typhi*. From Table III, the bacteria showed 100 % sensitivity to ciprofloxacin and gentamicin. Chloramphenicol, enrofloxacin, were the next set of antibiotics that the bacteria were most sensitive to. All the bacteria tested were resistant to erythromycin. The bacteria showed variable sensitivity to amoxycillin/clavulanicacid, neomycin, sulphamethoxazole/trimethoprim and tetracycline. The antibiotics susceptibility test carried out on *Citrobacter diversus* revealed that the widest zone of inhibition (28 mm) was shown by ciprofloxacin; this was followed by chloramphenicol that produced 26 mm zone of inhibition. Enrofloxacin produced 22 mm, gentamicin, neomycin and tetracycline

produced 16 mm each; amoxicillin, erythromycin and nitrofurantoin showed resistance. However, *E. coli* was resistant to erythromycin, sulphamethoxazole/trimethoprim and tetracycline; modearately sensitive to amoxicillin and neomycin but sensitive to chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin and nitrofurantoin. *Staph. aureus* was sensitive to chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, nitofurantoin and tetracycline; while it was moderately so to neomycin and sulphamethoxasole/trimethoprim but resistant to amoxicillin and erythromycin.

TABLE I: SOURCES AND FREQUENCY OF ISOLATION OF BACTERIA FROM POULTRY FEED SAMPLES COLLECTED IN ZARIA, NIGERIA

Bacteria	Number of bacteria isolated and frequency of isolation (%)			
	Feed mills	Self- compounded	Retail outlets	Total
Citrobacterdiversus	16 (100.00)	17 (94.44)	17 (85.00)	50(92.59)
Escherichia coli	12 (75.00)	18 (100.00)	9 (45.00)	39(72.22)
Klebsiellapneumoniae	5 (31.25)	4 (22.22)	4 (20.00)	13(24.07)
Morganellamorganii	6 (37.50)	6 (33.33)	4 (20.00)	16(29.62)
***Salmonella*Typhi**	0 (0.00)	4 (22.22)	0 (0.00)	4(7.40)
Staphylococcusaureus	1 (6.26)	0 (.00)	0 (0.00)	1(1.85)

TABLE II: GRAM'S REACTION AND BIOCHEMICAL TESTS ON THE BACTERIAL ISOLATES FROM POULTRY FEEDS IN ZARIA, NIGERIA.

Tests	*Citrobacter diversus*	*Morganella morgani*	*Escherichia coli*	*Salmonella typhi*	*Klebsiella pneumoniae*
Gram reaction	??	??	??	??	??
Oxidative Fermentative test	F	F	F	F	F
H_2S from TSI	??	??	??	+	??
Indole	+	+	+	??	??
Urease	+	+	??	??	+
Citrate	+	??	??	+	+
MR	+	+	+	+	+
Vp	??	??	??	??	??
Motility	+	+	+	+	??
Gas from glucose	+	+	+	??	+
Acid from lactose	+	??	+	??	+

Key: F= fermenter; O= Oxidizer; MR= Methyl Red; VP= Voges-Proskauer.

Staphylococcusaureus was haemolytic on blood agar, Gram positive cocci occuring in grape-like clusters, catalase positive and coagulase positive.

DISCUSSION

One Gram-positive bacterium (*Staphylococcus aureus*) and five Gram-negative bacteria (*Citrobacter diversus, Escherichia coli, Klebsiellapneumoniae, Morganella morganii and Salmonella* typhi*)* were isolated from poultry feeds in Zaria, Nigeria. These aerobic bacteria were found in both commercial and self-compounded poultryfeeds.This widespread distributioncould be attributed to the ubiquitous nature of the bacteria. The number andtype of food-borne microorganisms isolated can be used todetermine the quality and purity of poultry feeds.In this research, members of the family *Enterobacteriaceae* were the most frequently isolated bacteria. This may be due to the activities of humans, faecal contaminaton, the ability of the organism to survive in a wide range of habitats. Ezekiel et al., 2011;Furuta et al.,1980; Obi and Ozugbo, 2007 and Okonko et al.,2010 all found members of the family *Enterobacteriacae* to be the most frequently isolated bacteria in their works. According to Maciorowskiet al.(2007),the primary mode of contamination of feed materials is the transference of soil by wind, rain, mechanical agitation, or insects.Dust, according to Butcher and Richard, (2011) is the major source of *Salmonella* contamination in feed mills. *Staphylococcus* is known to be easily carried in thenasopharynx, throat, skin, cuts, boils, nails of man and as such humans can easily be a source of poultry feed contamination.

TABLE III: ANTIMICROBIAL SENSITIVITY OF BACTERIA ISOLATED FROM POULTRY FEEDS IN ZARIA, NIGERIA

Antibiotic	Concentration (µg)	*C. diversus*	*M. morganii*	*E. coli*	*S. aureus*	*S.* Typhi	*K. pneumoniae*
		Zone of inhibition in mm (Interpretation)					
AMC	30	12(R)	16(I)	16(I)	14(R)	18(S)	10(R)
C	30	26(S)	16(I)	20(S)	28(S)	20(S)	24(S)
CIP	5	28(S)	32(S)	30(S)	30(S)	22(S)	26(S)
ENR	5	22(S)	22(S)	24(S)	28(S)	12(R)	20(S)
E	5	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
CN	10	16(S)	24(S)	18(S)	18(S)	22(S)	20(S)
N	10	16(S)	18(S)	14(I)	12(I)	18(S)	16(S)
F	50	10S(R)	20(S)	22(S)	26(S)	20(S)	10(R)
SXT/TMP	25	20(S)	20(S)	0(R)	12(I)	0(R)	20(S)
TET	20	16(S)	8(R)	7(R)	28(S)	16(S)	16(S)

Key:AMC=amoxicillin/clavulanic acid, C=Chloramphenicol, E=Erythromycin, TE=Tetracycline, SXT=Sulphamethoxazole, N=Neomycin, F=Nitrofurantoin, ENR=Enrofloxacine, CIP=Ciprofloxacine, CN=Gentamycin, S= Susceptible/Sensitive, I= Intermediate, R= Resistant.

Escherichia coli and *Salmonella typhi* were observed more in self-compounded feeds. Locally compounded poultry feedshave been implicated in the spread of *Salmonella sp*. among poultry and possible transmission to humans (Okonko *et al.,* 2010). Thepresence of these microorganisms in thepoultry feeds suggestedpoor hygienic and sanitary practicesemployed in the processing and packagingof the feeds.The presence of these

two organisms (*E. coli* and *Salmonella typhi)* demonstrated a potential healthrisk as the organismsare pathogenic and can cause disease in humans and animals; even though *S.* Typhi is a strict human pathogen and has not been reported to cause disease in animals. Different strains of *E. coli* can cause a wide variety of disease syndromes in animals including septicemia, swollen head syndrome, cellulitis, and airsacculitis (Blood and Radostits, 1989). *E. coli* and *Salmonella* infections are usually associated with contaminated water and feeds and their presence suggests faecal contamination of both human and animal origin (Okonko *et al.,* 2010 and Prescott *et al*., 2005). Contamination may also be due to direct handling of the feed or feed ingredients without protective clothing during processing and/or packaging. Since most animal based protein ingredients are obtained from market sources with little or no sterilization, it is thus a strong possibility that contamination of these ingredients prior to compounding by the farmer could occur. The fact that no form of sterilization is usually carried out by the farmers during compounding allows for survival and proliferation of these bacteria. The presence of *Salmonella* and/or *E. coli* in poultry feeds observed in this study agreed with the works of Ezekiel, 2011; Furuta *et al.,*1980; Obi and Ozugbo, 2007; Okoli *et al.,* 2006; Okonko et al., 2010 and Maciorowski *et al.,* 2004 who also isolated same bacteria types in their works. Aside the two pathogens already discussed, other pathogens isolated in this study that could cause disease in poultry are *Morganella morganii* and *Staphylococcus aureus*. *Morganella morganii* has recently been reported to be associated with fatal infections in poultry (Zhao et al., 2012; Mamman *et al.,* 2014). *Staph. aureus* causes a few conditions in poultry the most important of which is bumble foot (Todar, 2011).

Pelletized commercial feeds showed the least isolation rate of *Escherichia coli* and none for *Salmonella typhi.* This is probably due to the heating process involved in pelletizing poultry feeds and the mechanised nature of processing which involves less human contact. This finding is in accordance with the work of Furuta et al., (1980). The type and incidence of bacteria isolated was not different in the paired samples collected. This was probably because the prevailing climatic condition during both sampling periods was the same (i.e. dry, harmattan season).

The antibiotic sensitivity tests generally showed resistance to erythromycin as all the organisms isolated were resistant to it. Since antibiotics were usually incorporated into poultry feeds, it would be logical to assume that every bacterial cell isolated from poultry feeds has the genes for emerging drug resistance (Okonko *et al.,* 2010). Susceptibility to the flouroquinolones was observed in all the organisms except resistance against enrofloxacin exhibited by *Salmonella typhi*. The bacteria isolated in this study were most suceptible to ciprofloxacin and gentamicin. This is probably due to less usage of these antibiotics in feeds leading to less exposure of the bacteria to the antibiotics. In addition, the drugs are bactericidal in their modes of action. Also, ciprofloxacin being a third generation drug; is known to maintain a high concentration in tissues and has an intracellular action (Aliu, 2007).

CONCLUSION and RECOMMENDATIONS

Poultry feeds in Zaria, Nigeria were found to be contaminated with aerobic bacteria such as: *Citrobacter diversus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Salmonella typhi* and *Staphylococcus aureus*. These bacteria were both sensitive to chloramphenicol, ciprofloxacin, enrofloxacin, and gentamicin but resistant to erythromycin. The bacteria showed variable sensitivity to amoxycillin/clavulanic acid, neomycin, sulphamethoxazole/trimethoprim and tetracycline. It is recommended that the farmer and manufacturers of commercial poultry feeds should ensure properhygiene when handling

poultry feed materials since most pathogenic bacteria contamination of poultry feeds could occur either during processing, packaging, and/or storage. They should ensure proper disposal of litter material, dust control in the feed milling facility, proper cleaning of equipments, proper dressing and personal hygiene of all involved in order to prevent contamination. Every feed mill should identify each critical control point, monitor that point for pathogens on a regular basis, and have a plan for corrective action if contamination is discovered. Management of poultry diseases should include antibiotic sensitivity tests. This is because the incidence of resistance is attributed to improper and indiscriminate use of antibiotics. This is important in preventing the occurence and spread of multi-drug resistant strains of bacteria.

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